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DECOLOURISATION OF REACTIVE ORANGE 16 (RO16) UNDER DIFFERENT PHYSICOCHEMICAL PARAMETERS BY LOCALLY ISOLATED DYE DEGRADING MICROBE BACILLUS SP UMK DG-1

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ABSTRACT

In this present study, a locally isolated dye degrading microbe *Bacillus* sp. UMK DG-1 was investigated for its capability to decolourise Reactive Orange 16 (RO16). The decolourisation assay was carried out under different physicochemical parameters, namely pH, temperature, agitation, and dye concentrations. Based on the results observed, the optimum condition for decolourisation of RO16 is at the alkaline condition (pH8–9), with a temperature of 37°C, and under the microaerophilic condition. Decolourisation activity also increased when a lower concentration of RO16 was used. Furthermore, the decolourisation of RO16 was analysed by a UV-Vis spectrophotometer to see changes in the UV-Vis spectra for untreated and decolourised RO16.

Keywords: decolourisation, RO16, dye degrading microbe, physicochemical parameters.

INTRODUCTION

The textile industry is heavily dependent on the application of chemical substances, especially synthetic dyes, which are used in the dyeing process. More than 8,000 chemical substances such as dyes and pigments are involved during the process. 10%–15% of dyes used during the dyeing process are not fixed to the fibre and thus released into effluent streams. This represents 2.8 x 10^5 tonnes of dyes discharged into the ecosystem yearly (Jin *et al*., 2007). The uncontrolled release of dyes to the environment draws a major concern due to their toxic nature. The untreated dye effluent is high in colour, biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, total organic carbon, pH, temperature, turbidity, and toxicity. It can cause adverse effects to the aquatic ecosystem by decreasing the photosynthesis rate and exhausting dissolved oxygen. The colourless amines, which are the breakdown products of dyes, are also toxic, carcinogenic, and mutagenic (Pang *et al*., 2013; Singh & Singh, 2017; Xu *et al.*, 2005).

Azo dyes, being the largest group of synthetic dyes used in the textile industry, are classified based on the number of azo groups present. They are monoazo, disazo, trisazo, tetrakisazo, and polyazo (Asad *et al.*, 2007; Lavanya, Dhankar, Chikara, & Sheoran, 2014). Approximately 80% of dyes used in the textile industry are azo dyes with more than 3,000 different varieties. Azo pigments are colourless pigments that form various colours by adding azo compounds. The biosynthesis of azo dyes is relatively easy as compared to natural dyes. Chemical stability and variety of colours are also properties that make azo dyes a more preferable choice (Chang *et al.*, 2004). The stability towards chemicals, heat, and temperature was conferred by the azo bonds, -N=N-, which is very hard to break (Joseph, Ogola, & Ashida, 2015; Lavanya *et al.*, 2014). Reactive Orange 16 (RO16) is classified to a group of reactive dyes or vinyl sulfone dyes. It is mainly used for the dyeing process of cellulose fibres (Anouar, Anouar, & Kacemi, 2014). RO 16 consists of two sulfonate groups that form negative charges when dissolved in water. Reactive dye is anionic in nature and soluble in water. Reactive dyes have a good washing capability and light fastness properties, and are relatively cheap as compared to natural dyes. During the dyeing process, approximately 10% to 20% of reactive dyes are hydrolysed, which contribute to coloured textile effluent (Chinta & Vijaykumar, 2013).

Microbial degradation of dyes has generated a lot of interest due to its potential for a complete degradation of dye waste as compared to other physical and chemical methods such as membrane filtration, coagulation, and flocculation. Major setbacks of physicochemical treatment of dyes are formation of sludge and membrane fouling due to the accumulation of dyes and organic matter (Dawood & Sen, 2014; Kharub, 2012; Verma, Dash, & Bhunia, 2012; Sharma, Saxena, & Gaur, 2014). Biological decolourisation and degradation of dyes can be achieved by several pathways, which are biosorption on cell biomass, biodegradation by cells, and biodegradation by enzymes. Biodegradation is considered as the most effective way for the decolourisation of dyes, which involves the breakdown or fragmentation of synthetic dyes into smaller or less harmful compounds (Joseph, Ogola, & Ashida, 2015). The aim of this study is to evaluate the decolourisation of RO16 by a locally isolated dye degrading bacteria, *Bacillus* sp. UMK DG-1. The decolourisation of RO16 by *Bacillus* sp. UMK DG-1 was carried out in several physicochemical parameters, which are pH, temperature, agitation, and dye concentration, to determine the optimum condition for the decolourisation activity.

MATERIALS AND METHOD

Bacterial strain

Bacillus sp. UMK DG-1 is a dye degrading bacteria isolated from a traditional batik factory in Kota Bharu. Kelantan, Malaysia. The strain was maintained on nutrient agar and minimal salt media supplemented with 0.1% (w/v) RO16 (Sigma Aldrich).

Minimal salt media

Minimal salt medium (MSM) was prepared using 2.0 g/L sodium chloride (NaCl), 0.42 g/L magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$), 0.29 g/L potassium chloride (KCl), 1.27 g/L dipotassium phosphate (K_2HPO_4), 0.42 g/L sodium nitrate ($NaNO_3$), 0.85 g/L monopotassium phosphate (KH_2PO_4), and 0.5 mL of 0.5 M ethylene diamine tetraacetic acid (EDTA). After that, 0.1% (w/v) of RO16 was added into the media. Then, 0.1 M sodium hydroxide (NaOH) and 0.1 M hydrochloric acid (HCl) were used to adjust the pH of the media to pH8.0, whereas distilled water was used to mark up the solution to 1 L. The media were sterilised by autoclaving at the temperature of 121°C and pressure of 15 psi for 15 minutes.

Inoculum preparation

Prior to the decolourisation assay, a loopful of bacterial colony from freshly grown culture was inoculated into 40mL Tryptic Soy Broth (TSB). The culture was incubated overnight at 30°C and 150 rpm until the culture reached optical density (OD) of 0.6 at 600nm.

Decolourisation of Reactive Orange 16 under different physicochemical parameters

The decolourisation of RO16 was carried out under different physicochemical parameters, namely pH, temperature, agitation, and dye concentration. 2% (v/v) of *Bacillus* sp. UMK DG-1 overnight culture (OD_{600} = 0.6) was inoculated into 200 mL of Luria broth supplemented with RO16. The culture was incubated for 72 hours. The optical density for RO16 was taken at a 12-hour interval. The percentage of decolourisation was calculated by the equation below:

$$\% \text{ Decolourisation} = \frac{A-B}{A} \times 100\%$$

A: Initial Absorbance
B: Final Absorbance

a. pH

Bacterial overnight cultures were inoculated separately into 200 mL of Luria broth (pH6, pH7, pH8, and pH9) supplemented with 0.1% (w/v) RO16. The cultures were incubated for 72 hours at 30°C under the static condition.

b. Temperature

Bacterial overnight cultures were inoculated into 200 mL of Luria broth supplemented with 0.1% (w/v) RO16. The cultures were incubated separately for 72 hours at 30°C and 37°C under the static condition.

c. Agitation

Bacterial overnight cultures were inoculated into 200 mL of Luria broth supplemented with 0.1% (w/v) RO16. The cultures were incubated separately for 72 hours at 30°C with150 rpm agitation and 30°C under the static condition.

d. Dye concentration

Bacterial overnight cultures were inoculated into 200 mL of Luria broth supplemented with 0.01%, 0.1%, and 1% (w/v) RO16. The cultures were incubated for 72 hours at 30°C under the static condition.

Decolourisation analysis

The UV-Vis spectrophotometer was used to analyse the UV-visible spectra (200–800nm) before and after the decolourisation of RO16 by *Bacillus* sp. UMK DG-1. Changes in the absorption spectrum in a visible range (400–700nm) were recorded and graphs were plotted.

RESULTS AND DISCUSSIONS

a. Decolourisation of RO16 at different physicochemical conditions

The decolourisation of RO16 by *Bacillus* sp. UMK DG-1 was carried out in different physicochemical parameters, namely pH, temperature, dye concentration, and agitation. The decolourisation of RO16 at a range of pH6–9 showed that the highest decolourisation percentage was achieved at the alkaline condition. 66.71% of RO16 was decolourised at pH9 after 24 hours and 66.68% of RO16 was decolourised at pH8 after 36 hours (Figure-1). This observation suggested that *Bacillus* sp. UMK DG-1 is an alkalophilic bacterium. The dyeing process involves a lot of alkaline substances and uses a very high concentration of NaCl. This condition only permitted the growth of extremophilic bacteria in the textile effluent. Several alkalophilic bacteria such as *Bacillus subtilis*, *Bacillus badius*, *Bacillus halodurans*, and *Sphingomonas paucimoboilis* were reported to be responsible for the decolourisation of Reactive Red M8B, Amaranth, Acid Black 24, and Methyl Red (Arulzhagan, 2016; Misal *et al.*, 2011; Prasad & Rao, 2014; Ayed *et al.*, 2011).

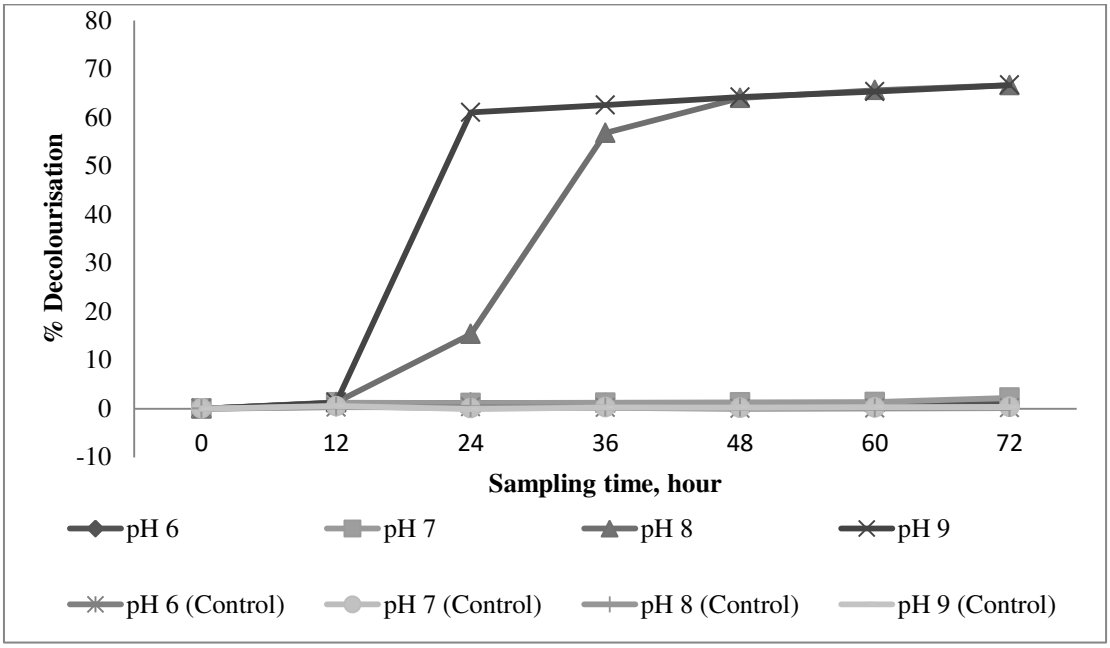

Figure-1. Decolourisation of RO16 by *Bacillus* sp. UMK DG-1 at different pH.

Incubation temperature is an important parameter in the biological treatment of textile effluent. This is because temperature affects the growth rate of cell and enzymatic reaction of dye degrading microbes in the removal of dyes (Gomaa, 2016; Shah, Patel, Nair, & Darji, 2013). Two different temperatures were analysed on the decolourisation activity of RO16 by *Bacillus* sp.UMKDG-1, which are 30°C and 37°C. It was found that a higher decolourisation rate was observed when the temperature was increased from 30°C to 37°C, which are 64.04% and 68.59%, respectively (Figure-2). The increase in dye removal efficiency might be due to higher kinetic energy or collision frequency and stronger sites of affinity for azo dye molecules to bind to active sites of enzymes that are responsible for the decolourisation of dye. Another possibility is that the increase in temperature causes higher number of binding sites on the adsorbent (Zuraida, Nurhaslina, & Halim, 2013). This is also due to the decolourisation rate of azo dyes that depend on the activation energy, in which the increase in temperature leads to an increase in kinetic energy of dye molecule to overcome the minimum energy that is required for a reaction to occur (Njuguna, 2013).

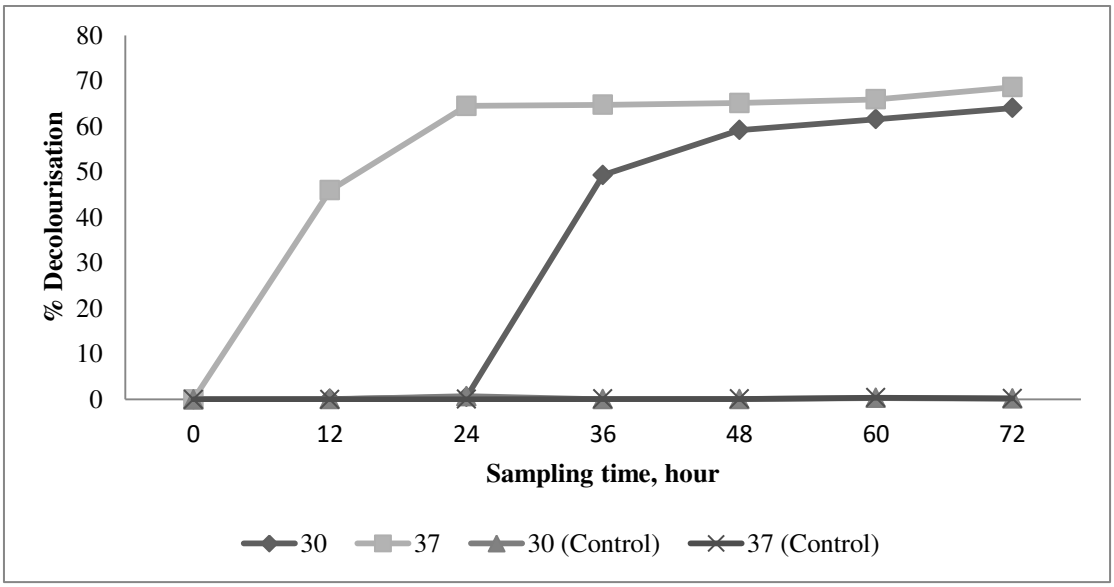

Figure-2. Decolourisation of RO16 by *Bacillus* sp. UMK DG-1 at different temperatures.

The decolourisation of RO16 by *Bacillus* sp. UMK DG-1 under agitation (150 rpm) and static conditions showed that the decolourisation activity was extremely reduced under the agitated condition. 69% of

RO16 was decolourised after 24 hours under the static condition, while only1.52% of RO16 was decolourised when the culture was agitated at 150 rpm (Figure-3). Gomaa *et al.*, (2016) reported the same observation for the decolourisation of reactive dyes by *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus cereus*, and *Pseudomonas* sp. isolated from environmental sludge. Decolourisation under a microaerophilic condition is commonly related to the activity of azoreductase. Azoreductase catalyses the reductive cleavage of azo bond (-N=N-) that occurs in the absence or low concentration of oxygen (Shah, Patel, Nair, & Darji, 2014).

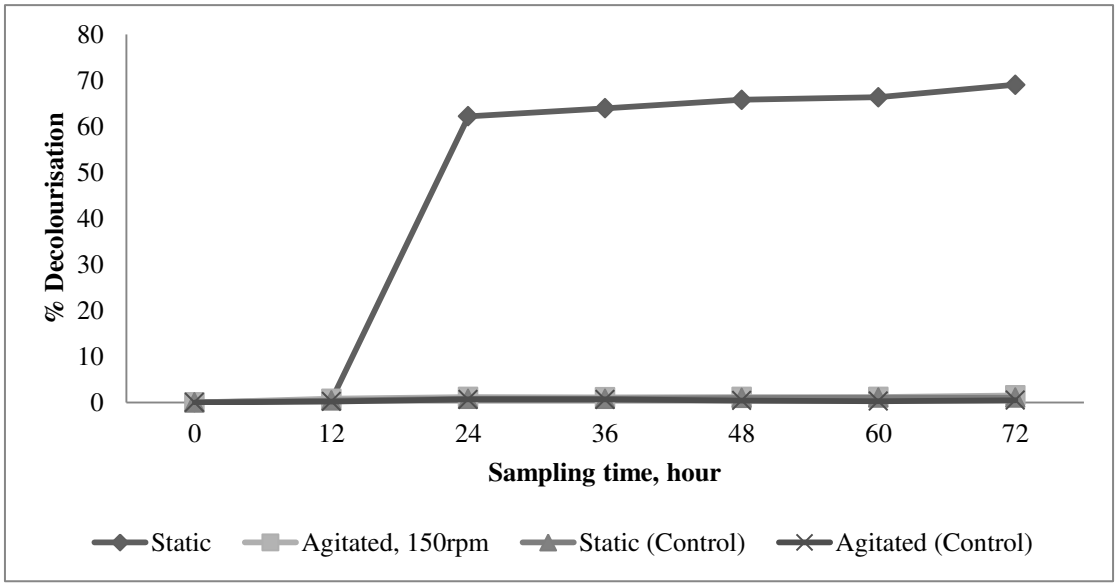

Figure-3. Decolourisation of RO16 by *Bacillus* sp. UMK DG-1 under static and agitated condition.

The decolourisation activity highly depends on dye concentration due to the toxic nature of azo dyes. The presence of sulphonic-acid group in aromatic rings of azo dyes can act as detergents to inhibit microbial growth (Chen *et al*., 2003). The microbial growth might also be retarded by the presence of heavy metal complex or non-hydrolised reactive groups in the dye (Sponza & Isik, 2005). As for *Bacillus* sp. UMK DG-1, the efficiency of colour removal was reduced when the concentration of RO16 was increased from 0.01% (w/v) to 0.10% (w/v) and 1.00% (w/v). The highest colour removal was recorded for 0.01% (w/v) RO16 (90%), followed by 0.1% (w/v) (61.67%) and 1% (w/v) (0.23%) (Figure-4).

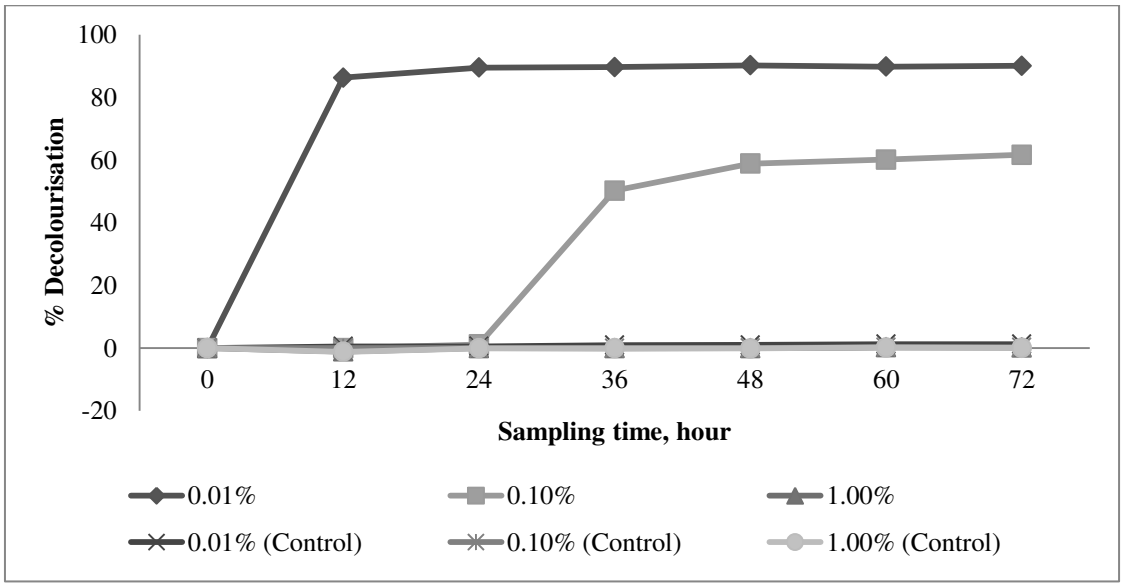

Figure-4. Decolourisation of different RO16 concentrations by *Bacillus* sp. UMK DG-1.

b. Decolourisation analysis

The decolourisation of RO16 was analysed by the UV-Vis spectrophometer. Decolourisation was confirmed by the disappearance of peaks in the visible region (400-700nm). The aromatic rings, chromophore, and precursor of RO16 were represented by different peaks in the UV-Vis spectra. Gomes, Miwa, Malpass, and Motheo (2011) stated that the peak at ~295nm was due to the presence of gamma acetylated acid structure, which acts as a precursor for RO16 synthesis. The peak at ~390nm might attribute to the aromatic rings that are bonded to the azo group, while the peak at ~500nm was due to the chromophore of the azo group that is responsible for the formation of colour (Migliorini, Braga, Alves, Lanza, Baldan, & Ferreira, 2011). Maximum absorbance at the chromophore peak is commonly used for monitoring the decolourisation activity (Gomes, Miwa, Malpass, & Motheo, 2011). As shown in Figure-5, remarkable changes can be observed in the UV-Vis spectra for control and bacterial culture with decolourised RO16. The peaks at ~390nm and ~500nm disappeared from the culture, suggesting the possible degradation of aromatic rings and chromophore of RO16.

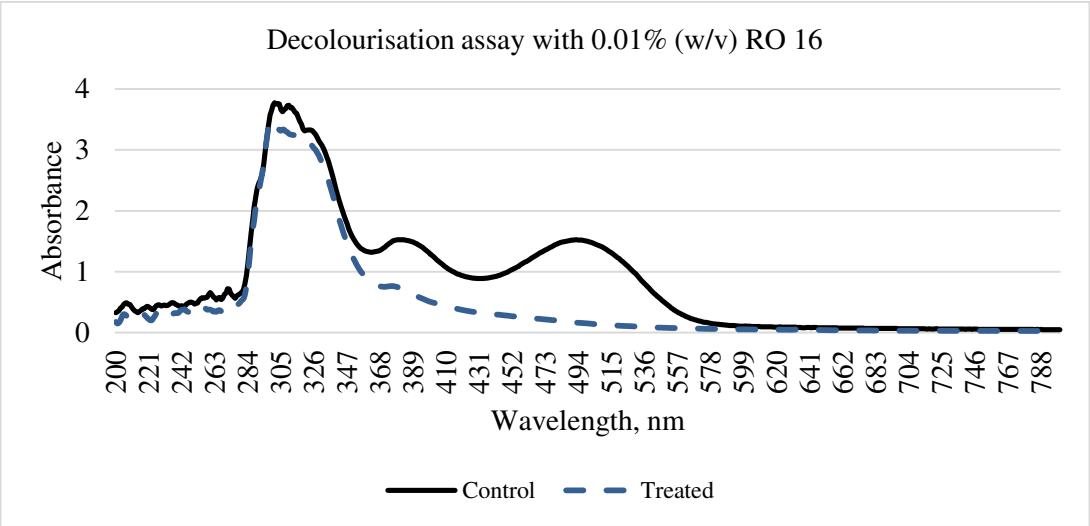

Figure-5. UV-Vis spectra for control and bacterial culture with decolourised RO16 (treated).

CONCLUSIONS

As a conclusion, a locally isolated dye degrading bacteria *Bacillus* sp. UMK DG-1 is able to decolourise Reactive Orange 16 at different physicochemical parameters. Based on the results observed, the highest decolourisation activity was recorded at pH8–9, temperature 37^{o}C, and incubation under the static condition. The decolourisation activity also increased when a lower concentration of RO16 was used. Furthermore, the decolourisation of RO16 was analysed by a UV-Vis spectrophotometer. The absence of peaks at ~390nm and ~500nm from the bacterial culture with decolourised RO16 suggested the possible degradation of aromatic rings and chromophore of RO16.

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