PROGRESS IN COLOR, COLORANTS AND COATINGS

available online @ www.pccc.icrc.ac.ir

Progress in Color Colorants Coating 18 (2025), 99-111

Ministry of Science, Research and Technology
Institute for Color Science & Technology

Decolorization of Textile Dye by Isolated Bacterial Strains from Textile Waste and Their Ability to Produce Polyhydroxyalkanoate

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ARTICLE INFO

Article history:
Received: 23 Mar 2024
Final Revised: 20 Jun 2024
Accepted: 3 Jul 2024
Available online: 24 Aug 2024

Keywords:
Dye removal
PHAs
FTIR
Disperse Red 73
Adsorption isotherm

ABSTRACT

Polyhydroxyalkanoate (PHA) is a type of polymer that is biodegradable, biocompatible, and eco-friendly. In this research, certain bacteria from textile waste, such as curing and bleaching, leather, and sizing waste, were found to produce PHA and remove color from textile dyes. Three types of bacteria were identified as Bacillus cereus SH2, Priestia megaterium SH3, and Bacillus cereus SH4 through genetic sequencing. These bacteria were grown in a medium with Disperse Red 73 to assess their ability to remove color and produce PHAs. The presence of PHA in the extracted biopolymer was confirmed using Fourier transform infrared spectroscopy (FTIR). Among the bacteria, SH4 was the most efficient at producing PHA (49.09 %), with a concentration of 0.54 gL^{-1}. SH2 showed significant color removal ability (98.91 %), indicating its potential in the treatment of dye wastewaters. The isotherm, kinetics, and thermodynamics of the process were also investigated. The findings indicated that the absorption kinetics and isotherms were most accurately described by the pseudo-first-order and Langmuir models, respectively. The thermodynamic analysis of the adsorption process revealed that the adsorption occurred spontaneously and was endothermic in nature. This is the first report of the newly isolated strains from textile waste and their ability in degradation of Disperse Red 73 and produce PHA. Prog. Color Colorants Coat. 18 (2025), 99-111© Institute for Color Science and Technology.

1. Introduction

Plastics possess characteristics such as strength, lightness, durability, and resistance to degradation, making them a crucial commodity for improving comfort and quality of life [1]. A significant challenge in the environmental realm of this century is the gradual replacement of fossil fuel-based plastics with biodegradable plastics sourced from nonfood renewable sources [2]. The release of plastics and harmful chemicals into soil and groundwater stands as a major concern in today's society [3]. Bio-based materials present a promising option for substituting non-degradable plastics [2]. Polyhydroxyalkonates (PHAs) represent a class of biodegradable and biocompatible intracellular biopolymers synthesized and produced by various microorganisms (Alcaligenes, Azotobacter, Bacillus, Pseudomonas) and certain genetically modified plants [4]. In conditions where nitrogen, phosphorus, and oxygen are scarce while there is an excess of carbon source, some microorganisms produce and store PHAs within themselves [5]. A PHA is made of 600-35,000 (R)-hydroxy fatty acid monomer units [6]. PHAs are divided into two groups; 1) short-chain length (SCL) consists of 3-5 carbon atoms in the chain, such as poly(3-hydroxybutyrate) P(3HB) and 2) medium-chain length (MCL) consists of 6-14 carbon atoms, such as poly (3-hydroxyhexanoate) P(3HHx) [7]. PHAs are intracellular-carbon and energy storage compounds that

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https://doi.org/10.30509/pccc.2024.167299.1292

their properties are similar to synthetic plastics, such as polyethylene and polystyrene. Therefore, they are considered to be a suitable alternative to petroleum plastics in many applications [8]. PHAs have large applications in medical (wound management, sutures, surgical meshes, cardiovascular fabrics, scaffolds for cartilage engineering), tissue engineering, agricultural, conventional fibers, filters and other areas [9]. PHB and its copolymers with 3-hydroxyvalerate (PHBV) are the most prevalent of this family [10]. These can be derived from renewable sources like plant oils, sugars, and carbon dioxide [11]. The utilization of mixed microbial cultures to produce PHAs and the use of organic waste as a carbon source enable cost reduction and valorization of available renewable resources such as food waste and sewage sludge [12]. Textile industries are the largest of sources of pollution of water and the production of textile waste has increased [13, 14]. About 70 % of textile dyes are azo due to their cost-effectiveness and ease of use. The use of bacterial methods can be useful in degrading synthetic dyes, such as azo dyes. Bioremediation is a non-hazardous, simple, cost-efficient, environmentally friendly, with good dye removal efficiency, high removal of biochemical oxygen demand and suspended solids, and an effective alternative to traditional methods for treating textile waste. The challenge with bacterial methods is that bacteria can be easily affected by environmental factors like pH, temperature, and initial dye concentration, among others. This can lead to reduced dye decolorization. Other drawbacks include a slow process, needing management and maintenance of micro-organisms and/or physicochemical pre-treatment [15-17]. Textile dyes can be decolorized by bacteria through biosorption, biodegradation, or a combination of both mechanisms. In biosorption, the dye is absorbed by the bacteria cells (adsorption on microbial biomass), changing the color of the bacterial cells. On the other hand, biodegradation breaks down the dye structure into simpler compounds, leading to the disappearance of absorption peaks in the dye spectrum or the formation of new peaks if new metabolites are produced during the biodegradation process [18, 19]. In this study, multiple bacterial strains were isolated from contaminated textile wastewater. These strains not only produce biopolymers but also have the ability to remove color from the wastewater. Due to their resilience in toxic environments, they exhibit superior capabilities compared to conventional bacteria. The purpose of this study is to isolate bacteria from textile waste and also to investigate their ability to to decolorize and to produce PHA. The isotherm (Langmuir and Freundlich), kinetics (first and second-order) and thermodynamics of dye adsorption is also investigated.

2. Experimental

2.1. Sample collection and bacteria isolation

Samples (curing and bleaching, leather and sizing waste) were provided of Textile and Weaving Co. (Yazd. Iran) for the bacteria isolation. These samples were stored at -20 °C. To isolate bacteria from textile waste, serial dilutions of the waste were made in sterile phosphate-buffered saline. Subsequently, 100 µL of enriched broth was spread on nutrient agar (NA) plates (Merck) and then incubated at 35°C for 48 hours. Pure colonies were then isolated and stored at 4 ºC on NA plates [20]. The textile dye, Disperse Red 73, utilized in this study was sourced from Khoshpak company in Iran.

2.2. Primary screening for PHA-producing bacteria

The presence of PHA as intracellular granules was confirmed by staining with Sudan Black B dye. For rapid screening of PHA producers, nutrient agar medium with 1 % glucose was autoclaved. Ethanolic solution of Sudan Black B dye (0.05 %) was spread on the colonies and the plates kept for 30 min. They are washed with ethanol (96 %) and examined under a light microscope with objective lens 100X. Colonies exhibiting a dark blue color were identified as positive for PHA production.

2.3. PHA production

The isolation of PHA-producing bacteria from waste was carried out using an enrichment technique. Samples were enriched by culturing in a medium containing yeast extract (5 gL^{-1}), tryptone (8 gL^{-1}), and NaCl (2.5 gL^{-1}). The bacteria were then inoculated in E2 medium and incubated in a rotary shaker (120 rpm) at 35°C for 24-72 hours. The shake flask cultures were conducted in 250 ml Erlenmeyer flasks containing 50 mL of medium. E2 medium, a nitrogen limited medium, was used as PHA production medium (gL^{-1}): 3.5 $NaNH_4HPO_4.4H_2O$, 7.5 $K_2HPO_4.3H_2O$, 3.7 KH_2PO4, 100 mM $MgSO_4.7H_2O$, and 10 mL trace elements with 10 gL^{-1} (w/v) glucose as

carbon source. The trace element solution consisted of (gL^{-1}): 2.78 $FeSO_4.7H2O$, 1.98 $MnCl_4.4H_2O$, 2.81 $CoSO_4.7H_2O$, 1.47 $CaCl_2.2H_2O$, 0.17 $CuCl_2.2H_2O$, and 0.29 $ZnSO_4.7H_2O$ (Sigma, USA) [21].

2.4. Measurement of decolorization efficiency

100 mgL^{-1} of Disperse Red 73 was inoculated in the PHA-producing medium. Three newly isolated strains were transferred to the PHA-producing medium. After incubation, samples were collected every 24 hours and centrifuged at 10,000 rpm for 10 minutes at 40 ºC. A nutrient broth (without bacterial culture) supplemented with the respective dyes served as a control. Decolorization efficiency was determined by measuring the absorbance of the culture supernatant at 438 nm. The decolorizing efficiency was expressed as the percentage of decolorization using equation 1 [22].

$$\% \text{ Decolorization} = \frac{\text{Initial absorbance-Final absorbance}}{\text{Initial absorbance}} * 100 \quad (1)$$

The effects of four factors pH (3, 7, 10), temperature (25, 37, 50 ºC), dye concentration (20, 50, 100 ppm) and incubation time (24, 48, 72 h) were investigated.

2.5. Fourier Transform Infrared (FTIR) spectroscopy analysis

The chemical composition of the extracted PHA was assessed using an FTIR spectrometer (Bruker-Equinox 55). Spectra for each sample were recorded within the range of 4000-500 cm^{-1}.

2.6. Extraction and quantitative analysis of PHA

A sample was collected from the production medium and centrifuged at 5000 rpm for 15 minutes. The supernatant was discarded and kept for drying. The cell dry weight (CDW) was taken as biomass weight. The pellet was resuspended in 5 mL of a 2 % sodium hypochlorite solution and incubated at 37 °C for 1 hour to completely digest cellular components. Following centrifugation and washing with an acetone:alcohol mixture (1:1 v/v), the PHAs were dissolved in boiling chloroform to eliminate any remaining organic solvents in the PHA pellets, followed by evaporation through air drying to obtain the extracted PHAs in dried form. Standard PHAs (PHB) from Sigma were utilized for comparison. The PHA dry weight (gL^{-1}) was determined after drying the resulting polymer to a constant weight. The PHA content was calculated using equation 2 [4].

$$\text{PHA production } (\%) = \frac{\text{Weight of PHAs (g/L)}}{\text{Weight of bimass (g/L)}} * 100 \quad (2)$$

2.7. DNA extraction, and phylogenetic tree construction

Bacterial DNA was extracted using the boiling method and centrifuged at 7000 rpm for 10 minutes. The supernatant containing the extracted DNA was amplified via polymerase chain reaction (PCR) using bacterial universal primers 27F forward and 1492R reverse. The PCR was conducted for 30 cycles with the following conditions: 1 minute at 94 °C (denaturation), 1 minute at 56 °C (annealing), and 1 minute at 72 °C (extension), followed by a final extension of 7 minutes at 72 °C. The PCR products were visualized on 1 % (w/v) agarose gel electrophoresis. The gel was stained with ethidium bromide, visualized using Gel doc, and photographed. The sequences were aligned with previously published sequences of bacterial strains using the BLAST function in the National Center for Biotechnology Information (NCBI) database. Evolutionary analyses were carried out using the Neighbor-Joining method with MEGA 7 software [23, 24].

3. Results and Discussion

3.1. Molecular detection and phylogenetic analysis

The amplification (1500bp) of the 16SrRNA gene was successfully depicted in Figure 1a. The sequences of isolates (*SH2*, *SH3*, and *SH4*) have been submitted to GenBank with the accession numbers OR635439, OR635822, and OR636055, respectively. Figure 1b illustrates the phylogenetic tree of all isolates.

3.2. PHA production by isolates

The production of PHA by isolates involved a process where bacterial cells were cultured, stained with Sudan Black B, and subsequently examined under a microscope. The microscopic analysis revealed the presence of lipophilic black granules within pink cells, as illustrated in Figure 2.

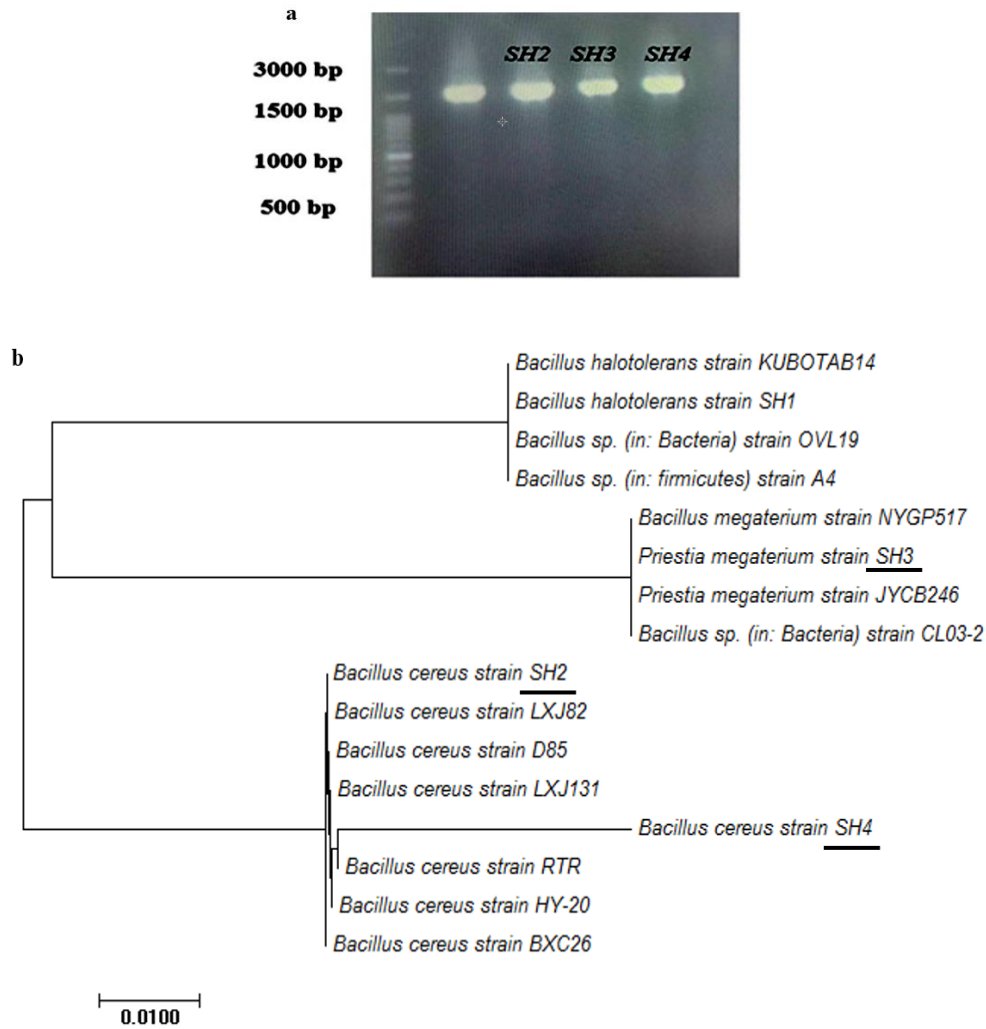

Figure 1: (a) DNA bands visualized on agarose gel electrophoresis with 1.5 % agarose, (b) Phylogenetic tree.

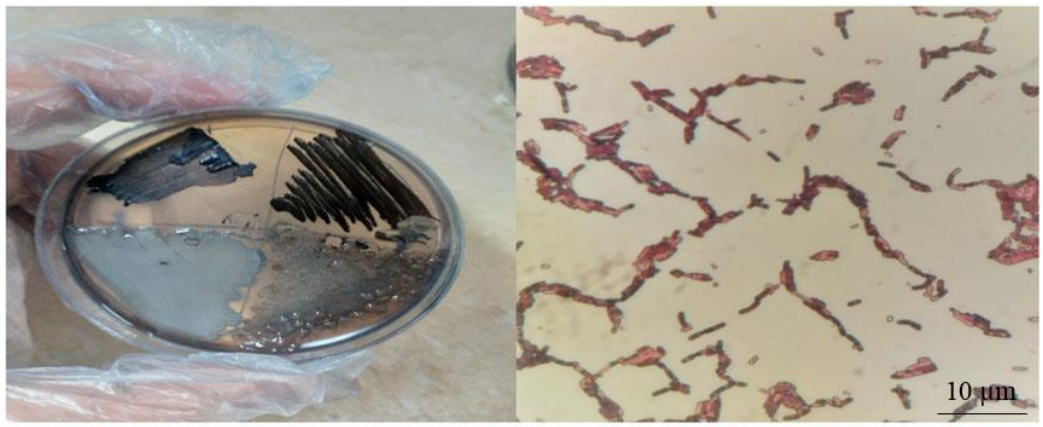

Figure 2: Rapid screening of isolates for production by plate assay method.

All the isolated strains were examined for PHAs production. The strain SH4 exhibited the highest PHA yield (49.09 %) with a polymer concentration of 0.54 gL^{-1} (Figure 3). Figure 4 illustrates the bioplastic derived from isolated strains from textile waste.

3.3. Textile dye removal

3.3.1. Effect of temperature on decolorization

Temperature plays an important role in degradation of dyes. Bacterial need optimum temperature to their growth, and metabolic activities. Low temperatures or temperatures above optimum levels might lead to cell inactivation in the bacteria [19]. The results showed that the optimal temperature for decolorization by all bacteria was found to be 37 °C, except for isolates *SH2* (Figure 5 and 6). Maximum of dye removal was recorded by *Bacillus cereus SH2* (98.91 %) at 25 °C.

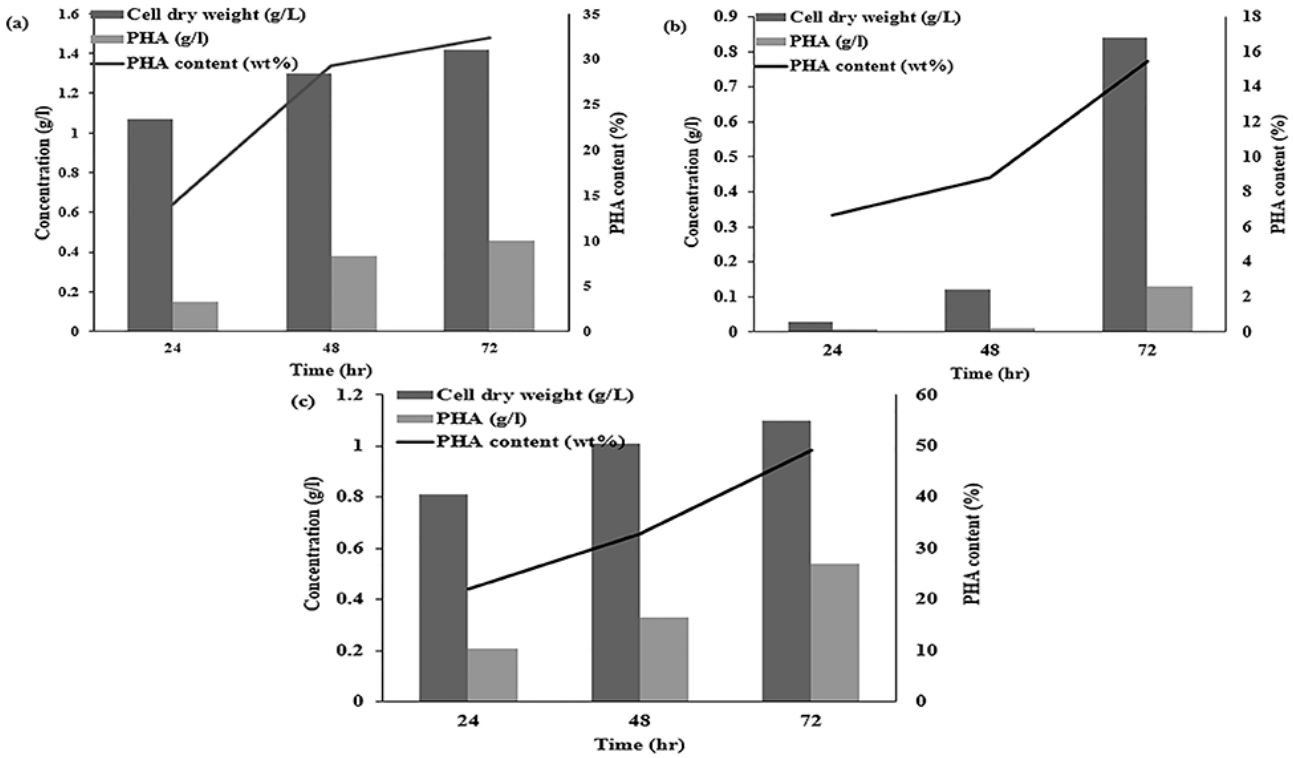

Figure 3: PHA content (%) and CDW (gL^{-1}) production by strain (a), *SH2 (*b) *SH3 and (c) SH4.*

Figure 4: Bioplastic obtained from *Bacillus cereus SH4.*

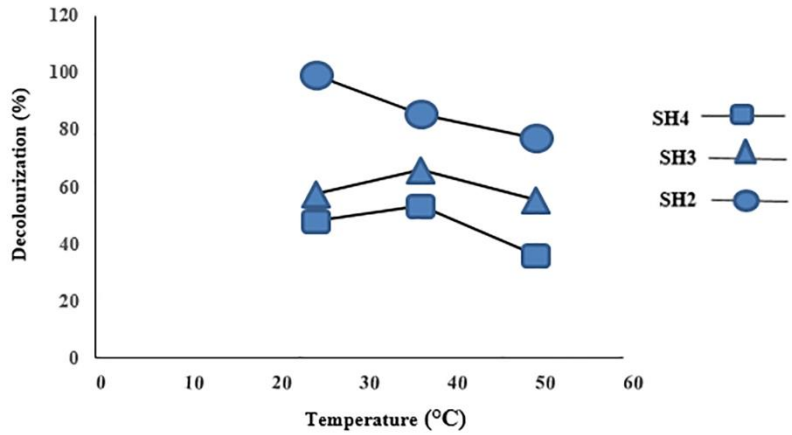

Figure 5: Effect of temperature on removal of dye by bacteria isolates (pH 7, 72 h, 100 ppm).

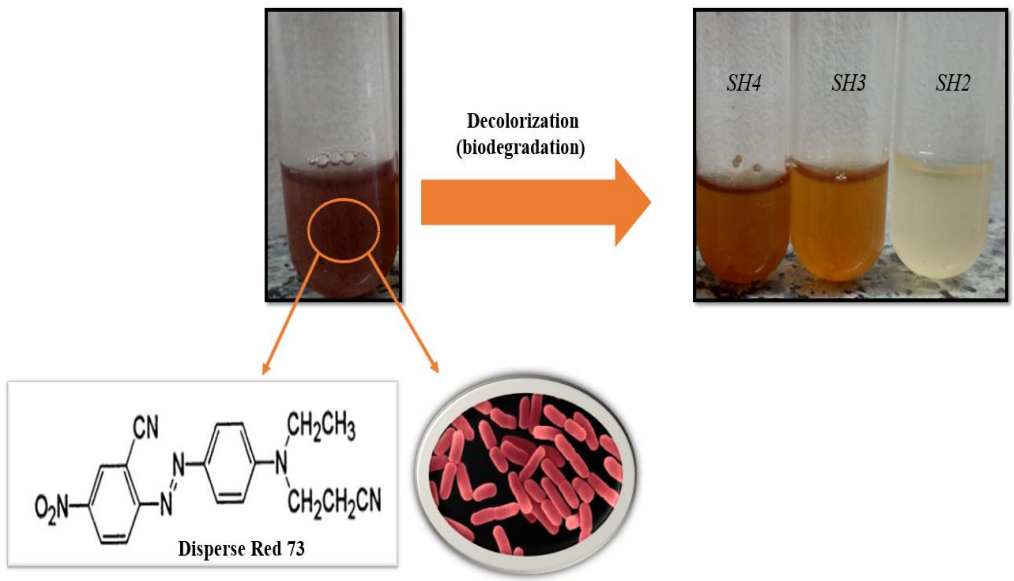

Figure 6: Dye degradation by isolates after incubation at 25 °C (pH 7, 72 h, 100 ppm).

3.3.2. Effect of pH on decolorization

The pH of the solution has essential effects for optimum bacterial growth in culture media on dye removal [19]. The effect of solution pH on Disperse Red 73 removal is presented in Figure 7. The results show that the highest removal amount of dye was at pH 7.

3.3.3. Effect of time on decolorization

The length of contact time can influenced the biosorption ability and plays an important role in dye removal [25]. As time increases, the degradation of dye also increases, to a certain extent (Figure 8).

3.3.4. Effect of dye concentration on decolorization

The dye concentration affects the efficiency of decolorization process. Various studies have shown that increasing the concentration of dye leads to a decrease in the efficiency of decolorization by bacteria, due to the toxicity of the dyes which inhibits the metabolic activities [19, 26]. The results showed that the highest dye removal was obtained at a concentration of 100 ppm and the lowest at a concentration of 20 ppm (Figure 9).

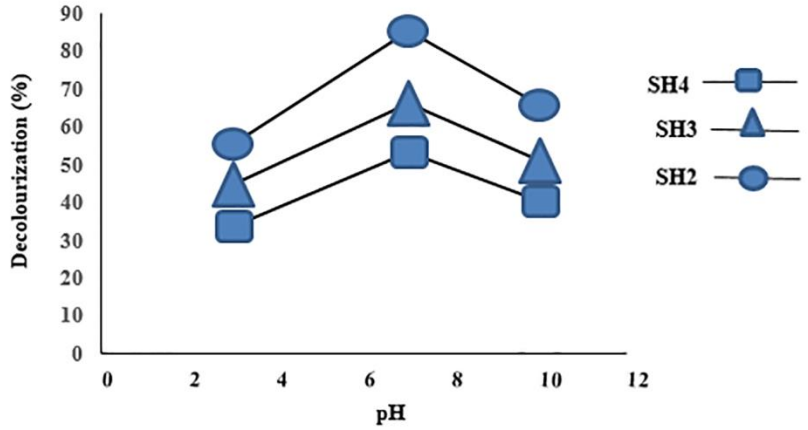

Figure 7: Effect of pH on removal of dye by bacteria isolates (37 °C ,72 h, 100 ppm).

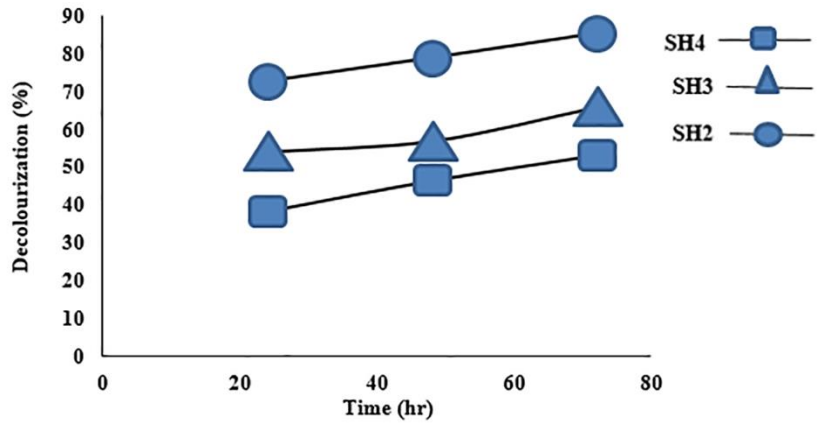

Figure 8: Effect of time on removal of dye by bacteria isolates (pH 7, 37 °C, 100 ppm).

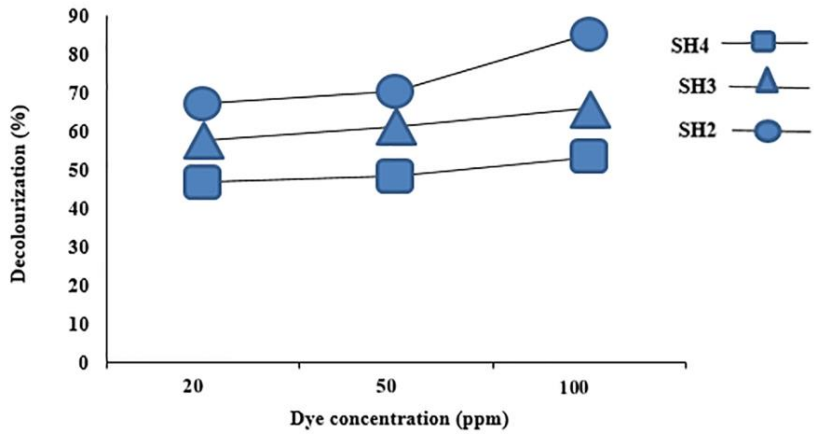

Figure 9: Effect of dye concentration on removal of dye by bacteria isolates (pH 7, 37 °C, 72 h).

3.4. Adsorption isotherm models

The adsorption isotherm shows how molecules distribute between liquid and solid phases when reaching equilibrium [27]. Different models like Freundlich and Langmuir were used for analysis. The Langmuir isotherm theory assumes that adsorption occurs on a homogeneous surface with identical sites, equally available and with equal energies of adsorption [28].

Equation 3 represents the linearized form of Langmuir isotherm.

$$\frac{C_e}{q_e} = \frac{1}{K_L q_{max}} + \frac{C_e}{q_{max}} \quad (3)$$

where q_{max} is the maximum adsorption capacity (mg g^{-1}), q_e is the solid-phase equilibrium concentration (mg g^{-1}), C_e is the liquid equilibrium concentration of dye in solution (mg L^{-1}), and K_L is the Langmiur constant.

The Freundlich adsorption isotherm is an empirical equation used to describe heterogeneous adsorption systems. It refers to reversible adsorption and multilayer adsorption [29, 30]. Equation 4 represents the linearized form of Freundlich isotherm.

$$\mathrm{Log}q_e = \mathrm{Log}K_f + \frac{1}{n}\mathrm{Log}C_e \quad (4)$$

K_F is the Freundlich constant and n is the heterogeneity factor depicting the adsorption intensity. The linear plot of Freundlich and Langmuir isotherm models are shown in Figures 10. The isotherm parameters are shown in Table 1. As seen, the Langmuir model yields a somewhat better fit R^2 than the Langmuir model.

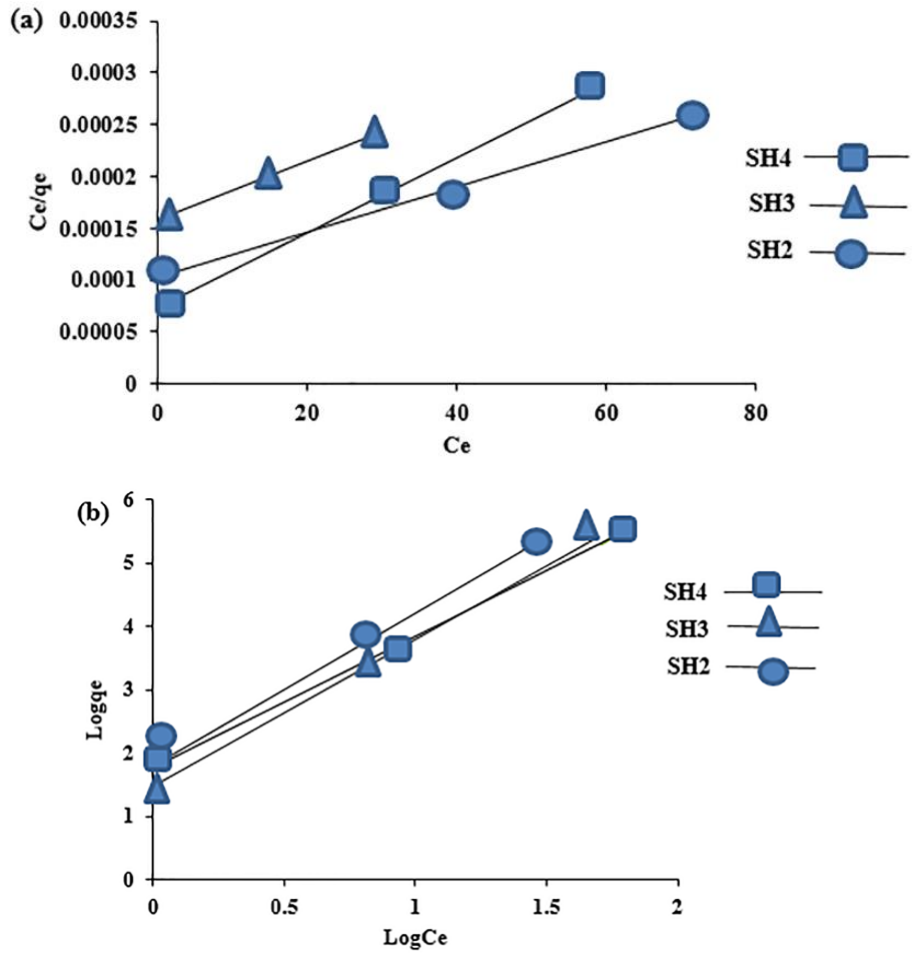

Figure 10: (a) Langmuir, and (b) Freundlich isotherms.

Table 1: Langmuir and Freundlich constants.

Bacterial isolates	Freundlich			Langmuir		
	R^2	K_F	n	R^2	K_L	q_{max}
SH2	0.84	63.28	0.41	0.99	0.02	500
SH3	0.97	58.98	0.48	0.93	0.015	333.33
SH4	0.98	29.30	0.43	0.94	0.058	250

Table 2: Kinetics parameters values.

Bacterial isolates	First order			Second order		
	q_{e1} (mg g^{-1})	K_1 (min^{-1})	R^2	q_{e2} (mg g^{-1})	K_2	R^2
SH2	109.65	0.0001	0.99	166.67	2.86×10^{-5}	0.98
SH3	57.11	0.0003	0.99	111.11	7.8×10^{-5}	0.99
SH4	26.10	0.0002	0.94	25.00	5.22×10^{-4}	0.87

3.5. Adsorption kinetics models

In dyeing, it is important to control the required time to achieve high bath exhaustion. This requires kinetic studies. For this purpose, pseudo-first-order and pseudo-second-order equations were applied to determine the controlling mechanism of the adsorption process [31]. The pseudo-first-order model proposed is presented by equation 5:

$$\mathrm{Ln}\left(q_e - q_t\right) = \mathrm{Ln}\, q_e - k_1 \quad (5)$$

Where q_e and q_t are the amount of dye adsorbed (mg g^{-1}) at equilibrium and time t (min), respectively. k_1 is the rate constant of adsorption (min^{-1}). The pseudo-second order model proposed is presented by equation 6:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (6)$$

where k_2 (g mg^{-1} min^{-1}) is the rate constant of pseudo-second-order.

Figures 11a and b explain the findings of Pseudo-first and second order models were used to fit the experimental data, respectively. Table 2 contains the results for the kinetic model parameters.

These results imply that the adsorption system studied obeys to the pseudo-first-order kinetic model.

3.6. Thermodynamic parameters

The following equations have been utilized to calculate the thermodynamic parameters like enthalpy (ΔH), Gibbs free energy (ΔG), and entropy (ΔS) [32].

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (7)$$

$$\mathrm{Ln}K_C = \frac{-\Delta G^0}{RT} = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (8)$$

where R is the gas constant (8.314 J mol^{-1} K^{-1}), T absolute temperature (K), and K_C denotes the equilibrium constant.

From the slope and intersection point of the ln Kc versus 1/T linear plot, the values of ΔH^0 and ΔS^0 can be obtained (Figure 12). The values of the thermodynamic parameters are presented in Table 3. The negative values of ΔG^0 suggested that the adsorption process was spontaneous. The positive ΔH^0 indicates that the process is endothermic in nature. The positive value of ΔS^o suggested that the increasing randomness during the adsorption process.

3.7. Functional group of PHA analysis

Figure 13 shows the FT-IR spectrum of the bacterial isolates. The bands at 1742 cm^{-1} and 1000–1300 cm^{-1} corresponded to the stretching of the C=O and C–O bonds, respectively [33]. Therefore, the polymer extracted was confirmed to be PHA.

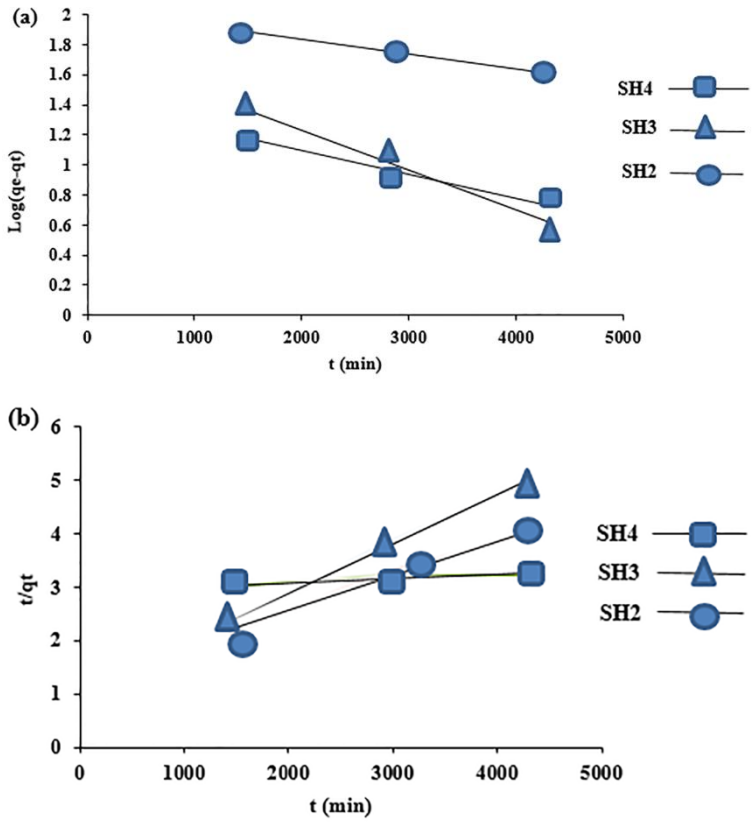

Figure 11: a) pseudo- first and b) second-order model kinetics.

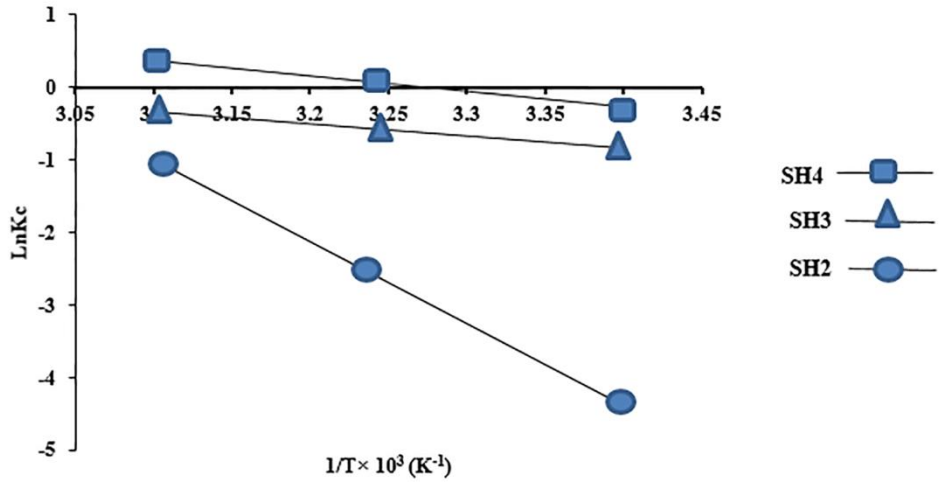

Figure 12: Plot of ln Kc verses 1/T.

Table 3: Thermodynamic parameters.

Bacterial isolates	ΔG			ΔH (kJ mol^{-1})	ΔS (J mol^{-1}K^{-1})	R^2
	293K	310K	323K			
SH2	-82.995	-87.816	-91.502	94.123	283.582	0.97
SH3	-12.084	-12.786	-13.323	14.192	41.291	0.74
SH4	-17.248	-18.249	-19.016	17.994	58.929	0.61

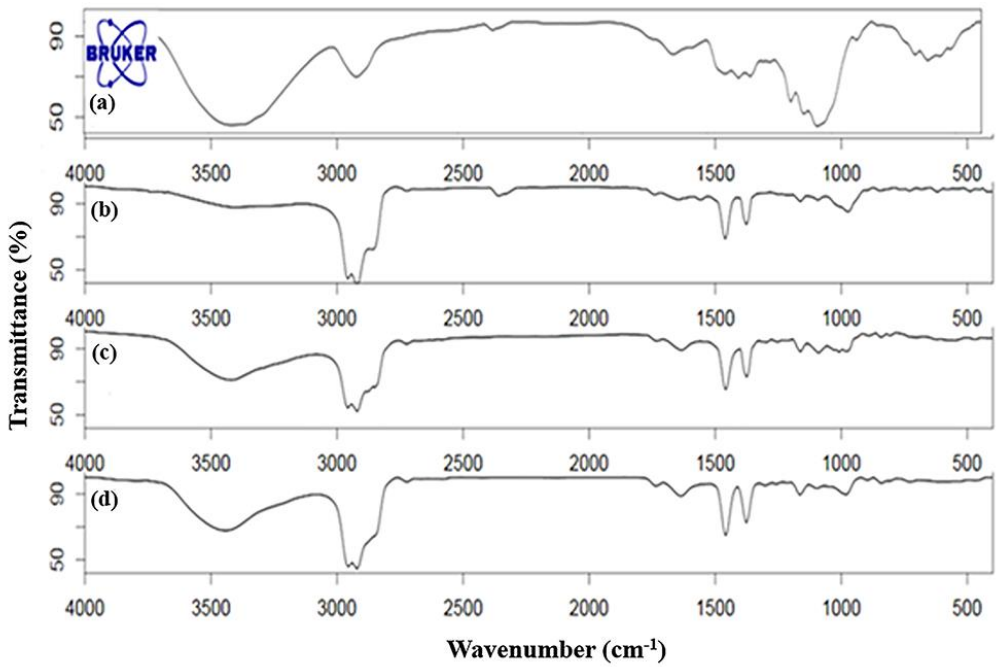

Figure 13: FTIR spectra of: (a) PHB standard, (b) SH3, (c) *SH2*, and (d) *SH4.*

4. Conclusion

Textile industries are a major contributor to water pollution, with textile waste production on the rise. Bioremediation offers a non-hazardous, cost-effective and environmentally friendly for treating textile waste. Also, many bacteria produce PHAs when exposed to stressful conditions that have the potential to replace petrochemical-based plastics. Three isolated strains were obtained from types of textile waste and their ability to produce PHA and removal dye was investigated. This study found that the isolated strain of *Bacillus cereus SH4* was able to produce more PHAs (49.09 %). Maximum of Disperse Red 73 dye removal was recorded by *Bacillus cereus SH2* with a degradation rate of 98.91 % (25 °C, pH 7, 72 h, 100 ppm). The adsorption of dye was best fitted with the Langmuir adsorption isotherm. The adsorption kinetics can be predicted by pseudo-first-order kinetic, and the thermodynamic parameters suggest that the process is spontaneous, random, and exothermic. These isolated bacteria can be used for the biodegradation of textile dyes and PHAs production.

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How to cite this article:
Shahi Z, Khajeh Mehrizi M, Mirbagheri Firoozabad MS. Decolorization of Textile Dye by Isolated Bacterial Strains from Textile Waste and Their Ability to Produce Polyhydroxyalkanoate. Prog Color Colorants Coat. 2025;18(1):99-111. https://doi.org/ 10.30509/pccc.2024.167299.1292.