Biodegradation of Textile Dye Wastewater with the Application of Response Surface Methodology (RSM): A Factorial Design Approach

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Abstract

The increasing demands in textile industries has created huge production of textile coloured products globally, leading to large production of textile effluent. Textile effluent is often discharged to the environment without proper treatment by the textile factories. The untreated textile effluent typically contains harmful chemicals and is hazardous to the environment, due to the toxicity of the dyes used. In this study, biological treatment is applied to the textile effluent. A 2-level full factorial design from response surface methodology (RSM) was applied to find the optimized treatment process condition for the textile wastewater degradation. Sixteen runs of experiment with 4 factors were performed; bacterial inoculum (%, v/v), temperature (°C), agitation (rpm), and pH were tested. It was observed highest decolourization obtained (91.95 % with pH 4, low concentration of bacterial inoculum (5 %), agitation speed (200 rpm) and temperature (40 °C)) meanwhile lowest decolourization was achieved at 73.47 % with pH 10, low concentration of bacterial inoculum (5 %), agitation speed (100 rpm) and high temperature (40 °C). It was observed that low concentration of bacterial inoculum (%, v/v) gave more favourable dye degradation and COD removal, while pH ranged from low to high in the textile dye treatment.

Keywords: Biological treatments, COD removal, Dye degradation, Full factorial design, Textile wastewater

Introduction

In 2017, United Nation World Water Development Report stated that over 80 % of the world's wastewater, and over 95 % in some least developed countries is discharged to the environment without proper treatments [1,2]. Wastewater can be categorized into few which are domestic wastewater, industrial wastewater and agricultural wastewater. Additionally, to domestic waste, agricultural waste is one of the major factors in contributing to water pollution. [3] reported that globally, 380 billion m^3 of wastewater are being produced annually. It is estimated for wastewater production to reach 470 billion m^3 by the end of sustainable development goals (SDG) in 2030, which indicates the increase of 24 % with current wastewater production and continue to reach 51 % increment by 2050. In the agricultural aspect, the use of fertilizer increases the nitrogen content in the water bodies such as rivers, lakes, ponds and sea. Higher nitrogen content can lead to the eutrophication where nitrogen is over accumulated in the water bodies that leads to the degradation of water quality and loss of habitat. It also degrades the esthetical quality of coastal waters, endangers the recreational applications of waterways as well as affects the commercial harvest of finfish and crustaceans [3,4].

Textile dye wastewater production has become 1 of the major concerns in Kuala Terengganu, as the production of *batik* textiles is 1 of the largest contributors to economic development, aside from other businesses. As the demand got higher, the production of *batik* textiles has immensely increased, resulting in a larger usage and application of textile dyes. *Batik* industries is one of the traditional decorative textile

processes in producing painted and colored textiles using wax, which are later made into clothing [5]. Similar to other textile industries, batik processing consumes a large amount of water for dyeing and washing [6,7]. In Terengganu, most of the *batik* textiles are produced from small factories, a very few of which apply the proper treatment of textile dyes wastewater. The wastewater is normally discharged directly into the river [8]. In this study, river water from Sg. Hiliran is chosen as the sampling area. The daily discharge of the textile dyes wastewater is approximately 100,000 m^3 that continuously pollutes Sg. Hiliran. The polluted water from this river eventually disseminates into the ocean, potentially endangering the water bodies and aquatic life.

Textile dye wastewater, of textile effluent is produced from textile industries. It is composed of various chemicals or pollutants from textile washing, dyeing and waxing processes, which are potentially hazardous to the environment [9]. One of the main components in textile dye wastewater are azo dyes, which are dangerous to the environment since they cannot be degraded easily [10,11]. The complex molecules in azo dye compounds make it harder to be digested and degraded, and proper treatment processes are needed. Even though there are common practices of wastewater treatment methods being carried out, the degradation of complex molecules of azo dyes still takes a longer processing time to get completely degraded [8,12,13]. Azo dyes are known to be toxic and potentially carcinogenic that can affect the environment and are dangerous to living things especially humans and [14-16]. The most common practices include adsorption and filtration methods. However, these practices caused another problem to arise, which was the production of by-products / generation of sludge [17], which later needed to be disposed of. The current methods of wastewater treatments are also being cost-inefficient, causing the local factories to abandon these textile wastewater processes to cut off the maintenance costs. Unfortunately, it eventually leads to the free discharge of untreated textile wastewater into the water bodies without proper monitoring from the authorities, such as Sg. Hiliran that leads into a bigger river, the Sg. Terengganu.

Biodegradation of dyes using bacteria has been displayed to be highly effective [18], and the bacterial consortium is more favourable than a single culture of bacteria due to the restrictions of pure culture in degrading dyes. They are often specific to certain dyes under consideration and could not be easily scaled up and maintained in large scale operations of textile effluent treatment plants [19]. In microbial degradation of textile dye activities, it can be divided into aerobic and anaerobic degradation. There are bacterial strains that are identified for the ability to decolorize azo dyes under aerobic conditions, namely *Xenphylusazovorans* KF46F, *Bacillus* strain, *Kertersia* sp. Strain VKY1 and *Staphylococcus* sp. while *Sphingomonasxenophaga* BN6, *Eubacterium* sp. *Clostridium* sp., *Butyrvibrio* sp., or *Bacteroides* sp., as reported by [20-22]. In research by [23], newly isolated *Lysobacter* sp. has shown a remarkable potential in degrading textile dye Congo Red (CR) and Yellow HEGR, where 80.4 and 80.1 % of the textile dyes were degraded, respectively. In a study conducted by [24], a bacterial consortium consisting of bacterial species *Providencia* sp. SDS and *Pseudomonas aeruginosa* strain BCH shows a complete decolourization of textile effluent within 20 h compared to a single bacterial decolourization process. In another study on microbial degradation of dyes conducted by [25], *Bacillus* spp. ETl-1979 was collected from activated sludge of textile wastewater treatment plants. The microbes could decolorize and detoxify the synthetic reactive dyes used in the research, and enzymes responsible for the degradation were investigated. The present study focuses on the removal of colour and chemical oxygen demand (COD) from the industrial textile wastewater collected from local factories situated adjacent to the Sg. Hiliran using the biological approach with the optimization of process conditions. The potential bacterial isolates used in this study later will undergo identification and characterization studies in future research.

Materials and methods

Textile dye effluent

The untreated textile dye effluent was obtained from a local backyard textile producing factory in Kuala Terengganu, where the wastewater is directly discharged into Sg. Hiliran. Approximately 5 L of textile wastewater was collected and stored in a < 3 °C chiller for the study purposes. The typical composition of the untreated textile wastewater is shown in **Table 1** [26,27].

Bacterial isolation and identification

The bacterial samples were collected at 3 main source points of the textile wastewater treatment plant at the local *'batik'* factory, and each were differentiated and labeled. The 3 source points are raw textile dye wastewater (RTW) (sampling point 1), anaerobic-aerobic tank (AAT) (sampling point 2) and

multimedia filtration tank (composed of sand and pebbles) (sampling point 3). 100 mL of each raw textile wastewater (RTW) and filtered wastewater (AAT and SF) were collected and maintained at < 3 °C for further isolation and cultivation. A 1 mL of each textile dye wastewater at the 3 collection points were dropped onto the nutrient agar (NA) (peptone (5.0 g/L), meat extract (3.0 g/L), agar (9.0 - 18.0 g/L)) and nutrient broth (NB) (peptone (5.0 g/L) and meat extract (3.0 g/L)) in triplicates. After the textile dye wastewater was spread onto the agar with a hockey stick, it was then incubated at 37 °C (Memmert) overnight. As for the nutrient broth, 1 mL of textile dye wastewater was pipetted into the sterile broth and incubated at 37 °C with 150 rpm in the incubator shaker (Jeio Tech) overnight [28].

Bacterial inoculum preparation

Bacterial inoculum of 3 isolates (C1, 3K and 3W) were prepared with nutrient broth (NB (peptone (5.0 g/L) and meat extract (3.0 g/L)) (Merck). 20 mL of nutrient broth were initially sterilized at 121 °C for 15 min (Hirayama) and cooled down. One loopful of each isolate was inoculated into the nutrient broth under aseptic conditions and incubated overnight at 30 °C, 150 rpm.

2^k factorial design

The 2^k factorial designs are widely used in the experiments that account for several factors and study the interaction effect of those factors on the response(s) with each variable is being investigated at 2 levels and determine which factors that have important effects on the study as well as the variations of the effect have on each factor [25,29,30]. In research conducted by [31], it was stated that a 2^k design is a useful tool in studying the response surface due to the screening experiments that are carried out in order to find the critical process and system variables. The k factors, each at 2 levels, are described as $2 \times 2 \times 2 \times 2 \dots \times 2^k$ factorial design. The advantages of using this design are needing fewer numbers of runs for each factor as well as the indications of significant trends in the particular experiment that can be observed efficiently. A fraction of factorial design also can be used to screen many factors that can be applied as building blocks of the experiments [32].

In this experiment, 4 factors were examined to optimize the process conditions of decolourization and chemical oxygen demand (COD) of textile wastewater by bacterial isolates. The factors investigated were the initial pH of the textile wastewater, bacterial consortium inoculum (%) (v/v), temperature (°C), as well as agitation (rpm). These factors were investigated with full factorial designs, resulting in a total of 16 experiments. The model of experimental designs is shown in **Tables 2** and **3**. The variables were measured in 2 levels, i.e. low and high. The initial pH of the media was measured with pH of 4 and 10, and the bacterial consortia inoculum was 5 and 15 % (v/v). The agitation speeds as measured in rotation per minute (rpm) were 100 and 200 rpm and the temperature (°C) was set at 20 and 40 °C. All the experiments had undergone 10 days of the incubation period [33].

Table 1 Typical properties of untreated textile dye wastewater

Parameters	Values
pH	7.0 - 12
Biochemical oxygen demand (BOD) (mg/L)	80 - 1,600
Chemical oxygen demand (COD) (mg/L)	150 - 12,000
Total Suspended Solids (TSS) (mg/L)	15 - 8,000
Total Dissolved Solids (TDS) (mg/L)	2,900 - 3,100
Chloride (mg/L)	1,000 - 1,600
Ammoniacal Nitrogen (mg/L)	70 - 80
Colour (Pt-Co)	50 - 2,500

Table 2 Experimental range and variables for decolourization and chemical oxygen removal

Variables	Coded symbol	Actual and coded values	
		−1	+1
pH	A	4	10
Bacterial consortium inoculum concentration (%) (v/v)	B	5	15
Agitation (rpm)	C	100	200
Temperature (℃)	D	20	40

Table 3 Full factorial design for decolourization and chemical oxygen demand removal.

Run No.	Factor A: pH	Factor B: [Inoculum] (%) (v/v)	Factor C: Agitation speed (rpm)	Factor D: Temperature (℃)
1	−1 (4)	+1 (15)	−1 (100)	−1 (20)
2	−1 (4)	+1 (15)	+1 (200)	+1 (40)
3	+1 (4)	+1 (15)	−1 (100)	−1 (20)
4	+1 (10)	+1 (15)	+1 (200)	−1 (20)
5	+1 (10)	+1 (15)	−1 (100)	+1 (40)
6	+1 (10)	−1 (5)	+1 (200)	−1 (20)
7	−1 (4)	+1 (15)	−1 (100)	+1 (40)
8	−1 (4)	−1 (5)	−1 (100)	+1 (40)
9	+1 (10)	+1 (15)	+1 (200)	+1 (40)
10	−1 (4)	−1 (5)	−1 (100)	−1 (20)
11	−1 (4)	−1 (5)	+1 (200)	−1 (20)
12	−1 (4)	−1 (5)	+1 (200)	+1 (40)
13	−1 (4)	+1 (15)	+1 (200)	−1 (20)
14	+1 (10)	−1 (5)	+1 (200)	+1 (40)
15	+1 (10)	−1 (5)	−1 (100)	−1 (20)
16	+1 (10)	−1 (5)	−1 (100)	+1 (40)

Results and discussion

Decolourization and COD removal from textile dye effluents was done with the microbial application through a statistical method called response surface methodology (RSM). Full factorial design follows the principal of 2^k design, where k factors are being studied at 2-levels [34]. There are 4 parameters involved in this study, i.e. initial pH, initial bacterial consortia inoculum concentration (%) (v/v), agitation speed (rpm), and temperature (℃). Initially, the aeration was also included in the study, but due to the insignificance of the preliminary screening effect, it was omitted [35,36]. Four parameters were selected based on the factors that affect the viability of the microbial cells. In bacterial growth, nutritional contents, pH of the media, agitation speed, aeration, temperature play a vital role in

determining its growth, thus explaining the selection of the parameters [37-40]. The parameters were measured with 2 levels of (+ve) and (−ve), indicating low and high values with no central levels. In **Table 4**, all the factors with their levels are displayed and the decolourization and COD removal are shown in % with the actual and predicted values.

Table 4 Decolourization and COD removal (%).

Run No.	Factor A: pH	Factor B: [Inoculum (%)]	Factor C: Agitation (rpm)	Factor D: Temperature (°C)	Decolourization (%)		Chemical oxygen demand (COD) removal (%)	
					Actual	Predicted	Actual	Predicted
1	−1 (4)	1 (15)	−1 (100)	−1 (20)	85.60	85.56	41.52	46.37
2	−1 (4)	1 (15)	1 (200)	1 (40)	85.60	88.56	61.01	46.24
3	1 (10)	1 (15)	−1 (100)	−1 (20)	89.00	88.96	54.99	54.37
4	1 (10)	1 (15)	1 (200)	−1 (20)	89.00	89.04	52.90	52.16
5	1 (10)	1 (15)	−1 (100)	1 (40)	79.93	79.89	63.47	56.45
6	1 (10)	−1 (5)	1 (200)	−1 (20)	77.77	77.71	73.40	70.99
7	−1 (4)	1 (15)	−1 (100)	1 (40)	82.77	82.81	40.35	48.46
8	−1 (4)	−1 (5)	−1 (100)	1 (40)	86.17	86.21	67.14	67.29
9	1 (10)	1 (15)	1 (200)	1 (40)	80.50	80.46	52.18	54.24
10	−1 (4)	−1 (5)	−1 (100)	−1 (20)	89.23	89.19	70.70	65.21
11	−1 (4)	−1 (5)	1 (200)	−1 (20)	87.76	87.80	65.07	62.99
12	−1 (4)	−1 (5)	1 (200)	1 (40)	91.95	91.99	63.97	65.07
13	−1 (4)	1 (15)	1 (200)	−1 (20)	82.99	83.03	36.03	44.16
14	1 (10)	−1 (5)	1 (200)	1 (40)	84.23	89.19	64.36	73.07
15	1 (10)	−1 (5)	−1 (100)	−1 (20)	82.16	82.14	74.85	73.21
16	1 (10)	−1 (5)	−1 (100)	1 (40)	73.47	73.51	73.63	75.29

Decolourization

A total of 16 experiments were performed, following the factorial design 2^4, and 2 responses were investigated, namely R1 (colour removal, %) and R2 (chemical oxygen demand (COD) removal, %). The highest percentage of colour removal from textile dye effluent was recorded at 91.95 %, performed by experiment no. 12 (R12) with process conditions of low initial pH (−1, 4), low initial concentration of

bacterial consortia inoculum (−1, 5 %), high agitation speed (+1, 200 rpm) and low temperature (−1, 20 ℃). In the study carried out by [41], high decolourization was achieved with low pH (pH 4.0) for natural textile dye (effluent (Remazol Turquiose Blue G (94 %)) that reached 59 % of decolourization with synthetic dye effluent (Lanaset Blue 2R). However, [36] reported the decolourization of only 52 % in their study with the same conditions. According to factorial design, the highest value of colour removal is expected to be 91.99 %, containing very little difference in the recorded values. The R10, R4 and R3 show the second-highest percentage of colour removal with 89.23 and 89.00 % for both later runs, respectively. The predicted colour removal values for these experiments are 89.19 (R10), 89.04 (R4) and 88.95 % (R3), showing little fluctuation in their real values obtained experimentally. In a recent study [42], it was observed that degradation of reactive textile dye (Direct Red 81) achieved more than 95 % with pH 7.0, at 30 ℃ within 24 h of experiment. The rapid decolourization process was achieved with optimum nutrient and environmental parameters, as to compare. Similar result was achieved by [43] with similar process conditions, where dye degradation achieved was > 90 %. It shows that all parameters studied gave significant effects in achieving maximum dye degradation. In another report by [44] new technology of dye degradation was studied, as photocatalytic dye degradation was studied, in order to investigate and enhance the ability of microbial cells in the dye degradation process. It was observed that the dye degradation took faster time with maximum dye degradation (~ 100 %). However, in this new technology, the process conditions are different with the current study.

The lowest percentage of colour removal was recorded at 73.74 % in R16 with the process conditions of high pH (+1, 10), low initial concentration of bacterial inoculum (-1, 5 %), low agitation (-1, 100 rpm), and at high temperature (+, 40 ℃). Nonetheless [45] reported that dye RG-19 was effectively decolourized (at 55 %) in the pH range of 5 - 10 with maximum decolourization achieved (94.10 %) at pH 8 with the optimized temperature of around 25 - 35 ℃. Meanwhile [46] reported 70 - 75 % of dye removal was achieved at pH 9 and the same amount of dye removal was obtained during normal pH (6 - 7). The predicted value for colour removal by R16 was 73.51 %, indicating very little fluctuation in the actual value obtained. Using the colour removal results shown in **Table 3**, the analysis of the main effects affecting the dye degradation activities by the local bacterial isolates through factorial design is illustrated in **Figure 1**. In addition, the analysis of variance (ANOVA) of the 4 parameters are presented in **Table 5**.

Table 5 ANOVA test and the main effects of colour removal by factorial design.

Source	Standardized effects	Sum of squares	F-value	*p*-value Prob > F	% Contribution
Model		371.36	1,004.52	0.0247*	
A	−3.89	60.41	2,287.78	0.0133*	16.27
B	−3.57	51.02	1,931.94	0.0145*	13.74
C	1.30	6.80	257.48	0.0396*	1.83
D	−0.83	2.73	103.41	0.0624	0.74

*p-value indicating model is significant

Table 5 exhibits the ANOVA test of the factorial design with the *p*-value of model 0.0247, indicating that the model is significantly large in this study. Three factors or parameters also exhibit a *p*-value less than 0.0500, with factor A (0.0133), factor B (0.0145) and factor C (0.0396), indicating the significance of these variables during the decolourization by local bacterial isolates using factorial design. On the other hand, the *p*-value of factor D (temperature) is 0.0624, which is rather insignificant since it is > 0.0500. This indicates that the parameter can be disregarded in the study since its *p*-value is more than 0.005. Even though this relatively low *p*-value has also been reported by [9], the higher temperature in their study was able to increase the rate of decolourization process of methylene blue dye.

Figure 1 shows the main effects of removing colour from textile wastewater by factorial design. As seen in this figure, 3 parameters show an impressive effect as compared to 1 factor, which is factor D (temperature). According to [47,48], the main effect is defined as the effect of an independent variable averaged across the levels of all independent variables. The analysis of main effects of the parameters allows the determination process of the general trends of the factor's influence in the experiment. Both initial pH and initial concentrations of bacterial inoculum (factor A and B) display a high adverse effect as compared to the temperature. All 3 effects are favourable in the negative effect of this experiment. From these results, it can be understood that the lower the concentrations or values of the investigated factors, the higher amount of removal of textile dyes can be obtained.

On the contrary, the factor C agitation shows a positive effect in this model, indicating that the higher set values of agitation speed can contribute to higher quantities of colour removal in the treatment process. The R-squared value obtained from this design is 0.9999, with an adjusted R-squared value of 0.09989. The linear equation obtained from the design is expressed in coded and actual factors as presented in the following equation;

$$R1 \text{ (Decolourization \%)} = + 84.57 - 1.94A - 1.79B + 0.65C - 0.41D - 1.67AB - 0.028AC + 0.16AD + 0.72BC + 0.45BD - 1.73CD + 1.12ABC + 1.98ABD - 0.99ACD + 1.71BCD \quad (1)$$

whereas in the actual factors, the equation is expressed as follows:

$$R1 \text{ (Decolourization \%)} = 84.56687 - 1.94313 \times pH - 1.78562 \times [\text{Inoculum}] + 0.65188 \times \text{Agitation} - 0.41313 \times \text{Temperature} - 1.67062 \times pH \times [\text{Inoculum}] + 0.028125 \times pH \times \text{Agitation} + 0.15688 \times pH \times \text{Temperature} + 0.72437 \times [\text{Inoculum}] \times \text{Agitation} + 0.45437 \times [\text{Inoculum}] \times \text{Temperature} - 1.72813 \times \text{Agitation} \times \text{Temperature} + 1.11938 \times pH \times [\text{Inoculum}] \times \text{Agitation} + 1.98438 \times pH \times [\text{Inoculum}] \times \text{Temperature} - 0.99313 \times pH \times \text{Agitation} \times \text{Temperature} + 1.71438 \times [\text{Inoculum}] \times \text{Agitation} \times \text{Temperature} \quad (2)$$
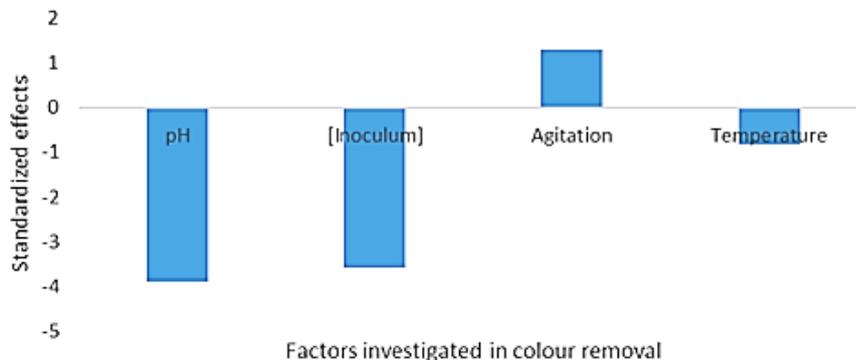

Figure 1 Main effects in colour removal by factorial design.

Chemical Oxygen Demand (COD) removal

The R2 dependent response for chemical oxygen demand removal (%) to 4 variables in factorial design is investigated and the results are elaborated in this section. Based on the values reported in **Table 3**, the highest value of COD removal is recorded at 74.85 % in the experiment no.15 (R15) with process conditions of high initial pH (+1, 10), low initial concentration of bacterial inoculum (−1, 5 %), low agitation speed (−1, 100 rpm) and in low temperature (−1, 20 °C). The predicted value gives 73.21 % of the COD removal which is about 2.19 % of the design's fluctuation value. In a study conducted by [49], optimum COD removal was achieved at pH 7.2 at the temperature from 28 to 29 °C and agitation speed of 150 rpm. This shows that optimum COD removal can be achieved from a pH range of 7 - 10 (neutral to alkaline) [50,51]. The lowest value of COD removal is recorded at the level of 36.03 % in (R13) with process conditions of low initial pH (−1, 4), high initial concentration of bacterial inoculum (+1, 15 %), and agitation speed of (+1, 200 rpm) at low temperature (−1, 20 °C).

Nevertheless, factorial design predicts that the highest COD removal should be achieved with high (+1) initial pH, low initial concentrations of bacterial inoculum, low agitation speed as well as high temperature to give a value of 75.29 % (R16). The actual value of COD removal that is achieved in the experiment is in fact not too different from R15, which is 73.63 % with only 1.629 % difference that is a result of the difference of the temperature during the incubation process. Meanwhile, the lowest value of COD removal can still be maintained by R13 within 36.03 %, with predicted values of 44.16 %, which only differs by 22.396 % compared to the actual value. This occurrence could happen during the experiments and could be contributed by the viability of the bacterial cells. In this run, the bacteria consortium could have been dead due to the excess nutrition; thus, hindering the occurrence of degradation activities. The excess uptake of nutrients can lead to stress of the cell, affecting the level of reactive oxygen species produced by mitochondria, thus potentially causing damage to cellular components and leading to the cell death [52-56]. For further details on the main effects of the factors investigated in this study, analysis of variance (ANOVA) is performed and presented in **Table 5**, while the main effects affecting the process of COD removal are illustrated in **Figure 2**.
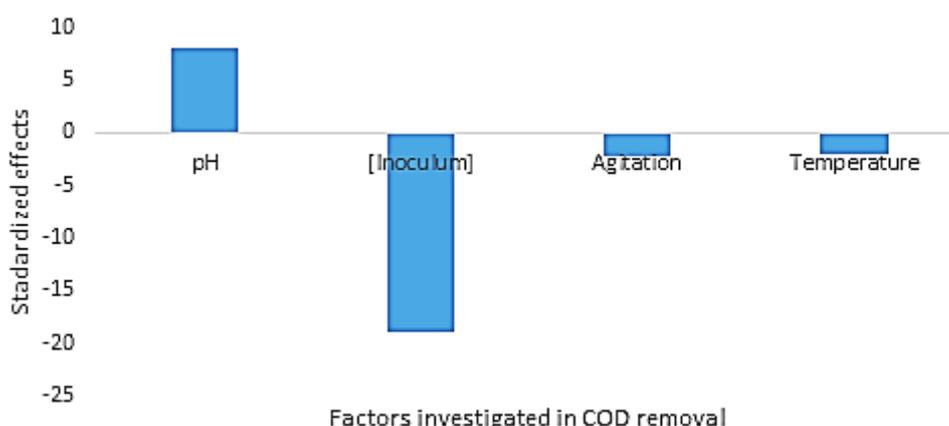

Figure 2 The factors that are investigated in the COD removal.

Table 6 ANOVA and main effects of COD removal by factorial design.

Source	Standardized effects	Sum of squares	F-value	p-value Prob > F	% Contribution
Model		1711.73	8.55	0.0022*	
A	8.00	255.92	5.11	0.0450*	11.37
B	-18.83	1418.84	28.34	0.0002*	62.71
C	-2.22	19.65	0.39	0.5438	0.87
D	-2.08	17.33	0.35	0.5682	0.77

*p-value indicating model is significant

Table 6 illustrates the ANOVA and primary model in removing COD from textile dye effluents by factorial design. From the ANOVA test, it is discovered that the model results in a significant COD removal, with the p-value of 0.0022, which is < 0.0500. Factors A and B (initial pH and initial concentrations of bacterial inoculum) also display the p-values of less than 0.0500 (0.0450 and 0.002, respectively), indicating that the parameters used in this study result in a significantly large removal of the chemical oxygen demand from textile dye wastewater. In contrast, the COD removal values of the other 2 parameters, namely, factors C and D (agitation and temperature), are insignificant due to the p-values being more prominent than 0.0500 (0.538 and 0.5682, respectively). Moving forward from the ANOVA test, these parameters' main effects are determined and illustrated in **Figure 2**.

The main effects of this model are significant due to the *p*-value, which is less than 0.05. The F-value of 8.55 indicates that the model is rather significant. The other significant model terms are model A (the initial pH) and model B (the initial bacterial inoculum concentrations). **Figure 2** provides a better perspective at the main effects' contributions to the COD removal by the local bacterial isolates using factorial design. It was discovered that C and D (agitation and temperature) have a slight influence in maximizing COD removal from textile effluent compared to the pH and bacterial inoculum concentration, where the lower concentration significantly affects the initial concentration of the bacterial inoculum. The initial pH, the positive effect is displayed in this figure, indicating that the COD removal is preferred and maximized in neutral and alkaline conditions. The R-squared for this model is recorded as 0.7566, and the adjusted R-squared is recorded as 0.6680. The regression equation of both coded and actual factors can be observed in the following equations;

$$R2 \text{ (COD removal, \%)} = + 59.72 + 4.00 \times A - 9.42 \times B - 1.11 \times C + 1.04 \times D \tag{3}$$

As for final equation in actual factors,

$$R2 \text{ (COD removal, \%)} = + 59.72313 + 3.99937 \times pH - 9.41688 \times [\text{Inoculum}] - 1.10813 \times \text{Agitation} + 1.04062 \times \text{Temperature} \tag{4}$$

Conclusions

The use of bacterial consortium was examined in the study, together with the 4 process conditions (pH, initial bacterial consortium concentrations, agitation speed and temperature). Based on the study, low pH to neutral pH is favourable in the process of decolourization of textile dye effluent, with normal speed of agitation and low concentration of bacterial consortium in the media (91.95 %). The main effect of parameters was determined, and temperature is determined to be insignificant in this study, based on the ANOVA test as it does not show any significant effect to the bacterial degradation process of the textile effluent. The decolorization model was significant with $p < 0.05$ ($p < 0.0247$). In chemical oxygen demand (COD) removal, higher range of pH is more favourable (neutral to alkaline) with a low concentration of bacterial consortium concentrations in the media (74.85 %), whilst the agitation speed and temperature are discarded, based on insignificant effect shown, by ANOVA test. The *p*-value of the model is $p < 0.0022$, indicating that the model is significant. The application of full factorial design in this decolourization and COD removal is found to be beneficial to the wastewater treatment technologies and application studies.

Acknowledgements

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