Objective: This study evaluated and identified the removal of colors and chemical oxygen demands from textile dye effluents by Bacillus cereus isolated from the local textile wastewater treatment plant.

Methods: Central composite design (CCD) from response surface methodology (RSM) was applied in order to achieve the optimized treatment process condition for the textile dyes wastewater degradation. Two-level of three process parameters with six center points resulted a total of twenty runs of experiments were performed. Bacterial inoculum (-1,+1) (%v/v), agitation (-1, +1) (rpm), and pH (-1, +1) were tested.

Results and Discussion: During the ten days of biodegradation process, highest decolourization achieved was 88.67% with low pH and agitation; and medium level of initial concentration of bacterial inoculum. Highest chemical oxygen demand (COD) removal was achieved with 99.20% from high pH (pH 10), low agitation (100 rpm) and high initial concentration of bacterial inoculum (15%, v/v).

Conclusions: The biological treatments was able to remove colour and chemical oxygen demand with application of CCD, giving the optimum settings of the three process parameters studied.

Keywords: Biodegradation, Central Composite Design, Textile Wastewater, Wastewater Treatment
1. Introduction

1.1. Background

Water pollution comes from domestic, agricultural and industrial waste. In 2014, an estimated amount of 2 million tons of agricultural and industrial sewage had been discharged into water bodies without being treated and an estimation of approximately around 1,500 km³ of wastewater being produced all over the world annually, as it is six times more of water exists in the world. Furthermore, an estimation of 380 billion m³ of wastewater are being produced globally each year. It is expected to increase by 24% in ten years and 51% in another thirty years. The production of textile dye wastewater annually was reported to be 700,000 - 800,000 tonnes metric globally and it is estimated about 10-20% are being released through industrial effluents, into the water bodies. The extensive application of dyes material especially in the textile industries have become a significantly concerning issue worldwide. In the past natural dyes were used for the dyeing and colouring of textiles.

1.2. Textile dye application

Batik, as one of the national identities of Malaysia has been produced mainly from a few states in Malaysia, that are to say Kelantan, Terengganu and Sarawak. As one of the major producers in batik textile industries in Terengganu, the production of its wastewater which is mainly composed of commercial dyes is also getting bigger. Commercial dyes are known to be composed of complex chemical compounds which make them toxic, difficult to degrade and resist the biodegradation over time. Few types of commonly found commercial dyes are chromophores dyes which are azo, anthraquinone and indigo dyes. They are known to be slow degrading because of their complex structures and their breakdown processes and by-products are potentially toxic, carcinogenic and mutagenic. The potential danger that the textile dyes are causing include the harming of the aquatic life such as fish, plants and thus leading to the disruption of the ecosystem. The biological and chemical oxygen demands will be high and therefore cause the water bodies to become polluted and endanger the lives involved.

1.3. Central composite design (CCD)

Central composite design (CCD) is a method under the response surface methodology (RSM) used extensively in designing an experiment. It follows a second-order polynomial degree, depending on the variables used in the experiment. It is the most commonly used second-order design, other than 3 k factorial design and Box-Behnken design. Central composite design consists of three crucial parts; (1) a full factorial or fractional factorial design, (2) an additional design, where experimental points are at a distance from its center and (3) a center point. Following the essential points mentioned, the total number of design points in the central composite can be described as \( n = 2k + 2k + n_0 \). As an example, a central composite design (CCD) of \( k = 2 \), \( a = \sqrt{2} \) and \( n_0 = 2 \) has the form:

\[
D = \begin{pmatrix}
-1 & -1 \\
+1 & -1 \\
-1 & +1 \\
+1 & +1 \\
-\sqrt{2} & 0 \\
\sqrt{2} & 0 \\
0 & -\sqrt{2} \\
0 & 0 \\
0 & 0
\end{pmatrix}
\]

The response \( Y \) is always represented by the following second-order polynomial equation as described:

\[
y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_i x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j \quad \text{Eq. (1)}
\]

Where \( Y \) is the predicted response, \( \beta_0 \) is offset term, \( \beta_i \) is the linear effect, \( \beta_{ii} \) is the squared effect, and \( \beta_{ij} \) is the interaction effect in the experiment. The central composite design has been applied widely in the optimization process, including dye removal from textile wastewater. CCD (central composite design) was applied to study the effects of variables and their interactions and maximize the production in the study. In their study, four main parameters/independent variable were identified and studied, resulting in thirty run of experiments. Maximum removal of COD and colour were achieved with 90.11% and 99.87% respectively. The main advantage of using CCD includes fewer experiment trials with multiple parameter assessments as compared to single factor experiments practised (one-variable-at-a-time method). This method offers disadvantages due to no interaction of effects among the variables studied. Three factors/independent variables were selected (COD load, aeration rate and hydraulic retention time) resulting in twenty runs of experiment. From FC-CCD, the maximum removal of COD, NH\(_4\)-N and Mn\(^2+\)
in drinking water is achieved (93.9%-95.5%). Both studies demonstrated the efficiency of applying response surface methodology in the process optimization of COD removal and decolourization of textile effluents. Before applying response surface methodology (RSM), a single factor optimization was applied and resulted in many experiments and the inability to determine the combined effects of the significant parameters (variables). Another advantage of using RSM/CCD is that the estimation of linear interaction and quadratic effects of the factors and a model prediction for response is proposed.28

This study focuses on the removal of colour and chemical oxygen demand (COD) from the industrial textile wastewater collected from local factory situated adjacent to the Sg. Hiliran. The backyard factory, as the locals referred to, is a small ‘batik’ textile producing factory, run by a local family. In the process of ‘batik’ dyeing, huge amounts of water is being used and the wastewater with the remaining textile dyes are discharged freely right into the river adjacent to the factory. The textile wastewater has been identified to have been directly discharged into the river without undergoing necessary treatments. The textile wastewater has undergone the biological treatment, where Bacillus cereus was applied in the wastewater treatment process. The effluent treatment process had been optimized using central composite design (CCD) for the optimization of process parameters in the textile dye wastewater treatment with high pH (pH 10), low agitation (100 rpm) and high initial concentration of bacterial inoculum (15%, v/v).

2. Research methodology

2.1. Optimization of fermentation conditions for decolourization and chemical oxygen demand (COD) removal

2.1.1. Textile dye effluent

Textile dye effluent was obtained from a local backyard textile producing factory in Kuala Terengganu, where the wastewater is being discharged directly into Sg. Hiliran. The textile wastewater was collected and stored in < 3 ℃ chiller for the study purposes.

2.1.2 Bacterial isolates

1 ml of each textile dye wastewater at three collection points were dropped onto the nutrient agar (NA) and nutrient broth (NB) and these were done in triplicates. After the textile dye wastewater was spread onto the agar with hockey stick, it then incubated at 37 ℃ (Memmert) overnight. As for the nutrient broth, 1 ml of textile dye wastewater was pipetted into the sterile broth and incubated at 37 ℃ with 150 rpm in incubator shaker (Jeio Tech) overnight. Bacterial inoculum of three isolates (C1, 3 K and 3 W) were prepared with nutrient broth (NB) (Merck). An approximate of 20 ml of nutrient broth were sterilized at 121 ℃ for 15 minutes (Hirayama) prior and cooled down. One loopful of each isolate were inoculated into the nutrient broth under aseptic condition and incubated overnight at 30 ℃, 150 rpm.

Three process parameters were selected to optimize colour and chemical oxygen demand (COD) removal from textile wastewater. Following the screening process done with full factorial designs, one factor was omitted, which was the temperature (℃) due to an insignificant effect that contributed to the decolourization and chemical oxygen demand removal. The suggested experiments of central composite design (CCD) can be observed in the following table that illustrates the removals of colour and chemical oxygen demand (COD) with three levels. (-) represents the low level, (0) represents the centre level and (+) represents the high level. As listed in Table 1, pH was tested at pH 4, pH 7, and pH 10. For bacterial consortia inoculum, the concentrations used in the study were 5%, 10%, and 15% in (v/v) measurement. Lastly, for agitation speed, measured in rotation per minute (rpm), 100 rpm, 150 rpm, and 200 rpm were applied in the optimization process.

Meanwhile in Table 2, it represents the central composite design (CCD) with a total of twenty number of experiments with all the parameters followed the set values. There are six (6) centre points experiments which labelled as Run No. 3, 7, 10, 15, 16 and 19. Run No. 1 represents all factors in high value (+), and Run No. 13 represents all factors in low value (-), while others are the combination of all values, including the centre values. The values in brackets represent the actual values in the experiments i.e. pH +1 is pH 10, inoculum

| Variables | Coded symbol | Actual and coded values |
|-----------|-------------|-------------------------|
| pH        | A           | 4 7 10                  |
| Bacterial inoculum concentration (%) (v/v) | B | 5 10 15 |
| Agitation speed (rpm) | C | 100 150 200 |
3. Presentation of findings and discussion

3.1. Process optimization of textile dye effluent and COD removal by central composite design (CCD)

Central composite design (CCD) is one of the experimental designs available in Response Surface Methodology (RSM) (Design Expert 7.0). CCD was chosen to investigate the optimization process of decolourization and the removal of chemical oxygen demand (COD) in the textile wastewater effluent. Following the three (3) investigated factors/variables, initial pH of textile effluent, initial bacterial consortium inoculum’s initial concentration (%) (v/v), and agitation speed (rpm), a total of 20 experiments, including six centre points, were designed and performed. The incubation period for these conducted experiments was ten days. The completed design of central composite design (CCD) with actual and predicted results can be observed in Table 3. Again, the actual value of factors studied are in the bracket; i.e Run 1: pH -1 is pH 4, Inoculum concentration +1 is 15%, v/v, agitation speed +1 is 200 rpm.

Table 2. Central composite design for three factors at three levels measurement.

| Run No. | Factor A: pH | Factor B: [inoculum] (%) (v/v) | Factor C: agitation speed (rpm) |
|---------|--------------|---------------------------------|-------------------------------|
| 1       | +1 (10)      | +1 (15)                         | +1 (200)                      |
| 2       | 0 (7)        | -1 (5)                          | 0 (150)                       |
| 3       | 0 (7)        | 0 (10)                          | 0 (150)                       |
| 4       | -1 (4)       | 0 (10)                          | 0 (150)                       |
| 5       | +1 (10)      | -1 (5)                          | -1 (100)                      |
| 6       | -1 (4)       | +1 (15)                         | +1 (200)                      |
| 7       | 0 (7)        | 0 (10)                          | 0 (150)                       |
| 8       | -1 (4)       | +1 (15)                         | -1 (100)                      |
| 9       | 0 (7)        | -1 (5)                          | 0 (150)                       |
| 10      | 0 (7)        | 0 (10)                          | 0 (150)                       |
| 11      | 0 (7)        | 0 (10)                          | +1 (200)                      |
| 12      | 0 (7)        | 0 (10)                          | -1 (100)                      |
| 13      | -1 (4)       | -1 (5)                          | -1 (100)                      |
| 14      | +1 (10)      | 0 (10)                          | 0 (150)                       |
| 15      | 0 (7)        | 0 (10)                          | 0 (150)                       |
| 16      | 0 (7)        | 0 (10)                          | 0 (150)                       |
| 17      | -1 (4)       | -1 (5)                          | +1 (200)                      |
| 18      | +1 (10)      | -1 (5)                          | +1 (200)                      |
| 19      | 0 (7)        | 0 (10)                          | 0 (150)                       |
| 20      | +1 (10)      | +1 (15)                         | -1 (100)                      |

3.2. Decolourization

The textile dyes removal process from textile effluent or decolourization was one of the responses in central composite design (CCD). Following the equation (1), the second-order polynomial equation was obtained. In this design, the highest colour removal was observed at 88.67% by experiment no.15 (R15), with the design of (-1) for initial pH, (0) for bacterial consortium inoculum’s initial concentration (%) (v/v), and (+1) for agitation speed (rpm) with the same value for the predicted value of textile dye removal by the design. Meanwhile, the lowest removal of colour from textile dye effluent was recorded at 2.18% by R18 with the design of (+1) for all parameters with a predicted value of 5.91%. The highest value of textile dye removal was predicted to be 89.68% by R10 with parameters condition of low level for initial pH (-1) and high level for both bacterial consortium’s initial inoculum concentration (+1) and agitation speed (+1). In comparison, the lowest was predicted to be performed by R18, with 5.91% of the colour removal from textile wastewater. The remaining actual and predicted dye removal (%) in each experiment’s textile effluent can further be observed in Table 3. Six experiments of centre points each gave values of textile dyes removal starting from 43.99% (R11), 69.39% (R2), 69.86% (R3), 73.81% (R12), 78.23% (R17) and 79.48% (R14). According to designated components in the experimental design, each centre point experiment has predicted values should be 64.85%. Again, the fluctuation values can be contributed by the process conditions during the experimental procedures and, importantly, the bacterial cell’s viability during the process.

Equation (2) and (3) represents the percentage of dye removal both in coded and actual factors.

\[
\text{R}_2 = 64.85 + 69.68A - 22.87B + 11.82C - 55.91AB + 29.84AC - 52.77BC + 0.07A^2 - 28.69B^2 - 23.34ABC - 62.15AB
\]

The equation in actual factors;

\[
\text{R}_2 = 64.85 + 69.68A - 22.87B + 11.82C - 55.91AB + 29.84AC - 52.77BC + 0.07A^2 - 28.69B^2 - 23.34ABC - 62.15AB
\]

The response, R2, or textile dye removal depended on the experimental design parameters. From the equation (2) and
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R-squared value (0.8420) for this model indicated that 84.20% of the variables supported the response while the adjusted R-squared was 0.6665. Analysis of variance (ANOVA) of this model is presented in Table 4. In this ANOVA model, all sources excluding C-C (agitation speed) and interaction AC (pH x agitation) were significant with model F-value 4.80, indicating that model was significant. The p-values less than 0.05 indicating that model terms were significant, wherein this model, source of A, B, AB, BC, A2, B2, ABC, and AB2 were significant model terms with 0.0315, 0.0126, 0.0175, 0.0221, 0.0064, 0.0245, 0.0235 and 0.0460, respectively. Meanwhile, factor C-C presented a p-value of more than 0.05 (0.143), pointing out that the factor was insignificant in the study of decolourization by local bacterial isolates using CCD. Factor C was joined with model AC, which showed the interaction between initial pH and agitation with p-value of 0.1326.

Fig. 1(a) and (b) represent normal plot of residuals and residuals vs. predicted from color removal in CCD. From Fig. 1(a), it was observed that R7, R8, R18, and R20 has a slight deviation from the line, suggesting departures while in the overall picture, the data were distributed normally. Since the residual plots were distributed normally, no transformation of the model was needed. The normal plot is usually plotted to identify outliers from the design of experiments, and it can be formed from raw data, estimated parameters, and residuals.
from model fits and in Fig. 1(a), the normality plot was assumed to be satisfied as the residuals plot approximated along a straight line. 30 Fig. 1(b) portrayed an illustration of residual data versus the predicted values. It can be observed that almost all values obtained have clumped towards higher response values, and the proposed model can be said as inadequate distribution of the data. A regression model versus the predicted values that were an adequate model due to even distribution of data in the model proposed by the study. 31 A similar model of equal data distribution was proposed according to the internally studentized residuals model, as normal and random distribution of data was observed. 32

Fig. 2(a) represents the actual values against predicted values obtained from the central composite design for decolourization. The actual values had small standard deviations according to normal probability plots. The point distribution of data should be around the line on the predicted-actual graph for the data to be considered that the model satisfied the ANOVA assumption. 33,34 Box-Cox plot family transformation is a linear regression technique when the linear model’s assumptions were violated. 35,36 Box-Cox plot is a tool for transformation in the data’s response, and it is widely used because of transformation acts symmetrically in the models with intercepts. 37,38 Box-Cox is plotted by lambda (λ) versus ln of residuals, as presented in Fig. 2(b).

Fig. 3(a) displays the contour plot between factor A (initial pH) and factor C (agitation). As ANOVA explained, both initial pH and agitation were significant in this study of decolourization by CCD. Linear expressions were portrayed in the figure indicating the effects of pH values and agitation speed on decolourization of textiles dyes. Linear expression expression was supported, indicating as the factor t (pH) increase, the yield, y also increased in the textile dye removal from textile wastewater. 39 Fig. 3(b) shows the interaction between factor A (initial pH) and factor B (initial concentrations of bacterial inoculum). The interaction between these two factors showed that centered values were preferred to obtain an optimized decolourization value. The same pattern was
shown by factor B (initial concentrations of bacterial inoculum) and factor C (agitation speed) in Fig. 3(c). The optimized value obtained was slightly lower in the initial concentrations and upper level for the agitation speed. This means the lower concentration of the bacterial inoculum and the higher agitation speed in the fermentation conditions; the higher value of decolourization could be achieved. Reduced amount of initial concentrations of bacterial inoculum consortium and increased agitation speed could contribute to the increased yield of decolourization of the textile effluent.

3.3. Chemical oxygen demand (COD) removal

Chemical oxygen demand (COD) removal (%) or symbolized as R1 in this experimental design follows the quadratic model second-order polynomial equation. Three potential parameters in maximizing the removal of COD were investigated. Referring to Table 3, the actual and predicted values of chemical oxygen demand (COD) removal (%) were recorded. The highest reduction of COD was achieved with 99.2% by R20 following design (+1) of initial pH, (-1) initial bacterial consortia inoculum concentration (%) (v/v), and (-1) of agitation (rpm). It was then followed closely by R9 with 99.18% of COD removal with (+1) of initial pH, (+1) bacterial consortia inoculum concentration (%) (v/v), and (-1) of agitation speed. The lowest removal of COD was obtained with 86.77% by (-1) of initial pH, (0) of inoculum concentration (%) (v/v), and (0) of agitation speed. Uniformed values of COD removal were not able to be achieved in the centre point experiments as the values range from 88.65%, (R2), 88.77% (R3), 94.57 (R17), 94.67% (R11), and 94.97% by R12. Both R9 and R20 recorded the highest COD removal’s predicted value as it gave 99.80%, and the lowest values were recorded by R1 and R5, both 90.35%, respectively.

For COD removal by CCD, there were two models suggested by the design. Following the lack of fit test, a linear
model was suggested. However, only the model A and C² are significant in the linear model, while all three variables were not significant. Another model suggested by CCD is the quadratic model, with modification. In this model, the final equation in coded and actual factors can be observed as follows

\[ R1 = +92.88 + 2.53^A - 1.44^C + 2.94^C^2 \] \hspace{1cm} \text{Eq. (4)}

while the equation on actual factors:

\[ R1 = +92.88273 + 2.53364^A - 1.44427^C +2.93700^C^2 \] \hspace{1cm} \text{Eq. (5)}

R-squared value obtained is 0.5447 while adjusted R-squared is 0.4594. Analysis of variance of this model can be observed in the following table.

According to Table S1 (Supplementary material), Model F-value was 6.38, showing that the model was significant with a p-value obtained 0.0048, thus indicating a p-value < 0.0500. In this analysis, only two factors and its interaction were significant as compared to the other parameters. Model terms labelled A denotes factor pH, and model C indicates the factor agitation. Model C2 indicates the agitation x agitation interactions were also significant with p-values of 0.0128 and 0.0262, respectively. The interaction of factor A (initial pH) and factor C (agitation) and C2 (agitation*agitation) will be discussed and presented in the next sub-topics.

According to the contour plots, A:A refers to the factor A pH and C:C referring to factor C agitation. It was labeled according to the input in the design during the initial setting of the design. Fig. 4(a) shows the normal plot residuals of chemical oxygen demand (COD) removal by bacterial isolates using CCD. In this model, it was shown that all the values were plotted closely to the linear graph. The studentized residuals measured the standard deviations between the actual and predicted values obtained in the experiments. As for Fig. 4(b), it was apparent that the data were distributed randomly, and no such abnormalities were discovered. Based on the ANOVA, it justified the assumptions that the model was significant. The actual and predicted graph was plotted and presented in Fig. 4(c). As for the Box-Cox transformation graph, it can be observed that the plot was already in linear expression, as compared to the Box-Cox plot in Fig. 4(d). According to ANOVA, factor A (initial pH) and factor C (agitation speed) were significant. The interaction of these two factors can be observed through the contour plot, indicating that the higher values of the initial pH of the solution (which in this study, the textile dye effluent) and a moderate amount of agitation speed could lead to the maximum value of COD removal by the bacterial isolates. The contour plots the response was described on how the response was affected when the level of factors changes, while other factors were at a fixed optimum level.³⁹

3.4. Effect of process parameters

3.4.1. Effect of initial pH

Initial pH in the media is crucial and vital in the microorganisms’ fermentation process. The acidity and alkalinity of the solutions will affect the performance of the microorganisms in the process. As initial pH increased, COD removal will decrease, and the equation in linear equation (4) exhibits that pH has a direct relationship with COD removal from textile effluent. However, for dye and COD removal, the different effects of initial pH had been observed, as illustrated by Fig. S1(a) and (b). Higher pH contributed to the increased amount of color removal from textile effluent. An increase in pH from pH 2 to pH 10 resulted in a 12.03% increase in adsorption, and due to higher pH values, the surface was negatively charged, which contribute to the increment of the electrostatic attraction of the positively charged cationic dye.⁴⁰ An optimal pH of 5.8 was able to achieve maximum degradation of synthetic Malachite Green (89%) by Hypocrea lixii, a species of marine Trichoderma.⁴¹ It worked well near the normal pH conditions for marine microorganisms, reported that the optimized pH value in the adsorption of methyl violet (MV) from aqueous solution was between 8 and 10, indicating that the activity of static interactions between ammonium groups in the adsorbents. Ammonium groups in adsorbents will attract the negatively charged phenolic compounds (potential active sites for NH₃) in strongly basic conditions, thus influencing the removal of methyl violet. However, lower initial pH was desired."
Fig. 4. (a) Normal plot of residuals (b) residuals vs. predicted (c) actual values vs. predicted and (d) Box-Cox plot (e) contour plot showing interactions between pH and agitation and (f) 3D surface of pH-agitation interactions.
3.4.2. Effect of initial concentrations of bacterial inoculum

Bacterial inoculum initial concentration is critical in the process of decolourization from textile wastewater. As presented in Fig. S2(a) and (b), bacterial inoculum concentration played a vital role in biodegradation. Inoculum size played a difference in removing color from aqueous solution. It was observed that an optimum concentration within 10% of *P. chrysosporium* gave a higher decolourization percentage. A range of 5% to 50% inoculum size of *P. aeruginosa* had been applied in decolourization of Reactive Red BS, and an optimum concentration of 25% was determined with a dye removal rate of 57.53 mg/l/h. In another study, an initial concentration of 10% of *P. aeruginosa* resulted in 97.10% decolourization of textile dye Direct Blue 71. It corroborated the result of previous research, stating that a median concentration if initial bacterial inoculum could lead to higher achievements of color removal. High concentrations of initial bacterial inoculum could cause population density in a set volume in an experiment, leading to cell toxicity due to the bacterial cells’ death due to the excess amount of carbon sources and an insufficient amount of essential nutrients during the chemical removal processes.

Bacterial inoculum is one of the factors that is studied in the process of decolourization and COD removal from textile wastewater by CCD. The highest decolourization activity was achieved with the median level in the design (10%, v/v), while the highest COD removal was achieved with a low level (5%, v/v) of the bacterial inoculum. According to ANOVA, bacterial inoculum concentration is one of the significant factors in removing color from textile wastewater. Lower initial pH and medium concentration of bacterial inoculum in the textile wastewater contributed to the maximum amount of color removal, as presented in the contour plot (Fig. 3(b)). Lower initial pH value (pH 4) demonstrated that bacterial consortia (*Bacillus* sp.) worked well in acidic conditions. An increase in inoculum concentration gave an increase in the dye decolorization process. It was observed that the optimum concentration of bacterial inoculum (10%) with 1% of glucose gave higher decolourization. Fig. 3(c) shows the interaction of inoculum concentration and agitation speed. It was observed that a higher speed of agitation and a lower concentration of bacterial inoculum, biodegradation of color from textile effluent could be obtained in the maximum amount and this might be due to the contact time and speed between the cell cultures and textile effluents, increasing the possibilities of the color removal process.

3.4.3. Effect of agitation

Agitation, one of the parameters investigated in this study, showed an average effect in the process of color and chemical oxygen demand removal from textile wastewater. Presented in Fig. S3(a) and (b) are the agitation effects in textile dye effluent and COD removal. Agitation was preferred at low speed because that it can lower the damage and injuries to the bacteria cell wall. Increased agitation speed can cause disruption and detrimental to the cell wall, thus damaging the bacteria itself. Therefore, in this study the lower speed is preferable. A lower speed of agitation was proposed, as the textile dye removal efficiency was dropped, causing no significant decolourization activities by *Planctobacterium* strain. Agitation at 150 rpm was designed in the textile wastewater treatment by microbial consortium in bioreactor indicated lower speed of agitation was considered as to avoid...
disruptions to the cell wall, thus improve the efficiency of the dye removal by the microbes.\(^\text{60}\) In the achievement of 88.67% of decolourization by CCD, agitation was set at 100 rpm (lower level). Both R9 and R20 that had lower agitation speed in the design contributed to a high decolourization percentage, with 74.83% and 83.90%, respectively. However, pH values in these two designs were at a higher level (pH 10), pointing that in alkaline conditions, the highest decolourization was achieved with acidic conditions (pH 4). Agitation is significant in submerged fermentation, as it is vital in the mixing and heat transfer process, thus creating shear forces that can change the morphology and growth of the microorganisms and the product formation.\(^\text{61,62}\) The application of filamentous white-rot fungus *Ganoderma* sp. Agitated cultures for 8 h were found to achieve almost 75% of decolourization while about 27% decolourization was able to be obtained in stationary cultures.\(^\text{63}\) In a recent study, agitation was set at 120 rpm and give 70-80% of textile decolourization within 22 h of fermentation process.\(^\text{64}\) In another research conducted, agitation at 175 rpm gave a better yield of laccase production, increasing the dye decolourization yield.\(^\text{65}\) An increment in agitation rate leads to a better aeration rate, thus increasing the production of laccase enzyme in *S. psammoticus*, resulting in higher decolourization of the dye solution. The increased mass transfer between cell cultures and the medium and increased the amount of laccase production thus contributed to higher color removal in the media. It represents the positive influence of agitation in microorganism growth and its interaction with the textile effluent components. In COD removal, the effect of agitation was significant as indicated by the p-value is less than 0.0500 and was represented by linear reaction where when agitation is increased, higher removal of COD is obtained. Few numbers of runs displayed a high percentage of COD removal with a high level of agitation. R4 gave 89.75% of COD removal, R10 gave 92.99%, R16 gave 90.66%, and R19 with 94.92%, respectively. However, the interaction effects between all three parameters that contributed to the maximum achievement of COD removal were not able to be determined since parameter B (bacterial inoculum concentration (% (v/v)) was deemed insignificant in this model.

### 3.4.4. Effect of temperature

In this study, temperature is the last factor studied in this factorial design to remove color and COD from textile dye wastewater. It was observed that during the study, the effect of temperature is insignificant due to the p-value is over 0.05. There was an increase of 12% in decolourization with an increase in incubation temperature from 30°C to 35°C suggesting that the profound effect this parameter could have on the dye removal capabilities by the integrated cultures of the microorganisms.\(^\text{29}\) No significant amount of decolourisation was obtained at a temperature below 25°C or above 40°C. An optimum temperature of 30°C to 35°C was found to be best for decolourization to occur, and no further decolourization activities occurred at a higher temperature than as stated.\(^\text{66}\) The effect of temperature in removing textiles by the adsorption process.\(^\text{67}\) When the temperature increased, there was no significant decolourization could be observed during the process. Further, it was suggested that the adsorption activity was slowed down and halted due to its physical characteristics unable to stand with the higher temperatures (> 55°C).

**Fig. S4(a)** and **(b)** illustrate the relation of temperature and color, and as well as COD removal from factorial design. From the figures, it can be observed that the effect of temperature was insignificant, because only slight deviations from the graph, therefore the factor of temperature was discarded in the central composite design for optimization of process conditions, both in the activities of decolourization and chemical oxygen demand (COD) removal.

The application of microbial isolates in this study indicates the biological approach in treating textile effluents. Besides this approach, physical and chemical treatments also applied in order to treat the textile effluents. For example, new wastewater treatment technologies being introduced that demonstrates an integration of biological and chemical methods in the treatment. Microbial electrolysiss cells (MEC)\(^\text{68,69}\), microbial fuel cells (MFC)\(^\text{70,72}\) and microbial electrochemical coupled systems\(^\text{73,74}\) technologies had emerged as new wastewater treatments approach due to capability of treating wastewater with low-cost and having value-added by-products.

### 4. Conclusions

The effects of process parameters applied in the decolourization and chemical oxygen demand (COD) removal using central composite design (CCD), were studied. Central composite design (CCD) is a method that is usually used for process optimization, proving the effectiveness in terms of
cost-friendly, time constrains as well as the focusing on the factors to be analyzed. It is a very useful tool in designing lab analysis and has been widely used in the process optimization strategy. Among four process parameters that were initially studied, pH, agitation speed and initial concentration of bacterial inoculum are significant in higher percentage of decolourization and COD removal, with p-value is < 0.05. The result obtained shows that, during the ten days of biodegradation process highest decolourization achieved was 88.67% with low pH and agitation; and medium level of initial concentration of bacterial inoculum. Highest chemical oxygen demand (COD) removal was achieved with 99.20% from high pH (pH 10), low agitation (100 rpm) and high initial concentration of bacterial inoculum (15%, v/v).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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