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Color and Chemical Oxygen Demand (COD) Removal by Bacteria Consortium Using Factorial Design

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***Abstract*:** *The present study is focused on the effect of bacteria consortium in removing color and reducing chemical oxygen demand (COD) in the textile dye wastewater. There were three bacterial isolated involved in this study namely C1, C3W and C3K. Factorial design was applied into this study with five factors to be observed. They were pH, initial bacterial consortium concentration, temperature, agitation and aeration. Following seven days of incubation period, it was observed that the chemical oxygen demand removal was achieved from 79.21% to 86% in the designated process conditions whilst decolorization occurred from 32.27% up to 52.74%. The highest decolorization achieved was 52.74% with 15% of bacterial inoculum, low initial pH and no aeration present while highest chemical oxygen demand removal (86%) was achieved with high initial pH, low initial bacterial inoculum and no aeration present during the incubation period.*

Keywords: *Textile dye wastewater, color removal, COD reduction, bacteria consortium, factorial design*

1. Introduction

Textile industries are one of the major factors that contribute to the water pollution with the textile wastewater production [1, 3-4, 8, 21]. Textile wastewater is a toxic discharge from textile industry, which contains colouring agents that can cause danger to the environment, especially the water bodies such as river, ponds, lakes and sea [20]. Synthetic dyes are widely utilized in textile, paper-made, dyeing and other industries leading to a large-scale development of dye industries [22]. Dyes are most hazardous chemical compound classes found in industrial effluents which need to be treated since their presence in water bodies reduce light penetration, precluding the photosynthesis of aqueous flora [2]. Dye removal is an important but challenging area of wastewater treatment because some dyes and their degradation products may be carcinogenic and toxic to animals [9, 18]. It is characterized by strong colour, high salinity, high temperature, variable pH and high chemical oxygen demand (COD) [10]. Recently, global economic prosperity has caused rapid growth and extensive development of the textile industry. Over 100, 000 dyes has been synthesized worldwide and more than 700,000 tonnes are produced annually. More than 10,000 dyes are commercially available, but over 5% is discharged into aquatic environments by plants and users [5]. Wide application of biological treatments since it is cost-effective, reduce energy usage and environmental friendly [1, 8, 14]. Azo dyes are used for producing majority of textile dyestuffs and also synthetic formulations in many industries [10]. Azo dyes are the most widely used dyes and represent over 60% of the total dyes [14]. Textile dyes and finishing wastewater is known to contain strong colour, high pH, temperature and COD and low biodegradability [19]. Anoxic or anaerobic hydrolysis-acidification process could not only remove the colour and a portion of the chemical oxygen demand (COD) [26]. The azo and other chromophoretic groups in the dye matrix render geno toxicity to the biodiversity in the environment [7]. Color is usually the first contaminant to be recognized in wastewater. The discharge of less than 1 ppm for some dyes is aesthetically displeasing, which impedes light penetration, and affecting gas solubility thus damaging the quality of the receiving streams and may be toxic to microorganisms utilized in the treatment processes, to food chain organisms and to aquatic life [17].

The study is aimed to observe the color and chemical oxygen demand (COD) removal as well as isolation and identification of environmental microbes that are capable in performing the tasks. In this study a complete biological treatment process is applied under certain parameters in order to determine the best process condition for these potential microbial isolates in order to remove color and chemical oxygen demand from the textile dye effluent. The process conditions that to be examined include pH, temperature, bacterial inoculums concentrations, agitation speed, aeration and initial dye concentration for the simulated dye wastewater.

2. Materials and Methodology

2.1. Raw Materials

Raw textiles wastewater used in this study was obtained from a local backyard textile industry located in Kuala Terengganu, Malaysia. The textile wastewater was stored at 4^oC in a chiller. Bacteria consortium applied in this study was collected from activated sewage (IWK Malaysia) and have been labeled in previous screening studies. They are labeled as C1, C3K and C3W respectively. The isolates are maintained on nutrient agar (NA, Merck) and nutrient broth (NB, Merck) alternately and stored at $<3^oC$ for further studies.

2.2. Experimental procedure

The factorial design (Stat-Ease Design Expert v7.0.0) was carried out with five factors to be studied namely pH, concentration of bacterial inoculum, temperature, agitation and aeration. There are two levels (-1 and +1) to be observed in the design which sum up to total eight run of experiments. The details of the factorial design can be referred in Table 1. The initial textile wastewater was set as 150ml, sterilized at 121^oC and inoculated with the designed bacterial consortium concentrations (5% and 15%) and incubated for seven (7) days. The samples then collected and analysis of pH (Thermo Scientific), COD (method 435 HACH DR2800), color (method 125 HACH DR2800), total suspended solids (method 8006 HACH DR900), turbidity (HACH 2100Q01) and dissolved oxygen (YSI Multiparameter) were conducted.

2.3. Calculation

In reduction of chemical oxygen demand (COD), it can be calculated using the equation as follows:

$$\text{COD removal (\%)} = [(P_0 - Pt) / P_0] \times 100$$

Where P_0 is the initial COD value, Pt is COD value after treatment time, t [25]. The calculation of color reduction also goes as follows;

$$\text{Color reduction (\%)} = [(I - F) / I] \times 100$$

With I representing the initial absorbance of the sample, and F is the absorbance of decolorized sample.

TABLE 1: Experimental design of 2-Level Factorial Design

Test (T)	Variable level in coded form					Decolorization (%)	COD Removal (%)
	X1 (pH)	X2 ([Inoculum] (%))	X3 (Temperature (^{o}C))	X4 (Agitation (RPM))	X5 (Aeration)		
1	-1	-1	1	1	-1	47.88	83.14
2	-1	1	-1	-1	1	52.74	80.80
3	1	1	-1	1	-1	50.43	79.21
4	1	1	1	1	1	47.45	81.67
5	-1	-1	-1	1	1	32.27	83.61
6	-1	1	1	-1	-1	43.88	82.85
7	1	-1	-1	-1	-1	45.04	86.00
8	1	-1	-1	-1	1	44.00	83.57
Coded values	Actual values	X2	X3	X4	X5		
-1	X1	5	30	200	0		
1	4.0	15	40	100	1		
	10.0						

3. Results and Discussion

3.1. NOVA (Analysis of Variance)

Table 2: ANOVA for COD Removal

Source	Sum of Squares	dF	Mean Square	F-value	p-value Prob > F
Model	24.01	5	4.80	1.76	0.4011
A: pH	0.22	1	0.22	0.08	0.8043
B: [Inoculum]	19.72	1	19.72	7.22	0.1151
C: Temperature	2.43	1	2.43	0.89	0.4455
D: Agitation	5.48	1	5.48	2.01	0.2922
E: Aeration	5.638E-003	1	5.638E-003	2.064E-003	0.9679
Residual	5.46	2	2.73		
Cor Total	29.47	7			

TABLE 3: ANOVA for decolorization

Source	Sum of Squares	dF	Mean Square	F-value	p-value Prob > F
Model	112.89	5	22.58	0.30	0.8783
A: pH	12.84	1	12.84	0.17	0.7185
B: [Inoculum]	64.76	1	64.76	0.87	0.4499
C: Temperature	8.210E-003	1	8.210E-003	1.100E-004	0.9926
D: Agitation	9.21	1	9.21	0.12	0.7588
E: Aeration	16.37	1	16.37	0.22	0.6856
Residual	149.22	2	74.61		
Cor Total	262.10	7			

Table 2 and 3 both shows the analysis of variance (ANOVA) of chemical oxygen demand (COD) removal and decolorization. According to both analysis, these two models are not significant since the p-value >0.05.

Although the end results showed that decolorization and COD removal did occur during the process, however the interaction between the effects were not fully observed and analyzed thus resulting the insignificant models. The maximum COD removal achieved was 86.00% by experiment number 7 which was affected by the initial pH of 10, initial concentration of inoculum was 5%, 100 rpm, temperature at 30^{o}C and anaerobic process. The lowest COD removal was achieved by experiment number 3 with initial pH 10, 15% concentration initial inoculum concentration, temperature at 30^{o}C, 200rpm and under anaerobic process (no aeration). As for decolorization, maximum value was obtained at experiment number 2 which gives 52.74% of color removal under the condition of initial pH 4, initial inoculum concentration 15%, Temperature at 30^{o}C, 200rpm and under anaerobic process while the lowest color removal was achieved by experiment number 5 with only 32.27%. These explain the insignificant model of experiment obtained as the p-value > 0.05. Final equation in terms of coded factors is described as below:

COD =+82.77 + 0.17 * A - 1.64 * B + 0.66 * C - 0.86 * D - 0.028 * E while final equation in terms of actual factors come as

COD = + 82.77227 + 0.17227 * pH - 1.63977 * [Inoculum] + 0.66409 * T - 0.86477 * Agitation - 0.027727* Aeration.

3.2. Main effects

It was observed that from ANOVA, the interaction between the effects was not fully analyzed since only single effect occurred. For the optimum chemical oxygen demand removal and decolorization, each factor responded differently.

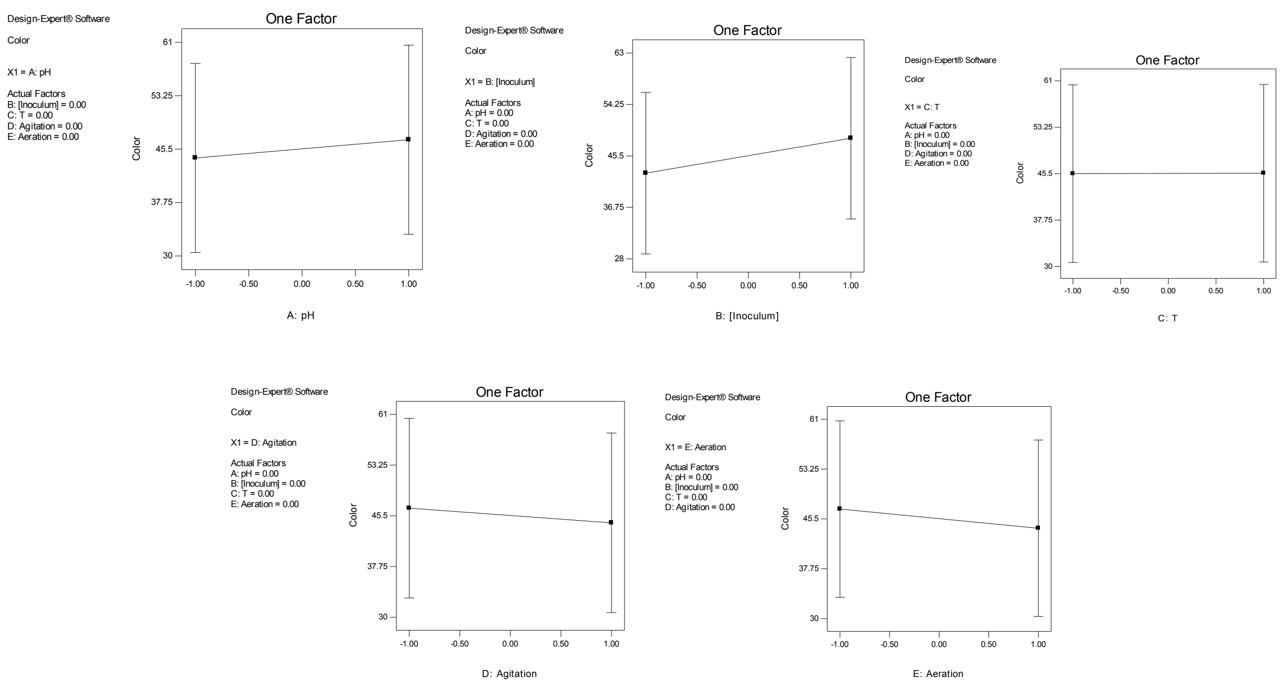

Fig 1: One factor effect for decolorization

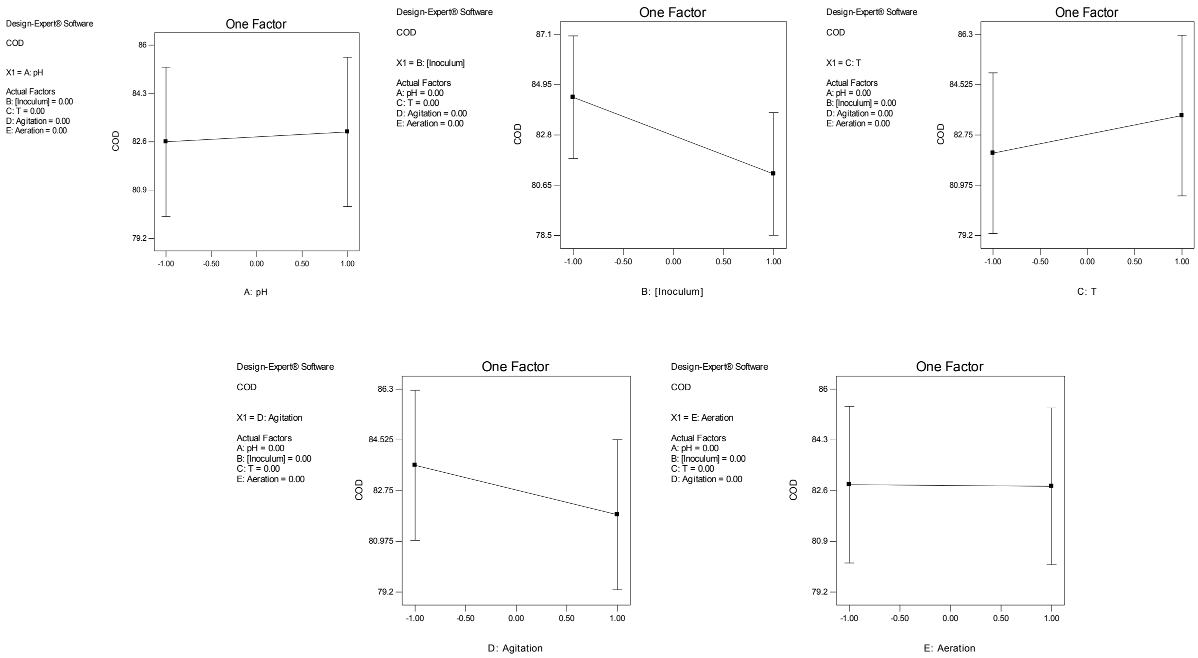

Fig. 2: One factor effect for chemical oxygen demand (COD) removal

The effects of each factor for decolorization were explained through Fig. 1. There were five factors that involved in the decolorization process which contributed to the maximum decolorization of the textile dye wastewater. Among five factors, there were factors that influenced the color removal process slightly which are factor C (temperature (T)) and factor E (aeration). It was observed that during the seven days of incubation period, high and low temperature did not give any significant effect to the removal of the color in the textile wastewater. Aeration also did not play major role in decolorization process as bacterial consortium was able to survive under anaerobic condition. This was proved with the maximum value of decolorozation achieved under the anaerobic condition. The most significant effect that was observed was the initial concentration of bacterial inoculum which was at high value (15%).

Chemical oxygen demand (COD) removal was described with the single effect of each factors involved in the process. In this removal activity, initial pH (A) and aeration (E) were not significantly affecting the removal process. This was because as can be observed in Fig. 2, there were only slight effects observed in the plot graph as compared to the other three factors (B, C and D). In COD removal, lower concentration of initial bacterial inoculum (5%) gave a higher value of COD removal as well as low temperature during incubation period. Apparently it gave a significant effect since maximum value of COD removal is achieved (T at 30^{o}C).

4. Conclusion

In this eight experiments conducted based on the factorial design (2^5), the interaction between effects were not fully analyzed. The response decolorization and chemical oxygen demand (COD) removal also were affected by different factors in order to maximize the removal process. However two factors were identified which are factor C (temperature, T) and factor E (aeration) that both give maximum amount of color and COD removal. It can be concluded that the bacterial consortium perform better under these anaerobic and low temperature condition.

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