INFLUENCE OF AGITATION, pH AND TEMPERATURE ON GROWTH AND DECOLORIZATION OF BATIK WASTEWATER BY BACTERIA *LACTOBACILLUS DELBRUCKII*

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ABSTRACT

Batik industries which are the Malaysian cottage textile industry are among the rapidly growing textiles industries in Malaysia and most of these industries can be found in East Coast states such as Kelantan and Terengganu. Batik industries engender a huge contribution to Malaysia's economy development due to high demands from local and abroad. However, wastewater from these industries causes a vast pollution to the environment due to the dye content because the manufacturer commonly discharge their effluents into environment without appropriate treatment. Therefore, treatments on batik effluent pollution to the environment are very crucial and get an enormous attention from the researchers. This study investigates the effect of agitation, pH and temperature on the microbial (*Lactobacillus Delbruckii*) growth and decolorization efficiency of Batik wastewater. The bacteria were incubated under aerobic conditions in the presence of 30 % (vol. /vol.) Batik wastewater in MRS broth in different temperature (25, 30, 37, and 42 °C) and pH (4, 5, 6, 7 and 8) for 84 hours, and growth of microbial and decolorization of batik wastewater were monitored. A microbial showed good growth in agitation culture but the color removal was best in static culture with 45 – 60 % color removed in less than 72 hours. Temperature and pH had a significant effect on the growth of microbial and also decolorization of the Batik wastewater. However, the optimum decolorization of Batik wastewater did not coincide with the growth of bacteria. The optimum pH and temperature value for growth of microbial were 7.0 and 37 ℃ and for decolorization of batik wastewater were 6.0 and 37 ℃.

Keywords: Batik wastewater, growth, decolorization, pH, Temperature

1. INTRODUCTION

Nowadays, Batik industry has become very commercialized and contributed positively to the economic growth for some states such as Kelantan and Terengganu. Batik manufacturing is mostly produced in a small scale industry which is known as cottage industry and commonly manufacturers prefer to build its industrial unit in their home backyard or alongside of the river [11]. However, less awareness on the importance of clean practices in the production of batik among the batik industry entrepreneurs cause the improper action taken by them by discharge the effluents without proper treatment. Apparently, textile industries consume large amount of water and chemicals during their wet processing [1]. Based on the preliminary studies from Abdul Latif (2002) shows that the wastewater from these homemade textile industries contains grease, wax, heavy metal, surfactant, suspended solid, and dyes (organic and inorganic). Consequently, the existence of dyes in the wastewaters will cause severe environmental problems and also cause serious health hazard because dyes have a synthetic origin and a complex molecular structure which makes them more stable and can remain in the environment for an extended period of time [4]. As this water pollution issues gain more attention from the community, the Kelantan Department of Environment (DOE) had done a studied on water quality perspective on Batik effluent in Kelantan by Syuhadah (2011) and she had found that the batik industry in Kelantan has the lowest level of compliance with the department's law and regulations. From the study, it has revealed that between January and September 2010, the batik industry in Kelantan only recorded a five per cent level of compliance compares to other manufacturing industries [11].

Due to this serious water pollution, there is an insistent need from the researchers to find an alternative method to preserve the environment and at the same time keep up the growth of our economic. Currently, there are many technologies available as the solution to the problems caused by the textile industry such as chemical precipitation, ultrafiltration, carbon adsorption, coagulation, flocculation and oxidation with ozone [3]. However, these treatments were either costly to apply in reality or huge chemical sludge production [5]. Therefore, it is necessary to protect the water resources by finding environmental friendly and cheaper solution to remove color from the batik wastewater. Thereby biological method by applying microbial degradation and decolorization has been studied extensively and believed to be a potential technology to treat textile wastewater. Various microorganisms can be performed in textile

decolorization, including fungi, actinomycetes, algae and bacteria [15]. However, there are still a lot of microorganisms that had an immense potential in improve the decolorization efficiency but still not been established yet. Therefore, this study was to identify the efficiency of *Lactobaciillus delbruckii* on decolorizing of batik wastewater. Additionally, in biological treatment processes, various physicochemical parameters such as the level of agitation, pH, temperature, oxygen, dye structure and dye concentration, directly influence the competence of bacterial decolorization of dye wastewater and also effects on microbial growth [15]. Hence, in particular, the objective of this present study was to investigate the effects of agitation, pH and temperature on the growth of bacteria as well as its relationship in decolorizing batik wastewater.

2. MATERIALS AND METHODS

2.1 Measurement of maximum absorbance of Batik wastewater

The Batik wastewater was kindly supplied by Kraf Holdings located at Rawang, Malaysia for research purpose. The samples had been collected after the dyeing processes had finished and its maximum absorbance by using UV-vis spectrophotometer (Uviline 9400, SECOMAM) was measured.

2.2 Bacteria strains and growth media

Figure 1 shows the bacteria stains, *Lactobacillus delbruckii* ATCC 12315 and the microbial was kindly supplied by Bioprocess Laboratory, Faculty of Chemical Engineering, UiTM Shah Alam and were kept frozen for long term storage at -20 °C in 10 -15% glycerol stocks and invigorated as needed. MRS broth was used for decolorization by dissolving 5.22 g of MRS Broth containing [(g l^{-1}): peptone from casein 10.0, meet extract 10.0, yeast extract (4.0), D+ Glucose 20.0, di-Potassium hydrogen phosphate 2.0, Tween 80 (1.0), di-Ammonium hydrogen Citrate 2.0, Sodium Acetate 5.0, Magnesium Sulfate 0.2, Manganesesulfate 0.04] in 100 ml of distilled water. Before their use, the microorganisms were subculture from a loopful of growth from stock culture and cultivated in 3 mL liquid broth for each bottles and the cells were cultivated at 37 °C, in a rotary shaker (150 rpm) under aerobic condition within 24 hours. They were routinely cultured in MRS Broth or on MRS Agar medium at 37 °C and 150 rpm under aerobic conditions.

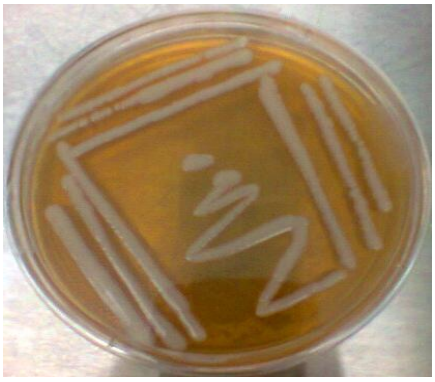


Figure 1: Lactobacillus delbruckii strains

2.3 Experimental of *Lactobacillus delbruckii* efficiency in the Batik wastewater

The experiment was initiated by carried out in Erlenmeyer flask (250 mL) containing 50 mL of 30% (vol. /vol.) Batik wastewater amended with MRS Broth. The initial pH was adjusted in the range of pH 4 til pH 8 before sterilization in an autoclave (Top Loading Autoclace - Hirayama) for 15 min. After the medium cooled to the room temperature, 3 mL of inoculum were added to each flask, aseptically under laminarflow (ESCO Horizontal Laminar Flow). These flasks were incubated for 84 hours at temperature, 37 °C and under shaking at 150 rpm in an incubator shaker (ECOTRON incubator shaker with cooling). The samples were withdrawn at regular intervals and analyzed for growth and decolorization for every 24 hours and centrifuged (Sigma 3-18K) at 10,000 rpm for 20 min. After that, the supernatant were collected and analyzed for the adsorption of dye. The decolorization effectiveness was carried out through absorbance readings at the maximum wavelength (λ_{max}) of 288 nm by using UV-vis spectrophotometer (Uviline 9400, SECOMAM). The efficiency of color removal was expressed as the percentage ratio of the decolorized dye absorbance to that of initial one based on the following equation 1 [7].

$$\text{Color removal (\%)} = \frac{Dye_{(i)} - Dye_{(f)}}{} \times 100\% \quad (1)$$

$Dye_{(i)}$

Where $Dye_{(i)}$ = initial dye absorbance, $Dye_{(f)}$ final dye absorbance. The growth of microbial was recorded by using UV-vis spectrophotometer (Uviline 9400, SECOMAM) at the optical density OD_{600} and regularly monitored for every 24 hour.

3. RESULTS AND DISCUSSIONS

3.1 Growth and and decolorization of Batik wastewater by *Lactobacillus delbruckii*

Table 1 shows the kinetic parameters that had been obtained from the experiment on the effects of pH, agitation speed and temperature on growth of microbial and color removal effectiveness by *Lactobacillus delbruckii* during batch fermentation. Based on the data presented, the optimum color removal of Batik wastewater corresponds with the maximum biomass. Based on Figure 1(a,b), 2(a,b) and 3(a,b), the color removal of Batik wastewater were detected early in the exponential growth phase and continuously shown color removal during this phase until start stationary phase. Besides, it shows that under controlled pH and temperature the color removal percentage showed an optimum level at the end of the exponential growth phase. During the growth of *Lactobacillus delbruckii*, $t_{stationary}$ (beginning of the stationary growth phase) corresponded to t_{Cmax} (time in which maximum color removal was observed) but the color removal during no agitation showed a slightly diverse because the color removal stopped earlier even during the exponential phase.

Table 1: Growth of microbial and decolorization of Batik wastewater by Lactobacillus delbruckii

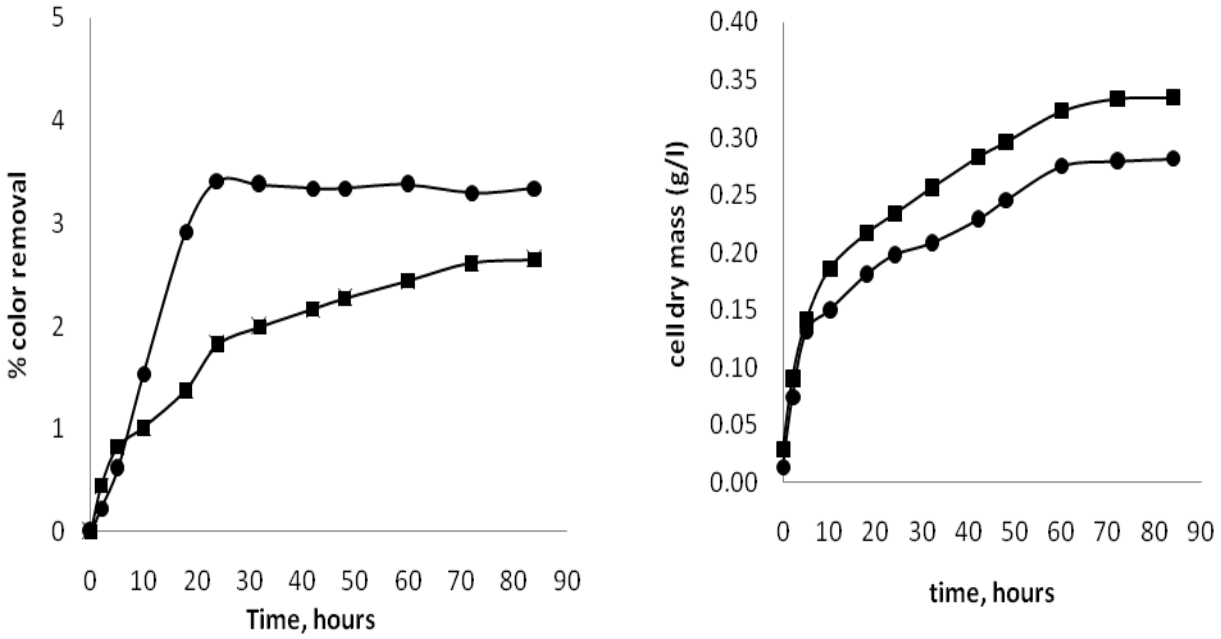
Fermentation conditions [a]		$t_{stationary}$ [b], (hr)	$t_{C\,max}$ [c], (hr)	X [d], (g/L)	$C_{removal}$, (%)
Agitate (rpm)	0	60	24	0.334	3.337
	150	60	60	0.281	2.647
pH	4	48	72	0.089	39.27
	5	72	72	0.213	45.04
	6	72	72	0.329	51.74
	7	72	72	0.340	42.15
	8	72	72	0.161	32.97
Temperature (° C)	25	72	72	0.226	12.89
	30	72	72	0.251	36.72
	37	72	72	0.333	46.06
	42	72	72	0.175	28.09

1. Fermentation at the different pH were carried out at 37 °C and at different temperature were carried out at pH 6 ($C_{removal}$) and pH 7 (cell dry mass) at Batik concentration 30 % (vol./vol.) but for different agitation speed were carried out as previous condition but at undiluted Batik wastewater.
2. The beginning of the stationary phase.
3. The time of maximum color removal percentage achievement.
4. Cell dry mass (g/l) at the moment of achievement of the maximum color removal percentage

3.2 Effects of agitate on growth of *Lactobacillus delbruckii* and decolorization of Batik wastewater

The effects of oxygen on cell growth and dye reduction were one of the important factor that need to be consider. During the cell growth stage, oxygen will have a significant effect on the physiological characteristics of the cells [13]. In this experiment, the growth of microbial and the color removal effciency were not really impressive because the dye concentration were too high and not been diluted first but for the next experiment, the dye concentration were diluted to 30% (vol. /vol.). However, as showed in Figure 2 (a) and (b), the *Lactobacillus delbruckii* still showed good growth in agitation culture but the color removal was better in static culture with 3.5% of color was removed. When tested in agitated culture at rate of 150 rpm, less than 2.6% color was removed. Static conditions were essential for bacterial decolorization even the growth microbial was less than that under agitate conditions. Therefore, for the next experiments static conditions were adopted to investigate bacterial decolorization. A similar observation was reported by a research done by Jadhav (2011) which the results showed that static condition was necessary for Remazol Red (RR) degradation but the culture grown showed significantly increased at shaking condition (120 rpm) and no dye decolorization was observed even at 24 hours of incubation. The growth of microbial were decrease during static incubation because transfer of oxygen is limited to the broth surface, and the

cell cultures will most likely sediment to the bottom flasks and become rapidly oxygen depleted [7]. Moreover, a detail study on analysis of the correlation between DO and the decolorization rate of C.I. Reactive Red 22 by using *Escherichia coli* by Chang (2000) showed that, under agitation at a rate of 200 rpm the DO level slightly decreased and no significant color removal was observed but under static condition, the DO level in the culture dropped instantaneously and the decolorization occurred. Furthermore, a study done by Khadijah (2009) also had indicated that decolorization is not dependent on biomass concentration but is significantly correlated with dissolved oxygen levels. Significantly, these previous study was correspond with the result presented in Figure 2 (a) and (b) where the microbial showed good growth in agitation culture but the color removal was best in static culture.



(a) (b)

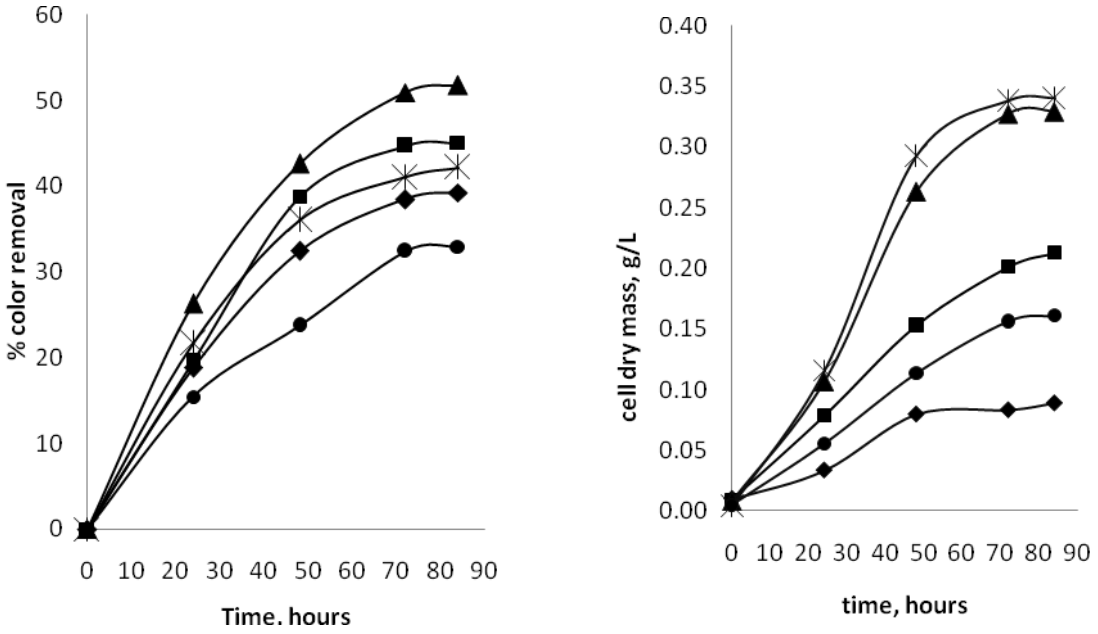
Figure 2: Effects of different agitate on the growth of Lactobacillus delbruckii on (a) growth rate and (b) decolorization of Batik wastewater at pH = 7 and T = 37 °C. (■) agitation = 150 rpm (●) agitation = 0 rpm

3.3 Effects of pH on growth of *Lactobacillus delbruckii* and decolorization of Batik wastewater

The changing effect of the initial media pH from the range 4 to 8 on Batik bioremoval was studied and the other parameters were kept stable. The medium pH has a major effect on the effectiveness of dye decolorization [15] and previous research done by Kodam *et al*. stated that, the effects of pH maybe related to the transport of dye molecules across the cell membrane. According to Chen *et al*. (2003), the optimal pH for dye decolorization is often between pH 6 and 10. Based on the Figure 3 (a), at the medium adjusted to pH 4, 5 and 8, the *Lactobacillus delbruckii* decolorized the effluents with lower efficiency between 45% until 32% while the optimum decolorization were at pH 6 with 52% color was removed. It means that *Lactobacillus delbruckii* can work in acidic medium and in a manner similar to a study by Phisit *et al*. (2007) stated that, the *Lactobacillus casei* strain TISTR 1500 was found to completely degrade Methyl orange at pH 6. According to Saratale (2011), rate of color removal is higher at the optimal pH, and tends to decrease rapidly at strong acid or strongly alkaline pH. Furthermore, a study by Ola (2010) stated that, the optimum pH to decolorize two reactive azo dyes by Bacillus cereus isolated from dye industrial waste was at pH 7. Besides, according to Moosvi *et al*. (2007), the consortium containing bacterial cultures *P. Polymyxa*, *M. Luteus* and *Micrococcus* sp. exhibit good decolorization ability in mixed form with ability to decolorize various dyes at pH from 6.5 to 8.5 has also previously been reported.

On the other hands, the growth of microbial was affected by pH change during bioremoval process as showed in Figure 3 (b) and the optimum growth with the highest cell numbers at pH 7 after 72 hours. An almost analogous observation were recorded with earlier research such as a by Mataragas (2003), *Lactobacillus curvatus* L442 and *Leuconostoc mesenteroides* L124 showed the optimum pH for growth occurred to be between pH 6 and 6.5. At lower pH as a pH value of 4, growth of the microbial was dramatically decreased and the growth of the cell stopped earlier during the exponential phase. This observation could be explained by the fact that the growth of the

lactic acid bacteria is lower at low pH values (< 5.0) [9]. Referred on the Figure 3 (a) and (b) it can be concluded that, the optimal pH value for color removal efficiency did not coincide with the optimum pH for growth and increased of growth rates do not automatically ensure satisfactory of color removal efficiency.



(a) (b)

Figure 3: Effects of different pH on the growth of Lactobacillus delbruckii on (a) growth rate and (b) decolorization of Batik wastewater at T = 37 °C. (♦) pH 4, (■) pH 5, (▲) pH 6, (*) pH 7, (●) pH 8

3.4 Effects of temperature on growth of *Lactobacillus delbruckii* and decolorization of Batik wastewater

The temperature is an important factor that was required to produce the maximum rate of color removal and corresponds with the optimum cell culture growth temperature [13]. The influence of temperature on bacterial growth and color removal is presented in Figure 4 (a) and (b) with four temperature were tested; 25, 30, 37 and 42 ℃. The optimum temperature for color removal (46%) and the growth of cell culture were at 37 ℃ and thus there was a direct relationship between color removal and growth temperature. However, a study by Ola (2010) has reported that optimum decolorization of azo dye by *Bacillus cereus* was more favorable at 35 ℃ compared to 37 ℃. The maximum color removal at temperature 25, 30 and 42 ℃ was 13%, 37% and 28% respectively, lower than that removed at 37 ℃. As the temperature increased from 25 ℃ to 37 ℃, the removal efficiency of Batik wastewater were increased probably due to the increase in the mobility of the large dye ions and also due to higher affinity of sites for dye or an increase in number of binding sited on absorbent [16]. However, as the temperature increased the color removal efficiency were decreased due to the decreased on surface activity [2] and thus it will contribute to the loss of cell feasibility or denaturation of an azo reductase enzyme and will cause the decline in color removal [15]. Based on the results, the decolorization rate of azo dyes increases to the optimal temperature and afterwards there is insignificant reduction in the decolorization activity. As a conclusion, the results obtained from this study showed that the efficiency of color removal and growth of cell was affected by the manipulation of temperature.

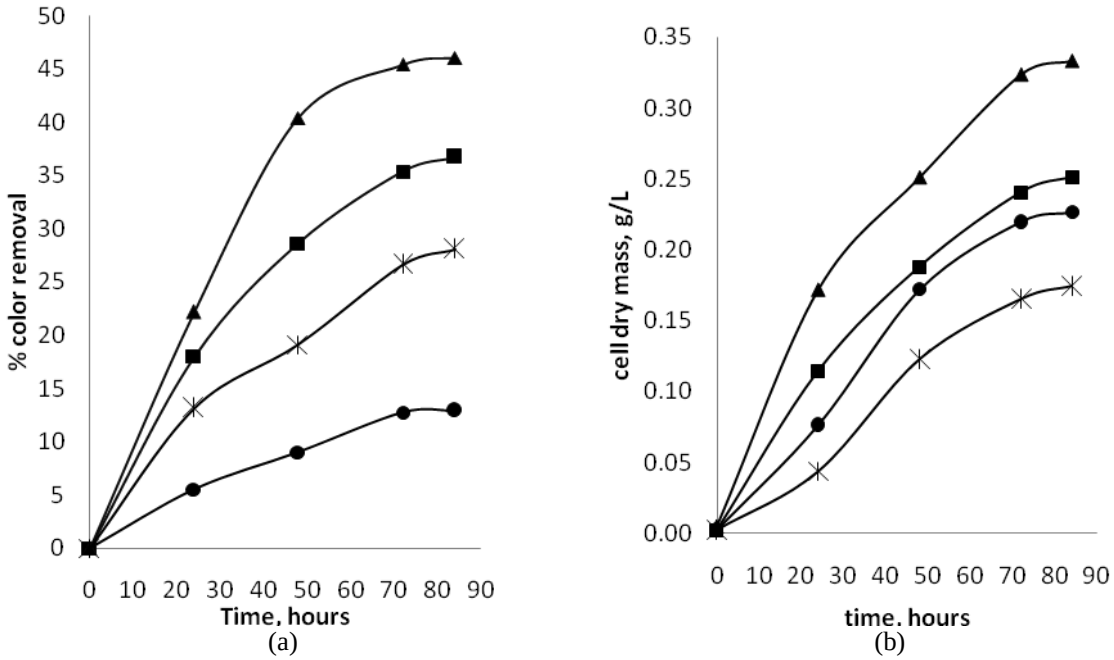


Figure 4: Effects of different agitate on the growth of Lactobacillus delbruckii on (a) growth rate and (b) decolorization of Batik wastewater. (●) T = 25 °C, (■) T = 30 °C, (▲) T= 37 °C, (*) T = 42 °C

4. CONCLUSIONS

The present study revealed that the *Lactobacillus delbrukjii* exhibited great potential in decolorizing the wastewater from Malaysian cottage textile industry. From the presented data, the efficiency of color removal and microbial growth is affected by the agitation speed, pH and temperature. The performance of *Lactobacillus delbrukii* shows that 52% of color removal at the optimum condition at pH 6 and temperature at 37 °C while the microbial growth shows optimum condition at pH 7 and temperature 37 °C. However, the cell growth increased as the agitation speed increased but color removal decreased as the agitation speed was increased. It is important to find the optimum growth condition of the microbial in order to achieve maximal color removal by *Lactobacillus delbruckii*. Last but not least, even the research on biodegradration of textiles wastewater by microbial have been eminent internationally but a limited studies in this research area reported locally. Looking at the current scenario of environment pollution, there is a great need to encourage and also doing more research on the decolorization of textiles industry effluents by using biological method to protect our environments.

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