

## Removal of Synthetic Dyes from Wastewater by Using Bacteria, *Lactobacillus delbruckii*

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**ABSTRACT** : The existence of various types of industries in Malaysia, making it the country's fastest-growing emerging in Asia. There are many industries that use the latest technology such as the use of synthetic dyes for the textile, leather tanning, paper and pulp as well as in the food industry. In Malaysia, the textile industry is the most widespread industrial use dyes as a fibre colouring. However, a variety of synthetic dyestuff released by the textile industry has been posing a threat to the safety of the environment caused by the presence of a large number of toxic contaminants such as organic waste, acids, bases and organic pollutants. Therefore, the authorities began to control the pollution created by the industry to tighten the regulation and enforcement by forcing the industry to treat waste before discharge to the environment. There are many methods have been used to treat this waste. However, it requires a treatment that really works not only at low cost with require minimal or no pre-treatment at all, but it must also be environmentally friendly, minimum sludge production and cleaner. This study used biological method to explore the usability of the microorganisms i.e. bacteria, *Lactobacillus delbruckii* for the removal of dyes from aqueous solutions. It involves the use of two commercial synthetic dyes i.e. Reactive orange 16(RO 16) and Reactive black 5(RB 5). The effects of different parameters such as pH, temperature and initial dye concentration were studied and the effectiveness of this method to remove the dye solution was determined by measuring the percentage of colour removal. The results showed that the bacteria are able to decolorize this two reactive dyes and the optimum pH, temperature and initial dye concentration were found to be 10 ppm, 6 and 37°C, respectively. Therefore, *Lactobacillus delbruckii* is a tremendous potential strain for decolourization of reactive textile dye effluent, and it can be used as a practical alternative in the treatment of textile wastewater to achieve effluents that congregate the Malaysian emissions standards.

**Keyword** - Synthetic dyes; textile industry; decolourization, bacteria

### I. INTRODUCTION

Color is the most visible pollutant that can be easily recognized in wastewater and it should be treated properly before discharging into water bodies or on land. The presence of color in wastewater either in industrial or domestic needs is considered as the most undesirable. Besides, the occurrence of various coloring agents like dyes, inorganics pigments, tannins and lignin which usually impart color [1] become among the main contributor for these environmental matter with dyes wastes are predominant. Dyes are widely used in many industries such as textile dyeing, food, cosmetics, paper printing, leather and plastics, with textiles industry is the major consumer. The number of synthetic dyes presently utilizes in textile industry is about 10 000, representing an annual consumption of around  $7 \times 10^5$  tonnes worldwide [2]. Synthetic dyes were classified based on their chromophores and it can be divided into several groups such as azo, anthraquinone, sulphur, indigod, triphenylmethyl and phthalocyanine derivatives [3]. Moreover, azo dyes are the most important class of commercial dye and versatile colorants which have been used excessively in industries worldwide due to their wide variety of color shades, high wet fastness profiles [4], ease and cost effectiveness compared to natural dyes [5].

Typically, dyes wastewater are the most problematic to be treated due to their chemical stability, high chemical oxygen demand (COD), toxic and some of these dyes are suspected as carcinogens [6]. Fahmi (2010) have reported that, the presence even on a very small portion of dyes in water bodies is highly noticeable by human eye and alters the aquatic ecosystem by reducing the penetration of sunlight [7]. Previously, there are many researchers have summarized a number of studies on physicochemical method for the removal of dyes from wastewater such as coagulation and flocculation [8], advanced oxidation [1], activated carbon [6],

ozonation and photocatalysis[9]. Eventhough these methods are effective in dye removal but several drawbacks are either costly to apply in industries or produce secondary sludge, limits their application [10]. By these reason, currently more researchers have been focusing their studies on exploring the alternatives approaches on color removal from dye wastewater.

In recent years, a wide range of studies have raising a enormous attention on biological methods with some microorganisms such as fungi, bacteria and algae [11] are highly capable to biodegrade and biosorb dyes wastewater. The application of microorganisms for dye wastewater removal offer considerable advantages such as the process is relatively low cost, environmental friendly, produce less secondary sludge and the end products of complete mineralization are not toxic [3]. Numerous research works has been conducted and proven the potential of microorganisms such as *Cunninghamella elegans* [12], *Aspergillus niger* [2], *Bacillus cereus* [13], *Chlorella sp.* [14] and also *Citrobacter sp.* [4] on dye wastewater removal. The adaptability and the activity of each microorganism are the most significant factors that influence the effectiveness of microbial decolorization [15]. Hence, in order to develop a practical bioprocess for the dye wastewater treatment, it is compulsory to continuously investigate microorganisms that capable to degrade azo dyes.

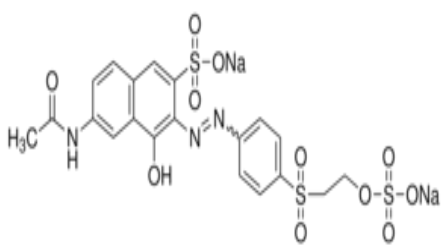
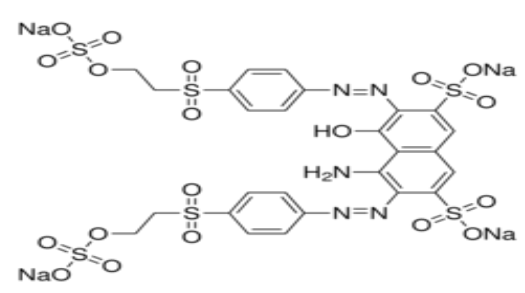
Therefore, this paper puts forward the potential of a bacterial strain, *Lactobacillus delbruckii* on dye wastewater treatment and evaluates their capability on decolorizing the dye solution. Lately, there are a few reports about lactic acid bacteria ability to degrade azo dyes has been published. Previous study by Phisit *et al.* (2007) had proved that *Lactobacillus casei* able to decolorize an azo dye with the optimum pH at pH 6 [5]. Besides, Cervantes and Santos (2011) have summarized that, the first studies documenting the bacterial reduction of azo dyes were reported on lactic acid bacteria by Brohm and Frohwein since 1937 [16]. Generally, *Lactobacillus delbrueckii* is under groups of bacteria and the genus *Lactobacillus* which contains a diverse assemblage of 140 species. Beyond that, *Lactobacillus* includes gram-positive, catalyse negative, non-motile, non-sporulating, and facultative anaerobes [17]. The genus *Lactobacillus* is one of the largest groups of lactic acid bacteria used in food fermentation processes [18] and mostly used in the production of yoghurt [19]. In addition, the effects of various parameters (such as temperature, pH and initial dye concentration) on dye decolorization by *Lactobacillus delbruckii* were also investigated.

## II. MATERIALS AND METHODS

### 2.1 Chemicals

The dyes used in this study were kindly provided by Bioprocess Laboratory, Faculty of Chemical Engineering, UiTM Malaysia and they were Reactive orange 16 (RO 16) and Reactive black 5 (RB 5). Their properties are given in Table 1. Stock solutions 100 ppm of dyes were prepared in distilled water and was diluted as required according to the working concentrations. All chemicals and reagents used for experiments were of analytical grade.

Table 1: Properties of dyes

Dye	RO 16	RB 5
Structural		
$\lambda_{\max}$	493 nm	596 nm
$M_w$ (g mol <sup>-1</sup> )	617.54	991.82

## 2.2 Microorganisms and cultivation method

*Lactobacillus Delbrueckii subsp. Lactis* ATCC: 12315 with the ability of degrading azo dyes were used throughout this study. Stock cultures were stored at  $-20^{\circ}\text{C}$  in 20% glycerol. In degradation studies, the organism is grown under anaerobic condition within 48 hours at its optimum temperature,  $37^{\circ}\text{C}$  and pH around 4.6 til 5.4 where maintained routinely. The used medium, MRS Broth was composed in 1000 ml of distilled water; containing [(g l<sup>-1</sup>): 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, Manganesulfate 0.04].

## 2.3 Measurement of dye concentration

The dye concentrations were measured with a spectrophotometer UV-vis (Uviline 9400, SECOMAM) at intervals during the decolorization process. The concentration dye was detected by spectrophotometer by reading the culture supernatant at specific maximum absorbance wavelength,  $\lambda_{\text{max}}$  after centrifugation (10,000 rpm,  $4^{\circ}\text{C}$  for 20 mins). The efficiency of color removal was determined by the following equation: Color removal (%) =  $\frac{C_i - C_f}{C_i} \times 100\%$ , where  $C_i$  and  $C_f$  were initial and final concentrations, respectively.

## 2.4 Decolorization of azo dyes by *Lactobacillus delbrueckii*

The ability of bacterial decolorization of two azo dyes with different molecular structure (RO 16 and RO 5) was investigated to compare their biodegradability. In order to evaluate the effects of environmental factors, such as pH (3-8,  $37^{\circ}\text{C}$ , 50 ppm), temperature ( $30^{\circ}\text{C} - 42^{\circ}\text{C}$ , pH 7, 50 ppm) and initial dye concentrations (10 – 100 ppm, pH 7,  $37^{\circ}\text{C}$ ) on bacterial decolorization were investigated. The required pH was adjusted by 1 mol NaOH or 1 mol HCl while dye concentrations was measured using spectrophotometer UV-vis at a wavelength corresponding to the maximum absorbance of each dye. The dye solution at desired concentration, pH and temperature taken in 250 ml Erlenmeyer flasks was contacted. The flasks were sterilized before incubated and kept under agitation in a rotating orbital shaker at 150 rpm and  $37^{\circ}\text{C}$  for desired time. Samples (5 ml) were withdrawn at regular time intervals and analyzed for color removal. All the experiments were conducted triplicate and the values of data are presented.

# III. RESULTS AND DISCUSSION

## 3.1 Initial time course studies

Regarding on the adsorption kinetics, it was discovered that contact time influenced the biosorption of RO 16 and RB 5. Fig. 1 shows the results of initial time course on decolorization of RO 16 and RB 5 by *Lactobacillus delbrueckii*. Based on the results, the maximum decolorization for both reactive dyes were recorded at the 48 hours of incubation time at pH 6 and  $37^{\circ}\text{C}$ . The maximum rate of color removal of RB 5 (51%) kept on enhancing within first 48 hours and no further decolorization occurred to 72 hours. However, maximum decolorization of RO 16 (57%) by *Lactobacillus delbrueckii* followed almost the same pattern as decolorization of RB 5 but have slightly increasing in color removal during 48 hours till 72 hours of incubation under the same conditions. Sandra (2012) had stated that, the length of contact time can influenced the biosorption ability and it can be vary in accordance with properties of the dye and the activity of the microorganisms [12]. This statement is substantiated by the results achieved in our study using *Lactobacillus delbrueckii*. As a conclusion, the maximum decolorization of the RO 16 and RB 5 were at 48 hours, thus, this optimum time period was used for the subsequent experiments.

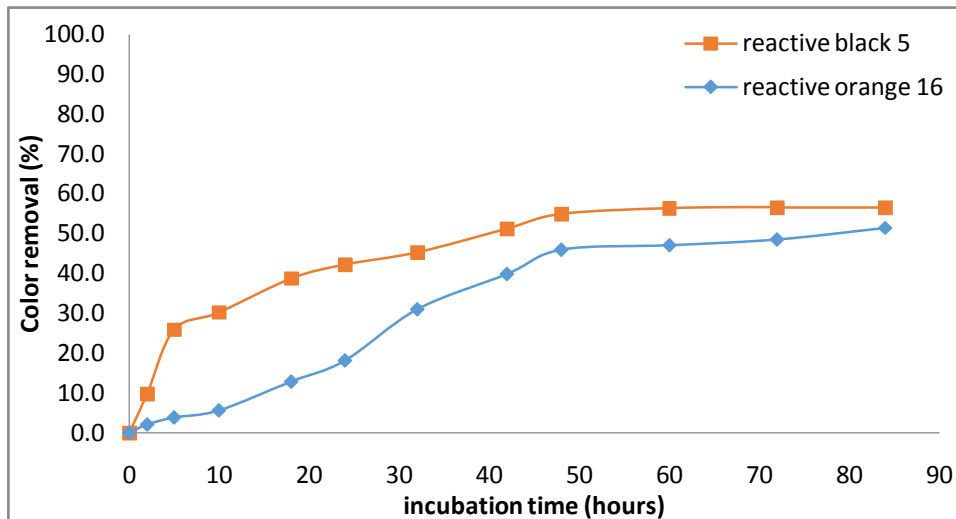


Figure 1: Effect of incubation time on decolorization of RO 16 and RB 5 by *Lactobacillus delbruckii*

### 3.2 Effects of pH on dye decolorization

The study evaluated the effects of pH on the decolorization of RO 16 and RB 5 over a wide range of pH (3.0–8.0) after 48 hours cultivation. The influence of pH on adsorption of the two dyes is shown in Fig. 2. For both dyes, the best color removal was achieved at pH 6 with 60% for RO 16 and 53% for RB 5, respectively. The data presented were associated with study by Hui (2009), the optimal pH for color removal is often between 6.0-10.0 [4]. Thus, for further studies, the optimum pH was adopted at pH 6. Moreover, as the pH was increased from pH 5.0 to 6.0, the percent removal of RO 16 was increased from 52% to 60%. However, rate of decolorization decreased at lower pH (3.0–4.0) and also at higher pH (7.0–9.0). The results showed the similar pattern for RB 5 decolorization with the rate of decolorization increased from pH 5.0 to 6.0 with the percent removal increased from 33% to 53% whereas the rate of color removal were much lower at strongly acidic (3.0–4.0) or strongly alkaline (8.0–9.0) conditions. According to Phisit *et al.* (2007) study, maximum decolorization of Methyl orange by *Lactobacillus casei* strain TISTR 1500 was found to completely degrade at pH 6 [5]. Besides, 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 been reported [20]. The pH tolerance of decolorizing dye by bacteria is important because azo dyes bind to cotton fibers by addition or substitution mechanisms under alkaline conditions and at high temperature [4]. Therefore, the results clearly indicate that decolorization of both reactive dyes by *Lactobacillus delbrueckii* were significantly affected by the pH value of the dye and acidic pH was favourable for the adsorption for both the dyes.

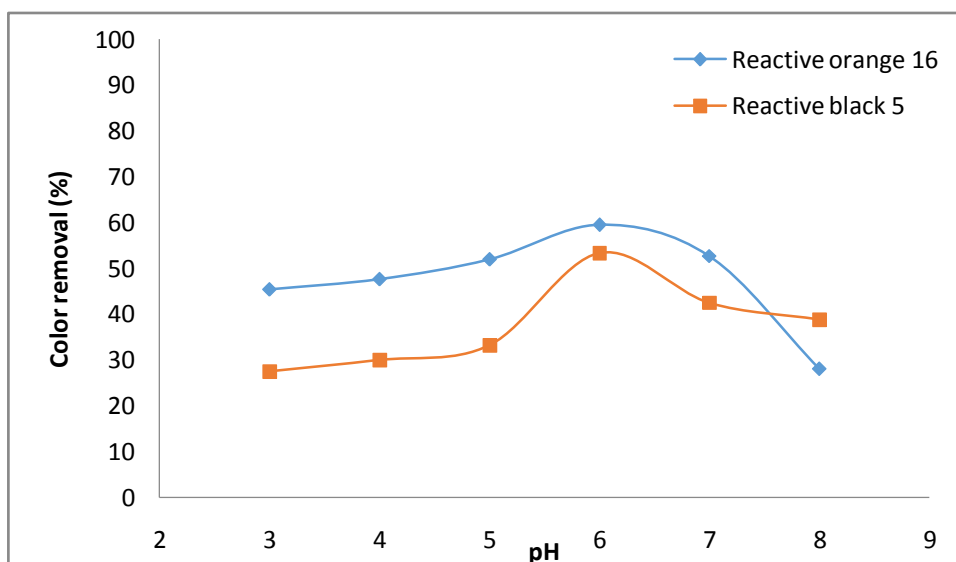


Figure 2: Effect of pH on decolorization of RO 16 and RB 5 by *Lactobacillus delbruckii*

### 3.2 Effects of temperature on dye decolorization

During color removal of RO 16 and RB 5 by *Lactobacillus delbruckii*, the temperature was significant effect on the decolorization efficiency for both dyes. Based on Fig. 3, the decolorization of the dyes was tested for wide range of temperature from 30°C to 42°C. It was observed that the optimum temperature for RO 16 and RB 5 were at 37°C, with 63% and 55% color removal, respectively. Besides, increase in decolorization of RO 16 and RB 5 were sequentially with increase of temperature from 30°C to 37°C, the percent removal of RO 16 and RB 5 was increased from 46% to 63% and 45% to 55%, respectively. However, when further increase in the temperature above the optimum conditions (37°C), a decreased in both dye decolorization were noticed and decolorizing activity was significantly suppressed from 37°C to 42°C. According to Anjaneya (2011) study, a decrease in dye decolorization at high temperature because decline in microbial activity that lead to the inactivation of the enzyme and loss the cell viability [21].

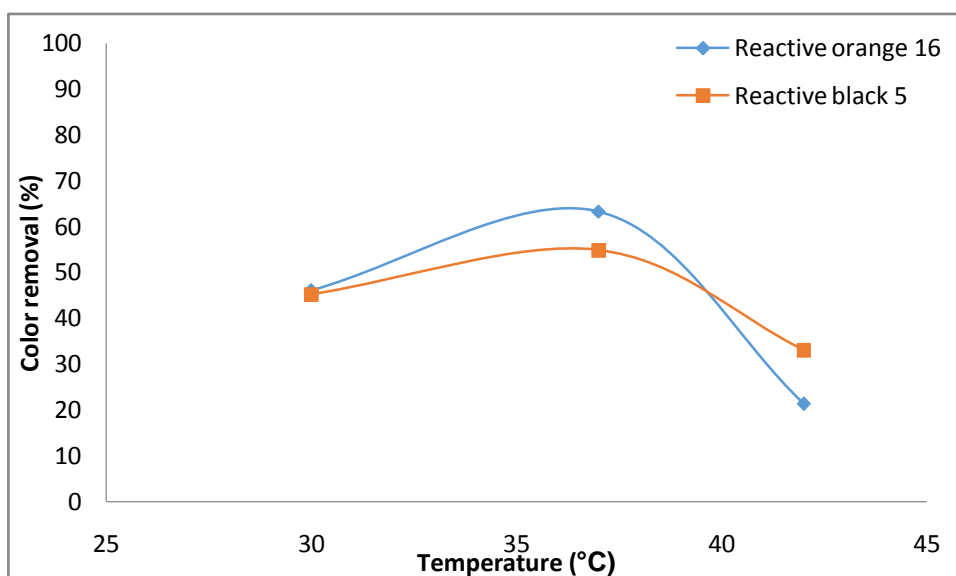


Figure 3: Effect of temperature on decolorization of RO 16 and RB 5 by *Lactobacillus delbruckii*

### 3.3 Effects of initial dye on dye decolorization

The percentage decolorization of RO 16 and RB 5 by *Lactobacillus delbruckii* was carried out at different initial dye concentrations (10 ppm-100 ppm) and Fig. 4 shows the effect of initial dye concentration on dye removal of RO 16 and RB 5 by bacteria. The microbes could effectively decolorize RO 16 and RB 5 with decolorization percentage of 46% and 49% for 10 ppm, respectively. Besides, it was observed that decrease in percentage color removal of RO 16 and RB 5 by *Lactobacillus delbruckii* until only 27% and 31% removal, respectively with increase in concentration condition. According to Anjaneya (2011) , lower decolorization percentage at high dye concentration was reported and it was expected to happen because the inhibitory effects of high dye concentrations [21]. Initial concentration provides a significant driving force to overcome all mass transfer resistances of the dye between the aqueous and solid phases and thus, this suggests that initial dye concentration affected both dye decolorization percentages.

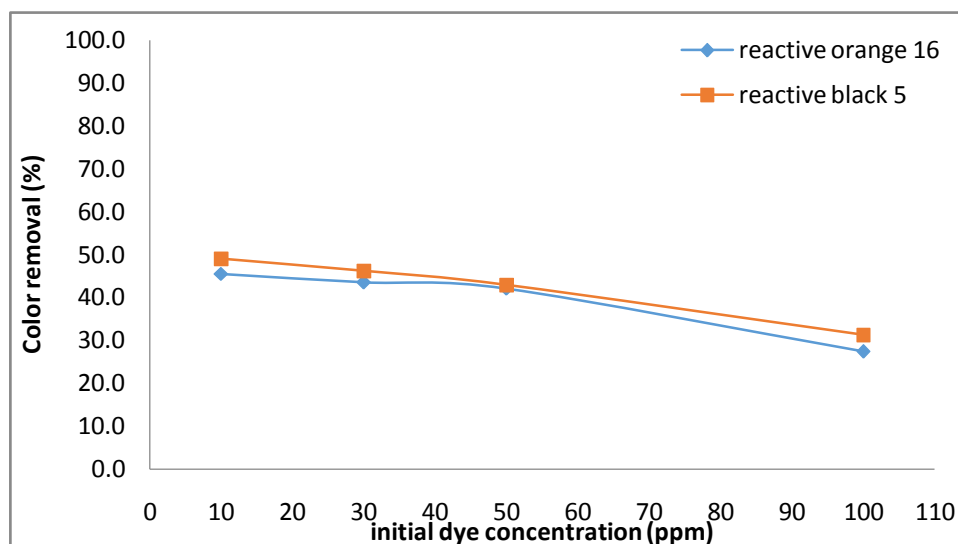


Figure 4: Effect of initial dye concentration on decolorization of RO 16 and RB 5 by *Lactobacillus delbruckii*

#### IV. CONCLUSIONS

The results obtained from this work showed that bacteria species, *Lactobacillus delbruckii* possessed impressive decolorization efficiency. The color removal was dependent on the dye concentration, pH and temperature and the optimal conditions for dye removal were 10 ppm, 6 and 37°C, respectively. It can be concluded that, *Lactobacillus delbruckii* is the highly promising and suitable microorganisms to use in the treatment of solution containing dye. However, more studies are essential prior to employing this method commercially in the real textile industry

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#### REFERENCES

- [1] Muhammad Ridhwan, Che zulzikrami and Nazerry, Characteristics of color removal of azo dyes by advanced oxidation process and biological treatment, *2011 International Conference on Biotechnology and Environmental Management*, vol. 18, 2011, 13-18.
- [2] Karthikeyan, K., Nanthakumar, K., Shanthi, K. And Lakshmanaperumalsamy, P., Response surface methodology for optimization of culture conditions for dye decolorization by a fungus, *Aspergillus niger* HM11 isolated from dye affected soil, *Iranian Journal of microbiology*, Vol. 2, No. 4, 2010, 213-222.
- [3] Forgacs E., Cserhati T., & Oros G., Review article: Removal of synthetic dyes from wastewater., *Environmental International*, 30, 2004, 953-971.
- [4] Hui, W., Jian, Q. S., Xiao, W. Z., Yun, T., Xiao, J. X., and Tian, L. Z., Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by *Citrobacter* sp. CK3. *International Biodeterioration & Biodegradation*, 63, 2010, 395-399.
- [5] Phisit, S., Shinji, T., Ampin, K., K., S., Shuichiro, M., & Kenji, A. (2007). Metabolism of azo dyes by *Lactobacillus Casei* TISTR 1500 and dyes by effects of various factors on decolorization. *Water Research*, 41, 985-992.
- [6] Syafalni S., Ismail, A., Norli, I. And Tan, S.K., Production by *Chryseomonas Luteola* and its application in dye wastewater treatment, *Modern Applied Science*, Vol. 6, No. 5, 2012, 13-20.
- [7] Fahmi, Che Zulzikrami, and Nazerry Rosmady, Multi stage ozonation and biological treatment for removal of azo dyes industrial effluent, *International Journal of environmental science and development*, vol. 1, No. 2, 2010, 193-198.
- [8] Yuan, Y., Wen, Y.Z., Li, Y.Z. and Luo, S.Z., Treatment of wastewater from dye manufacturing industry by coagulation, *Journal of Zhejiang University SCIENCE A, Suppl. II*, 2006, 340-344.
- [9] Hasnain, M.I., Lee, S.L., Faridah, A.H., Hamidi, A.A., Azam, R. and Jaya, P., Low cost removal of disperse dyes from aqueous solution using palm ash, *Dyes and Pigments*, 74, 2007, 446-453.
- [10] Ventaka, S.M., Chandrasekhara R. And Sarma, P.N., Simulated azo dyes wastewater treatment using suspended growth configured sequencing batch reactor(SBR) under anoxic-aerobic-anoxic microenvironment, *Applied ecology and environmental research* 7(1), 2005, 25-34.
- [11] Yuzhu, F. and Viraraghavan, T, Fungal decolorization of dye wastewaters: a review, *Bioresource Technology*, 79, 2001, 251-262.
- [12] Sandra, T.A., Jose, C.V.J., Carlos, A.A., Kaoru, O., Aline, E.N., Ricardo, L.L. and Galba, M. C., A biosorption isotherm model for the removal of reactive azo dyes by inactivated mycelia of *Cunninghamella elegans* UCP542, *Molecules*, 17, 2012, 452-462.
- [13] Lamia, A., Chaieb K., Ceheref A. and Bakhrouf A., Biodegradation and decolorization of triphenylmethane dyes by *Staphylococcus epidermidis*; *Desalination*, 260, 2010, 137-146.
- [14] Daneshvar N., Ayazloo M., Khatee A.R. & Pourhassan M. Biological Decolorization of Dye solution containing Malachite Green by Microalgae *Cosmarium* sp. *Bioresources Technology* 98, (2007), 1176-1182.

- [15] Kuo, C.C., Jane, Y.W., Dar, J.L. and Sz, C.J.H., Decolorization of the textiles dyes by newly isolated bacterial strains, *Journal of biotechnology*, 101, 2003, 57-68.
- [16] Cervantes, F.J. & Santos A. B. D., Reduction of azo dyes by anaerobic bacteria : microbiological and biochemical aspects. *Rev Environ Sci Biotechnol* DOI 10.1007/s11157-011-9228-9, 2011.
- [17] Wang, S., Kong, J., Gao C., Guo, T., & Liu, X. Isolation and characterization of a novel phage (phiLdb) of *Lactobacillus delbrueckii*. *International Journal of Food Microbiology*, 137, (2010) 22-27.
- [18] Ravin, V. & Alatossava, T. (2003). Three new insertion sequence ISLdl2, ISLdl3, and ISDdl4 in *Lactobacillus delbrueckii* : Isolation, molecular characterization, and potential use for strain identification. *Plasmid* 49, 253-268.
- [19] Karina, C.P., Gustavo, V.T, Fabian, R.M., Enrique, D.P., (2010). Viability of *Lactobacillus delbrueckii* Under Human Gastrointestinal Conditions Simulated in Vitro. *American Journal of Agricultural and Biological Sciences* 5 (1), 37-42.
- [20] Moosvi S., Kher X., & Madamwar D., (2007). Isolation, characterization and decolorization of textile dyes by a mixed bacterial consortium JW-2. *Dyes and Pigments*, 74, 723-729.
- [21] Anjaneya, O. And S. Yogesh, et al., Decolorization of sulfonated azo dye Metanil Yellow by newly isolated bacterial strains: *Bacillus* sp. Strain AK1 and *Lysinibacillus* sp. strain AK2, *Journal of Hazardous Materials*, 2011, 351-358.