Biodecolorization of Textile Industrial Waste by Thermophilic Bacteria *Anoxybacillus rupiensis* TS04 and *Anoxybacillus flavithermus* TS15

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Abstract

Wastewater of textile industries contained a high content of synthetic dyes that could damage the aquatic ecosystem and environmental aesthetics. The use of microorganisms on the biodecolorization of textile industrial waste had advantages such as low cost and environmentally friendly. The purpose of the study was to determine biodecolorization capability of Anoxybacillus rupiensis TS04 and Anoxybacillus flavithermus TS15 for industrial waste of tie-dye fabrics. Completely randomized design with factorial pattern was used in the research; factor I and II were concentrations of wastewater and types of bacteria, respectively Anoxybacillus rupiensis TS04 showed the highest decolorization ability by 83.25% for wastewater concentration of 80% (v/v) and Anoxybacillus flavithermus TS15 by 69% at 40% (v/v) waste concentration.The highest cell number of Anoxybacillus rupiensis TS04 was obtained as 1.52 x 106 cfu/mL and Anoxybacillus flavithermus TS15 3.70 x 105cfu/mL.

Keywords: biodecolorization, textile industrial waste, thermophilic bacteria,

1. Introduction

The textile industry became one of the most important and fast growing industries of a country. The development of the textile industry led to a good benefits to people's lives, but also increased the risk of environmental damage if the waste product was not handled properly. The existence of textile waste in waters could disrupt the penetration of sunlight, which could lead to the disruption of organism life and threaten the preservation of aquatic ecosystems. Textile industry waste discharged into landfill still had high contents of dye and COD (Chemical Oxygen Demand). The condition occurred due to the lack of cheap waste treatment technology (Guswandhi et al., 2007).

In the textile industry, synthetic dyes were commonly used because they had better properties than natural dye compounds. The advantages of synthetic compounds were easy to obtain with a fixed composition, had a variety of colors, more durable, easy to use, and the price was relatively cheap (Awaluddin et al., 2001).

However, synthetic dyes might have health effects such as skin and eye irritation and as mutagenic agents (Mathur, 2006).

Textile waste treatment chemically and physically was effective enough to remove color, but there were some drawbacks such as relatively high cost and less environmentally friendly. Therefore, it was necessary to look for waste processing technology that was cheaper and environmentally friendly. Currently, the emerging technology was biologically processing, by utilizing microorganisms to degrade textile dye molecules that had complex structures into simpler molecules (Manurung et al., 2004).

The use of biological systems in waste treatment was by utilizing the activity of microorganisms to degrade existing materials in wastewater into a material that was easily separated or had a low pollution effect. The use of bacteria in the treatment of effluent efficiently could absorb toxic heavy metals and radionucleotides from the environment (Gadd, 1992). Successful processing of textile dye waste was biologically highly dependent on capability of microorganisms and concentrations of waste containing synthetic dyes. Therefore, this study was conducted to test the biodecolorization capa-

bility of *Anoxybacillus rupiensis* TS04 and *Anoxybacillus flavithermus* TS15 (Yohandini et al., 2015) in textile industry waste with different concentrations.

2. Materials and Method

Randomized Complete Design (RCD) with factorial pattern was used in this research. Factor I was the concentration of textile waste (0%, 40%, 60%, 80% and 100%) and factor II was bacterial type (*Anoxybacillus rupiensis* TS04 and *Anoxybacillus flavithermus* TS15) and each treatment was repeated 4 times.

Sampling

Sample of wastewater was taken from tie-dyes fabrics (kain jumputan) industry at Kertapati, Palembang-South Sumatra, by using stratified random sampling method. From each sampling site, 1 L of wastewater was taken, temperature and pH of waste were measured.

Making of Inoculum

Five oses of each thermophilic bacterium were inoculated into 250 mL of NB (Nutrient Broth) medium containing 50% of textile industry waste. The cultures were incubated in the shaker incubator at 150 rpm for 72 hours, 55°C. The number of cells determined by the plate count method, cell with an amount of about 1 x 10^6 cfu/mL was ready used as a source of inoculum.

Cultures Treatments

Each bottle of cultures contained 90 mL of NB medium with varying concentration of textile industry waste (0%, 40%, 60%, 80% and 100%). Ten milliliters of each inoculum *Anoxybacillus rupiensis* TS04 and *Anoxybacillus flavithermus* TS15 were poured into a culture bottle, then incubated in a shaker incubator at 150 rpm, 55°C, for 8 days. Absorbance of samples were measured using a UV-Vis spectrophotometer at 380nm, before and after fermentation. The percentage of biodecolorization was calculated using the formula (Tripathi and Srivastava, 2011):

$$\% \text{ decolorization} = \frac{\text{Initial absorbance value} - \text{final absorbance value}}{\text{Initial absorbance value}} \times 100\%$$

Data Analysis

The data of the percentage of biodecolorization and the number of thermophilic bacterial cells were processed statistically using Analysis of Variance (ANOVA) at the significant level (α) 5%. If the results were significantly different, the analysis then proceed with Duncan New Multiple Range Test (DNMRT) (α) 5%.

3. Results And Discussion

Biodecolorization Capability of Thermophilic Bacteria

The result of statistical analysis on biodecolorization ability of thermophilic bacteria showed that there was interaction between treatment of waste concentration and bacteria type with p-level value of 0.0013 <0.05. This showed that the concentration of textile industry waste could influence the activity of thermophilic bacteria in dye degradation. As a result, the interaction of these two treatments could affect the biodecolorization ability. Based on proceeded test with Duncan New Multiple Range Test (DNMRT) (α) 5%, it was known that the biodecolorization ability of each type of thermophilic bacteria differ in each concentration of textile industry waste, as shown in Table 1.

Table 1.Biodecolorization capability of thermophilic bacteria with different concentration of textile industrial waste after 8 days incubation

No.	Treatment Interaction[*]	Biodecolorization ability (%)[**]
1.	K0*B2	0,00 [a]
2.	K0*B1	0,00 [a]
3.	K4*B2	13,00 [ab]
4.	K4*B1	23,50 [abc]
5.	K2*B2	25,50 [abc]
6.	K3*B2	36,75 [bcd]
7.	K1*B1	44,25 [cd]
8.	K2*B1	56,75 [de]
9.	K1*B2	69,00 [ef]
10.	K3*B2	83,25 [f]

[*] K0, K1, K2, K3, K4: conc. of wastewater 0%, 40%, 60%, 80%, and 100%, respectively. B1 and B2: *Anoxybacillus rupiensis* TS04 and *Anoxybacillus flavithermus* TS15
[**] The numbers followed by the same letter indicate the results are not significantly different

Based on the data in Table 1, the highest biodecolorization ability was obtained at interaction 80% waste concentration by *Anoxybacillus rupiensis* TS04, i.e 83%, but no significantly different with the interaction 40% waste concentration by *Anoxybacillus flavithermus* TS15. The lowest biodecolorization ability was obtained at 100% concentration of waste by *Anoxybacillus rupiensis* TS04 by 13%, and no significantly different with interaction 100% waste concentration by *Anoxybacillus flavithermus* TS15 of 23.50%. The data indicated that the biodecolorization ability was highly determined by the type of bacteria and the concentration of waste used. At high waste concentrations, decolorization ability decreased, because it could inhibit bacterial growth. Saratale et al. (2009) suggested that high concentration of waste could affected bacterial growth due to the toxic effects of dyes on bacterial cells, and might inhibit the active side of the azoreductase enzyme by dye molecules. While at low concentrations of waste, decolorization ability could also decreased because

the waste of the dye became a source of nutrients for the bacterial growth. Textile industry waste contain organic materials that could be used by bacteria for their growth (Nugroho and Ikbal, 2005).

Degradation the dye of textile industry waste by thermophilic bacteria for 8 days incubation was indicated by the change of color from dark to brighter as shown in Figure 1. Dark colors in the textile industry waste was resulted from a combination of some synthetic dyes such as direct bordox, direct green, direct blue and erionyl yellow, which were commonly used in tie-dye fabric industry. The dyestuff degradation process of textile industry waste in the research was used aerobic thermophilic bacteria. Aerobic bacteria were able to reduce azo compounds with the aid of oxygen catalyzed by azoreductase enzyme and produced aromatic amines (Lin et al., 2010).
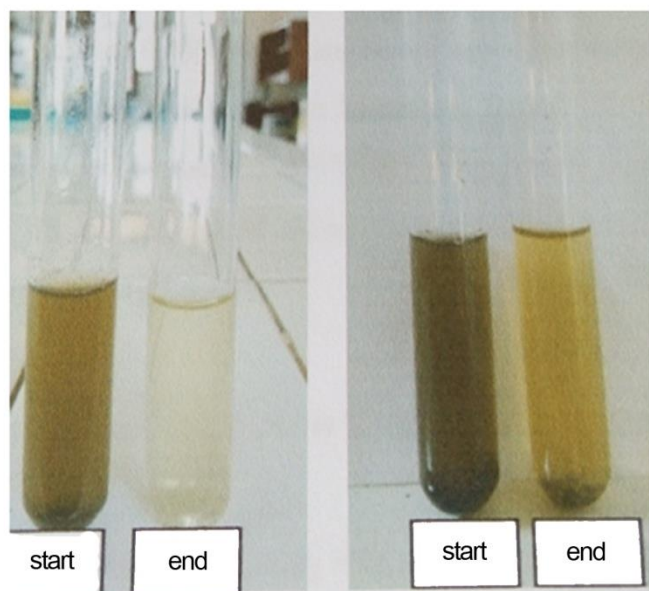

Figure 1. Change in color of textile industry waste by thermophilic bacteria during 8 days incubation
     (a). *Anoxybacillus rupiensis* TS04
     (b). *Anoxybacillus flavithermus* TS15

Number of Bacterial Cells

The interaction treatment between the concentration of textile waste and bacteria type did not significantly affect the number of bacterial cells with p-level of $0.4465 > 0.05$, whereas the result of variance analysis showed that the treatment of waste concentration had p-level equal to $0.0266 < 0.05$, which mean significantly effect on bacterial cells number, DNMRT (α) 5% test as shown in Table 2.

Table 2. The number of bacterial cells at different concentrations of waste after 8 days incubation in the biodecolorization process of textile industry waste

No.	Concentration of Textile waste (v/v)	Cells number (cfu/mL)[*]
1.	0	$1.62 \times 10^{5\,a}$
2.	40	$1.23 \times 10^{6\,ab}$
3.	60	$1.18 \times 10^{6\,ab}$
4.	80	$1.68 \times 10^{6\,b}$
5.	100	$4.75 \times 10^{5\,a}$

[*] The numbers followed by the same letter indicate the results are not significantly different

In Table 2, it could be seen that the highest cells number was achieved on treatment of 80% waste concentration with cells number 1.68×10^6 cfu/mL but no significantly different with treatment of waste concentration 40% and 60%. While the lowest cell number was achieved on treatment of 0% concentration of waste with cells number 1.62×10^5 cfu/mL and no significantly different with treatment of waste concentration 40%, 60% and 100%. This showed that the concentration of textile industry waste greatly affected the growth of bacterial cells. According to Chen et al. (2003), the reactive group of azo dyes greatly inhibited the growth of microorganisms at higher concentrations. Optimal waste concentration increased bacterial cells growth, in this case the concentration of textile industry waste 80% was the optimum concentration for bacterial cells growth.

The analysis of variance on the bacteria type treatment resulted in p-level $0.0008 < 0.05$ which mean significantly different to the number of bacterial cells. Further test results with DNMRT (α) 5% were shown in Table 3.

Table 3. The number of thermophilic bacterial cells after 8 days incubation in the biodecolorization process of textile industry waste

No.	Type of Bacteria	Number of Cells (cfu/mL)[***]
1.	*Anoxybacillus flavithermus* TS15	$3.70 \times 10^{5\,a}$
2.	*Anoxybacillus rupiensis* TS04	$1.52 \times 10^{6\,b}$

[***] The numbers followed by the same letter indicate the results are not significantly different

In Table 3, the number of *Anoxybacillus flavithermus* TS15 cells (3.70×10^5 cfu/mL) was significantly different from *Anoxybacillus rupiensis* TS04 (1.52×10^6 cfu/mL). The results showed that Anoxybacillus rupiensis TS04 was better to utilize textile industry waste for its growth. Handayani et al. (2016) suggested that an increase in the number of bacterial cells indicated that the bacteria were

capable to utilize textile waste as a carbon source for its growth.

4. Conclusion

Anoxybacillus rupiensis TS04 had the highest biodecolorization ability of 83.25% at a textile waste concentration of 80% (v/v), while *Anoxybacillus flavithermus* TS15 had 69% biodecolorization ability at waste concentration 40% (v/v). *Anoxybacillus rupiensis* TS04 had the highest cells number of 1.52 x 106cfu/mL whereas *Anoxybacillus flavithermus* TS15 had cells number of 3.70 x 105cfu/mL.

5. Acknowledgement

References

Awaluddin R., Darah S., Ibrahim C.D andUyub A.M. 2001.Decolorization of Commercially Available Synthetic Dyes by The White Rot Fungus Phanerochaetechrysosporium ME 446 (ATCC 34541). *Proc. NSF workshop*. Kuala Lumpur.

Chen, K.C., Wu, J.Y., Liou, D.J. and Hwang, S.C.J. 2003. 'Decolorization of the textile dyes by newly isolated bacterial strains', *Journal of Biotechnology*, 101(1), pp. 57–68. doi: 10.1016/S0168-1656(02)00303-6.

Gadd, G.M. 1992. *Metal Tolerance Initiating Microbiology of Extreme Environment*. University Press, Milton Keynes.

Guswandhi, Panjaitan, J.S.P., Suhardi, S.H., Niloperbowo, W. dan Setiadi, T. 2007. Penghilangan Warna Limbah Tekstil dengan *Marasmius* sp. dalam Bioreaktor Unggun Tetap Termodifikasi (*Modified Packed Bed*). *Prosiding Seminar NasionalRekayasa Kimia dan Proses*. ISSN: 1411-4216

Handayani, N.I., Moenir, M., Setianingsih,N.I., Malik, R.A. 2016. Isolasi Bakteri Heterotrofik Anaerobik pada Pengolahan Air Limbah Industri Tekstil. *Jurnal Riset Teknologi Pencemaran Industri*. 7(1): 37 - 45

Lin, J., Zhang, X., Li, Z. and Lei, L. 2010. 'Biodegradation of Reactive blue 13 in a two-stage anaerobic/aerobic fluidized beds system with a *Pseudomonas* sp. isolate', *Bioresource Technology*, 101(1), pp. 34–40. doi: 10.1016/j.biortech.2009.07.037.

Manurung, R., Rosdanelli, H., dan Irvan. 2004. *Perombakan Zat Warna Azo Reaktif Secara Anaerob-Aerob*. USU: Medan.

Mathur, N., Bhatnagar, P. and Bakre, P. 2006. 'Assessing mutagenicity of textile dyes from pali (Rajasthan) using ames bioassay', *Applied Ecology and Environmental Research*, 4(1), pp. 111–118.

Nugroho, R. dan Ikbal. 2005. Pengolahan Air Limbah Berwarna Industri Tekstil dengan Proses AOPs. *JAI*. 1(2): 163 - 172

Saratale, R.G., Saratale, G.D., Chang, J.S. and Govindwar, S.P. 2009. 'Decolorization and biodegradation of textile dye Navy blue HER by Trichosporon beigelii NCIM-3326', *Journal of Hazardous Materials*, 166(2–3), pp. 1421–1428. doi: 10.1016/j.jhazmat.2008.12.068.

Tripathi, A. and Srivastava, S.K. 2011. 'Ecofriendly Treatment of Azo Dyes: Biodecolorization using Bacterial Strains', *International Journal of Bioscience, Biochemistry and Bioinformatics*, 1(1), pp. 37–40. doi: 10.7763/IJBBB.2011.V1.7.

Yohandini, H., Julinar, and Muharni.2015. Isolation and Phylogenetic Analysis of Thermophile Community Within Tanjung Sakti Hot Spring, South Sumatera, Indonesia, *HAYATI Journal of Biosciences,* 22(3): 1-6.