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

Muharni,1,* Heni Yohandini,2* M. Yunus Rivai 1

1Department of Biology, Faculty of Mathematic and Natural Sciences, Sriwijaya University, South Sumatra 30662, Indonesia.
2Departement of Chemistry, Faculty of Mathematic and Natural Sciences, Sriwijaya University, South Sumatra 30662, Indonesia

*Corresponding author.
E-mail address: muharni_bio@unsri.ac.id (Muharni).
Peer review under responsibility of Biology Department Sriwijaya University

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 10^6 cfu/mL and *Anoxybacillus flavithermus* TS15 3.70 x 10^5 cfu/mL.

Keywords: biodecolorization, textile industrial waste, thermophilic bacteria.

Received: 12 February 2018, Accepted: 20 May 2018

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-

Muharni et al, 2018 | Abstract
bility of Anoxybacillus rupiensis TS04 and Anoxybacil-
lus flavithermus TS15 (Yohandini et al., 2015) in textile
industry waste with different concentrations.

2. Materials and Method

Randomized Complete Design (RCD) with fac-
torial 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)
each treatment was repeated 4 times.

Sampling
Sample of wastewater was taken from tie-dyes
fabrics (kain jumputan) industry at Kertapati, Palem-
bang-South Sumatra, by using stratified random sam-
pling 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
106cfu/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 bi-
decolorization was calculated using the formula (Tripa-
thi and Srivastava, 2011):

\[
\text{Biodecolorization} = \frac{\text{Initial absorbance value} - \text{final absorbance value}}{\text{Initial absorbance value}} \times 100\%
\]

Data Analysis
The data of the percentage of biodecolorization and
the number of thermophilic bacterial cells were pro-
cessed statistically using Analysis of Variance (ANOVA)
at the significant level (α) 5%. If the results were signifi-
cantly 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 biodecoloriza-
tion 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 treat-
ments 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
collection of textile industry waste, as shown in Table 1.

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment Interaction(^{*)}</th>
<th>Biodecolorization ability (%)(^{**})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>K0 B2</td>
<td>0,00 *</td>
</tr>
<tr>
<td>2.</td>
<td>K0 B1</td>
<td>0,00 *</td>
</tr>
<tr>
<td>3.</td>
<td>K4 B2</td>
<td>13,00 *</td>
</tr>
<tr>
<td>4.</td>
<td>K4 B1</td>
<td>23,50 **</td>
</tr>
<tr>
<td>5.</td>
<td>K2 B2</td>
<td>25,50 **</td>
</tr>
<tr>
<td>6.</td>
<td>K3 B2</td>
<td>36,75 **</td>
</tr>
<tr>
<td>7.</td>
<td>K1 B1</td>
<td>44,25 **</td>
</tr>
<tr>
<td>8.</td>
<td>K2 B1</td>
<td>56,75 **</td>
</tr>
<tr>
<td>9.</td>
<td>K1 B2</td>
<td>69,00 *</td>
</tr>
</tbody>
</table>

\(^{*}\) K0, K1, K2, K3, K4: conc. of wastewater 0%, 40%, 60%, 80%, and 100%,
respectively. B1 and B2: Anoxybacillus rupiensis TS04 and Anoxybacillus fla-

\(^{**}\) The numbers followed by the same letter indicate the results are not signifi-
cantly different

Based on the data in Table 1, the highest biodecol-
oration ability was obtained at interaction 80% waste
collection 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%
collection 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 concen-
trations, decolorization ability decreased, because it could
inhibit bacterial growth. Saratate 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 en-
zeyme by dye molecules. While at low concentrations of
waste, decolorization ability could also decreased because

Muharni et al, 2018 | 2. Materials and Method
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).

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Concentration of Textile waste (v/v)</th>
<th>Cells number (cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1.62 x 10^5</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>1.23 x 10^6</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>1.18 x 10^6</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>1.68 x 10^6</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>4.75 x 10^5</td>
</tr>
</tbody>
</table>

*) 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 x 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 x 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

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of Bacteria</th>
<th>Number of Cells (cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anoxybacillus flavithermus TS15</td>
<td>3.70 x 10^4</td>
</tr>
<tr>
<td>2</td>
<td>Anoxybacillus rupiensis TS04</td>
<td>1.52 x 10^5</td>
</tr>
</tbody>
</table>

**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 x 10^5cfu/mL) was significantly different from Anoxybacillus rupiensis TS04 (1.52 x 10^6cfu/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
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 10^6 cfu/mL whereas Anoxybacillus flavithermus TS15 had cells number of 3.70 x 10^5 cfu/mL.

5. Acknowledgement

The research was funded by PNBP Unsri through Competitive Research Scheme 2016.

References


