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Isolation And Decolorization Of Indigenous Bacteria For Eco-Friendly Treatment Of Textile Waste Pollutants

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ABSTRACT

The boiling process is the one of the methods used to treat fabric materials, which can result in non-dissolvable waste. One of the approaches to overcome industrial waste is through the implementation of for decolorization. Commonly, waste management frequently used for decolorization are coagulation and filtration. But this management produce side waste like sludge. The using potency of microorganism is developing of all the aspect in life. The degradation with eco friendly and doesn't produce side waste. This microorganism has important role at the cycle of biogeochemistry and living of metabolism in the world naturally. It have a capability to reduce the toxic compound and many pollutants.It can have benefit in bioremediation. All of those of microorganism is a part of biodiversity of Indonesia. It can isolated from cover land and the ocean. Isolate of potencial microorganism can isolated from industrial waste, include industry of textile. Isolated bacteria of indigenous from the waste is the bacteria which have ability the good adapt in waste condition, ithave enzyme activity for decolorization process to change into the simply compound. It can be separated onto sourceof nutrition for its development. This research aims to isolate of potencial bacteria to decolorize waste textile. The bacteria is isolated from the land in waste textil area at Bandung city. Getting isolate of bacteria with the growth of sample in Nutrient Agar Medium. That isolate will grow and identified in morphology form. This research to get indigenous potencial bacteria and to know the optimum condition for decreasing the pigment from the waste textil. The decolorization using the concentrateof 50, 100 and 200 mg/L with Submerged Fermentation (SmF) or submerged fermentation, according Hernandez, (2008) and Bergsten-Torralba, (2009). The efficiency of decolorization from the various of concentration follow the comparison of pigment from starting concentration in % form. Finally, getting the genus *Bacillus* spp, it can reduce the level of pigment until 90% at the concentration pigment at 100 mg/L.

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1. INTRODUCTION

The textile industry must meet wastewater quality standards in accordance with the Regulation of the Minister of the Environment of the Republic of Indonesia Number 5 of 2014 concerning Wastewater Quality Standards. The combined wastewater from textile factories in Indonesia contains an average of 750 mg/l suspended solids and 500 mg/l BOD.

Waste and emissions are non-product outputs from textile industry activities. Especially for the textile industry, which in the production process has a finishing-dyeing unit, it has the potential to cause water pollution with a high ammonia content. The industry in general is still making efforts to manage the environment by treating waste (treatment) by building a waste treatment plant. However, in practice the waste treatment requires a large amount of money and then the industry must also incur operational costs so that the waste can meet quality standards.

Wastewater that is simply dumped into the environment causes pollution, among others, polluting water sources such as rivers, lakes, springs, and wells. Liquid waste gets more serious attention than other forms of waste because liquid waste can cause environmental pollution in the form of physical pollution, chemical pollution, biological pollution and radioactive pollution.

Textile waste is the dominant liquid waste produced by the textile industry because there is a dyeing process which in addition to requiring chemicals also requires water as a solvent medium. The textile industry is an industry that is engaged in the garment sector by processing cotton or synthetic fibers into fabrics through the following process stages: Spinning and Weaving. Textile industry waste is classified as liquid waste from the coloring process which is a synthetic chemical compound, has a strong pollutant power. These dyes have been proven to be able to pollute the environment. Textile dyes are all dyes that have the ability to be absorbed by textile fibers and are easily removed from color (chromophores) and groups that can bond with textile fibers (auxochromes).

Good and correct waste treatment requires a relatively large amount of money. (Novitrianiingsih & Titah, 2016). Government in PP RI No. 82 of 2001 has set water quality standards in the context of controlling environmental pollution. The waste generated should be at a minimum according to the quality standards that have been set. Still under the same regulation, the lowest level of water quality is class four (IV) that the water is at least safe for irrigating crops.

In the water treatment process, there are several ways to make the water meet the specified quality standards. The process that is passed is based on physical, chemical and biological aspects.

These processes include filtering (physical aspects), adding chemicals (chemical aspects) and adding bacteria (biological aspects). There are several chemicals that are commonly used, easy to obtain, and have low prices, including chlorine and activated carbon. Chlorine is widely known to the public as a water purification agent, besides that it also functions as a disinfectant (germ killer). Activated carbon can absorb toxic substances and as a purification agent. Meanwhile, the addition of Bacillus bacteria can play a role in reducing the levels of COD, BOD, and ammonia in the waste.

Biological methods as an alternative that are considered cheap and more environmentally friendly, one of them is using bioremediation techniques. Bioremediation is defined as the biological recovery process of polluted environmental components into a non-toxic form (Munir, 2006). Waste treatment with the help of microbes has been widely used, this process is often referred to as the biodegradation process. Biodegradation is defined as a process of oxidation of organic compounds by microbes due to the metabolic process of organic substances through enzymes to produce carbon dioxide, water and energy to be used in synthesis, mortality and respiration (Suarsini and Fidiastuti, 2017). Bacteria are important biological agents that have the ability to biodegrade waste (Utari et al., 2015). According to Blumel et al. (1998), bacteria capable of decolorizing dyes can generally be found in places exposed to dye waste. The process of biodegradation of waste generally utilizes a population of microorganisms or other products.

such as enzymes produced by microorganisms themselves in overhauling organic compounds that exist in naturally polluted environments (Jusfah, 1995). There are three kinds of biological waste treatment, namely, aerobic, anaerobic and facultative. The choice of treatment depends on the characteristics of the wastewater, the conditions and purposes and objectives of the treatment (Sarjoko, 1991).

2. MATERIALS AND METHODS

Study area

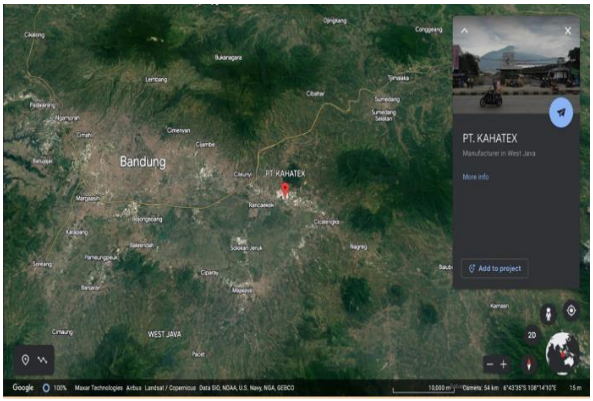


Figure 1. Location of Kahatex Industry Textile, Rancaekek. Bandung, Jawa Barat (6°57'07"S 107'47'20"E)

Procedures

Materials

The tools used in this study include: aluminum foil, autoclave, sample bottles, winkler bottles, pipette bulbs, petri

dishes, funnels, Erlenmeyer, measuring cups, heaters, incubators, object glass, filter paper, gas stoves, cuvettes, labels, air flow laminators, refrigerators, micropipettes, microscopes, loops, ovens, Bunsen burners, water baths, dropping pipettes, volume pipettes, tube racks, spatulas, spectrophotometers, test tubes and balances.

The materials used in this study included: distilled water, 70% alcohol, 96% alcohol, Whatman filter paper no.41 Macherey-Nagel, chloramphenicol Colsancetine, McFarland 3 solution, 0.9% physiological NaCl, river waste, Yeast and Mold Agar medium (YMA), Yeast and Mold Broth medium (YMB), test substances for parameter measurement include: measurement of COD and BOD: H2SO4, starch indicator, MnSO4, Na-thiosulphate, O2 reagent, sulfuric acid; COD measurement: H2SO4K2Cr2O7, [NH4)2Fe(SO4)2} 0.1N.

Sampling

Waste samples were obtained from the waste treatment unit of PT Kahatex, Rancaekek 6°57'07"S 107'47'20"E

A total of one ml of textile waste liquid samples were taken using a sterile bottle. Each sample was placed in sterile bottles and stored in the refrigerator at 4°C until the sample is used. Meanwhile, the comparison isolates of *B. subtilis* and *P. aeruginosa* were obtained from the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Padjadjaran. Azo dyes were obtained from the Food Technology Laboratory of the Faculty of Agriculture, Universitas Padjadjaran.

Isolation and screening of textile dye degrading bacteria Growth and Selection

The isolation of textile dye-degrading bacteria depends on the sample, nutritional requirements, and the dye targeted, the process chosen for bacterial isolation may vary. Experimental dyes should Experimental dyes should be performed (Jamee & Siddique, 2019). In this study, the sample was grown in a nutrient medium supplemented with a test dye. The choice of medium depends on the growth requirements of the bacteria in the sample. Nutrient broth added with a 1% concentration dye is suitable for many bacteria. Bushnell Haas's medium (BH) consisted of 0.1% KH_2PO_4, 0.1% K_2HPO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 0.002% $CaCl_2 \cdot 2H_2O$, 0.1% NH_4Cl, 0.1% NH_4NO_3, 0.01 NaCl %, and 0.005% $FeCl_3$–$6H_2O$ at pH 7 was used. Mineral salt media (MSM) containing Na_2HPO_4 (3.6 g), $(NH_4)_2SO_4$ (1.0 g), KH_2PO_4 (1.0 g), MgSO4 (1.0 g), Fe(NH_4) citrate (0 .01g), $CaCl_2 \cdot 2H_2O$ (0.10 g) and 10.0 mL of trace element solution per liter can also be used. For enrichment of dye decolorizing bacteria, the effluent sample and dye mixture should be added to a conical flask containing suitable media, incubated under suitable conditions (eg, 37°C, 100–150 rpm). The media must be supplemented with glucose, yeast

extract, etc. If the sample microorganisms cannot use the dye as a carbon source. (Jamee & Siddique, 2019). After incubation, the solution is monitored for decolorization at predetermined intervals (eg, 6 h). For positive results with certain dye samples, results are confirmed for individual colonies using the plate method on filter media (Jamee & Siddique, 2019).

Data Analysis

Samples collected from contaminated sites may be textile waste or soil affected by the waste. The sample should be diluted with sterile distilled water or sterile saline. Each dilution should be spread or streaked on a plate to keep the nutrients at optimum temperature for 24 hours. Pure colonies are then isolated, and these isolates must be tested for their ability to decolorize the dye on the media added with the dye. Furthermore, bacteria can be identified by biochemical tests, genomic DNA, or 16S rRNA. (Jamee & Siddique, 2019).

The method used in the process of isolating indigenous bacteria is a descriptive method by means of a Pour Plate or a pouring cup through a series of sample dilutions. 1 ml of liquid waste sample was diluted with 9 ml of sterile physiological NaCL up to 108 dilution. Liquid waste samples from the last three dilutions, namely dilutions 106, 107 and 108, were taken 1 ml each and poured into a petri dish containing 20 ml of Nutrient Agar liquid medium. (NA) containing textile dyes at a concentration of 80 mg/l. Liquid waste samples and medium were mixed until homogeneous by rotating the petri dish slowly on the table. After the medium is frozen, the petri dish is then turned upside down so that the condensation water does not fall on the agar because the surface of the agar must be dry. All the petri dishes were then wrapped in paper and incubated in an incubator at 37^0C for 1x24 hours. Bacterial colonies growing in petri dishes were isolated for each different colony. Colonies that have been isolated are then purified by taking an ose of a bacterial colony, then streaking it on the NA agar medium in a test tube. The test tube that already contains the bacterial culture is back

Persentase Dekolorisasi % :

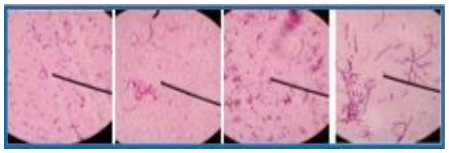
$$\frac{Initial\ Absorbance\ Value - Final\ Absorbance\ Value}{Initial\ Absorbance\ Value}\ x\ 100\%$$

3. RESULTS AND DISCUSSION

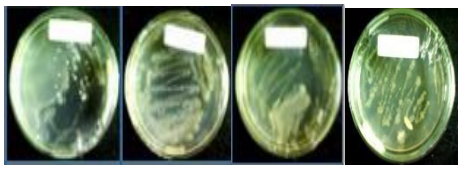
Isolation, Selection and Identification of Indigenous Bacteria in Batik Liquid Waste

Based on the results of the isolation of batik liquid waste in a preliminary test through a series of dilutions, eleven isolates of indigenous bacteria were obtained which have the potential to reduce the color content of batik wastewater. The eleven bacterial isolates were then selected using the Submerged Fermentation (SmF) method and four superior bacterial

isolates were obtained which have the potential for the dye waste decolorization process. In the advanced decolorization process by adjusting various variations of dye concentration, from the four indigenous bacterial isolates it will be known that one of the best isolates is the most superior for the batik wastewater decolorization process. The superior bacterial isolates were selected based on the fastest decolorization time and the highest level of decolorization efficiency in reducing the color content of batik wastewater. Based on the results of identification by staining method, the four superior bacterial isolates belonged to the genus Bacillus. as shown in Figure 4.1



Bacillus spp1. *Bacillus* spp2. *Bacillus* spp3. *Bacillus* spp4.



Bacillus spp1. *Bacillus* spp2. *Bacillus* spp3. *Bacillus* spp4.

Figure 4.1. (A) Colony of *Bacillus* spp.. (B) Microscopic of *Bacillus* spp. (Source of Figure :Personal Documentation, 2012)

Growth of Bacillus spp. on Variations in Concentration of Dyes

Based on the bacterial growth curve in Figure 4.2(A,B,C), it can be seen that at dye concentrations of 50 mg/l, 100 mg/l and 200 mg/l there was an adaptation phase (lag phase) for the growth of Bacillus sp. at the beginning of the fermentation until the second day of fermentation. Growth of Bacillus spp. entered the exponential phase with the highest amount of growth on the 3rd day of fermentation

Remazol Blue Dye Decolorization

At the beginning of the fermentation there was a decolorization process with a higher average efficiency at a dye concentration of 50 mg/l and 100 mg/l (Figure 4.3AB), but the highest decolorization efficiency was found at a dye concentration of 100 mg/l using bacteria Bacillus spp., which is up to 90.88%. The decolorization efficiency at a dye concentration of 200 mg/L (Figure 4.3C) was lower than the decolorization effectiveness at a concentration of 50 mg/l and 100 mg/l. This is because too high a dye concentration will affect the ability of bacteria to grow and decolorize to reduce dye levels, as shown in graph 4.3 Remazol blue dye decolorization efficiency based on variations in dye concentration.

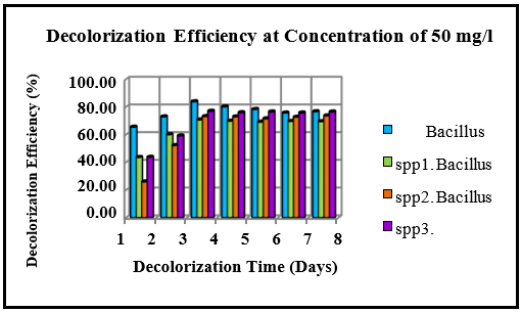
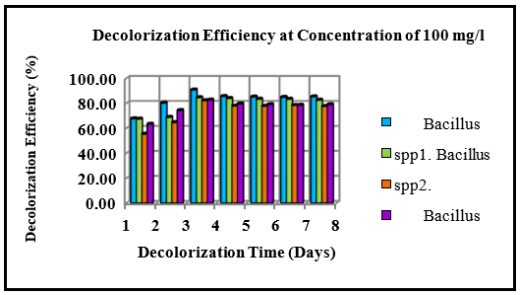


Figure 4.3.A Decolorization Efficiency at Concentration of 50 mg/l (Jurnal Sumber Daya Alam dan Lingkungan)



Graph 4.3B Decolorization Efficiency at Concentration of 100 mg/l (Sumber : Jurnal Sumber Daya Alam dan Lingkungan)

Effect of Bacterial Growth on COD (Chemical Oxygen Demand) and pH Value of Remazol Blue Dye

The efficiency of the decolorization of COD changes is shown in Figure 4.6. The average value of each COD in remazol blue dye at the end of fermentation showed a very significant decrease, namely 30.38-65.29%. This indicates that each type of Bacillus spp. These substances have a high ability to remodel dyes and reduce COD levels contained in remazol blue dye, even though the COD value after processing through decolorization still exceeds the permitted waste quality standard, so further research is still needed to optimize the decrease in COD values contained in the dye. color in accordance with the allowable quality standards ranged from 6.54-8.28. This is due to remazol blue dye, which is a synthetic dye with a higher chemical compound content, so that the pH value is alkaline as the results of measurements that have been carried out. From these observations, the bacteria Bacillus spp. The potentials found in this study are neutrophilic and alkalophilic, because they can live in a medium above pH 7 to pH 8.28 (Sumarsih, 2003).

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