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Microbial degradation of batik waste water treatment in Indonesia

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Abstract The existence of the batik industry is economically enough to provide a great livelihood for the community. The increasing demand of batik by the community has an impact on the growth of the batik industries in various regions in Indonesia. However, this industrial activity has a negative impact in production of liquid waste disposal. In batik production process, the liquid waste was produced by more than 95% which is emitted from process of soaking, heating and rinsing. Liquid waste has the potential to cause environmental pollution and affect the condition of living organism, including humans. One of efforts to reduce pollutants in batik waste water is by using biodegradation. Biodegradation is usually carried out by a consortium of a number of microbes. The objective of this study was to evaluate the potential of microbes for batik waste water degradation. Samples were taken from water and sludge contaminated batik waste water in the sewerage and river at Banyumas. Isolation and purification using the dilution planting method were conducted, to obtain pure culture using the pour plate technique, and maintenance of bacteria using streak culture. About 8 genus of microbes found in water bodies that have been contaminated by batik waste water are *Mesophilobacter*, *Methylococcus*, *Agrobacterium*, *Neisseria*, *Xantobacter*, *Deinococcus*, *Sporosarcina*, and *Bacillus*. The result showed that BOD degradation in batik waste water by microbial consortium was about 85.71%. Regarding on the result, it can be concluded that bacteria found in the sewerage and river were able to degrade pollutant from batik wastewater.

1. Introduction

The existence of batik industry is economically enough to provide a great livelihood for the community. Increasing demand of batik by consumers has an impact on the growth of the batik industries in various regions in Indonesia [1]. However, this industrial activity has a negative impact due to its waste water disposal [2]. In the production process, the liquid waste produced more than 95% [3].

The waste water produced comes from the process of soaking, heating and rinsing. Waste water has potentially the environment and affect the condition of living organism, including humans. Indicators of polluted waters can be seen from changes that are easily observed, such as temperature, pH, color, odor, sediment, and the presence of certain microorganism [2,4].



Indicators used to determine polluted waste water are BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), DO (Dissolved Oxygen), pH, nitrogen, phosphorus, heavy metals, CO₂, suspended solid material organic and inorganic, and total solids both organic and inorganic [5]. These indicators are the standard for polluted or uncontaminated waters [6].

In addition to pollution of aquatic environment, batik industry waste water can also cause odor, especially waste water in the form of liquid. The odor is due to the suspension of solids from coloring material used in the batik process. High temperatures will result in decreasing Dissolved Oxygen (DO) content in water which kill many organism so that it disturbs the balance of the water ecosystem [7,8].

The problem of managing batik waste water in several batik industry centers in Banyumas are to dispose the waste directly into the river without any treatment. Batik waste water discharged into the river without treatment has potentially polluted the environment and disrupt the life of aquatic biota.

The ability of microorganisms in organic matter in batik liquid waste has indeed been recognized by many researchers. Reliability of microbes including bacteria, fungi, and protozoa in waste water treatment and its role in maintaining the ecological balance of the waters has been widely elaborated. One of the commonly used bioremediatory agents is bacteria. Bacteria have an important role in the decomposition of organic matter. In general there are two types of bacteria based on the need for oxygen, it's aerobic bacteria and anaerobic bacteria. The objective of this research was to evaluate the BOD degradation by using microbial isolated from water body contaminated by batik waste water.

2. Material and Methods

Bacteria was isolated from river and sludge contaminated with batik waste. Isolation and purification using the dilution planting method or sample dilution were carried for the identification. In order to obtain pure culture bacteria the pour plate technique for maintain bacteria using streak culture (culture of scratching) was used. Dilution were carried for the identification. In order to obtain of samples was carried out up to level 10⁻³. Samples from dilutions of 10⁻¹.10⁻², 10⁻³ as many as 1 ml to be grown on nutrient agar by pour plate. Then the culture was incubated 24 hours at room temperature.

The colonies are separated and show different features, each of which is grown into nutrient agar medium in a streak quadrant. Culture was incubated for 24 hours at room temperature. Separate colonies that show different characteristics are transferred back to the nutrient agar medium in a streak quadrant, and so on until the colony is obtained with uniform characteristics (pure culture).

Pure isolates obtained from the isolation were grown on nutrient agar medium in a spread plate, and incubated at room temperature for 24 hours. The culture observed characteristics included colonies, colony shape, elevation, colony surface texture, colony color, and colony size. Observation of bacterial microscopic characteristics include: gram staining, endospore staining and bacterial cell measurements. Bacterial identification was carried out by matching its characteristics with the bacterial identification book from Bergey's manual of determinative bacteriology 9th. This is to find out the name of indigenous bacteria produced from the isolation of batik waste water. After identifying the bacterial genus, the next step is to multiply the bacterial culture as needed. The multiplication of bacteria is added to batik waste water to evaluate the growth and BOD degradation.

3. Results and Discussion

Based on the identification activities that have been carried out, bacteria from the genera *Mesophilobacter*, *Methylococcus*, *Agrobaacterium*, *Neisseria*, *Xantobacter*, *Deinococcus*, *Sporosarcina*, and *Bacillus* were obtained. These are types of aerobic or facultative anaerobes which are effective as biological agents in processing organic waste.

The results of identification of bacterial isolates by observing the macroscopic characteristics of bacterial colonies were based on colony morphology which included colony shape, colony color, colony edge, and elevation of bacterial colonies that had been cultured on nutrient agar plates.

Observation of the microscopic characteristics of bacterial cells includes the shape and size of cells, observation of the results of gram staining, and observations of endospores staining results. Based on observations of microscopic characteristics. Biochemical test of bacterial cell activity including

carbohydrate fermentation test, TSIA test, methyl red test, indole test, proskauer voges test and citrate use test.

Table 1. Identification of bacteria from river and sludge contaminated with batik waste

No	Code of isolates	Colony shape	Colony edge	Elevation of bacterial	Surfave properties	Colony color	Size of colony	Cell shape	Size of cell	Gram staining	Biochemical test					Name of genus
											TSIA test	MR test	VP test	Sitrat test	Indol test	
1	A1(1)	Irregular	Undulate	Corvex	Wrinkled	Opaque	Large	Basil	78,43135 µm	-	-	-	-	-	+	Mesophilobacter
2	A1(2)	Irregular	Entire	Raised	Glistening	Transluent	Large	Cocci	65,18269 µm	-	-	+	-	+	-	Methylococcus
3	A2	Irregular	Undulate	Pulvinate	Rough	Transluent	Large	Basil	8,603492 µm	-	-	+	-	+	+	Agrobacterium
4	A3	Rhizoid	Undulate	Pulvinate	Wrinkled	Opaque	Moderate	Cocci	46,94431 µm	-	-	+	-	-	+	Neisseria
5	B2	Rhizoid	Undulate	Pulvinate	Rough	Transluent	Moderate	Cocci	112,1552 µm	-	-	-	+	+	+	Methylococcus
6	B3	Irregular	Entire	Pulvinate	Rough	Transluent	Large	Basil	6,769941 µm	+	+	+	-	+	+	Xanthobacter
7	B4	Irregular	Undulate	Crateriform	Wringkel	Transluent	Large	Cocci	88,16432 µm	+	-	+	-	-	+	Deinococcus
8	C1	Irregular	Undulate	Flat	Wrinkled	Transluent	Large	Cocci	113,2664 µm	+	-	+	-	-	+	Sporosarcina
9	C2	Irregular	Undulate	Crateriform	Wrinkled	Opaque	Large	Cocci	90,3688 µm	+	-	+	+	+	+	Methylococcus
10	C3	Irregular	Undulate	Flat	Rough	Opaque	Moderate	Basil	16,77075 µm	+	-	+	+	-	+	Bacillus
11	D	Irregular	Undulate	Umbonate	Wrinkled	Transluent	Large	Cocci	7,039983 µm	-	-	+	-	-	-	Methylococcus

Determination of bacterial isolates was carried out based on the macroscopic characteristics of colonies, microscopic cells and biochemical tests of bacterial cell activity. Testing the characteristics of bacterial isolates was carried out based on the book Bergey's manual of determinative bacteriology 9th [9]. Bacterial culture is an attempt to increase the amount of bacteria isolated.

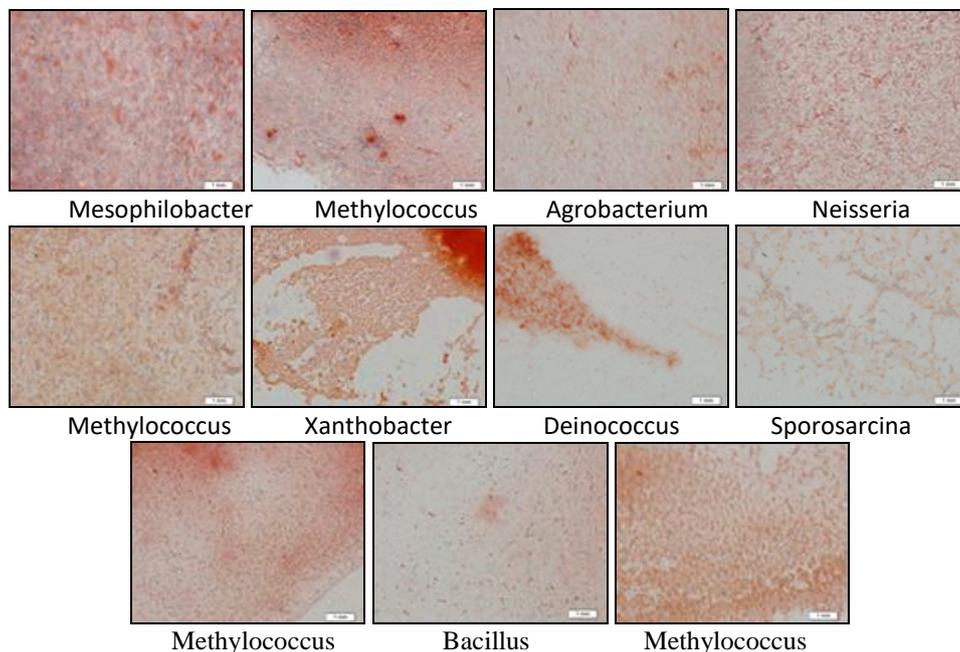


Figure 1. Bacteria culture (with endospores staining) can reduce pollution from batik

Bacterial culture is carried out on nutrient broth medium which has been added with batik waste water as much as 2%. The culture is then in sheaker for approximately 2x24 hours at room temperature.

Observations were made to determine the resistance and ability of living bacteria by looking at the color of the medium, that the medium experienced turbidity which showed bacterial growth in the medium.



Figure 2. Observation bacterial growth on medium; (a) before adding bacteria, (b) after adding bacteria (6 days)

Typical oxidation or decomposition of organic matter, especially in some types of industrial waste water containing for example phenols, detergents, oils, etc., bacteria must be given a time of adjustment (adaptation) [10] several days through contact with the waste water (acclimatization) [5], before it can be used as seeds in the water BOD analysis. Conversely some organic and inorganic substances can be toxic to bacteria (such as cyanide, copper, etc.) and must be reduced to the desired extent [5].

The parameters used in the research activities are based on the parameters contained in the Minister of Environment Regulation Number 5 of 2014 Republic of Indonesia concerning waste water quality standards, one of which is BOD. These parameters are used to determine the content of organic matter in batik wastewater [11].

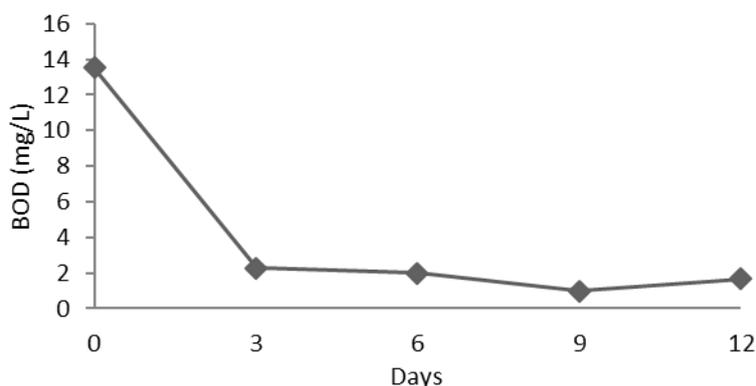


Figure 3. BOD degradation of batik waste water by bacteria

The results of the observation showed that the old treatment of contact had a significant influence on the BOD value, resulting in a decrease of up to 85.71%. The ability of bacteria to decompose organic matter must be supported by other biological agents that can reduce or eliminate residual decomposition of organic matter by bacteria [12].

In decomposing organic matter in batik liquid waste, bacteria release enzymes to decompose organic compounds and produce by-products in the form of carbon dioxide (CO_2), methane (CH_4), hydrogen (H_2) and water (H_2O), and energy to support metabolic activity [13]. Decomposition of azo compounds which are organic pollutants in batik liquid waste by bacteria produce CO_2 , H_2O and NH_3 compounds and energy in the form of biomass [13]. Decomposition of azo compounds by bacteria was carried out using the enzyme azo reductase [2]. Enzymatic azo reduction is catalyzed by an enzyme called azo reductase [14]. Inorganic materials resulting from decomposition of organic matter by bacteria will then be absorbed by plants with the content of the enzyme phytochelatin in their roots and leaves [5], [12].

4. Conclusion

The study evaluated the use of microbial consortium to degrade batik waste water. From the observation result, we obtained about 8 bacterial in a colony symbiosis of mutualism that take an important role of cleaning pollutants from batik waste water. The degradation of BOD in batik waste

water was 85.71% and it also proved that the batik waste water can be degraded by using microbial bioremediation method

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