Utilization of probiotic bacteria as an effort to handle liquid waste from the palm oil processing industry

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Abstract. The research aims to determine pollutant content in oil industry wastewater by using probiotic bacteria in reducing the content of pollutants in the palm oil industry. This study conducted in Laboratory of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar and Health Laboratory Center (BBLK). The sample was determined using a random sampling method. The data were analyzed variance (ANOVA) method. The results of the research indicate that there are two types of bacterial isolates, namely, isolate A was Lactobacillus bulgharicus and isolate B was Lactobacillus plantarum. Probiotic bacteria isolated from cow’s milk can reduce BOD content from 250.25 mg/L to 95.56 mg/L. COD value decreases from 530.25 mg/L to 245.81 mg/L. TSS value decreases from 420 mg/L to 232 mg/L. The initial fat content decreases from 30.25 mg/L to 21.65 mg/L. The initial measurement of value decreases from 7.5 to 6. Biodegradation process of the palm oil industry wastewater with the addition of the probiotic bacterial isolates can reduce organic content and pollutant content of the liquid waste of the palm oil industry. The most effective rate of waste degradation is bacterial isolate Lactobacillus plantarum with McFarland 1.5

1. Introduction

Wastewater is one source of pollutants that can cause a significant decrease in water quality in water bodies such as rivers, reservoirs, and lakes. The high level of pollutants causes waste from household waste, agricultural industry, hospital waste, and the commercial sector [1]. Pollution is caused by waste water being discharged directly into the environment without going through the processing [2]. One of the most dangerous pollutants is the palm oil industry liquid waste that contains high organic compounds. Palm oil industry produces liquid waste generally consists of organic and inorganic waste. Palm oil industry wastes that enter the waters contain high levels of organic compounds and chemical compounds that can cause environmental pollution [3].

Palm oil is one of Indonesia’s mainstay commodities, which is developing so rapidly. The rapid development of oil palm in Indonesia is supported by land conditions that are very suitable for oil palm plants. Palm oil also has a higher productivity advantage compared to other sources of vegetable oil. Palm oil can produce about 7 tons/ha of palm oil production, while soybeans produce oil of 3 tons/ha of soybean production [4].

The rate of development of the palm oil industry in Indonesia is increasingly rapid, both an increase in the area of oil palm and an increase in the number of palm oil processing factories [5]. The increase in palm oil production causes the high potential of byproducts in the processing of fresh fruit
bunches (FFB) into palm oil. During the process of processing palm fruit into palm oil, the palm oil industry produces liquid waste that still contains oil and other organic components [6].

Liquid waste generated from the Palm Oil processing plant (PKS) can have a negative impact on the environment because it has a very high biochemical oxygen demand (BOD) and chemical oxygen demand (COD), so before it is discharged into the waters, BOD and COD from the liquid waste must be reduced [7]. One environmentally friendly method is bioremediation using microorganisms. Most microorganisms including bacteria, can use oil/fat as a source of carbon and energy. So that the processing of palm oil liquid waste can take place effectively, what needs to be done is to look for microorganisms that have the potential to degrade oil/fat [8].

Probiotic bacteria are very effective in helping to manage liquid waste in palm oil mills. These probiotic bacteria can eliminate waste odors and reduce pollutant content to prevent environmental pollution. Probiotic bacteria can decompose glucose or carbohydrates that produce lactic acid will lower the pH and cause a sour taste. Biological treatment of waste has many advantages compared to chemical and physical treatment. Excess biological treatment can neutralize the burden of pollutants and not endanger the environment through probiotic bacteria[9]. Probiotic bacteria are easily found in dairy products. Milk as the main raw material for making dangke is a very important source of animal protein for the human body because it has a complete and balanced nutritional content [10].

Addition of inoculum to palm oil processing industry waste contains several microorganisms that have the ability to reduce levels of organic matter such as decreased biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS) and oil/fat [11].

Based on the description above, this research was conducted to reduce the pollutant content in the liquid waste of the palm oil processing industry by utilizing probiotic bacteria isolated from dangke cow's milk which is a typical food from Enrekang district, South Sulawesi Province.

2. Methods

2.1. Research location and design
The study conducted on December 2018. The research location was located in the Microbiology Laboratory of the Faculty of Medicine, Hasanuddin University, Makassar, and the Makassar Medical Laboratory (BBLK) Tamalanrea Makassar.
This type of research is an experimental study using probiotic bacterial isolates as bio activators in the biodegradation process of palm oil processing industry wastewater. The research variable used was treatment with the addition of bacterial isolates with McFarland standard 0.5, 1 and 1.5. As control is palm oil liquid waste without the addition of bacteria. The study design used was a completely randomized design (CRD) consisting of 7 treatments, where each treatment was repeated 2 times. The intended treatment is P0: (Palm oil liquid waste without bacterial addition (control)), P1 :( Palm oil liquid waste 108 ml + 12 ml bacterial isolate A MF 0.5), P2 :(Palm oil liquid waste 108 ml + 12 ml bacterial isolate A MF 1), P3 :(Palm oil liquid waste 108 ml + 12 ml bacterial isolate A MF 1.5), P4 :(palm oil liquid waste 108 ml + 12 ml bacterial isolate B MF 0.5), P5 :(Palm oil liquid waste 108 ml + 12 ml bacterial isolate B MF 1), P6 :(Palm oil liquid waste 108 ml + 12 ml bacterial isolate B MF 1.5).

2.2. Sample and population
The population used in this study is the Oil Palm Plantation in Kota Baru Regency, South Kalimantan Province. The research sample to be used is the palm oil processing industry liquid waste and probiotic bacterial strains isolated from dangke cow's milk.

2.3. Research implementation stage
Liquid waste is taken from the initial settling basin at the wastewater treatment plant (IPAL) of the palm oil processing industry by purposive sampling with 4 points at the pond outlet, then isolates bacterial isolates from dangke by dilution techniques up to 10-9, and obtained two bacterial isolates
obtained then gram staining and biochemical testing were performed. Pure bacterial culture obtained from isolation is a starter. The starter is then propagated using the McFarland standard on a scale of 0.5 (1.5 x 10^8 bacteria / mL), 1 (3 x 10^8 bacteria / mL) and 1.5 (4.5 x 10^8 bacteria / mL). This scale shows the concentration of bacteria / mL. Then the starter is inoculated into a sample bottle containing waste with a comparison of starter volume: waste volume = 12:108. A total of 12 ml probiotic microbial inoculums with a scale of 0.5, 1, 1.5 were inserted into each test bottle containing 108 ml of palm oil liquid waste, then the process of observation and biodegradation test of the organic content of palm oil liquid waste was carried out for 5 days for BOD parameters and COD parameters, TSS, oil / fat content and degree of acidity (pH) were carried out until the 10th day.

2.4. Data analysis
Data obtained from all measurements were analyzed using Analysis of Variance (ANOVA) using SPSS Software.

3. Results

3.1. BOD (Biological Oxygen Demand)
The results of the observation of wastewater samples ie on day 0 were obtained BOD values of 250.25 mg / L, then on the 5th day there was a process of waste degradation which was marked by a decrease in BOD content at P4, P5 and P6 experiencing a degradation rate that faster at 94.88 mg / L, 92.85 mg / L and 92.76 mg / L compared to P3 at 25.58 mg / L and P2 at 26.19 mg / L. However, the most effective is P6. While in P0 and P1, they experience a slower degradation process with a value of 26.74 mg / L and 26.62 mg / L, as in Table 1.

| Table 1. BOD (Biochemical Oxygen Demand) test results |
| Treatment | Day | H-0  | H-5  |
|-----------|-----|------|------|
|           |     |      |      |
| P0        | 250.25 | 97.16 |
| P1        | 250.25 | 96.85 |
| P2        | 250.25 | 96.54 |
| P3        | 250.25 | 95.88 |
| P4        | 250.25 | 96.76 |
| P5        | 250.25 | 96.43 |
| P6        | 250.25 | 95.56 |

3.2. COD (Chemical Oxygen Demand)
In the treatment results obtained P0 has the lowest degradation compared to P1, P2, P3, P4, P5, and P6. While the treatment that has the highest degradation ability is P6 which consists of a mixture of waste and B bacteria, where the COD value on the 0th day is 430.25 mg / L then decreases on the 5th day to 362.14mg / L and on the 10th day decreased by 245.81 mg / L, as in Table 2.

| Table 2. COD (Chemical Oxygen Demand) test results |
| Treatment | Day | H-0   | H-5   | H-10  |
|-----------|-----|-------|-------|-------|
|           |     |       |       |       |
| P0        | 530.25 | 363.69 | 251.74 |
| P1        | 530.25 | 362.48 | 248.62 |
3.3. **Content of TSS (Total Suspended Solid)**

Based on research that has been done, the average amount of TSS in wastewater is 420 mg / L, but after adding a number of bacteria and incubated for 10 days, it has decreased in all treatments ranging from P0 to P6, which is 259 to 232mg / L, as in Table 3.

| Treatment | Day | H-0  | H-5  | H-10 |
|-----------|-----|------|------|------|
| P0        | 420 | 378  | 259  |
| P1        | 420 | 374  | 245  |
| P2        | 420 | 375  | 236  |
| P3        | 420 | 373  | 234  |
| P4        | 420 | 376  | 235  |
| P5        | 420 | 374  | 237  |
| P6        | 420 | 372  | 232  |

3.4. **Fat content**

Based on the results of research at the beginning of the process of degradation of palm oil waste or on the 0th day the average fat content of 30.25 mg / L, then on the 5th day the content decreased, this indicates the process of waste degradation by bacterial activity, which is about 27.08 - 23.36 mg / L. The fat content continues to decrease on the 10th day, and the lowest content is P6, as in Table 4.

| Treatment | Day | H-0  | H-5  | H-10 |
|-----------|-----|------|------|------|
| P0        | 30.25 | 27.08 | 25.54 |
| P1        | 30.25 | 25.84 | 24.97 |
| P2        | 30.25 | 25.61 | 23.85 |
| P3        | 30.25 | 24.96 | 22.19 |
| P4        | 30.25 | 24.98 | 24.52 |
| P5        | 30.25 | 23.54 | 23.36 |
| P6        | 30.25 | 23.36 | 21.65 |
3.5. Acidity
The pH value during the degradation process is a change from pH 7.4 to 6.5-6.0. This shows the activity of microorganisms in degrading organic material in liquid waste and metabolizing, as in Table 5.

| Treatment | Day | H-0 | H-5 | H-10 |
|-----------|-----|-----|-----|------|
| P0        | 7.5 | 7   | 6,5 |
| P1        | 7.5 | 7   | 76,5|
| P2        | 7.5 | 6,5 | 6   |
| P3        | 7.5 | 6,5 | 6   |
| P4        | 7.5 | 7   | 6,5 |
| P5        | 7.5 | 6,5 | 6   |
| P6        | 7.5 | 6,5 | 6   |

4. Discussion
Based on the research that has been done, this study shows the results that the bacterial isolates isolated from dangke cow's milk, can reduce the pollutant content contained in the liquid waste of the palm oil processing industry and the most effective degradation rate in the process of waste biodegradation by the addition of bacterial isolates from dangke milk cattle are isolates of the bacterium Lactobacillus plantarum with McFarland 1.5 standard.

The palm oil industry wastewater treatment process using microorganisms is one of the effective ways to use because it has economic value that is relatively inexpensive and environmentally friendly compared to physical and chemical methods. In general, the results obtained from this study are in accordance with the Republic of Indonesia's Ministry of Environment regulation number 5 of 2014 covering the quality standards for wastewater so that they can be discharged into the environment.

In dangke, two types of bacterial isolates were obtained, isolates A and B based on the results of gram staining and biochemical tests compared with the book Bergey's Determinative Bacteriology found that the isolation of bacterial A was Lactobacillus bulgharicus namely gram-positive rod-shaped with the results of a negative catalase biochemical test, TSIA acid-acid, negative indole, negative urease, negative citrate and able to ferment carbohydrates (maltose, glucose and sucrose). Whereas isolate B was Lactobacillus plantarum, in the form of gram-positive rods with negative catalase biochemical test results, TSIA acid-acid, negative indole, negative urease, negative citrate and able to ferment carbohydrates (maltose, glucose, lactose and sucrose).

The results of the research into the effectiveness of decomposition of organic matter with the addition of bacterial inoculums are higher when compared to decomposition of organic materials without the addition of inoculums. The fastest decrease in BOD value was P6 compared to P0 with 108 mL of palm oil industrial wastewater treatment added 12 mL of bacterial isolate B with M.F 1.5, so that bacterial isolate B was more effective in reducing BOD levels compared to bacterial isolate A.

The results of the research that have been done, from all treatments have different COD content degradation abilities. In the treatment results obtained P0 has the lowest degradation compared to P1, P2, P3, P4, P5 and P6. This is because in the treatment, P0 only consists of a mixture of palm oil liquid waste without the addition of probiotic bacteria into the sample bottle, in contrast to the treatments P1, P2, P3, P4, P5, and P6, each of which has a mixture of bacteria A and B into the sample bottle. While the treatment that has the highest degradation ability is P6 which consists of a mixture of waste and B bacteria, where the COD value on the 0th day is 430.25 mg / L then decreases on the 5th day to...
245.81 mg / L and on the 10th day decreased by 245.81 mg / L. Addition of bacteria to liquid waste can accelerate the decomposition process of organic content. The greater the number of microbes under certain conditions will increase the ability of degradation.

The results of the research that have been done, the number of suspended solids that have decreased significantly is P6 compared to other treatments, where the initial TSS value of 420 mg / L then decreased to 372 mg / L on the 5th day and on the 10th day the amount TSS becomes 232 mg / L. The TSS value of an industrial liquid waste has decreased because the suspended solids which are mostly made from organic substances are decreasing. The decrease in TSS value is caused by the activity of degrading organic compounds by a bacterial consortium. The decrease in TSS content is influenced by an increase in the number of microorganisms.

Based on statistical test results the oil and fat content had no significant effect, but the results of the treatment with the addition of these bacteria met the waste quality standard standards based on the 2014 Government Regulation, which was under 25.00 mg / L with results ranging from 24.97 - 21.65 mg / L. Both types of bacterial isolates A and B give different levels of fat reduction. B bacteria in the treatment experienced a higher decrease in fat content, compared to bacterium A. This is presumably because bacterium B (Lactobacillus plantarum) has amylolytic properties greater than bacterium A (Lactobacillus Bulgaricus) where this bacterium is able to utilize the fat content as a source of nutrients, and the fat will split into simpler compounds. In addition it is suspected because the enzymes produced by B bacteria are able to break down simpler compounds so that they can be utilized for the process of cell metabolism.

Based on the results of statistical tests changes in pH have no significant effect on the process of degradation of palm oil liquid waste, but the final pH produced, which is 6.0 - 6.5, the results are in accordance with the Government Regulation of 2014 concerning the quality standards of industrial liquid waste which ranges from 6.0 - 9.0.

5. Conclusion and recommendation

Based on the results of a series of identification and biochemical tests on two isolates from dangke cow's milk, it can be stated that isolates A and B are lactic acid bacteria respectively as bacterium A is Lactobacillus bulgharicus and bacterium B is Lactobacillus plantarum. Probiotic bacteria isolated from dangke cow's milk can reduce BOD content from 250.25 mg / L to 95.56 mg / L. Decreased COD value from 530.2 mg / L to 245.81 mg / L. TSS value from 420 mg / L decreased to 232 mg / L. The initial oil / fat content is 30.25 mg / L and decreases to 21.65 mg / L. At the measurement of the initial pH value is 7.5 experiencing a decrease in pH value to 6. In the biodegradation process of palm oil industry liquid waste by the addition of probiotic bacterial isolates, able to reduce the content of organic matter and pollutant levels in the palm oil industry liquid waste. The most effective rate of waste degradation is Lactobacillus plantarum isolate with McFarland 1.5.

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