Identification of Faecal and Urinary Bacteria in Margodadi Embung, South Lampung

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Abstract. Margodadi Embung (retention basin) is located in South Lampung, Indonesia, which is designed for irrigation use and water conservation area. This Embung is surrounded by different types of farm fields, such as corn plant, cassava plant, palm tree and paddy fields. The farmers were using manure and chemical fertilizers for their fields, and they also cleaned their cattle in Embung after plowing at the farm. Those various activities around Embung can be the source of pollutant that decrease the water quality in Margodadi Embung. This study was aim to identify the water quality of Margodadi Embung from the parameter of faecal and urinary bacteria. Water sampling were carried out during the rainy and dry season conditions, at three sampling points at Margodadi Embung. The methods for calculating the number of faecal bacteria were using Most Probable Number (MPN), while biochemical test of faecal bacteria were using Eosin Methylene Blue (EMB) media. Afterwards, to find out the other contaminating bacteria from urine, the plate count method in selective media were conducted. The results show that the total of faecal coliform bacteria were 2604 MPN/100 mL in dry season, and 1340 MPN/100 mL in rainy season, and the urinary bacteria were 100 CFU/mL. It can be concluded that the water quality of Embung was suitable for irrigation use.

1. Introduction

Embung or retention basin was made for irrigation use and water conservation area. Retention basin carries pollutants that include nutrients, sediments, organic matter and microorganisms as the main source of pollution in the waters [1]. Margodadi Embung is located in South Lampung, Indonesia, which was surrounded by agricultural area, such as paddy fields, corn plant, and palm tree. In addition, farmers usually use manure and chemical fertilizer for their agricultural fields. Those activities can allow the pollutant get into the Embung water through run off when they were watering the plantation [2].

Residues of fertilizer that flow into the water body can be a source of nutrients for aquatic biota, especially aquatic plants [3], so that eutrophication process were happened in the embung. There were increased number of plant in water because of the large number of nutrient and pollutants from agricultural products. Other than that, more nutrients in the water will increase the growth of bacteria that affecting the water quality of Embung [4].

Identification of bacteria in embung is one of the tools to assess the risk of pollutant in water body and monitor the water quality. Gram positive and gram negative bacteria may cause digestion and urinary tract infection [5]. This study aims to identify the bacteria as water pollutant sources indicator.
in Margodadi Embung, South Lampung. The result of the study can be used by the local government as a tool to manage and monitor the water quality of Margodadi Embung, South Lampung.

2. Methodology

2.1. Water Sampling
Water sample were taken from Margodadi Embung from three sampling location: 1) inlet, 2) middle and 3) outlet. Grab sampling methods were used using 100 mL sterile bottle. The samples were put into the ice box. The sampling were conducted in two different weather conditions, the rainy and dry seasons.

2.2. Media Preparation for Faecal Coliform
The LB (Lactose Broth) media and BGLB (Brilliant Green Lactose Broth) were sterilized in test tube, but EMB (Eosin Methylene blue) agar were sterilized in the petri dish with autoclave for 30 minutes [6].

2.3. Media Preparation for Urinary Bacteria
The media CLED (Cysteine Lactose and Electrolyte Deficient) agar were sterilized in petri dish with autoclave for 30 minutes [6].

2.4. Identification of Bacteria
Identification of faecal coliform by using MPN method and urinary bacteria by using plate count method [6].

3. Results and Discussion

3.1. Faecal Coliform Bacteria
Faecal Coliform can be identified by three methods, first is presumptive test using LB media, confirmed test by BGLB media and complete test by EMB media.

3.1.1. LB Media
LB media was conducted to determine the presence of coli group bacteria from water samples, as well as to obtain the index or value of the nearest approximate amount of coli group organisms. This test is a specific test for detecting coli group bacteria [6].

The result of LB media are showed in Table 1. Figure 1 is Coliform bacteria in LB media. Positive tube is indicated that coliform is exist by the presence of bubbles in the tube.

![Figure 1. LB media for presumptive test](image-url)
Table 1. Result of positive tube in LB media

| Sample                  | Tube   |
|------------------------|--------|
|                        | A1    | A2  | A3 | B1 | B2 | B3 | C1 | C2 | C3 |
| 1st sample (Outlet point) | +     | +   | -  | +  | -  | +  | +  | +  |    |
| 2nd sample (Middle point)  | +     | +   | +  | +  | +  | +  | +  | +  | +  |
| 3rd sample (Inlet)        | +     | -   | -  | +  | +  | +  | -  | -  | -  |

Most of the tube test were in positive result, except tube of B1 and B3 in 1st sample, A2 and C3 in 3rd sample.

3.1.2 BGLB Media

Confirmed tests were conducted to BGLB media to ensure the presence of *coli* group bacteria in water samples that have yielded positive results in the estimation tests [6]. If this tests showed a positive result, then the water cannot be consumed. The result of BGLB media was showed in Table 2. Figure 2 is faecal coliform bacteria in BGLB media. Positive tube indicated that *coliform* were exist by the presence of bubbles in the tube.

![BGLB media for confirmed test](image)

Table 2. Result of positive tube in BGLB media

| Sample                  | Tube   |
|------------------------|--------|
|                        | A1    | A2  | A3 | B1 | B2 | B3 | C1 | C2 | C3 |
| 1st sample (Outlet point) | +     | +   | +  | -  | +  | -  | +  | +  | +  |
| 2nd sample (Middle point)  | +     | +   | +  | +  | +  | +  | +  | +  | +  |
| 3rd sample (Inlet)        | +     | -   | +  | +  | +  | +  | +  | +  | -  |

The tube was negative result is B1 and B3 in 1st sample, and also A2 and C3 in 3rd sample.
3.1.3 **EMB Media**

EMB media was carried out to confirm the presence of faecal coliform in a water sample, or if necessary to ensure that the results were doubtful or unclear. This test was the latest analysis of water sample. In this test using selective and fermented Eosin Methylene Blue (EMB) or Endo agar which was scratched culture that gave positive results [6]. The results of EMB media are showed in Table 3. Figure 3 is faecal *coli*form bacteria in EMB media. Positive petri dish indicated by the complex colour of dark purple or green metallic. This colour can indicate the bacteria that ferment lactose or sucrose like faecal coliform.

![Picture of EMB media](image)

**Figure 3.** EMB Media for complete test

| Sample                  | Petri Dish |  |  |  |  |  |  |  |
|-------------------------|------------|---|---|---|---|---|---|---|
| 1st sample (Outlet point) | +          | + | + | - | + | - | - | - |
| 2nd sample (Middle point) | +          | + | + | - | - | + | - | - |
| 3rd sample (Inlet)      | -          | - | - | - | + | + | - | - |

In EMB media showed in 1st and 2nd sample, only 4 petri dish were positive faecal coliform, in 3rd sample only 3 petri dish were positive faecal coliform.

3.1.4 **MPN Media**

MPN method was developed for the enumeration of *Eschericia coli* in water samples. Beside that MPN can identify the number of total faecal coliform by quality standard of water quality [6]. MPN analysis results showed in the Table 4.

| Sample               | Quality Standard (MPN/100 mL) | Dry Season (MPN/100 mL) | Rainy Season (MPN/100 mL) |
|----------------------|-------------------------------|------------------------|---------------------------|
| 1st sample (Outlet point) | 2000                          | 160                    | 100                       |
| 2nd sample (Middle point)   | 2000                          | 2400                   | 1200                      |
| 3rd sample (Inlet)         | 2000                          | 44                     | 40                        |

This results were similar with the research in Lake Michigan which showed the total number of *Eschericia coli* were higher in water around agricultural area [7]. According to Government Regulation
No. 82 about Water Quality, this Embung water coliform parameter average are still below the standard (2000 MPN/100 mL), so the water are still can be used for irrigation but not for consumption use.

However, if the irrigation water has a higher concentration of faecal bacteria, the raw vegetables that using those water need more disinfection after farming [8].

3.2. Urinary Bacteria
The result of identification urinary bacteria in Embung water are showed in Table 5. Urinary bacteria can be showed in petri dish and can be counted during 24 hours after incubated in CLED selective agar. The picture of urinary bacteria are showed in Figure 4 (a) – (c) below.

![Figure 4](image-url)

**Figure 4.** Urinary bacteria in: (a) 1st sample; (b) 2nd sample; (c) 3rd sample

| No | Parameter                  | 1st sample | 2nd sample | 3rd sample |
|----|----------------------------|------------|------------|------------|
| 1  | Shape of colony            | Circle     | -          | Circle     |
| 2  | Colour of colony           | Glowing blue| -         | Glowing blue|
| 3  | Surface                    | Smooth     | -          | Smooth     |
| 4  | Elevated of colony         | Flat       | -          | Flat       |
| 5  | Total of colony            | 5          | -          | 1          |

Table 5. Result of Urinary bacteria

The calculation result of CFU (Colony Forming Unit) are:

\[
CFU = \frac{(total \ colony \ of \ bacteria)}{(dilution \ factor)} \tag{1}
\]

- 1st sample: \( CFU = \frac{(5)}{10^{-2}} = 500 \text{ CFU/mL} \)
- 2nd sample: \( CFU = 0 \text{ CFU/mL} \)
- 3rd sample: \( CFU = \frac{(1)}{10^{-2}} = 100 \text{ CFU/mL} \)

The urinary bacteria were showed not so higher than the faecal coliform bacteria. But the presence of this urinary bacteria may be comes from faecal pollution from the livestock [9]. Urinary bacteria was responsible to Urinary Tract Infection and mostly caused by the gram negative bacteria *Escherichia coli* and *Klebsiella pneumonia* [10].
4. Conclusion
Based on the results of bacterial identification, faecal *coli*form bacteria and urine bacteria were detected. The amount of bacterial urine were not as much as the faecal *coli*form bacteria in water body. In dry seasons the total number of faecal *coli*form bacteria in Margodadi Embung were above 2000 MPN/100 mL, the water quality standard Class III PP No. 82, the total of faecal *coli*form bacteria were 2604 MPN/100 mL, and in rainy season conditions were below 2000 MPN/100 mL, only 1340 MPN/100 mL. Water in Margodadi Embung can still be used as a function of water for irrigation but not for daily consumption.

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