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Wastewater Quality Test Using The MPN (Most Probable Number) Method To Detecte Total Coliform Bacteria

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ABSTRACT

This study aims to Test Wastewater Quality Using the Mpn (Most Probable Number) Method to Detect Total COLIFORM Bacteria. This study uses laboratory analysis, which aims to provide an overview of the microbiological quality of wastewater based on the presence and number of Coliform bacteria. The working principle of the Most Probable Number (MPN) method in detecting total Coliform bacteria in wastewater is carried out semi-quantitatively by detecting microbial growth through lactose fermentation which produces gas or turbidity in selective liquid media. The implementation procedure includes a presumptive test stage using Lactose Broth (LB) media, a confirmatory test with Brilliant Green Lactose Bile Broth (BGLB) media, and determining the final value by matching the combination of positive tubes with the standard MPN table. From the results of laboratory testing on three domestic wastewater samples (1224 AL, 1225 AL, and 1226 AL), it was found that all samples showed negative results with a combination of 0-0-0 tubes in the presumptive test. This indicates that the tested sample does not contain Total Coliform bacteria or has a value of less than 1000 MPN/100 mL, so that its microbiological quality is declared very good and has met the quality standard threshold set by the government through the Minister of Environment Regulation No. 5 of 2014 and PP RI No. 22 of 2021.

Keywords: Quality Test, Wastewater, Mpn (Most Probable Number) Method, Total Coliform Bacteria

INTRODUCTION

Water is an essential compound for all living things and plays a vital role in agriculture, industry, fisheries, households, and recreation, making it an irreplaceable resource (Organization, 2022). Water is one of the basic human needs because humans are highly dependent on it for both drinking and daily life. Approximately three-quarters of the human body is composed of water, and no one can survive more than 4-5 days without drinking water (Precha et al., 2022). Furthermore, water is also used for cooking, washing, bathing, and other daily needs. It is crucial for us to protect water sources from pollution. (Gufran & Mawardi, 2019). Water pollution itself is the entry of living creatures, substances, energy and other components into water by human activities, so that the water quality decreases to a certain level which causes the water to not function according to its intended use (Amelia et al., 2023) .

Wastewater or liquid waste is a waste liquid that is no longer used and comes from households, trade, offices, industries and other public places (Trisnaini et al., 2018). This waste contains materials or substances that are harmful to the health or life of living things and disrupt environmental sustainability. Based on the Regulation of the Minister of Environment of the Republic of Indonesia Number 7 of 2014 concerning Wastewater Quality Standards, that health service facilities carry out the processing of domestic waste, hazardous and toxic waste and are required to meet the wastewater quality standards as stipulated in the established ministerial regulations (Shams et al., 2023). On this basis, a wastewater treatment installation is needed that functions to process all waste products. Waste that will be discharged into the environment is waste that has been managed properly and has met quality standards and does not cause disturbances to the surrounding environment (Busyairi et al., 2016) .

One of the wastewater tests is by looking at its microbiological parameters. The bacteria used as an indicator of water pollution are coliform bacteria (Sillberg & Rungratanaubon, 2022). Coliform bacteria are a group of intestinal bacteria, non-spore organisms that are motile or non-motile, rod-shaped, have the ability to ferment lactose and produce acid and gas at a temperature of 37°C within an incubation time of 48 hours. Total coliforms that exceed the standard limit for wastewater quality are an indicator of the presence of contamination that can cause dangers such as the spread of disease through water media when the wastewater is discharged into the environment (Gufran & Mawardi, 2019) .

One of the most commonly used methods for the detection and quantification of coliform bacteria is the Most Probable Number (MPN) method, which is able to estimate the number of bacteria based on growth in selective media. (Hadiansyah et al., 2021) . The Most Probable Number (MPN) is a technique used to estimate microbial density in a sample indirectly. Unlike the plate method, which uses solid media, the MPN approach uses liquid media contained in a test tube. (Mayangsari et al., 2023) . The calculation depends on the number of tubes showing positive results, which indicates microbial growth after incubation at a certain temperature and time period (Hamid, 2019) .

According to Sabila and Setyaningrum (Acreman et al., 2021) the advantages of the MPN method lie in its simplicity and flexibility. This method allows for the estimation of microorganism concentrations in samples with a high level of sensitivity, even at very low concentrations. This test can be applied to various sample types, including water, food, and soil (Baranyanan et al., 2024). Furthermore, this method does not require sophisticated equipment, making it easy to implement in various laboratories, especially for basic microbiology testing (Barus et al., 2022).

Therefore, regular monitoring of wastewater quality is crucial to ensure a healthy and safe environment for the public. Inadequate waste management can increase the risk of environmental pollution and endanger public health. This study aims to present a comprehensive review of wastewater quality testing using the MPN method to detect total coliform bacteria, based on previous research. (Jiwintarum et al., 2017) .

Wastewater and its Types

Waste is the result of various human activities, such as industrial, agricultural, and household activities. Waste is divided into several variations based on its form, including solid, liquid, and gaseous waste. Waste management is very important due to its negative impact on the environment and human health if not handled properly (Syafri et al., 2020a). Water, soil, and air can be polluted by waste if not managed properly (Kholifah, 2022) . In addition to being divided based on its form, waste can also be classified based on its source, namely: industrial waste, medical waste, agricultural waste, and domestic waste. Domestic waste is waste originating from various household activities, such as food scraps, plastic, paper, liquid waste from the kitchen or bathroom, and human waste (Sitepu, 2024).

Wastewater or liquid waste is unused waste liquid originating from households, trade, offices, industry and other public places, which contain materials or substances that are harmful to the health or life of living things and disrupt the sustainability of the environment, such as chemicals, pathogenic bacteria, and heavy metals, which can cause water pollution, ecosystem damage, and the spread of disease, so that it requires proper and effective processing to reduce its negative impacts and maintain environmental balance (Mayangsari et al., 2023) .

Domestic waste, or household liquid waste, is divided into two types: blackwater and graywater. Blackwater refers to waste from toilets, while graywater is waste produced from washing clothes, washing dishes, and bathing. (Sebayang et al., 2025) . Of all domestic waste produced, graywater accounts for the largest share, approximately 50% to 80%. Its pollution levels are low to moderate, while blackwater has moderate to high levels. Household waste is the largest contributor to water pollution in Indonesia, with approximately 85% of waste entering the waters. Prolonged wastewater treatment in aquatic ecosystems has the potential to trigger environmental pollution (Misrofah & Purwantisari, 2021) .

Coliform Bacteria as an Indicator of Water Pollution

Coliform bacteria are *a* group of microorganisms in the Enterobacteriaceae family *that are* often used as indicators of water pollution. This is due to their ability to reflect the presence of pathogenic microorganisms in aquatic environments (Makwinja et al., 2019). These bacteria can be found in various habitats, both in the digestive tract and in environments such as soil and water (Zainuddin *et al.*, 2024).

Morphologically, *coliform bacteria* are rod-shaped, non-spore-forming, and can be motile or nonmotile. These bacteria are capable of fermenting lactose, producing acid and gas at 37°C within a 48-hour incubation period. This characteristic is the basis for testing for the presence of *coliform bacteria* in water samples using the MPN method (Mubyarso et al., 2017) .

Coliform bacteria are classified into two groups: fecal and non-fecal. *Fecal coliforms,* such as *E. coli,* originate from the feces of humans and warm-blooded animals (Syafri et al., 2020b). The presence of *E. coli* in water is an important indicator of fecal contamination to monitor because its presence is often correlated with the presence of other pathogens that can potentially harm human health. (Said, 2006). Non-fecal *coliforms such as Aerobacter* and *Klebsiella* usually come from dead animals or plants and do not always come from human feces (Nanda et al., 2024) .

Most Probable Number (MPN) Method

Definition and Basic Principles

The Most Probable Number (MPN) is a semi-quantitative technique commonly used to detect and estimate the number of bacteria, such as *coliforms* and *E. coli,* in water samples. This method consists of three main stages: a presumptive test *to* detect coliforms in general, a confirmatory test to confirm the presence of specific coliforms, and a completed *test.* (Nurbaya & Sari, 2023) .

The working principle of MPN involves incubating a diluted sample in a test tube containing a selective medium. After a period of incubation at a specified temperature, tubes showing microbial growth are indicated by turbidity or gas formation in the Durham tube (positive). The frequency of these positive tubes is then used to estimate the concentration of microorganisms in the sample by referring to the available MPN table. The MPN value is an estimate of the growth units or colony-forming units in the sample (Hastuti, 2024). The units used are usually per 100 mL or per gram (Pratiwi et al., 2019) .

Media Used

In the MPN method, there are two main types of media used. First, *Lauryl Tryptose Broth* (LTB) media is used in the presumptive test stage. LTB works by inhibiting the growth of

coliform microorganisms, while the lactose content in this medium plays a role in encouraging the growth *of coliforms.* Lactose fermentation can be observed through the formation of gas in the Durham tube, which is an indicator of the presence *of coliforms.* (Rahayu & Nurwitri, 2012) .

Brilliant Green Lactose Bile Broth (BGLB) media is used in the confirmation test stage. BGLB media contains peptone, oxgall, lactose, and brilliant green, each of which has a specific function. (Supriyatno, 2000) . Peptone acts as a nutrient source, lactose is used as a fermentation substrate, while brilliant green and oxgall act as selective agents that inhibit the growth of Gram-positive bacteria and microorganisms other than *Coliform.* BGLB media is able to inhibit the growth of bacteria other than *Coliform bacteria* so that the results of the confirmation test are more accurate and specific (Rahmawati et al., 2024) .

MPN Dilution Range and Series

The MPN method uses several dilutions adjusted to the estimated bacterial concentration in the sample. Variation I uses 5 tubes of 10 ml, 1 tube of 1 ml, and 1 tube of 0.1 ml, suitable for specimens that have been processed or with an estimated low bacterial count. Variation II uses a 5-5-5 series, namely 5 tubes of 10 ml, 5 tubes of 1 ml, and 5 tubes of 0.1 ml, intended for specimens that have not been processed or with an estimated high bacterial count. Variation III uses 3 tubes for each dilution series as an alternative when the number of tubes or media supplies is limited (Rahmiani, 2022) .

The more tubes used in the MPN calculation, the higher the precision. The MPN method has a 95 percent confidence level, so for each MPN value, there is a range between the lowest and highest MPN values. The lower the MPN value, the better the water quality (Rophi, 2022) .

Wastewater Quality Standard Regulations in Indonesia

Based on the Regulation of the Minister of Environment of the Republic of Indonesia Number 7 of 2014 concerning Wastewater Quality Standards, health care facilities that process domestic waste, hazardous and toxic waste and are required to meet the wastewater quality standards as stipulated in the established ministerial regulations (Rezagama et al., 2020). Based on the Regulation of the Minister of Environment of the Republic of Indonesia Number 5 of 2014 concerning wastewater quality standards, health care facilities that process domestic waste are required to meet the wastewater quality standards for the *total parameters. coliform,* namely 5,000 cells/100 ml. Based on this, a wastewater treatment plant is needed that functions to process all waste products. Waste that will be discharged into the environment must be waste that has been properly managed and has met quality standards and does not cause disturbances to the surrounding environment (Mayangsari et al., 2023).

The MPN/100 ml index is used to measure bacterial concentration and is compared with the quality standards based on Appendix VI of the Republic of Indonesia Government Regulation No. 22 of 2021. For *E. coli* (*fecal coliform*), the maximum limit is 100 MPN/100 ml (class I), 1,000 MPN/100 ml (class II), and 2,000 MPN/100 ml (class III and IV). For total *coliform,* the limits are 1,000 MPN/100 ml (class I), 5,000 MPN/100 ml (class II), and 10,000 MPN/100 ml (class III and IV). Interpretation of the results is done by comparing the MPN value with the established quality standards, to assess the quality of the water being tested. (Hasibuan, 2022) .

Factors Affecting Coliform Bacteria Contamination

coliform bacteria contamination in wastewater is not uniform because it is influenced by various environmental factors. These factors include temperature, pH, organic matter content, salt content, and salinity (Mubyarso *et al.*, 2017).

Coliform bacteria can survive in temperatures ranging from 12°C to 44°C. Temperatures that are too high can damage the bacterial protein structure, while temperatures that are too low can slow down the bacteria's metabolic activity (Suriadikusumah et al., 2021). Regarding pH, *coliform bacteria* grow optimally at a neutral pH (7.0). A pH that is too acidic or alkaline can disrupt the biological function of bacterial cells, thus inhibiting their growth (Sabila & Setyaningrum, 2023) .

The availability of organic matter as a nutrient source also affects the abundance of *coliform bacteria. Coliform bacteria* tend to be abundant in wastewater derived from food waste due to the abundant availability of nutrients. Furthermore, *coliform bacteria* are generally found in environments with low salt concentrations, as high salt concentrations can cause osmotic pressure that inhibits their growth. High salinity will result in low abundance of *coliform bacteria,* and vice versa (Pratiwi *et al.*, 2019).

METHOD

Types of research

This study uses laboratory analysis, which aims to provide an overview of the microbiological quality of wastewater based on the presence and number of *Coliform bacteria.*

Time and Place of Activity

This research was conducted from January 5 to February 5, 2026, at the Regional Health Laboratory of North Sumatra Province (Mar'atusholikha et al., 2020). The research location was selected based on the availability of microbiology laboratory facilities and the implementation of wastewater quality testing according to health standards (Saputri & Makhfud, 2020) .

Objects and Activity Samples

The object of this study was wastewater, whose microbiological quality was tested. Domestic wastewater samples were collected using sterile sample bottles. Sampling was carried out aseptically to avoid contamination from the external environment. The tested samples were identified using the laboratory sample codes 1224 AL, 1225 AL, and 1226 AL (Susilowati et al., 2020).

Tools and materials

The tools used were incubators, volume pipettes, sample bottles, Erlenmeyer flasks, test tubes, Durham tubes, beakers, cotton, test tube racks and spirit lamps, while the materials used were distilled water, Lactose broth (LB) media, Brilliant Green Lactose Blue (BGLB) media and wastewater samples.

Work procedures

Initial Test (Prediction Test)

Objective: To form gas bubbles in a Durham tube at a temperature of 35°C for 48 hours. (Sari et al., 2019) .

1. Samples were taken and homogenized first.
2. Prepare 3 dilution tubes to carry out the dilution.
3. Pour 45 mL of distilled water into a 10^1 dilution tube, 9 mL into a 10^2 and 10^3 dilution tube. In the first dilution tube, 5 mL of sample is added, then 1 mL is taken and put into a 10^2 and 10^3 dilution tube. From each dilution, homogenization is carried out so that the sample is mixed evenly.
4. Prepare 9 sterile tubes, filled with Durham tubes and each tube filled with 5 ml of lactose broth.
5. The tubes are arranged on a tube rack and the tubes are marked with sample numbers.
6. Using a sterile pipette, 10 ml of sample from the 10^1 dilution tube was placed into tubes 1-3 which had been filled with Lactose Broth.
7. In tubes 4-6, 1 ml of sample from the 10^2 dilution tube was inserted and tubes 7-9 were filled with 0.1 ml from the 10^3 dilution tube.
8. Then the tube was incubated in an incubator at a temperature of 35°C for 1 x 24 hours.
9. After incubation, see if there is gas formation from the Durham tube, then proceed to the confirmation test. (Sebayang et al., 2025) .

Confirmation Test (Confirmation Test)

Objective: To confirm whether the sample contains bacteria with gas formation in the initial test caused by *Coli group bacteria* (Juwana & Nugroho, 2019).

1. From the tubes that were positive in the initial test, they were planted in BGLB media, each tube containing 5 ml of BGLB.
2. Incubate in an incubator at 35°C for 1 x 24 hours.
3. Then observe the positive tube in BGLB media at a temperature of 35°C, indicated by the presence of growth and gas formation in the Durham tube.
4. Then record the positive BGLB tube after incubation for 1 x 24 hours according to the MPN table.

Determination of MPN Value

The number of tubes showing positive results in each series was recorded and converted to an MPN/100 mL value using a standard MPN table (Sendrós et al., 2020). This value is used to assess the level of *coliform bacteria contamination* in wastewater samples.

Data collection technique

Data obtained through:

1. Direct observation, namely observation of the growth of bacterial colonies in lactose broth and BGLB media.
2. Documentation, in the form of recording test results and visual documentation during testing activities in the laboratory.

Data Analyst Technical

The test results data were analyzed descriptively, by observing the presence of bubbles in the Durham tube indicating the presence of *Coliform bacteria* found in wastewater samples with microbiological wastewater quality standards. Data obtained from the results of testing *Coliform bacteria* in outlet waste using the MPN method. The principle of this method is that *Coliform bacteria* can ferment lactose which is indicated by the formation of gas in the Durham tube in *Lactose Broth* (LB) and *Brilliant Green media. Bile Lactose Broth* (BGLB). The MPN value is calculated based on the number of positive tubes (gas production) and adjusted according to the MPN table value

RESULTS AND DISCUSSION

coliform bacteria in three domestic wastewater samples conducted at the UPTD Regional Health Laboratory of North Sumatra Province, the three samples with codes 1224 AL, 1225 AL, and 1226 AL all showed negative results. *The Most Probable* Method used was *Number* (MPN) with a 3–3–3 scheme, namely three tubes for each series of dilutions of 10 mL, 1 mL, and 0.1 mL. The combination of positive tubes from the entire sample is recorded as 0–0–0 on *Lactose media. Broth* (LB) after incubation at 35°C for 48 hours. The results of the Prediction Test are shown in Table 1 (Serra-Llobet et al., 2022).

Table 1. The presence of Coliform bacteria in domestic wastewater samples at the UPTD Regional Health Laboratory of North Sumatra Province

No	Sample	10 mL 3 tubes (10^1)	1 mL 3 tubes (10^2)	0.1 mL 3 tubes (10^3)	Combination Pool	Lactose Broth Test Results
1.	1224 AL	0 (+) 3 (-)	0 (+) 3 (-)	0 (+) 3 (-)	0-0-0	Negative Negative Negative
2.	1225 AL	0 (+) 3 (-)	0 (+) 3 (-)	0 (+) 3 (-)	0-0-0	Negative Negative Negative
3.	1226 AL	0 (+) 3 (-)	0 (+) 3 (-)	0 (+) 3 (-)	0-0-0	Negative Negative Negative

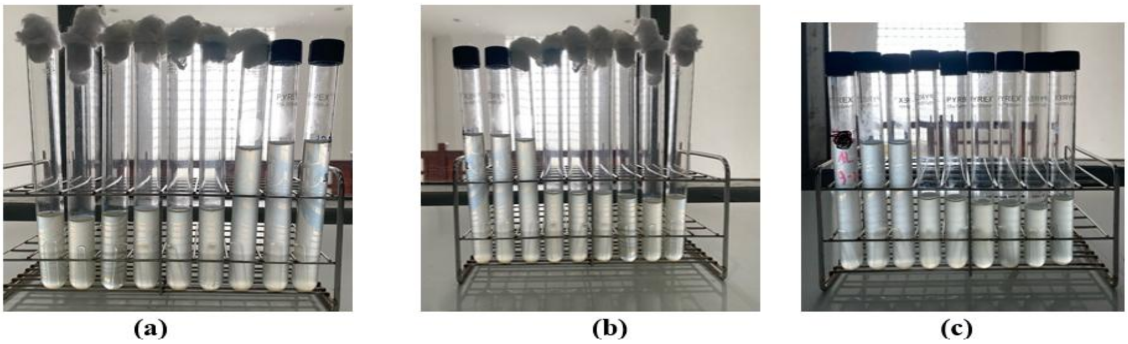

Figure 1. Image of the Predictor Test Results; a) All tubes are negative (1224 AL), b) All tubes are negative (1225 AL), c) All tubes are negative (1226 AL).

Discussion

Presumptive *Test*

The microbiological quality testing of wastewater in this study was conducted using *the Most Probable Number* (MPN) method. The MPN method is a technique used to estimate microbial density in a sample indirectly. Unlike the plate method, which uses solid media, the MPN approach uses liquid media contained in test tubes, and the calculation relies on the number of tubes showing positive results, indicating microbial growth after incubation at a specific temperature and time (Sitepu, 2024) .

The first stage carried out is a presumptive test *using* Lactose *Broth* (LB) media. LTB media works by inhibiting the growth of non- *coliform microorganisms,* while the lactose content in this media plays a role in encouraging the growth *of Coliform,* and lactose fermentation can be observed through the formation of gas in the Durham tube which is an indicator of the presence *of Coliform.* Positive tubes are identified by observing turbidity or gas formation in the Durham tube, and usually for each dilution level a series of 3, 5, or 7 tubes are used (Suastuti, 2017) .

In this study, the incubation process was carried out at 35°C for 48 hours. Observations on all tubes from the three samples showed no gas formation in the Durham tubes or turbidity in the LB medium. The combination of positive tubes from the three samples (1224 AL, 1225 AL, and 1226 AL) was recorded as 0-0-0, meaning that not a single tube showed a positive result from the entire dilution series tested.

This is in line with the statement that the predictive test focuses on detecting the presence of the *Coliform bacteria group* which is known to be able to ferment lactose within 48 hours at 37°C with the results in the form of acid and gas production, so that if there is no gas formed in the Durham tube, it can be concluded that there is no lactose fermentation activity by the *Coliform bacteria* in the sample (Rahmiani, 2022). Insufficient bacterial concentration can result in insufficient lactose fermentation to produce detectable gas in the Durham tube (Wardani, 2021).

Reasons for Not Carrying Out a Confirmative Test (*Confirmative Test*)

Based on the results of the presumptive test, which all showed a combination of 0-0-0, the test was not continued to the confirmatory test stage. This is based on the basic principle of the MPN method, where the confirmatory test is only carried out if there is a tube that shows a positive result in the presumptive test first. There are 3 stages of the MPN test, namely the first presumptive test *where* the sample is incubated in LTB media to detect gas in the Durham tube as an indication of the presence of total *Coliform bacteria* ; if gas is formed, the test continues to the second stage, namely the confirmatory test *using* BGLB media to confirm the presence of total *Coliform* through gas and acid production; and finally the complementary test *(* Azhar, 2021).

The confirmatory test aims to ensure that the positive result of the presumptive test is indeed caused by *Coliform bacteria,* not by non- *Coliform bacteria.* (Wardani, 2021) . Therefore, because the presumptive test yielded no positive tubes from all the samples tested, no tubes need further confirmation with BGLB media. Proceeding to the confirmatory test when the presumptive test results are all negative lacks scientific basis and is procedurally unnecessary (Elrayah, 2024).

Positive results in the presumptive test are continued with a confirmatory test, where in this test the media used is BGLB media to ensure contamination of *fecal coli bacteria* or non-fecal bacteria by seeing the presence or absence of gas in the Durham tube. Therefore, because there were no positive results in the presumptive test in this study, the confirmatory test was not relevant to be carried out and the test was declared complete at the presumptive test stage with the conclusion that the three domestic wastewater samples did not contain *Coliform bacteria.*

MPN Value and Interpretation of Results

The results of the 0-0-0 tube combination in all samples indicate that the MPN/100 mL value in the three wastewater samples is 0 or less than the 1000MPN/100 mL requirement or can be stated as negative. The smaller the MPN value, the better the water quality and the more suitable it is for use, and the MPN method has a confidence level of 9.5 % so that for each MPN value there is a range of the lowest and highest MPN values (Rahayu, 2012).

When compared to applicable regulations, these results are far below the established threshold. Based on Regulation of the Minister of Environment of the Republic of Indonesia Number 5 of 2014 concerning wastewater quality standards, health care facilities that process domestic waste are required to meet wastewater quality standards for total *coliform parameters,* namely 5,000 cells/100 mL (Mayangsari et al., 2023). Furthermore, based on Appendix VI of Government Regulation of the Republic of Indonesia No. 22 of 2021, the maximum limit for total *coliform* is 1,000 MPN/100 mL (class I), 5,000 MPN/100 mL (class II), and 10,000 MPN/100 mL (class III and IV).

Thus, the three domestic wastewater samples tested (1224 AL, 1225 AL, and 1226 AL) were declared to meet the quality standards set by the government. The number of *coliform bacteria colonies* in water correlates with the presence of pathogenic bacteria, so the lower the *coliform bacteria content,* the better the water quality (Amelia et al., 2023). These results indicate that the microbiological quality of the tested wastewater samples is classified as very good in terms of total *coliform content.*

Factors Causing Negative Results in All Samples

Several factors may explain why the three domestic wastewater samples tested showed negative results or no *coliform bacteria were detected* at all. The level of *coliform bacteria contamination* in wastewater is variable and is influenced by various environmental factors, including temperature, pH, organic matter content, salt content, and salinity (Zainuddin et al., 2024) .

Coliform bacteria can live in a temperature range of 12°C to 44°C, where high environmental temperatures can damage the structure of bacterial proteins and temperatures that are too low can cause bacterial metabolic activity to slow down. So that *Coliform bacteria* can be small in number if it comes from domestic wastewater that has a temperature above 44°C or below 12°C (Saputri & Makhfud, 2020). If the temperature of the wastewater at the time of sample collection or storage is outside the optimal range for *Coliform growth,* the number of living bacteria can be greatly reduced to the point of being undetectable.

Second, the pH factor. *Coliform bacteria* grow optimally at a neutral pH (7.0), and a pH that is too acidic or alkaline can disrupt the biological function of bacterial cells, thus inhibiting their growth (Mubyarso et al., 2017). Therefore, *Coliform bacteria* can be low in number if they come from domestic wastewater that has a non-neutral pH. Unsuitable wastewater pH conditions can be one of the causes of undetected *Coliform bacteria* in the tested samples (Surya et al., 2020).

Third, low organic matter content. The availability of organic matter as a nutrient source also affects the abundance of *coliform bacteria,* where *coliform bacteria* tend to be high in wastewater originating from food waste due to the abundant availability of nutrients (Pratiwi *et al*., 2019). If the wastewater sample tested does not originate from activities that produce high levels of organic waste or has undergone a treatment process that reduces organic matter, the number of *coliform bacteria* will decrease significantly.

Fourth, the waste processing factor through the IPAL. Based on the results of research in reference journals, the decrease in the number of *Coliform bacteria* is because the outlet sampling point is the end point of the IPAL storage tank and the liquid waste has gone through a series of *treatments* to reduce *Coliform bacteria,* where one of the treatments given is the addition of chlorine in the IPAL treatment before the last tank, and the addition of chlorine can cause damage to bacterial cells, including damaging the ability of cell permeability and damaging nucleic acids and enzymes in the bacteria (Busyairi *et al.,* 2016). If the wastewater sample tested is a sample from the outlet point that has gone through the IPAL treatment process, it is very possible that the number of *Coliform bacteria* is no longer detectable.

Fifth, salinity and salt concentration factors. *Coliform bacteria* are generally found in environments with low salt concentrations, as high salt concentrations can cause osmotic pressure that inhibits their growth, and high salinity will result in low abundance of *Coliform bacteria* (Pratiwi *et al., 2019). The chemical composition of the tested wastewater, including its mineral and salt content, can be a determining factor in whether or not Coliform* bacteria grow. in the sample (Forestier et al., 2020).

Sixth, the sampling location factor is far from the direct source of pollution. Samples from locations farther from the direct source of pollution, such as areas with vegetation or agricultural land, may show lower total *Coliform concentrations. This is caused by natural processes such as filtration by soil or decomposition by microorganisms that can reduce the number of Coliform bacteria* before the water reaches the sampling location. As well as the presence of a better waste management system or less domestic activity around the sampling location can also contribute to lower total *Coliform levels.* (Suastuti, 2017) .

Implications of Research Results on Wastewater Quality

The study's findings, which showed all samples were negative for total *coliform bacteria,* have important implications for wastewater quality management. Regular wastewater quality monitoring is crucial to ensuring a healthy and safe environment for the public, and inadequate waste management can increase the risk of environmental pollution and endanger public health.

The absence of *coliform bacteria* in the three domestic wastewater samples tested indicates that the wastewater meets applicable Indonesian microbiological quality standards. This indicates that the wastewater treatment or management system at the sampling location is operating effectively, resulting in no detectable *coliform bacteria contamination.*

However, negative results in one testing period cannot be used as a permanent guarantee that the wastewater is free from *Coliform bacteria contamination.* forever. Variations in the level of *Coliform contamination* in samples are influenced by several environmental factors, such as temperature, pH, organic matter content, salt content, and salinity. Therefore, the presence of high levels *of Coliform bacteria indicates fecal contamination and the possible presence of other pathogens* that can cause water-borne diseases. Better waste treatment and regular water quality monitoring are needed to prevent negative impacts on health and aquatic ecosystems (Saputri & Makhfud, 2020) . Therefore, regular testing is still necessary to ensure the consistency of wastewater quality in accordance with applicable regulations

CONCLUSION

The working principle of *the Most Probable method Number* (MPN) in detecting total *Coliform bacteria* in wastewater is carried out semi-quantitatively by detecting microbial growth through lactose fermentation that produces gas or turbidity in selective liquid media. The implementation procedure includes a presumptive test stage using *Lactose Broth (LB) media, a confirmatory test using Brilliant Green Lactose Bile Broth (BGLB)* media, and determining the final value by matching the combination of positive tubes with the standard MPN table. From the results of laboratory testing on three domestic wastewater samples (1224 AL, 1225 AL, and 1226 AL), it was found that all samples showed negative results with a combination of 0-0-0 tubes in the presumptive test. This indicates that the samples tested did not contain *Total Coliform bacteria. Coliform* or has a value of less than 1000 MPN/100 mL, so that the microbiological quality is declared very good and has met the quality standard threshold set by the government through the Minister of Environment Regulation No. 5 of 2014 and PP RI No. 22 of 2021.

Suggestion

Although current test results indicate that wastewater quality meets requirements, facility managers are still advised to conduct regular microbiological quality monitoring because bacterial contamination levels can be affected by changes in environmental factors such as temperature, pH, and organic matter content. Wastewater treatment systems, or WWTPs, must also be optimally maintained to ensure their effectiveness in eliminating pathogenic bacteria before discharge into the environment.

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