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RESEARCH ARTICLE

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# UTILIZATION OF *Moringa oleifera* SEEDS AS A NATURAL COAGULANT IN REDUCING *TOTAL COLIFORM* BACTERIA AND *Escherichia coli (E. coli)* IN ANIMAL SLAUGHTERHOUSE WASTEWATER

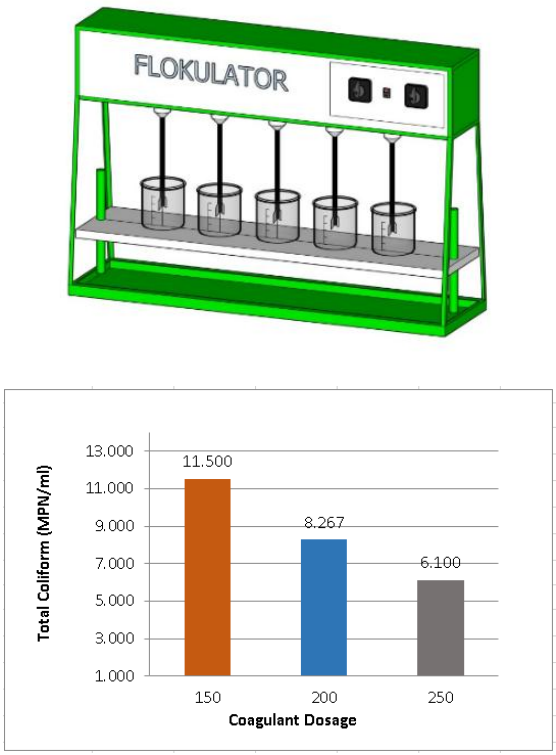
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**Graphical abstract**




**ABSTRACT**
Livestock slaughterhouse wastewater has the potential to pollute the environment because it contains pathogenic bacteria, especially Escherichia coli and total coliform, which are indicators of fecal contamination. This study aims to analyze the effect of varying doses of natural coagulant from moringa seeds (*Moringa oleifera*) on the reduction of E. coli and total coliform in RPH wastewater with a settling time of 40 minutes. The method used was coagulation-flocculation with varying coagulant doses of 150 gr, 200 gr, and 250 gr, followed by a sedimentation process. Microbiological analysis was conducted to determine the number of E. coli and total coliform bacteria, with each treatment repeated three times. The results showed that increasing the dose of moringa seed coagulant affected the reduction in the number of bacteria. For the total coliform parameter, the number of bacteria decreased from 11,500 MPN/mL at a dose of 150 gr to 6,100 MPN/mL at a dose of 250 gr. Meanwhile, the number of E. coli decreased from 3,100 CFU/mL to 1,550 CFU/mL at the same dose. These results indicate that moringa seeds have the potential to be used as an effective, simple, and environmentally friendly natural coagulant in reducing pathogenic bacterial contamination in slaughterhouse wastewater.

**Keywords:** Escherichia Coli, Coagulation–Flocculation, *Moringa Oleifera*, Slaughterhouse Wastewater, Total Coliform.

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## 1. 1 INTRODUCTION

Liquid waste produced from slaughterhouses has the potential to pollute the environment because it contains organic matter, suspended solids, and pathogenic microorganisms such as *Total Coliform* and *Escherichia coli* (E. coli), which are indicators of fecal contamination. The increase in slaughterhouse activity in line with the growing demand for meat has led to a larger volume of liquid waste being produced, while the waste treatment systems available in many slaughterhouses are still unable to reduce the bacterial content to meet the established quality standards. In addition, the presence of resistant E. coli bacteria in slaughterhouse wastewater has also been reported to increase the risk of ongoing environmental pollution (Bachmann et al., 2025).

Livestock slaughterhouse wastewater contains various types of microorganisms, including indicator bacteria such as *Total coliform* and *Escherichia Coli (E.Coli)*, which reflect the level of fecal contamination and risks to environmental health. High total coliform content indicates serious microbiological contamination, as this group of bacteria is generally associated with the presence of other pathogens originating from animal feces. Meanwhile, Escherichia coli, which is part of the total coliform group but more specifically originates from warm-blooded animal feces, is often used as the main indicator of fecal contamination. Several studies show that liquid waste from slaughterhouses in various regions of Indonesia has E. coli and total coliform concentrations that exceed quality standards, thus potentially causing gastrointestinal diseases if they contaminate community water sources (Nkansah et al., 2022).

Effective management of slaughterhouse wastewater is crucial because microbiological parameters, particularly total coliform and E. coli, are directly related to public health and the sustainability of aquatic ecosystems. Wastewater containing high levels of indicator bacteria can be a source of pathogen spread to water bodies, which in turn can affect the quality of irrigation water, recreational waters, and drinking water sources. Research by Suleiman and Mbawala (2021) shows that inadequate wastewater treatment systems are closely related to an increase in waterborne diseases, making the control of total coliform and E. coli levels a key focus in the design of microbiological wastewater treatment systems.

Total coliforms are a group of Gram-negative bacteria that occur naturally in environments such as soil, water, and vegetation, and originate from human and animal waste. The presence of these bacteria is often used as an indicator of water and liquid waste pollution levels because it correlates with organic content and the presence of other pathogenic microorganisms. The Most Probable Number (MPN) method is widely used to determine the total coliform count in water samples because it is highly sensitive to variations in bacterial concentration and can effectively provide an overview of the level of microbiological contamination.

The presence of high levels of total coliform in water or waste samples indicates contamination by organic sources, particularly fecal matter, which can originate from domestic, agricultural, or industrial waste. Mardizal et al. (2024) showed that variations in total coliform levels in rivers are influenced by poorly managed human activities, making monitoring of this parameter an early indicator of the risk of river water and aquatic ecosystem pollution.

*Escherichia coli (E. coli*) is part of the total coliform group that specifically originates from the intestines of humans and warm-blooded animals, so its presence is often used as a more specific indicator of fecal contamination than total coliform. E. coli is highly relevant in assessing water and

wastewater quality due to its ability to reflect the possible presence of other fecal-transmitted pathogenic bacteria, which can potentially cause health problems such as diarrhea and gastrointestinal infections if exposed to poorly treated water.

The concentration of *Escherichia coli* in wastewater or water sources is often much more sensitive to changes in pollution conditions than total coliforms. A global study report by Li (2025) shows that E. coli can remain detectable in high concentrations in the effluent of many wastewater treatment plants in various countries, because the treatment process does not eliminate fecal bacteria. This confirms that the E. coli parameter is a very important indicator in evaluating the effectiveness of microbiological wastewater treatment, especially in the context of public health and safe water quality.

Monitoring these two parameters, namely total coliform and Escherichia coli, is not only important for assessing the quality of liquid waste but also has direct implications for public health. The presence of E. coli in drinking water and water sources indicates fecal contamination that can potentially cause gastrointestinal illness if consumed or exposed to directly. Therefore, water quality standards for both sanitation and overall water quality often set threshold values for total coliform and E. coli as indicators of pathogenic bacteria that must be met to maintain health and a safe environment.

*Moringa oleifera* seeds are known to contain cationic proteins that function effectively in the coagulation-flocculation process by attracting negatively charged particles in water or wastewater, thereby binding suspended particles and microbes into flocs that easily settle. Research shows that in addition to their strong coagulant properties, moringa seeds also exhibit antimicrobial activity against various bacteria, including Escherichia coli, thereby potentially reducing the load of pathogenic bacteria directly, rather than just settling solid particles. This makes moringa seeds superior not only in reducing turbidity but also in suppressing the number of indicator bacteria such as total coliforms and E. coli in liquid waste.

The use of Moringa oleifera seeds as a natural coagulant in water and wastewater treatment has been increasingly studied in the last five years due to its environmentally friendly properties and effectiveness in reducing physical and microbiological parameters. (Ali et al. 2021). The cationic proteins contained in Moringa oleifera seeds can neutralize the negative charge of suspended particles and bacterial cells, thereby supporting the formation of larger flocs that settle more easily (Narasiah, 2020).

Various efforts to treat slaughterhouse wastewater have been developed to reduce pathogenic bacteria content, but the success rate may vary depending on the operational conditions applied. Differences in coagulant type, dosage, and settling time have the potential to produce inconsistent bacterial reduction effectiveness. In addition, studies on the effect of variations in natural coagulant dosage on the reduction of Total Coliform and Escherichia coli (E. coli) at specific settling times are still relatively limited, especially in simple and easily applied approaches. These conditions indicate the need for research that more specifically examines the relationship between natural coagulant dosage and settling time on the reduction of pathogenic bacteria in slaughterhouse wastewater.

The main problem in managing slaughterhouse wastewater is the high content of microbiological indicator bacteria, particularly Escherichia coli and total coliform, which have the

potential to pollute the environment and endanger public health. Therefore, a study is needed to determine the extent to which natural moringa seed coagulants can reduce the levels of these two bacterial parameters in slaughterhouse wastewater. In addition, variations in coagulant dosage and contact time are thought to affect the effectiveness of the coagulation-flocculation process, making it important to analyze the comparative effects of these two factors on the reduction of E. coli and total coliform levels. This problem formulation is aimed at gaining a more comprehensive understanding of the performance of moringa seeds as an alternative natural coagulant in the treatment of slaughterhouse wastewater.

## 2. MATERIALS AND METHODS

This study used liquid waste from a slaughterhouse in Malang as the object of research, with parameters analyzed including Total Coliform bacteria and Escherichia coli (E. coli). The main material used as a natural coagulant was moringa seed powder (Moringa Oleifera), while the equipment used included glass beakers, volumetric pipettes, an oven, a flocculator, and microbiological analysis devices.

Moringa seeds used as natural coagulants are first peeled, dried in an oven at $105^0$C for 1 hour, then ground into powder. The moringa seed powder is then sieved using a 60mesh sieve to obtain homogeneous particle sizes, thereby increasing the effectiveness of the coagulation-flocculation process.

The liquid waste treatment process was carried out using the coagulation-flocculation method with varying doses of moringa seed coagulant at 150 mg/l, 200 mg/l, and 250 mg/l, with a settling time of 40 minutes. The liquid waste sample was stirred rapidly at a speed of 200 rpm, then continued with slow stirring at a speed of 60 rpm for 5 minutes to form flocs. After the stirring process, the sample was left to settle under sedimentation conditions.

Total Coliform and Escherichia coli (E. coli) bacteria analysis were performed using standard microbiological methods. Each treatment was repeated three times to ensure the reliability and consistency of the data obtained. The test results were then analyzed descriptively to determine the effect of coagulant dose variation on the reduction of pathogenic bacteria in slaughterhouse wastewater.

The coagulation-flocculation process in this study was carried out using a flocculator, as shown in Figure 1.

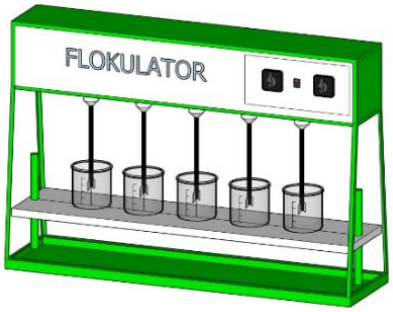



**Figure 1.** Flocculator used in the coagulation-flocculation process of slaughterhouse wastewater. Tool length 80 cm, Tool width 30 cm, Tool height 55 cm, Number of beakers 6 pieces, Beaker capacity 1,000 mL (1 L).

The flocculator was used in this study to support the coagulation-flocculation process in the treatment of liquid waste from slaughterhouses. The flocculator functions to produce slow and controlled agitation that allows suspended particles, including microorganisms, to collide and combine to form larger flocs. This uniform agitation process is important to ensure that the distribution of moringa seed coagulant is evenly distributed throughout the wastewater sample, so that the interaction between the coagulant and pollutant particles can occur optimally.

Microbiological analysis was conducted to determine the number of indicator bacteria, namely total coliform and Escherichia coli, in slaughterhouse wastewater before and after coagulation-flocculation treatment. The testing was conducted in a laboratory using a culture method with selective media, which allowed for the growth and visual observation of indicator bacteria colonies. The purpose of this analysis process was to obtain quantitative data on changes in the number of bacteria as a basis for evaluating the effectiveness of the treatment applied.

## 3. RESULT AND DISCUSSION

Researchers have used various approaches in this study, resulting in quantitative data on the effect of varying doses and contact times of moringa seeds (Moringa oleifera) on the reduction of pathogenic bacteria in slaughterhouse wastewater. The data obtained show that changes in coagulant dosage and contact time produce different responses in terms of the reduction in Escherichia coli and total coliform levels, as reflected in the variation in bacterial concentration values after treatment. In general, an increase in the dose of moringa seeds was followed by a greater reduction in the number of bacteria, while the contact time setting played a role in optimizing the coagulation-flocculation and sedimentation processes. These results confirm that the effectiveness of bacterial reduction is not determined by a single parameter, but by a combination of coagulant dose and contact time, which influence each other.

This study produced quantitative data on the effectiveness of using moringa seeds (Moringa oleifera) as a natural coagulant in reducing the levels of Escherichia coli and total coliform bacteria in liquid waste from slaughterhouses. The results were obtained through laboratory testing of wastewater samples before and after coagulation-flocculation treatment with varying doses of moringa seeds and contact times. The test results showed changes in bacterial concentration after treatment, enabling an evaluation of the effect of coagulant dosage and contact time on the efficiency of reducing fecal contamination indicator bacteria. Thus, the results of this study directly answered the research objectives, namely, to assess the ability of moringa seeds to reduce E. coli and total coliform levels, and to compare the effectiveness of treatments based on variations in dosage and sedimentation time used.

### Initial Condition of Waste

The initial condition of liquid waste from slaughterhouses before treatment is an important aspect in evaluating the level of pollution and its potential impact on the environment. Liquid waste from slaughterhouses is produced from a series of operational activities that involve direct contact with blood, animal tissue, organic waste, and the possibility of mixing with feces, thereby contributing to the high microbiological load in the waste. The initial characteristics of this waste reflect the sanitation quality of the production process and form the basis for determining the requirements and effectiveness of the treatment methods to be applied.

Microbiological quality standards are set as a reference to protecting aquatic environments from the negative impacts of liquid waste disposal. These limits serve to ensure that wastewater discharged into water bodies does not contain microorganisms in quantities that could harm aquatic organisms or human health. Therefore, a study of the initial condition of RPH wastewater, particularly in relation to total coliform and Escherichia coli parameters, is important as a basis for assessing the urgency of implementing treatment processes and evaluating the success of the methods used in reducing the microbiological pollutant load.

Laboratory results before treatment and comparison with quality standards based on applicable regulations are shown in Table 1.

**Table 1** Initial characteristics of waste

| Parameter | Concentration | Quality standard* |
|---|---|---|
| Total Coliform | 49.300 MPN/ ml | 10.000 MPN /100ml |
| *Escherichia Coli* | 12.733 CFU/ml | 2.000 CFU/100ml |

*Government Regulation No. 22 of 2021 concerning the Implementation of Environmental Protection and Management Appendix VI Class

Based on these initial conditions, the implementation of wastewater treatment processes is a necessity that cannot be ignored. Treatment efforts are aimed at reducing the microbiological load to near or meet established quality standards, thereby minimizing negative impacts on the environment. In the context of this study, the coagulation-flocculation process using moringa seeds was selected as the treatment method to evaluate its ability to reduce the number of indicator bacteria, particularly total coliform and Escherichia coli, from the initial contaminated conditions to conditions that are safer for the environment.

The laboratory processes carried out during the research can be seen in the following figure



**Figure 2.** The process of bacterial analysis using the culture method on agar media in Petri dishes for testing total coliforms and Escherichia coli.

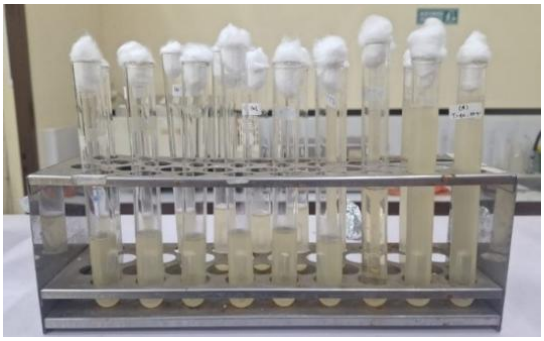


**Figure 3.** Results of culturing animal slaughterhouse wastewater samples on agar media in Petri dishes during total coliform and Escherichia coli analysis in the laboratory.

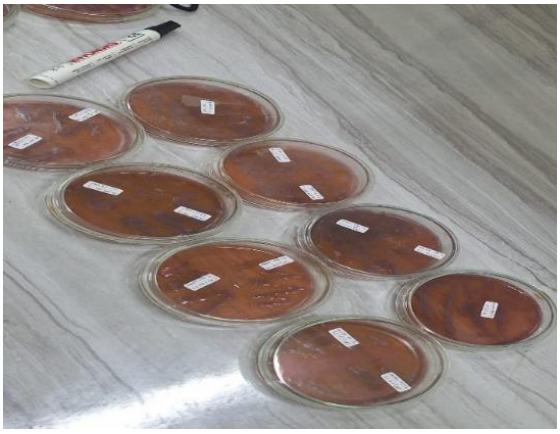


**Figure 4.** The process of bacterial analysis using the culture method on agar media in Petri dishes for testing total coliforms and Escherichia coli.

The images presented serve as visual documentation of the stages of microbiological analysis conducted during the study, particularly in testing for total coliform bacteria and Escherichia coli in liquid waste from slaughterhouses. These images aim to clarify the analysis methods used, so that readers can concretely understand the laboratory testing process that forms the basis for obtaining quantitative data. In addition, the images of Petri dishes and test tubes illustrate the application of culture methods and the Most Probable Number (MPN) method in determining the presence and number of indicator bacteria. This visualization reinforces the validity of the research methods, as it shows that the testing process was carried out in accordance with standard microbiological procedures commonly used in wastewater quality analysis.

**Test Parameter Analysis**

***Reduction of Total Coliform bacteria in RPH liquid waste***

Total coliform is one of the microbiological parameters commonly used to assess the level of liquid waste pollution, particularly in relation to fecal contamination and environmental sanitation conditions. The presence of high levels of total coliform indicates that the wastewater contains microorganisms originating from the digestive tract of animals or humans and has the potential to carry other pathogens that are harmful to health. Therefore, the total coliform parameter is used as an initial indicator to evaluate the effectiveness of a wastewater treatment process, including in animal slaughterhouse wastewater, which has high microbiological load characteristics.

The results of total coliform testing in slaughterhouse wastewater after coagulation-flocculation treatment using moringa seeds at a settling time of 40 minutes are presented in Table 2.

**Table 2** Results of total coliform and E. coli testing at a settling time of 40 minutes

| Coagulant dosage (gr) | Settling time (minute) | Total Coliform |
|---|---|---|
| 150 | 40 | 11.500 MPN/ml |
| 200 | 40 | 8.267 MPN/ml |
| 250 | 40 | 6.100 MPN/ml |

The results of the study show that increasing the dose of moringa seed coagulant during a 40-minute settling period affects the reduction in the number of total coliform bacteria in slaughterhouse wastewater. In general, the higher the dose of coagulant administered, the greater the reduction in the number of bacteria observed. The reduction in the total number of coliforms along with an increase in the coagulant dose indicates that moringa seeds play a role in improving the performance of the coagulation-flocculation process. The positively charged proteins contained in moringa seeds can neutralize the negative charge on suspended particles and bacterial cells, thereby forming larger flocs that settle more easily during the sedimentation process. The higher the coagulant dose administered, the more active sites are available to bind particles and bacteria, thereby reducing the total coliform count in the liquid phase more effectively.

These results show that coagulant dosage variation is an important factor in controlling total coliform contamination in slaughterhouse wastewater. Although there has been a decrease in the number of bacteria, the total coliform values obtained indicate that the effectiveness of treatment is still influenced by other operational conditions, such as settling time and initial wastewater characteristics. Thus, the use of moringa seeds as a natural coagulant has good potential in reducing total coliform, but it needs to be combined with optimal process control to meet the established environmental quality standards.

The results of testing the total coliform count in slaughterhouse wastewater are presented in Table 1. To clarify the effect of varying doses of moringa seed coagulant on the total coliform count, the results are further presented in Graph 1.

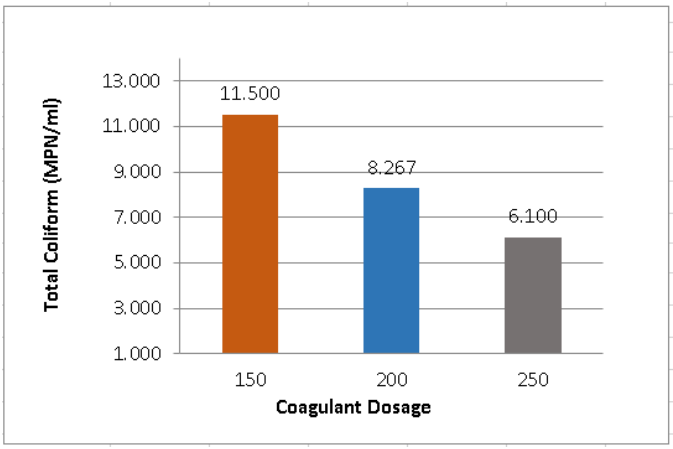



**Figure 5.** Percentage decrease in Total Coliform bacteria in slaughterhouse wastewater

Based on the graph, an increase in the dose of moringa seed coagulant was followed by a decrease in the total number of coliform bacteria at a settling time of 40 minutes.

***Reduction of E.Coli bacteria in RPH liquid waste***

House wastewater discharges into the aquatic environment. *Escherichia coli* is an indicator bacterium specifically used to identify fecal contamination originating from the digestive tract of warm-blooded animals. The presence of this bacterium in slaughterhouse wastewater indicates relatively recent fecal contamination, given that E. coli generally cannot survive long in aquatic environments without a fresh supply of organic matter. Therefore, the detection of E. coli not only reflects the sanitary conditions of the wastewater but also provides more specific information about the potential entry of other fecal pathogenic microorganisms. The presence of high levels of E. coli is directly related to an increased risk of health problems, such as gastrointestinal diseases, making this parameter an important indicator in assessing the safety and health impact of slaughter

The results of total coliform and E. coli testing for several variations of moringa seed coagulant dosage are presented in Table 3.

**Table 3** Results of Escherichia coli (E. coli) testing at a sedimentation time of 40 minutes

| Coagulant dosage (gr) | Settling time(minute) | E.Coli |
|---|---|---|
| 150 | 40 | 3.100 CFU/ml |
| 200 | 40 | 2.200 CFU/ml |
| 250 | 40 | 1.550 CFU/ml |

Based on the data presented in Table 3, the number of bacteria decreased with increasing doses of moringa seed coagulant at a settling time of 40 minutes. In the case of Escherichia coli (E. coli), an increase in the coagulant dose was followed by a decrease in bacterial concentration. This shows that the dose of moringa seed coagulant plays a role in increasing the effectiveness of bacterial reduction in slaughterhouse wastewater.

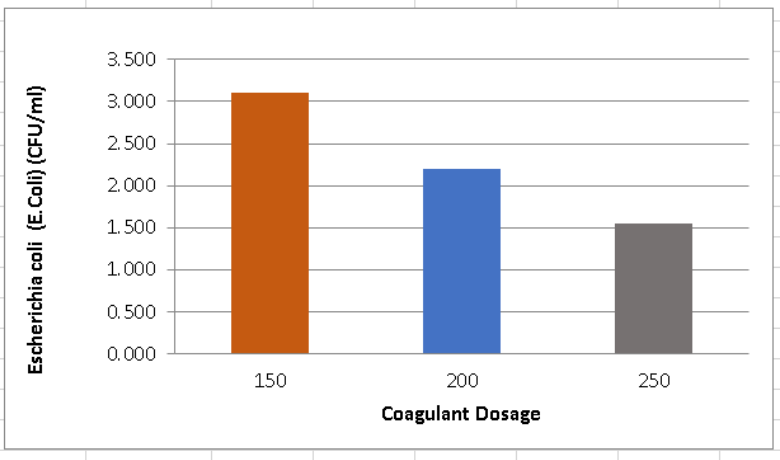



**Figure 6.** Variations in the reduction of E. coli bacteria.

The decrease in the number of bacteria is related to the cationic protein content in moringa seeds, which can neutralize the negative charge on bacterial cells and suspended particles, thereby supporting the formation of larger flocs that settle more easily during the sedimentation process.

Thus, the use of natural coagulants from moringa seeds at the appropriate dosage can be a simple and environmentally friendly alternative for treating liquid waste from slaughterhouses to reduce pathogenic bacterial contamination.

The graph shows that variations in the dosage of moringa seed coagulant affect the reduction in the number of Escherichia coli at a settling time of 40 minutes. Higher coagulant doses resulted in lower bacterial counts in both parameters tested, indicating an increase in the effectiveness of the coagulation-flocculation process. Thus, the coagulant dose is one of the factors that determine the success of slaughterhouse wastewater treatment in reducing pathogenic bacterial contamination.

## 4. CONCLUSION

Moringa seeds (*Moringa oleifera*) have been proven to have potential as a natural coagulant in reducing E. coli and total coliform contamination in slaughterhouse wastewater through a coagulation-flocculation process with a settling time of 40 minutes. Increasing the coagulant dose from 150 g to 250 g showed a reduction in bacteria, marked by a decrease in Total Coliform from 11,500 to 6,100 MPN/mL and E. coli from 3,100 to 1,550 CFU/mL. These results confirm that the coagulant dose is an important factor in process performance and indicate that moringa seeds can be a simple and environmentally friendly alternative for wastewater treatment, especially for laboratory-scale systems.

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