Production of Powdered Bio-coagulant from *Moringa oleifera* Seeds Using Vacuum Drying Method

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ABSTRACT

Laundry wastewater is classified as domestic wastewater that has a turbidity of 25 – 150 NTU and can cause an environmental problem. Moringa seeds are plants that can be used as natural coagulants for the treatment of wastewater containing suspended colloids. The main purposes of this study are to extract proteins from moringa seeds into a bio-coagulant, determine the optimum time for drying the extract form into powder form, calculate the percentage of protein in the extract form and powder form and determine of turbidity removal efficiency using the extract form and powder form in condition low turbidity, medium turbidity, and high turbidity. Powdered bio-coagulant is obtained through several stages including moringa seeds size reduction into 250μm, oil extraction from solids using n-Hexane, protein extraction from solids using NaCl solution, protein separation by centrifugation process, and drying the protein using vacuum drying method at 40°C and 70 mbar. The best results were obtained by the extraction process using 2 M NaCl solution and an optimal drying time of 3 hours. Protein percentage in the extract form of bio-coagulant was 1.09% with a turbidity removal efficiency of 76.15%, while the protein percentage in the powder form was 80.75 % with a turbidity removal efficiency of 30.35%.

Keywords: Moringa Seeds, Bio-Coagulant, Vacuum Drying.

1. INTRODUCTION

The urban culture that tends to want something practical encourages the formation of businesses that focus on serving basic needs, such as food, clothing, and cleanliness. One of the growing businesses is laundry services which contribute to increasing the amount of wastewater discharged into the environment. Laundry waste is classified as domestic wastewater which has a turbidity of 25-150 NTU depending on the condition of the clothes, while the maximum turbidity allowed to enter the water body is 25 ppm or the equivalent of 75 NTU [1]. Therefore, the process of laundry wastewater treatment is needed, including through the coagulation process.

The common chemical used as a coagulant is alum [(Al₃)(SO₄)₂·18H₂O], but the sludge coagulated using alum contains aluminium metal which can pollute the environment. Moringa (*Moringa oleifera*) is one of the plants that can be used as a natural coagulant for the treatment of TSS-containing wastewater. Moringa seeds have the advantage of being a natural bio-coagulant, namely biodegradable, the treatment of coagulated sludge will be easier and safer when the waste enters the biological treatment unit. Moringa plant is an alternative resource and can thrive in tropical areas such as Indonesia and is not the main food ingredient so that when used for coagulants in water treatment it does not interfere with food needs.

In the separation of protein fractions in moringa seeds, albumin and globulin are proteins that capable of acting as natural coagulants and can be extracted using water (H₂O) and solution (NaCl) [2]. These proteins can bind to negative ions and act as a pH buffer [3]. The protein content in moringa seeds can capture organic compounds, both those that can be decomposed by microorganisms or not. Even some heavy metals such as lead can be agglomerated [4].

So far, the bio-coagulant from moringa seeds is used in extract or supernatant form (liquid). Moringa seed extract in liquid form with a concentration of 70 ppm, can reduce TSS by 62.05% from the initial turbidity of 120 ppm in textile wastewater [5]. This
liquid bio-coagulant has a storage period of 24 hours at 200°C, if it is more than that storage time, the protein contained therein will be degraded [6].

Until now there has been no research that converts liquid moringa seed extract into powder form, this requires the development of a further method capable of converting this supernatant bio-coagulant into the powder form. One method that can be used is the vacuum drying method, by varying the drying time, the best drying time will be obtained which will produce bio-coagulants in powder form which have a longer shelf life and are more practical and safe in storage. The objectives of this study were to extract proteins from moringa seeds into a bio-coagulant, determine the optimum time for drying the extract form into powder form, calculate the percentage of protein in the extract form and powder form and determine of turbidity removal efficiency using the extract form and powder form in condition low turbidity, medium turbidity, and high turbidity.

2. METHOD

This research was conducted in the Chemical Engineering laboratory of Politeknik Negeri Bandung. The raw material used is Moringa seeds from Surabaya. Bio-coagulant is obtained through several stages including grinding and sizing, oil extraction, protein extraction, centrifugation, and drying the protein.

2.1. Grinding and Sizing

Grinding and sizing were carried out on moringa seeds to obtain a pass size of 250 μm [5]. Grinding was conducted by using a grinder for dry solids, while sizing was conducted by using a vibrator screen.

2.2. Oil Extraction

Oil extraction was carried out using hexane solvent with a ratio of solids to hexane 1:5 w/v for 1 hour [2]. The oil obtained was then separated by precipitation and filtering.

2.3. Protein Extraction and Centrifugation

Globulin and albumin protein extraction was carried out using 2 M NaCl solvent. Solid I was extracted with a ratio of solid I: NaCl solution 1:20 w/v [2]. The supernatant was separated using a centrifugation process at 4000 rpm for 30 minutes.

2.4. Drying of product

At this stage, the supernatant from the centrifugation process was dried using the method vacuum drying (temperature of 40°C and pressure of 70 mbar absolute) to produce bio-coagulant in the powder form. Variation of drying time was 1, 2, 3, and 4 hours.

2.5. Protein Content Measurement

The spectrophotometric method was conducted to determine the protein contained in bio-coagulant powder products by counting the number of peptide bonds that were released due to the activity of Cu²⁺ ions in the solution. The absorbance was measured at a maximum wavelength of 540 nm.

2.6. Performance Testing of Coagulant

At this stage, the performance of bio-coagulant from moringa seed was tested by adding the coagulant to the laundry wastewater with low turbidity (25-30 NTU), medium turbidity (50-60 NTU), and high turbidity (100-120 NTU). The best dose of bio-coagulant is determined by the jar test method. Coagulation in the jar test was conducted at 120 rpm for 1 minute, while flocculation was at 40 rpm for 20 minutes.

3. RESULT AND DISCUSSION

The production of bio-coagulant powder from moringa seeds was conducted by taking the main active ingredients, namely albumin and globulin protein. The steps taken were grinding and sizing, extraction to separate the oil, then extraction to take protein, centrifugation, and vacuum drying. The mass balance of all processes of bio-coagulant powder production can be seen in Figure 1.

Figure 1. Mass balance of the process of bio-coagulant powder production
Figure 2 shows the moringa seed solid resulted from the grinding and sizing process as raw material for bio-coagulant. The purpose of this process is to expand the contact during extraction so that an extract that has a high enough protein content is produced.

Figure 2. Moringa seeds solid resulted from the grinding and sizing process

3.1. Oil Extraction Result from Moringa Seed Solid

The oil in moringa seeds has the highest solubility in n-hexane compared to other organic solvents, namely ethanol or ether with an extraction time of 30 minutes [7]. The oil content in the ingredients used is quite high, so the extraction time is longer, which is 1 hour. The presence of oil content can be seen from the change of clear colour of hexane to yellow which can be seen in Figure 3.

Figure 3. The oil extraction process in moringa seeds uses n-hexane. a) Extraction process, b) n-hexane before extraction, c) n-hexane after extraction

After extraction, the solid can settle by gravity and the solution is filtered using filter paper and a funnel manually to separate the oil slowly. The retained solid is then dried by drying in the sun because n-hexane is volatile and so that the protein in the solid is not damaged by high temperatures. From the experiment, the average extracted oil content was 22.9% separated from solids, when compared to all the oil content in moringa which is 34.6% [8]. It can be determined that the extraction efficiency is 66.19%.

3.2. Protein Extraction and Separation Process Result from Moringa Seed Solid

The protein extraction process can be seen in Figure 4. From the experiment, it was obtained that the 2 M NaCl solution gave the highest protein concentration; this can be seen from the change in the solution to dark purple when the extract was added with biuret. The protein concentration in the extract was 0.9% [9]. The results obtained by extraction using a 2 M NaCl ratio of 1:20 (w/v) for 1 hour gave dissolved protein levels of 1.09% (w/v) or the equivalent of 8.63 g of protein extracted from 50 g of solids. According to Baptista [2], protein extract from defatted seed of moringa seeds was contained of albumin and globulin at fraction range of 20-44% with the value of zeta potential of 10-26 mV.

Figure 4. The protein extraction process using 2 M NaCl solution. a) Extraction Process, b) Bio-coagulant extract

From the results of the mass balance calculation, impurity is also suspended in a solution of 12.97 g, so it is necessary to carry out a centrifugation process to separate impurity from dissolved protein. In the centrifugation process, 100 mL of supernatant was produced with a concentration of 0.68% (w/v) dissolved protein. Some of the undissolved protein will settle with the impurities, while other proteins are still carried away by the remaining solvent with a level of 0.36% (w/v). This occurred because the effective supernatant is separated at 6000 rpm for 30 minutes [7], while in the research conducted, centrifugation at 4000 rpm due to the limited capabilities of the available tools.

After being stored in the refrigerator at 4°C for a day, the protein in the supernatant will settle so that it can be filtered and dried. The remainder of the supernatant filter still has a protein content of 0.4% so that the protein in the solids is obtained about 0.3 g when dried.
3.3. Effect of Drying Time to the Bio-coagulant Protein Content

The effect of drying time to the protein content can be seen in Figure 5.

Based on Figure 5, the product/powder has the highest protein content at 3 hours drying with a level of 80.75% with a total powder of 0.3857 g. The evaporated water is 48.83% of the total wet solids. At the drying time of 1 and 2 hours, the amount of water in the material was still high, thereby reducing the percentage of protein in solids. Meanwhile, when the drying time is increased to 4 hours, the protein will degrade due to the addition of heat.

3.4. Bio-coagulant Performance Test Results

The bio-coagulant performance test was carried out by the jar test method using bio-coagulant extracts in 3 turbidity conditions, including low turbidity (32.46 NTU), medium turbidity (59.95 NTU), and high turbidity (107.8 NTU) using laundry wastewater. The dosage ranges used were 40.88; 81.75; 122.63; 163.50; 204.38 and 245.25 ppm. The best dose obtained in this performance test is used for performance tests on powdered bio-coagulants.

3.4.1. Effect of Bio-Coagulant Extract (Liquid) Dosage on Settling Velocity

The settling velocity is calculated from the ratio of the total volume of sediment formed to settling time. A heavy floc will settle more quickly than a light floc. However, under certain conditions the lighter floc will produce more sediment volume because the floc formed does not compact. The effect of bio-coagulant extract dosage on settling velocity can be seen in Figure 6.

At low turbidity conditions, the resulting sediment is light so that the volume of sediment produced is more but not dense, this affects the settling velocity, while in medium and high turbidity the resulting floc is relatively heavier. The settling velocity will increase with bio-coagulant dosage increasements. By increasing the dosage, it will increase the number of polypeptides that are able to bind the floc thus increasing the settling velocity. At low doses of bio-coagulants, a smaller number of ions are formed, resulting in a long settling velocity. The highest settling velocity was obtained at the highest bio-coagulant dosage, namely 245.25 ppm.

3.4.2. Effect of Bio-Coagulant Extract (Liquid) Dosage on Turbidity Reduction Efficiency

The effect of bio-coagulant extract dosage on turbidity reduction efficiency can be seen in Figure 7.

The decrease in turbidity is due to the presence of positive zwitterions which can produce hydrogen bonds with suspensions in water. Positive zwitterion will stabilize the suspended colloid which tends to be negatively charged so that it forms a floc and settles [10].

Based on Figure 7, at low and medium turbidity conditions, when the bio-coagulant dose is low, the floc formed is difficult to settle, causing turbidity to increase. This is due to the low suspension so that ion-bound floc floats and unable to form large and heavy flocks. At low turbidity conditions, the efficiency of turbidity reduction reaches 67.04% at a bio-coagulant dose of 245.25 ppm. In medium turbidity, the efficiency of turbidity reduction reached 54.06% at a bio-coagulant dose of 204.38 ppm. Meanwhile, at high turbidity conditions, a low bio-coagulant dose can form a heavy flock and easily settles. In high turbidity conditions, the efficiency of turbidity reduction
reached 76.15% at a bio-coagulant dose of 242.25 ppm.

3.4.3. Performance of Powdered Bio-Coagulant

Moringa seed extract works well at high turbidity (100 - 120 NTU) due to more frequent contact of the ions in the bio-coagulant with the colloid and form heavy floc thus providing high efficiency. Therefore, the performance test of powdered bio-coagulants was carried out under high turbidity conditions (107.8 NTU) and used the best dose of extract form. The powder was added to two different addition conditions, namely 247 ppm with dissolution and 255.37 ppm without dissolving before being affixed to wastewater. The comparison of the efficiency of reducing turbidity between powdered bio-coagulants and extracts can be seen in Figure 8.

![Figure 8](image_url)

**Figure 8.** Comparison of the efficiency of reducing turbidity between powdered and extracts form bio-coagulant

Based on Figure 8, the use of powdered bio-coagulant without dissolution resulted in a turbidity reduction efficiency of 30.39%, while with the dissolution resulted in a turbidity reduction efficiency of 18.11% and the use of extract form of bio-coagulant resulted in a turbidity reduction efficiency of 76.15%. The efficiency of reducing the turbidity in coagulation-floculation using powdered bio-coagulant did not provide better efficiency than when using extract form. This is due to the ability to form zwitterions of the powder is not evenly distributed compared to the extract so that the powder does not have the ability to bind the floc and decrease the sediment. Fast dissolving, causing the peptides to recombine, so that the ions formed have little contact with the waste because they have undergone binding between the peptides [10] and provide a lower efficiency of turbidity reduction.

4. CONCLUSION

The protein in moringa seeds has the potential to be used as a bio-coagulant. The best concentration of NaCl solution to extract the protein in moringa seeds is 2 M. The optimum time for drying the bio-coagulant extract into powdered bio-coagulant at a 40°C and 70 mbar is 3 hours. The bio-coagulant extract contains a protein content of 1.09% with a volume of 792 mL and the powdered bio-coagulant contains a protein content of 80.75% with a mass of 0.3857 g. The use of bio-coagulant extract resulted in an efficiency of 76.15% reduction in turbidity with a settling rate of 0.5 mL/min and the use of powdered bio-coagulant resulted in a reduction in turbidity efficiency of 30.39% with a settling rate of 0.12 mL/min at 107.8 NTU turbidity. The performance of bio-coagulant powder is still below the performance of bio-coagulant extract.

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