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Characterization of Physico-Chemical Properties of Nano-Sized Moringa oleifera Seed Powder and Its Application as Natural Coagulant in Water Purification Process

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Abstract
Several studies stated that the seed powder of *Moringa oleifera* is proven effective as natural coagulant in water purification process. Through milling process using HEM, the present study developed two size of *Moringa oleifera* filtered seed powder, i.e. 100 mesh (MoM) and nano particle (MoN), where the measurement of which was carried out using PSA. The two size seed powder was then characterized based on physical and chemical properties to application as biocoagulant in water purification process. Characterization indicated that the size of MoM and MoN was around 2300 nm and 300 nm, respectively. The major MoM compound content was 44.65% total protein, 27.05% fat, 10.86% water content, and 3.79% ash content; while for MoN was 44.41% total protein, 26.98% fat, 9.38% water content, and 3.53% ash content. Molecul weight measurement was carried out using SDS-page and the seed powder weight ranged was around 13-28 kda and 52-63 Kda. Dissolved protein content for MoM and MoN was 0.062% and 0.290%, respectively. The optimum dosage for MoN to decrease the turbidity of ground water sample and waste water was 30 ppm and 40 ppm, respectively; while for MoM was 80 ppm and 100 ppm, respectively. MoN is more effective than MoM.
Keywords: nano particle, natural coagulant, *Moringa oleifera*, water purification.

1. Introduction

The treatment of standard water into clean water requires several steps, one of which is the addition of coagulant such as Poly-Aluminum Chlorida (PAC) and sulphic aluminum (alum) to precipitate polluter. PAC and alum continuos usage in large amount is seen as potential threat for the health of processed water consumers because coagulant may still remains, although in small amount. Ecologically, the use of synthetic coagulant produces a certain amount of sludge sediment that is a pollutant for environment because the sludge is relatively difficult to degrade and can change the component of soil and water minerals from normal condition (Amagloh & Benang 2009). In addition, the use of such coagulant in continuous manner leads to dependency on the producers of synthetic coagulant.

In contrast with synthetic coagulant, biocoagulant is a natural coagulant from plants such as moringa seed (*Moringa oleifera*), tamarind seed, winged bean seed and flamboyant seed. Moringa seed powder is the most effective coagulant for water treatment compared to alum. Studies on moringa seed is developing. Dolcas Biotech LLC (2008) discussed about the uniqueness of *Moringa oleifera*. Moringa is used as water purifier (Schwarz 2000); moringa plants have various benefits (HDRA 2002); parts of moringa plants can function as medicine (HSU R *et al*. 2006); moringa is an effective natural coagulant and equal to alum and PAC (Amagloh & Benang 2009); moringa seed can be used as natural coagulant in water treatment process (Ali *et al.* 2009). Other researchers stated that moringa seed is not toxic (Grabow *et al*. 1985) so that it is recomended to be used as biocoagulant in developing countries. Various studies conducted stated that moringa seed is an effective biocoagulant to improve physical-chemical properties of polluted water (Esti & Haryanto 2000); (Dewi Dwirianti 2004). *Moringa oleifera* functions as coagulant trough adsorption and neutralization mechanisms (Bhatia *et al*. 2007). *Moringa oleifera* is potential as organic pollutant absorben in simulation solution (Akhtar *et al*. 2007). *Moringa oleifera* is reported can eliminate the turbidity and dissolved organic matters of river water (Sánchez-martín *et al.* 2010). Saulawa *et al*. (2011) conducted moringa seed extract preservation using trona solution. Damayanti *et al*. (2011) made membrane consists of *Moringa oleifera*, PAC and zeolite for palm oil effluent tretment.

Affecting factor for the effectivity of moringa seed powder as biocoagulant is the active compound of the powder. When used as biocoagulant, the active compounds are dissolved in water and eventually react to the polluters within. The size of moringa seed powder is, surely, affect the amount of dissolved active compound. Smaller size moringa seed powder has larger amount of dissolved active compunds in water. In this study, various sized moringa seed powder were used to determine the effect of optimum particle size. Nano sized particles were obtained from crud sized powder. *Moringa oleifera* crude powder that pass through 100 mesh filter was labeled as MoM, while the nano sized one was MoN. It is expected that small sized powder developed using nano technology will be more efficient and remain effective as coagulant. Prior to application as biocoagulant in water purification process, moringa seed powder needs to be characterized to determine the different properties of MoM and MoN.

2. Study Method

2.1 Material and Tools

Materials used were moringa seeds (*Moringa oleifera*) and waste water and soil water. All of the materials were obtained from Tangerang, Banten, Indonesia. Tools used were Philips HR1757 blender, filter 100 mesh in size, pH meter (Myron L ARH1), digital thermometer, portable conductymeter (Myron L ARH1), portable turbidity meter (HANNA Instrument), High Electro Milling (HEM) PW 7001, Scaning Electron Microscope (SEM), X-Ray Diffraction (XRD), X-Ray Fluorosence (XRF), PSA (Particle Size Analyzer).

2.2 Procedure

2.2.1 Biocoagulant Preparation

Biocoagulant was prepared in several steps, i.e. harvesting, making of moringa seed powder in various sizes using blender and HEM, followed by analysis of particle size using Psa, powder profile using SEM and mineral content using XRF.

Mature moringa fruits, indicated with blackish and dry fruit skin, were harvested and the seeds of which were obtained. Dry and high quality seeds were selected prior to peeling to obtain the content of moringa seed, i.e. white yellowish granules. The contents were then mashed using blender to obtain the powder of moringa seeds.

Moringa seeds were mashed using blender to obtain moringa powder prior to filtered using filter 100 mesh in size. The outcome was then labelled as MoM sample (*Moringa oleifera* Mesh partikel). A certain amount of the powder was then milled using HEM to obtain nanometer sized powder and the outcome was labeled as MoN (*Moringa oleifera* Nano partikel). Milling using HEM was conducted for 4x30 minutes at 1000 rpm.

2.2.2 Characterization

The characteristics of moringa seed powder was examined, among others particle size using Psa, the major component of moringa seed powder using a series of Proximat Analysis consisted of total protein content (Kjeldahl method AOAC, 1990), fat content using soxhlet extraction method, and water and ash contents following AOAC 1984 method.

2.2.3 Application

The application of moringa seed as coagulant to improve water sample quality was tested in laboratory scale using jar test method. Moringa powder was weighed according to already determined dosages and put into beaker glass with 1000 mL water samples (waste and soil water). Solution consisted of water sample and coagulant was mixed and rapidly stirred (100 rpm) for 1 minute prior to stirring process in moderate speed (50 rpm) for 15 minutes and slow speed (30 rpm) for 15 minutes to help floc formation. The suspension was allowed for 1 without any disturbance. The supernatant of which was then obtained for parameter examination purpose. After the parameters examined, the percentage of the change was calculated as follow.

$$\%\ change = \frac{Initial\ value - Final\ value}{Initial\ value} \times 100\%$$

3. Result and Discussion

3.1 The Physical Characteristics of Moringa Seed Powder

Seeds of *Moringa oleifera* were mashed using two different way, i.e. blended using blender and milled using HEM. The particle size of the output was then measured using PSa. The result of dry blending and the substances succed passing through the 100 mesh filter that has powder size of 2361.4 nm or around 2300 nm is labelled as MoM; while the result of milling using HEM was 336.5 or around 300 nm in size and labelled as MoN. See Table 1 for the physical appearance of moringa powder, particle size and polydispersity index (PI) measured using PSA.

Table 1. The particle size of moringa seed powder

Moringa Sample	Physical Appearance	Polydispersity Index (PI)	The Average of Particle Size (nm)
MoM	Powder	0.167	2,361.4
MoN	Powder	0.964	336.5

Physical appearance of MoM is powder with PI value of 0.167. such small value (<1) indicates that MoM particles are uniform in size. On the other hand, MoN physical appearance is also powder with particle size of 336.5 nm and PI value of 0.964. The particle size of MoN is already considered small and PI value <1 indicates that MoN particles are already uniform in size. However, the PI value of MoN is greater than MoM, meaning that MoN particles are less uniform than MoM. This is due to the milling process of moringa seed powder using HEM for 4x30 minutes caused the seeds already produced oil, making the physical appearance of which slightly clumpy.

MoM powder size is too big compared to MoN powder and cannot be chategorized as nano size, while MoN already belongs to nano sized particle. This is in line with definition by Buzea (2007) which stated that the size of nano particle is 1 to 1000 nanometer.

The particle profile of moringa seed powder was examined using Scanning Electron Microscope (SEM). See Figure 1 for the SEM image of moringa seed powder. SEM result showed that the particle size was not highly uniform where some was big and others were already small sized. In addition, the profile of the powder is hollowed granule. XRD test result indicates that moringa seed powder is amorphous, not crystal.

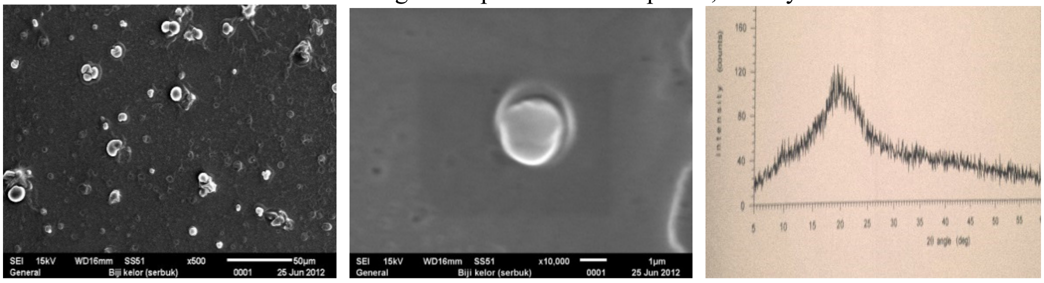

Figure 1. The image of Scanning Electron Microscope (SEM) and X-Ray Diffraction (XRD) Result of moringa seed powder

In conclusion, the physical properties of moringa seed powder morphologically are hollowed granule and amorphous. Such physical properties cause the powder become pasta if added with certain amount of water. Other study about SEM result of moringa seed powder indicates that the powder tends to form agglomerate morphologically (Kumari *et al*. 2006).

3.2 Major Composing Compound of Moringa Seed Powder

In this study, moringa seed powder was differed by particle size, i.e. MoM and MoN. The followings are the test result of the major component of moringa seed powder.

3.2.1 Main Component of Moringa Seed Powder

The main component of MoM moringa seed powder is 44.65% protein, 27.05% oil/fat, 10.86% water content, 3.79% ash content, and 13.65% others; while MoN is 44.41% protein, 26.98% oil/fat, 9.38% water content, 3.53% ash content, and 15.70% others (Table 2). There is no significant difference in the main component of MoM and MoN.

Table 2. The result of proximat analysis on moringa seed powder

Parameter	MoM	MoN
Protein Content (%)	44.65 ± 3.73	44.41 ± 1.29
Fat Content (%)	27.50 ± 0.00	26.98 ± 0.03
Water Content (%)	10.86 ± 0.09	9.38 ± 0.17
Ash Content (%)	3.79 ± 0.01	3.53 ± 0.05
Others (%)	13.65	15.70

Total protein as the greatest component of MoM and MoN moringa seed powder was 44.65% and 44.41%, respectively. This in line with a study conducted by Ruttarattanamongkol et al. (2014) who stated that protein in the major component that makes up moringa seed powder. The powder can be used as natural coagulant because of its high cationic protein content. Protein in moringa seed can be used as an effective coagulant and floculant to improve water qaulity (Hellsing *et al*. 2013).

The total protein content of MoM and MoN was of relatively the same figure. This indicates that different sized powder has the same major component.

Moringa seed powder can be used as natural coagulant because it contains high dissolved protein content in water. The protein can bind colloidal polluter in turbid/dirty water. The high protein content makes moringa seed powder potential as natural coagulant in the purification process of dirty water (Folkard *et al*. 1993).

3.2.2 Dissolved Protein Content of Moringa Seed Powder

A certain amount of MoM and MoN was dissolved in distilled water and filtered prior to measurement of the dissolved protein content using Lowry method. See Figure 2 for the result of the measurement. MoM protein content in water was lower than MoN, i.e. 0.062% and 0.29%, respectively.

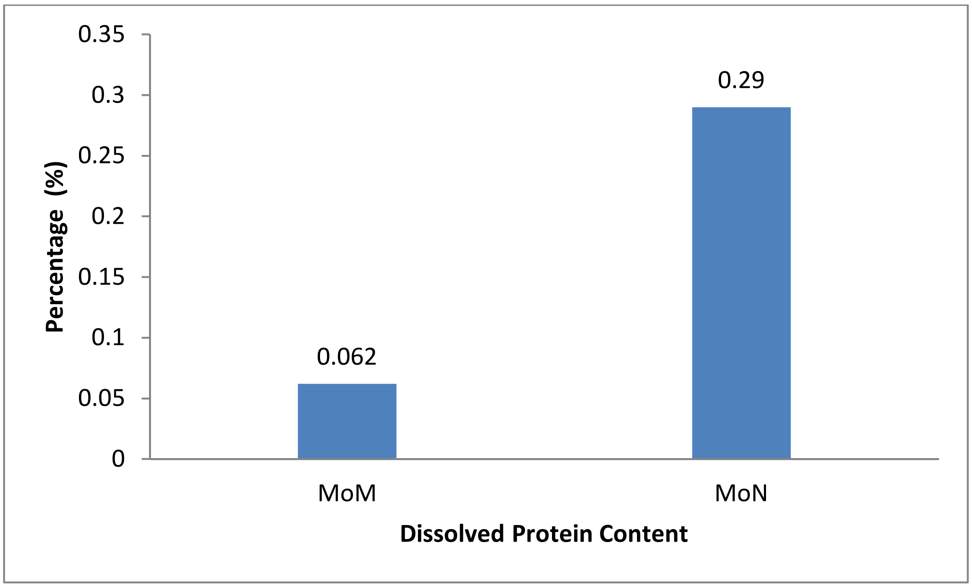

Figure 2. Dissolved protein content of MoM and MoN moringa seed powder

The difference in particle size brought about significant effect on the dissolved protein content of moringa seed powder, or in another word, making moringa seed powder smallerin size up to nano particle size resulted in the dissolved protein content increased up to 4.7 times. Therefore, because MoN powder is smaller in size, the dissolved protein content of which was higher. In contrast, MoM powder is bigger in size, so that the dissolved protein of which was relatively lower. Small sized powder of MoN has wider surface area for reaction. Powder in small size allows higher chance for optimum reaction between composing substance and its solvent so that the dissolved protein content shows greater figure when being measured. This is in line with the theory of chemical reaction rate which states that surface area affects chemical reaction rate. Sharma *et al*. (2006) conducted test to determine the effect of different particle size on the effectivity of moringa seed powder as coagulant. The test indicates that small sized powder (105 micron) is more effective than 210 and 420 micron sized powder.

3.2.3 The Molecular Weight of Protein in Moringa

Protein is the major substance of moringa seed powder. Total protein content of MoM and MoN was 44.65% and 44.41%, respectively. Based on SDS-Page analysis test (Figure 3), protein molecular weight in MoM and MoN was around 13-28 kda and 52-63 Kda. There is no significant difference between the protein molecular weight of MoM and MoN. The difference in the particle size of MoM and MoN powder leads no difference in the major substance of the powder.

There is no difference in protein molecular weight of MoM and MoN moringa seed powder. This indicates that grinding process using HEM for 4x30 causes no change in the protein type of moringa seed powder. Protein molecular weight in this study is similar with other study, i.e. 10 kDa and 17-26 kDa (B. Gracia-Fayos *et al.* 2010). However, other study by Ghebremicael *at al.* (2005) stated that protein in moringa is of low molecular weight, i.e. 6.5 kDa.

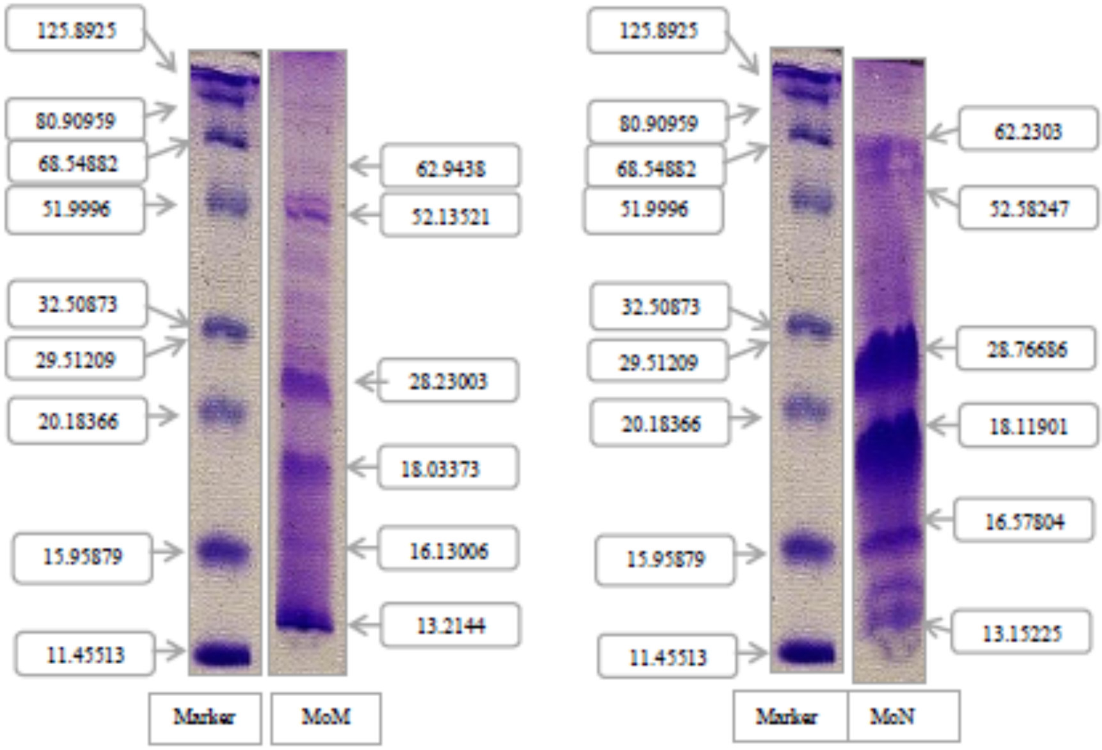

Figure 3.The profile of protein molecular weight (kilodaltont) shown by the gel image result of SDS-Page process

3.2.4 Major Mineral Compound of Moringa Seed

Based on analysis for measuring protein molecular weight and determining the mineral level of moringa seed powder, it is concluded that the different particle size of MoM and MoN causes no difference in the composition of their substance. It, however, causes difference in the content level of the substance.

Table 3. Mineral compound in moringa seed powder

No	Formula	Concentration in MoM (%)	Concentration in MoM (%)
1	Na_2O	40.66	43.10
2	SO_3	30.46	20.84
3	MgO	8.69	10.26
4	K_2O	7.39	10.08
5	P_2O_5	7.06	5.43
6	CaO	1.46	3.03
7	SiO_2	1.22	2.36
8	Al_2O_3	1.27	1.61
9	Cl	0.83	1.12
10	La_2O_3	0.24	0.71
11	Fe_2O_3	0.40	0.43

Mineral contents of MoM and MoN tested using XRF were Na_2O, SO_3, MgO, K_2O, P_2O_5, CaO, SiO_2, Al_2O_3, Cl, La_2O_3, and Fe_2O_3 (Table 3). There was no difference between the compound of MoM and MoN. All the major compounds were found in MoM and MoN moringa seed powder; the only difference noticed was the compund level. Na_2O level in MoM was 40.66%, while in MoN 43.10%. SO_3 level in MoM was 30.46%, while in MoN 20.84%. Because all the compounds can be found in MoM and MoN, it indicates that there is no different compound between MoM and MoN. The different size of powder, i.e. 2,361.4 nm and 336.5 nm, leads to no difference in the major compound of the powder.

3.3 Application of Moringa Seed Powder as Biocoagulant

The application of moringa seed powder as biocoagulant was tested using Jar-test series against waste water (high turbidity) and soil water (low turbidity). Parameters measured were turbidity, temperature, pH and conductivity. See Table 4 for the determination data of the optimum dosage of MoM moringa seed powder in wastewater and ground water.

Table 4. The determination of the optimum dosage of MoM moringa seed powder for waste and sground water

MoM Concentration (mg/L)	Wastewater				Ground Water			
	Turbidity Decrease (%)	Conductivity (µS/cm)	pH	Temperature (°C)	Turbidity Decrease (%)	Conductivity (µS/cm)	pH	Temperature (°C)
40	90.2	1104.7	4.85	28.3	91.3	225	7.25	28.5
80	97.9	1052.5	5.67	28.7	97.5	219	7.38	28.9
100	98.6	1004.65	6.20	28.8	97.4	216.35	7.39	29
120	98.1	1005.7	6.28	29.1	96.6	223	7.39	28.7
140	97	1109.7	6.34	28.3	96.3	228.33	7.41	28.5

In this study, the water treatment used *Moringa oleifera* seed powder as coagulant and the optimum dosage required by waste water and ground water was different. For MoM the dosage of 100 mg/L brought about the best results in wastewater, shown by 98.6% turbidity decrease, pH value near normal and the conductivity value of which was the lowest (Table 4). For ground water, the highest decrease turbidity belonged to coagulant dosage of 80 mg/L.

The optimum dosage in this study was lower than of other study by Katayon *et al.* (2006) who stated the optimum dosage of 160 mg/L is for water with medium turbidity value and Nishi *et al.* (2012) reported the optimum dosage of 175 mg/L is for water with 350 NTU turbidity. Water with initial turbidity higher than 150 NTU may use dosage of 50-200 mg/L, while dosage of 10-50 mg/L is for initial turbidity less than 50 NTU (Schwarz 2000). This indicates that the optimum dosage of *Moringa oleifera* coagulant is influenced by the level of initial turbidity of water samples that will be processed with *Moringa oleifera* coagulant.

For MoN, dosage of 40 mg/L brought about the best result in waste water, seen from the decrease of the highest turbidity level, pH value near normal, and the lowest conductivity value (Table 5). In ground water, such the optimum values were obtained at coagulant dosage of 30 mg/L.

See Table 4 and 5 for the effect of MoM and MoN coagulant on the other water quality parameters, i.e. temperature. The addition of MoM and MoN as a coagulant in wastewater treatment processes did not affect the temperature significantly. The initial temperature of the samples was 28.3 °C and the highest temperature after the addition of coagulant was 29,1 °C. The use of a coagulant in water treatment process did not change the temperature drastically. The temperature of each sample was still in the range of normal temperature for water because the change was still lower than ± 3°C.

The addition of MoM and MoN decreased conductivity value. High conductivity value is determined by the presence of dissolved mineral ions and non-organic compounds. The addition of coagulant led parts of such ions and compounds be dispersed into floc that eventually precipitated and discarded from the solution.

Table 5. The determination of the optimum dosage of MoN moringa seed powder for waste and ground water

MoM Concentration (mg/L)	Wastewater				Ground Water			
	Turbidity Decrease (%)	Conductivity (µS/cm)	pH	Temperature (°C)	Turbidity Decrease (%)	Conductivity (µS/cm)	pH	Temperature (°C)
20	90.6	1109.7	4.83	29.1	92.8	225.33	6.70	28.7
30	96.4	1102.7	5.10	28.6	98.6	221.33	7.41	28.9
40	98.3	1005.7	6.18	29.1	98.3	223	7.39	28.7
60	98.1	1109.7	6.14	28.3	97.3	228.33	7.41	28.5
80	97.4	1136	6.20	28.6	96	227	7.42	28.9

See Table 4 and 5 for the effect of MoM and MoN coagulant on water sample pH value. In general, pH value increased where ground and waste water changed from acidic to neutral. This is because polluters in water sample were already precipitated and the water become purer and the pH of which changed to neutral in line with clean water natural pH. Although without any reagent addition, the use of moringa seed coagulant is capable of improve the pH value of water sample. Unlike alum where coagulant activity is highly influenced by the natural alcalinity of water to be coagulated so that other addition material is required such as lime to increase the alcalinity or pH of the water. It results in the precipitation residual sludge of which as the side outcome is big in volume, more acidic and has residual synthetic compounds that are difficult to degrade. On the other hand, sludge produced by moringa seed coagulant is more neutral and smaller in volume, in addition to biodegradable organic materials.

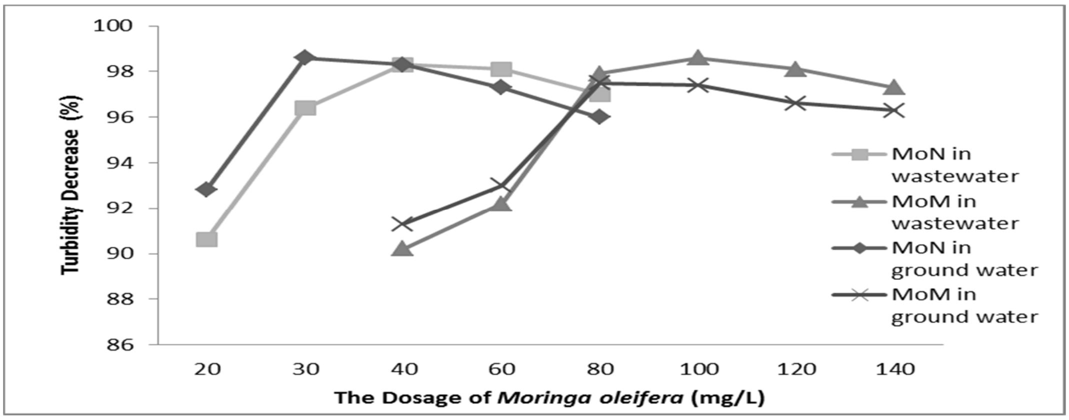

Figure 4. The comparison of MoN and MoM optimum dosage in wastewater and ground water

See Figure 4 for the comparison of MoM and MoN optimum dosage in ground water and wastewater. MoN was capable to decrease turbidity optimally at 30-40 mg/L dosage, while MoM was 80-100 mg/L. Concentrations higher than the optimum dosage lead to the lower percentage of turbidity decrease, meaning that turbidity increases. This was because all colloidal polluters in water were neutralized and precipitated at optimum dosage. Therefore, excessive coagulant leads to turbidity because it does not interact with other colloidal

MoN is more effective in reducing the turbidity of waste water and ground water compared to MoM. This is because MoN has smaller powder size than MoM so that when being dissolved, active substances in MoN were more soluble than MoM. It is shown by the data of soluble protein content (Lowry method) in MoN that is higher than MoM. MoN protein content was 0.29%, while MoM 0.06175%.

The effectivity of moringa seed is determined by protein content of which. Protein in moringa seed powder is cationic protein that reacts with colloid polluter with negative charge in waste water. Sahni and Srivastava (2008), who conducted electrophoresis examination on protein in *Moringa oleifera* stated that the protein content of which is 79.3% cationic and 20.7% anionic. Solution from moringa seed powder is known as cationic polyelectrolite (Broin 2002). The voltage value of 5% moringa seed without seed was around +6 mV (positive). This indicates that the solution is dominated with positive voltage although the solution is of a complex heterogenous mixture. The zeta potential of waste water was around -46 mV (negative). This resulted in the coagulation between suspended particle and moringa seed is influenced with destabilization process of coloid negative voltage by cationic polyelectrolite (Broin 2002).

The most possible mechanism in coagulation process is adsorption and voltage neutralization or adsorption and unstable bonds among particles. Of the two mechanisms, determining which one is occurring is really difficult because the two mechanisms may simultaneously occur. However, coagulation mechanism with moringa seed is commonly adsorption and coltage neutralization (Sutherland *et al.* 1994).

The presence of polluter in water is because of suspended solid substance, both organic and non-organic. Non-organic substances are commonly the outcome of decayed rocks, sands, sludges, and dissolved metals. Non-organic substances are from domestic and industrial effluent and can become the source of food and reproduction for bacteria. In addition, microbes, algae, and planktons can make water turbid. After coagulant addition into sample followed with rapid stirring, cationic protein produced by *Moringa oleifera* was distributed to the solution thoroughly prior to interaction with negative charged-particles the cause of dispersed turbidity. Such interaction affected force and disrupt particle stability so that the particle can bond with small particles and form sediment. The process is known as coagulation. Therefore, *Moringa oleifera* can be called as coagulant. Because the coagulant is from plant, it is also called natural coagulant or biocoagulant. The size of powder affects the effectivity of moringa seed as biocoagulant. Moringa seed powder in nano sized particle (MoN) is more effective than MoM.

4. Conclusion

The major compound composition in moringa seed powder biocoagulant is protein as the largest component. There is no difference in the compound of MoM and MoN, where the only difference seen is the content level of the compounds. Moringa (*Moringa oleifera*) seed powder as biocoagulant is proven can be applied to improve the quality of wastewater and groundl water. MoM and MoN seed powders are capable of improving water quality. MoN was capable to decrease turbidity optimally at lower dosage than MoM. Therefore *Moringa oleifera Nano size particle* (MoN) is more effective than *Moringa oleifera Mesh size particle* (MoM) to improve standard water quality by turbidity parameter.

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