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COMBINATION OF PHYTOCOAGULANT MORINGA OLEIFERA SEEDS AND CONSTRUCTED WETLAND FOR COFFEE PROCESSING WASTEWATER TREATMENT

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Abstract

Wastewater on coffee processing contains high BOD, COD, TSS, NH_4^+-N, and TP but low pH. Overall, these effluents did not meet the standard for discharged effluent in accordance with the East Java Province Regulation No. 72 of 2013. This research aims to determine the effectivity of the combination between *Moringa oleifera* seeds as a natural coagulant in flocculation coagulation unit and vertical subsurface flow constructed wetland to treat the wastewater on coffee processing. Adding *Moringa oleifera* seeds powder of 100 mg/L on vertical subsurface flow constructed wetland with retention time for 8 days was proven effectively to produce pH parameter content close to neutral or 6.65 ± 0.04, reduce TSS to 99.63 ± 0.10%, COD to 98.06 ± 0.04%, and BOD to 97.67 ± 0.24%. Thus, it would be safely discharged off in the waterbody. However, for NH_4^+-N and TP contents, the discharged effluents still have not met the standard set as both increased up to 8.16 ± 0.02% for NH_4^+-N and 11.47 ± 0.11% for TP with the retention time of 8 days. Further researches are necessary to be conducted in order to reduce both NH_4^+-N and TP contents on the wastewater on coffee processing.

Keywords: Coagulant, Coffee processing, *Moringa oleifera* seeds, Subsurface flow constructed wetland, Wastewater.

1. Introduction

The industry has an important role in Indonesian economy. One of the industries that significantly contribute to economic development and the expansion of employment opportunities in the coffee processing industry. The majority of the coffee processing industries are small and medium enterprises (SMEs). These industries employ less than 250 employees and have limited capital, knowledge, as well as equipment [1, 2]. Those limitations cause them unable to process their wastewater before discharging into the waterbody.

Wastewater on coffee processing (WWCP) is one of the biggest problems faced by many coffee industries due to the amount of water used in the production process. The main pollutants are from organic wastes during the pulping process when the mesocarps are peeled and the mucus covering parchment is partially removed [3]. For each kilogram, coffee beans generate 40-45 litre wastewater [4, 5]. In general, coffee manufactures directly discharge wastewater without any prior treatment. This wastewater contains high organic contents such as pectin, protein and sugar, nutrients, and dissolved organic with low pH as well as has brownish colour [6-8], which may cause serious health issues such as eyes problems, skin irritation, as well as indigestion and respiratory disorders [7, 9].

Many prior researches were conducted by implementing various technologies to treat WWCP, such as by using up-flow anaerobic sludge blanket [10], anaerobic digestion [11], advanced oxidation process [12], expended granule sludge blanket [7], and up-flow anaerobic hybrid reactor [13]. However, those technologies were inconvenient to be implemented in SMEs, because they prefer a simple, cost-effective and safe technology [9]. Constructed wetland is a system designed by employing natural process using wetland vegetation, soils, and macrobiotics in wastewater treatment [14-16] or it can be concluded that this system is based on biological processes [17, 18]. The constructed wetland has high potential to be implemented in the SMEs due to its simple construction resulting in low cost in operation and maintenance as well as eco-friendly [19-21]. However, constructed wetland had several inefficiencies, such as clogging in the inlet due to the solid entrapment and sedimentation, biofilm growth, plant decay products, granular medium properties and chemical precipitation [22]. To obtain high efficiency, the constructed wetland is possibly combined with physical, chemical, and other biological processes as a pre-treatment unit.

Pre-treatment technology is needed to reduce the pollution load [16, 23, 24]. One of the pre-treatment technologies possibly implemented is flocculation coagulation. Most chemical coagulants are used to form agglomeration with suspended solids to sediment [22, 25]. Generally, coagulants used are iron salts, polymer, and lime [9, 22]. Yet, these coagulants have weaknesses, for examples: expensive, rare, and dangerous due to toxic contained [26]. Therefore, an alternative coagulant is needed in the coagulation unit as pre-treatment of constructed wetland to process WWCP. Using natural coagulants is believed to be more environmentally friendly and more cost-effective [25]. Natural coagulant with the potential to be developed is *Moringa oleifera seeds*. *Moringa oleifera seeds* are produced from the *Moringa oleifera* tree is indigenous vegetation in Indonesia, which is easily found and abundantly available. *Moringa oleifera* seeds has anti-bacterial effect [27-29], antioxidant [30, 31], anti-inflammatory [32-35], immune booster [36, 37], and nutrition source [36, 38]. In addition, many researchers found that *Moringa* *oleifera* seeds powder has coagulating properties used in various water treatment such as turbidity, total alkalinity, dissolved solids and hardness [39-43]. Moreover, *Moringa oleifera* seeds have additional benefits such as widely available and easily stored, not requiring special care, environmentally friendly and eliminate microorganisms [39, 42, 44, 45]. In wastewater treatment at Baluk river, adding 3% of *Moringa oleifera* seed in the coagulation process successfully reduced several parameters such as turbidity from 67.25 ± 0.07 NTU to 5.07 ± 0.04 NTU (with 85-94% efficiency), Fe from 1.414 ± 0.07 mg/L to undetectable (with 100% efficiency), Cu from 1.401 ± 1.28 mg/L to 0.028 ± 0.06 mg/L and Cd from 1.887 ± 1.04 mg/L to 0.036 ± 0.012 mg/L (with 96-98% efficiency), Furthermore for pH, it increased the alkalinity from 5.48 ± 0.01 to 7.15 ± 0.01 or in reaching the standard neutral range [39].

In another treatment, by combining sediment method and two-step clarifier tanks using 150 mg/L *Moringa oleifera* seed to treat tapioca industrial wastewater, they successfully increased pH from 6.7 to 7 and BOD from 1.702 mg/L to 913.05 mg/L or had 46.35% removal efficiency reduced COD from 63.14 mg/L to 51.47 mg/L or had 34.32% removal efficiency and TSS from 206.6 mg/L to 90.20 mg/L or had 56.34% removal efficiency [9]. In addition to BOD, COD, TSS, and metal, 750 mg/L *Moringa oleifera* seeds were able to reduce the blue dye to 95%, black dye to 85%, and orange dye to 80% in the textile industrial wastewater [46].

Another research in textile industrial wastewater treatment showed that adding *Moringa oleifera* seeds was also able to reduce the alkalinity from 700 mg/L to 600 mg/L, eliminate hardness from 460 mg/L to 50 mg/L [47], and reduce coliform bacteria up to 77% [48, 49]. In conclusion, the study proves that *Moringa oleifera* seeds are very effective in reducing TSS, metals, alkalinity, hardness and coliform bacteria and increasing pH but are not effective in reducing BOD and COD contents. However, the effectiveness of using *Moringa oleifera* seeds for industrial wastewater treatment still requires additional and further research because various types of industrial wastewater are very likely to provide different efficiency. A processing combination may be needed to optimize the decrease in BOD and COD removal efficiency. The combination of flocculation coagulation *Moringa oleifera* seeds as natural coagulant with constructed wetlands built definitely requires further research. This study aims to determine the effectiveness of the combination of flocculation coagulation processing with *Moringa oleifera* seeds as natural coagulants and constructed wetlands to treat the WWCP so that it is safely discharged into the waterbody.

2. Material and methods

2.1. Raw wastewater

WWCP was collected from small-scale coffee industry in Pasuruan, East Java, Indonesia and stored at approximately 4 ºC.

2.2. Coagulant

Moringa oleifera seeds used were from Sumenep, Madura, East Java. The *Moringa oleifera* fruits were peeled to get the seeds, then dried. Dried kernels were grinded into powder using mortar and laboratory pestle, which should be filtered using 50-mesh sieve. The *Moringa oleifera* seeds powder was dissolved in distilled water to make 50 g/L of solution, then stirred for 30 min using a magnetic stirrer, and filtered using Whatman no filter. 40 to separate the seed particles from the solution [40, 48]. This solution was then stored in the refrigerator with a temperature of 20 ºC to prevent pH changes and a viscosity [45].

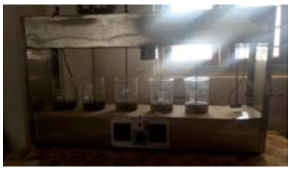
2.3. Coagulant experiment

The test was performed using a six-jar test. Prior to the experiment, temperature and pH on WWCP were measured using digital pH-meters. In each test tube, 1 L WWCP was given *Moringa oleifera* seeds powder. Coagulant *Moringa oleifera* seeds powder was added at the beginning of the mixing step. The flocculation coagulation experiments were performed with an initial 1-minute rotation at 300 rpm, followed by 5-minutes rotation at 150 rpm and then a 15-minutes rotation at 80 rpm [22]. The sample was then preserved for 2 hours for the sedimentation process, after which, the 50 mL sample was withdrawn from 5 cm below the liquid surface to measure pH, BOD, COD, TSS, NH_4^+-N and TP contents.

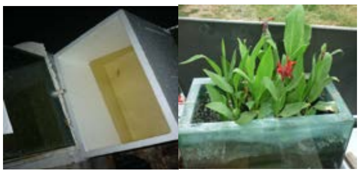
2.4. Constructed wetland, media, and plant

Constructed wetland used was vertical subsurface flow constructed wetland (VSSFCW). The reactor was designed using glass box with rubber sealer sized 1 × 1.5 m^2. It was filled with coarse gravel (3-6 cm) to a depth of 15 cm, topped with 45 cm of small gravel (1 cm) and was planted with *Canna indica* was planted at 25 cm intervals. For plants acclimatization, wastewater was diluted to gradually increase its concentration from 30% to 65% and then 100% within 4 weeks. The influent and the effluent were collected and analysed in 5 days, 8 days, and 15 days to measure pH, BOD, COD, TSS, NH_4^+-N and TP contents. The schematic diagram of this research can be seen in Fig. 1.

(a) *Moringa oleifera* seeds cake.



(b) Flocculation coagulation *Moringa oleifera* seeds powder on wastewater coffee processing (WWCP).



(c) Vertical subsurface flow constructed wetland (VSSFCW).

(d) Raw WWCP, WWCP after flocculation coagulation process and WWCP in the effluent of VSSFCW.

Fig. 1. Experimental diagram.

2.5. Experimental setup

WWCP was first set up using an equalization tank and inserted into a tube added with *Moringa oleifera* seeds powder. Instant mixing using a jar test was done and followed by further mixing, then it was sedimented in a high-efficiency flocculation coagulation reactor. The supernatant of the flocculation coagulation reactor was discharged to a constructed wetland for post-treatment with varying loading levels and fed intermittently. Every day, pilot experiments were operated continuously.

2.6. Analytical method

BOD was measured on 5 days of BOD test where the sample was incubated at 20 ºC and the oxygen consumed was measured using the Wrinkler method [50]. COD was determined by the dichromate closed reflux and measured using a spectrophotometer. TSS was measured by a gravimetric where the residue dried into a constant weight for at least 1 h at 103 ºC to 105 ºC. pH was measured by pH meter. NH_4^+-N was analysed using a spectrophotometer and TP was analysed using colourimetric methods [49]. Measurement was performed on the influent before coagulant was added to the flocculation coagulation process and effluent after constructed wetland. Table 1 shows the influent analysis of WWCP.

Table 1. WWCP characteristic compared with standard effluent set by government regulation.

Parameter	Unit	Raw water (WWCP)	Discharge standards (East Java Province Regulation No. 72/2013)
pH	-	4.32 ± 0.4	6-8
BOD	mg/L	1,229 ± 2.12	50
COD	mg/L	1,728 ± 1.61	50
TSS	mg/L	2,063 ± 0.00	30
NH_4^+-N	mg/L	5.87 ± 0.04	-
TP	mg/L	4.32 ± 0.02	-

2.7. Statistical analysis

The data were tested using an experimental design with SPSS 18 software. The dependent variables (pH, BOD, COD, TSS, NH_4^+-N and TP) were analysed in advanced for their homogeneity using Lavene Test. Variables considered homogeneous if they had significance value more than 0.05. Furthermore, the independent variables (*Moringa oleifera* powder seeds dose and retention time to the dependent variables both independently and together) were tested. The results were then further proved using the Pillai's Trace test results, Wilks Lambda, Hotelling Trace and Roy's Largest Root in the multivariate test. The significant influence of independent variables on the dependent variables as indicated by a significant value < 0.05.

3. Results and Discussion

Table 1 shows that initial pH, BOD, COD, TSS, NH_4^+-N and TP in WWCP were higher than the standard set by the government of East Java (East Java Province Regulation No.72 of 2013). If this WWCP continues to be discharged into the water body without going any prior treatment, it will undeniably pollute the environment. This research was conducted with an experimental test using a combination of flocculation coagulation with VSSFCW to treat WWCP. The innovation was by using *Moringa oleifera* seeds powder as a natural coagulant in the flocculation coagulation unit as a substitute for chemicals that have been normally used.

Flocculation coagulation as a pre-treatment unit in a combination of flocculation coagulation treatment with VSSFCW by adding *Moringa oleifera* seeds powder varied on the dose of 50 mg/L, 100 mg/L and 150 mg/L to be flown to VSSFCW. These doses were measure for its effects on pH, BOD, COD, TSS, NH_4^+-N and TP. Table 2 shows the test results of Pillai's Trace, Wilks Lambda, Hotelling's Trace, and Roy's Largest Root on *Moringa oleifera* seeds powder dose has a significance of 0.000 or < 0.05 and can be seen in Table 2 column 6. It indicated that additional doses of *Moringa oleifera* seeds powder on 50 mg/L, 100 mg/L, and 150 mg/L significantly influenced to pH, BOD, COD, TSS, NH_4^+-N and TP. The effect of the dose of *Moringa oleifera* seeds powder doses on each parameter (pH, BOD, COD, TSS, NH_4^+-N and TP) will be described further.

As seen in Table 2, adding *Moringa oleifera* seeds powder dose affected the pH parameter, proved by the pH increase after adding the dose of 50 mg/L, 100 mg/L, and 150 mg/L) (see Fig. 2). On 50 mg/L pH increased from 4.32 ± 0.4 to 6.3 ± 0.14, on 100 mg/L pH increased to 6.65 ± 0.04, and increased to 7.2 ± 0.13 on 150 mg/L.

Based on Table 1 the pH value as determined by government regulations is in the range of 6.5 to 8.5 [47]. The combination of flocculation coagulation and VSSFCW treatment with adding the doses of *Moringa oleifera* seeds powder on 50 mg/L, 100 mg/L and 150 mg/L to make WWCP successfully met the effluent standards set by the government.

Table 2. Results of multivariate test on *Moringa oleifera* seeds powder doses, retention time, and interaction between *Moringa oleifera* seeds powder doses and retention time to parameters.

Effect (1)		Value (2)	F (3)	Hypothesis df (4)	Error df (5)	Significance (6)
M.O Dose	Pillai's Trace	1.940	173.833	12.000	64.000	.000
	Wilks' Lambda	.000	775.490[a]	12.000	62.000	.000
	Hotelling's Trace	1357.16	3392.91	12.000	60.000	.000
	Roy's Largest Root	1341.15	7152.82	6.000	32.000	.000
Retention time	Pillai's Trace	1.995	2309.657	12.000	64.000	.000
	Wilks' Lambda	.000	5873.166	12.000	62.000	.000
	Hotelling's Trace	5962.403	14906.007	12.000	60.000	.000
	Roy's Largest Root	5737.842	30601.826	6.000	32.000	.000
Retention time* M.O dose	Pillai's Trace	3.341	28.734	24.000	136.000	.000
	Wilks' Lambda	.000	152.704	24.000	109.356	.000
	Hotelling's Trace	798.887	981.965	24.000	118.000	.000
	Roy's Largest Root	773.862	4385.217	6.000	34.000	.000

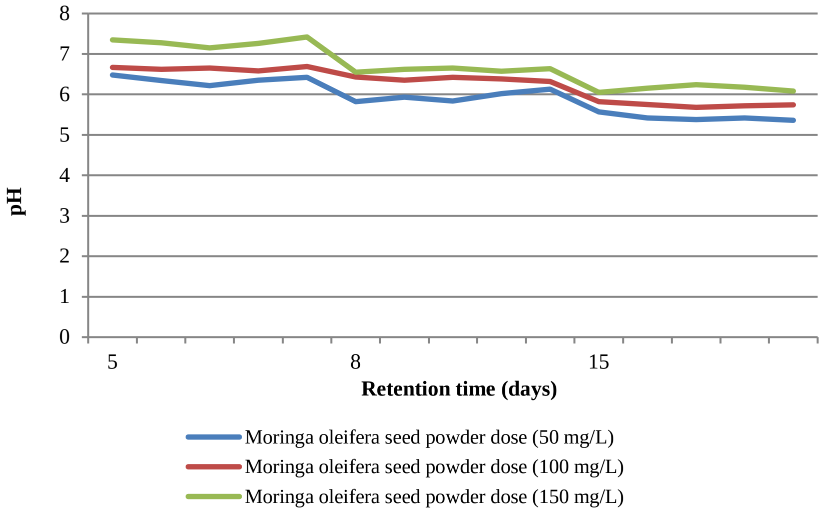


Fig. 2. pH to coagulant dose and retention time.

This result was different with research conducted by Shan et al. [39], Arnoldson and Bergman [40], Amagloh and Benang [51] mentioned that adding *Moringa oleifera* seeds did not significantly change pH. However, this result in line with previous research [45], which found that adding *Moringa oleifera* seeds on the wastewater of textile manufacture increased pH from 4 to 5-6.5. The increase in pH parameters in both researches was due to amino acid contained in *Moringa oleifera* seeds, which roled as proton-acceptor released a group of functional hydroxyl to make alkaline solution [9, 44]. pH increase along with increasing doses of *Moringa oleifera* seeds powder caused by the increase of turbidity level, which was essentially a protein polyelectrolyte [42]. This research found that coagulation caused pH increase averagely on 9.78 ± 0.01%.

The increase in pH value is inversely proportional to TSS, which tends to reduce along with the increasing doses of *Moringa oleifera* seeds powder as seen in Fig. 3. From the TSS initial value of 2.063 ± 282 mg/L reduced into 1.082.66 ± 86 mg/L by adding 50 mg/L *Moringa oleifera* seeds powder or 52.48 ± 0.4%. A very significant increase occurred after adding 100 mg/L *Moringa oleifera* seeds powder where the TSS reduced to 20.42 ± 34 mg/L or 99.63 ± 0.16% and adding 150 mg/L reduced TSS into 18 ± 2.6 mg/L or 99.83 ± 0.22%.

Although adding 150 mg/L TSS resulted in high removal percentage, it could not refer as significant; thus, the effective dose was 100 mg/L of *Moringa oleifera* seeds powder. pH increase became a more alkaline or in 9.78 ± 0.01% and TSS removal was in 99.63 ± 0.16% or similar to previous research in which, by adding *Moringa oleifera* seeds powder to the well-water increased pH as 8.8% and reduced TSS to 86.2% [52]. The purification mechanism was due to the role of proteins especially polypeptides with a molecular weight of 6000 to 16,000 Daltons. Polypeptides acted as a cationic polyelectrolyte or colloidal positive charges through adsorption and neutralization, which attached to the negatively charged impurities in water, leading to large flocs in the water [25, 53, 54]. Aside from reducing TSS, *Moringa oleifera* seeds powder on WWCP was able to purify water colour.

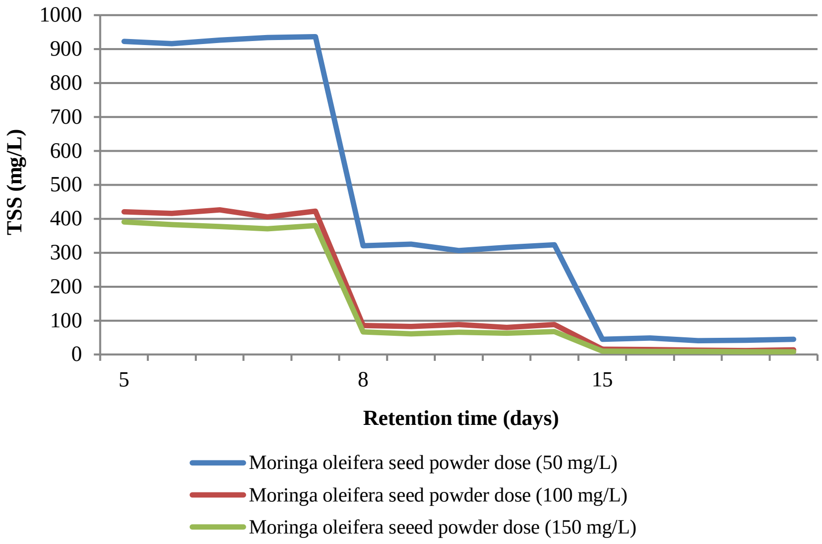


Fig. 3. TSS to coagulant dose and retention time.

In addition to pH increase, *Moringa oleifera* seeds were able to reduce organic content in WWCP, which can be seen from BOD and COD contents. Results of Pillai's Trace, Wilks Lambda, Hotelling's Trace, and Roy's Largest Root on *Moringa oleifera* seeds dose had a significance of 0.000 or < 0.05 to COD and BOD. It showed that adding *Moringa oleifera* seeds powder dose significantly affected the COD removal.

Result indicated that COD removal as 46.21 ± 0.18% on *Moringa oleifera* seeds powder dose of 50 mg/L or from 1.728 ± 426 mg/L to 798.50 ± 126 mg/L, then increased into 98.06 ± 0.04% on *Moringa oleifera* seeds powder dose of 100 mg/L or 48.26 ± 28 mg/L and increased further into 99.60 ± 0.23% on *Moringa oleifera* seeds powder dose of 150 mg/L or 46.88 ± 16 mg/L as seen in Fig. 4.

The difference in the increase in the COD removal percentage by adding *Moringa oleifera* seeds powder doses of 100 mg/L and 150 mg/L was less significant (1.27 ± 0.19%) so the most effective dose of *Moringa oleifera* seeds powder was 100 mg/L.

The results were in line with previous research in which, adding 100 mg/L and 150 mg/L *Moringa oleifera* seeds powder were able to reduce COD in car wash wastewater by 74% and 80% [55].

Similar to COD removal, the significant effect of *Moringa oleifera* seeds powder dose was indicated by the gradual decrease in BOD removal. Aside of COD, BOD dramatically reduced as well with a removal efficiency of 29.83 ± 0.03% on dose 50 mg/L or from 1.229 ± 2.12 mg/L to 366.61 ± 11.32 mg/L.

However, the efficiency increased significantly in the dose of 100 mg/L as 97.67 ± 0.24% to 28.63 ± 8.16 mg/L; and it became less significant on dose 150 mg/L as 97.96 ± 0.18% or 25.07 ± 8.37 mg/L. The removal efficiency of COD and BOD are seen in Fig. 4 and 5.

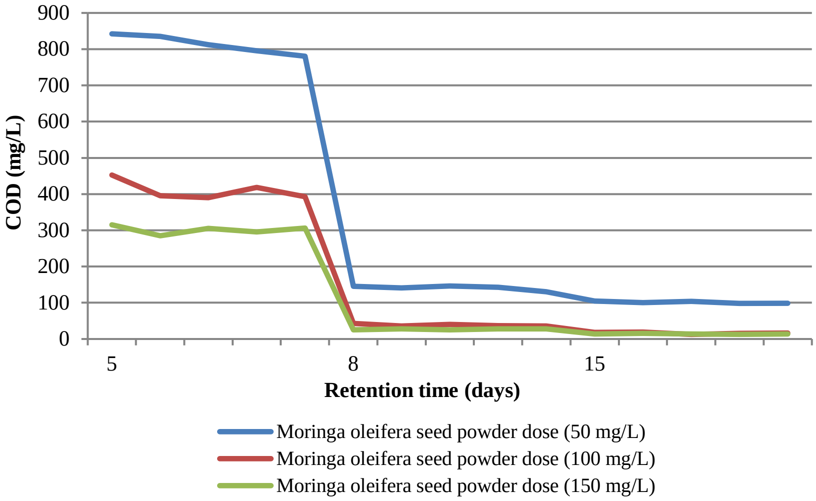


Fig. 4. COD to coagulant dose and retention time.

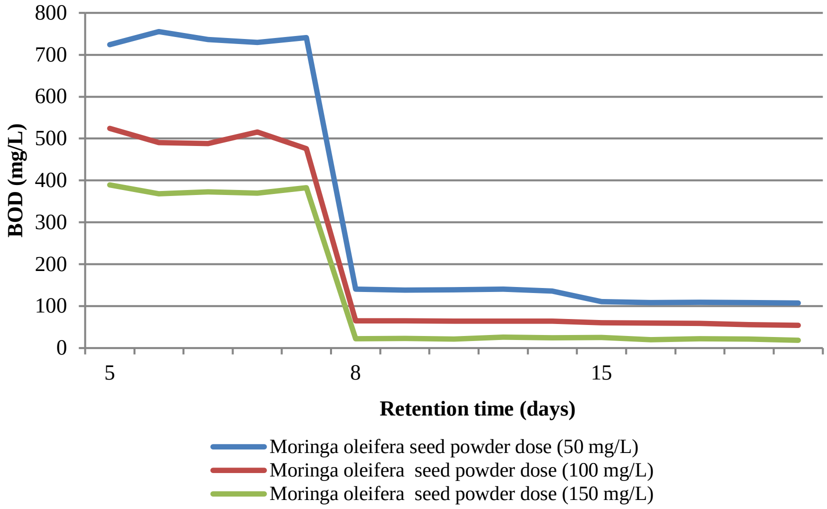


Fig. 5. BOD to coagulant dose and retention time.

Figure 5 shows BOD removal is significant on dose 100 mg/L and decreased on dose 150 mg/L. BOD, COD, and TSS removal were not accompanied by NH_4^+-N and TP removal. The results of the multivariate test shown by Results of Pillai's Trace, Wilks Lambda, Hotelling's Trace, and Roy's Largest Root on *Moringa oleifera* seeds powder doses had significance of 0,000 or <0.05 to NH_4^+-N and TP as indicated by an increase in NH_4^+-N and TP along with increasing doses of *Moringa oleifera* seeds powder. Figure 6 shows that adding 50 mg/L *Moringa oleifera* seeds powder resulted in an increase in NH_4^+-N and TP concentration comparable to 100 mg/L and 150 mg/L. Adding 50 mg/L of *Moringa oleifera* seeds powder increased to 1.38 ± 0.28% or from 5.87 ± 1.36 mg/L to 5.95 ± 2.13% then increased significantly to 8.16 ± 0.03%; at the dose of 100 mg/L, it increased into

6.42 ± 1.12 mg/L and continued to 12.37 ± 0.21% at 150 mg/L to 7.16 ± 0.9 mg/L. Similar condition occurred in TP removal as well in which, from the initial 4.32 ± 0.4 mg/L increased to 7.48 ± 0.17% to 4.64 ± 0.8 mg/L at the dose of *Moringa oleifera* seeds powder by 50 mg/L; 11.47 ± 0.11% or increased to 4.8 ± 0.2 mg/L at 100 mg/L, and 5.049 ± 0.4 mg/L at dose of 150 mg/L or 16.88 ± 0.26% (seen in Fig. 7).

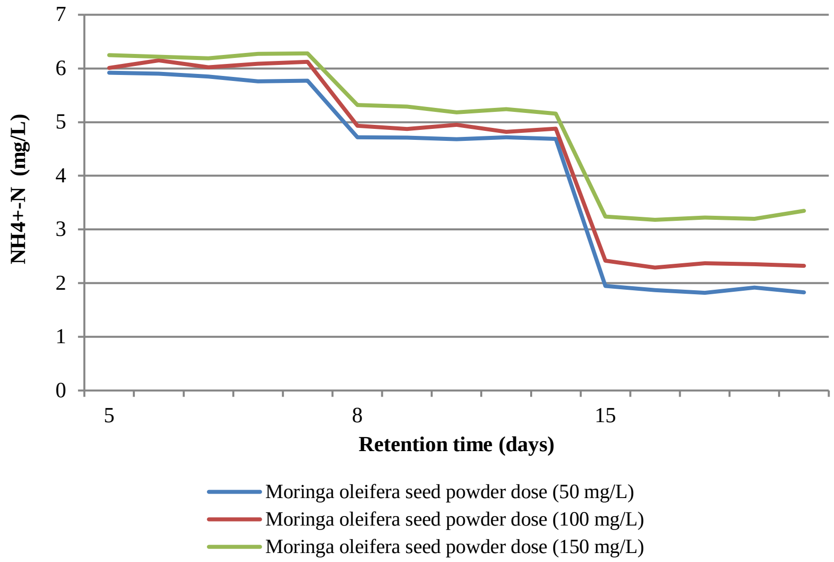


Fig. 6. NH_4^+-N to coagulant dose and retention time.

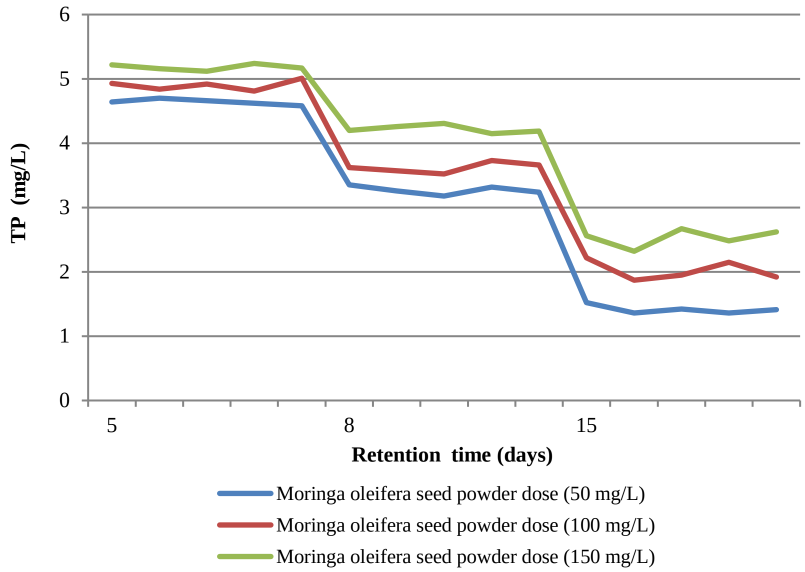


Fig. 7. TP to coagulant dose and retention time.

After flocculation coagulation process, WWCP was flown to vertical flow constructed wetland with *Canna indica*. The flow was 20 cm/d with intermittently fed (5 minutes on and 55 minutes off). The observation was conducted on day 5, 8, and 15 to measure parameters (pH, BOD, COD, TSS, NH_4^+-N and TP) percentages. The temperature was maintained on 20 ºC considered to the temperature of coagulant for optimal result. Moreover, temperature 14-20 ºC was recommended for the efficient of nitrogen removal [56]. In VSSFCW units, retention time is varied to 5 days, 8 days and 15 days. This retention time variation is intended to determine the effect of pH, BOD, COD, TSS, NH_4^+-N and TP removal in the WWCP. The effect of retention time variations on VSSFCW on decreasing various parameters is shown in Table 2 column 6.

The test result of Pillai's Trace, Wilks Lambda, Hotelling's Trace, and Roy's Largest Root on the retention time of VSSFCW showed the significance of 0.000 or < 0.05. It proved that prolonging retention time at VSSFCW significantly influenced pH, BOD, COD, TSS, NH_4^+-N and TP levels. The significant influence of retention time at pH as indicated by a pH decrease along with the addition of retention time. The result indicated that retention time significantly influenced parameters (pH, BOD, COD, TSS, NH_4^+-N, and TP) as well as pH reduced from 6.65 ± 0.04 to 6.36 ± 0.14 on day 5, to 6.39 ± 0.12 on day 8, and to 6.13 ± 0.14 on day 15. pH reduced on each retention time was due to the nitrification process in effluent [57, 58]. Although the pH decreased in the VSSFCW treatment process, the pH effluent was within the permissible range to be discharged under the government regulations of East Java Province No. 72 of 2013.

The significant level of VSSFCW retention time to various parameters in WWCP is shown in all parameters including at BOD and COD as indicators of organic content as well. System effectiveness in improving water quality is seen from organic content, nutrient, and faecal bacteria indicator [19], which is seen represented by the COD and BOD contents. Organic nutrient removal is controlled by similar factors as BOD defined by the organic substances in wastewater. This research showed that the removal efficiency of BOD increased along with retention time as 24.91 ± 0.22 %, 97.67 ± 0.24%, and 98.19 ± 0.11% at a retention time of day 5, 8, and 15 respectively. According to the results, the highest significance showed on day 8, while on day 15 it showed relatively less compared to day 8. The result indicated that effluent met the standard set by the government on day 8 and 15 (for 100 mg/L dose).

BOD removal was due to sedimentation and adsorption during the metabolic process. *Canna indica* roots helped sedimentation process more effective than gravels as it enlarged the surface area of food source attached for microbial populations [59]. Plants oxygenated the media, so microorganisms attached or located in the medium were able to support the degradation of the organic molecule aerobically. These microorganism communities were called "periphyton". The periphyton and natural chemical processes were responsible for almost 90% of pollutant removal and waste breakdown [20]. This research showed removal efficiency as 70-80% meaning that organic decomposition by *Canna indica* was high as this produced carbon dioxide and acid, which lowered pH on effluent [60]. Similar to BOD removal, the significant of VSSFCW retention time to COD was showed from the increase of COD removal efficiency along with the longer of retention time. The greatest removal efficiency was on day 15 as 97.06 ± 0.04%, while removal efficiency on day 5 and day 8 was 70.29

± 0.03% and 94.53 ± 0.18% respectively. The efficiency of COD removal was higher than BOD from 80-90%. The highest efficiency was on day 8 retention time; although there was an increase on COD and BOD removal efficiency on the day 15 of retention time, those were less significant compared to day 5 day and day 8 of retention time. Although the efficiency of COD removal efficiencies was high, the effluent standard set by the government was obtained on day 8 and day 15 of retention time. This removal efficiency was less than COD standard set on the 100 mg/L dose.

On NH_4^+-N and TP removal, the analysis results of the multivariate test showed that the variation of retention time in VSSFCW significantly influenced both parameters. The test result of Pillai's Trace, Wilks Lambda, Hotelling's Trace, and Roy's Largest Root on the retention time of VSSFCW showed the significance of 0.000 or < 0.05. At NH_4^+-N removal efficiency of 5.12% was on day 5 of retention times, then increased to 45.69% on day 8 retention time, and 48.75% on day 15 retention time. NH_4^+-N removal efficiency increased along with the retention time in VSSFCW. Although this research was set for the temperature of nitrogen or phosphor removal at 20 ºC [21] to obtain a better result, it has not yet met the effluent standards set by the government. Similar to NH_4^+-N, in TP removal, the removal efficiency increased along with retention time. The results indicated that phosphate removal efficiency was 5.54% on day 5; 42.82% on day 8, and 46% on day 15 of retention time. In this research, the longest retention time was 15 days with phosphate contained was 1.42 mg/L. Similar to ammonia nitrogen, effluent phosphate was not able to meet a standard set by the government. The increase of removal efficiency along with retention time was similarly proved in previous research using *Vetivera zizaniodes* in VSSFCW with a removal efficiency of 44.16%, 66.63% and 67.97% on day 0, 5, and 21 of retention times respectively. The TP removal process was due to the adsorption process (binding to electrostatic exchange sites on clay particles and clay-humus aggregates), assimilation process, and plant uptake into plant biomass [20]. The removal efficiency of NH_4^+-N and TP in this study was less than 50%. This result was similar to the previous study where removal nutrients were low in a constructed wetland and it was less than 50% [24, 56]. To obtain higher removal efficiencies, it is recommended to use HFCW as it provides suitable conditions for nitrate reduction formed during nitrification in VF beds [16]. The phosphate removal efficiency was obtained in this research lower than nitrogen. It was similar to the results of previous researches where phosphorous removal in vertical flow constructed wetland was very limited and it was impossible to obtain a high volume of bind phosphorous for a prolonged period [61].

Combining WWCP treatment using flocculation coagulation with varied doses of *Moringa oleifera* seeds powder and VSSFCW retention time resulted on significant effect on the pH, BOD, COD, TSS, NH_4^+-N and TP removal indicated by the value of the Test Result of Trace's, Wilks Lambda, Hotelling's Trace, and Roy's Largest Root on retention time and *Moringa oleifera* seeds powder significance of 0,000 or <0.05 as shown in Table 2 Column 6. Increasing or decreasing the *Moringa oleifera* seeds powder doses or VSSFCW retention time greatly affected the VSSFCW parameters removal efficiency. This research shows that the highest effect was on the 100 mg/L and 150 mg/L dose of *Moringa oleifera* seeds powder, which was able to make WWCP parameters meet the effluent standards set by the government; although it did not occur on NH_4^+-N and TP.

However, due to the insignificant difference of removal efficiency between 100 mg/L and 150 mg/L, the most effective dose to use was 100 mg/L. Likewise, 15 days of the VSSFCW retention time was not able to make NH_4^+-N and TP meet the effluent standards set by the government. Therefore, further research is needed by increasing the extension of VSSFCW retention time at a dose of 100 mg/L until the ideal retention time to meets the effluent standards set by the government is determined.

4. Conclusions

Combining *Moringa oleifera* seeds as natural coagulant with VSSFCW was only able to increase pH and optimize the decrease in BOD, COD and TSS parameters but not for NH_4^+-N and TP parameters. The most effective processing combination was obtained at the concentration of addition of *Moringa oleifera* seeds powder of 100 mg/L and VSSFCW retention time for 8 days. This combination resulted in pH increase from 4.32 ± 0.4 to 6.65 ± 0.054, TSS removal from 2.063 ± 282 mg/L to 20.42 ± 34 mg/L or 99.63 ± 0.10%, COD removal from 1.728 ± 1.61 mg/L to 48.26 ± 28 mg/L or 98.06 ± 0.04% and BOD removal from 1.229 ± 2.12 mg/L to 28.63 ± 8.16 mg/L or 97.67 ± 0.24%. These three parameters have met the effluent standard set by the government. However, NH_4^+-N and TP in WWCP increased to 6.42 ± 1.12 mg/L in 8 days retention time as 8.16 ± 0.02% and TP increased to 4.81 ± 0.2 mg/L or 11.47 ± 0.11 % on day 8 retention times. These increases made both NH_4^+-N and TP parameters were not able to meet the effluent standards set by the government. For future research, prolonging retention time of VSSFCW is needed in order to find the ideal time using 100 mg/L *Moringa oleifera* seeds dose to increase the removal efficiency of both NH_4^+-N and TP in WWCP.

Abbreviations

BOD	Biological Oxygen Demand
BOD_5	5-day Biological Oxygen Demand
COD	Chemical Oxygen Demand
CW	Constructed Wetland
HFCW	Horizontal Flow Constructed Wetland
pH	Power of Hydrogen
TP	Total Phosphate
TSS	Total Suspended Solid
VSSFCW	Vertical Sub Surface Flow Constructed Wetland
WWCP	Wastewater on Coffee Processing

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