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Aquaculture wastewater treatment using plant-based coagulants: Evaluating removal efficiency through the coagulation-flocculation process

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ABSTRACT

The ability of mixed neem, cassava, and wild betel plant leaves to act as plant-based coagulants was tested in real aquaculture wastewater through the coagulation-flocculation process. The performance of plant-based coagulant (PBC) was compared with that of chemical coagulant (alum) to observe the removal efficiency of turbidity, TSS, color, and COD. Ratio, as a new approach, was used by selecting TSS as an indicator or index to substitute for the dosage term and calculated based on a simplified equation. The extracted medium for these coagulants was prepared by using water extraction. Initial characterizations showed that the PBC had some carbon and amine groups (as also confirmed by LC-MS) with an average zeta potential of −22.83 ± 0.62 mV. Results indicated both coagulants (plant-based and chemical coagulants) successfully removed the measured polluted parameters of turbidity (85.17 %), TSS (80.28 %), color (59.42 %), and COD (54.63 %) for PBC with dosage of 0.79 mg/L and coagulant mass of 3.94 mg, while alum gained a higher removal with turbidity (99.08 %), TSS (98.71 %), color (97.29 %), and COD (75.31 %) with dosage and coagulant mass of 698.4 mg/L and 349.2 mg, respectively. This signaled that alum still acted as the best coagulant, but PBC showed a promising opportunity as a potential substitute for chemical coagulant.

1. Introduction

Coagulation-flocculation are considered to be one of the most essential methods for wastewater treatment [34]. The idea of having this process is to improve the water quality by removing colloidal or suspended particles in downstream processes, namely sedimentation and filtration [1]. This coagulation and flocculation process is perceived as an efficient and environmentally friendly method and can be an appealing option due to its relatively simple operation and considerable cost [17,19,37]. Raw materials to be used in this method can be inorganic, synthetic organic polymers, and natural coagulants. For example, aluminum sulfate (alum) is the common coagulant that is widely used in the treatment process [7]. The inorganic coagulants are well-known for their efficiency in wastewater treatment, but further studies imply possible health risks to humans over long periods of exposure [4]. Thus, alternative coagulants from natural sources (plants, animals, and microorganisms) are being explored to seek potential sources to be a substitute for inorganic coagulants.

In terms of plant-based coagulants, most studies focus on process effectiveness, mechanisms, active compounds or properties, and applications. One essential key finding from the literature specifies that active compounds from the plants contribute to the coagulation and flocculation process [20,29]. Several plants previously studied include banana peels, beans, okra, nirmali, moringa, bubble nut [30], neem, and cassava [2] in various of wastewater such as aquaculture, raw water, textile, pharmaceutical, domestic, dairy and palm oil mill effluents (POME) in which the removal performance reported to be as high as 99.2 % (turbidity), 97.28 % (COD), 99.2 % (TSS), 99.86 % (color), 92.9 % (TDS)

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and 91.5 % (BOD) [4,30]. Different plant species and their respective selected parts in the application of wastewater treatment might deliver different removal capabilities.

The selection of plant parts is a vital element due to the amount of active compounds within the designated parts contributing to the removal efficiencies. The most popular parts of the plants are the seeds (69.57 %), mucilage/grains (6.52 % each), leaves/fruit/gum (4.35 % each), and lastly, the root/sclerotium (2.17 % each) [4,32]. These data showed that studies on plant-based coagulants using leaves are still limited. In addition, studies on native Malaysian plants such as neem, cassava, and wild betel for coagulants are still rare. Current studies mostly use dosage for the application of coagulants, while the use of ratios as a novel approach should be encouraged. The use of ratio approach can save coagulant use compared to dosage.

The aim of the study was to investigate the performance of mixed plant leaves (neem, cassava, and wild betel) as plant-based coagulants to treat real aquaculture wastewater. In this study, the mixed coagulant was examined to see the potential of removing polluted parameters (turbidity, TSS, color, and COD), and the obtained result was compared with alum to see the relative removal efficiency between these two coagulants. Also, a new approach called "ratio" was used as a new indicator of measurement instead of using dosage in this study. The ratio is defined as the mass ratio [TSS (mg)/coagulant (mg)], and this approach is based on selected polluted parameters as an index or indicator, which in this experiment was TSS. The details of the ratio discussion are presented in subsection 2.3.

2. Materials and methods

2.1. Sample of aquaculture wastewater

The wastewater sample was collected from a local small-medium enterprise (SME) company located in Labu, Negeri Sembilan, Malaysia, approximately 35 km from the university campus. Various freshwater fish were farmed in this location using open-pond cultures. For the purpose of the experiment, the selected wastewater was from a freshwater catfish pond due to its high levels of polluted parameters such as turbidity and suspended solids. The collected wastewater was transferred into a 25 L plastic gallon and brought back to the lab to be analyzed for initial characteristics and experimentation purposes.

2.2. Preparation and characterization of PBC

Plant selection was done based on the findings from the previous study by Ahmad et al., (2022), which three best plants were selected as plant-based coagulant (PBC) in this study. The selected plants, namely neem, cassava, and wild betel, were mixed with a composition ratio of 1:1:1, and leaves were utilized as usable parts for the coagulant preparation. As these are local plants that can be easily found, the plant leaves were collected from Bangi, Selangor, Malaysia. The collected leaves were dried at 50 °C for 48 h in an oven (1370 GX Shel Lab, USA). The dried leaves were then crushed into powder by using a mechanical blender (Panasonic, Malaysia) and filtered to get a fine powder (<38 μm). Each collected powder was then weighted and mixed at a composition ratio of 1:1:1, followed by adding distilled water and stirring for 30 min to ensure the mixture was homogeneously mixed. After the stirring was done, the mixture was filtered using 10-μm filter paper (Double Rings, China) and directly used for the experiment. The coagulant was freshly prepared before the experiment ran to ensure that there was no deterioration of the coagulant solution.

The characterization of PBC was conducted individually to obtain clear identification for each composition. Functional group analysis was conducted using Fourier-transform infrared spectroscopy (FT-IR), and the obtained results were compared to the Sigma-Aldrich IR spectrum database (https://www.sigmaaldrich.com/MY/en/technical-documents/technical-article/analytical-chemistry/photometry-and-reflectometry/ir-spectrum-table). A liquid chromatography-mass spectrometry (LC-MS) (UltiMate 3000 UHPLC) analysis was conducted to identify the organic molecular and intermediate organic compounds following the described protocol described in Kurniawan et al. [23]. Zeta potential analysis was conducted to confirm the reading by FT-IR using the Zetasizer (Malvern Instrument, UK). Zeta potential analysis was done by diluting 1 g/L of coagulant (individual and PBC) in distilled water without any pH adjustment. Scanning electron microscopy (SEM) equipped with Energy Dispersive X-Ray (EDX) (Zeiss Model Supra VP, Germany) to obtain the surface morphology of the dried coagulant and its elemental composition.

2.3. Experiment run for jar test

The experiment was demonstrated with a jar-test lab scale run in 500 mL beakers (Iwaki, Germany) with five separated beakers. The run used 450 mL of aquaculture wastewater and 50 mL of coagulant to sum up the total volume of 500 mL. Two different coagulants were used for this experiment, specifically PBC and a chemical-based coagulant (alum).

In this experiment, a new approach was suggested, known as "ratio" to replace the dosage application. The ratio is defined by selecting TSS as an index for aquaculture pollution to determine the ratio value that can be applied in this experiment. The discussion related to the ratio approach versus dosage application was provided by Ahmad et al. [5]. The definition of the ratio is the mass of TSS (mg) per mass of coagulant (mg), as stated in the equation below:

$$\text{Mass ratio } (R) = \frac{TSS_{ww}V_{ww}}{C_{coagulant}V_{coagulant}} = \frac{m_{TSS}}{m_{coagulant}} \quad (1)$$

$$C_{coagulant} = \frac{m_{coagulant}}{V_{distilledwater}} \quad (2)$$

where ww = wastewater, C = concentration (mg/L), V = volume (L) and m = mass (mg).

The jar test operation was run based on two different coagulants: PBC and chemical-based. For the PBC, leaves of neem, wild betel, and cassava were mixed at a composition of 1:1:1, while for the chemical coagulant, alum was used. A ratio approach was introduced to replace the dosage application in this experiment as a new term. The ratio started at 0.1, 1.0, 10, 100, and 1000 mg TSS/mg coagulant (noted as R:0.1, R:1, R:10, R:100, R:1000) based on the calculation from Eq. (1) for the corresponding 5 beakers for each coagulant. Based on the calculation, this indicates that the higher the ratio number, the lower the coagulant dosage and mass used, and vice versa.

While for the operation condition of the experiment, rapid mixing and slow mixing were run at 180 rpm (3 mins) and 10 rpm (20 mins), respectively, followed by sedimentation for 30 mins [3,16] using a flocculator (VELP, Malaysia). To analyze the removal performance, a 25-mL sample of supernatant was taken at a point 5 cm below the surface after the coagulation-flocculation process was completed. The polluted parameters (turbidity, TSS, color, and COD) were analyzed, and the data were compared in terms of removal performance for both plant-based and chemical coagulants.

2.4. Analysis of statistics using ANOVA

Statistical analysis was done using SPSS Software v21 (IBM, USA). In this study, analysis of variance (ANOVA) was applied to investigate the correlation between factors (variables used in this run) and responses (polluted removals). A post-hoc test using Turkey HSD was run to understand the significant differences from the data findings. All conclusions were determined based on the p-value of $p < 0.05$, indicating a significant difference [3,21,22].

3. Results and discussion

3.1. Aquaculture wastewater characteristics

Real aquaculture wastewater was obtained from the sampling site of a freshwater catfish pond, then analyzed and presented in Table 1. The obtained data specified that some findings exceeded the removal effluent standards as outlined by the Department of Environment Malaysia related to the Environmental Quality (Industrial Effluent) Regulations 2009. In this experiment, the aquaculture showed high-turbidity water with a turbidity range of 392.67–657.33 NTU, which implies a high presence of colloidal particles. This data agreed with the TSS value observed, which is likely high, around 776–876.00 mg/L, and above the threshold level of Malaysian standard measurement for effluent discharge. The color also indicated exceeded the threshold level with 1736–1789 ADMI compared to the established Malaysian effluent discharge standard, implying very high color detection in the aquaculture wastewater. The exceeded values of these present polluted parameters in the aquaculture wastewater showed the presence of high nutrients that originated from food residues and fish feces [6]. By comparing the analyzed data from this experiment with other studies from Igwegbe et al., [14] and Ohale et al., [28], the pH value of the aquaculture wastewater was the only parameter found to be within the standard limits.

3.2. Characteristics of PBC

The results of the FTIR analysis for each individual PBC are tabulated in Table 2. Based on Table 2, cassava functional groups were dominated by carbons (including alkane, alkene, and aromatic compounds). In addition, alcohol vibrational waves were detected twice (indicating intermolecular bonded and primary alcohols), and aldehyde was detected once. For neem, detected functional groups were more varied as compared to cassava, including the carbons (alkane and aromatic compounds), alcohol (intermolecularly bonded, free, and tertiary alcohols), amines, carboxylic acid, and isocyanate. For wild betel, the vibrational waves showed similar functional groups with cassava, including carbons (alkane and alkene) and alcohols (intermolecularly bonded and secondary alcohols).

Alcohol is dominant in most compounds, indicating that each of the dried plants may show a negative charge while diluted in water. Similar to Kurniawan et al. [24], the dominant functional groups of alcohol and amine indicated the negative charge during hydrolysis with water. The presence of alcohol (hydroxyl groups) in PBC facilitates the compound's interactions with water molecules, allowing higher solubility to occur [10]. In addition, the presence of hydroxyl groups also facilitates aggregation as the result of adsorptive forces [36]. As confirmed by the LC-MS readings (Table 3), most of the detected compounds were amine-based compounds, like N-benzyl-N-ethyl-dodecan-1-amine, bucharidine, pentoxifylline, and 11-alpha-hydroxygalanthamine. Protonated amines facilitate the flocculation that occurs via the sweep coagulation mechanism [12]. LC-MS readings also showed glucoronate-base compounds, which may indicate glucose-related compounds. Some C-H stretching, carboxylic acid groups, and detection of glucose-related compounds might be indications of the presence of polysaccharides,

Table 1
Aquaculture wastewater characteristics.

Parameter	Unit	This research	Igwegbe et al., [14]	Ohale et al., [28]	Malaysia industrial effluent standard*
Turbidity	NTU	392.67 – 657.33	404	502	–
TSS	mg/L	776 – 876	695	992.19	50
Colour	ADMI	1736 – 1789	Yellowish green	Green solid	100
COD	mg/L	711.00 – 727.33	758	593	80
pH	–	7.13 – 7.21	7.9	6.9–7.9	6–9
TDS	mg/L	293.33 – 338.33	650	–	–
EC	µS/cm	604.33—693.33	1963	–	–

TSS: total suspended solids, TDS: total dissolved solids, EC: electrical conductivity.
*Department of Environment Malaysia [24].

Table 2
Functional groups of the coagulants.

Plant species	Wavenumber (cm^{-1})	Appearance	Group	Identified compound class
Cassava	3353.48	Broad	O–H stretching	Intermolecular bonded alcohol
	2920.62	Medium	C–H stretching	Alkane
	2851.1	Strong	C–H stretching	Alkane
	1977.65	Medium	C–H bending	Aromatic compound
	1732.04	Strong	C=O stretching	Aldehyde
	1651.83	Medium	C=C stretching	Alkene
	1456.16	Medium	C–H bending	Alkane
	1065.35	Strong	C–O stretching	Primary alcohol
	719.37	Medium	C = C bending	Alkene
Neem	3864.96	Medium	O–H stretching	Free alcohol
	3520.77	Medium	O–H stretching	Intermolecular bonded alcohol
	3288.48	Medium	O–H stretching	Carboxylic acid
	2917.51	Strong	C–H stretching	Alkane
	2849.29	Strong	C–H stretching	Alkane
	2361.69	Strong	O=C=O	CO_2
	2286.75	Strong	N = C = O	Isocyanate
	1878.53	Medium	C–H bending	Aromatic compound
	1726.63	Strong	C=O stretching	Aldehyde
	1605.59	Medium	N–H bending	Amine
	1516.79	Strong	N–O stretching	Nitro compound
	1436.1	Medium	C–H bending	Alkane
	1242.29	Medium	C–N stretching	Amine
	1157.29	Strong	C–O stretching	Tertiary alcohol
	1033.02	Strong	S=O stretching	Sulfoxide
	654.01	Strong	C–Br stretching	Halo compound
Wild betel	3341.71	Broad	O–H stretching	Intermolecular bonded alcohol
	2920.74	Strong	C–H stretching	Alkane
	2851.69	Strong	C–H stretching	Alkane
	1622.39	Medium	C=C stretching	Conjugated alkene
	1417.21	Medium	C–H bending	Alkane
	1316.65	Strong	C–F stretching	Fluoro compound
	1102	Strong	C–O stretching	Secondary alcohol
	780.6	Strong	C–H bending	1,2,3-trisubstituted

Table 3
LC-MS readings of major compounds.

Cassava	Neem	Wild betel
N-benzyl-N-ethyl-dodecan-1-amine	Septentriodine	Septentriodine
Bucharidine	11alpha-Hydroxygalanthamine	11alpha-Hydroxygalanthamine
Pentoxifylline	dTDP-D-glucuronate	Salidroside

which are also preferred for the flocculation process [36]. In addition to that, septentriodine and salidroside, which are not directly related to coagulation and flocculation mechanisms, are common metabolites found in plants.

The previous claim was also confirmed by zeta potential analysis, as depicted in Fig. 1. As predicted, the tested zeta potential for all compounds resulted in a negative charge (-25.57 ± 0.82 mV for cassava, −16.33 ± 0.66 mV for neem, −17.75 ± 1.15 mV for wild betel, and –22.83 ± 0.62 mV for PBC). The obtained result was in accordance with previous study which stated that *Artocarpus heterophyllus* [31], and *Trigonella foenum-graecum* [26] also showed negative charge for zeta potential. The negatively charged coagulants will only perform charge neuralization on a differently charged particle (in this case, positively charged wastewater) [13,25]. Since aquaculture wastewater also possesses a negative charge [23], the mechanisms of charge neutralization are impossible to occur.

The SEM images for individual compounds of PBC (Table 4) showed that all dried plants showed an irregular, intact structure, with the particle size of neem being bigger as compared to the other two species. Further magnification to 5000 × showed similar structures for all plant species. All images showed coarse surfaces with irregular, porous shapes. The obtained result was similar to Lim et al. [27], stating that fenugreek powder showed a rough, irregular, and porous cluster. In another study, Mohammad Lanan et al., (2020) stated that fenugreek powder showed non symmetrical structure with a deep void structure (porous). *M. oleifera* also showed a heterogeneous surface with an irregular porous shape, as stated by El Gaayda et al. [11].

Compositional analysis by EDX (Table 5) revealed that all species contain a similar composition of elements, with cassava having the highest carbon percentage. Unlike cassava and wild betel, neem did not contain Mg, while wild betel did not contain any Ca. In addition to those differences, wild betel also showed the content of Si and Cl, which were not found in the two other species. Differences in elemental composition are highly related to the different species [11]. However, different extraction methods may also result in variations in elemental composition in the final plant-based coagulant product [4,25].

3.3. Removal performance

3.1.1. Turbidity removal

The efficiency of turbidity removal using PBC and alum is presented as per Fig. 2. Overall, the data indicated for both coagulants offered a good turbidity removal rate of over 50 % for the overall established ratio ranges. For PBC, all introduced ratios showed a high removal rate of over 80 % with the increase of the ratio from R:0.1 to R:1000. At a low ratio of R:0.1, the removal was 83.82 % with a dosage of 7884 mg/L (coagulant mass 3942 mg), and by statistic, the removal significantly increased when the ratio increased to R:1 (removal at 84.86 % and at a dosage of 788.4 mg/L with a coagulant mass of 394.20 mg), and then remained not significant up to R:1000 (removal at 85.17 % at a dosage of 0.79 mg/L with a coagulant mass of 0.39 mg). With the given findings, the optimum ratio for PBC is to be at R:1000 due to the lowest coagulant mass used for the considerable removal efficiency.

While for alum, the indication of findings based on the removal trends in Fig. 2, the turbidity removal significantly improved with the increase ratio from R:0.1 to R:1 and reduced again after increasing the ratio from R:10 to R:1000. At R:0.1, the obtained removal was 92.58 % with a dosage of 6984 mg/L and a coagulant mass of 3492 mg, and increased to 99.08 % at R:1 with a dosage and coagulant mass of 698.4 mg/L and 349.2 mg, respectively. The removal efficiency dropped to 89.63 % when the ratio was added to R:10 with a dosage of 69.84 mg/L (coagulant mass of 3.49 mg). At the final ratio of R:1000, the removal was the lowest at 71.73 % with a dosage of 0.70 mg/L and a coagulant mass of 0.35 mg. The optimum condition for alum in terms of turbidity removal can be concluded at R:1, with the highest removal performance. Comparing the optimum condition at the same ratio, PBC showed significantly higher removal of turbidity as compared to alum at R:1000, while alum showed significantly higher removal at R:1. Based on this result, a chemical coagulant such as alum required a high mass of coagulant to be efficient in removing the turbidity as compared to PBC.

There were studies conducted to investigate the plant-based coagulant in removing turbidity in wastewater treatment, done by Asrafuzzaman et al., [9] to evaluate the efficiency of three different plants (*Moringa oleifera* (seeds), *Cicer arietinum* (industrial powder), and *Dolichos lablab* (seeds)) for turbidity removal in synthetic turbidity water. There are three separate ranges of turbidity: higher (90–120 NTU), medium (40–50 NTU), and lower (25–55 NTU). The findings showed *M. oleifera* managed to remove turbidity from 86.9 % to 94.1 % (higher), 65.62 %–69.37 % (medium), and 56 %–60 % (lower), while *C. arietinum* achieved a removal efficiency of around 93.78 %–95.89 % (higher), 74.28 %–81.63 % (medium), and 62.58 %–71.29 % (lower), and lastly, turbidity removal was obtained by *D. lablab* at 84.5 %–88.9 % (higher),

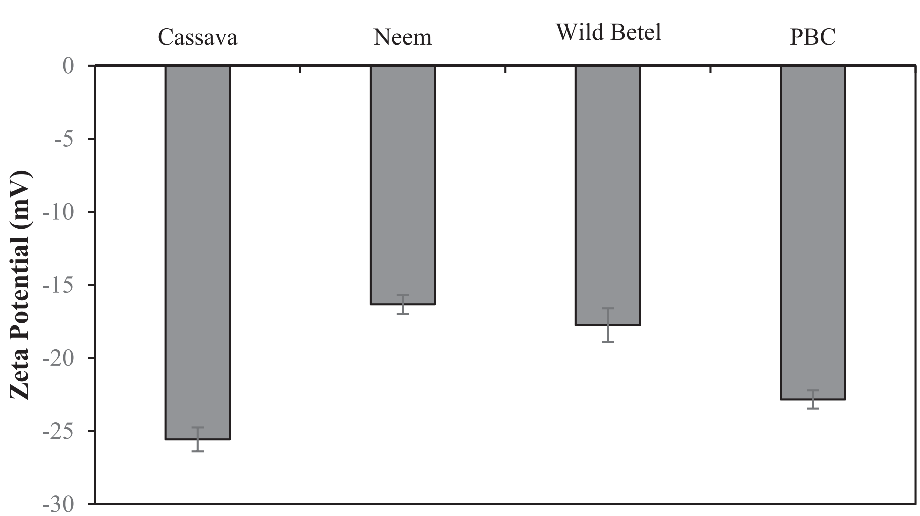


Fig. 1. Zeta potential of PBC.

Table 4
SEM images of the coagulants.

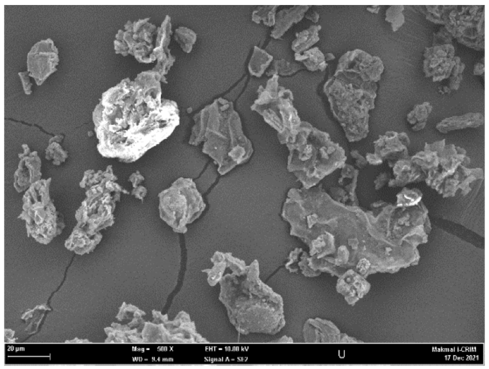
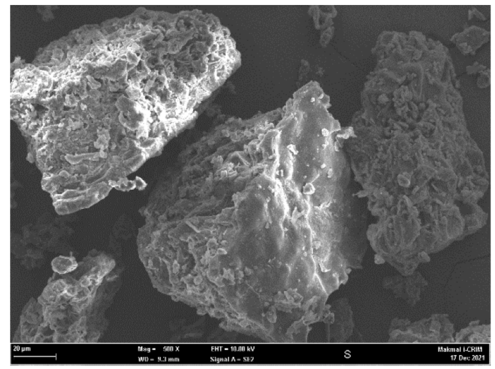
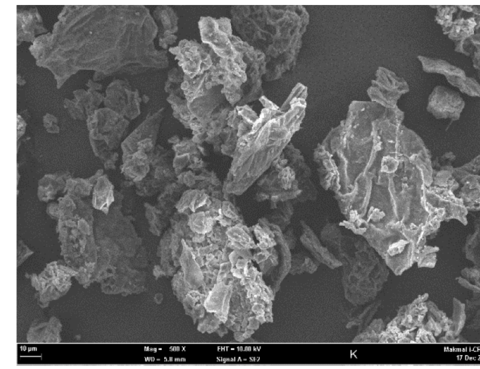
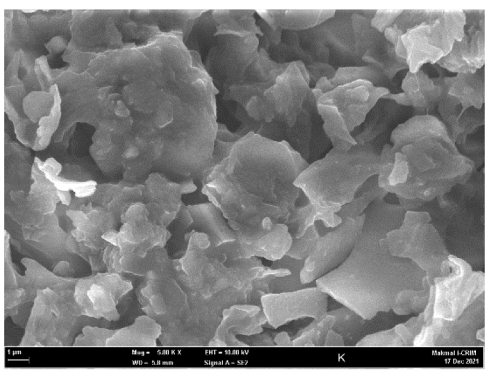
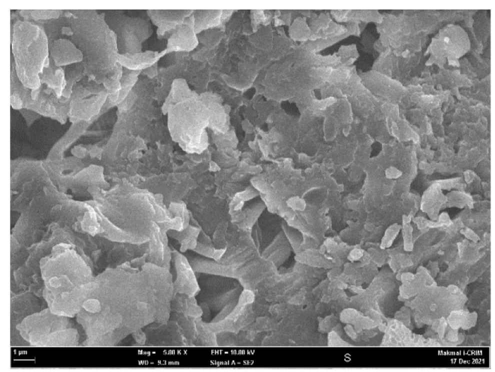
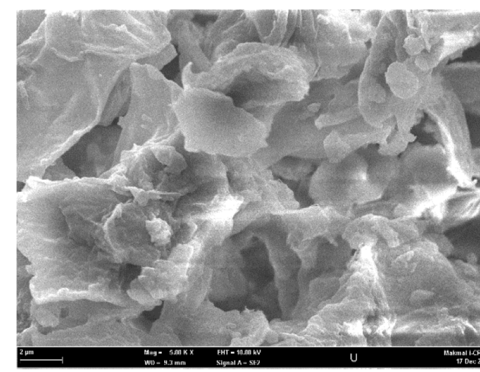
Type Magnification	Cassava	Neem	Wild betel
500×			
5000×			

Table 5
Elemental composition of the PBC.

Component	Composition (%)		
	Cassava	Neem	Wild betel
C	71.4	59.7	59.6
O	20.6	36.7	30.6
K	5.1	1.7	6.6
Ca	2.2	1.8	–
Mg	0.6	–	0.4
Si	–	–	0.7
Cl	–	–	2.1

65.10 %–68.19 % (medium), and 49.71 %–60.85 % (lower). The reported result of this experiment concluded that all plants showed good removal efficiency, especially to treat higher-turbid water.

3.1.2. TSS removal

For the TSS removal, the acquired findings are presented in Fig. 3, where the TSS removal was compared for PBC and alum. The figure indicated that for PBC, the TSS removal was estimated at 73.59 %–80.2 % with a dosage range of 7884 mg/L decreased to 0.79 mg/L and coagulant mass reduced from 3942 mg to 0.39 mg within the range of R:0.1 to R:1000. The removal efficiency to be expected was lower at an initial ratio of R:0.1 but significantly increased with the increase in ratio. But the TSS removal showed no significant difference statistically when the ratio started increasing from R:10 towards the final ratio with a removal range of 79.68 % to 80.28 %. At this point, the optimum ratio to be suggested was R:1000 with a removal efficiency of 80.28 %, a dosage of 0.79 mg/L, and a coagulant mass of 0.39 mg.

For alum, the same trend was shown in Fig. 3, indicating the same removal performance obtained from turbidity removal. Over 90 % TSS removal was attained from R:0.1 to R:1, with a TSS removal range of 93.13 %–98.71 % at the dosage range (6984 mg/L to 698.4 mg/L) and coagulant mass range (3492 mg to 349.2 mg), and significantly reduced less than 87 % (dosage: 69.84 mg/L, coagulant mass: 3.49 mg) from R:10 towards R:1000, as low as 68 % (dosage: 0.70 mg/L, coagulant mass:

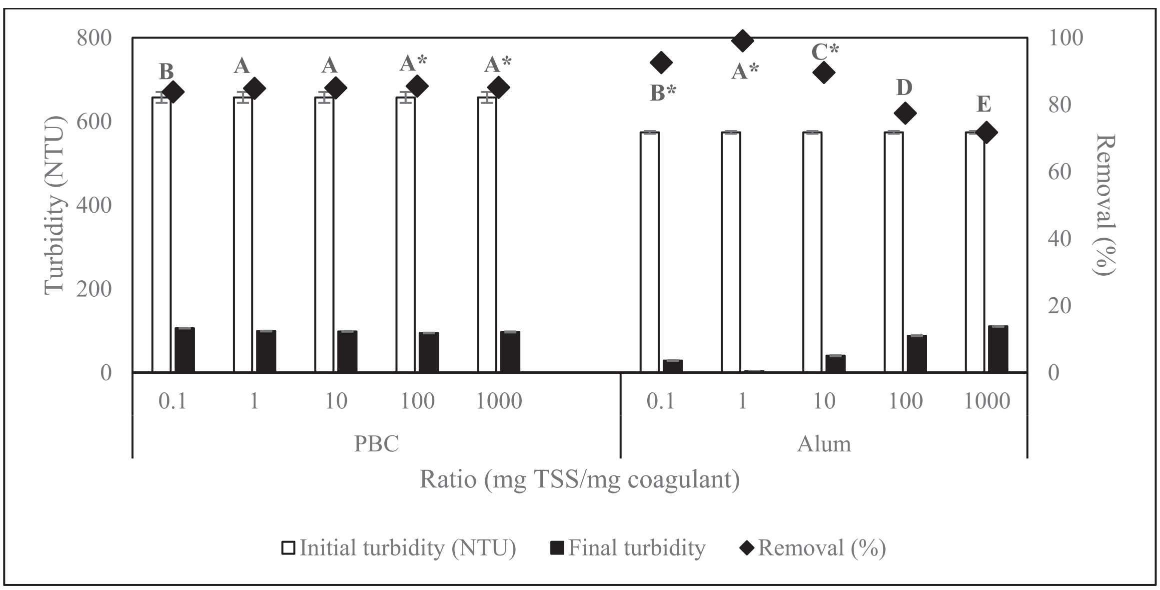


Fig. 2. Performance of turbidity removal for PCB and alum. The letters A–D indicate a significant difference in turbidity removal for the same coagulant type among different ratios. The asterisk symbol (*) indicates a significantly higher turbidity removal between PBC and alum in the same ratio.

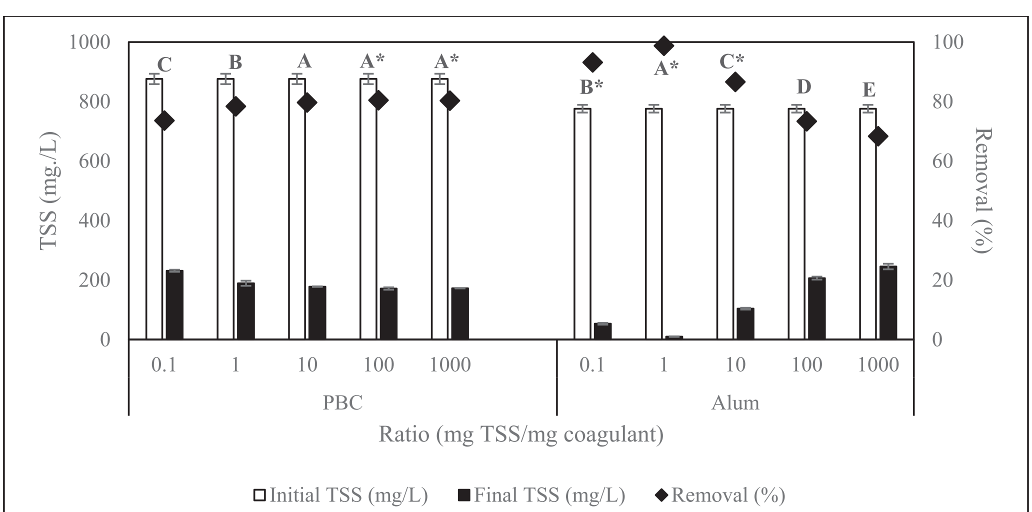


Fig. 3. Performance of TSS removal for PCB and alum. The letters A–D indicate a significant difference in turbidity removal for the same coagulant type among different ratios. The asterisk symbol (*) indicates a significantly higher TSS removal between PBC and alum in the same ratio.

0.35 mg). Thus, R:1 can be suggested to be the best optimum point of TSS removal for alum, even with a high amount of coagulant mass used. Similar to the results for turbidity removal, at the optimum PBC ratio (R:1000), PBC showed significantly higher TSS removal. While at R:1, as the optimum ratio of alum, PBC showed significantly lower TSS removal. From these results, it can be concluded that PBC requires a lower coagulant mass as compared to alum to achieve similar TSS removal.

Studies on TSS removal using natural coagulants have also been investigated in the treatment of wastewater for coagulation-flocculation. A study was done by Asharuddin et al., [8] to investigate the performance of cassava peels to be used as the main coagulant and coagulant aid in raw water treatment. The findings revealed the cassava peels alone had substantial removing capability, with a removal rate of 42.59 %, and by changing the cassava peels to act as coagulant aid, the removal rate had boosted to 90.48 %. This was reported under optimized conditions of cassava peels to alum ratios of 100 mg/L to 7.5 mg/L at the basic pH of 9.75, rapid mixing at 200 rpm (1 min) and 100 rpm (2 min), slow mixing at 25 rpm (30 min), and settling time under 30 min. A treatment of domestic wastewater was investigated by Wan Kamar et al., [35] to determine the capability of sago starch in removing TSS, color, and COD. An estimated 82 % of TSS was removed by using the coagulant at the optimum dosage of 2000 mg/l at pH 7. Other polluted parameters (color and COD) were also reported to be removed by over 70 %. This signifies that sago starch has great potential to act as a coagulant to treat domestic wastewater and is possible to be used for other wastewater treatments.

3.1.3. Color removal

In terms of color removal for real aquaculture wastewater treatment, the data was reported and presented as per Fig. 4. The removal of color shows lower removal performance compared to the previous results of turbidity and TSS removal for PBC. The color removal was obtained as low as 24.09 % (coagulant mass: 3942 mg with a dosage of 7884 mg/L) at an initial ratio of R:0.1 and as high as 59.42 % at R:1000 (coagulant mass: 0.39 mg with a dosage of 0.79 mg/L). The trend of removal shown that at the initial ratio, the removal showed a significant increase from 24.09 % to 58.99 % from R:0.1 to R:10 and gradually maintained from R:10 to R:1000 with a color removal range of 58.99 %–59.42 %. At this point, as there was no significant increase in color from R:10 to R:1000, this implies that the optimum ratio should be taken from R:1000 with high removal (59.42 %) and the lowest coagulant mass usage.

The use of alum as a chemical coagulant in removing color indicates better performance compared to PBC. Overall color removal for alum is stated to be over 53 % and goes up to 97.29 % for the ratio range. At first, the removal was measured within 84 %–88 % for R:0.1 to R:1 (with a dosage and coagulant mass of 6984 mg/L and 3492 mg for R:0.1 and 698.4 mg/L and 349.2 mg for R:1, respectively). Then in R:10-R:1000, the removal kept decreasing from over 84 % previously to 75.98 %, and at R:1000, the removal was at 53.13 %. Statistics show a significant difference in color removal for each point of the ratios from the initial to the end. The data demonstrated that the optimum point of alum should be selected at point R:1, with the highest removal gained.

Comparing the optimum conditions and removal trend, the results are still similar with turbidity and TSS removals, in which the opimum ratio for PBC is R:1000 and that for alum is R:1. From Fig. 4, it can be clearly seen that the increase in ratio (lowering the used coagulant mass) had a positive effect on color removal until R:10 for PBC, while only up to R:1 for alum. The increase in ratio for alum (reducing its coagulant mass to R:1000) showed inclines in terms of color removal. For color removal, PBC outperformed alum only at R:1000, while alum showed significantly higher removal from R:0.1 to R:10.

Jaeel and Ali [15] had run a study on color removal from textile wastewater treatment by using extracted powder from *Capparis spinosa*. A set of experiments was executed to investigate the effects of plant concentration, dye concentration, acidity function, and settling time. They discovered that by using Capparis spinosa to remove color, up to 96 % removal could be obtained with an optimum settling time of 20 min. The findings also showed that the color removal tends to decrease after exceeding 100 mg/L of coagulant dosage due to the potential for *C. spinosa* to crease back after this concentration.

3.1.4. COD removal

COD removal for real aquaculture wastewater treatment is shown in Fig. 5. For the PBC removal performance of COD, the data showed that the removal ratio generally increased. However, at R:0.1 (with dosage and coagulant mass use of 7884 mg/L and 3942 mg), no removal of COD was detected, then increasing significantly to 34.28 % at R:1 (dosage: 788.4 mg/L, coagulant mass: 394.2 mg), and going up to 53 %–55 % for the ratio range of R:10–R:1000 (dosage from 78.84 mg/L to 0.79 mg/L, and coagulant mass from 39.42 mg to 0.39 mg). The finding of no removal at R:0.1 might be due to the high amount of coagulant mass used, which contributes to the addition of organics in the wastewater, as shown by the increase in COD after treatment.

While for alum, the overall performance can be observed to be over 50 % removal of the listed ratios. The COD removal was identified to be

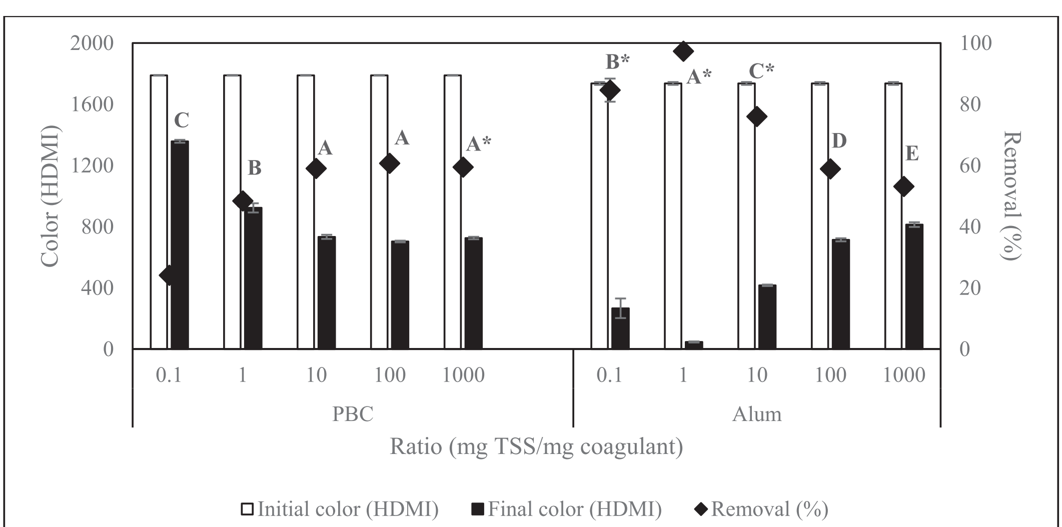


Fig. 4. Performance of color removal for PCB and alum. The letters A–D indicate a significant difference in turbidity removal for the same coagulant type among different ratios. The asterisk symbol (*) indicates a significantly higher color removal between PBC and alum in the same ratio.

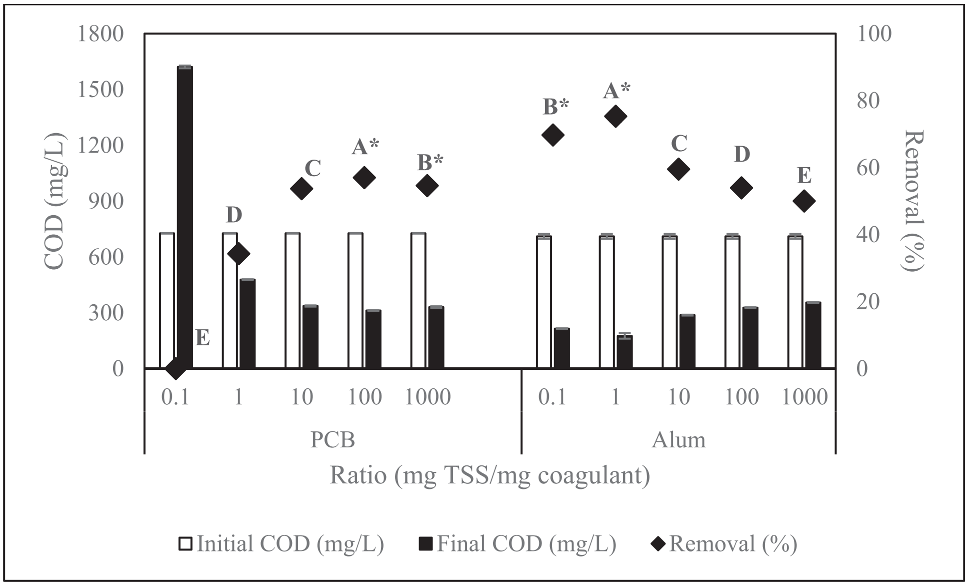


Fig. 5. Performance of COD removal for PCB and alum. The letters A–D indicate a significant difference in turbidity removal for the same coagulant type among different ratios. The asterisk symbol (*) indicates a significantly higher COD removal between PBC and alum in the same ratio.

most effective at R:1 (dosage and mass coagulant at 698.4 mg/L and 349.2 mg), indicating the highest removal obtained at 75.31 %, and the lowest was noted at R:1000 (dosage and mass coagulant at 0.70 mg/L and 0.35 mg) with the removal of 50.01 %. Even though a high amount of alum was used in this treatment, especially for R:0.1 (dosage and coagulant mass used at 6984 mg/L and 3492 mg), the COD removal still provided reliable removal.

Different from previous results of turbidity, TSS, and color, the optimum ratio for PBC was obtained at R:100, while for alum at R:1. It can be seen clearly from Fig. 5 that the increase in ratio (use of less coagulant mass) increases the COD removal efficiency for PBC while decreasing it for alum. Combining all the obtained results, it can be concluded that PBC works better in a higher ratio (lower coagulant mass), while alum performs better in a lower ratio (higher coagulant mass).

Among the studies related to COD removal using natural coagulant, Khader et al., [18] conducted an experiment for COD removal with three different plants' seeds (radish, *Cicer arietinum*, and eggplant). The study was demonstrated by using synthetically produced wastewater (from the petroleum industry) by adding crude oil, clay, and salts. Based on the study, the COD removal reported for radish, *C. arietinum*, and eggplant was 93.48 %, 95.2 %, and 92.18 %, respectively. The findings revealed a great deal of COD removal (over 92 % for all plants used in this experiment), which suggested the potential of these plant-based coagulants to be applied to real wastewater. In a separate study, Sethu et al., [33] demonstrated the efficiency of using *Opuntia cactus* in the treatment of real POME wastewater. The experiment was run by selecting *O. cactus* pads as the used part for coagulant and okra waste as flocculant. The study reported that COD removal with *O. cactus* acting as coagulant alone managed to achieve 91.2 %, while by adding okra as flocculant, the COD removal could improve as high as 93.9 %. The suggested dosage for *O. cactus* was 8 g/L with a pH of 9 and a reaction time of 240 min. Also, the findings discussed the mechanism involved, which was the polymeric bridge mechanism.

5. Conclusions

PBC (a mix of neem, cassava, and wild betel powders) showed good efficiency for the treatment of real aquaculture wastewater. A new approach called "ratio" was successfully introduced to replace the common application of "dosage" by selecting TSS as an indicator or index from the polluted parameters. The removal efficiency of PBC was achieved at optimum ratio of R:1000 with 85.17 % (turbidity), 80.28 % (TSS), 59.42 % (color), and 54.63 % (COD), while that of alum was reported at optimum conditions of R:1 for 99.08 % (turbidity), 98.71 % (TSS), 97.29 % (color), and 75.31 % (COD). Even though the findings showed alum with better removal performance, PBC is still an effective option that is very promising to treat aquaculture wastewater, especially in relation to turbidity and TSS removal. This result indicated that PBC can be an option for wastewater treatment and can be expanded for treatment applications in industrial wastewater.

CRediT authorship contribution statement

Azmi Ahmad: Investigation, Formal Analysis, Data Curation, Writing - Original Draft, Visualization. **Siti Rozaimah Sheikh Abdullah:** Supervision, Finding acquisition. **Hassimi Abu Hasan:** Supervision, Finding acquisition. **Ahmad Razi Othman:** Supervision, Finding acquisition. **Setyo Budi Kurniawan:** Data Curation, Writing – original draft, Writing – review & editing, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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