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Coagulation–flocculation of aquaculture effluent using biobased flocculant: From artificial to real wastewater optimization by response surface methodology

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ABSTRACT

Coagulation–flocculation is currently the best practice for aquaculture effluent treatment, and biobased compounds are emerging as coagulant/flocculants. This study aimed to characterize the bioflocculant produced from *Serratia marcescens* and applied it to treat artificial turbid water (kaolin substrate) and real aquaculture effluent using the combination of one variable at a time (OVAT) and response surface methodology (RSM) analyses. The bioflocculant produced by *S. marcescens* was characterized as anionic flocculant with isoelectric point at pH 1.7 and 13.3. At pH 7, its protein content was 1.3 μg/mL, and its total carbohydrate level was 0.53 mg/L. The bioflocculant consisted of various carboxylic acids and enzyme intermediates, indicating the presence of polysaccharides and protein. Comparison of optimized treatment conditions between OVAT and RSM showed that rapid mixing speed, slow mixing time, and sedimentation time were the most influential factors for coagulation–flocculation. The aquaculture effluent required lower rapid mixing speed (125 rpm) and shorter sedimentation time (39 min) than artificial wastewater (160 rpm and 67 min, respectively). The low performance of the bioflocculant in treating aquaculture effluent was due to the more complex characteristics of real aquaculture effluent compared with those of kaolin substrate.

Environmental implications: The characterization of bioflocculant produced by *Serratia marcescens* in terms of its protein level, total carbohydrate content, and isoelectric point has never been reported. The obtained results may provide an insight into the potential of this compound to substitute widely used chemical flocculants with reliable performance. The findings may also be used as a basis to upscale coagulation–flocculation from being applied to artificial wastewater in the laboratory to treating real wastewater, especially with the use of biobased compounds.

1. Introduction

Aquaculture is one of the sectors that contribute to surface water pollution [1,2]. Aquaculture effluent has high turbidity, high organic content, high suspended solid amount, and a specific color [3,4]. It also contains high levels of nutrients, mainly nitrogen and phosphorus [5,6]. These elements cause eutrophication when discharged into surface water without proper treatment [7,8].

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The current best practice in aquaculture effluent treatment is coagulation and flocculation [9,10]. This technology was proven to be effective in treating most pollutant parameters in aquaculture effluents. Heiderscheidt et al. [11] reported the high performance of poly-aluminum chloride in removing up to 98 % of turbidity, suspended solids, and phosphates from aquaculture effluents. Gibson et al. [9] also demonstrated the successful treatment of suspended solids from aquaculture effluent using polyacrylamide (PAM) polymers. In addition to the type of coagulant, several factors are influence the performance of coagulation–flocculation. Ebeling et al. [12] stated that the optimum dose of alum was 60 mg/L for drinking water treatment. Jangkorn et al. [13] mentioned that alum concentration of 400 mg/L provided the best performance of wastewater treatment. Demirbas and Kobya [14] stated that the optimum pH for aquaculture effluent treatment was pH 7. In addition, rapid mixing, slow mixing, and sedimentation influence the performance of alum [15].

Some of the above factors also influence the overall performance of biocoagulants/bioflocculants [16,17]. Chitosan shows the best performance at a dose of 0.5 g/L, rapid mixing speed of 100 rpm, rapid mixing time of 15 min, settling time of 20 min, and pH 4 [18]. Eggshell extract works optimally at a rapid mixing of 150 rpm for 2 min and a slow mixing of 30 rpm for 2 min [15]. The highest performance of *Moringa oleifera* can be achieved through optimization using response surface methodology (RSM) with a dose of 0.34 mg/L, pH 6.93, rapid mixing of 135 rpm for 13.52 min, and sedimentation time for 113.15 min [19].

Optimization studies of coagulation–flocculation conditions have been conducted for different types of chemical coagulants/flocculants. In addition, reports on optimization for biobased coagulants/flocculants are currently increasing. However, research on these materials is still limited to the types of plant- and animal-based compounds. Optimization for bacteria-based bioflocculation, especially that from *S. marcescens*, has never been attempted. In addition, studies comparing the performance of bioflocculants in treating artificial versus real wastewater are limited. The current work aimed to analyze the characteristics bioflocculant produced by *S. marcescens* and optimize the coagulation–flocculation conditions to treat artificial wastewater (kaolin substrate) and real aquaculture effluent. Treatment comparison between the two types of wastewater was carried out through a two-level test comprising one variable at a time (OVAT) and RSM. The results benefit the science of wastewater treatment, especially in providing alternative biobased compounds to substitute currently used chemical-based coagulants/flocculants.

2. Materials and methods

2.1. Preparation of artificial wastewater, aquaculture effluent, and bioflocculation

Two types of wastewaters were used: artificial wastewater derived from kaolin substrate and real aquaculture effluent. Artificial wastewater was prepared by mixing 4 g of kaolin into 1 L of distilled water [20]. A sample of aquaculture effluent with pH of 7.6 ± 0.5, turbidity of 1112 ± 250 NTU, and total suspended solid (TSS) of 582 ± 125 mg/L was obtained from freshwater fishpond cultivating catfish in Negeri Sembilan, Malaysia. A bioflocculant solution was prepared by vortex mixing 1 mg of bioflocculant powder into 1 L of distilled water using a vortex (Stuart Scientific, UK) for ±2 min or until the solution was completely mixed. Details on the extraction bioflocculant from *S. marcescens* can be found in previous studies [21,22]. $CaCl_2$ solution with a concentration of 10 g/L was also prepared as a coagulant compound.

2.2. Characterization of bioflocculant

The bioflocculant was characterized by its charge (zeta potential) and protein, total carbohydrate, and organic contents. Zeta potential was analyzed by using a Zetasizer (MALVERN Instrument, UK). In brief, 1 mg of bioflocculant powder was added into 10 mL of distilled water to obtain 100 mg/L concentration. The sample was then pH adjusted using 1 M HCl (R&M Chemicals, Malaysia) or 1 M NaOH (R&M Chemicals, Malaysia) until the desired pH (1–14) was achieved [23] and transferred into a disposable zeta cell for analysis. Zeta potential charges were expressed in mV. An isoelectric curve was drawn by plotting between the obtained charges and the pH of the solution. The isoelectric point was then determined from the point where the pH shows a zeta potential of 0 Mv [24,25].

Protein content was analyzed using the protein–dye binding method [26]. A protein standard curve was drawn by preparing seven dilutions of bovine serum albumin (BSA) with amount varying from 0.05 to 0.5 mL. Distilled water was added to each tube until 0.5 mL of volume was achieved. Afterward, 2.5 mL of Bradford reagent was introduced, and the mixture was incubated on each tube for 5 min at room temperature until a blue solution appeared as a result of the formation of a protein–dye complex under acid conditions. The intensity of the blue color was measured with a UV spectrophotometer DR 6000 (HACH, USA) at 595 nm. Distilled water was used as control and given the same treatment. Total protein concentration was determined by mixing 0.5 mL of sample and 2.5 mL of Bradford reagent and then quantifying the protein using a spectrophotometer. The optical density (OD) reading from the sample was then plotted in the protein standard curve to determine the protein content in the sample, namely, a solution comprising 1 mg of bioflocculant in 10 mL of distilled water. The protein content was analyzed under pH 1 to 14. The readings of the protein content were then plotted in the isoelectric curve to obtain a comparison in various pH levels.

Total carbohydrate levels were analyzed using the phenol and sulfuric acid method of Swaroopanand et al. [27]. The total carbohydrate standard curve was drawn by preparing as many as 10 glucose standard solutions in 10 tubes (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 mL) with a concentration of 0.1 mg/mL. Each tube was then diluted to a volume of 1 mL. In brief, 1 mL of 5 % phenol (R&M Chemicals, Malaysia) and 5 mL of 96 % sulfuric acid (R&M Chemicals, Malaysia) were dropwise added to each tube, which was then shaken so that the phenol and sulfuric acid were well mixed. After 10 min, all tubes were placed in a water bath at 25 °C–30 °C for 15 min. Distilled water (1 mL) given the same treatment was prepared as control. The optical density (OD) of each tube was taken at a wavelength of 490 nm with a spectrophotometer and then plotted into a carbohydrate standard curve. Samples were prepared by mixing 1 mg of bioflocculant in 10 mL of distilled water. Afterward, 0.2 mL of the solution was taken and treated with the above-mentioned procedure. The OD reading from the sample was then plotted in the carbohydrate standard curve to determine the total carbohydrate content in the sample. Carbohydrate content was also analyzed under pH 1 to 14. The total carbohydrates readings were also plotted in the isoelectric curve to obtain a comparison of the total carbohydrate content in various pH levels.

Gas chromatography-mass spectrometry (GC–MS) was used to analyze the composition of monosaccharides and the type of linkages in the polysaccharide chain [28]. In brief, 1 mg of bioflocculant was dissolved in 1 mL of acetone (R&M Chemicals, Malaysia), vortexed (Memert+, Germany) for 30 min, and centrifuged (Memert+, Germany) at 5000 rpm for 15 min. The supernatant was filtered using a 0.22 μm membrane (Whatman, UK), and the clear filtrate was placed in a GC vial for later reading. GC–MS analysis of samples (1 μL) was carried out using an Agilent 7890A (GC)/Agilent 5975C (MS) and a liquid injection HP-5MS (P.N. 19091S-433UI) column. The GS conditions were as follows: the temperature was increased from 50 °C to 210 °C at a rate of 3 °C/min with an injection temperature of 280 °C, and helium was used as a carrier gas with a flow rate of 1.0 mL/min. The MS parameters were as follows: ionization energy 70 eV, transfer line temperature 280 °C, and scan mode range 40–250 *m/z*.

Liquid chromatography-mass spectrometry (LC-MS) analysis was

also performed to determine the content of organic molecular compounds and intermediate organic compounds [29]. The sample was prepared at a concentration of 1 mg/1 mL in distilled water. Separation was performed using a Thermo Scientific C18 column (Acclaim™ Polar Advantage II, 3 × 150 mm, 3 μm particle size) on an UltiMate 3000 UHPLC system (Dionex). Gradient elution was conducted at a flow rate of 0.4 mL/min and a column temperature of 40 °C using H2O + 0.1 % formic acid and 100 % acetonitrile with a run time of 22 min. The sample injection volume was 3 μL. Gradients started at 5 % acetonitrile (0–3 min); 80 % acetonitrile (3–10 min); 80 % acetonitrile (10–15 min) and 5 % acetonitrile (15–22 min). The system was equipped with a reversed-phase C18 column (Acclaim RepMap RSLC, 75 μm × 15 cm × 2 μm, 100A) and a HILIC column (LUNA HILIC, 150 × 4.6 mm, 3 μm 200 A). High-resolution MS was carried out using a Bruker Daltonic Micro-TOF QIII using ESI positive ionization with the following settings: capillary voltage 4500 V; nebulizer pressure 2.0 bar; drying gas 8 L/min at 300 °C, and range 50–1500 *m/z*. Compound readings were performed using the MetFrag web tool based on the PubChem database referencing MS and MS2 values.

2.3. Optimization of coagulation–flocculation conditions using one variable at a time (OVAT) analysis for artificial wastewater and aquaculture effluent

For each experiment, glass beakers (Pyrex, USA) with a volume of 500 mL filled with 200 mL a mixture of wastewater + coagulant + biocoagulant were used. In the optimization test using OVAT, only turbidity was read because it represents the state of total pollutant. Initial parameter reading was performed a second after the mixing of the three compounds, and final reading was conducted after the sedimentation time was over. Two types of wastewater, namely, artificial wastewater from kaolin substrate and aquaculture effluent, were used. These two types of wastewater were chosen to compare the performance of bioflocculant in treating wastewater that contains identified pollutants and real pollutants.

Seven factors, namely, bioflocculant dose, bioflocculant concentration, rapid mixing speed (RMS) and time (RMT), slow mixing speed (SMS) and time (SMT), and sedimentation time (ST), were analyzed at this stage. pH and temperature affecting the process were eliminated from the experiment because their evaluation require the use of energy and the addition of chemical compounds that could later have an influence when the wastewater is being reused in the aquaculture system. Turbidity removal was calculated using Eq. (1). The entire analysis was carried out using the jar test method.

$$Turbidity\ removal\ (\%) = \frac{Initial\ turbidity - Final\ turbidity}{Initial\ turbidity} \times 100\% \quad (1)$$

2.3.1. Influence of bioflocculant dose

Ten variables, namely, doses of 1 %, 2 %, 3 %, 4 %, 5 %, 6 %, 7 %, 8 %, 9 %, and 10 % of the total volume of the solution in the reactor, were tested at this stage. The dose of bioflocculant was adopted from literature s [20,30,31]. Dose is important in coagulation–flocculation to provide enough particles for mechanism interaction [25,32,33]. For each dose, a ratio of 3 ($CaCl_2$) to 2 (bioflocculant) was used. For example, for a bioflocculant dose of 1 %, 3 mL $CaCl_2$ + 2 mL bioflocculant +195 mL wastewater were used. The mixing sequence was performed by placing the wastewater first in a beaker, followed by $CaCl_2$ and then the bioflocculant. Other factors were locked at the values of 1 mg/L for bioflocculant concentration, 150 rpm RMS for 1 min, 10 rpm SMS for 10 min, and ST for 30 min. The optimum dose was analyzed statistically and then locked in the next stage.

2.3.2. Influence of bioflocculant concentration

The optimum biocoagulant dose was determined from previous OVAT test results. Five variations were tested for bioflocculant concentration, namely, 1, 10, 100, 1000, and 10,000 mg/L. The selected concentrations were based on previous studies, and a log range was used to cover their extremely large differences [34–36]. Similar to dose, the optimum concentration of the bioflocculant is required to facilitate the interaction mechanism [37,38]. The dose of $CaCl_2$ used remains the same ratio, with the factor of RMS locked at 150 rpm for 1 min, SMS of 10 rpm for 10 min, and ST for 30 min. The determination of the optimum bioflocculant concentration was done by statistical analysis.

2.3.3. Influence of RMS

Four variations were selected to be tested at this stage which includes 100, 150, 200, and 250 rpm based on the capabilities of the tool and the results of previous research [39–41]. RMS is very important factor to provide sufficient contact for bioflocculants and dissolved solids in wastewater [42,43]. The dose and concentration of the bioflocculant were locked at the optimum values, while the RMT was locked at 1 min, slow mixing at 10 rpm for 10 min, and ST at 30 min. The determination of the results of the optimum RMS in this experiment was done statistically.

2.3.4. Influence of RMT

After the optimum speed of rapid mixing was determined, as many as four variations (1, 2, 3, and 4 min) of RMT were selected for testing [44,45]. In addition to forceful collisions, time is important to facilitate the collision [42,43]. The dose and concentration of the bioflocculant and the RMS were locked at their optimum values, and the SMS was set at 10 rpm for 10 min and ST for 30 min. Statistical analysis was then applied to determine the optimum RMT.

2.3.5. Influence of SMS

At this stage, the dose and concentration of the bioflocculant, the speed and time of rapid mixing were locked in their optimum values. The influence of SMS, which refers to the capabilities of the tool and the results of previous research [44,46], was analyzed using four variations, namely, 10, 15, 20, and 25 rpm. SMS is important in providing interaction between the microflocs that form into macroflocs [47]. The optimum SMS was determined by statistical analysis and then locked as the optimum condition in the next stage.

2.3.6. Influence of SMT

Testing the influence of SMT was carried out after previous factors had been optimized. At this stage, as many as 4 variations of 5, 10, 15, and 20 min are used in the analysis. The determination of this variation was based on the results of previous research [18,44,48]. Sufficient time is required for the particles to form macroflocs, thus a SMT is very important in the flocculation process [49]. The optimum SMT was then also determined by statistical analysis.

2.3.7. Influence of ST

As the final stage of the OVAT test, as many as six variations of the ST were selected for testing, covering 15, 30, 45, 60, 120, and 180 min [50,51]. Optimum ST is required to provide sufficient duration for macroflocs to settle [11,43,52]. At this stage, the dose and concentration of bioflocculant, the speed and time of rapid mixing, and the speed and time of slow mixing were locked at their optimum values. The optimum ST was then determined by statistical analysis.

2.4. Optimization of coagulation–flocculation conditions using RSM analysis

Readings of turbidity, TSS, and flocculating activities were carried out during RSM. For the reading of flocculating activity, a control in the form of wastewater + $CaCl_2$ + distilled water only (with the same composition as the experimental reactor) was used. After the OVAT test, three factors were selected to be further analyzed as factors in RSM optimization. These factors were chosen through a comparison of *p*

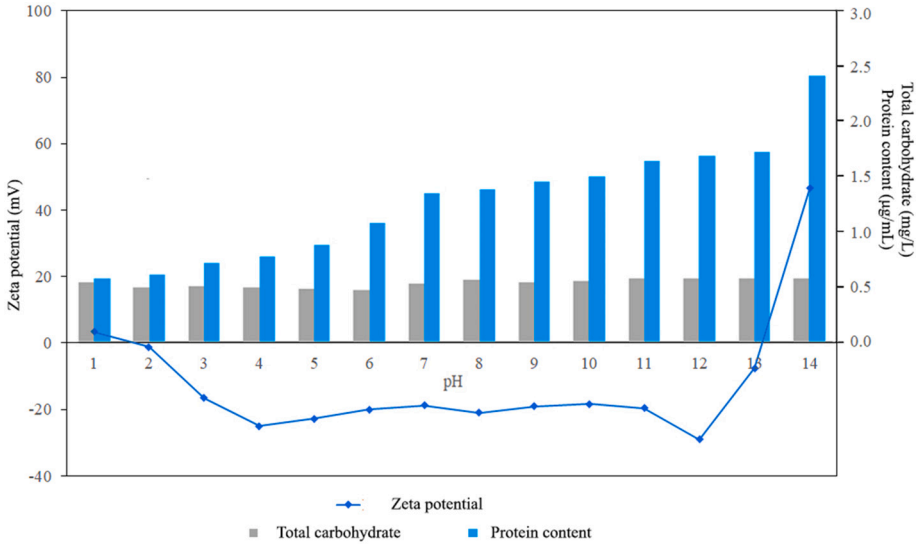

Fig. 1. Isoelectric curve, protein content, and total carbohydrate of bioflocculant from *Serratia marcescens*.

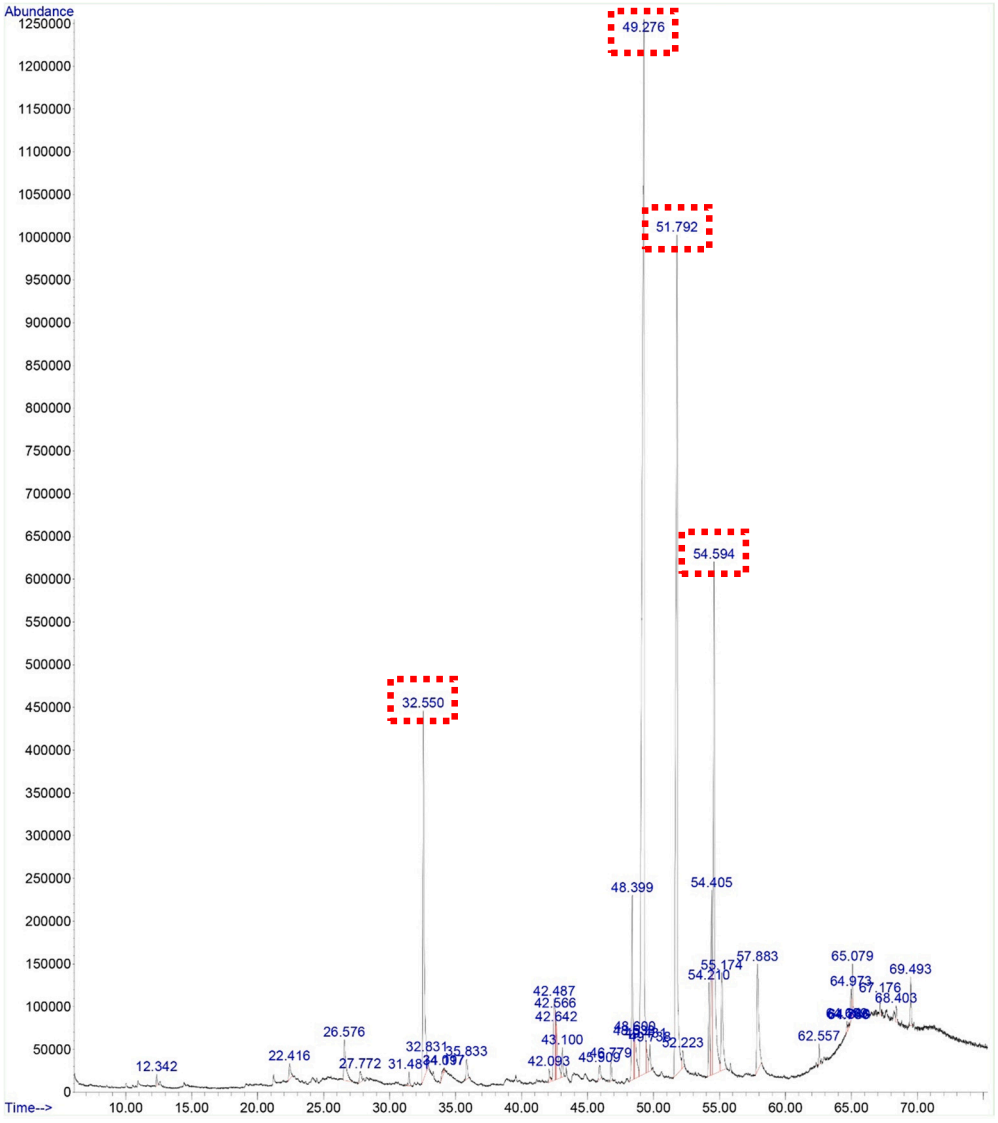

Fig. 2. GC–MS spectrum of bioflocculant from *Serratia marcescens*.

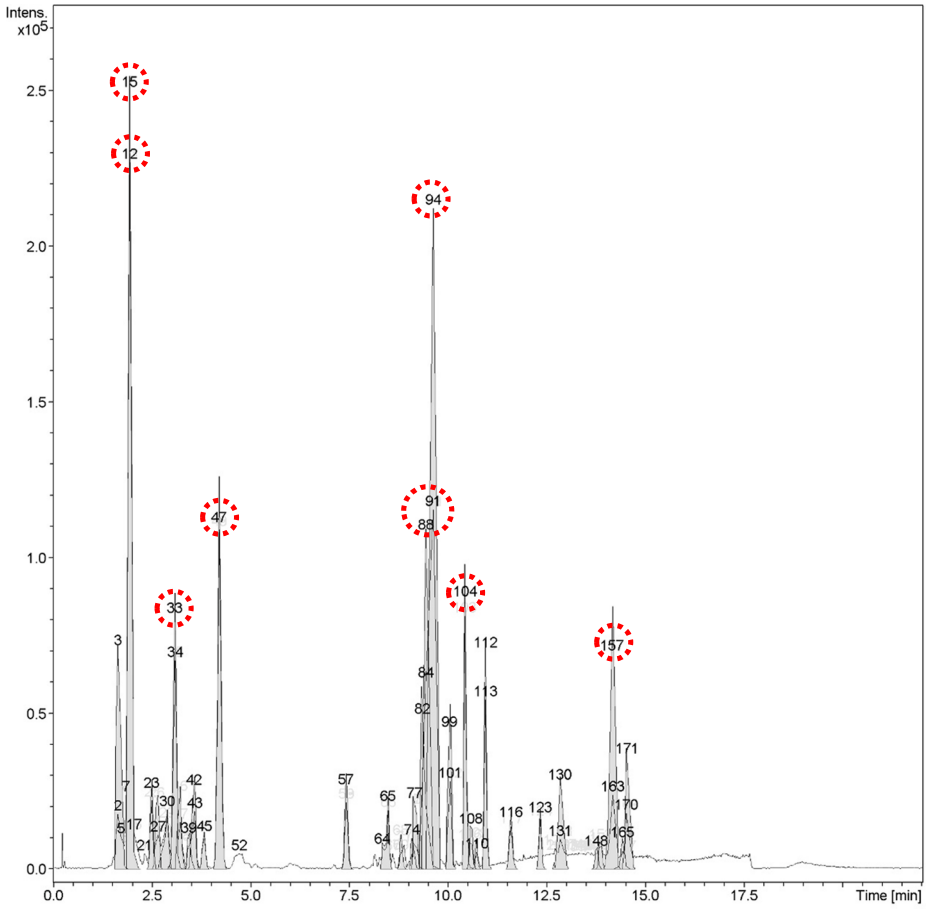

Fig. 3. LC-MS spectrum of bioflocculant from *Serratia marcescens*.

Table 1
Summary of dominant compounds detected by GC–MS.

Retention time (min)	Identified compound	Molecular weight (g/mol)
32.550	Phenol, 2,4-bis-(1,1-dimethylethyl)	278.5
49.276	n-Hexadecanoic acid	256.4
51.792	9-Hexadecenoic acid	254.4
54.594	Oleic acid	282.5

Table 2
Summary of dominant compounds detected by LC-MS.

Wave number	Compound	Molecular weight (g/mol)
12	6-[2-(Methylamino)-2-oxoethyl] sulfanylhexanoic acid	219.3
15	Benzyl-hydroxy-dimethyl-lambda4-sulfane	170.27
33	(1-Methylpyridin-1-ium-3-yl)methyl carbamimidothioate	182.27
47	3-(3-Methylidenepentyl)thiophene	166.29
88	N-butyl-3,3-dimethylbutan-2-amine	157.3
91	4,5-Dichloro-6-(trichloromethyl)triazine	267.3
94	3-Ethyl-4,5-diiodothiazole-2(3H)-thione	397.0
104	Hydroxy-trimethyl-pentadecyl-lambda5-phosphane	304.5
157	4-(Octylsulfonylamino)butanoic acid	279.4

values, where a smaller value indicates a more significant influence [53]. At this stage, RSM was chosen because it can optimize and analyze interactions between factors. Meanwhile, the Box Behnken Design (BBD) was adopted because it can reduce the number of test reactors. In addition, the range to be applied was determined beforehand through the OVAT test. The RSM model with BBD was run with three responses, namely, turbidity removal (Eq. (1)), TSS removal (Eq. (2)), and flocculating activity denoted in Eq. (3) as *y1*, *y2*, and *y3*, respectively. TSS concentration was determined using a spectrophotometer (HACH, USA). After the optimum conditions for all factors were determine, a comparison was made between artificial wastewater and aquaculture effluent.

$$TSS\ removal\ (\%) = \frac{Initial\ TSS - Final\ TSS}{Initial\ TSS} \times 100\% \quad (2)$$

$$Flocculating\ activity\ (\%) = \frac{Final\ turbidity\ in\ control - Final\ turbidity}{Final\ turbidity\ in\ control} \times 100\% \quad (3)$$

2.5. Statistical analysis

Statistical tests were performed to determine the optimum conditions for each factor in the OVAT test. All obtained data were tested for distribution, homogeneity, and independence and identified as parametric. One-way ANOVA was conducted for each factor at a confidence interval of 95 % ($\alpha = 5$ %). Significance was detected using *p*-values, and comparison of *p*-values was applied to determine the most influential factors [53]. A post-hoc test was performed for the significant factor using Tukey HSD analysis to determine the optimum condition in each factor. For the RSM test, the statistical analysis built into the model was used.

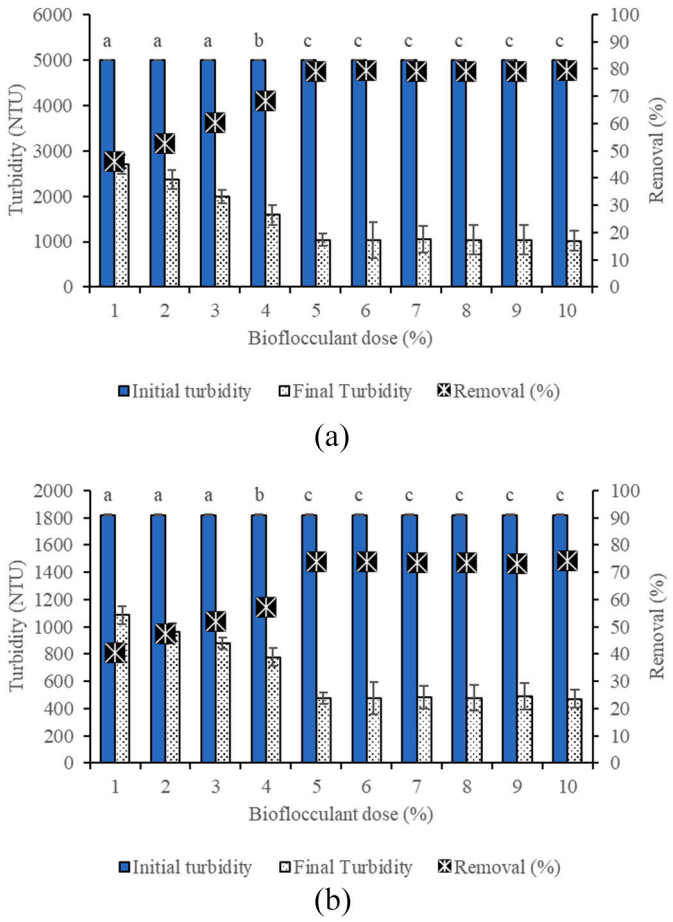

Fig. 4. Influence of bioflocculant dose on (a) kaolin and (b) aquaculture effluent treatment. Values shown are mean ± SD. Different letters above the graph (a–c) indicate significant differences in turbidity removal between bioflocculation doses based on ANOVA ($p \leq 0.05$).

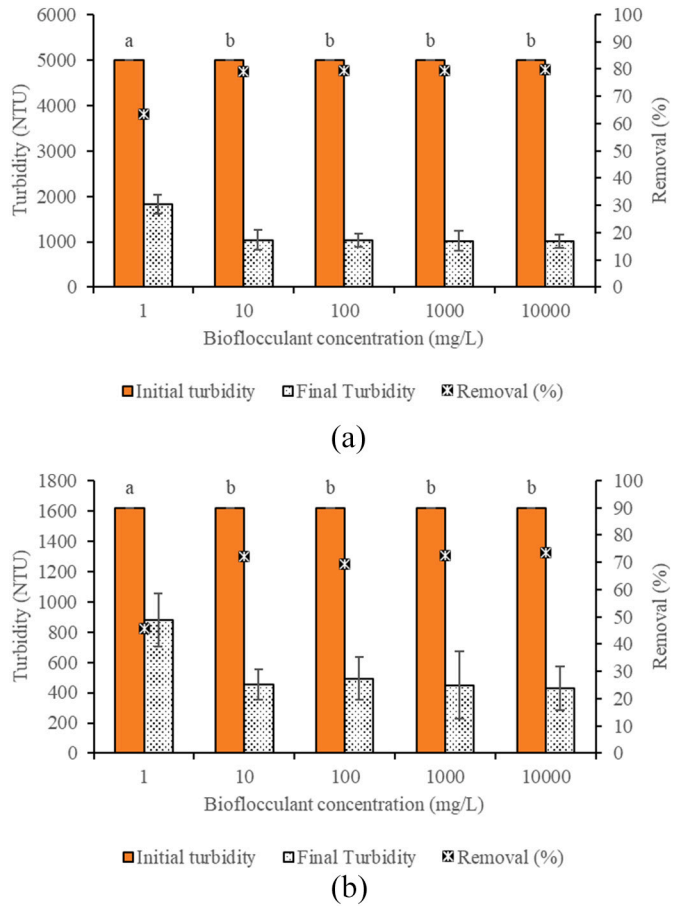

Fig. 5. Influence of bioflocculant concentration on (a) artificial wastewater and (b) aquaculture effluent treatment. Values shown as mean ± SD. Different letters above the graph (a–b) indicate significant differences in turbidity removal between bioflocculation concentration based on ANOVA ($p \leq 0.05$).

3. Results and discussion

3.1. Bioflocculant characteristics

3.1.1. Zeta potential

The results of zeta potential, protein content, and total carbohydrate are shown in an isoelectric curve illustrated in Fig. 1. The bioflocculant produced by *S. marcescens* was an anionic type of flocculant with ζ potential of −18.77 ± 1.45 mV in pH 7. The charge of the obtained bioflocculant was negative, although it was moderately negatively charged compared with other bioflocculants produced by bacteria. *Enterobacter* sp. ETH-2 produces bioflocculants with a charge of −28.7 ± 8.23 mV [54], *Bacillus amyloliquefaciens* DT produces bioflocculants with a zeta potential of −33.67 ± 0.90 mV [55], and *Bacillus licheniformis* produces bioflocculants with a charge of −48.3 mV [56]. Kaolin substrate and aquaculture effluent have a pH range of 6–8 [57], so the zeta potential results in this range were further discussed.

As shown in Fig. 1, the isoelectric point of the bioflocculant was at pH 1.7 and 13.3. Therefore, its working mechanism changes in systems with pH <1.7 or >13.3 because it has a positive charge at these pH levels. For other pH ranges, the bioflocculant shows a negative charge so it works as an anionic flocculant, such as for kaolin substrate and aquaculture effluent. Most anionic flocculants work through bridging and patching mechanisms when applied to systems that also have a negative charge [1,58]. Bridging facilitates agglomeration by entangling the particles into a large matrix [59], and patching facilitates agglomeration by providing pin connection-like interaction between particles to form large sized ones [60]. The bioflocculant can work through different mechanisms (most probably via charge neutralization) on acid mine wastewater (which has a highly acidic pH) [61] or tannery wastewater (which has a highly alkaline pH) [62,63] because of the opposite charge between particles and the bioflocculant.

3.1.2. Protein content

The standard curve for protein content can be found in supplementary materials (Fig. S1). As shown in Fig. 1, the protein content in the bioflocculant produced by *S. marcescens* increased with the pH. At neutral pH (pH 7), the measured protein content was 1.3 μg/mL. The highest protein content of 2.4 μg/mL was obtained at pH 14. The increase in protein content was observed because the solubility of proteins increases with the pH. Sharma et al. [64] tested protein solubility at pH 4 to 10 and found an increase in protein content from 5 % to 82 %. Singh et al. [65] also noted an increase in protein content from pH 5.5 (3.5 mg/mL) to pH 8 (4.2 mg/mL). At neutral pH, proteins can contribute to flocculation through a bridging mechanism, in which they act as high-molecular-weight compounds. At base pH, they can contribute to coagulation through a sweep mechanism due to deprotonation [66].

3.1.3. Total carbohydrates

The standard curve for total carbohydrate content is shown in Fig. S2. As illustrated in Fig. 1, the amount of total carbohydrates decreased when the pH was close to neutral and then increased again at base pH. The highest total carbohydrate content of 0.58 mg/L was obtained at pH 14, and the lowest content of 0.47 mg/L was obtained at pH 6. Hameed et al. [67] stated that total carbohydrate content tends to

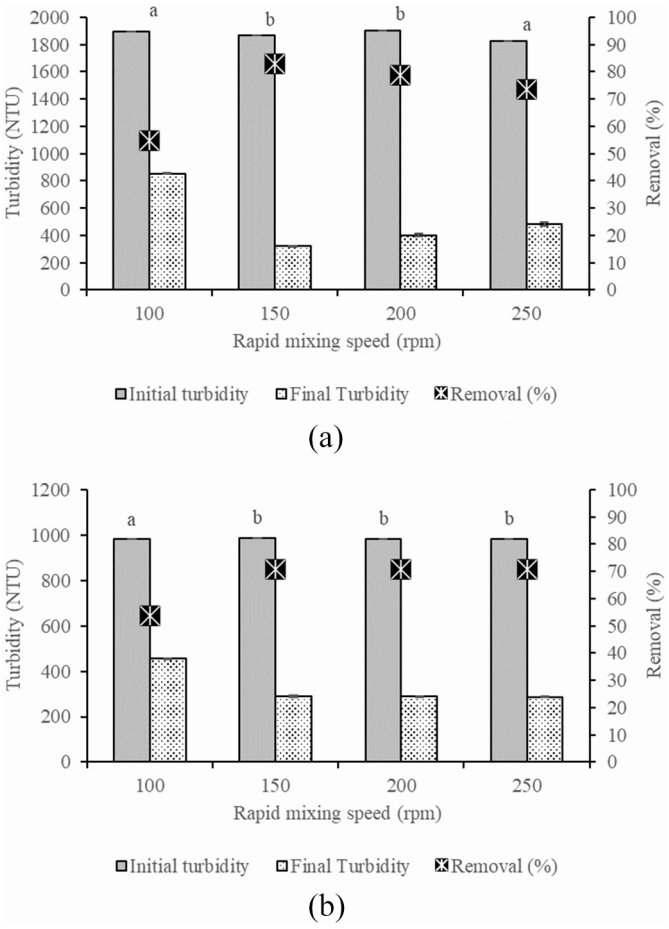

Fig. 6. Influence of RMS on (a) artificial wastewater and (b) aquaculture effluent treatment. Values shown as mean ± SD. Different letters above the graph (a–b) indicate significant differences in turbidity removal between RMS based on ANOVA ($p \leq 0.05$).

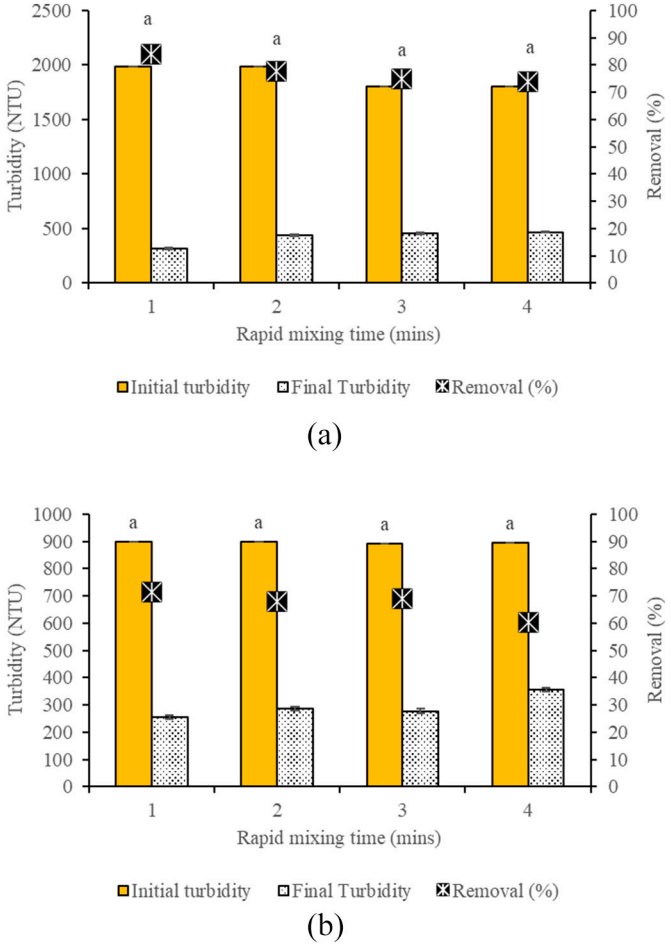

Fig. 7. Influence of RMT on (a) artificial wastewater and (b) aquaculture effluent treatment. Values shown as mean ± SD. Same letters above the graph (a) indicate no significant differences in turbidity removal between RMT based on ANOVA ($p > 0.05$).

decrease at near-neutral pH and increase again at base pH. Carbohydrates can contribute to coagulation or flocculation as surface-active compounds or high-molecular-weight particles [68].

3.1.4. Organic compounds

The GC–MS spectrum is shown in Fig. 2, and the LC-MS spectrum is displayed in Fig. 3. A summary of the compound readings can be found in Tables 1 and 2. GC–MS readings revealed four dominant compounds in the bioflocculant: one alcohol-based compound and three carboxylic acid-based compounds. The existence of alcohol may also contribute to the negative charge of the bioflocculant. Tyagi et al. [69] mentioned that sitosterol acetate is one of the compounds in extracellular polymeric substance (EPS) produced by *Parapedobacter* sp. ISTM3. Ali et al. [70] stated that hexadecanoic acid is the main content of EPS produced by *Pseudomonas aeruginosa*. Li et al. [71] mentioned that acetic acid is the main compound of EPS produced by *Agrobacterium* sp. M-503, and Bafana [72] also reported that galacturonic acid can be found in EPS produced by *Chlamydomonas reinhardtii*.

Similar to the GC–MS readings, LC-MS results showed the presence of compounds based on carboxylic acids (peak numbers 112 and 157). Bacterial EPS is a mixture of polysaccharides, proteins, and nucleic acids; its carboxylic acid content can indicate its polysaccharide content [73]. Carboxylic acid is an intermediate compound for sugar degradation and also the result of fermentation or enzymatic reactions [74]. Other compounds detected by LC-MS were degradation metabolites or intermediate compounds, which also constituted the protein content in bioflocculants. For example, the compound with peak number 33 is part of an enzyme that refers to a catabolism reaction (PubChem MESH: D004798). The compound with peak number 47 is also part of an enzyme that catalyzes molecular assembly (PubChem MESH:D013930).

All detected compounds are intermediate compounds that have a low molecular weight [75]; however, high-molecular-weight compounds, such as sugars and proteins, might have been present but not detected. The readings of these organic compounds can support previous readings about the presence of proteins and polysaccharides and their characterization into specific compounds. Feng et al. [76] and Guo et al. [77] mentioned that a high-molecular-weight compound can provide a strong bridging action for pollutant particles and facilitate agglomeration via bridging mechanisms.

3.2. OVAT analysis

3.2.1. Influence of bioflocculant dose

The results of the influence of bioflocculant dose on the treatment of artificial wastewater can be found in Fig. 4a. Significant difference in removal was found under doses of 1 %, 4 %, and 5 %, ($p \leq 0.05$); insignificant differences were observed among the other doses (5 % up to 10 %). This finding indicated that increasing the bioflocculant dose beyond 5 % can no longer provide a significant impact on the agglomeration process. The removal value decreased at doses of 7 % (79 ± 4.72 %) and 8 % (79.16 ± 5.24 %) compared with that at a dose of 5 % (79.2 % ± 2.32 %), showing that the optimum dose of bioflocculant was 5 %. Further increasing the dose of bioflocculant will not improve and can

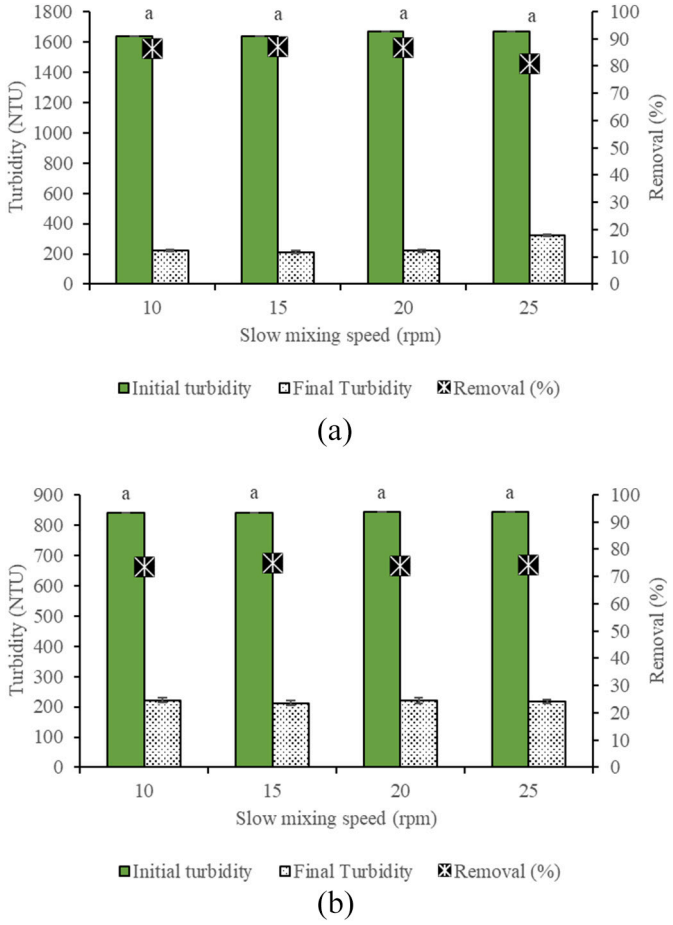

Fig. 8. Influence of SMS to (a) artificial wastewater and (b) aquaculture effluent treatment. Values shown are mean ± SD. Same letters above the graph (a) indicate no significant differences in turbidity removal between SMS based on ANOVA ($p > 0.05$).

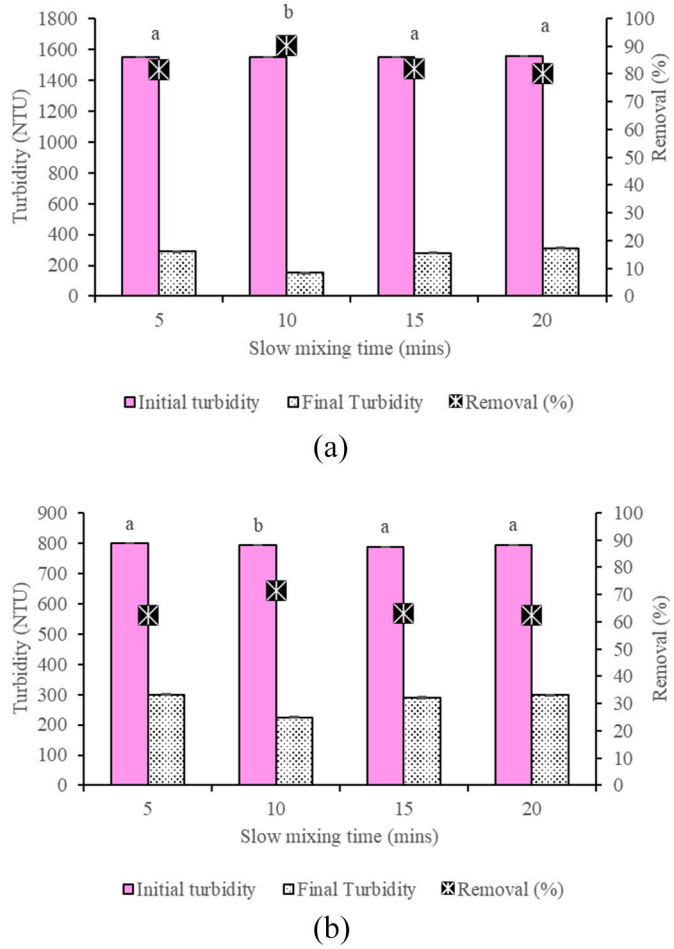

Fig. 9. Influence of SMT to (a) artificial wastewater and (b) aquaculture effluent treatment. Values shown as mean ± SD. Different letters above the graph (a-b) indicate significant differences in turbidity removal between SMT based on ANOVA ($p \leq 0.05$).

even decrease the performance of turbidity removal because the non-interacting bioflocculant will remain as suspended compounds that can increase turbidity [78]. Excess dosage can also cause a repulsive force that appears due to excess charge similarity and can reduce the performance of turbidity removal [12,47].

Similar results were obtained for the influence of bioflocculant dosage on the treatment of aquaculture effluent (Fig. 4b). Increasing the dose up to 5 % had a significant impact on the removal of turbidity ($p \leq 0.05$). Turbidity removal of 73.77 % ± 1.88 % was obtained at a dose of 5 %, but increasing the dose up to 10 % showed a removal of 74.15 % ± 2.96 %. The use of an excessive dose of bioflocculant does not have a positive impact on turbidity removal and can even lower the performance because the residues of the bioflocculant failing to interact actually contribute to the turbidity in the suspension [39].

3.2.2. *Influence of bioflocculant concentration*

For bioflocculant concentration, 10 mg/L showed a significant influence on the treatment of artificial wastewater ($p \leq 0.05$) (Fig. 5a). No significant increase in turbidity removal was observed at concentrations higher than 10 mg/L. This finding indicated that 10 mg/L was the optimum concentration that can produce turbidity removal of 79.2 % ± 3.46 %. Further increasing the bioflocculant concentration beyond this value does not affect the removal because the particles present are enough to support the interaction mechanisms during flocculation [79,80].

Similar results were also obtained for aquaculture effluent treatment (Fig. 5b). The optimum concentration was found to be 10 mg/L, which showed a significant difference in turbidity removal compared with 1 mg/L ($p \leq 0.05$) and an insignificant difference compared with 100, 1000, and 10,000 mg/L. Turbidity removal at the optimum concentration reached 71.98 % ± 5.14 %, and increasing the concentration to 10,000 mg/L only lead to an increase of only 73.52 % ± 7.16 %. An increase in the concentration of bioflocculants can have an impact on the charge of the solution due to the addition of compounds [81,82], but the presence of excess charge can cause a repulsive force that has a negative impact on the removal of turbidity [60,83]. In this research, $CaCl_2$ was used as coagulant to facilitate the initial agglomeration of particles. The substitution of $CaCl_2$ with a biocoagulant compound is highly suggested to provide a complete biobased treatment and to assess the performance of combined biocoagulant and bioflocculant in treating wastewater.

3.2.3. *Influence of RMS*

The variation of RMS had a significant effect on the removal of turbidity ($p \leq 0.05$) for the treatment of artificial wastewater (Fig. 6a). Variation of 150 rpm showed a significant difference in the removal of turbidity (82.8 % ± 4.4 %) compared with 100 rpm but not compared with 200 rpm. A significant decrease was found at the RMS of 250 rpm (73.65 % ± 11.65 %). This finding showed that the speed of 150 rpm was enough to facilitate the particles to collide in the system to form microflocs [84]. Increasing the speed up to 250 rpm even inhibits the formation of flocs caused by too much shear force [85]. Excessive shear force can break the bond between the thickener and the particles in the system, thus lowering the coagulation–flocculation performance [86].

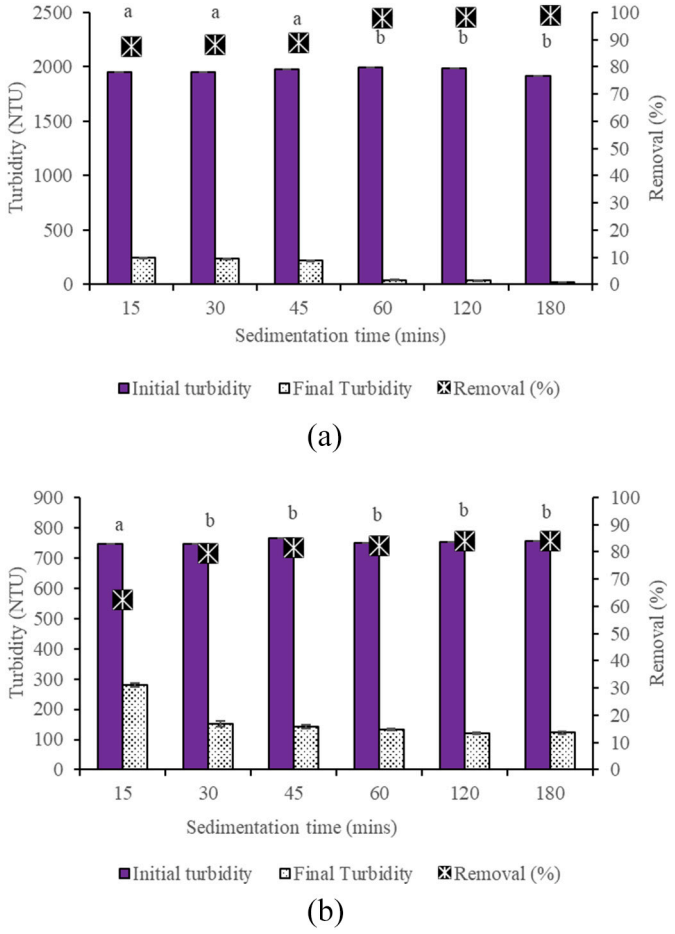

Fig. 10. Influence of ST to (a) kaolin and (b) aquaculture effluent treatment. Values shown are mean ± SD. Different letters above the graph (a-b) indicate significant differences in turbidity removal between ST based on ANOVA ($p \leq 0.05$).

Table 3
Recapitulation of OVAT results.

Factor	Selected optimum condition for artificial wastewater	*p*-Value	Selected optimum condition for aquaculture effluent	*p*-Value
Bioflocculant dose (%)	5	0.026	5	0.029
Bioflocculant concentration (mg/L)	10	0.039	10	0.032
RMS (rpm)	150	0.022	150	0.001
RMT (min)	1	0.447	1	0.378
SMS (rpm)	10	0.728	10	0.998
SMT (min)	10	0.001	10	0.002
ST (min)	60	0.000	30	0.004

For the treatment of aquaculture effluent (Fig. 6b), the optimum RMS was at 150 rpm, which showed significant difference compared with 100 rpm ($p \leq 0.05$) and but not with 200 and 250 rpm. The results were quite similar to those for the treatment of artificial wastewater where the optimum point was also found at an RMS of 150 rpm (70.65 % ± 4.4 %). Therefore, 150 rpm can provide enough force for particles to collide; further increasing the RMS will not increase the performance of floc formation [85].

3.2.4. Influence of RMT

The results of the influence of RMT on artificial wastewater treatment can be found in Fig. 7. No significant differences were found for each time variation, and a decrease was found for turbidity removal performance. Turbidity removal performance was 83.85 % ± 6.49 % for 1 min and 74.04 % ± 5.52 % for 4 min. This phenomenon may have occurred because the optimum RMS had been applied, and the collision between particles occurred without difficulty. However, a long RMT can break the agglomeration that have formed between particles [86]. On the basis of these results, an RMT of 1 min was chosen as the optimum condition because it provides the highest removal performance. Further increase in mixing time can lead to an increase in energy consumption.

RMT also did not have a significant impact on turbidity removal for aquaculture effluent treatment ($p > 0.05$). Increasing RMT even decreased the turbidity removal performance (Fig. 7b). Turbidity removal at 1 min of RMT reached 71.49 % ± 6.49 %, and increasing the time up to 4 min even resulted in a reduced turbidity removal of 60.18 % ± 5.52 %. This finding showed that the shearing force that occurred during coagulation–flocculation at 4 min RMT was too much and caused a disconnection from the agglomeration that had occurred [87].

3.2.5. Influence of SMS

Similar to the RMT, the variation of the SMS also did not have a significant impact on the removal of turbidity for artificial wastewater treatment ($p > 0.05$) (Fig. 8a). The turbidity removal was 86.41 % ± 6.49 % at a speed of 10 rpm and decreased to 80.79 % ± 5.52 % at a speed of 25 rpm. Therefore, the SMS of 10 rpm provides the optimum force for macrofloc formation; increasing the value to 25 rpm provides too high shear force that can reduce the performance of turbidity removal [39].

SMS also did not have a significant impact on the removal of turbidity in aquaculture effluent treatment (Fig. 8b). Optimum conditions were chosen at a SMS of 10 rpm because increasing the speed did not provide a significant increase in removal. Turbidity removal reached 73.57 % ± 6.49 % at a speed of 10 rpm and 74.11 % ± 5.52 % at a speed of 25 rpm. This finding showed that the speed of 10 rpm was sufficient for providing the force for the interaction between particles in the formation of macroflocs [78].

3.2.6. Influence of SMT

In testing the influence of SMT for artificial wastewater treatment, it was found that a time of 10 min had a significant impact as compared to 5 min ($p \leq 0.05$), while increasing the time up to 20 min even decreased the turbidity removal performance (Fig. 9a). The highest turbidity removal was 90.19 ± 0.45 %. This shows that 5 min was not enough to facilitate the interaction between particles and that excessive time has a negative impact on the formation of flocs. Similar to the previous discussion that too much shear force can cause the interaction between particles to be broken which lowers the turbidity removal performance [39].

The results of the influence of SMT for aquaculture effluent treatment were similar to those for artificial wastewater, where the optimum point was found at 10 min (Fig. 9b). The removal of turbidity was obtained as much as 71.59 ± 1.22 % obtained at optimum conditions. SMT has an influence on floc formation due to low gradient velocity conditions which can facilitate aggregation in floc formation [87].

3.2.7. Influence of ST

ST has an important role in providing time for the flocs that have formed to settle. A significant difference was found for the increase in ST from 45 to 60 min for artificial wastewater treatment ($p \leq 0.05$), while the other results showed no significant difference (Fig. 10a). Turbidity removal of 97.99 ± 2.26 % was obtained at optimum conditions, while adding time up to 180 min only increased removal up to 98.85 ± 1.9 %. This showed that the entire floc that can settle has managed to settle optimally within 60 min [43,88]. Referring to this result, then 60 min

Table 4
Results of analysis based on BBD suggestion for artificial wastewater treatment.

Std	Run	RMS (rpm)	SMT (min)	ST (min)	Turbidity removal (%)	TSS removal (%)	Flocculating activity (%)
9	1	150	7.5	50	89.2	92.6	78.1
6	2	175	10	50	88	91.3	77
17	3	150	10	60	99.2	99	81.2
8	4	175	10	70	100	100	82.4
11	5	150	7.5	70	95.6	97	81.6
16	6	150	10	60	99.6	99.5	82.2
10	7	150	12.5	50	89.2	95	78.7
12	8	150	12.5	70	99.2	99.6	81.8
3	9	125	12.5	60	94	96	80.6
5	10	125	10	50	90.8	95	78.6
15	11	150	10	60	99.4	99	82.3
7	12	125	10	70	93.6	94.2	79.2
2	13	175	7.5	60	94.2	96.2	81
14	14	150	10	60	99.4	99.5	81.8
13	15	150	10	60	99.6	99.5	81.2
4	16	175	12.5	60	96.6	98.9	80.4
1	17	125	7.5	60	92.1	94	78.2

was taken into account as the optimum ST for artificial wastewater treatment.

The results of the influence of ST for aquaculture effluent treatment can be seen in Fig. 10b. In contrast to the OVAT results for artificial wastewater, the optimum ST for aquaculture wastewater treatment was found to be 30 min. Adding time up to 180 min did not show a significant impact ($p > 0.05$). The shorter time indicates that the sedimentation speed for flocs in aquaculture wastewater was higher compared to artificial wastewater [89,90]. The removal of turbidity at a ST of 30 min was 79.60 ± 8.02 %, while increasing the ST up to 180 min only shows 83.86 ± 2.8 %.

3.3. OVAT results

The overall OVAT results for artificial wastewater and aquaculture effluent showed similar outcomes, that is, the variations of bioflocculant dose and concentration, RMS, SMT, and ST induced significant difference in turbidity removal as summarized in Table 3. According to the highest significance value (based on the lowest p value) [91,92], the three factors selected to be detailed further in RSM were RMS, SMT, and ST. The optimum conditions based on the results of OVAT for the treatment of artificial wastewater were bioflocculant dose of 5 % with a concentration of 10 mg/L, RMS of 150 rpm for 1 min, SMS of 10 rpm for 10 min, and ST of 60 min with a turbidity removal of 97.99 % ± 2.26 %. For aquaculture effluent treatment, the optimum conditions were bioflocculant dosage of 5 % with a concentration of 10 mg/L, RMS of 150 rpm for 1 min, SMS of 10 rpm for 10 min, and ST of 30 min with a turbidity removal of 79.60 % ± 8.02 %.

3.4. Optimization using RSM

3.4.1. Optimization of artificial wastewater treatment conditions

On the basis of the OVAT results, RSM was conducted with three factors, namely, RMS, SMT, and ST. The respective ranges used were 125–175 rpm for RMS, 7.5–12.5 min for SMT, and 50–70 min of ST. Other factors were run under optimum conditions based on OVAT. Seventeen reactors were suggested by BBD. The results of the analysis for each response according to the run on RSM can be found in Table 4.

All data obtained were then run without transformation with $y1$ = turbidity removal, $y2$ = TSS removal, and $y3$ = flocculating activity. RSM suggested the quadratic model to be fit with the results. For all responses, ANOVA showed significant value for the selected model and nonsignificant values for lack of fit. For turbidity removal, the R^2 value was 0.9978 with difference between the adjusted R^2 and predicted R^2 at 0.025. For TSS removal, the R^2 value was 0.9872 with difference between the adjusted R^2 and predicted R^2 at 0.1406. For flocculating activity, the R^2 value 0.9701 with the difference between the adjusted R^2 and predicted R^2 at 0.0688. According to the RSM criteria, the selected model showed good results.

ANOVA also showed that the factor of RMS and SMT did not have a significant influence on the flocculating activity. Meanwhile, all other factors had a significant influence on the response. For turbidity removal, the interaction between the factor of RMS and SMT did not have a significant influence on the response. For the response of TSS removal and flocculating activity, only the interaction between RMS and settling time had a significant influence on the response. The interaction between factors in the 3D models are shown in Figs. 11–13. The optimized equations obtained for turbidity removal ($y1$), TSS removal ($y2$), and flocculating activity ($y3$) referring to the quadratic model for artificial wastewater are expressed as Eqs. (4) to (6).

$$y1 = 99.44 + 1.0375*A + 0.9875*B + 3.9*C + 0.125*AB + 2.3*AC + 0.9*BC - 2.7075*A2 - 2.5075*B2 - 3.6325*C2 \quad (4)$$

$$y2 = 99.3 + 0.9*A + 1.2125*B + 2.1125*\mathrm{C} + 0.175*AB + 2.375*AC + 0.05*BC - 1.975*A2 - 1.05*B2 - 2.2*C2 \quad (5)$$

$$y3 = 81.74 + 0.525*A + 0.325*B + 1.575*C - 0.75*AB + 1.2*AC - 0.1*BC - 1.22*A2 - 0.47*B2 - 1.22*C2 \quad (6)$$

Solutions of optimum conditions for the three factors were run under the criteria of RMS: in range, SMT: in range, ST: in range, turbidity removal: maximum, TSS removal: maximum, and flocculating activity: maximum. RSM suggested 100 solutions for the optimum conditions of artificial wastewater treatment, and the one with the highest desirability of 1000 was chosen as shown in Fig. 14.

As shown in Fig. 14, the optimum conditions obtained were RMS of 160.865 rpm, SMT of 11.4688 min, and settling time of 67.3273. These results were then summarized and rounded into a bioflocculant dose of 5 % (v/v), bioflocculant concentration of 10 mg/L, rapid mixing 160 rpm for 1 min, slow mixing 10 rpm for 12 min, and settling time 67 min. A validation test was then performed in accordance with the summary results of the optimum conditions. The findings showed that turbidity removal was 99 % (error 2.1 %), TSS removal was 99 % (error 1.8 %), and flocculating activity was 72.8 % (error 11.7 %). Ribardo and Allen [93] stated that the error obtained from the maximum model was as much as 20 % referring to the Harrington function evaluation scale, which still shows an acceptable and good value and represents exceptional quality and performance.

3.4.2. Optimization of aquaculture effluent treatment conditions

On the basis of the OVAT results for aquaculture effluent treatment,

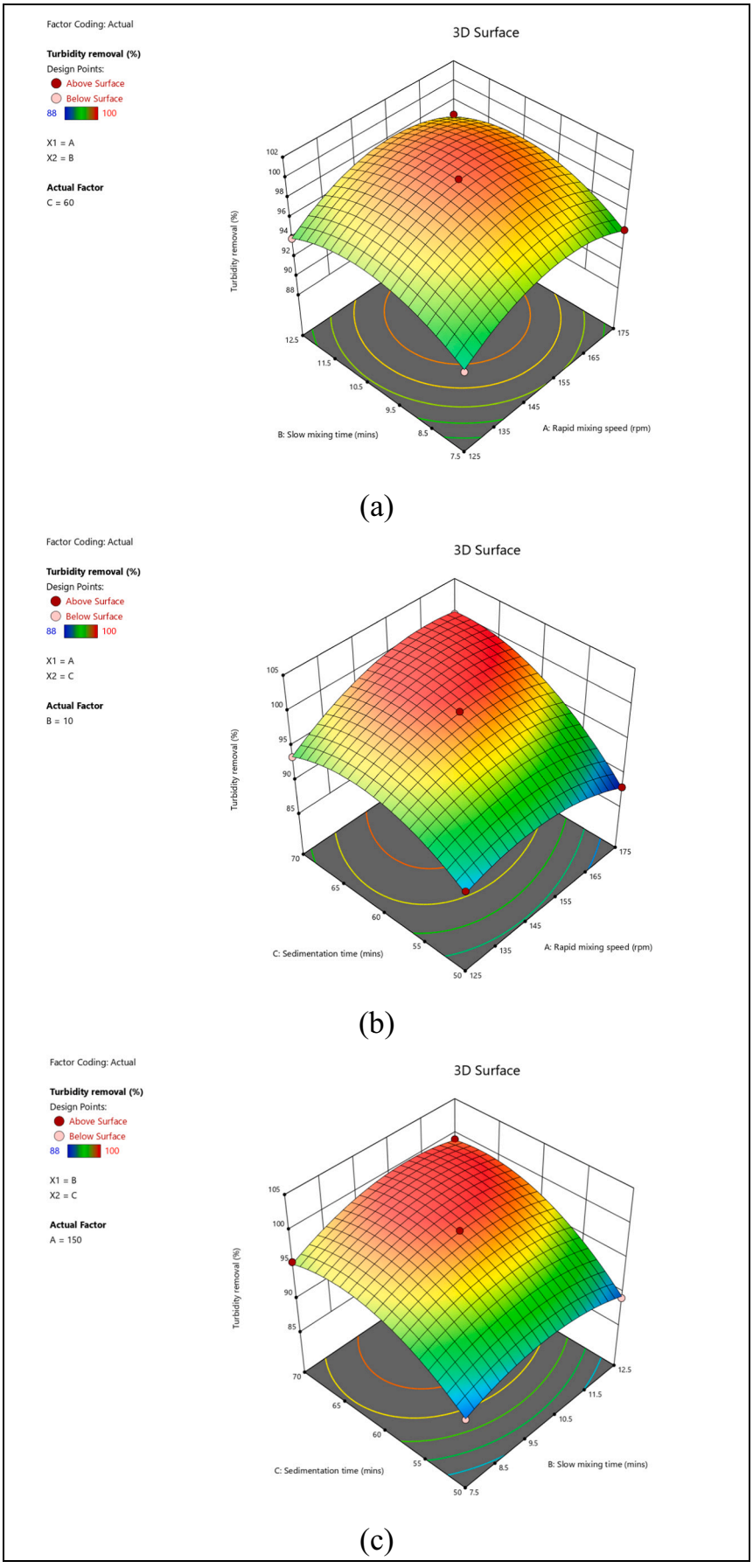

Fig. 11. Interaction between (a) RMS and SMT, (b) RMS and settling time, (c) SMT and settling time for turbidity removal.

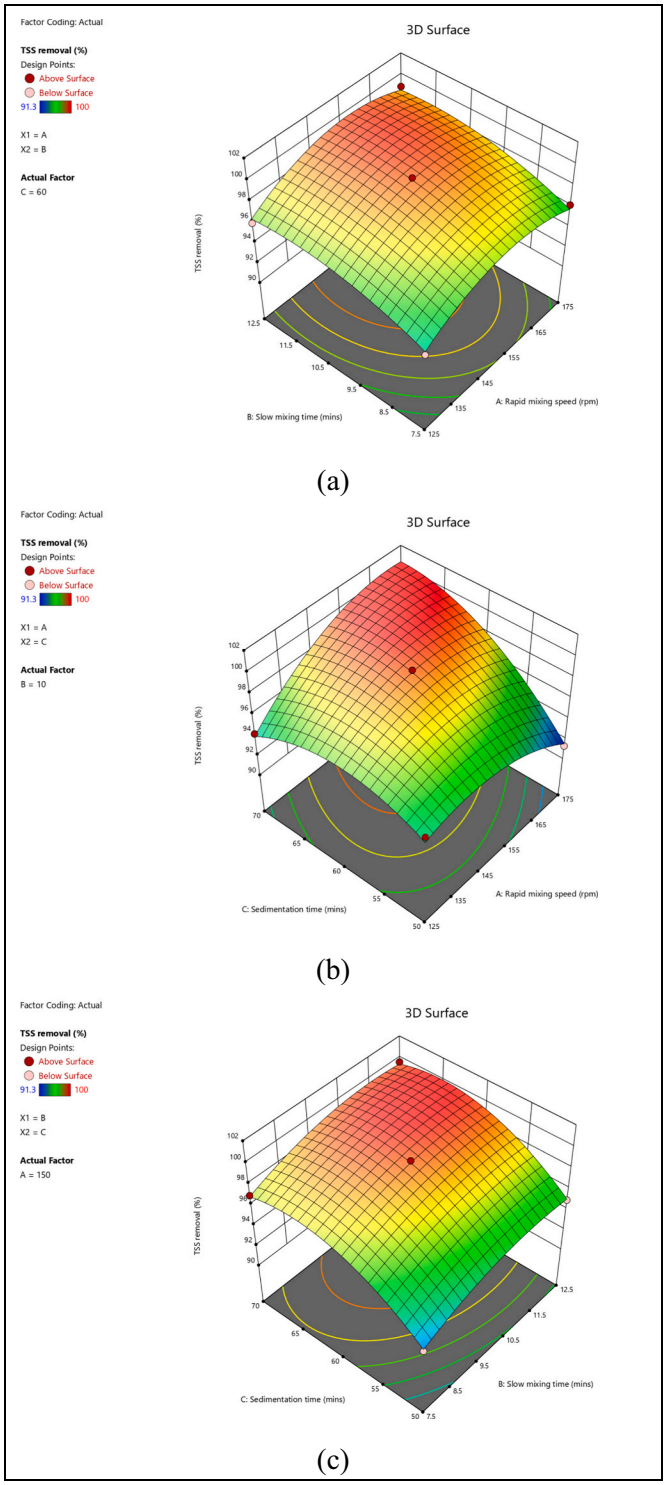

Fig. 12. Interaction between (a) RMS and SMT, (b) RMS and settling time, (c) SMT and settling time for TSS removal.

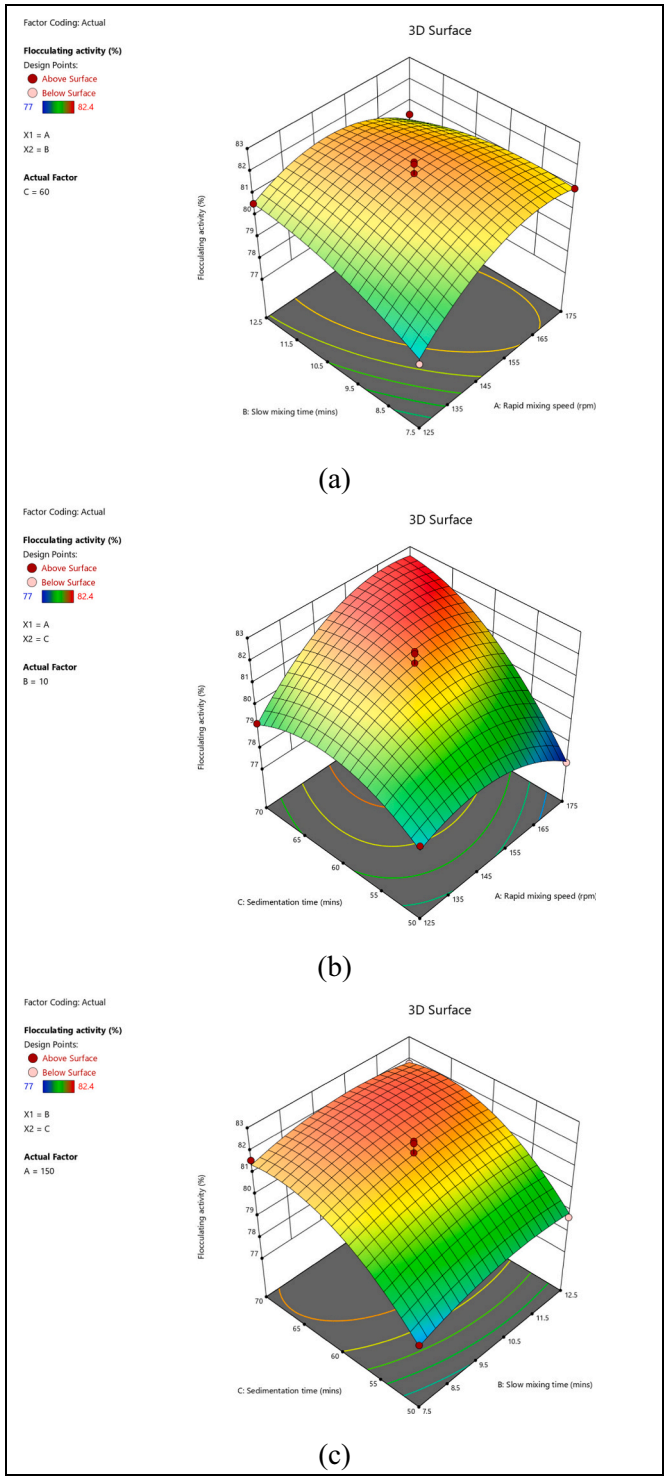

Fig. 13. Interaction between (a) RMS and SMT, (b) RMS and settling time, (c) SMT and settling time for flocculating activity.

RSM was carried out with three factors, namely, RMS, SMT, and ST with the ranges of 125–175 rpm, 7.5–12.5 min, and 20–40, respectively. Seventeen reactors were suggested by BBD. The analysis results for each response according to the run can be referred to in Table 5.

All obtained data were then run without transformation with the same criteria as RSM for artificial wastewater treatment. RSM showed that turbidity removal and flocculating activity showed a good fit to quadratic model, while the TSS removal showed a good fit with a linear model. Because of the value of the lack of fit was still not significant, the difference between the adjusted R^2 and the predicted R^2 was still below 0.2 after changing from linear to quadratic model, and in order to obtain interactions between factors, therefore the models were chosen to run with the quadratic model for all responses. Differences between models with other responses can cause differences in desirability during the optimization process [94]. All models showed significant fit values with non-significant value for lack of fit. For turbidity removal, an R^2 value of 0.9995 was obtained with a difference of adjusted R^2 and predicted R^2 of

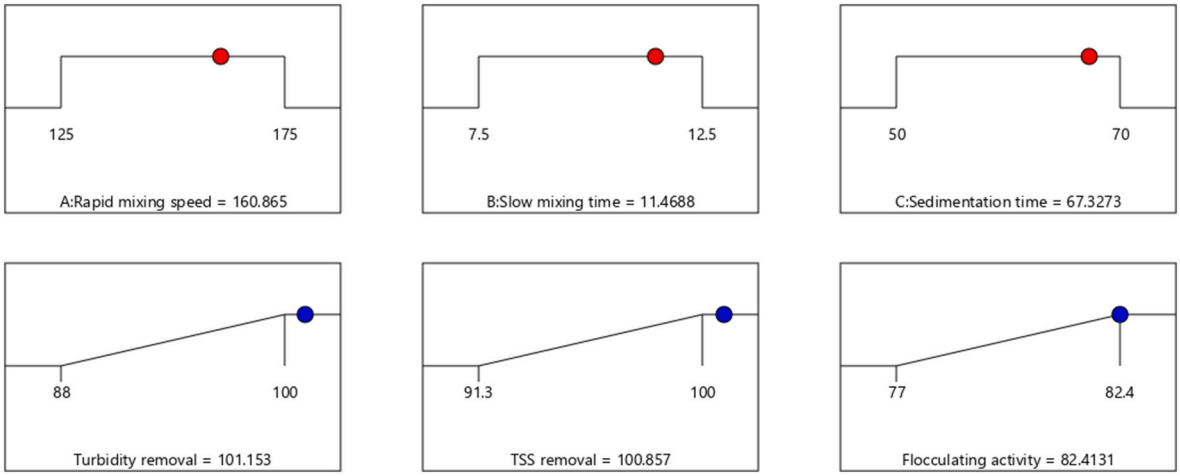

Fig. 14. Chosen solution and predicted value for responses for artificial wastewater treatment.

Table 5
Results of analysis based on BBD suggestion for aquaculture effluent treatment.

Std	Run	RMS (rpm)	SMT (min)	ST (min)	Turbidity removal (%)	TSS removal (%)	Flocculating activity (%)
17	1	150	10	30	79.6	96.5	65.4
10	2	150	12.5	20	70.2	92.2	61.2
5	3	125	10	20	71.6	92.3	63.4
1	4	125	7.5	30	78.5	95.8	66.5
15	5	150	10	30	79.5	96.7	67.3
9	6	150	7.5	20	73.2	93.1	62.1
8	7	175	10	40	80.1	98.2	68.2
13	8	150	10	30	79.5	97	70.2
2	9	175	7.5	30	78	96	68.2
16	10	150	10	30	79.7	95.3	69.9
3	11	125	12.5	30	79.2	96.1	68.3
7	12	125	10	40	82	98.4	68.4
14	13	150	10	30	79.5	94.5	70.1
4	14	175	12.5	30	76.5	95.2	66.9
11	15	150	7.5	40	81.2	97.8	68.2
6	16	175	10	20	70.3	92.4	63.5
12	17	150	12.5	40	83.2	98	67.9

0.0045. For the TSS removal, the R^2 value was 0.9318 with an adjusted R^2 and predicted R^2 difference of 0.0115. For flocculating activity, the R^2 value was 0.8362 with the difference between the adjusted R^2 and predicted R^2 of 0.0685. According to the RSM criteria the whole model has a good fit. ANOVA revealed that the factor of RMS and SMT did not have a significant influence on TSS removal and flocculating activity. The interaction between RMS and settling time did not have a significant influence on the turbidity removal, while the interaction between all factors did not have a significant influence on the TSS removal and flocculating activity. The interaction between factors in the 3D models are shown in Figs. 15-17. As for the equations obtained for turbidity removal ($y1$), TSS removal ($y2$), and flocculating activity ($y3$) based on the quadratic model for aquaculture effluent treatment can be referred to Eqs. (7)–(9).

$$y1 = 79.56 - 0.8*A - 0.225*B + 5.15*C - 0.55*AB - 0.15*AC + 1.25*BC - 1.23*A2 - 0.28*B2 - 2.33*C2 \quad (7)$$

$$y2 = 96 - 0.1*A - 0.15*B + 2.8*C - 0.275*AB - 0.075*AC + 0.275*BC - 0.0875*A2 - 0.1375*B2 - 0.5875*C2 \quad (8)$$

$$y3 = 68.58 + 0.025*A - 0.0875*B + 2.8125*C - 0.775*AB - 0.075*AC + 0.15*BC - 0.04*A2 - 1.065*B2 - 2.665*C2 \quad (9)$$

Solutions for optimum conditions of aquaculture effluent treatment were constructed using the criteria of RMS: in range, SMT: in range, ST: in range, turbidity removal: maximum, TSS removal: maximum, and flocculating activity: maximum. RSM suggested 20 solutions with the chosen highest desirability of 1000 can be referred to in Fig. 18.

According to Fig. 18, the optimum conditions for aquaculture effluent treatment were obtained at RMS of 125.8 rpm, SMT of 11.81 min, settling time of 39.4 min which was then summarized and rounded into bioflocculant dose of 5 % (v/v), bioflocculant concentration of 10 mg/L, RMS 125 rpm for 1 min, SMS 10 rpm for 12 min, and ST 39 min. The validation test was then carried out referring to optimum conditions that have been summarized and rounded, which resulted in turbidity removal of 80.1 % (error 3.5 %), TSS removal of 92.2 % (error 6.3 %), and flocculating activity of 60.2 % (error 12.6 %). Similar to the result obtained for artificial wastewater treatment, the maximum error value obtained from the model was 20 % which is based on the Harrington function evaluation scale and the obtained error value in this research are acceptable and very good also represent exceptional quality and performance [93].

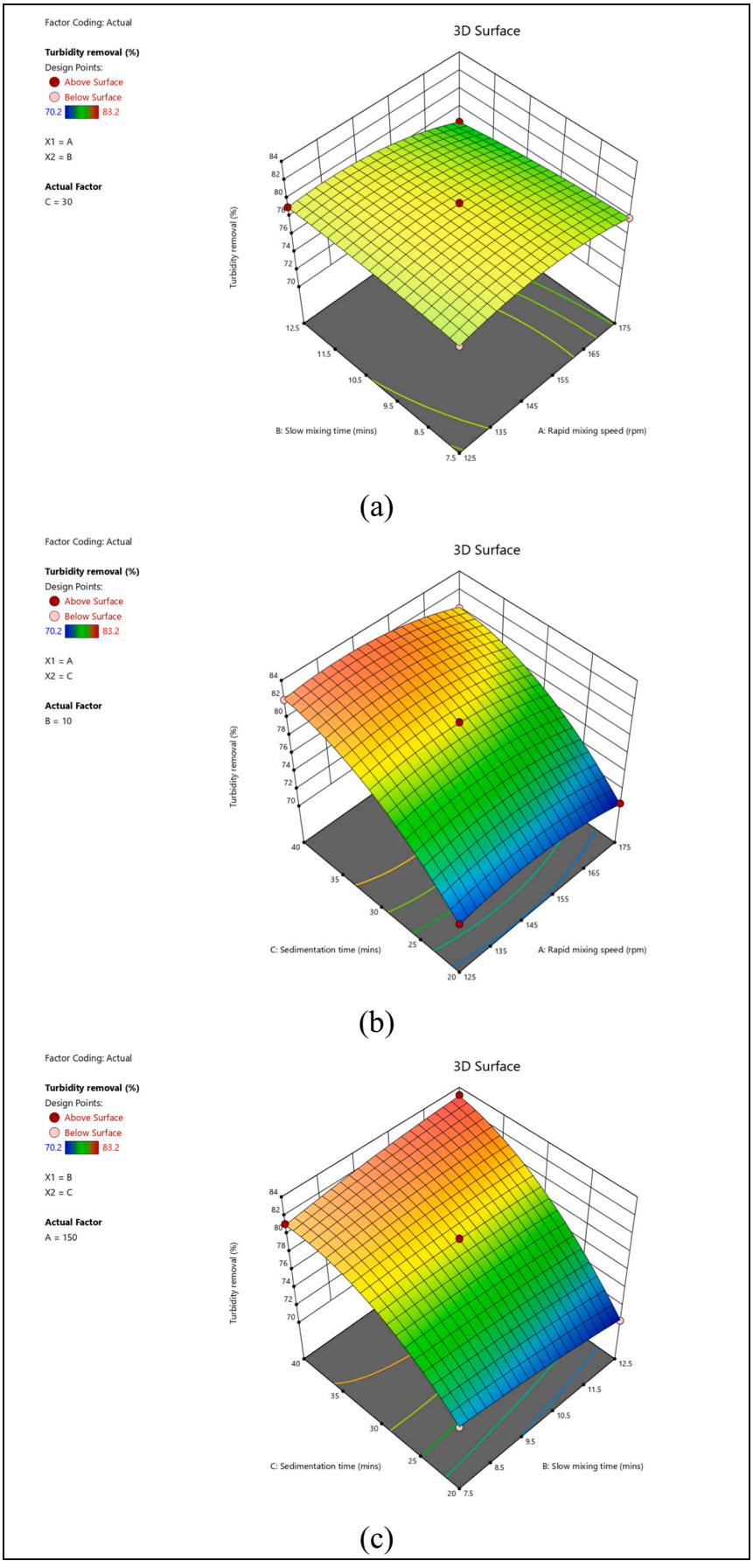

Fig. 15. Interaction between (a) RMS and SMT, (b) RMS and settling time, (c) SMT and settling time for turbidity removal.

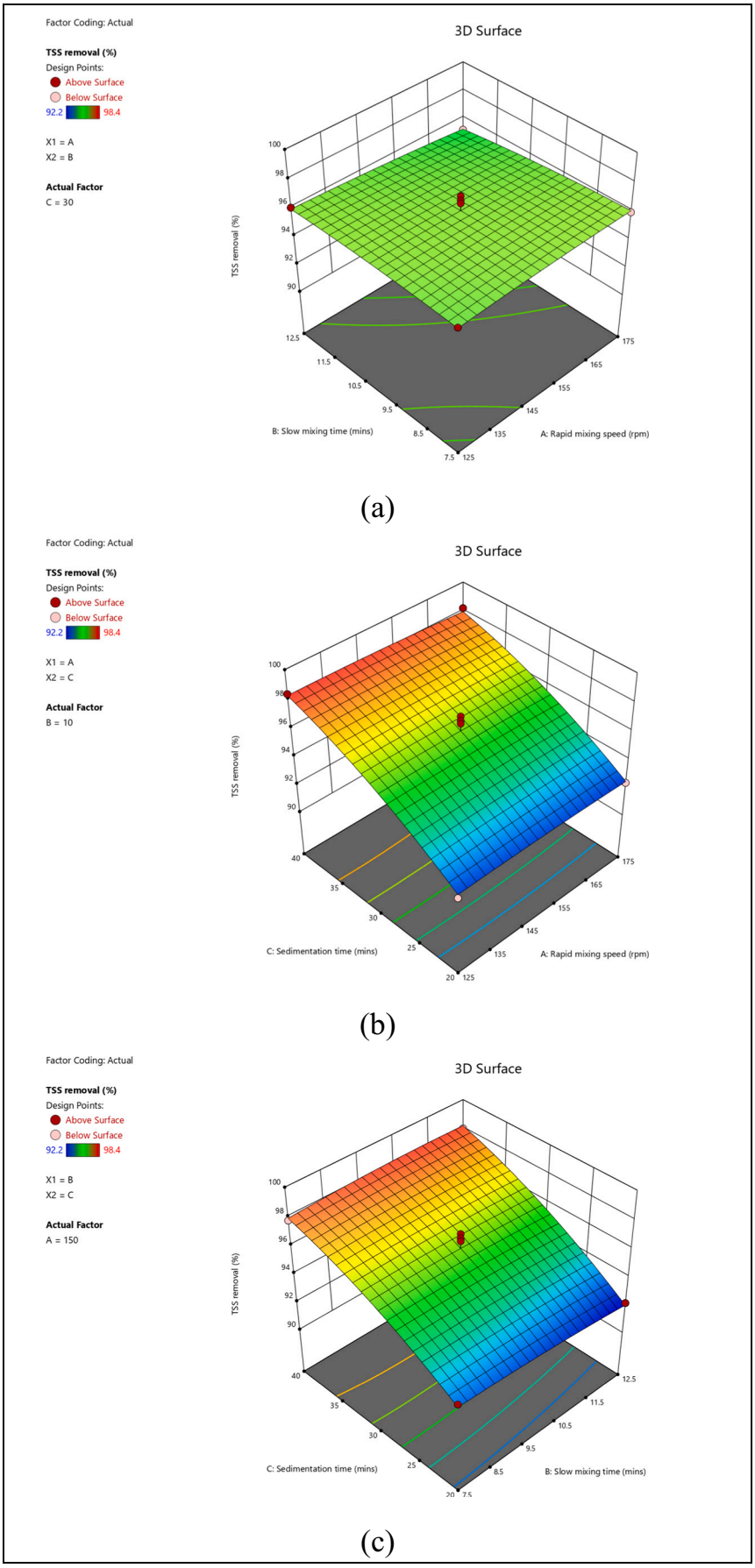

Fig. 16. Interaction between (a) RMS and SMT, (b) RMS and settling time, (c) SMT and settling time for TSS removal.

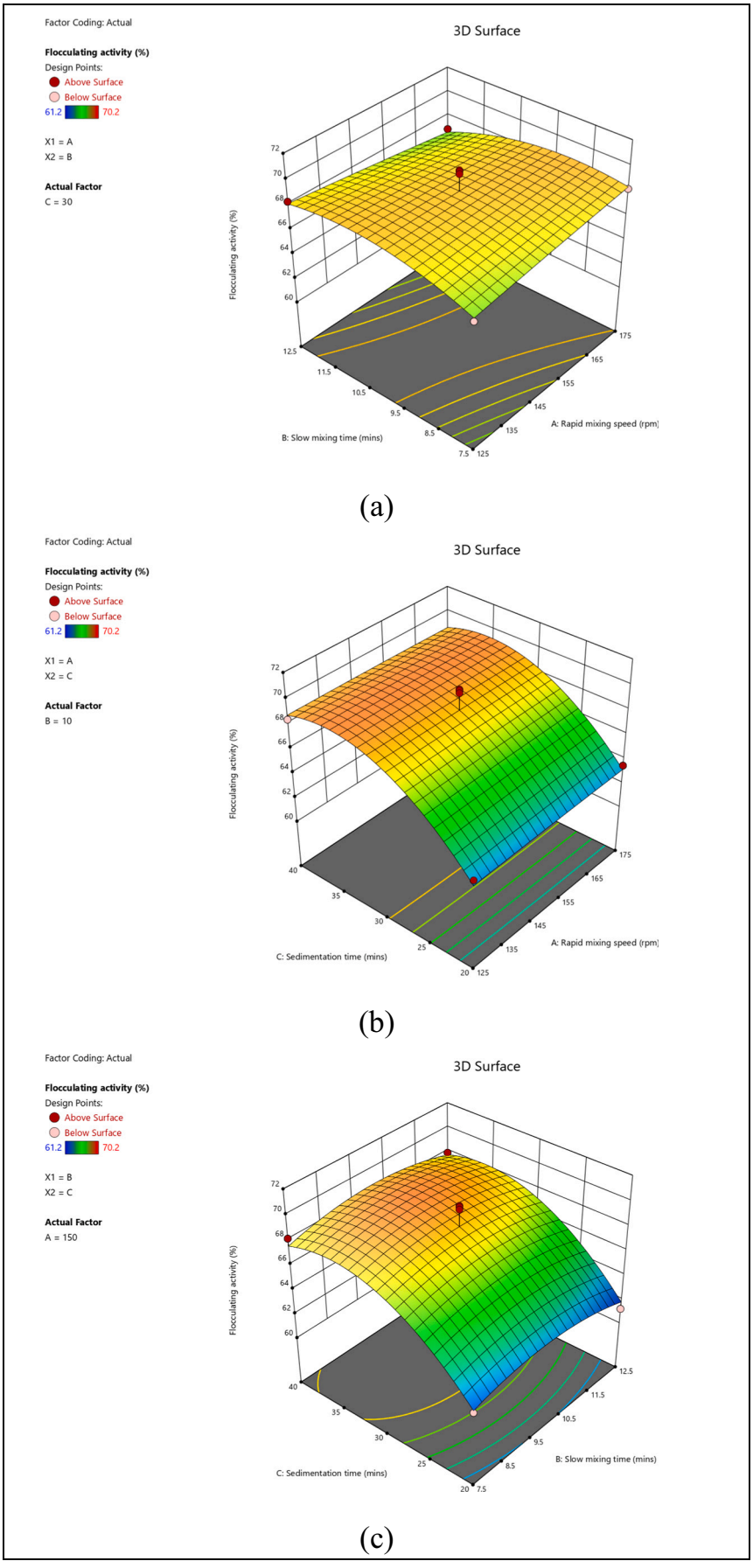

Fig. 17. Interaction between (a) RMS and SMT, (b) RMS and settling time, (c) SMT and settling time for flocculating activity.

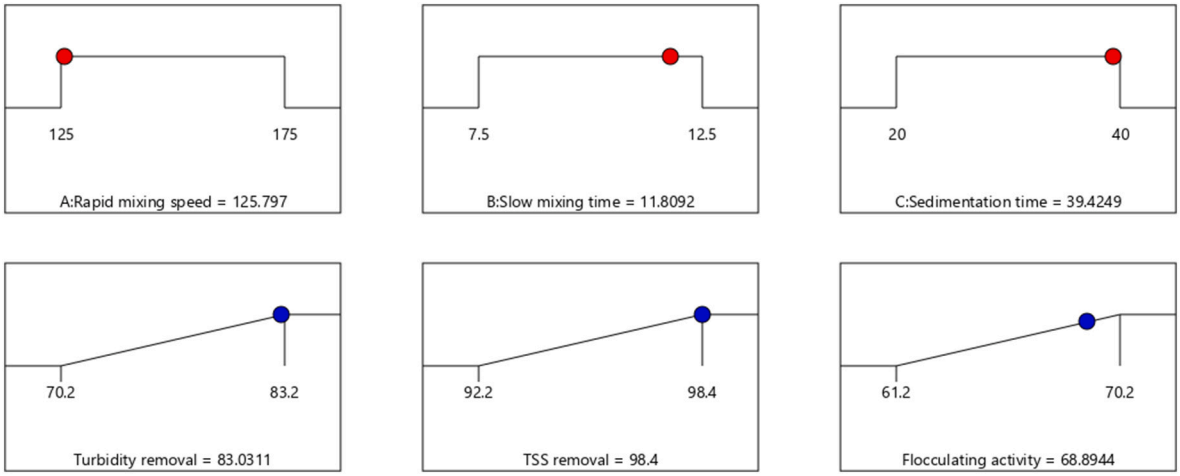

Fig. 18. Chosen solution and predicted value for responses for aquaculture effluent treatment.

Table 6
Comparison of optimized coagulation–flocculation conditions.

	Bioflocculant dose (%)	Bioflocculant concentration (mg/L)	RMS (rpm)	RMT (min)	SMS (rpm)	SMT (min)	ST (min)
Artificial wastewater	5	10	160	1	10	12	67
Aquaculture effluent	5	10	125	1	10	12	39

	Turbidity removal (%)	Error (%)	TSS removal (%)	Error (%)	Flocculating activity (%)	Error (%)
Artificial wastewater	99	2.1	99	1.8	72.8	11.7
Aquaculture effluent	80.1	3.5	92.2	6.3	60.2	12.6

3.5. Juxtaposition of optimized coagulation–flocculation conditions

The optimum treatment conditions for artificial wastewater and aquaculture effluent are listed in Table 6. RMS and settling time exhibited difference and were higher for artificial wastewater than for aquaculture effluent. This finding can be attributed to the characteristics of artificial wastewater: its particles are fabricated suspended solids and tend to be smaller than those in aquaculture wastewater [95,96]. As a result, a relatively high speed is needed to facilitate collision between particles to obtain the same performance. Small particle sizes require a long time to settle, so the required settling time for artificial wastewater was longer than that for aquaculture effluent [97]. Furthermore, the bioflocculant treatment achieved lower performance of turbidity removal, TSS removal, and flocculating activity for real aquaculture effluent than for artificial wastewater. This finding was due to the more complex characteristics of aquaculture effluent compared with those of kaolin substrate that may have affected the removal mechanism during the treatment [98].

3.6. Future research directions

In this work, RSM showed exceptional performance in optimizing the treatment conditions for artificial and real aquaculture wastewater. However, some other optimization methods/models may suggest different results, such as artificial neural network (ANN) [99,100]. Comparison of optimized conditions between RSM and ANN would be an interesting topic [101] to enrich the knowledge on the optimization of wastewater treatment conditions.

The adsorption rate and affinities of the bioflocculant onto particles may also have a significant impact on the agglomeration processes [102–104], especially during rapid mixing, slow mixing, and sedimentation time. Analyzing the adsorption affinity may help elaborate the flocculation process using the bioflocculant and depict the flocculation mechanism [104].

Cost–benefit analysis can be performed by juxtaposing both compounds to assess the viability of using this bioflocculant for wastewater treatment as a substitute for currently available compounds, such as PAM [105]. Economic feasibility analysis focusing on the overall cost-effectiveness [106] while considering the social return of investment with environmental impact benefits may also be carried out [107].

4. Conclusion

The use of biobased flocculant produced by *S. marcescens* for the treatment of turbid water was proven to be feasible. The performance of this bioflocculant in pollutant removal from aquaculture effluent was found to be considerably high. Zeta potential analysis revealed that the bioflocculant contains various carboxylic acid and shows anionic characteristics. Comparison between OVAT and RSM analyses showed that RMS, SMT, and ST played significant roles during the treatment. Optimization using BBD revealed that artificial wastewater requires 160 rpm of RMS, and aquaculture effluent requires only 125 rpm. Furthermore, the required ST is 67 min for artificial wastewater and only 39 min for aquaculture effluent. The lower treatment performance for aquaculture effluent compared with that for artificial wastewater was due to the complex characteristics of real wastewater that might have affected the removal mechanism during the treatment.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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