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RESEARCH PAPER

The Optimal Performances of Starches from two Cassava varieties as Bioflocculants for the Treatment of Textile Wastewater

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Abstract
The optimal performances of starches produced from two cassava varieties–*Manihot aipi* (SMA) and *Manihot palmate* (SMP) as bioflocculants for the treatment of textile wastewater were investigated in this study. The central composite rotatable design was used to investigate the effects of varying dosages of each cassava starch, wastewater-pH, and settling time on the turbidity removal from the wastewater with alum as the primary coagulant. Highly significant second-order multilinear quadratic regression models were developed from the experimental data, resulting in a very high coefficient of determination (r^2) values of 0.999 for the SMA and 1.000 for the SMP models. The optimum cassava doses of 50 and 150 mg/L, pH-values of 6.5 and 8.0, and settling times of 95 and 77 minutes led to predictive maximum turbidity removals of 98.35 and 88.87%% with desirability functions of 0.95 and 0.63 for the SMA and SMP, respectively. The corresponding observed turbidity removal recorded at these optimum conditions were 88.72% and 88.52% for the SMA and SMP, respectively. At these optimum conditions, there was no significant difference between the predicted and observed turbidity removed from the wastewater at a $p \leq 0.05$ significance level. Verification of the Jar tests showed a good agreement between the experimental data and the models and confirmed that the SMA was superior to the SMP in supporting the alum to remove turbidity from the textile wastewater. As a result, the study revealed that *Manihot aipi* starch has more flocculating capability than *Manihot palmate* for the treatment of textile wastewater.

Keywords: Cassava-starch; Coagulation-flocculation; Optimal performance; Textile-wastewater; Turbidity-removal

INTRODUCTION

The potential effects of the pollutant loads of wastewater from the various wet operations in textile manufacturing on water resources is of great concerns to environmentalists (Verma *et al*., 2012; Yusuf and Sonibare, 2004). Their effects on fauna and flora occasioned by indiscriminate discharge of the untreated textile wastewaters into natural water bodies have been reported in Nigeria (Awomeso *et al*., 2010; Durotoye *et al*., 2018; Odjegba and Bamgbose, 2012; Yusuf and Sonibare 2004). Dense colouration and high salinity from the usage of dyes for textile production are among the notable pollutants in textile wastewater (Verma *et al*., 2012). Several of these pollutants are nonbiodegradable and are rarely settleable under natural conditions because of their electrochemical properties *(Mokif et al*., 2020; Zangooei *et al*., 2016).

Presently, textile wastewater cannot be completely treated with a single-step method (Kaavessina and Distantina, 2017). Several treatment methods including electrochemical (Río *et al*., 2011), combined electrocoagulation (Can and Kobya, 2006), and coagulation-flocculation

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(Badrus 2018; Mohd-Salleh *et al*., 2019; Mokif *et al*., 2020; Padhiyar *et al*., 2020; Saravanan *et al*., 2017) that are based on the principle of colloids and turbidity removals have been applied to treat textile wastewaters. However, coagulation-flocculation has become the technique of choice in recent times because of its low operational cost, ease of operation, and relatively higher efficiency. During coagulation, rapid agitating device, aided by suitable coagulant distabilises the suspended colloidal particles in the wastewater. This is followed by flocculation that slowly mixes the dispersed pollutant to form large floc aggregates through adsorption, precipitation, entrapment, and bridging processes with or without the aid of flocculant (Badrus, 2018; Lugo-Arias *et al*., 2020; Mokif *et al*., 2020; Usefi and Asadi-Ghalhari, 2019; Zaman *et al*., 2020). The resulting flocs can then be removed from the water during subsequent sedimentation, flotation, and filtration operations (Verma *et al*., 2012).

The success of wastewater treatment by coagulation-flocculation process depends on the nature of the coagulants/flocculants used and the operational conditions applied (Mohd-Salleh *et al*., 2019). With respect to flocculant, the two commercially available types for wastewater treatment are polymeric and inorganic flocculants. The polymers available for wastewater treatment are either synthetic or natural, but both are capable of forming large floc aggregates that are resistant to shear forces. Polyacrylamide is one of the synthetic polymers that has been extensively used for the treatment of textile wastewater (Kaavessina and Distantina, 2017). However, natural polymers like balsa, guasimo, and cocklebur have become trendy due to their superior shear stability, high biodegradability, and non-toxicity (Kaavessina and Distantina, 2017; Lugo-Arias *et al*., 2020).

Cassava (*Manihot esculenta* Crantz) tuber is one of the precursors from which bioflocculant can be produced, just as it has also been found useful in several other techno-functionalities including adhesive (Akpa, 2012), bioethanol (Shanavas *et al*., 2011), glutamic acid (Jyothi *et al*., 2005), and hydrogen (Su *et al*., 2009). A tuber of cassava, if processed can produce about 15–20% fibrous slurry that contains 50–70% starch on dry weight basis (Jyothi *et al.*, 2005). Cassava are broadly divided into two varieties: *Manihot aipi* (sweet) *and Manihot palmate* (bitter), and are distinguished from one another by their properties. The *Manihot aipi* has less than 50 ppm of cyanogenic contents and has reddish-green leaves, red stems, brownish unpeeled tubers, and dark shade milky white peeled tubers. The *Manihot palmate* has more than 100 ppm hydrogen cyanide contents and has green-yellow leaves, brownish stems, and white peeled tubers (Obueh and Kolawole, 2016; Sarkiyayi and Agar, 2010). Apart from these features, each cassava variety contains varying proportions of cellulose, hemicellulose, proteins, polysaccharides, Fe_2O_3 and Al_2O_3, and a range of multi-functional groups that determine their level of affinity for colloidal impurities in wastewater (Othman and Rahim, 2018; Kumar *et al*., 2020a; Kumar *et al*., 2020b; Vigneshwaran *et al*., 2020).

The coagulating and flocculating abilities of cassava starch for wastewater treatment have been extensively investigated (Kumar *et al*., 2020a; Kumar *et al*., 2020b; Lugo-Arias *et al*., 2020). However, there is a dearth of information about the extent to which the inherent properties of cassava starch, resulting from the taxonomical features of its precursor affect its performance as biocoagulant, bioflocculant, and/or as partial replacement of chemical coagulant for the treatment of textile wastewater. The aim of this study is to investigate the bioflocculant performance of starches from two cassava varieties (*Manihot aipi and Manihot palmate*) in the treatment of textile wastewater with alum as the primary coagulant. To demonstrates the novelty of the research, emphasis was placed on the potential best cassava variety to be used to produce the bioflocculant. The experiments were designed using the central composite rotatable design, a subset of the surface response methodology to investigate the effects of the process parameters like cassava starch dose, wastewater-pH, and settling time on the effectiveness of turbidity removal from the wastewater.

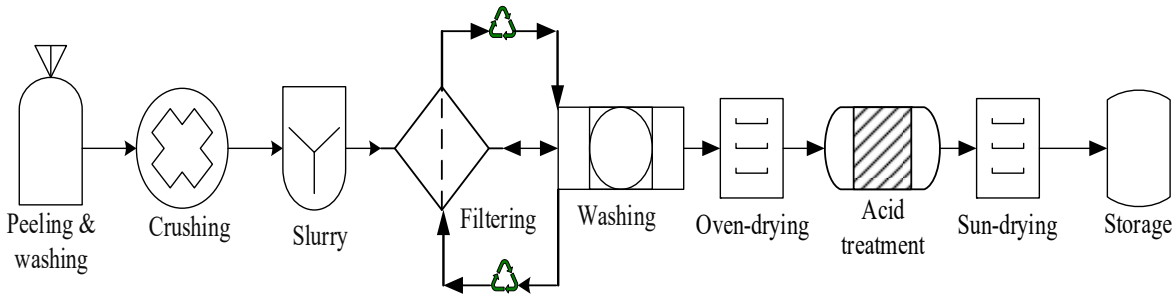

Fig. 1. Flow diagram for processing the starches from the cassava varieties

MATERIALS AND METHODS

The two cassava varieties *Manihot aipi* and *Manihot palmate* were procured from a private farm located in Makurdi, Benue State – Nigeria. Each cassava starch was prepared from the corresponding tubers by following the standard protocols for starch preparation (Akpa, 2012; Rasool *et al*., 2020). Figure 1 is the flow diagram for processing the cassava starches. In each case, 10 kg of the tubers were manually peeled using a kitchen knife, the peels were cleaned with tap water, and then sun-dried for 1 hour to reduce their excess moisture contents. Slurries were produced from each cassava peel through mortar and pestle. The starches were extracted from each cassava slurry by sieving through a latex transparent cloth while the fiber was retained on the cloth. The retained cassava fiber was repeatedly washed 4 to 5 times with tap water on the cloth to extract any remaining inherent starches as much as possible. The extracted starch was introduced into a transparent plastic container, securely placed in an undisturbed location, and allowed to settle for 4 hours. The supernatant was carefully decanted from the solution and the starch was washed with distilled water to eliminate any remaining fiber. The starch was oven-dried for 6 hours at 45°C. The extracted starches were modified via acidic treatment (Adamu *et al*., 2014; Wang *et al*., 2022). In doing this, 200 grams of each category of starch was dissolved in 0.3 M of HNO_3 inside a beaker and was carefully placed in an undisturbed location for 24 hours and then thoroughly washed in distilled water until a neutral pH was achieved. Finally, the starches were dried under bright sunlight for 4 hours, labelled as starch from *Manihot aipi* (SMA) and starch from *Manihot palmate* (SMP), and stored in firmly covered plastic jars to prevent contamination and moisturization.

The grain size analyses of the starches were performed using sieves of varying mesh sizes. The starches were grounded into finer powder using a laboratory blender and 50 grams of each starch was sieved on a mechanical shaker with a standard set of seven (7) sieves (Pharmacopoeia, 2022; Dehghanian *et al*., 2016). Diffuse reflectance infrared fourier transform (DRIFT) spectroscopic analyses were performed on each starch to determine the variabilities in the surface chemistry with respect to the available functional groups. This was accomplished by placing 2 grams of each starch powder on the KBr disc of the spectrophotometer (Shimadzu Model IRPrestige-21) and recording the infrared spectra for the starches in the specified range of 500 to 4000 cm^{-1} at a resolution 4 cm^{-1}, a scan speed of the 30s, and scan number 64 (Kumar *et al*., 2020a). The spectra images obtained were redrawn using OriginPro version 2021b (OriginLab Corporation, Northampton, MA, USA).

Samples of the textile wastewater were collected from a clothing Ventures located in Makurdi, Benue State–Nigeria. The company deals in laundry services, knitting, dyeing, printing, and finishing processes on clothes and wrapprers. The wastewater was collected daily in two separate icepacked ten (10) liters plastic containers that were sealed with two (2) mm cellophane and transported to the Chemistry Lab of the Department of Chemistry, Benue State University–

Nigeria, which is located about two (2) kilometres away from the sampling location. The raw water quality was assessed using the APHA, (2017) standardized techniques. Using a turbidimeter (2100p Hach), the pH of the water samples were measured according to the nephelometric method (2130). The chemical oxygen demand was determined in line with the closed reflux colorimetric method (5220D) using a standard COD (Hach Lange, 200 LT) meter, and the total suspended solids was determined using the 2540D method with the aid of Hach Dr 2010 TSS meter.

Using the two starches independently as bioflocculants with aluminum sulfate hydrate (alum) as the primary coagulant, the performances of the starches in the coagulation-flocculation studies were assessed. The alum was in powdered form with specifications (chemical formula, $Al_2(SO_4)_3 \cdot 18H_2O$; molecular weight, 666.44 g/mol; grade, 48%; pH, 3–3.3; density, 1.69 g/cm^3). The alum stock solution of 5000 mg/L was prepared by diluting 63 grams of the powdered alum in 1 liter of double-distilled water (Regti *et al*., 2017). On the other hand, the 1000 mg/L stock solutions of each of the starches was separately prepared by dissolving 50 grams of each cassava starch in 1 liter of double-distilled water (Adamu *et al*., 2014). Each suspension was thoroughly mixed using a magnetic stirrer for 1 hour to achieve uniform mixtures. From a preliminary investigation conducted using the alum as a coagulant for the removal of both turbidity from a synthetic Congo-red wastewater, the optimum dose of alum needed to achieve responsive turbidity reduction was 30 mg/L. This optimum dose of alum was introduced into each designated beaker, as the primary coagulant and varying concentrations ranging from 0 to 200 mg/L of each cassava starch were prepared from each corresponding stock solutions by serial dilution as bioflocculants to enhance the treatment process.

The Jar Tests involving the use of each starch category were designed and planned with JarTestWizard (version 1.01) and conducted using a standard Jar test apparatus (CIV61L, 6-position paddles on which standard beakers of 1000 ml were placed) (dos Santos *et al*., 2018; Saravanan *et al.*, 2017). The experiments were perfromed by selecting a set of 20 labelled beakers partly filled with 500 ml of the wastewater, after the pH was adjusted to the designed values using either 0.1 M of HCl or NaOH for addition of acid or base, respectively. The wastewater was dosed with the corresponding fixed concentration of the alum before agitating rapidly on the testing paddles and the corresponding designated cassava starch dosage was added, while maintaining the same volume of the wastewater (i.e., 500 ml) in all the beakers during the flocculation process. The rapid agitation was performed at 100 rpm for 1 minute, followed by slow agitation at 10 rpm for 20 minutes. Each designated beaker was allowed to settle for the designed settling time. At the end of which, 10 ml of the coagulated water sample was carefully abstracted at exactly 5 ml depth from the surfacewater level to determine the final concentration of turbidity.

The treatment of the textile wastewater was optimized using cassava starch dosages, wastewater-pH, and settling time, as the independent variables and turbidity removal, as the dependent variable. The coded and actual levels of the independent treatment variables are shown in Table 1. The central composite rotatable design (CCD), a subset of the response surface methodology (RSM) was employed in designing the experimental runs using Design-Expert

Table 1. Coded and Actual Values of the Process Factors used in the Experiments

Treatment factors	Symbol	coded and actual values				
		-α	**-1**	**0**	**+1**	**+α**
Starch dosage (mg/L)	A	0	50	100	150	200
Wastewater-pH	B	3.5	5.0	6.5	8.0	9.5
Settling time (minutes)	C	40	45	70	95	120

version 10.0.1.0 (Stat-Ease, Inc., Minneapolis, USA) software.

In each case, five levels of the three independent process factors that include starch dosages ranging from 0 to 200 mg/L, wastewater-pH ranging from 3.5 to 9.5, and settling time ranging from 40 to 120 minutes that resulted to a total experimental run of 20 were carefully selected in conformity with the CCD (Kumar *et al*., 2020a; Prasad & Yadav, 2021). In all cases, the experimental run was performed three times to satisfy the statistical principle. The turbidity removal was calculated using Equation 1.

$$\text{Turbidity Removal}(\%) = \left(\left(T_o - T_f\right) / T_o\right) \times 100 \quad (1)$$

where T_o and T_f are the initial and final turbidity of the wastewater before and after the Jar test.

Statistical multiple linear regression analyses were conducted on the data obtained from the coagulation-flocculation experiments to investigate the main and interactive effects of the independent process factors on the turbidity removed from the wastewater. The same Design-Expert version 10.0.1.0 software was used to determine the significant level of the independent and interactive factors using analysis of variance (ANOVA) with a significance level set at $p \leq 0.05$ for all variables. The data generated were further subjected to multiple linear regression analyses to produce coded mathematical models in the form given in Equation 2. This can be further expanded into Equation 3.

$$Y = \alpha_0 + \sum_{i=1}^{k} \alpha_i X_i + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \alpha_{ij} X_i X_j + \sum_{i=1}^{k} \alpha_{ii} {X_i}^2 + \varepsilon \quad (2)$$

where Y is the theoretical function for the predicted response factor, X_i and X_j are the independent process factors, α_o is the constant coefficient, and α_i, α_{ii}, and α_{ij} are the linear, quadratic, and interactions coefficients, respectively. In addition, ε is the residual error and k represents the number of factors in the model.

$$Y_{tub} = \alpha_0 + \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_{12} AB + \alpha_{13} AC + \alpha_{23} BC + \alpha_{11} A^2 + \alpha_{22} B^2 + \alpha_{33} C^2 \quad (3)$$

where Y_{tub} is the theoretical response functions for turbidity removal from the wastewater, α_o is the intercept or global mean of the experimental design, α_1, α_2, and α_3 are the coefficients of the main factor effects, α_{12}, α_{13}, and α_{23} are the coefficients of the interaction effects, and α_{11}, α_{22}, and α_{33} are the coefficients of the quadratic effects of the independent process factors, respectively. A, B, and C represent the cassava starch dosage (mg/L), wastewater-pH, and settling time (in minutes) used for the coagulation-flocculation experiments, respectively.

The numerical optimization technique based on the desirability function was adopted to ascertain the levels of each of the independent process factors needed to achieve the maximum turbidity removal from the textile wastewater (Dawood & Li, 2013). This was done by setting each independent factor within the range of its designed level and turbidity removal to the maximum, with 75% being the lower limit and 100%, as the upper limit. The levels of each starch dose, wastewater-pH, and settling time required to achieve the predicted maximum turbidity removal were determined. They are thereafter, combined in a separate coagulation-flocculation Jar test to determine the actual extent of turbidity removed from the wastewater.

The validation of the optimum turbidity removals from the wastewater was conducted by determining percentage errors and performing F-Tests on the standard deviations and variances between the predicted and observed values of the responses recorded at the optimum levels of the independent factors (Ahmad *et al*., 2021; Ogbeh *et al*., 2019).

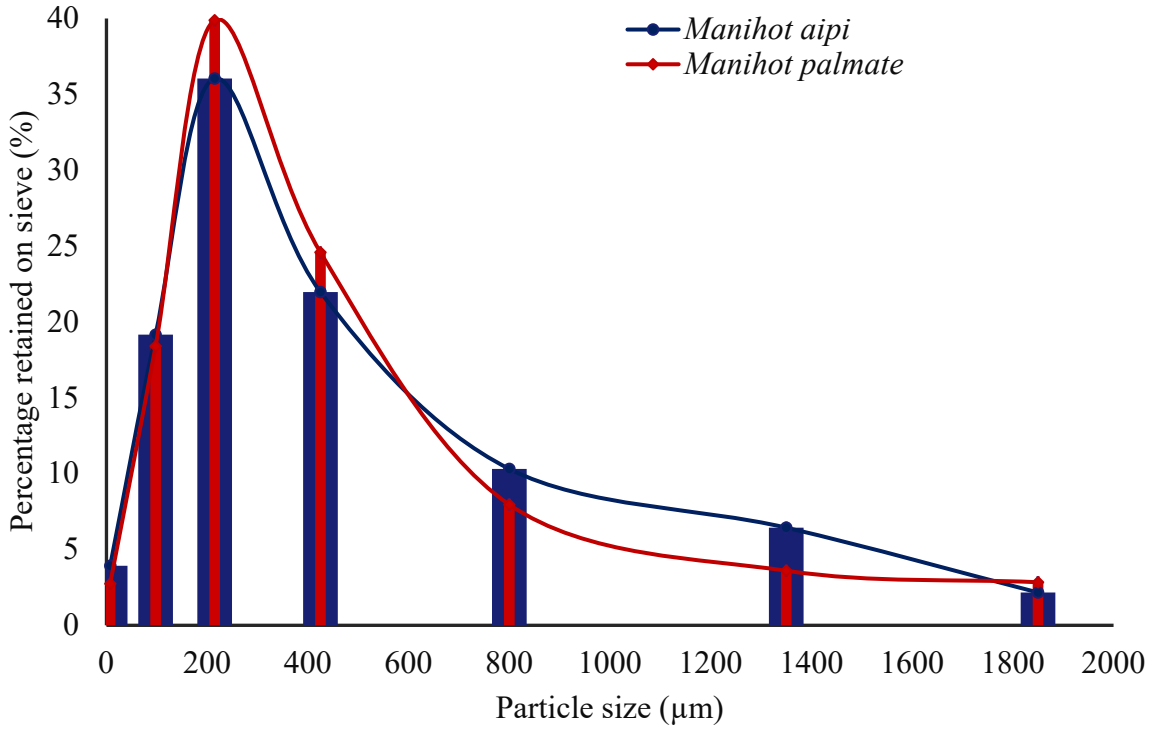

Fig. 2. Particle size distribution curves for the cassava peel starches

RESULTS AND DISCUSSION

The essence of analyzing the powder particles is to identify the potential behaviour of the starches in solution or in suspension. But because starch is highly soluble in water and regenerative (Yu & Fu, 2020), it is expected that by reducing its particle size, the rate of its dissolution in water can be increased because of the inverse relationship between its particle size and surface area (Arunarajeswari *et al.*, 2020). However, the average specific surface areas of each starch variety was never ascertained, as high resolution scanning microscopic images of th particles was never obtained.

The extracted cassava starches were of diverse particle sizes ranging from 7.5 to 1850 µm. Even though the starches were mostly of the same particle size range and were both polydisperse and positively skewed distributed (see Figure 2), the degree of their sizes distributions were not the same. The SMA's frequency distribution curve appears to be more skewed around the mean than the SMP's. On the other hand, further analyses of the powder particles indicate that about 20% of the particles were deemed as coarse ($D_{10/22}$) with an average particle diameter of 433 and 568 µm, 30% as moderately coarse ($D_{22/44}$) with an average particle diameter of 312 and 351 µm, and 50% as moderately finer ($D_{44/85}$) with a particle diameter of 305 and 270 µm for the SMA and SMP variety, respectively.

According to the Pharmacopoeia, (2022), a powder is said to be of coarse particles, if the particles pass-through sieve number 10 with 1700 µm sieve opening and not more than 40% of them pass-through sieve number 22 with 710 µm sieve opening. It is moderately-coarse, if the particles pass-through sieve number 22 with 710 µm, but not more that 40% of it pass through sieve number 60 with 250 µm. Moderately finer powder has particles that pass-through sieve number 44 with 355 µm sieve opening and not more than 40% of the particle pass-through sieve number 85 with 180 µm sieve opening. The moderately finer power particles constitute the samples used in conducting the Fourier transform infrared spectroscopy analysis on the

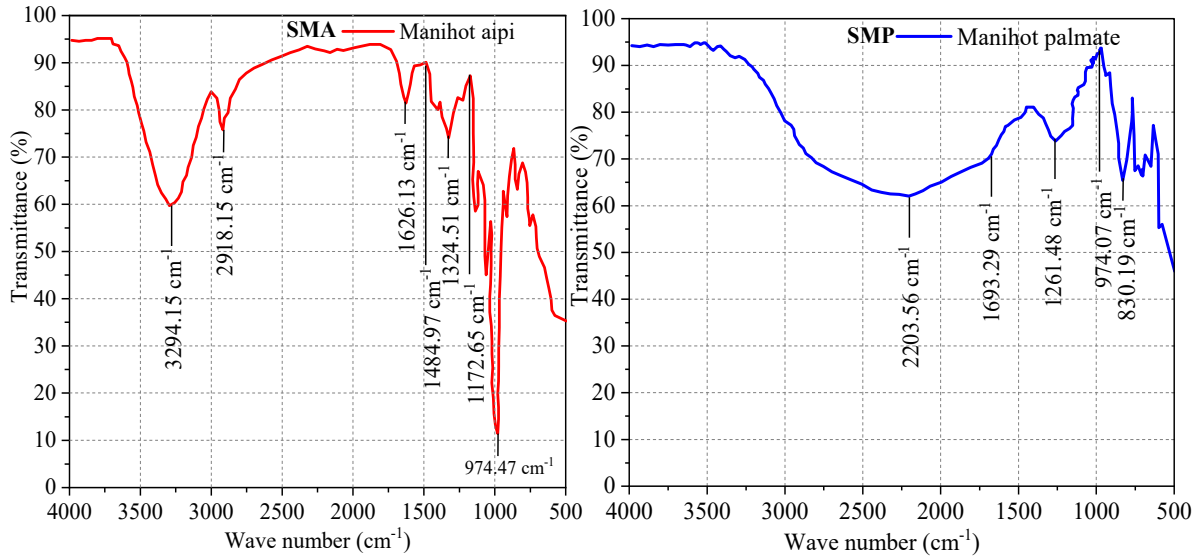

Fig. 3. Infrared Spectra for the SMA and SMP varieties obtained from the DRIIFT

starches produced from each cassava variety. More than 50% of the powder starch used for the coagulation-flocculation experiments were the ones retained on sieves number 44 and 85 with mesh openings of 250 and 180 µm.

The broadband for each cassava starch produced from the *Manihot aipi* and *Manihot palmate*, as determined by the DRIFT is shown in Figures 3 (SMA and SMP), respectively. The diverse range of adsorption peaks on each DRIFT spectra indicates the presence of several functional groups of different structural strengths on the starch extracts from the cassava varieties. As shown in Figure 3, there are strong absorption peaks at 3294 and 2918 cm^{-1} for the *Manihot aipi* and at 2203 and 1693 cm^{-1} for the *Manihot palmate*. These peaks are associated with O–H and N–H stretching vibrations in carboxyl and phenol groups (Vigneshwaran *et al*., 2020). This implies that both the aipi and the palmate starch contain varying proportion of protein molecules in the form of a polymer of OH and NH amine groups.

The symmetric and asymmetric bends shown by the bands between 2459 and 1740 cm^{-1} for the SMA and between 1610 and 1340 for the SMP in Figure 3 can be attributed to the stretching of the aliphatic C-H bond of the form $-CH_2-$ and $-CH_3-$ groups that are present in fatty acids (Vigneshwaran *et al*., 2020). There is therefore, a varying proportion of fatty acids in the starches produced from the aipi and palmate starch.

The weak absorption peaks at 1626 cm^{-1} for the SMA and 1261 cm^{-1} for the SMP in Figures 3 indicate the existence of C=O groups in the protein structures (Vigneshwaran *et al*., 2020). This confirms the existence of protein in both the aipi and palmate starches, but the protein of higher structural strength or proportion is present in the aipi starch than in the palmate starch. The presence of C–N groups on the aipi and the palmate starch are indicated by the weak asymmetric peaks at 1484 and 974 cm^{-1} in Figure 3 for the SMA and SMP, respectively. The presence of COOH groups on the aipi and palmate starches are indicated by the peaks at 1324 and 830 cm^{-1}, respectively.

The main quality parameters of the textile wastewater that was determined before performing the Jar Test include an average pH of 7.42±0.34, a turbidity level of 195±30 NTU, COD of 592±10 mg/L, and total suspended solids of 226±16 mg/L. The adjustments of the wastewater-pH through the addition of strong acid or base substantially altered the raw water quality. When the wastewater-pH was adjusted further within the alkaline range from its original value, the red-colored wastewater stabilized with slight changes in its colour. But the colour waned towards

transparency, as the wastewater was however conditioned to lower pH range. This indicates that the wastewater-pH plays a significant role in the structural stability of the wastewater colour. According to Krishna *et al*., (2017), the colour in dyes is characterized by the presence of a nitrogen-to-nitrogen bond. It is most likely that this bond might have been disrupted, as the solution pH increases towards the acidity range.

The CCD matrix and details of the observed and predicted levels of turbidity removed under the influence of the starch doses for both SMA and SMP, wastewater-pH, and settling time is shown in Table 2. In general, the influence of the varying concentrations of the three process factors on the extent of turbidity removed from the wastewater can be noticed across all the experimental runs. Whereas, extremely low turbidity was removed, when the wastewater was dosed with the lowest concentration of the SMP at the lowest levels of pH and settling time, the wastewater turbidity remained rather unchanged at these levels of the process factors for the SMA (see run 1 in Table 2). The reason for this remarkable difference is not clear, but it may be due to the aiding or hindering attributes of the starches, as bioflocculants. Even more remarkable is the fact that run 20, which does not involve the addition of any starch doses (i.e., control) for both sets of experiments resulted in moderately high turbidity removal of 63.30 and 53.26% for the experiment performed with the use of the SMA and SMP, respectively. These relatively high turbidity removed was likely due to the influence of the alum, as the primary coagulant. Table 3

Table 2. The CCD Matrix and the Observed and Predicted Turbidity removed by both SMA and SMP from the wastewater

Run No.	Dosage (mg/L)	pH	Time (min)	Turbidity Removed (%) by the SMA		Turbidity Removed (%) by the SMP	
				Observed	Predicted	Observed	Predicted
1	50	5.0	45	-2.59	-4.03	2.41	2.56
2	50	5.0	95	84.40	82.23	77.00	76.90
3	50	8.0	45	17.90	18.54	15.10	15.23
4	50	8.0	95	83.10	82.25	72.02	71.95
5	100	3.5	70	11.80	14.27	52.00	51.96
6	100	6.5	40	0.15	-0.72	-6.00	-6.31
7	100	6.5	70	84.70	84.96	72.95	72.72
8	100	6.5	70	83.80	84.96	72.65	72.72
9	100	6.5	70	85.70	84.96	77.12	77.26
10	100	6.5	70	84.20	82.21	77.12	77.26
11	100	6.5	70	82.90	82.21	71.68	71.90
12	100	6.5	70	83.10	84.96	72.65	72.72
13	100	9.5	70	52.80	51.23	79.41	79.35
14	100	6.5	120	82.90	84.66	71.68	71.90
15	150	5.0	45	21.70	21.91	22.52	22.65
16	150	8.0	45	64.30	65.84	60.00	60.17
17	150	8.0	95	80.30	81.11	78.90	78.82
18	150	5.0	95	61.00	59.73	59.00	58.93
19	200	6.5	70	87.20	86.13	75.96	75.90
20	0	6.5	70	63.30	65.27	53.26	53.22

Table 3. Analyses of Variances of the Experimental Data of Turbidity Removal from the Textile Wastewater

Original factor	Sum of Squares		DoF		Mean Square		F-value		p-value (Prob > F)		Status
	SMA	SMP	SMA	SMP	SMA	SMP	SMA	SMP	SMA	SMP	
Block	81.79	60.85	1	1	81.79	60.85					
Model	19300.54	13855.99	9	9	2144.50	1539.55	483.30	34866.29	< 0.0001	0.0001	significant
A-starch dose	525.12	620.66	1	1	525.12	620.66	118.35	14056.11	< 0.0001	< 0.0001	significant
B-pH	1648.48	905.24	1	1	1648.48	905.24	371.52	20501.02	< 0.0001	< 0.0001	significant
C-Settling time	8799.39	7382.84	1	1	8799.39	7382.84	1983.11	167199.16	< 0.0001	< 0.0001	significant
AB	228.02	308.39	1	1	228.02	308.39	51.39	6984.08	< 0.0001	< 0.0001	significant
AC	1173.46	724.47	1	1	1173.46	724.47	264.46	16407.12	< 0.0001	< 0.0001	significant
BC	254.14	155.32	1	1	254.14	155.32	57.27	3517.54	< 0.0001	< 0.0001	significant
A^2	76.24	290.39	1	1	76.24	290.39	17.18	6576.45	0.0025	< 0.0001	significant
B^2	4402.98	242.48	1	1	4402.98	242.48	992.30	5491.41	< 0.0001	< 0.0001	significant
C^2	2913.66	3559.88	1	1	2913.66	3559.88	656.65	80620.66	< 0.0001	< 0.0001	significant
Residual	39.93	0.40	9	9	4.44	0.04					
Lack of Fit	35.28	0.32	5	5	7.06	0.06	6.07	3.34	0.0523	0.133	not significant
Pure Error	4.65	0.08	4	4	1.16	0.02					
Cor Total	19422.27	13917.23	19	19							

SMA is starch from *Manihot aipi*; SMP is starch from *Manihot palmate*; DoF is the degree of freedom

Table 4. Coefficient of the Model Terms and Valuable Statistics for Model Evaluation

Response variable	Turbidity removal		Prob. Value		Model Parameters	Model Statistics	
	SMA	SMP	SMA	SMP		SMA	SMP
Intercept	+83.58	+74.99	-	-			
A	+6.20	+6.74	< 0.0001	< 0.0001	r^2	0.9979	1.0000
B	+10.99	+8.14	< 0.0001	< 0.0001	$r^2_{adjusted}$	0.9959	0.9999
C	+25.38	+23.25	< 0.0001	< 0.0001	$r^2_{predicted}$	0.9829	0.9998
AB	+5.34	+6.21	< 0.0001	< 0.0001	Adeq. precision	57.72	549.67
AC	-12.11	-9.52	< 0.0001	< 0.0001	CV (%)	3.47	0.36
BC	-5.64	-4.41	< 0.0001	< 0.0001	Standard Dev.	2.11	0.21

shows the summary of the analyses of variances of the experimental data obtained from using both starches and the other two independent factors for the Jar Test.

The measure of the significance of each model and its terms were determined using the F-test and probability value, with significance level set at $p<0.05$. The models F-values of 483.30 and 34866.29 obtained for the SMA and SMP, respectively implies that the models are both highly significant and that only about 0.01% probability exists that these large F-values could results from noise rather than from the combined process factors used in the Jar Test. Similarly, the F–values of 6.07 and 3.34 arrived at for the models' 'lack of fit' pertaining to the use of the SMA and SMP means that the 'lack of fit' of the models are not significant; about 5.23 and 13.3% chance exist that a 'lack of fit' this large could arise from noise, and that the models are perfect fits for each set of the experimental data. The 'lack of fit', which normally should not be significant, compares the experimental residual to the pure error that is associated with the centre points of the experimental design.

All the terms (i.e., A, B, C, AB, AC, BC, A^2, B^2, and C^2) in the predictive quadratic models for the two sets of data obtained from using the SMA and SMP, as coagulant aids were found to be highly significant. However, the significance of the terms in the model for the SMP were higher than those of the SMA, and this is attributable to the higher F-values associated with the use of the SMP. Equations 4 and 5 represent the multilinear regression models for predicating the removal of turbidity (Y_{SMA} and Y_{SMP}) from the textile wastewater using varying doses of SMA and SMP, respectively.

$$Y_{SMA} = 83.58 + 6.20A + 10.99B + 25.38C + 5.34AB - 12.11AC - 5.64BC - 2.30A^2 - 17.49B^2 - 14.22C^2 \quad (4)$$

$$Y_{SMP} = 74.99 + 6.74A + 8.14B + 23.25C + 6.21AB - 9.52AC - 4.41BC - 4.49A^2 - 4.10B^2 - 15.72C^2 \quad (5)$$

The coefficients of the models' terms and some valuable statistical measures for evaluating the precision of the two models are shown in Table 4. Even though they vary from one another, the positive coefficients of the main linear terms A, B, and C in Equations 4 and 5 mean that cassava starch dosage (p-value<0.0001), wastewater-pH (p-value<0.0001), and settling time (p-value<0.0001) contributed different levels of synergetic effects on the overall proportion of turbidity removed from the wastewater. In general, the linear contributions induced by using the SMP slightly outweighs those contributed by using the SMA as coagulant aid.

Similarly, synergistic contributions were induced from the interactions of the cassava starch dosage and the pH (AB) for the two models, but the synergistic influence obtained from using the SMP was slightly superior to that associated with the SMA, as indicated by its higher coefficient.

However, antagonistic interactive effects were noticed between the cassava starch dosage and settling time (AC), and between the pH and settling time (BC) for both models. The negative contributions induced from the interactions of these sets of process factors were much higher for the SMA than those from the SMP, as indicated by their higher coefficients. Similarly, all the contributions received from the quadratic terms in the two models (i.e., SMA and SMP) were antagonistic to turbidity removal from the wastewater.

The coefficient of determination (r^2) is a statistical measure of goodness of fit that relates to how the observed values correlate with the predicted values. The r^2 values of 0.9979 for the SMA model and 1.000 for the SMP model are high enough indications that 99.79 and 100% of the variances of the independent process factors were the major contributors to the extent of turbidity removed from the wastewater. Although the precision r^2 value of 0.9829 is in reasonable agreement with the adjusted r^2 of 0.9959 for the SMA model, in that the difference between them (i.e., 0.003) is far less than 0.2 (Prasad & Yadav, 2021). But the difference between the precision r^2 value of 0.9998 and adjusted r^2 of 0.9999 for the SMP model (i.e., 0.0001) is even far smaller than that obtained for the SMA. The adequate precision (AP) represents a ratio of forecasted values to their overall standard deviation. The AP values of 57.72 and 549.67 for the SMA and SMP models, respectively are both higher than the required AP value 4, which implies that both models have strong enough signals for optimization of the independent process parameters.

The coefficient of variance (CV) is a ratio of the standard deviation of any set of data to its means and is a measure of precision and reproducibility of the data. For any datasets to be repeatable, its CV value must be less than 10%. The CV values of 3.47 and 0.36% are indicatives of the repeatability of the SMA and SMP models, respectively. The standard deviation is a measure of the experimental error, and the values of 2.11 and 0.21 are indicatives of correct sizing of the experimental designs for both the SMA and SMP, respectively.

To further evaluate the accuracy of the models, the residual-based diagnostic plots were obtained from the data analyses performed by the Design Expert software. Only a few of the datasets deviated slightly from the straight line of the probability plots in Figures 4 (a and b) for both the SMA and SMP models, respectively, which validate the normality of the data. Figures 4 (c and d) are plots of the residuals versus the experimental run orders for both datasets obtained from using the SMA and SMP, as coagulant aids respectively. Whereas Figure 4c indicates an evenly and randomly scattered datasets without any lurking data for the SMA, the same cannot be said about the dataset for the SMP in which a lurking dataset (i.e., run number 13) that may influence the response variable can be noticed in Figure 4d. Figures 4 (e and f) are plots of the observed (actual) response values versus the predicted response values. It helps to detect group of values, that are not easily predicted by the model. The data points should be splatted evenly by the 45^o degree line. Both datasets obtained from using the SMA and SMP, as bioflocculant conform greatly with this condition.

To achieve the optimum combinations of the three independent process factors (i.e., cassava starch dose (CS, mg/L), wastewater-pH, and settling time (ST, minutes)) required to achieve complete or maximum removal of turbidity from the wastewater, numerical optimization strategy was applied. The impacts of the interactions between the process parameters on the turbidity removed are shown in the three-dimensional response surface plots in Figures 5, where the surface plots on the left-hand side represent the interaction effects on the turbidity removed from the wastewater between the cassava dosage and the wastewater-pH (AB), between the cassava dosage and the settling time (AC), and between the wastewater-pH and settling time (BC) for the SMA model and the corresponding plots on the right-hand side represent the interaction effects induced by SMP on the turbidity removed from the wastewater. With respect to the SMA, the general trend shown by the surface response plots (Figure 5) about the interaction effects is that by decreasing the cassava dosage and the wastewater-pH to the acidic range, the highest desired maximum turbidity removal was achieved, when the coagulated wastewater was allowed

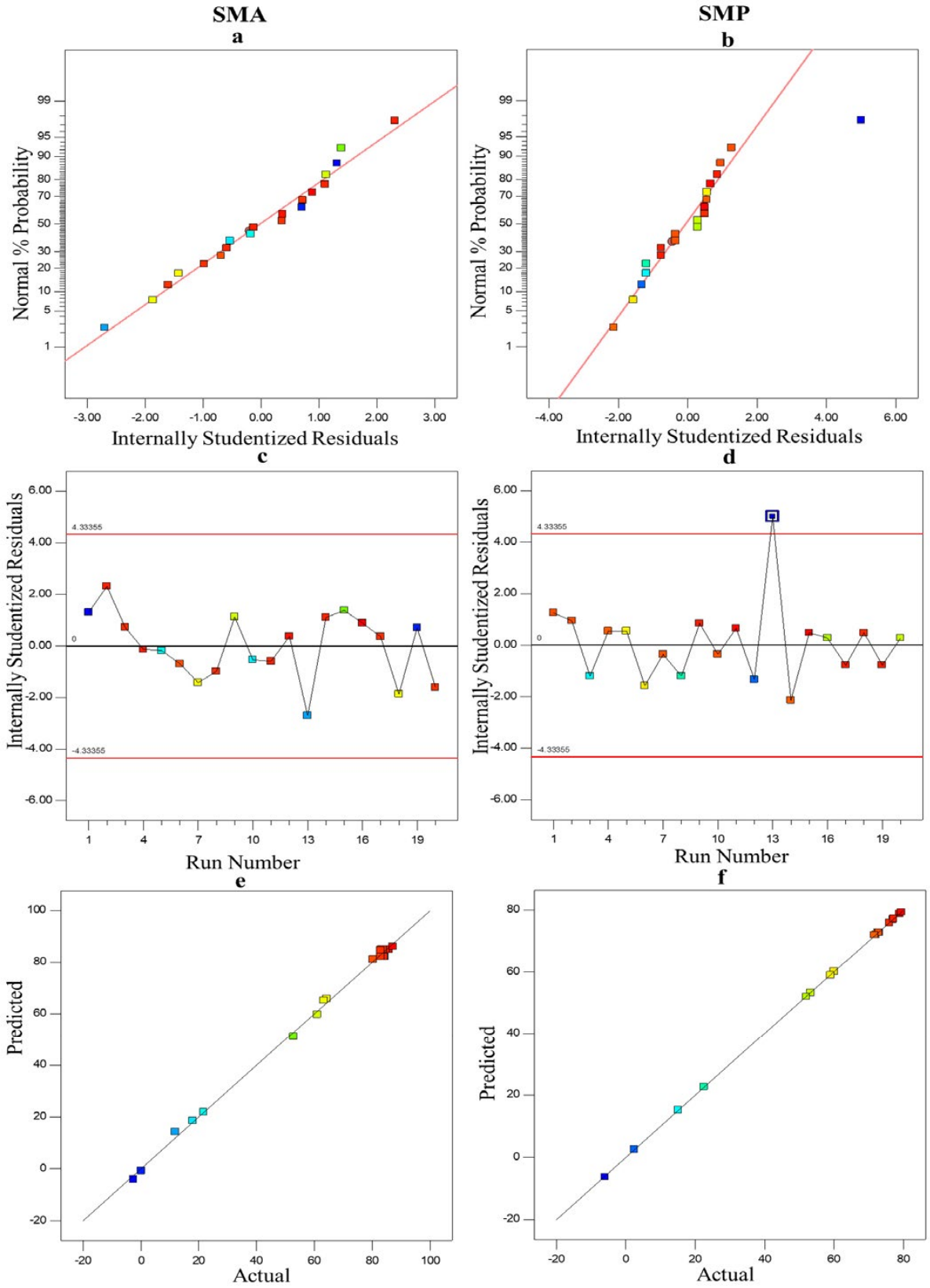

Fig. 4. Residual plots for the SMA and SMP with normal distribution (a and b), residual vs runs (c and d), and predicted vs actual (e and f)

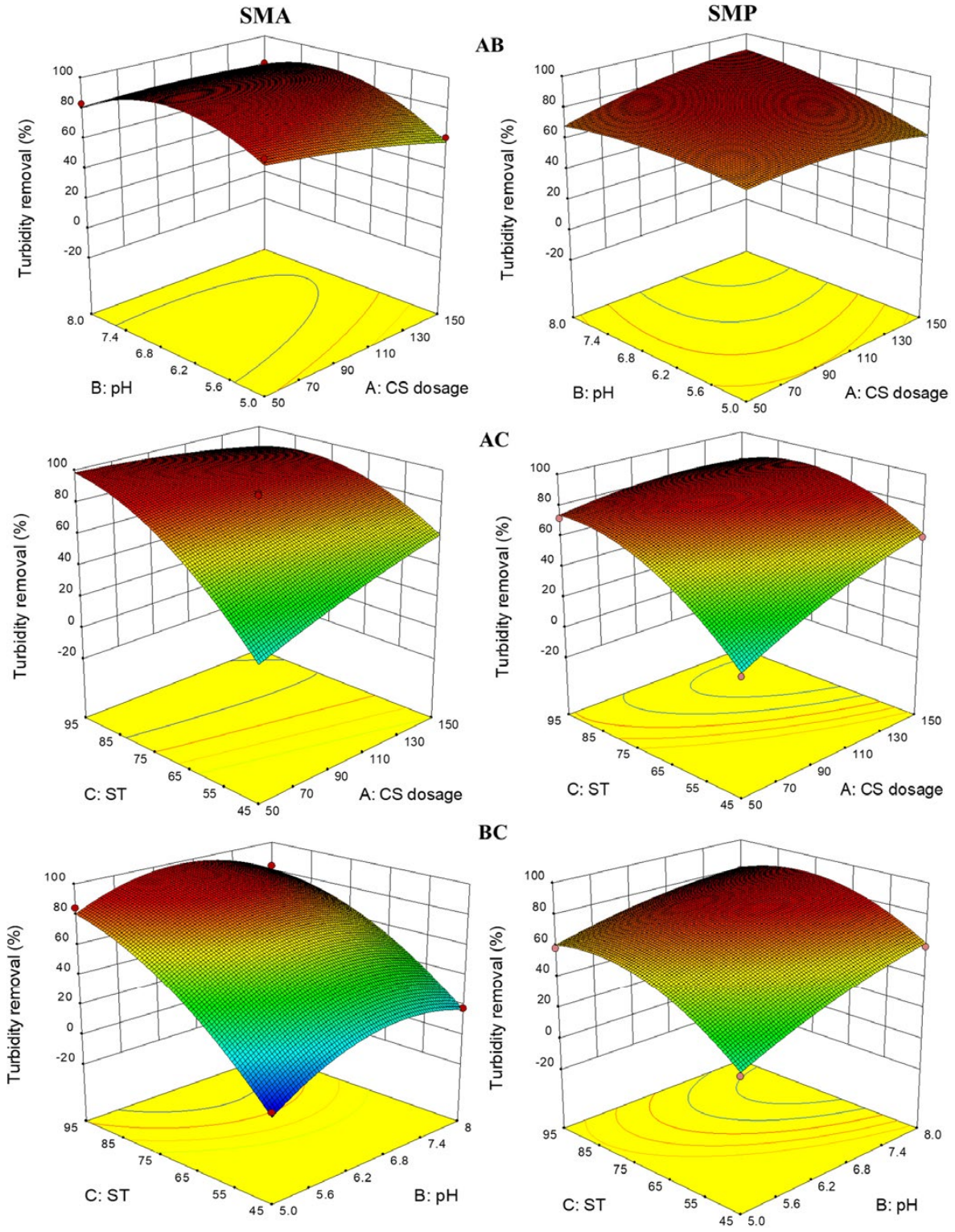

Fig. 5. Response Surface Plots for the Interactions Effects of the Process Factors on Turbidity removal for the SMA (left-hand side) and SMP (right-hand side)

sufficient time to settle. With respect to the SMP model however, a converse trend was obtained, as indicated between the process factors. Increasing the cassava dosage and wastewater-pH to the alkaline range were necessary for the attainment of maximum turbidity removal from the wastewater at a relatively moderate settling time.

The following optimum values: cassava dosage of 50 mg/L, wastewater-pH of 6.5, and settling time of 95 minutes were required to achieve a predictive 98.35% turbidity removal from the wastewater at a desirability function of 0.95 for the SMA model. But the optimum cassava dosage of 150 mg/L, wastewater-pH of 8.0, and settling time of 77 minutes were necessary for the achievement of the maximum predictive 88.87% turbidity removal from the wastewater at a desirability value of 0.63 for the SMP model. Validation and confirmation of these optimum levels of the independent process factors were performed by combining them on another separate Jar Tests and measuring the turbidity removed from the wastewater. For the Jar Test involving the use of the SMA, as a coagulant aid, three sets of experimental runs (i.e., 50, 100, and 150 mg/L cassava starch dose; 5.0, 6.5, and 8.0 wastewater-pH; 45-, 95-, and 95-minutes settling time) were performed with fixed concentration of the primary coagulant (alum). The observed turbidity removals at these optimum levels include -2.59, 87.22, and 80.30%, respectively.

For the SMP however, the three experimental runs performed involve the use of 50, 150, and 150 mg/L cassava starch dose; 5.0, 8.0, and 8.0 wastewater-pH; 45-, 77-, and 95-minutes settling time. The observed turbidity removed at these optimum levels include 2.40, 88.52, and 78.82%, respectively. The percentage error between the predicted and observed turbidity removal at these optimum levels of the process factors for the SMA was 12.13% with an F-Test value of 1.20 and p-value of 0.908, which implies no significant difference exist in the ratios of the variances and standard deviations between the predicted and observed turbidity removed at $p \leq 0.05$ significance level. For the SMP model however, the percentage error value of 0.20% with F-Test of 1.00 and p-value of 0.999 was recorded between the predicted and observed turbidity removed at the confirmation levels of the process factors. Similarly, there was no sufficient evidence that significant difference exists between the ratios of the variances and standard deviations between the predicted and observed values of turbidity removed from the wastewater treated with the SMP.

Generally, the acidic modification of the two cassava starch varieties helped them to attain the quality levels that are comparable with those of the natural and synthetic polymers, which aided the alum in reducing the wastewater turbidity (Yu & Fu, 2020). Whereas lower dosage of the SMA was needed to achieve maximum removal of turbidity from the wastewater that has low pH, a relatively higher dosage of the SMP was required to attain the maximum turbidity removed from the wastewater with higher pH. The highly significant positive effects of the doses of both SMA and SMP (i.e., $p < 0.0001$ in Table 3) imply that the cassava dosages do not only influence the degree of turbidity removed from the wastewater, but also affect the other process parameters. This is different from the findings of Usefi and Asadi-Ghalhari, (2019) that reported a significant negative interactive effect between wastewater-pH and rice starch dose for the coagulation-flocculation of wastewater containing kaolin powder. The relatively high coefficients of the main and interactive effects of the small cassava dosage as well as the higher value of the turbidity removed from the wastewater makes the SMA functions better than the SMP in aiding the coagulation-flocculation process. As expected, the cassava starch acts as a natural flocculant or polymer that helps to either neutralize the anionic charge on the particles or colloids or helps to adsorb turbid particles or helps to entrap the flocculation process and/or enable the bridging of particles to occur thereby leading to the formation of large flocs (Usefi and Asadi-Ghalhari, 2019).

The highly significant positive effects of the interactions of the cassava starch dosages and the wastewater-pH are indicated by the small p-value<0.0001 (Table 3) recorded for the SMA and SMP models. This implies that both starches influenced the protonation/deprotonation of

particles in the water depending on the pH value of the water (Dehghanian *et al*., 2016). With respect to the SMA model, the achievement of high turbidity removal from the wastewater at lower cassava dosage and lower wastewater-pH means that both the lower concentration of the cassava starch and lesser presence of protons preconditioned the rapid formation of flocs (Prasad & Yadav, 2021). Low pH is important for coagulation and precipitation of negatively charged impure particles in water, because the reduced concentration of hydrogen ions (H^+) allows for the positively charge primary coagulant (i.e., alum) to neutralize and precipitate the impure particles (Villabona-ortíz *et al*., 2021); hence, removal of impure particles is high. At lower pH, the cassava starch also creates conducive environment for the inherent organic compounds (humic and fulvic molecules) to stay together in the water and help to enhance the flocculation process (Jabbar & Alatabe, 2021). The presence of hydrogen bonds within the structure of the cassava starch might probably allow the occurrence of inter-particles bridging to take place through adsorption of particles on the starch surfaces. Also, cellulose's presence in the starch makes its molecular weight to be high, which creates an ideal environment for particle bridging to occur swiftly flocs (Usefi and Asadi-Ghalhari, 2019).

With respect to the SMP, the high turbidity removed from the wastewater at high cassava dosage and high wastewater-pH may probably be due to improved bridging of particles to form larger flocs because of the predominant presence of hydroxyl (OH^-) ions (Ali *et al*., 2022) and higher concentration of the starch polymer. This is in agreement with the suggestion of Usefi and Asadi-Ghalhari, (2019), that the zeta potential for rice starch decreases with increasing pH. Zeta potential is the distance between particle charge and the water surrounding it. In the absence of the cassava starch, the high presence of hydroxyl ions could overwhelm the impure particles for adsorption unto the surfaces of metallic ions or any other available coagulating species; thus making the removal of impure particles to be poor (Freitas *et al*., 2015). The impacts of the settling time on the degree of turbidity removed from the wastewater are profound.

In general, higher turbidity was removed from the wastewater when the coagulated water was allowed to settle for a longer period. The higher removal of turbidity from the coagulated wastewater at increased settling time is because the prolong settling time offers the fine flocs the chance to aggregate and form high-dense flocs that can rapidly be settled to the bottom of the beakers (Ahmad *et al*., 2021).

CONCLUSIONS

Highly polydisperse and acid-modified powder starches were produced from two cassava varieties–*Manihot aipi* and *Manihot palmate*. The cassava starches were used as bioflocculants for the treatment of textile wastewater in separate sets of coagulation-flocculation experiments involving the use of alum as the primary coagulant. The optimum effects of each cassava starch dosage, wastewater-pH, and settling time on the degree of turbidity removed from the wastewater were studied using central composite rotatable design, a subset of response surface methodology. Second-order multilinear quadratic regression models were developed from the experimental data obtained from using the aipi starch (SMA) and palmate starch (SMP), as coagulant-aids to alum for turbidity removal from the textile wastewater. The developed models for the SMA and SMP data were found to be highly significant with very high coefficient of determination (r^2) values of 0.999 and 1.000, respectively. The optimum process conditions for the aipi starch (SMA) dosage of 50 mg/L, pH of 6.5, and settling time of 95 minutes produced a predictive maximum turbidity removal of 98.35% with a desirability value of 0.95. While the optimum process conditions of cassava dosage of 150 mg/L, pH of 8.0, and settling time of 77 minutes produced a predictive maximum turbidity removal of 88.87% with a desirability value of 0.63 for the palmate starch (SMP). The corresponding observed turbidity recorded at these optimum conditions were 88.72% for the SMA and 88.52% for the SMP and there was

no significant difference between the predicted and observed turbidity removed from the wastewater at $p \leq 0.05$ significance level. Verification of the Jar tests showed a good agreement between the experimental data and the model values and confirmed that the SMA was superior to the SMP in aiding the primary coagulant (alum) in maximizing the removal of turbidity from the wastewater. Starch of better coagulating potentials can be extracted from the sweet cassava variety (*Manihot aipi*) than from the bitter cassava variety (*Manihot palmate*). In the future, it may be necessary to produce high resolution scanning electron microscopic images of the starches to help in the determination of the specific surface area of the starch particles, identify the various functional groups on them, and investigate how these functional groups relates to their performances as coagulants and flocculants for the treatment of diverse wastewaters.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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