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Research article

Response Surface Methodology optimization of chito-protein synthesized from crab shell in treatment of abattoir wastewater

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ABSTRACT

Abattoir wastewater generated from various meat processing operations in several developing countries pose a serious threat to the environment. Consequently, there is urgent need to reduce the impact of environmental pollution from it. Coagulation techniques have been recommended and used by many researchers successfully in treating wastewater, therefore an investigation of possible use of chito-protein extracted from crab shell (locally sourced) was used as a coagulant for treating abattoir wastewater. Coagulation experiments were carried out using jar-test procedure to investigate the influence of pH, time of settling, temperature and adsorbent dosage for coagulation of BOD, COD, Turbidity and Colour from the wastewater sample. To determine the interaction effect of the various process variables, Response Surface Method (RSM) was used in the optimization of the process variables. To determine the effectiveness of the coagulant, pre and post characterization of the wastewater samples were undertaken, the result of the post characterization of the wastewater sample indicated that most of the water quality parameters except Iron were within WHO standard. The Total Suspended Solid (TSS), for instance stood at 564.6 mg/L and 29 mg/L respectively for pre and post characterisation, the value of 29 mg/L of the post characterization was below the WHO recommended value of 30 mg/L. The predicted responses and the experimental values correlated significantly, an indicator that RSM optimization method used in this study is suitable in modelling the process variables. The result of the study further shows that optimum process variable is dependent on the solution pH (acidic), coagulant dosage of 2–3g, settling time of 25–30 min and operating temperature from 323K to 333K. The coagulant used in this study, when compared with previous studies have shown to have strong potential for use as a coagulant and as an alternative to chemical coagulants in the treatment of abattoir wastewater.

1. Introduction

The ever increasing need for protein through meat production as world population increases has resulted to several pollution problems (Bohdziewicz et al., 2003). Wastewater is derived from combination of industrial, domestic, agricultural or commercial activities, surface runoff or storm water, and from sewer inflow or infiltration (Tilley et al., 2014). Wastewater from abattoir is an effluent generated from cleaning operations in slaughter houses. In many countries, pollution arises from activities in meat production as a result of failure in adhering to Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP), this has resulted to severe pollution of water bodies (Bohdziewicz et al., 2003).

The major contaminants in slaughterhouse (abattoir) effluent are organic matter with relatively high level of suspended solid, liquid and fat (Eryuruk et al., 2014). Wastewater from abattoir is categorized under strong wastewater, since it possesses high concentrations of suspended solids, BOD, COD, soluble and insoluble organics. Wastewater putrefies fast, and is highly proteineous, this leads to environmental pollution problems (Altaher et al., 2011). Other characteristics of abattoir wastewater is the presence of suspended solids due to rumen contents, high volume of blood from the animals slaughtered, undigested food, flesh pieces, feathers, and pieces of bone. It is known to degrade the quality of water bodies and endangers public health, unless properly treated, wastewater can harm public health and the ecosystem.

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The impact of discharge of untreated abattoir wastewater on Nigerian water bodies has become a significant issue of environmental concern (Isehunwa and Onoveae, 2011; Chukwuma et al., 2018). In a recent study, risk assessment was applied to five abattoirs using fuzzy logic system in Anambra State of Nigeria, the overall fuzzy-based risk assessment from the study indicates that abattoir operations poses risk to the area ranging from moderate to severe risk (Chukwuma et al., 2020). Several studies have been done using various technologies such as biogas anaerobic technology as alternative in the treatment of abattoir wastewater (Chukwuma and Orakwe, 2014). Consequently, there is urgent need to reduce the impact of wastewater from abattoir operations in Nigeria using efficient method(s).

Coagulation/flocculation techniques have been recommended and used by many researchers successfully in treating wastewater however, with diverse views (Okey-Onyesolu et al., 2018a). Conventionally, industries employ the use of chemical coagulants in the treatment of wastewaters without considering the side effect of synthetic chemicals. Chemical coagulants are highly toxic, increases the pH of the treated water etc. Owing to demerits associated with the use of chemical coagulants, several studies have explored the use of alternative coagulants which are eco-friendly, biodegradable and can address the disadvantages of chemical coagulants. Also, several variables affect wastewater treatment, previous studies have limited the process variable investigated to two variables only, this is not adequate, for instance, Amudaa and Amoob (2007) investigated optimized dosage of coagulant and pH in the reduction of COD and Suspended Solids in beverage wastewater treatment, similarly, Marey (2019) investigated the same factors on turbid water. There is need to incorporate more factors, it has been asserted that several factors optimization is needed to enhance the study of the interaction effects of various process variable (such as pH, coagulant dosage, settling time and temperature) as done in this study (Okey-Onyesolu et al., 2017).

Crab shell is a natural carbohydrate biopolymer derived by deacetylation of chitin, a major component of the shells of crustaceans such as crab, shrimps and crayfish. Chitosan in crab shell waste has the advantage of low cost and high bio-compatibility (Menkiti et al., 2008). It has recently been recommended to be utilized for industrial effluent treatment, heavy metal sorption and drinking water treatment (Vishali et al., 2016). The use of crab shell chitosan in wastewater treatment has not received adequate attention especially in developing countries for treatment of waste water, there are scarce literatures on this also online, few studies on this however include the work of Devi et al. (2012), Okey-Onyesolu et al. (2018b), Rahmi et al. (2017) and Xu et al. (2019). Devi et al. (2012) utilized crab shell chitosan for diary wastewater treatment, Okey-Onyesolu et al. (2018b) extracted chito-protein from egg shell for deturbidization of abattoir wastewater, Rahmi et al. (2017) synthesized chitosan from crab shell for production of nano-particles, while Xu et al. (2019) used shell powder from oyster origin as adsorbent material for groundwater pollution control: the study considered oyster shell powder heavy metal ion adsorption properties in an aqueous solution to treat Lead, Copper and Cadmium. Although chitins and chitosan derived from natural coagulants have been applied in wastewater treatment for coagulation, there has been scanty of reported work on its application as a chito-protein in the treatment of abattoir wastewater. Hussain et al. (2019) reported that the measure of proteins expected to bind together all the distributed particles in the wastewater is essential in coagulation process, he asserted that the low application of active protein components in coagulant extract, is the major cause of reduction in coagulation efficiency. The novelty of this research hinges on the use of chito-protein obtained during the de-proteinization process of chitosan as coagulant for abattoir wastewater treatment. The objective of this study is to use chito-protein and optimization technique to determine optimum operating variables for pH, coagulant dosage, settling time and temperature as process variables in treatment of abattoir wastewater.

2. Materials and method

2.1. Materials source and collection

The major material utilized in this work include: chito-protein coagulants extracted from animal source: Crab Shell Chito-protein (CSC). The raw materials for the extraction of the natural coagulants (crab shell) were gotten from various restaurants/eateries around Awka, in Anambra State of Nigeria. Abattoir wastewater was collected from a slaughter house (Amansea) located in Anambra State of Nigeria.

2.2. Material characterization, extraction and estimation

The abattoir wastewater was characterized before and after the treatment at Biotechnology Laboratory, Nnamdi Azikiwe University, Awka, Anambra State. The following parameters were determined: Turbidity, pH, Total Solids (TS), Total Suspended Solids (TSS), Total Dissolved Solid (TDS), Total Hardness, Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Conductivity, Heavy Metals and Colour. The characterization was accomplished after American Public Health Association (APHA, 1998) prescribed standard methods. Heavy metals were analysed using Atomic Absorption Spectrophotometer (AAS) model AAS-240Z AA based on APHA (1998) standard. For extraction of bio-coagulant, 1000g raw samples for egg and crab shell were washed; sun dried, and ground using an industrial machine. The grounded samples were sieved using 0.6 mm sieve to maintain consistent surface area. 3.5 % (w/v) according to Al-Mutairi (2006) of dilute sodium hydroxide (NaOH) solution was used for the extraction of the active crude coagulant. The crab shell powder and the sodium hydroxide solution were mixed in solid - liquid ratio of 1:10 (w/v) in 500 ml beaker. It was afterwards stirred for 2 h using magnetic stirrer at a temperature of 65 °C. During this stage there was mild formation of foam which eventually subsided. Afterwards, the mixture was cooled to room temperature; while the resulting suspensions were separated from the crude extract using filter sack set-up via filtration process. A period of 30 min was given for the filtrate to settle, afterwards it was decanted to concentrate the bio-coagulant, the concentrated bio-coagulant was sun dried to minimize the moisture content, crushed using mortar and pestle to obtain the coagulant in powdered form and stored in air tight container for use. Determination of the protein content was done by the determination of total nitrogen using the micro Kjeldahl procedure, hence the amount of protein is obtained by multiplying the nitrogen content by 6.25 (a constant factor) (Kjeldahl, 1883). The method involves the digestion of sample with hot concentrated sulphuric acid in the presence of a metallic catalyst. Ash content (%), moisture content (%), crude protein (%), were determined by ASTM D629-15 (2015) lipid content (%) was extracted using soxhlet fat extraction method (FSA, 1998), and carbohydrates (%) was determined by Jatto et al. (2013).

The mass of the coagulant dosed is calculated using the following formula;

$$C_m = m/v \tag{1}$$

where C_m = Concentration of coagulant (g/1)

V = the volume of the wastewater solution used which is 250ml (0.25L)

Therefore, for 2g/1 of coagulant,

We have 2 g/l = m(g)/0.25L

m = 0.5g.

2.3. Process optimization variables for coagulation-flocculation experimental procedure

Jar-test apparatus was utilized for the coagulation experiments, the factors considered were pH, the dosage, the effect of temperature and time of settling. Coagulation in this study involves rapid mixing at a short time say, 2–5 min to effectively disperse the coagulant and encourage particle collision. This process is followed by a flocculation process involving a gentle mixing at a longer time which increases the particle size from sub-microfloc to visible suspended solids. Particles are thus bound together to produce larger macroflocs, to prevent the macroflocs from shearing, careful attention was given to the mixing velocity and energy. The mixing velocity and energy were abated when there appear to be an increase in floc formation. In the present work, the beakers were agitated at 250 rpm for 2 min (rapid mixing) and 30 rpm for 20 min (slow mixing), this was reported in a similar work by Nwabanne and Obi (2019).

2.3.1. The effect of coagulant dosage variation

The initial pH and Turbidity of the wastewater samples were measured at room temperature using pH and Turbidity meter. 1g–5g of CSC were added to 1000 ml of the abattoir waste water contained in five different 1000 ml beakers. The mixture was subjected to rapid mixing at 250 rpm for 2 min, followed by slow mixing at 30 rpm for 20 min using magnetic stirrer. After the slow stirring, the mixtures were allowed to settle by gravity, samples were withdrawn from the beaker and tested for the Turbidity, COD, BOD and Colour removal at different withdrawal times.

2.3.2. Effect of pH variation

The optimum dosage obtained from the result of section 2.3.1 was used for the evaluation of pH effect. Equal amount of the already established optimum coagulant dosages were dosed into 6 different beakers with each containing 1000 ml of the abattoir waste water samples. Consequently, pH adjustment of the samples was done using 0.1M HCl and 0.1M NaOH to obtain pH solutions at 2, 4, 6, 8 and 10. Two (2) minute fast stirring and 20 min slow stirring periods were allowed. After the slow stirring period, the mixtures were allowed to settle by gravity, samples were withdrawn from the beaker and tested for the Turbidity and Colour removal at different withdrawal time.

2.3.3. Effect of temperature variation

A set of jar tests experiments was carried out using the already determined optimum dosage and pH; while the solution temperature was varied at different temperatures; 303K, 313k, 323K and 333K using adjustable temperature magnetic stirrer (B.Bran Scientific model78HW-1). The residual Turbidity, COD, BOD and Colour were also measured and recorded with respect to temperature within the settling period of 60 min.

2.4. RSM optimization of physio-chemical properties of the wastewater

Quadratic regression model was used to predict Turbidity, BOD, COD and Colour responses of the experiment, with pH, settling time, dosage and temperature being the input parameters. Then optimization was carried out via interactive surface graphics after comparative analysis for each response to determine the input parameters that would yield minimal BOD, COD, Turbidity and Colour responses. RSM as a good experimental design system was used to evaluate the effect of the above several factors and their interaction on the system response. It is a combination of mathematical and statistical methods. Design Expert software version 10.0 was used in the experimental design, Central Composite Design (CCD) design was chosen to analyse the parameters. CCD provides high quality predictions over the entire design space because it generates new extremes for all factors outside the design bracket. The summary of the 4-level factor design used in this work is shown on Table 1. A-30 experimental run generated by 2^4 fractional factorial CCD technique of the RSM was performed to evaluate the mutual impact of the independent variables on the dependent variables.

The type of regression model used is quadratic regression model. The mathematical model is shown in Eqs. (2), (3), (4), and (5):

$$y_1 = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4 + a_5x_1x_2 + a_6x_1x_3 + a_7x_1x_4 + a_8x_2x_3 + a_9x_1^2 + a_{10}x_2^2 + a_{11}x_3^2 + a_{12}x_4^2 \quad (2)$$

$$y_2 = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4 + a_5x_1x_2 + a_6x_1x_3 + a_7x_1x_4 + a_8x_2x_3 + a_9x_1^2 + a_{10}x_2^2 + a_{11}x_3^2 + a_{12}x_4^2 \quad (3)$$

$$y_3 = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4 + a_5x_1x_2 + a_6x_1x_3 + a_7x_1x_4 + a_8x_2x_3 + a_9x_1^2 + a_{10}x_2^2 + a_{11}x_3^2 + a_{12}x_4^2 \quad (4)$$

$$y_4 = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4 + a_5x_1x_2 + a_6x_1x_3 + a_7x_1x_4 + a_8x_2x_3 + a_9x_1^2 + a_{10}x_2^2 + a_{11}x_3^2 + a_{12}x_4^2 \quad (5)$$

where; a_0 to a_9 are the regression coefficients, x_1 to x_4 are the input variables in the order: pH, settling time (min), dosage and temperature, y_1 to y_4 are the responses in the order BOD, COD, Turbidity and Colour.

2.5. Statistical validation

RSM result was subjected to error analysis, statistical analysis was carried out in order to assess the respective predictive performance and estimation capabilities by means of equation 6 statistical techniques (see Table 2).

3. Results and discussion

3.1. Characterization of coagulant and wastewater

The Chito-protein used as the coagulant was analysed, the result of the proximate analysis of the bio-coagulant is shown in Table 3.

From Table 3, crude protein consists of the major constituent of the bio-coagulant with about 43%. This is followed by the carbohydrate content of 36.5%, while lipid was the least constituent of the bio-coagulant.

The pre and post characterization results of the wastewater samples are presented in Table 4. The result of the characterization was compared with World Health Organization (WHO) standard. The abattoir wastewater possesses elevated amount of TSS, BOD, COD, Colour and Heavy Metals before treatment. Turbidity is the cloudy appearance in water caused by small suspended particles, the significant reduction in

Table 1. CCD summary for abattoir wastewater coagulation.

Independent variables		Coded variable levels				
		-α	-1	0	+1	+α
Settling time	minute	0	15	30	45	60
Temperature	K	303	313	323	333	343
Dosage	g	1	2	3	4	5
pH	-	2	4	6	8	10

Table 2. Error functions and its equations.

Error function	Equation	Reference	Equation numbers
Root mean square error, (RMSE)	$\sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_{i,exp} - y_{i,pred})^2}$	Okey-Onyesolu et al., (2017)	6

Turbidity value after treatment for both wastewater samples indicates the occurrence of efficient coagulation process (Nur and Rajesh, 2014).

The post characterization shows that most of the parameters were within WHO standard, TSS for instance stood at 564.6 mg/L before the coagulation, it reduced to 29 mg/L which is within the WHO standard after the coagulation experiment. Iron was however higher with a value of 1.074 mg/L, this is above the recommended value of 0.3 mg/L. Turbidity is a significant parameter in coagulation–flocculation assessment. At the point when the aim of coagulation–flocculation is to expel colloidal and suspended solids from water, the leftover Turbidity gives a pointer of how well the treatment has succeeded., the experiment indicated that Turbidity has a value of 310 NTU before coagulation and 10.6 NTU after coagulation, the result of post characterization was within the WHO recommendation, this shows the effectiveness of Chito-protein in abattoir wastewater treatment. COD is usually used as a measurement of pollutants in wastewater and natural waters, the COD value before coagulation was 692 mg/L and drastic reduction to 27.83 after coagulation occurred, an indicator that the coagulant contributed immensely in the wastewater treatment hence the Turbidity reduction. Generally, most of the heavy metals, BOD, COD etc where significantly reduced after the coagulation process in the experiment.

3.2. RSM optimization model result of abattoir wastewater treatment

RSM-CCD model was employed to obtain the optimal conditions, providing the highest removal of pollutants from the wastewater studied. RSM was used to model the parameters; the Analysis Of Variance (ANOVA) results for the models terms are given in Tables 5 and 6. ANOVA was applied to estimate the significance of the model at 5% significance level.

From Table 5, it was observed that all the linear terms and quadratic terms of the models for all the parameters was significant while only interactive term of settling time (x_2) with dosage (x_3) and dosage (x_3) with temperature (x_4) was significant for the BOD, the R^2 for the model is 0.987 indicating a good model prediction. For the COD, all the linear terms and quadratic terms of the model were significant, the interaction effect between the pH (x_1) and all other process variables were insignificant, the interaction between dosage and temperature was seen to be significant (P value of 0.000131). It therefore shows that dosage and temperature is a significant factor for BOD and COD coagulation. Table 6 shows the ANOVA table for Turbidity and Colour as response variable similar to Table 5.

From Table 6, it is observed that all the linear terms and quadratic terms of the models for all the parameters was significant for Turbidity and Colour, while the interactive term of settling time (x_2) with dosage (x_3) and dosage with temperature (x_4) was significant for both Turbidity and Colour, the R^2 for the model is 0.987 and 0.99 respectively for Turbidity and Colour, indicating a good model prediction. A model is regarded to be significant when the p-value is less than 0.05, in Tables 5 and 6., the p-values is presented, it is therefore infered that the linear terms x_1, x_2, x_3 and x_4, interaction terms x_2x_3 and x_3x_4 and all the quadratic terms (except x_2x_3 for COD) x_1^2, x_2^2, x_3^2 and x_4^2 for BOD, COD, Turbidity and Colour removal from abattoir wastewater treatment, are significant model terms. Based on this, the insignificant terms of the model were removed and the model reduces to the final equations (coded). Hence, the final equations for Y_{BOD}, $Y_{COD,}$ $Y_{Turbidity}$ and Y_{Colour} in coded values can be expressed as:

$$Y_{BOD} = -2295 + 8.55x_1 + 0.26x_2 + 66.99x_3 + 13.7x_4 + 0.05x_2x_3 - 0.13x_3x_4 - 0.53x_1^2 + -0.013x_2^2 - 4.12x_3^2 - 0.02x_4^2 \quad (7)$$

$$Y_{COD} = -2287.1 + 7.51x_1 + 0.368x_2 + 46.13x_3 + 14.46x_4 - 0.231x_3x_4 - 0.442x_1^2 - 0.0238x_2^2 - 5.226x_3^2 - 0.0342x_4^2 \quad (8)$$

$$Y_{turbidity} = -2281.7 + 8.6x_1 + 0.3x_2 + 16.77x_3 + 11.7x_4 + 0.05x_2x_3 - 0.13x_3x_4 - 0.52737x_1^2 - 0.012742x_2^2 - 4.1157x_3^2 - 0.02027x_4^2 \quad (9)$$

$$Y_{colour} = -1936.9 + 5.69x_1 + 0.45x_2 + 69.32x_3 + 11.47x_4 + 0.06x_2x_3 - 0.144x_3x_4 - 0.44x_1^2 + -0.011x_2^2 - 3.77x_3^2 - 0.017x_4^2 \quad (10)$$

3.2.1. RSM plot for the interaction effect on the responses variables

A-30 experimental run generated by 2^4 fractional factorial central composite design technique of the Response surface methodology were performed to evaluate the combined effect of the independent variables (settling time, solution temperature, dosage and pH) on the response (% Yield). The result of response surfaces and contours generated to investigate the effect of coagulant dosage and settling time is shown in Figures 1, 2, 3, and 4. Figure 1, shows the effect of coagulant dosage variation from 1g – 5g of CSC and settling time from 0 to 60 min on BOD reduction in percentage. It shows that increase in CSC dosage beyond 4.2g leads to decrease in BOD reduction, also CSC dosage below 2.5g resulted in decrease in BOD reduction. The Figure also shows that there is

Table 3. Results of proximate analyses of the bio-coagulant.

S/N.	Parameters	Value
1.	Moisture content (%)	7.05
2.	Crude protein (%)	43.10
3.	Ash content (%)	8.67
4.	Crude fibre (%)	3.08
5.	Lipid content (%)	1.60
6.	Carbohydrates (%)	36.5

Table 4. The Pre and Post Characterization results of the Wastewater Samples with CSC.

S/N	Parameter	Unit	Before Coagulation	After Coagulation.	WHO Standard
1.	Turbidity	NTU	310	10.6	<11.75
2.	TSS	mg/L	563.6	29	30.00
3.	COD	mg/L	692	27.83	NS
4.	BOD_5	mg/L	470	18.45	30
5.	Colour	mg/L	210.2	65.93	-
6.	pH	-	6.8	7.3	6.6–8.56
7.	TS	mg/L	1080	77	500
8	TDS	mg/L	516.4	48	50.00
9.	Total hardness	mg/L	80	50	500.00
10.	Sulphate	mg/L	12.63	1.72	-
11.	Iron	mg/L	4.790	1.074	0.3
12.	Potassium	mg/L	8.10	0.08	-
13.	Magnesium	mg/L	58.64	4.5	75
14.	Lead	mg/L	0.5	0.00	0.1

Remark: Nephelometric Turbidity Unit-NTU, Not Stated- NS.

Table 5. ANOVA table for BOD and COD for the wastewater treatment.

Coefficients	BOD			COD		
	Coefficient values	P-values	Error and prediction parameters	Coefficient values	P-values	Error and prediction parameters
a_o	-2295	1.45E-08		-2287.1	1.52E-08	
a_1x_1	8.55	0.0061	$R^2 = 0.987$	7.51	0.00609	$R^2 = 0.98657$
a_2x_2	0.2578	0.0065		0.368	0.0065	
a_3x_3	66.99	9.53E-07	$AdjR^2 = 0.97$	46.13	9.53E-07	$AdjR^2 = 0.97404$
a_4x_4	13.7	1.93E-08		14.46	1.92E-08	
$a_5x_1x_2$	0.0115	0.205	RMSE = 1.0286	0.0115	0.20046	RMSE = 1.0286
$a_6x_1x_3$	0.0722	0.583		0.0722	0.944	
$a_7x_1x_4$	-0.0113	0.394	p-value = 1.944e-11	-0.0113	0.3941	p-value = 1.9394e-11
$a_8x_2x_3$	0.0518	0.0086		0.0518	0.085	
$a_9x_2x_4$	0.00146	0.407	SSE = 15.871	0.00146	0.40802	SSE = 15.871
$a_{10}x_3x_4$	-0.131	0.000131		-0.231	0.000131	
$a_{11}x_1^2$	-0.527	1.94E-08		-0.442	1.94E-08	
$a_{12}x_2^2$	-0.0127	2.85E-10		-0.0238	2.85E-10	
$a_{13}x_3^2$	-4.116	1.60E-12		-5.226	1.60E-12	
$a_{14}x_4^2$	-0.02	3.33E-08		-0.0342	4.20E-08	

Table 6. ANOVA table for turbidity and colour.

Coefficient	Turbidity			Colour		
	Coefficient values	P-values	Error and prediction parameters	Coefficient values	P-values	Error and prediction parameters
a_o	-2281.4	1.57E-08		-1936.9	6.43E-09	
a_1x_1	8.551	0.0061	$R^2 = 0.987$	5.6896	0.01105	$R^2 = 0.99058$
a_2x_2	0.258	0.0065		0.45091	0.00328	
a_3x_3	16.77	9.53E-07	$AdjR^2 = 0.97404$	69.316	3.25E-08	$AdjR^2 = 0.98178$
a_4x_4	11.69	1.92E-08		11.469	9.53E-09	
$a_5x_1x_2$	0.0115	0.20046	RMSE = 1.0286	0.007146	0.31077	RMSE = 0.81741
$a_6x_1x_3$	0.0722	0.5828		0.00719	0.94485	
$a_7x_1x_4$	-0.0113	0.39412	p-value = 1.9394e-11	-0.00478	0.64655	p-value = 1.3938e-12
$a_8x_2x_3$	0.0518	0.0086		0.06046	0.0004792	
$a_9x_2x_4$	0.0015	0.407	SSE = 15.871	0.000594	0.6681	SSE = 10.022
$a_{10}x_3x_4$	-0.131	0.000131		-0.1441	3.94E-06	
$a_{11}x_1^2$	-0.527	1.94E-08		-0.4404	9.98E-09	
$a_{12}x_2^2$	-0.0127	2.85E-10		-0.01112	6.87E-11	
$a_{13}x_3^2$	-4.116	1.60E-12		-3.7678	2.00E-13	
$a_{14}x_4^2$	-0.02	3.29E-08		-0.01679	1.90E-08	

a general increase in BOD reduction with increasing settling time, a minimum of 5 min settling time is required for 65% BOD reduction. It also indicates that maximum BOD reduction of 77.85% is achieved at 3.176g dosage of CSC and settling time of 43.75 min.

This study aligns with a previous study by Hassan (2011), the study shows that percentage removal of Turbidity increases when the time of slow mixing with Alum and the time of final settlement of the water samples were increased for the same dosage of Alum used. Settling time is therefore essential for maximum removal of BOD in wastewater treatment. Similarly as shown in Figure 1, Figure 2 represents the COD reduction (in percentage) for the same process parameters of dosage variation and settling time. The COD reduction was considerably low at low dosage (1g) and settling time (less than 5 min), increasing dosage at 2g and settling time above 10 min, resulted in COD reduction up to 60%. Optimum dosage of 3.616g and settling time of 47.88 min revealed maximum COD removal of 85.23%, this is higher than the BOD removal of 77.85% observed in Figure 1. Since BOD and COD reflect the pollution degree of the water, and are the comprehensive index of the relative content of organics, 77.85% and 85.23% reduction of BOD and COD respectively from this study is in indicator that chito-protein is effective in abattoir wastewater treatment. A study by Abd Rahim et al. (2019) investigated on optimization process of coagulation of wastewater using cassava peels and alum, the authors reported that the coagulants removed TSS, Turbidity and COD up to 89.86%, 90.32% and 18.87%, respectively. The removal of COD up to 85.23% from this study in comparison to 18.87% as asserted by Abd Rahim et al. (2019) shows the effectiveness of chito-protein in wastewater treatment as against the use of Alum which could be detrimental to human health. Figures 3 and 4 shows the interaction effect of dosage variation and settling time in percentage removal of Turbidity and Colour respectively.

From the figure, a clear peak was observed, this indicates that the optimum conditions were inside the design boundary. Turbidity reduction was considerably low at low dosage (1g) and settling time (less than 5 min) with about 55% reduction. However, at coagulant dosage of 3.7g and settling time of 46.5 min, maximum Turbidity removal of 90.95% was achieved. The amount of Colour removal shown in Figure 4 was about 72.76%, this is less than the Turbidity removal. The dosage amount for maximum reduction for Colour removal is the same for the Turbidity removal. However, the settling time of 49.25 min, which is a bit longer, was the optimum settling time for the Colour removal. The surface and contour plots on the effect of settling time and pH on BOD, COD, Turbidity and Colour is shown in Figures 5, 6, 7, and 8. From Figure 5, it

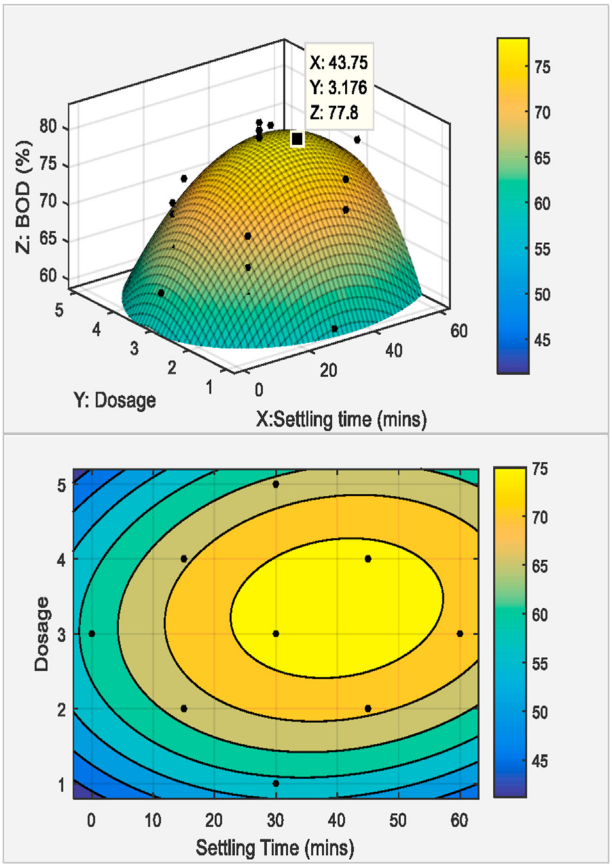

Figure 1. Surface and contour plot of interaction of settling time and dosage on BOD.

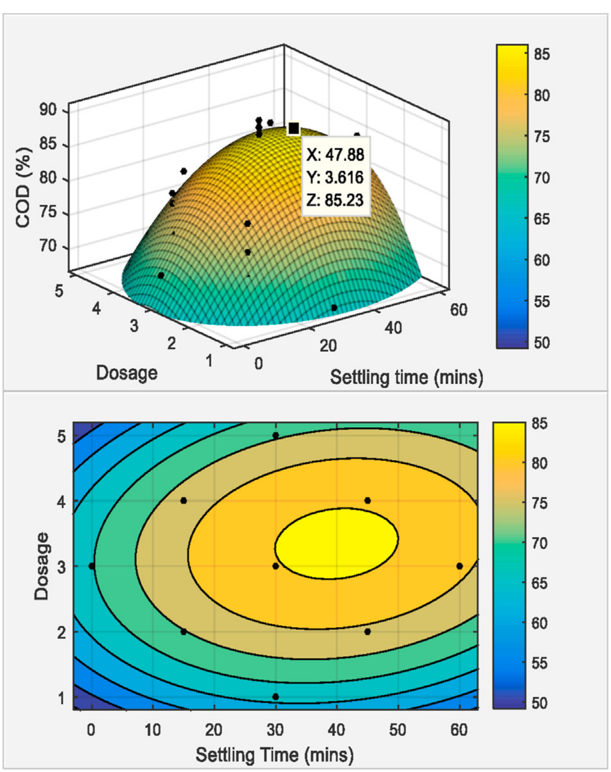

Figure 2. Surface and contour plot of interaction of settling time and dosage on COD.

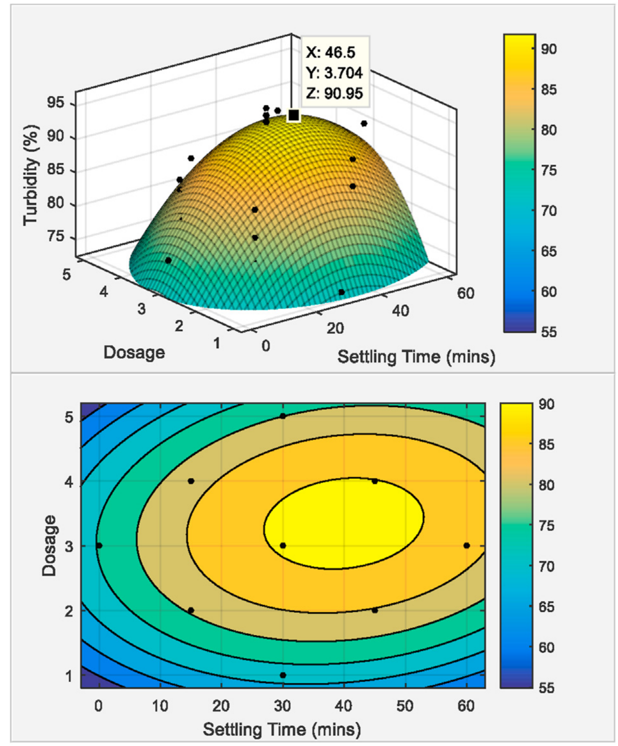

Figure 3. Surface and contour plot of interaction of settling time and dosage on Turbidity.

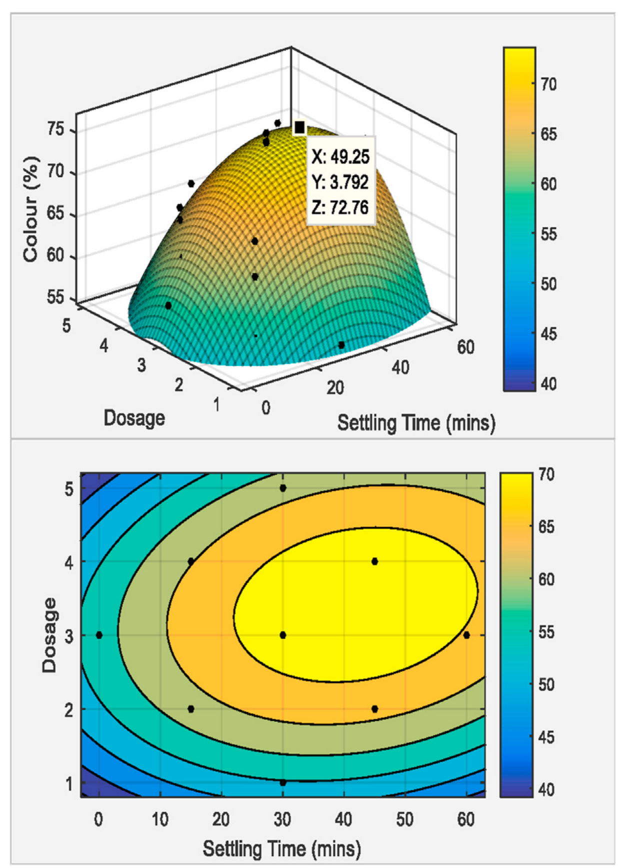

Figure 4. Surface and contour plot of interaction of settling time and dosage on Colour.

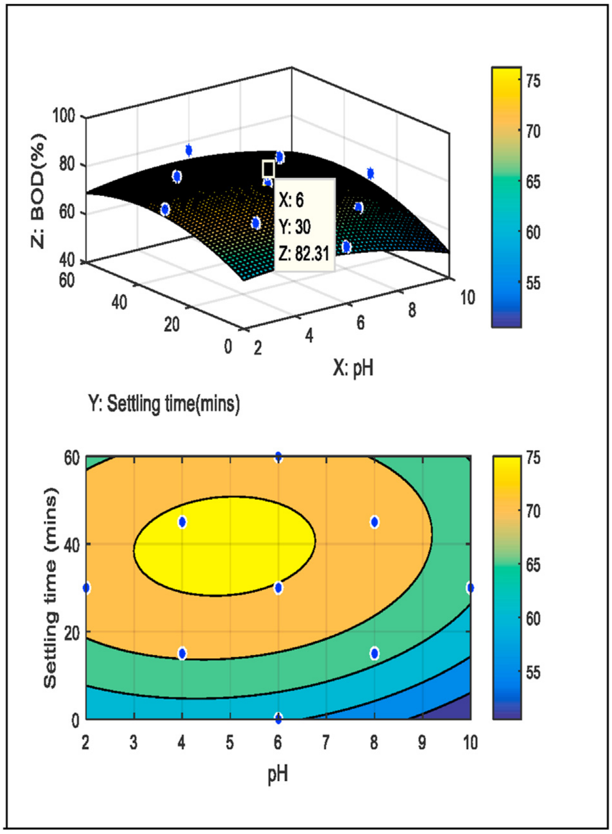

Figure 5. RSM Surface and contour plot of interaction of settling time and pH on BOD.

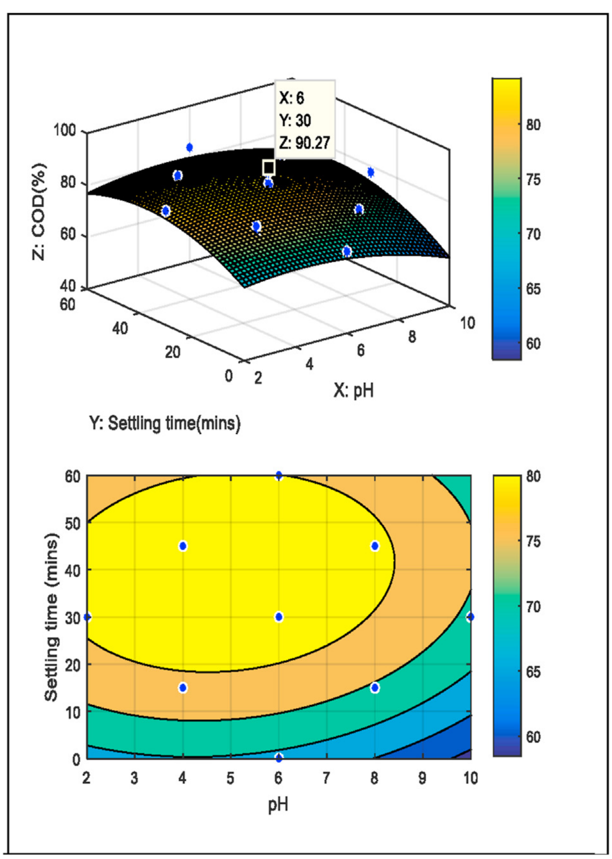

Figure 6. RSM Surface and contour plot of interaction of settling time and pH on COD.

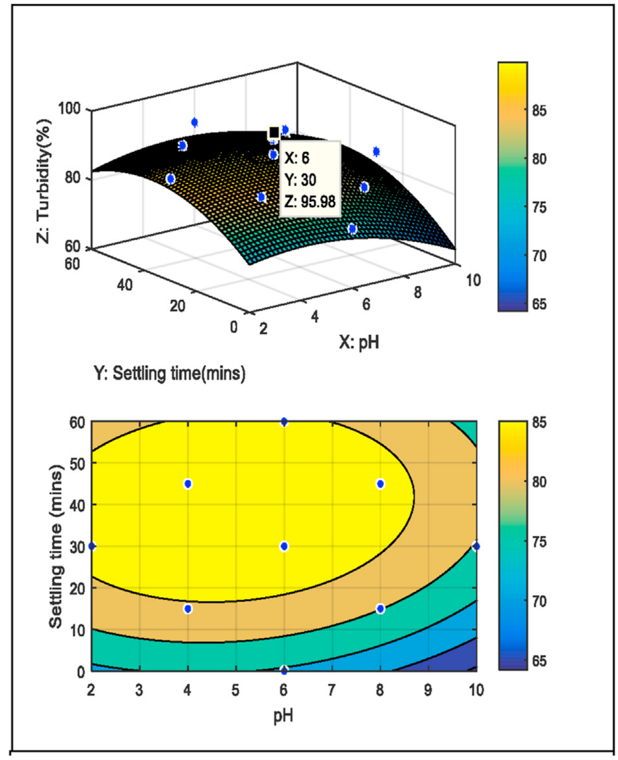

Figure 7. RSM Surface and contour plot of interaction of settling time and pH on turbidity.

was observed from the surface and contour plots that the percentage removal of BOD increased as settling time and pH increased, but decreased beyond pH of 6. This is similar to COD removal in Figure 6. Maximum BOD removal of 82.31% and COD removal of 90.27% removal was observed at pH 6 and settling time of 30 min for both response parameters.

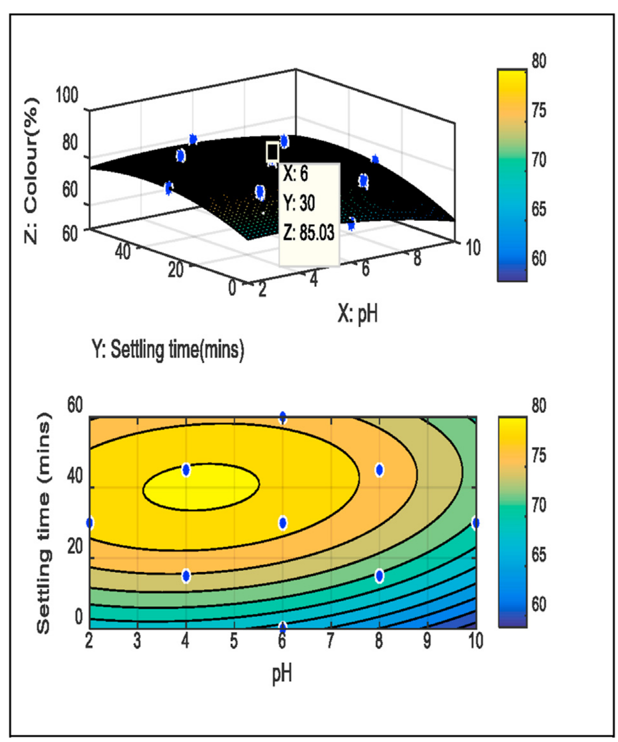

Figure 8. RSM Surface and contour plot of interaction of settling time and pH on color.

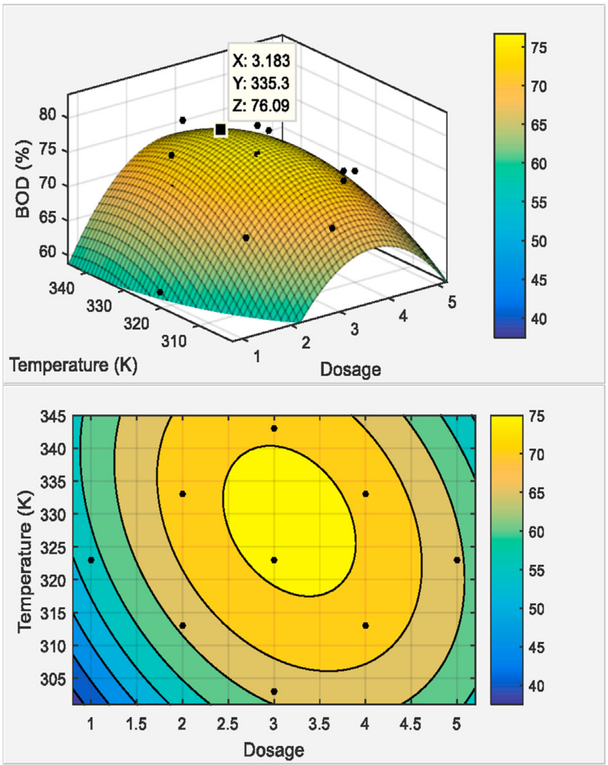

Figure 9. RSM Surface and contour plot of interaction effect of Temperature and dosage on BOD.

Apart from having higher COD removal as shown in the plots, the settling time and pH ranges for better COD reduction was higher than the BOD. This shows that pH of 6 should be maintained for optimum BOD and COD removal. Figures 7 and 8 shows surface and contour plot for

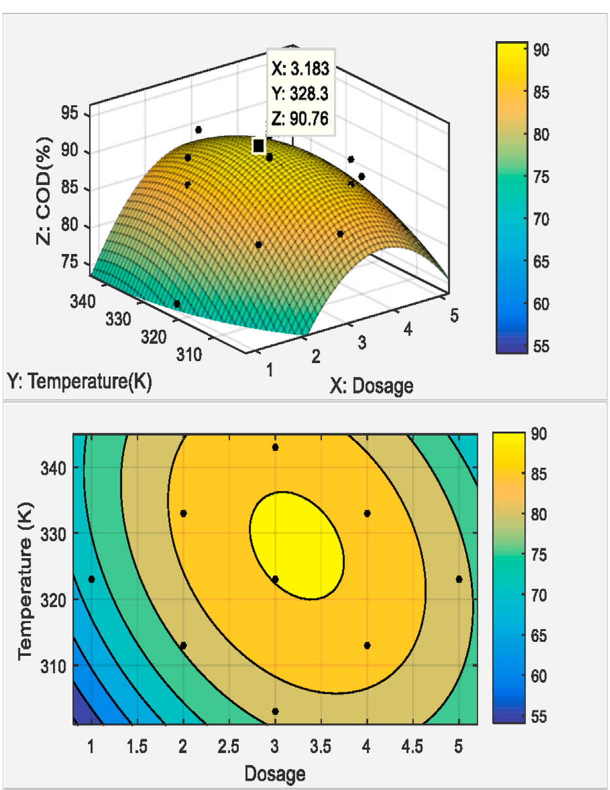

Figure 10. RSM Surface and contour plot of interaction effect of Temperature and dosage on COD.

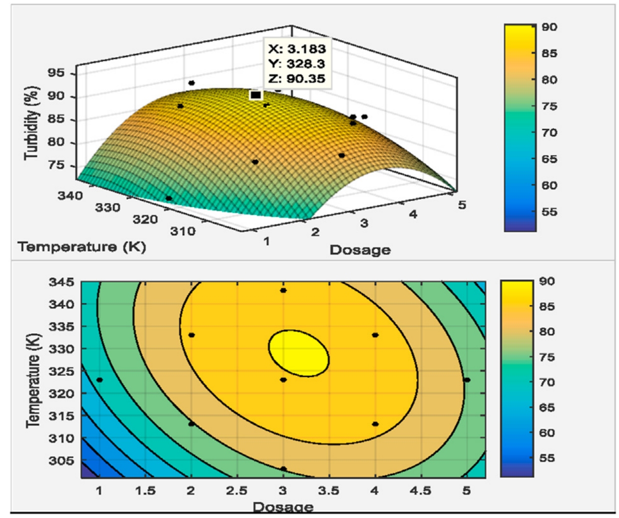

Figure 11. RSM Surface and contour plot of interaction of temperature and dosage on turbidity.

Turbidity and Colour reduction. The figures has similar trend with Figures 5 and 6; with settling time of 30 min and pH of 6.

This shows that the percentage removal of effluent parameters from abattoir wastewater using CSC coagulants is more effective when the solution is in acidic medium and gets saturated with increase in time. At high pH (alkaline medium), the charge of the coagulating species seems to become less positive and as a result, less attracted to anionic organic compounds. Therefore, acidic medium is suggested to be the most effective medium especially for Colour and BOD removal. The interaction effect of dosage and temperature is presented in Figures 9 and 10, Figure 9 depicts the effect of temperature and dosage on BOD removal, Figures 9 and 10 have similar contour, however, Figure 9 has wider range of peak contour.

The plot shows that low reduction of BOD and COD is observed at low temperature and dosage, also the coagulant dosage of 3.183g serves as the optimum dosage for both BOD and COD removal, the optimum temperature however differed, with 355.3k for BOD removal and 328.3k for COD removal. The maximum BOD and COD reduction is 76.06% and 90.76% respectively.

Figures 11 and 12 has similar trend with Figures 9 and 10, the maximum Turidity and Colour reduction is 90.35% and 72.16% respectively. From the Figures, it was observed that the percentage removal of the effluent parameters increases as temperature and

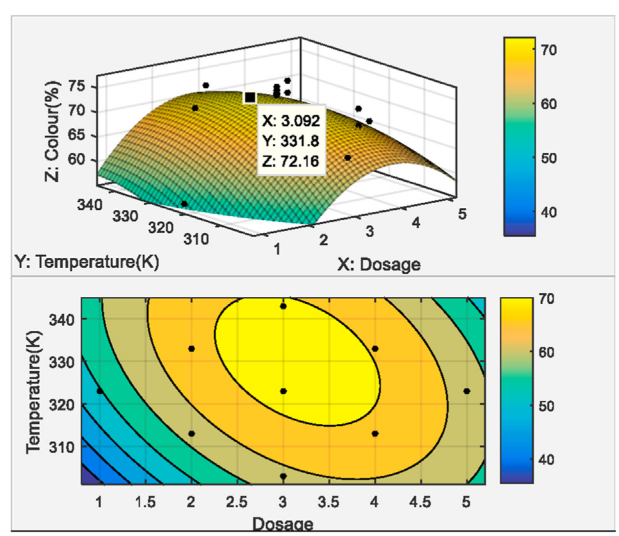

Figure 12. RSM Surface and contour plot of interaction of temperature and dosage on colour.

Table 7. RSM predicted conditions for optimum responses of abattoir wastewater treatment.

Parameters	pH (-)	Settling time (min)	Dosage (g)	Temperature (K)	Predicted optimum values (%)
CSC					
BOD	2	25	2	323	94.27
COD	2	28	2	313	95.42
Turbidity	4	30	3	333	99.32
Colour	4	30	3	333	92.15

Table 8. Validation of optimized RSM responses for abattoir wastewater treatment.

Parameters	pH (-)	Settling time (min)	Dosage (g)	Temperature (K)	Predicted optimum values (%)	Experimental values (%)
BOD	2	25	2	323	94.27	95.01
COD	2	28	2	313	95.42	96.14
Turbidity	4	30	3	333	99.32	98.93
Colour	4	30	3	333	92.15	94.48

coagulant dosage increased but decreased as temperature increases and dosage above 4g. This could be attributed to the fact that the coagulation capacity or strength of the coagulant was distorted by high temperature. In the optimization study conducted by Nwabanne and Obi (2019), an optimum Turbidity removal of 94.39 % was reported at pH of 2, 1000 mg/l of coagulant dosage, 50 min settling time and operating temperature of 50 °C, the result of this study seems to be similar to the study.

3.3. Optimization process of RSM using CCD

To determine the optimum process variables, the coagulation process was carried out with the assistance of design expert software. The optimization was performed using the numerical result in Matlab 2017. To maximize the responses of $Y_{Turbidity}$, Y_{Colour}, Y_{BOD} and Y_{COD}, Eqs. (7), (8), (9), and (10) were solved to determine the most suitable solutions, with the objective to the responses were maximized within the design space. A usual approach, which consists of selecting the best solution based on economic considerations, was therefore adopted and the optimal solutions presented in Table 7.

3.4. Validation of the results

Experimental runs were conducted under optimal conditions, to validate the accuracy of the models. The optimum predicted values from CCD were further confirmed by performing the experiment obtained from the predicted inputs condition, the results of the experimental values is as shown in Table 8. The verification experiments demonstrated a strong correlation between the experimental and RSM model, indicating that the RSM-CCD approach adopted in this study was appropriate for optimizing the coagulation-flocculation process.

4. Conclusion

In this research, coagulation-flocculation technique was studied to remove contaminants (BOD, Turbidity, COD and Colour) from abattoir wastewater using bio-extract as natural coagulants. Based on the investigations done, the following conclusions were drawn: it was observed that the RSM optimization correlated significantly with predicted responses and the experimental values.

The removal efficiency of Turbidity, BOD, COD and Colour from the wastewater were dependent on the solution pH that is acidic, coagulant dosage of 2–3g, settling time of 25–30 min and operating temperature from 323K to 333K. The CSC chito-protein, a novel coagulant was shown to have strong potential for use as a coagulant and as an alternative to chemical coagulants for the treatment of abattoir wastewater.

Declarations

Author contribution statement

Okey-Onyesolu, Chinenye Faith: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Chukwuma, E. C.: Analyzed and interpreted the data; Wrote the paper.

Okoye, C. C.: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Onukwuli, O. D.: Conceived and designed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Abd Rahim, N.S., Othman, N., Fahirah, S.N., Asharuddin, S.M., Malek, M.A., 2019. Turbidity, COD and total suspended solid removal: application of natural coagulant cassava peel starch. Int. J. Recent Technol. Eng. 8, 213–222.
Al-Mutairi, N.Z., 2006. Coagulant toxicity and effectiveness in a slaughterhouse wastewater treatment plant. Ecotoxicol. Environ. Saf. 65, 74–83.
Altaher, H., Qada, E., Omar, W., 2011. Pretreatment of wastewater streams from petroleum/petrochemical industries using coagulation. Adv. Chem. Eng. Sci. 1, 245–251.
Amudaa, O.S., Amoob, I.A., 2007. Coagulation/flocculation process and sludge conditioning in beverage industrial wastewater treatment. J. Hazard Mater. 141, 778–783.
APHA, 1998. Standard Methods for the Examination of Water and Wastewater, twentieth ed. Alpha/Awwa/Wef Washinton DC, USA.
ASTM D629-15, 2015. Standard for Quantitative Analysis of Textiles. ASTM International, West Conshohocken, PA, p. 2015.
Bohdziewicz, J., Sroka, E., Korus, I., 2003. Application of ultrafiltration and reverse osmosis to the treatment of the wastewater produced by the meat industry. Pol. J. Environ. Stud. 12, 269–274.
Chukwuma, E.C., Orakwe, L.C., 2014. Anaerobic co-digestion of cattle paunch manure and cow dung for biogas production. ARPN J. Eng. Appl. Sci. 9, 1–6.

Chukwuma, E.C., Orakwe, C.O., Ugwuishiwu, B.O., Nwoke, O.A., Igbokwe, E.C., 2018. GIS hotspot application and use of set-cover problem for centralized abattoir biogas plant treatment facilities in Anambra State of Nigeria. Agric. Eng. Int. 63–68. CIGR Journal Open access at. http://www.cigrjournal.org.

Chukwuma, E.C., Abdul Rashid, Okafor, G., Nwoke, A.O., 2020. Fuzzy based risk assessment of abattoir operations and treatment facilities: a case study of Onitsha North/South LGA of Anambra State of Nigeria. Elsevier J. Food Bio-prod. Process. 119, 88–97.

Devi, G., Shinoon, A.H., Sekhar, G.C., 2012. Treatment of vegetable oil mill effluent using crab shell chitosan as adsorbent. Int. J. Environ. Sci. Technol. 9, 234–244.

Eryuruk, K., Tezcanun, U., Bakir Ogutveren, U., 2014. Electrocoagulation in a Plugflow Reactor: the treatment of cattle Abattoir wastewater by Iron rod anodes. Int. J. Environ. Res. 8, 461–468.

FSA (Food Science Austria), 1998. Crude Fat Determination – Soxhlet Method. Meat Technology Information Sheet. Retrieved from. www.meatupdate.csiro.au.

Hassan, S.N., 2011. The effect of settlement time on reducing coagulant dosage in water treatment plants. J. Environ. Stud. 12, 25–32.

Hussain, S., Ghouri, W.S., Ahmad, A., 2019. Pine cone extract as natural coagulant for purification of turbid water. Heliyon 5, e01420.

Isehunwa, S.O., Onoveae, S., 2011. Evaluation of produced water discharge in Niger Delta. ARPN J. Eng. Appl. Sci. 6, 24–30.

Jatto, E.O., Asia, I.O., Egharevba, F., Odia, A., 2013. Kinetic studies of wastewater treatment from rubber factory using snail shell. N Y Sci. J. 6 (2), 25–33.

Kjeldahl, J., 1883. New method for the determination of nitrogen in organic substances. Zeitschrift Fur Analytische Chemie 22 (1), 366–383.

Marey, A.M., 2019. Effectiveness of Chitosan as Natural Coagulant in Treating Turbid Waters Researches. http://www.revistabionatura.com.

Menkiti, M.C., Igbokwe, P.K., Ugodulunwa, F.X.O., Onukwuli, O.D., 2008. Rapid coagulation/flocculation kinetics of coal effluent with high organic content using blended and unblended chitin derived coagulant (CSC). Res. J. Appl. Sci. 3, 317–323.

Nur, F.A., Rajesh, N., 2014. Wastewater Treatment by Using Natural Coagulant Wastewater Treatment by Using Natural Coagulant, 2nd eureka.

Nwabanne, J.T., Obi, C.C., 2019. Coag-flocculation performance of snail shell biomass in abattoir wastewater treatment. J. Chem. Technol. Metal. 54 (No. 6), 1177–1188.

Okey-Onyesolu, C.F., Ude, C.N., Onukwuli, O.D., Okoye, C.C., 2017. Optimization and characterization of adsorptive behaviour of pentaclethramacrophylla activated carbon on aqueous solution. Sigma J. Eng. Nat. Sci. 35, 213–226.

Okey-Onyesolu, C.F., Onukwuli, O.D., Okoye, C.C., 2018a. Deturbidization of vegetable oil refinery wastewater with extracted fish scale biomass via coagulation process; non-linear kinetics studies. J. Eng. Res. Rep. 2, 1–14.

Okey-Onyesolu, C.F., Onukwuli, O.D., Okoye, C.C., Ejimofor, M.I., 2018b. Deturbidization of abattoir wastewater with extracted egg shell chito-protein via coagulation process. In: International Conference Proceedings of the Faculty of Engineering. Nnamdi Azikiwe University, Awka, Nigeria, pp. 271–281.

Rahmi, C.N.A., Husnina, N.A., Trismiarni, E.M., Putri, D.C.M., 2017. Sythesis of Chitosan from the crab shell with encapsulation method. J. Eng. Appl. Sci. 12, 4725–4729.

Tilley, E., Ulrich, L.C., Reymond, P.H., Zurbrugg, C., 2014. Compendium of Sanitation Systems and Technologies –, 2nd Revised Edition. Swiss Federal Institute of Aquatic Science and Technology (Eawag), Duebendorf, Switzerland, p. 175.

Vishali, S., Rashmi, P., Karthikeyan, R., 2016. Evaluation of wasted biomaterial, crab shells (Portunus sanguinolentus), as a coagulant, in paint effluent treatment. Desal. Water Treat. 57 (28), 13157–13165.

Xu, X., Liu, X., Oh, M., Park, J., 2019. Oyster shell as a low-cost adsorbent for removing heavy metal ions from wastewater. Pol. J. Environ. Stud. 28, 2949, 2949.