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Characterization and FibreMetric Studiesof Natural Organic Polymer for Dye Degradation in Wastewater: Face-Centred Central Composite Design

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***Abstract*— Further researches have been on-going in the application of natural organic polymer (NOP) coagulants in contaminant removal from industrial wastewater. The relevance of using Moringaoleifera coagulant (MOC) as NOPfor colour removal from crystal Ponceau 6R dye was investigated in this study. The proximate analysis, structure, surface morphology, and fibre metric study of the precursor were investigated. Response surface methodology (RSM) using face-centred central composite design (FCCD) optimized four process variables including pH, coagulant dosage, dye concentration, and time. The proximate analysis showed a high protein content of 39.34% and fibre content of 1.16%. FTIR analysis showed the presence of O-H, N-H, C=H in the coagulant precursor. The SEM image revealed the image of the polymer rough surfaces,the most occurring pore size 0f 0.41 µm2, different fibre length between 948.44 nm -13.85µm, and a compact net structure. The pH has the highest influence on the colour removal, followed by time as indicated clearly in the main effects plots. Coagulant dosage and dye concentration have less influence on the process. The verification experiments agreed with the predicted values having a standard error value of 1.58%. Overlay contour plot established optimum areas where the predicted response variable is in an acceptable range (≥ 80%) with respect to optimum conditions. The FCCD approach was appropriate for optimizing the process giving higher removal efficiency when compared to the main effect plots. Therefore, protein extract from Moringaoleifera(MO)seed has potentials for application as efficient coagulant while showing significant polymeric characteristics.**

***Index Terms*— Coagulation-flocculation; natural organic polymer; Biebrich scarlet; fibre metric; face-centred central composite design; overlaid contour plot.**

I. INTRODUCTION

There has been an environmental public health problem across the globe in which one major causes is from wastewater discharge. Dyes and pigments are among the main contaminants generated from wastewater discharge [1]. Presently, about 10,000 commercial dyes and pigments are being used and over 7.11 X 10^7 kg/yr is produced worldwide [2]. Dye production industries including textile, rubber, pulp, paper, plastic, cosmetics, food, pharmaceutical, leather tanning, printing and medicine generate wastewater characteristically high in colour, organic and inorganic contents. These dye wastewaters are toxic, carcinogenic, slow down self-purification of streams by reducing light penetration, retard photosynthetic activity and inhibit growth of biota [3], [4]. Therefore, dye wastewaters generated from industrial applications have to be treated to accord with discharging limit [5].

Dyes could be classify as anionic (direct, acid and reactive dyes), cationic (basic dyes), and non-ionic (disperse dyes). Anionic dyes are the largest class of dyes used in the world [6]. In aqueous solution, anionic dyes carry a net negative charge due to the presence of sulphonate (SO_3^-) groups. Acid dyes are anionic compounds and mostly belonged to azo and anthraquinonic groups. Acid dyes are characterized by the existence of azo bond (R-N=N-R_2), amino group. Removal of contaminants from acid dyes wastewater is often difficult because they contain highly soluble and semi-soluble contaminants. The complex aromatic structure, synthetic origin and difficult to be biodegraded also make acid dyes difficult to treat. Biebrich scarlet or acid red 66 (AR 66) is a dark, brownish powdered azo dye. Applications of Biebrich scarlet are useful in fabrics dyeing including wool, silk, polyurethane fibres, nylon. It is also used as a plasma stain and to import colour to pharmaceutical preparations [7].

The methods used for contaminant removals from dye wastewater could be divided into three main categories; physical, chemical and biological. Physical treatments such as precipitation, ion exchange, membrane filtration, irradiation, ozonation and adsorption are widely used techniques. Physico-chemical treatment methods are coagulation-flocculation, precipitation, photo-catalysis, oxidation and chemical sludge oxidation. Biological treatment techniques used are aerobic degradation, anaerobic degradation, and living/dead microbial biomass [8]. Coagulation-flocculation is an already established technique for contaminant removal due to its vast applications in wastewater treatment ranging from wastewater containing: biochemical oxygen demand; BOD, colour, dissolved organic carbon; DOC, turbidity, chemical oxygen demand; COD, grease and oil, total suspended solids; TSS, heavy metals[9]. Coagulation-flocculation is considered a chemical treatment as it implies the addition of a coagulant. Typical coagulant agents are inorganic salt such as $Al(SO_4)_3$ or $FeCl_3$, as well as synthetic organic polymer [10],[11]. Although these chemicals are rather effective in removing dyes and suspended matters from the aqueous solution, several disadvantages have recently arisen, such as their impact on

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human diseases like Alzheimer's caused by inorganic salts and neurotoxin caused by acrylic amid [12].

Natural organic polymers are of emerging trend by many researchers because of their abundant source, low price, environmental friendly, multi-functional, and biodegradable in water. *MoringaOleifera* (horseradish or drumstick tree), a non-toxic (at low concentrations) is a tropical plant belonging to the family of *Moringaceae* which is cultivated across the tropical belt of India, Asia, Sub-Saharan Africa and Latin America. *Moringaoleifera*is esteemed as a versatile plant due to its multiple uses. It is arguably the most studied natural coagulant within the environmental scientific community. It is widely acknowledged as a plant with numerous uses. Almost every part of its plant system can be beneficial for medicinal purposes [13]. The seeds contain active coagulating agents characterized as cationic proteins, having molecular weight of 13 kDa and an isoelectric point between 10 and 11. The seeds also have antimicrobial activity and are utilized for wastewater treatmentbecause of their strong coagulating properties for sedimentation of suspended undesired particles, generate reduced sludge volumes in comparison to alum, soften hard waters and act as effective adsorbers of organic particles [14],[15].

Fig. 1.*MoringaOleifera*:a) seeds, b) seed powder.

Therefore, the potentials of using active proteins agent from MOCin decolourization of AR 66 (Biebrich scarlet) dye in aqueous solution was studied. Full characterization and fiber-metric studies of the coagulant were done. Newer approach of extracting active coagulant agent is adopted in the coagulation-flocculation process. The FCCD approach was utilized to determine the optimum operational conditions and to also determine the best area that satisfies the operating specifications.

II. MATERIALS AND METHODS

A. CoagulantPreparation and Extraction of Active Component

Dried seeds ofthe MOwere purchased from local market located in Enugu city, Nigeria. Matured seeds showing no sign of discolouration, softening or extreme desiccation were selected. The seeds were ground to fine powder (0.1 – 0.36 mm) using an ordinary food processor to achieve complete solubilization of the active ingredients. Extracting the active agent from precursor was achieved by preparing a stock solution of 20,000 mg of powder samples in 1 L distilled water. The suspension was stirred using a magnetic stirrer (Model 78HW-1, U-Clear England) for 20 min at room temperature to promote adequate extraction of the coagulant proteins and to enhance the cationic agent needed for the process. A local filter cloth was employed to filter the suspension to enable the presence of nano,micro, and macro-particles in the filtrate for an enhanced coagulation-flocculation/adsorption process. The resultant filtrate solution was used as coagulant at required dosages. Dilution formula was employed to get the required working coagulant dosages of 200 – 1000 mg/L. Fresh solutions were prepared daily and kept refrigerated to prevent ageing effects (such as change in pH, viscosity and coagulation activity). Before each experiment, solutions were shaken vigorously and used immediately for each sequence of experiment.

B. Characterization of the Coagulants

Yield, bulk density, moisture content, ash content, protein content, fat content, carbohydrate content and fibre content of the seed powder were determined by the standard official methods of analysis of the A.O.A.C [16]. Fourier-Transform Infrared (FTIR) spectrometer supplied by IR Affinity-1, Shimadzu Kyoto, Japan, was used to study the chemical structure and functional groups present in the sample. The spectra were measured in the mid-infrared range (4000 – 400cm^{-1}).Surface structure and morphology of the seed powder were studied using scanning electron microscope (SEM)supplied by Phenom Prox., world Eindhoven, Netherlands.

C. Buffer Solution Preparation

All assays were done in a pH-stable medium. Buffered solutions (pH 2, 4, 6, 7, 8 and 10) were prepared by the standards established according to the National bureau of standards (NBS) and were standardized using a digital pH meter. All reagents used were of analytical purity grade.

D. Preparation of Dye and Decolourization Procedures

Biebrich scarlet (AR 66), water soluble dye was provided by May & baker England with molecular structures as shown in Fig. 2. Physical characteristics of the acid dye are summarized in Table 1. Dye with commercial purity was used without further purification. The absorption spectrum of the dye was obtained by dissolving 1000 mg/L of AR 66 in distilled water. A sample of the solution was scanned against the blank of distilled water in the range of 250 – 850 nm using UV-Vis spectrophotometer (Shimadzu, Model UV - 1800). Maximum wavelength (λ_{max}) of 536 nm was obtained as shown in Fig. 3.Stock solution of 1000 mg/L of dye was prepared by dissolving accurately weighed amounts of AR 66 in separate doses of 1 L distilled water. The desirable

experimental concentrations of 10 – 50 mg/L were prepared by diluting the stock solution when necessary. The wavelength of maximum absorbance (λ_{max}) and calibration curve at λ_{max} were determined.

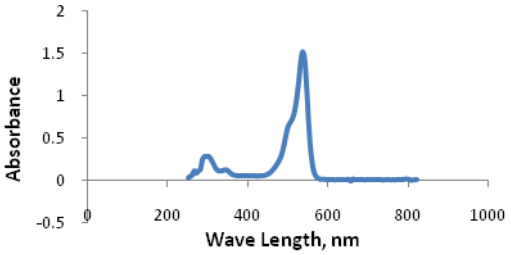


Fig.3. Spectrum Peak Report for AR 66

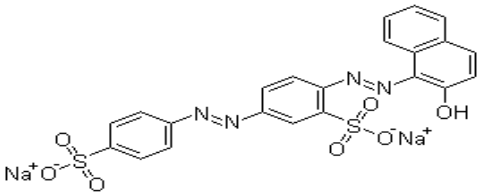


Fig.2. Structure of Biebrich scarlet dye (Acid red 66).

Table 1 Physical properties of Biebrich scarlet dye.

Property	Data
Chemical Name	Sodium 6- (2-hydroxyna phthylazo) – 3, 4' – azodibenzenesulfonate.
Chemical formula	$C_{22}H_{14}N_4Na_2O_7S_2$
Molecular Weight	556.48
CAS number	4196 – 99 - 0
ECC number	224 – 084 - 5
Melting point	181 - 188^{0}C.
UV /visible Absorbance	Max (water): 505+ 6nm.
C.I number	26905
Class	Azo
C.I name	Acid red 66
Common name	Biebrich scarlet.

E. Coagulation Studies

A conventional jar test apparatus supplied by Phipps and Bird, VA, USA, equipped with six beakers of 1 L capacity and six paddle stirrers was used to perform the coagulation-flocculation experiment. The jar test was conducted to evaluate the coagulation performance of the coagulants agent extracted and to establish the best operating condition for the coagulation-flocculation process[17]. The procedure involved 4 min of rapid mixing at 100 rpm. The mixing speed was reduced to 40 rpm for another 25 min. The study was conducted by varying few experimental parameters including pH, coagulants dosages, dye concentrations and time. The pH was adjusted to the desired value using 0.1 M HCl and 0.1 M NaOH. All the suspensions were left for settling (60 - 540 min). Supernatant samples were withdrawn after settling for absorbance analysis using UV/Vis spectrophotometer (Shimadzu, Model UV - 1800) set at maximum wavelength (λ_{max}) of 536 nm. Colour concentration measurement was determined by comparing absorbance to concentration on a calibration curve. The colour removal efficiency was obtained according to Eq. (1).

$$\text{Colour removal (\%)} = \left(\frac{C_0 - C}{C_0}\right) x\ 100 \quad (1)$$

where C_0 and C are the initial and final colour concentration (mg/L) in dye solutions before and after coagulation-flocculation treatment, respectively.

F. Experimental Design and Data Analysis

RSM tool was employ to develop a mathematical correlation between the operating variables affecting the dye removal. Central composite design (CCD), a very efficient design tool for fitting the second-order models, was used in the experimental design [18]. The CCD introduced by Box Wilson in 1951, is well suited for fitting quadratic surfaces, which usually works well for the process optimization [19]. Face-centred central composite design (FCCD) was implemented as a CCD. A CCD is made face-centred by the choice of $\alpha = 1$ which is having the position of the star points at the face of the cube portion on the design [9]. The face-centred option ensures that the axial runs will not be any more extreme than the factorial portion. The independent variables selected for this study were pH (A), coagulant dosage (B), dye concentration (C), and time (D). A 2^4 two-level factorial for four independent variables consisting of 16 factorial points coded to the usual ± notation, 8 axial points and 6 replicate at centre point where conducted, given a total of 30 experiments. Mathematically, Eq. (2) was used to determine the total number of runs performed. The total number of experiments, N is:

$$N = 2^k + 2k + n \quad (2)$$

where k is the number of factors and n is centre points.

The experimental design table is presented in Table 2. For statistical calculations, the variables Z_i (the real value of an independent variable) were coded as X_i (dimensionless value of an independent variable) according to Eq. (3):

$$X_i = \frac{Z_i - Z_i^*}{\Delta Z_i} \quad (3)$$

where Z_i stands for the uncoded value of ith independent variables, Z_i^* stands for the uncoded value of ith independent variables at centre point and ΔZ_i is a step change value.

Design-expert software (9. 0 State Ease, Minneapolis, USA) was used to analyze the experimental data and was fitted to a second-order polynomial model to optimize the variables in the coagulation-flocculation process. Design-expert also demonstrated the analysis of variance (ANOVA). The response was used to develop an empirical model which correlated the response to the dye coagulation-flocculation variables using a second degree polynomial equation as given by Eq. (4):

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} X_i X_j + \varepsilon \quad (4)$$

where Y is the predicted response, b_0 the constant coefficient, b_i the linear coefficients, b_{ii} the quadratic coefficients, b_{ij} the interaction coefficient, X_i X_j are the coded values of the variables, n is the number of independent test variables and ε is the random error.

Adequacy of the proposed model was revealed using the diagnostic tool provided by analysis of variance (ANOVA). The quality of the model fit was expressed by the coefficient of determination (R^2). The R^2 value provides the extent of variability in interaction between the response and the factors. Analyses were done by means of Fisher's 'F' test and P-value (probability). Model terms were evaluated by the P-value using 95% confidence level [18]. The optimum operating conditions of the colour removal were obtained by analyzing the main effects plots, contour plots and overlaid contour plotting using Minitab 16 software.

Table 3 - Levels and range of the variables tested in the CCD design.

Variables	Factors	Unit	Range and levels				
			Lowest -α	Low -1	Centre 0	High +1	Highest +α
pH (X_1)	A	-	2	2	6	10	10
Dosage (X_2)	B	mg /L	200	200	600	1000	1000
Dye concentration (X_3)	C	mg/L	10	10	30	50	50
Time (X_4)	D	min	60	60	300	540	540

III. RESULTS AND DISCUSSION

A. Characterization Result of the VSC

Proximate composition

The proximate analysis of coagulant precursor was summarized in Table 3. The moisture content values show water absorption ability of the coagulant. High crude protein 39.34% indicates the presence of protein, which is in agreement with the literatures that the protein contents of the precursor is cationic poly-peptides (have a long chains of amino acids held together by peptide bonds) [20]. Fibre content present established the precursor as an organic polymer with repeating small molecules that could extend as tails and loops when dispersed in water [21]. These characteristics lead to a new discovery of adsorption mechanism relevant in the process. The presence of protein, moisture, carbohydrate andfibre, justified the use of the seed powder as a coagulant in this work.

Table 3 - Proximate compositions of the MOseed

S/No.	Parameters	Values
1.	Yield	32.68
2	Bulk density (g/mL)	0.425
3.	Moisture Content (%)	5.02
4.	Ash content (%)	2.12
5.	Protein content (%)	39.34
6.	Fat content (%)	19.47
7.	Fibre content (%)	1.16
8.	Carbohydrate (%)	32.89

FTIR

The FTIR spectra of MOC before and after coagulation-flocculation were shown in Fig. 4 and Fig. 5, respectively. Noticed, is an absorption peak in the broad range of 3656.92-3683.93cm^{-1} before and after the process. This is attributed to the stretching vibration of –OH groups and to the vibration of water absorbed, or complexes in the coagulant [22]. The free hydroxyl groups present in the spectral confirms the presence of carboxylic acids, alcohols and phenols on the coagulant. This band also corresponds to the O-H stretching vibrations of cellulose, pectin and lignin [23]. The spectral studies revealed that the characteristic absorption peak for amines were evident in the range of 3656.92-3683.93cm^{-1} for aromatic primary amine (N-H stretch). The presence of N-H stretching signals in the peaks also confirms the presence of amino compounds, and this confirms high crude protein contents present in the coagulant

as also evident in Table 3. The medium peaks in the range of 2700.26-2113.92 cm^{-1}, was attributed to the bending vibration of O-H groups in the water molecule, namely the H–O–H angle distortion frequency. A major band of 1651.02 and 1678.03cm^{-1} present in the coagulant indicates the presence of a C=O group (carbonyl compound). The spectra show that some peaks were shifted or disappeared as can be seen in 2237.36cm^{-1} and 1311.56cm^{-1} and the new peaks were also detected. These changes observed in the functional groups of -C=C-H, C=CH, (C-H stretching), --CH_2- groups is an indication of the possible involvement of those functional groups on the surface of the polymer surface on the coagulation process. The diminished peaks in Fig. 5 showed that all the functional groups are completely involved in the process.In addition, there was a strong adsorption peak at 694.36cm^{-1}, which is the characteristic frequency for C-H out of plane deformation groups. The C-H, C≡C-H out-of plane bending is typically the most informative relative to the location and spatial geometry of the double bond. The FTIR spectral and proximate analysis suggest the presence of moisture, proteins and esters, which justify its use as potential source of coagulant and also adsorbing particles on to its polymer chains.

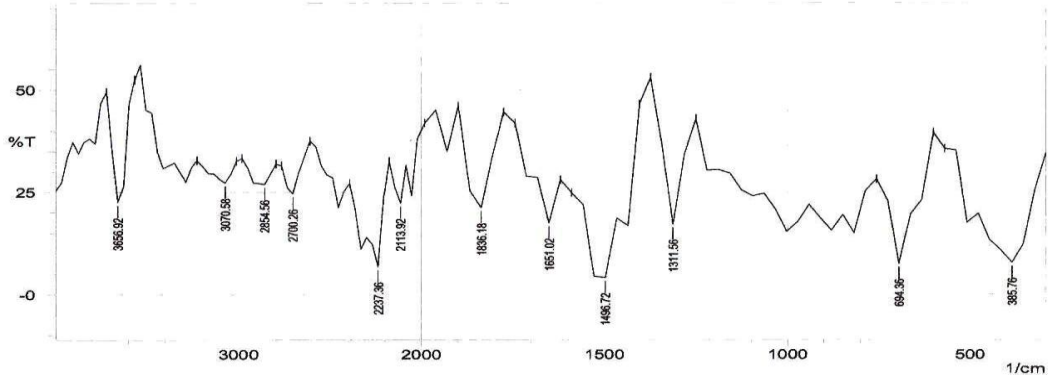


Fig. 4. Transmittance FTIR Spectrum of MObefore the coagulation-flocculation study.

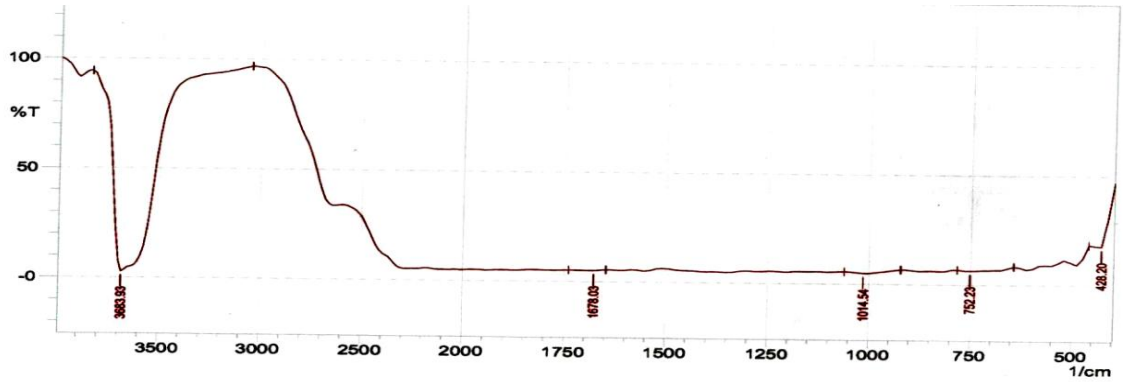


Fig. 5. Transmittance FTIR Spectrum of MOafter the coagulation-flocculation study.

SEM Result

The surface morphology of VSC in 350x magnification is shown in Fig. 6. The SEM image revealed well developed pores of different shapes and sizes.The pore size (micro-pores, macro-pores and meso-pores)and distribution are properties that are unique to natural organic polymers (NOPs).A major pore size of 0.41 $\mu m^2$was revealed in the histogram and fibre lengths between 948.44 nm -13.85µmwere captured as shown in Fig. 7. Different fibre lengths are unique features of NOPs which enhances their multifunctional utilization as coagulants and adsorbents [3].Rough surfaces as observed show that the coagulant isof coarse fibrous substances largely composed of cellulose and lignin which confirms its polymeric characteristics. Particles could be adsorbed or attached it selves on these polymer chains through inter-particle bridging or electrostatic contacts. The pores and rough surfaces observed on the coagulants morphologies confirm adsorption as an important mechanism in coagulation-flocculation process. The morphologies also possess compact-net structures which is more favourable to particle flocculation due to bridge aggregation.The compact-net structure was more favourablein flocculation and particle-bridge formation among flocs as compared with the branched structure [24].

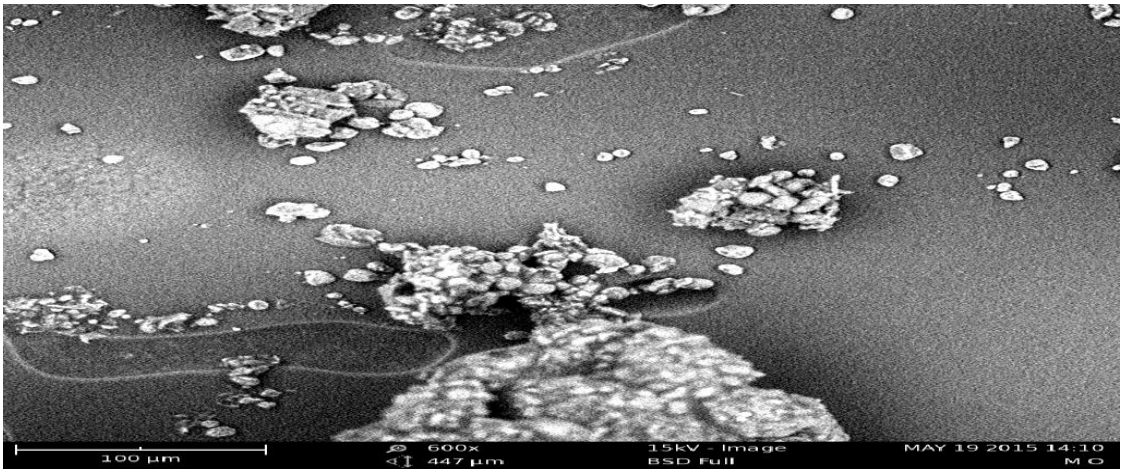


Fig. 7. SEM image of MOC

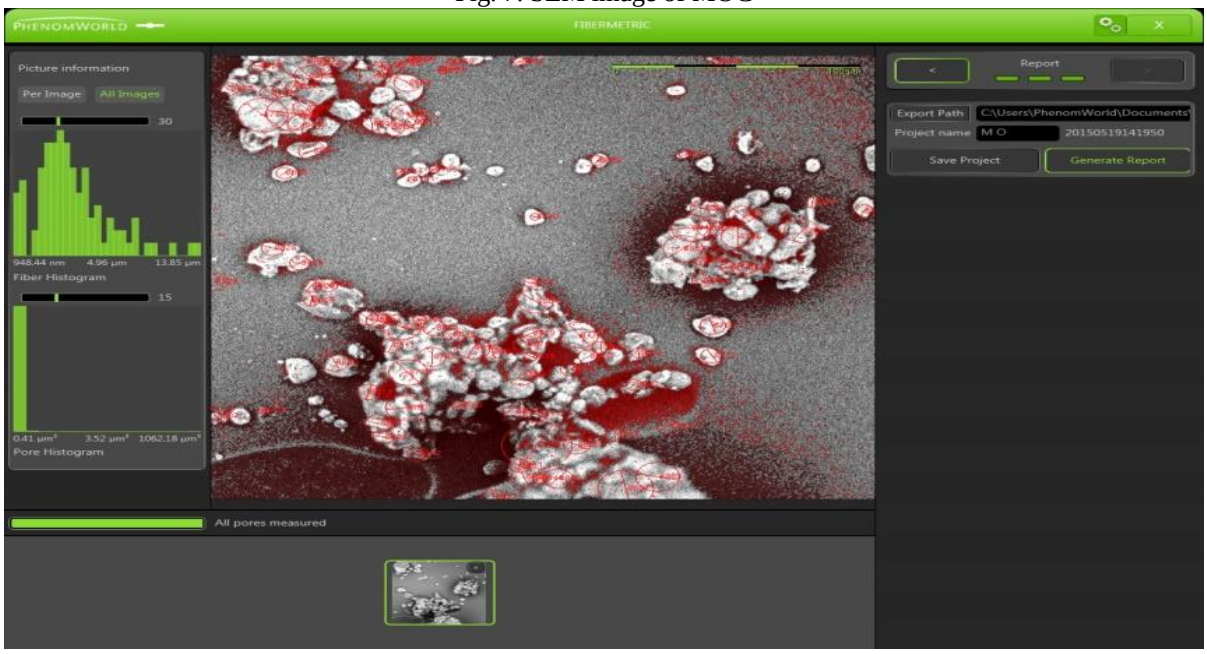


Fig. 7. SEM Fibre Metric Image Measurement; Fibre Histogram, Pore Histogram, and Pore Measurement for MOC.

B. Development of Regression Model

To study the factor interactions, experiments were performed for different combinations of the factors. The responses to the combinations were obtained. The experimental design matrix showing the coded and uncoded factor combinations together with the experimental (exp) and predicted (pre) decolourization efficiencies are shown in Table 5.

Table 4 -FCCD in coded and uncoded form and response result.

Std Order	Run Order	A: pH Coded	A: pH Uncoded	B: MOC Dosage (mg/L) Coded	B: MOC Dosage (mg/L) Uncoded	C: Dye Conc. (mg/ L) Coded	C: Dye Conc. (mg/ L) Uncoded	D: Time (min) Coded	D: Time (min) Uncoded	Y_{moc} (Colour removal (%) Experimental (exp)	Y_{moc} (Colour removal (%) Predicted (pre)
17	1	-1	2	0	600	0	30	0	300	97.6	96.36
28	2	0	6	0	600	0	30	0	300	43.8	45.89
20	3	0	6	1	1000	0	30	0	300	38.4	36.19
9	4	-1	2	-1	200	-1	10	1	540	95.4	95.63
8	5	1	10	1	1000	1	50	-1	60	8.7	8.88
16	6	1	10	1	1000	1	50	1	540	33.2	34.73
2	7	1	10	-1	200	-1	10	-1	60	10.7	11.79
7	8	-1	2	1	1000	1	50	-1	60	65.3	65.00
22	9	0	6	0	600	1	50	0	300	39.9	38.57
1	10	-1	2	-1	200	-1	10	-1	60	70.5	69.61
24	11	0	6	0	600	0	30	1	540	63.1	59.02
5	12	-1	2	-1	200	1	50	-1	60	58.7	60.12

12	13	1	10	1	1000	-1	10	1	540	40	38.99
4	14	1	10	1	1000	-1	10	-1	60	13.7	14.52
27	15	0	6	0	600	0	30	0	300	43.8	45.89
25	16	0	6	0	600	0	30	0	300	43.8	45.89
26	17	0	6	0	600	0	30	0	300	43.8	45.89
19	18	0	6	-1	200	0	30	0	300	33.9	31.92
18	19	1	10	0	600	0	30	0	300	41.1	38.16
14	20	1	10	-1	200	1	50	1	540	29.7	30.70
3	21	-1	2	1	1000	-1	10	-1	60	74.7	74.11
11	22	-1	2	1	1000	-1	10	1	540	98.8	98.9
10	23	1	10	-1	200	-1	10	1	540	34.4	35.34
15	24	-1	2	1	1000	1	50	1	540	94	93.32
13	25	-1	2	-1	200	1	50	1	540	87.7	87.51
30	26	0	6	0	600	0	30	0	300	43.8	45.89
29	27	0	6	0	600	0	30	0	300	43.8	45.89
23	28	0	6	0	600	0	30	-1	60	33.2	33.09
21	29	0	6	0	600	-1	10	0	300	48.3	45.45
6	30	1	10	-1	200	1	50	-1	60	7.4	5.78

The adequacy of the model was justified through ANOVA as shown in Table 5. The quadratic regression shows the model was significant at 95% confidence level by the Fisher's testing. This was confirmed having obtained the F-value of 236.51. The P-value result of less than 0.05 shows statistically significant model. The models did not exhibit lack-of-fit indicating insignificant lack-of-fit.

Coefficient of determination (R^2) measures the model's overall performance. Greater than 0.2 differences between predicted R^2 and adjusted R^2 indicate that non-significant term may be included in the model. A high R^2 value, close to 1, is desirable and ensures a satisfactory adjustment of the quadratic model to the experimental data. The R^2 value of 99.55% indicates the model could not explain 0.45% of the total variations. The value of predicted R^2 and adjusted R^2 were less than 0.2 indicating the accuracy of the model. It was observed from Table 6 that the model, all the coefficient terms (A, B, C and D), interaction terms (AB and AD) and square terms (A^2 and D^2) were significant to the model response whereas other non-specified terms were insignificant to the model response.

Table 5: ANOVA results for the response Y_{moc}.

Y_{moc}	Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F		R- Squared
	Model	19986.14	14	1427.58	236.51	<0.0001	significant	
	A-pH	15242.58	1	15242.58	2525.31	<0.0001		
	B-Dosage	81.92	1	81.92	13.57	0.0022		
	C-Dye Conc.	212.87	1	212.87	35.27	<0.0001		
	D-Time	3026.42	1	3026.42	501.40	<0.0001		
	AB	3.15	1	3.15	0.52	0.4811		
	AC	12.08	1	12.08	2.00	0.1777		
	AD	6.13	1	6.13	1.01	0.3297		
	BC	0.14	1	0.14	0.023	0.8807		
	BD	0.86	1	0.86	0.14	0.7118		
	CD	1.89	1	1.89	0.31	0.5840		
	A^2	1182.35	1	1182.35	195.89	<0.0001		
	B^2	363.07	1	363.07	60.15	<0.0001		
	C^2	39.16	1	39.16	6.49	0.0223		
	D^2	0.068	1	0.068	0.011	0.9167		
	Residual	90.54	15	6.04				
	Lack of Fit	90.54	10	9.05				
	Pure Error	0.000	5	0.000				
	Cor Total	20076.68	29					
	R - Squared							0.9955
	Adjusted R - Squared							0.9913
	Pred R - Squared							0.9240

Second-order quadratic Eqs (5 and 6) show the final empirical equation in terms of coded and actual factors, respectively after excluding the insignificant terms.Positive signs in front of the equations indicate an interactive positive

effect among the factors. In conclusion, the quadratic model for the response measured is significant and adequate.

$$Y_{moc} = 45.8939 + -29.1 * A + 2.13333 * B + -3.43889 * C + 12.9667 * D + 21.3623 * A^2 + -11.8377 * B^2 + -3.88772 * C^2 \quad (5)$$

$$Y_{moc} = +89.31547 -23.26273 * pH +0.094706 * Dosage +0.31754 * Dye Conc. +0.052611 * Time +1.33514 * pH ^2 -7.39857E-005 * Dosage ^2 -9.71930E-003 * Dye Conc. ^2 \quad (6)$$

A reliable model should have a good prediction with experimental data. There is a good agreement between the actual removal efficiency (%) and predicted removal efficiency (%) as shown in Fig. 8. The observed points reveal that the actual values are distributed relatively near the straight line in most cases, showing a good prediction by the model. A close relationship between predicted and actual data indicates a good fit.

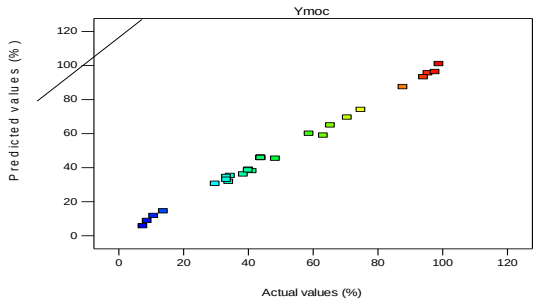


Fig. 8. Equality plot for the actual and predicted values of Y_{moc}colour removal (%).

C. Evaluation of Operational Parameters

Main and interactioneffects(data means) of the process parameters

A main effect occurs when the mean response changes across the levels of a factor. You can use main effect plots to compare the relative strength of the effects across factors. InFig. 9, the solution pH has more effect on the process. Its impact on the colour removal efficiency was highest at pH 2 having the efficiency of 82.52%.This trend will be explained further by charge on the hydrolysis products and precipitation of polymeric hydroxides which are both controlled by pH variations. As the functional groups of the acid dyes are anionic, hydrolyses products of the organic biopolymer can neutralize the negative charges on dye molecules followed by polymer adsorption, sweep-flocculation or inter-particle brigding. Charge neutralization, sweep-flocculation, polymer adsorption and inter-particle brigding could have played a predominant role in the coagulation-flocculation process due to its pH value. Inter-particle brigding and sweep-flocculation mechanisms could be present in the removal when polymer dosages are high (Shi et al., 2007). Generally, adsorption of the organic contaminants (OC) onto polymer hydroxide precipitate forming at high pH is also limited. As pH increases, OC become more negatively charged and polymer hydrolysis species become less positively charged, resulting in lower adsorption tendency[25]. Therefore, coagulation-flocculation of OC in wastewater is mainly performed under low pH conditions along with the presence of soluble cationic polymer hydrolysis species. These species react with anionic functional groups on OC to precipitate as a polymer-OC. In conclusion, high removal efficiency at low pH values are predominant in OC removal from acid dyes. Similar results were reported by Beltran and Sanchez [26]and Moghaddan [19]. The effect of MOC dosage on colour removal gave the highest mean removal efficiency of 51.87% at 1000mgMOC/L. Charge-neutralization and adsorption mechanism was observed to be predominant mechanism in the colour removal due to high efficiency at low dosage. The use of cationic polymer for coagulation-flocculation of negatively-charged colour particles is needed, because strong adsorption affinity and neutralization of the particle charges could occur. The high dosages of the organic polymer could also give rise to chain bridging and adsorption mechanism [27]. The concentration of dye provides an important driving force to overcome all the mass transfer resistance of the dye between the aqueous and solid phases. It plays an important role in the coagulation-flocculation process removal efficiency of 54.06% at 10 mg/L dye concentration. The studied dye concentration range has the least significance on the removal process. Flocs formation involves both interactions of coagulant hydroxide precipitate following hydrolyses reaction and contact with particles. Coagulation-flocculation performance is mostly evaluated through time-dependent decrease in particle concentration and consequently, coincides with the growth of aggregates. Fig. 8 also shows that the highest reduction in colour concentration was observed at 540 min resulting in the mean removal efficiency of 64.03%. This clearly shows that settling time has a huge effect on the removal process. In addition, flocculation time (60-540min) observed in this process confirmed further the presence of adsorption mechanism. In conclusion, the effects of pH and time on the process are much bigger than effects of coagulant dosage and dye concentration.

An interaction plot is a plot of means for each level of a factor with the level of a second factor held constant. An interaction between factors occurs when the change in response from the low level to the high level of one factor is not the same as the change in response at the same two levels of a second factor. That is, the effect of one factor is dependent upon a second factor. You can use interaction plots to compare the relative strength of the effects across factors. Notice on Fig. 10 below that the pH interacting with other factors gave the highest mean efficiency of 97.6%.

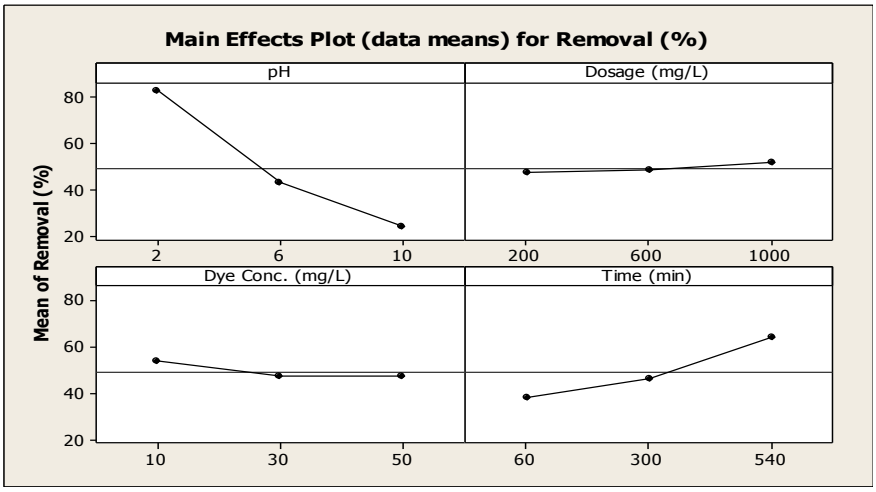


Fig. 8.Main effects plot for the colour removal (%).

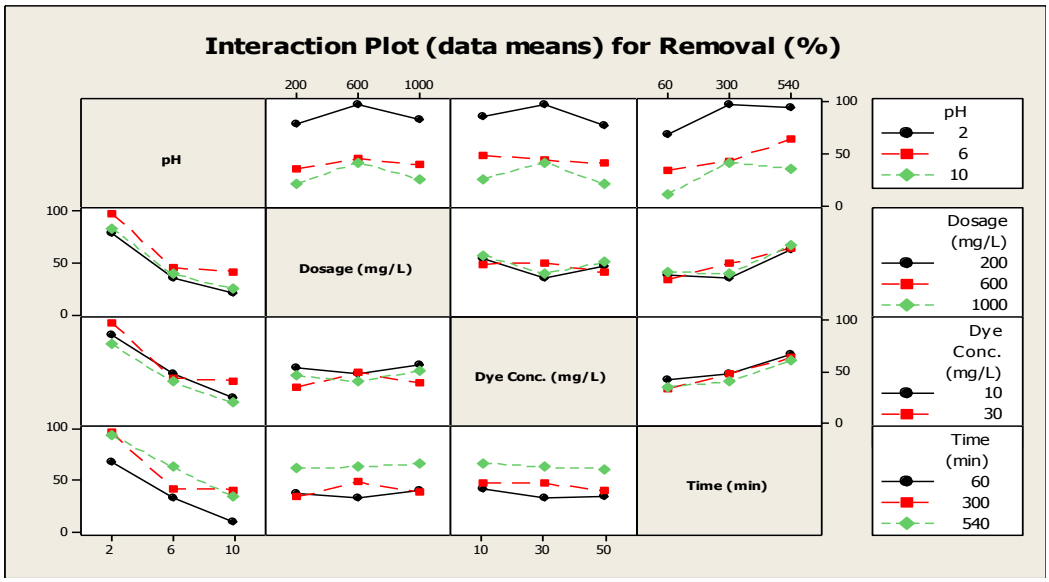


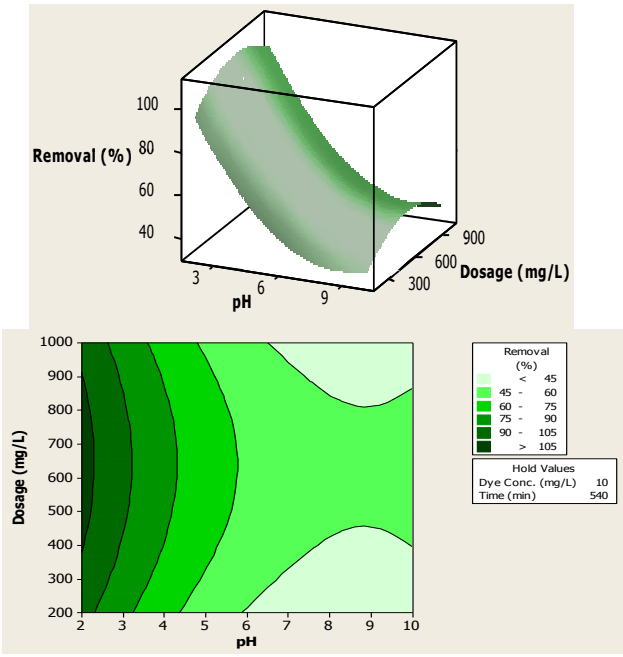
Fig. 9.Interaction plot for the colour removal (%): pH, VSC Dosage, Dye Concentration, and Time.

Response surface and its corresponding contour interaction.

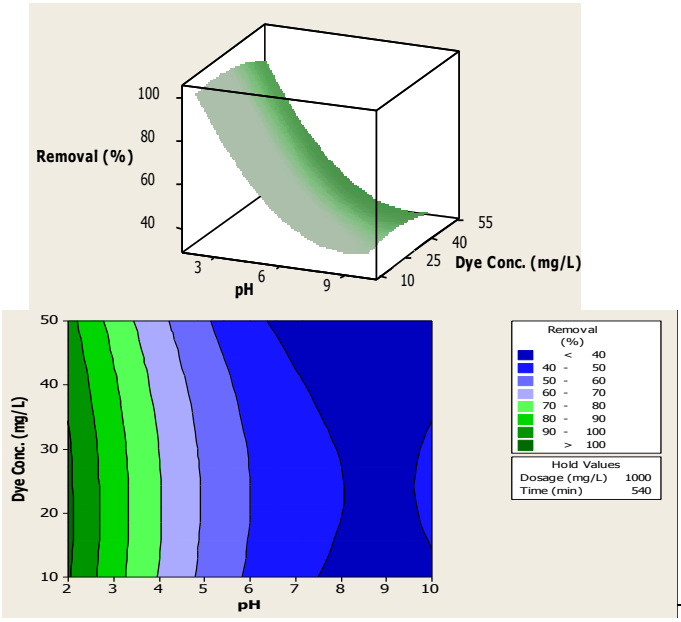
3D surface plots and its contour plots are represented in Fig. 11 for a better interactive factor effects on the decolourization of AR 66 dye at Optimum hold values of pH 2, dosage 1000mg/L, dye concentration 10mg/L and time 540min. The plots show that the maximum removal efficiency is in the range of pH 2 - 4, coagulant dosage from 500 – 900 mgMOC/L, at 10 – 20 mg/L dye concentration and settling time from 300 – 540 min. The response surface plots indicate that the maximum removal efficiencies are located inside the design boundary.The effectiveness of the organic polymeris highly dependent on pH as shown in Fig. 10. The polymers showed higher colour removals at very low pH values.

In addition, removal efficiencies increased more at higher coagulant dosages. Maximum efficiency was achieved at coagulant dosages between 800 – 1000 mgMOC/L. The high removal efficiencies of >82% found in the 1000 mgMOC/L dosages confirms charge-neutralization, adsorption, and particle sweep-flocculation mechanisms predominant in the studied dosage range. Positive charge species are responsible for removal of particles by charge neutralization. The use of cationic polymer for coagulation-flocculation of negatively-charged colour particles is needed, because strong adsorption affinity and neutralization of the particle charges could occur. High percentage removals observed at high dosages of the organic polymer were as a result of sweep flocculation and adsorption mechanisms, which are inclined to occur at high dosages [27]. In conclusion, settling time is of great importance for an effective coagulation-flocculation performance. The reduction in colour concentration did not vary significantly after 540 min showing equilibrium was achieved after 540 min. Destabilization of the aggregate flocs could set in after this time due to saturation of the active sites[3].

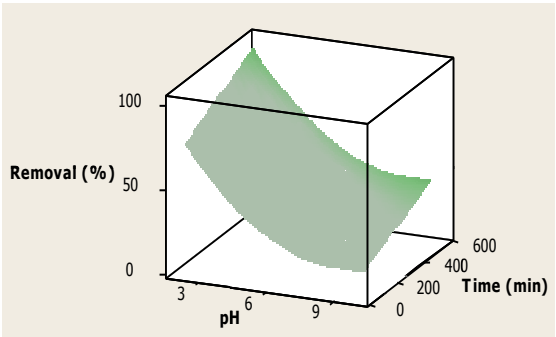
a.



b.



c.



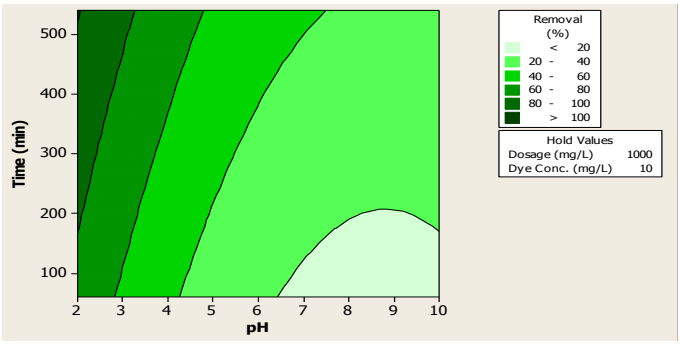


Fig. 11.3D Surface and its contour plots for the removal process as a function of: a) pH and dosage; b) pH and dye concentration; c) pH and time.

D. Optimization Analysis

Minitab 16.0 was used to optimize the colour removal efficiency. Process optimization searches for a combination of factor levels that simultaneously satisfy the criteria placed on each responses and factors. Numerical optimization was employed and the desired maximum goal was set for each factor and responses. These goals are combined into an overall desirability function, for effective maximization of the function. Optimal conditions and the optimization results are shown in Table 7.

1) Model validation and confirmation experiments.

The optimum predicted values were further validated by carrying out the experiment at the optimal predicted conditions and the results of the experimental values were also shown in Table 6. The experimental data confirms good agreements with RSM results. The verification experiments demonstrated a good agreement between the experimental and predicted, indicating RSM approach adopted was appropriate for optimizing the coagulation-flocculation process. The standard error (%) between the predicted and the experimental value of 1.58% was obtained. The value was less than 4% indicating very good prediction by the model. The adequacy of the model was once again verified effectively by the experimental data validation

Table 6 - Confirmation analyses of the predicted model.

Variables	Units	Optimum Values
pH	-	2
MOC Dosage	mg/L	689
Dye Conc.	mg/L	25.70
Time	min.	538
Colour removal efficiency (predicted)	%	98.80
Colour removal efficiency (experimental)	%	97.24
Standard Error	%	1.58

2) Overlaid contour plots

The overlaid contour plot (OCP) is used to visually identify an area where the predicted means of the response variable is in an acceptable range. Contour plots are useful for establishing operating conditions that produce desirable

response values.A compromise among the optimum conditions for response is desirable. Overlaid plots allow you to visually identify an area of compromise among the various responses.The desirability function approach together with graphical optimization was used to achieve this goal [18]. By defining the desired limits, the optimum condition can be visualized graphically by superimposing the factors in an OCP, as shown in Fig. 12. The white shades in Fig. 12 are the optimum predicted spot showing the areas that satisfy the response goal of greater than or equal to 80% colour removal for the factor interactions at the optimum hold values. The areas were in agreement with the response and contour ranges obtained. Areas that do not fit the optimization criteria were shaded grey. OCP is mostly applied when there is an emergency because it reduces preparation time and experimental cost. It shows clearly the high efficient region of the contour.

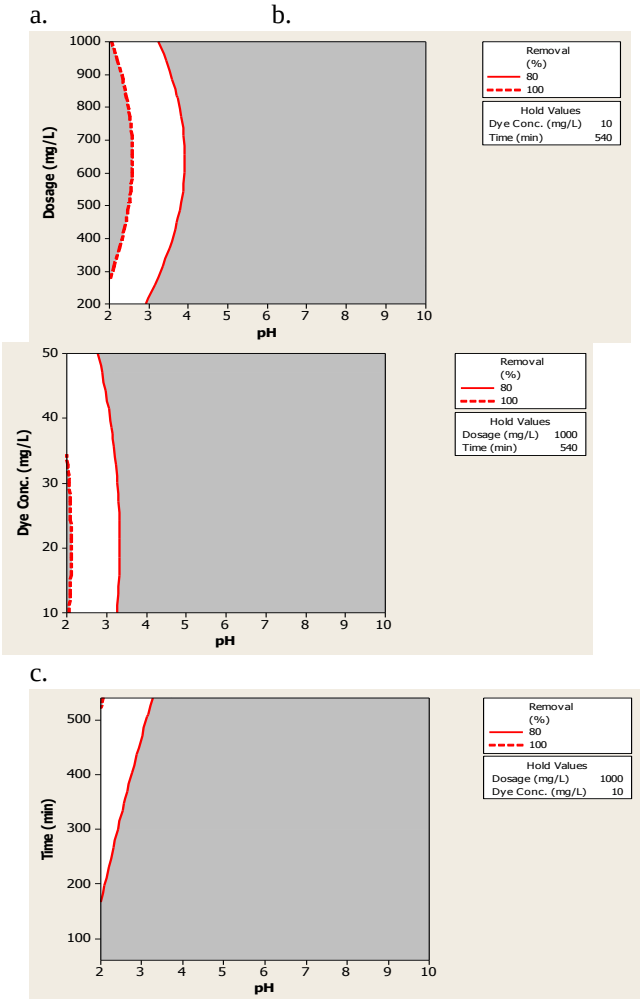


Fig. 12. Overlaid Contoursfor interactions of: a) dosage and pH; b) dye concentration and pH; c) time and pH.

IV. CONCLUSION

In this research, the proximate analysis, structure and morphology of MOC were investigated. Proximate analysis result showed that MOhas the characteristics of a potential coagulant. FTIR analysis indicated that some chemical bonds such as O-H, N-H, C=H were present in the coagulant precursor. SEM image revealed rough surfaces, different pores sizes, and compact-net structure. The pore sizes revealed the presence of micro-pores, meso-pores, and macro-pores. The characterization result showed the NOP ability; to destabilize contaminant particles, to enhance flocs formation due to its polymer characteristics and to adsorb particles on its surfaces due to its different pores and rough surfaces. The experimental results were statistically analysed with RSM technique by implementing FCCD. From the study, pH and time were the most influencing factors in the colour removal process. The optimum conditions from the main effects (data means) plot were pH 2, coagulant dosage 1000 mg/L, dye concentration 10 mg/L and time 540 min coagulation-flocculation with pH having the most significant effect on the process followed by settling time. Pore measurement and rough surfaces observed on the coagulant morphology confirmed that adsorption was an important mechanism in the coagulation-flocculation process. Optimal predicted process conditions of pH 2, coagulant dosage 689mg/L, dye concentration 25.70 mg/L and time 538min, giving a confirmation efficiency of 98.80% indicating a good agreement with the experimental efficiency earlier obtained. There was a higher removal efficiency using the FCCD model as compared to the efficiency obtained from the main effect plot. In other words, FCCD shows an effective optimization tool for the coagulation-flocculation process.

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