Progress in Energy and Environment

Journal homepage: https://www.akademiabaru.com/submit/index.php/progee

Volume 15 (2021) 8-30

Research Article

Performance of fenugreek and okra for the physico-chemical treatment of Palm Oil Mill Effluent – Modeling using Response Surface Methodology

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Abstract

Palm oil mill effluent (POME) is an odorous dark brownish solution that has high total suspended solids (TSS), turbidity (TUR) and chemical oxygen demand (COD). In this study, performance of fenugreek (*Trigonella Foenum-graecum*) and okra (*Abelmoschus Esculentus* (*L.*)) as a bio-coagulant and bio-flocculant respectively, were investigated for the treatment of POME. The objective of this study was to determine the optimum operating conditions for the bio-coagulant-flocculant in terms of pH, dosage and rapid mixing speed via Response Surface Methodology (RSM). Percentage removal of TSS, TUR and COD were measured. The study indicated that the optimum conditions to be 4.1 g/L of fenugreek, 58 mL of okra/500 ml POME and 197 rpm rapid mixing speed at pH 3.2 to obtain TSS, TUR and COD removal efficiencies of 92.7%, 94.97% and 63.11%, respectively. From the study, it is clearly showed that the combination of fenugreek and okra have the potential to be used as bio-coagulant-flocculant for the physico-chemical treatment of POME.

Article Info

Received 2 December 2020

Received in revised form 2 January 2021

Accepted 3 January 2021

Available online 6 January 2021

Keywords

Bio-coagulation

Bio-flocculation

Fenugreek

Okra

Palm Oil Mill Effluent

Response Surface Methodology

1 Introduction

Malaysian palm oil industry is growing steadily in recent years by becoming one of the key palm oil producers and exporters of the world, right after Indonesia. It is evident by the total recorded oil palm planted area has reached a total amount of 5.85 million hectares in 2018 while in 2017, it covered an area of 5.81 million hectares, an increase of around 0.7% in just a year. This is a good indication as it shows market demand for palm oil continues to rise despite the controversy over the plantation of palm oil leading to deforestation. However, the palm oil industry is required to step up its game in its milling and processing sectors to ensure a sustainable and profitable industry in the long run.

An increase in production will lead to an increase in the palm oil waste, particularly palm oil mill effluent (POME). Based on [1], an estimate of around 2.5 to 3.75 tonnes of POME is generated per tonne of crude palm oil (CPO) production. This thick-brownish liquid is a colloidal suspension of 95-96% water, 0.6-0.7% oil and 4-5% total solids produced from three main sources, namely sterilizer condensate, separator sludge and hydrocyclone wastewater [2]. As it is harmful to humans and has a detrimental effect to the environment, palm oil mills are not allowed to discharge raw POME into any watercourses to avoid aquatic pollution due to its high turbidity, suspended solids, contents of organic

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and oil. Table 1 shows the typical characteristics of raw POME and the prescribed discharge limit set by the Department of Environment (DOE), Malaysia.

Table 1 Typical characteristic of raw POME and the regulatory discharge limits [3].

Parameter	Raw POME	DOE Discharge Limit
Temperature (°C)	85	45
pH	4.7	5.0-9.0
Oil & grease (mg/L)	6,000	50
BOD (mg/L)	25,000	100
COD (mg/L)	50,000	-
TS (mg/L)	40,000	1500
TSS (mg/L)	18,000	400
TVS (mg/L)	34,000	-
TN (mg/L)	750	200
Colour	Above 500	200

The most conventional method for treating POME in Malaysia is the ponding system as more than 85% of the palm oil mills use this method [4]. They are low in cost and easy to operate but do have disadvantages like large treatment areas, long hydraulic retention time (HRT), bad odour and poses difficulty in maintenance [5]. Moreover, the anaerobic ponds of the ponding system emit huge amounts of greenhouse gas methane (biogas) while the pond effluent holds the nutrient possible for surface and groundwater pollution [6]. Hence, research on other means of treatment especially tertiary treatment or polishing systems are considered to improve the characteristic of the POME.

Physico-chemical treatment pulls attention as the recovered solids can be used as fertilizer or animal feed [4]. It consists of three phases whereby the first phase is coagulation. It is the addition of a certain chemical (coagulant) to destabilize the mutual repulsion of the colloidal particles causing the particles to bind together and become larger, heavier masses of solids called floc [7]. Its aim is to effectively let them settle down by creating a chemical reaction or eliminating the negative charges that cause particles to repel each other [8]. Then, flocculation takes place to encourage the flocs to further agglomerate into masses large enough for them to settle down faster. Lastly, settling takes place whereby mixing is terminated and the flocs are allowed to settle.

Currently, wastewater treatment industry uses chemicals to perform coagulation (alum, ferric chloride) and flocculation (acrylamide) due to its effective performance, high availability and cost-effectiveness. But it has its drawbacks. For example, overdosing of alum is not desirable as prolonged exposure to water with high aluminum concentration is linked to the development of Alzheimer's disease [9]. Additionally, acrylamide is also suspected to be a potential carcinogen which may lead to growth of cancerous cells in the human bodies [10]. The main aim of this study is to evaluate the potential and application of fenugreek-okra as bio-coagulant-flocculant for the treatment of POME. Based on Table 2, up to date, there is no published work on fenugreek-okra as bio-coagulant-flocculant in the treatment of POME.

Fenugreek is scientifically known as *Trigonella Foenum-graecum* and belongs to the *Leguminosae* family. It is a plant that is cultivated worldwide, particularly in Asia, Northern Africa and the Middle East. It adds flavour to dishes and is widely known as a folk medicine for its beneficial values which include appetite simulation, anti-inflammatory, antipyretic, antimicrobial, antioxidant, antidiabetic, anticancer and antiatherogenic properties as reported by Lu et al. [11]. As for the bioflocculant, okra, it is scientifically known as *Abelmoschus Esculentus* (*L.*) Moench, previously known as *Hibiscus esculentus* (*L.*) that belongs to the Malvaceae family. It is a fruit native to Africa and cultivated in tropical and subtropical countries. Fresh okra can only last for 3 to 4 days because they lose water easily, making them easily perishable.

In this study, variables include pH, fenugreek dosage, okra dosage and rapid mixing speed were manipulated to investigate their effects on the removal of total suspended solids (TSS), turbidity (TUR) and chemical oxygen demand (COD) of the treated POME. Design of experiment (DOE) was conducted using response surface methodology (RSM) to reduce the number of experiments required to perform using the jar test method. RSM generates the results in 3D-plots showing the interaction between factors and its target response. These interactions were used to relate the variables showing relationship and explain the choice of the optimum condition in order to achieve the best response. After determining the optimum condition, the experiment was repeated to verify the outcome of the RSM ensuring the suitability of the model. Characterization tests include Fourier-transform infrared spectroscopy (FTIR), field emission scanning electron microscopy (FESEM), energy dispersive x-ray spectroscopy (EDX), Brunauer, Emmett and Teller analysis (BET), zeta potential and bomb calorimeter were then carried out to further verify the effectiveness of fenugreek-okra as a bio-coagulant-flocculant in the treatment of POME.

Table 2 Combination of coagulant-flocculant with its respective removal efficiencies at optimum condition in the treatment of POME.

Coagulant	Flocculant	Optimum condition	Removal efficiency (%)			Reference
			TSS	TUR	COD	
Alum (chemical)	*C.obtusifolia* seed gum	At pH 4.7, 1.15 g/L then 2.47 g/L	81.58	-	48.22	[12]
Alum (chemical)	Rice starch	At pH 4, 0.2 g/L then 0.55 g/L	88.4	-	27.0	[13]
Chitosan	Magnetite	At pH 6, both at 0.25 g/L	98.8	97.6	62.5	[14]
Fenugreek	Banana Peel	22.44 g/L followed by 69 mins settling time	73.9	-	74.9	[15]
Moringa oleifera seed	NALCO 7751 (chemical)	At pH 5, 4 g/L then 7 g/L	99.3	-	52.5	[16]
Peanut	Okra	At pH 11.6, 1.000 g/L then 1.355 g/L	92.5	86.6	34.8	[3]
Wheat germ	Okra	At pH 12, 1.171 g/L then 0.100 g/L	86.6	87.5	43.6	[3]

2 Methodology

2.1 Preparation of Materials

2.1.1 Palm Oil Mill Effluent (POME)

Raw POME was collected from Jugra Palm Oil Mill, Banting, Selangor. It was collected at the end-pipe of the milling process, which is the effluent right before entering the first pond of the ponding system. The samples were kept in airtight plastic containers and stored in a dark, dry area of the laboratory to prevent microbial decomposition. Initial readings of pH, total suspended solids (TSS), turbidity (TUR) and chemical oxygen demand (COD) were taken for the raw POME samples prior to using them for jar test. As POME has fluctuating characteristics that vary according to time, the recorded values were averaged to obtain the results shown in Table 3. Notably, it was required to invert the plastic container five to ten times in order to homogenize the raw POME sample before conducting the jar test.

2.1.2 Fenugreek Powder Coagulant

Fresh fenugreek was purchased from Tesco situated in the Semenyih town, Selangor. Then, the seeds were dried using an electric oven at 70°C for 24 hrs. They were then blended using an electric blender until fine powder form and later, sorted using a sieve shaker (Fritsch). Sizes ranging between 200 to 500 µm was collected and kept in an airtight beaker using parafilm to prevent humidification and formation of mold. For every stock solution prepared, 10 g of fenugreek powder was dissolved in 500 ml of distilled water to obtain a concentration of 20 g/L. The solution was stirred for 10 mins and filtered using a cotton muslin cloth to remove solid residual in the solution. The solution was poured into a 500 ml volumetric flask and distilled water was further added to reach the 500 ml mark followed by inverting it a few times to ensure complete mixing of the solution. It was normally prepared right before the start of the jar test.

Table 3 Characteristic of raw POME prior to treatment.

Parameters	Range	Average Value
pH	4.5-4.9	4.7
TSS (NTU)	7,600-14,000	11,240
TUR (mg/L)	7,700-13,600	9,640
COD (mg/L)	19,700-41,400	30,550

2.1.3 Okra Mucilage Flocculant

Fresh okra was purchased from Tesco situated in the Semenyih town, Selangor. The okra was cleaned with distilled water to remove any impurities followed by cutting them into short, small pieces. It was mixed with distilled water in the weight ratio of 1:1 and later, soaked for 12 hrs. When the okra was soaked, it was filtered using a cotton muslin cloth to obtain the okra mucilage. The okra mucilage was left aside to settle in order to remove the unwanted bubbles at the top layer. After settling for a few hours, the bubbles were removed, and the okra mucilage was kept in an airtight beaker and stored in the fridge to extend its shelf life. It was taken out whenever necessary to conduct the experiments.

2.1.4 Jar Test Experiment [3]

The main equipment used to conduct jar tests was the Phipps & Bird flocculator, which consisted of six paddle mixers for beakers to be placed under. The control panel was used to adjust the stirring speed while the timer panel was used to adjust the stirring time. The back panel of the flocculator was illuminated to facilitate observation. 500 mL of raw POME sample was required for each litre beaker of jar test. The four variables, coagulant dosage, flocculant dosage, pH and stirring speed had different combinations that were designed by the response surface methodology (RSM) using Design Expert® version 6.0.5 software (Stat-Ease Inc., Minneapolis). It is based on the principle of CCD as shown in Table 4. pH of the raw POME was adjusted using either sodium hydroxide (1M) or sulphuric acid (1M) to be within the range of 3 to 8. After that, required amount of fenugreek stock solution was added into the raw POME followed by rapid mixing for 2 mins. The okra mucilage was added accordingly before undergoing slow mixing at a speed of 60 rpm for 30 mins. The treated POME was then left aside to settle for 240 mins so that supernatant can be formed at the top layer. After settling is completed, the supernatant layer was taken using a dropper after sedimentation and analyzed for removal of TUR, TSS and COD.

2.2 Analytical Method

2.2.1 Reading of TSS, TUR and COD [3]

TSS was measured using spectrophotometer (DR3900, Hach, USA) while TUR was measured using turbidity meter (MI415, Milwaukee, Hungary). As for COD reading, 2 mL of treated POME samples was required to mix with COD vials (High Range, Hach, USA) separately. The sample was then digested at 150°C for 2 hrs. Once it is cooled to room temperature, the COD reading was taken using the spectrophotometer (DR3900, Hach, USA). Each experiment was repeated thrice for accuracy. Hence, the value for each parameter was averaged and recorded as the final reading. Removal efficiency of TUR, TSS and COD were calculated based on the following equation [3]:

$$\text{Removal Efficiency (\%)} = \frac{\text{Final reading - Initial reading}}{\text{Initial reading}} \times 100\% \tag{1}$$

where the initial readings refer to the average value of the parameters of the raw POME shown in Table 3. Final reading refers to the average value of the parameter.

Table 4 Variables combinations designed by RSM.

No. of Jar Test	Run	pH	Fenugreek Dosage (g/L)	Okra Dosage (mL/500mL POME)	Rapid Mixing Speed (rpm)
1	3	6.3	4	26.4	150
2	16	8	4	100	150
3	19	3	4	20	150
4	1	8	5.4	48.8	168
5	5	8	5.4	48.8	168
6	7	8	5.4	48.8	168
7	2	5.4	4	68	180
8	23	5.4	4	68	180
9	14	5.4	6.42	20	180
10	18	5.4	6.42	20	180
11	6	7.8	8	44	200
12	10	3	8	34	200
13	22	4	8	100	200
14	25	8	8	100	200
15	8	7.8	5.2	100	200
16	11	8	4	20	200
17	13	3	4.6	99	200
18	24	3	5	40	200
19	4	5.4	6.4	68	150
20	12	5.4	6.4	68	150
21	15	8	8	20	150
22	9	3	8	100	155
23	17	3	5	100	163
24	20	3	8	41.2	165
25	21	7.8	8	100	200

2.2.2 Sludge Dewatering [3]

The experiment was repeated at the optimum operating condition as determined by RSM. After sedimentation, the supernatant was removed while sludge was distributed into six 50 mL plastic tubes. The plastic tubes were placed in the centrifuge (5430, Eppendorf, Germany) to further remove the supernatant. The rotation speed was set at 7830 rpm. After 7 min, the concentrated sludge was separated from the supernatant and oven dried at 70°C overnight. The dried solid sludge was then collected and sent for further analyses to determine its characteristics. The experiments include FTIR, FESEM, EDX, BET, Zeta Potential and Bomb Calorimeter.

2.2.3 Characterization Test [3]

Field emission scanning electron microscope (FESEM) (Quanta 400F, FEI, USA) coupled with with X-Max Detector (INCA 400, Oxford-Instrument, USA) was used to study the surface morphology of fenugreek powder, raw POME and treated POME sludge. Additionally, using the same equipment, the elemental composition of the samples was determined by performing energy-dispersive X-ray spectroscopy (EDX) analysis. FTIR spectroscopy of fenugreek solution and okra mucilage were obtained using the FTIR spectrometer (Frontier, Perkin-Elmer, USA) where the available functional groups of the sample adsorb the adsorbent material. Then, the spectroscopic data is recorded in the adsorption spectrum of the infrared region. The specific surface area for fenugreek powder was determined from adsorption isotherm using Brunauer, Emmett and Teller analysis (BET) analysis (ASAP Model 2000, Micromeritics, USA). Zeta potential analysis was conducted on the fenugreek solution and raw POME using a zetasizer (Nano, Malvern, UK). Its main aim is to measure the magnitude of the electrostatic charges particles which is vital to explain the attractive or repulsion force that exist between particles during POME treatment. Lastly, bomb calorimeter (6100, Parr Instrument Company, USA) is used to determine the calorific value of the treated POME sludge on whether it has potential to be used as a fuel or fertilizer.

2.3 Design of Experiment and Data Analysis

RSM comprised of mathematical and statistical techniques is especially useful for the optimization of chemical reactions, industrial processes and experimental designs [17]. It was carried out using the Design Expert software (version 11.1.2.0, Stat-Ease Inc., Minneapolis, USA) by assessing the relationship between the factors (fenugreek dosage, okra dosage, pH and rapid mixing speed) and the responses (TUR, TSS and COD removal efficiencies). The relevant factors were then optimized to predict the best responses. The range of the factors shown in Table 5 were determined by conducting preliminary experiments with reference to literature.

Table 5 Factors, their range and associated code in RSM.

Factor	Symbol	Range	Low Level (-1)	Zero (0)	High Level (+1)
Fenugreek Dosage (g/L)	A	4-8	4	6	8
Okra Dosage (ml/500 ml POME)	B	20-100	20	60	100
pH	C	3-8	3	5.5	8
Rapid Mixing Speed (rpm)	D	150-200	150	175	200

Optimal (custom) design was the chosen response surface design instead of the conventional central composite design (CCD) or Box-Behnken design as it provides more flexibility in the design space. It can be customized to fit a linear, quadratic or cubic model. It is also notable that this is the first time optimal (custom) design is chosen to optimize for a coagulation-flocculation process. In this case, the responses were predicted by a second order model in the form of quadratic polynomial equation as shown in the equation below. The design points generated were randomized and simply spread out in the design space due to the use of the coordinate exchange algorithm. In addition, I-Optimal was chosen over D-Optimal to achieve a greater precision in the estimated model as it minimizes the average variance of prediction over the design space [18]:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \left(\beta_{ii} X_i^2 + e_i \right) \quad (2)$$

where Y is the response variable X_i and X_j are the factors which influence Y; β_0 is the constant, β_0, β_{ij} and β_{ii} are the coefficients of linear, interaction and quadratic coefficients, respectively. k is the number of factors studied and e_i is the random error.

Analysis of Variance (ANOVA) was then used to evaluate the interactions between the factors and the responses. The determination coefficient, R^2 value was the determinant of the quality of fit for the polynomial model. Moreover, F value (Fisher's F-test) expressed the statistical significance while the p-value (probability) that has a 95% confidence level evaluated the model terms. The relationship between the factors and responses were visualized by generating 3D response surface and contour plots. Finally, the optimum operating conditions of the fenugreek-okra were determined.

3 Results and Discussion

3.1 Regression Analysis and Model Fitting

The experimental results based on the runs generated by the I-optimal design were conducted and their respective results are shown in Table 6. There are a total of twenty-five runs. Based on Table 6, the observed removal efficiencies of TSS, TUR and COD varied in the ranges of 64.77 to 98.37%, 79.25 to 97.16% and 35.35 to 83.79%, respectively.

Table 6 I-optimal design of the study involving factors (A, B, C and D) and responses (TSS, TUR and COD).

Run	Factor				Response		
	A	B	C	D	TSS (%)	TUR (%)	COD (%)
1	-0.3	-0.28	+1	-0.28	64.77	79.25	51.52
2	-1	+0.2	-0.06	+0.21	92.64	95.53	68.34
3	-1	-0.84	+0.32	-1	90.36	92.74	59.00
4	+0.2	+0.2	-0.05	-1	95.10	93.12	57.23
5	-0.3	-0.28	+1	-0.28	76.14	87.10	35.35
6	+1	-0.4	+0.9	+1	94.69	94.30	71.03
7	-0.3	-0.28	+1	-0.28	83.08	93.36	77.66
8	-0.4	+1	0.9	+1	92.55	90.62	73.43
9	+1	+1	-1	-0.79	95.98	96.97	70.00
10	+1	-0.65	-1	+1	96.77	97.12	71.96
11	-1	-1	+1	+1	84.19	88.53	43.72
12	+0.2	+0.2	-0.05	-1	96.01	95.45	67.67
13	-0.7	+0.977	-1	+1	96.01	97.16	68.12
14	+0.21	-1	-0.06	+0.2	84.31	94.28	55.08
15	+1	-1	+1	-1	84.31	87.48	69.67
16	-1	+1	+1	-1	94.29	95.49	52.50
17	-0.5	+1	-1	-0.47	88.49	89.67	49.86
18	+0.21	-1	-0.06	+0.2	94.97	95.69	66.60
19	-1	-1	-1	-1	93.97	96.11	63.16
20	+1	-0.47	-1	-0.5	93.75	95.03	44.13
21	+1	+1	+0.9	-0.4	93.64	95.05	75.62
22	+1	+1	-0.6	+1	97.16	97.07	69.03
23	-1	+0.2	-0.06	+0.21	73.31	88.86	42.75
24	-0.5	-0.5	-1	+1	98.37	97.06	83.79
25	+1	+1	+1	+1	94.85	95.57	79.70

The result of the analysis of variance using ANOVA are summarized in Table 7. The model recommended for each response is different where TUR uses a quadratic model while TSS and COD use a reduced 2Fl model. To improve the model, model terms with values greater than 0.1000 were omitted accordingly until it reached its statistical significance. Table 7 shows the F-value to be 124.88, 5.16 and 5.88 for TUR, TSS and COD removal, respectively. It implies the models are significant where overall, there is only a less than 1% chance that the F-value this large could occur due to noise. P-value is another indication to show the significance of the model terms, it has to be less than 0.0500. The Lack of Fit F-values are 0.46, 1.27 and 0.71 for TUR, TSS and COD, respectively. They are not significant relative to the pure error. There is a 76.51%, 60.38% and 73.11% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant Lack of Fit data is a good indication that the model fit in properly.

As for the fit statistics, they are shown in Table 8. High R^2 coefficients are achieved for TSS, TUR and COD which are 0.9977, 0.7667 and 0.8546, respectively. All R^2 values are greater than 0.75 indicating the model suitability in predicting experimental outcomes [19]. The Predicted R^2 is in reasonable agreement with the Adjusted R^2 for TSS due the difference between each other is less than

0.2. However, the Predicted R^2 for TUR and COD are not as close to the Adjusted R^2. Recommended solutions which include model reduction and removing outliers were performed but the difference is still more than 0.2, indicating a large block effect or a possible problem with the data. Results are considered reliable and precise when the coefficient of variation (C.V. %) is less than 10% [19]. Adequate (Adeq) Precision measures the signal to noise-ratio and a value greater than 4 is desirable as it indicates an adequate signal and the model can be used to navigate the design space. All the aforementioned target for C.V. % and Adeq Precision were achieved by all 3 responses.

Table 7 Analysis of variance.

Response		Sum of Squares	Degree of Freedom	Mean Square	F-value	p-value	Remark
TSS	Quadratic Model	428.12	14	30.58	124.88	0.0001	Significant
	Lack of Fit	0.5655	3	0.1885	0.4552	0.7651	Not significant
TUR	Reduced 2FL Model	122.44	7	17.49	5.16	0.0081	Significant
	Lack of Fit	34.54	10	3.45	1.27	0.6038	Not significant
COD	Model	2134.95	9	237.22	5.88	0.0073	Significant
	Lack of Fit	308.88	8	38.61	0.7085	0.7311	Not significant

Table 8 Fit statistics.

Response	Std. Dev.	Mean	R^2	Adjusted R^2	Predicted R^2	C.V. %	Adequate Precision
TSS	0.4949	92.10	0.9977	0.9897	0.9304	0.5373	34.2572
TUR	1.84	94.02	0.7667	0.6183	0.2235	1.96	8.3615
COD	6.35	64.72	0.8546	0.7091	0.2091	9.82	8.2737

After conducting ANOVA, the model equations for the predicted removal efficiencies of TUR, TSS and COD are obtained and expressed in equations as follows:

$$\text{TTS Removal (\%)} = 89.47 + 0.1517A + 3.08B - 3.23C + 0.1487D + 0.9927AB$$
$$+0.1758AC - 1.20AD + 3.15BC - 0.1888BD - 1.12CD + 2.50A^2 - 2.76B^2 - 1.87C^2 + 5.24D^2 \quad (3)$$

$$\text{TUR Removal (\%)} = 93.62 - 0.1670A + 0.6740B - 1.80C + 0.3496D + 1.41AB$$
$$+2.18BC - 1.96CD \quad (4)$$

$$\text{COD Removal (\%)} = 71.30 + 0.1331A + 0.3493B + 4.37C + 7.07D + 8.88AC$$
$$+5.43BC + 4.28BD - 6.78CD - 1.210B^2 \quad (5)$$

where *A*, *B*, *C* and *D* refer to the factors mentioned in Table 5.

Normal plot of residuals is displayed in Fig. 1 for removal efficiency of TSS, TUR and COD, respectively. It is an indication of model adequacy between the actual and measured data whereby the design points need to be distributed relatively close to the reference line. The plot of predicted versus actual values for each response is also shown in the same figure. A good distribution of design points is required to be either on or near the straight line ($y = x$). Based on the figure, all three plots are satisfactory proving the prediction capability of the model.

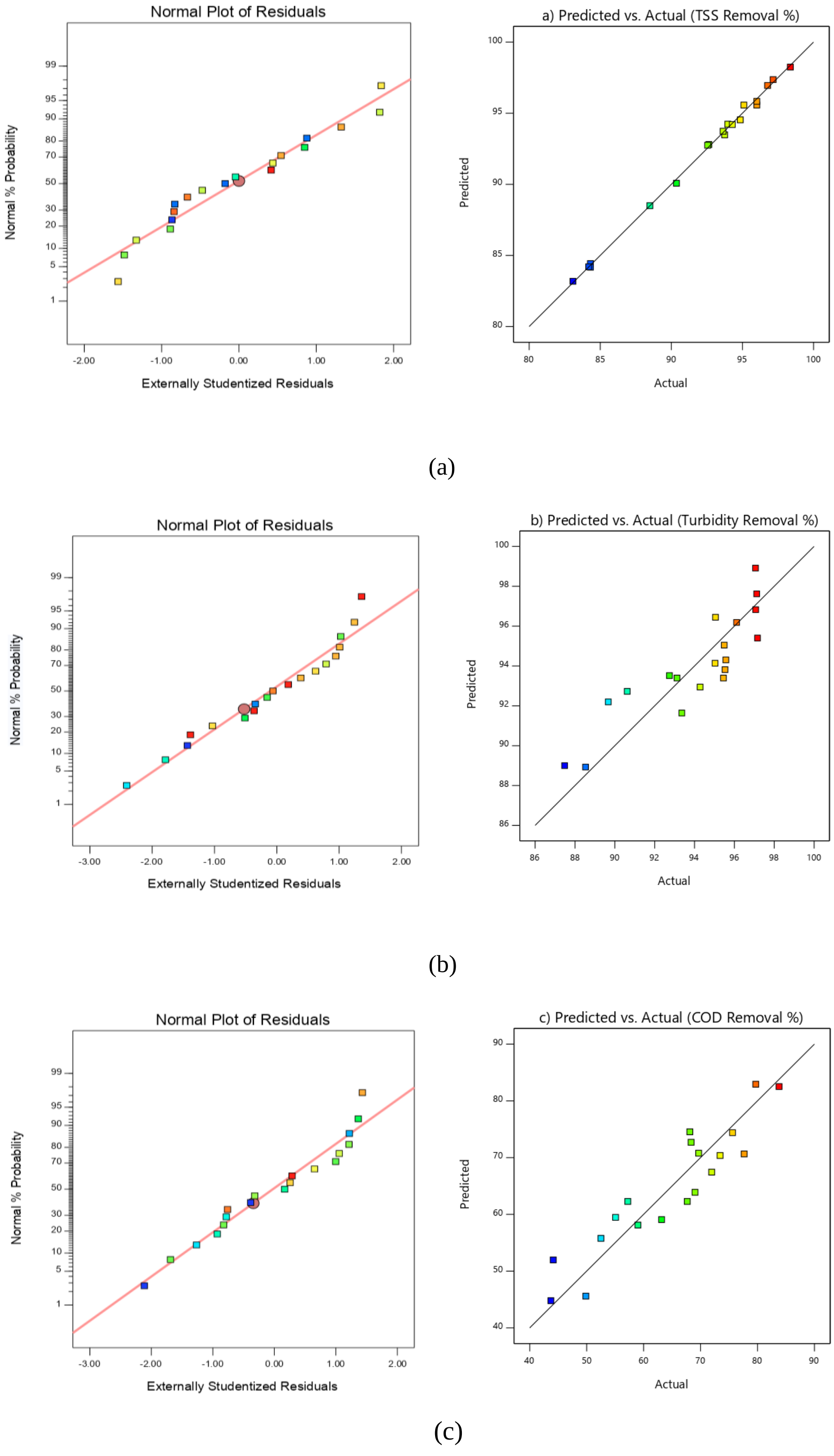

Fig. 1 Normal Plot of Residuals (on the left) and Predicted vs Actual Value Plots (on the right) for (a) TSS (b) TUR and (c) COD.

3.2 Three-dimensional (3D) Plot

The interaction between factors, fenugreek dosage (*A*), okra dosage (*B*), pH (*C*), rapid mixing speed (*D*) and responses, removal efficiencies of TSS, TUR and COD are investigated using the three-dimensional (3D) plots of the regression models. Their interactions are displayed in Fig. 2 and 3.

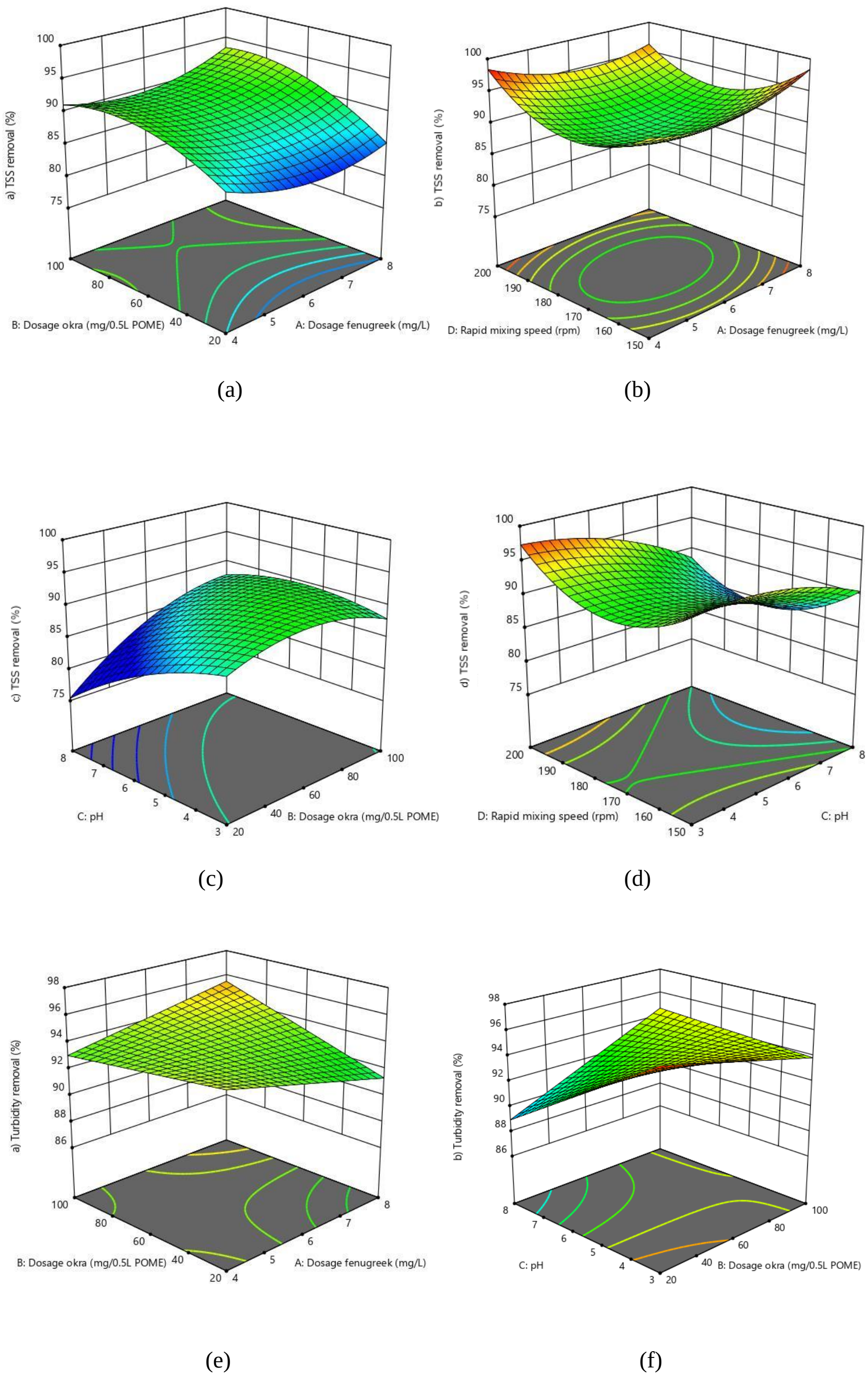

Fig. 2 Interaction related to TSS removal efficiency is (a) *A*-*B* (b) *A*-*D* (c) *B*-*C* (d) *C*-*D* while TUR removal efficiency is (e) *A*-*B* (f) *B*-*C*.

3.2.1 Interaction between Okra Dosage and pH (B-C)

The interaction of okra dosage (*B*) and pH (*C*) plays an important role in this experiment as it affects the removal efficiencies of TSS, TUR and COD from POME greatly. The okra dosage is in the range of 20 to 100 ml/500 ml POME while the pH of the initial POME is varied between pH 3 to 8. Factors of fenugreek dosage and mixing speed were kept constant at 6 g/L and 175 rpm, respectively. Based on Fig. 2(c), the TSS removal efficiency increases subsequently when the pH is decreased from 8 to 3 while the okra dosage is maintained within the range of 40 to 80 ml/ 500 ml POME. At these conditions, it is able to achieve a TSS removal efficiency of around 90%. Hence, TSS removal efficiency works best when the pH is low while okra dosage is within the predetermined range.

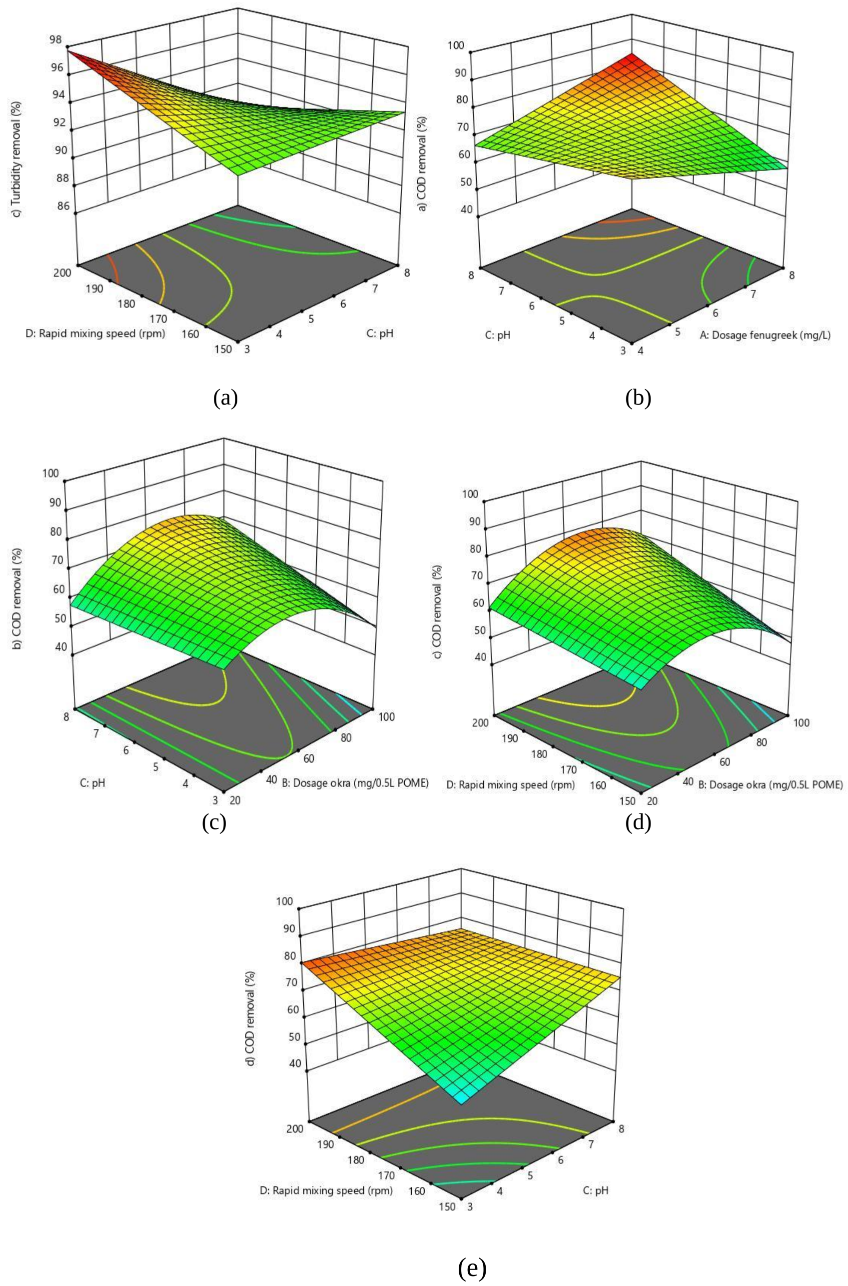

Fig. 3 Interaction related to TUR removal efficiency is (a) *C-D* while COD removal efficiency is (b) *A-C* (c) *B-C* (d) *B-D* and (e) *C-D*.

For the TUR removal efficiency, Fig. 2(f) shows it generally perform very well with the efficiency ranging between 90-96%. At the minimum okra dosage of 20 ml/ 500 ml POME, removal efficiency is the highest (96%) at pH 3 but subsequently decreases to the lowest (90%) when pH is adjusted from the acidic to the alkaline condition. Hence, a low pH is important for a high TUR removal efficiency while okra dosage, in this case, is not very relevant. However, the interaction between pH and okra dosage shows a different result on the COD removal efficiency displayed in Fig. 3(c). Overall, a maximum of around 70% removal efficiency is observed when pH is between the neutral to alkaline condition while okra dosage is within the range of 40 to 80 mL/500 mL POME. A high pH works best for COD removal efficiency while the okra dosage must be within the optimum condition of 40 to 80 ml/500 ml POME.

Okra is a natural anionic (polymer) polysaccharide. It has an electronegative property due to the presence of free carboxylic groups and displays a porous structure in the FESEM analysis [20]. Its flocs based on the adsorption and bridging mechanism. The adsorption occurs via electrostatic forces, van der Waal forces, hydrogen bonding and chemical bonding [21]. Bridging occurs when the segment of the okra polymer chain adsorbs onto the POME particles, thus, linking them together and increasing its size [22]. It works after the POME particles are destabilized during the coagulation process. Okra shows good flocculation capability is because the polysaccharide consists of D-galactose, L-rhammnose and D-galacturonic acid, whereby the active element, galacturonic acid, is well known for achieving 50% turbidity removal [23]. In addition, Kumar et al. [24] associated it with cactus Opuntia which has shown promising flocculating properties in previous studies as both of them have the presence of mucilage which is a sticky and complex carbohydrate that has high water retention capability.

The reason okra works better at a lower pH in removing suspended solids is due to the ionization of polymer. In acidic condition, the amide groups of the okra are protonated and became positively charged while the carboxyl group becomes neutral. This results a higher electrostatic attraction of okra and POME particles [25]. The okra polymer chain is able to adsorb more POME particles easily, thus, enlarging the flocs in the process. This allows more suspended solids to be removed during settling as they take in other tiny particles that could not form flocs inside them and settle along with them. But, as the pH increases, the electrostatic interaction becomes weaker as the carboxyl group becomes negatively charged while the amide group is deprotonated. This causes an electrostatic repulsion between the okra polymer and POME particles due to similar charge. As this prevents the flocs from aggregating, lesser suspended solids would be removed due to poor settling. However, it is notable that an increase in the supernatant layer was observed when settling time was extended. Additionally, a high TUR removal efficiency is also attainable at low pH is because it is closely related to TSS. Ahmad et al. [26] proved that suspended solid concentration is directly proportional to turbidity. So, reducing TSS reduces the TUR, simultaneously. As for the decrease in COD removal efficiency at low pH, it is not unexpected as the bioflocculant, okra, would have contributed to COD while aiding the removal of POME particles [27].

The polymer bridging mechanism also affects the okra dosage in order to achieve high removal efficiencies of TSS and COD. This is because increasing the okra dosage increases the adsorption of POME particles. As more available polymer binds with the POME particles, high aggregation allows the flocs to become larger and denser, thus, increasing their tendency to settle. However, overdosing occurs after the after the optimum condition (40 to 80ml/500ml POME) decreasing the TSS and COD removal efficiency. When excess POME particles are being adsorbed onto the surface of the polymer, it causes re-stabilization [28]. Repulsive energy between the okra polymer and POME particles increases and the aggregated particle is dispersed in the solution causing a hindrance in floc formation [29]. In conclusion, extreme pH cannot be chosen in order to achieve a good balance between the removal efficiency of TSS, TUR and COD while okra dosage needs to be within 40 to 80 ml/500 ml POME to prevent under or overdosing the POME that would reduce the performance of the okra as a flocculant.

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3.2.2 Interaction between pH and Rapid Mixing Speed (C-D)

The interaction between pH (*C*) and mixing speed (*D*) is another significant relationship in this experiment where all three removal efficiencies TSS, TUR and COD were greatly affected by it. The pH of the POME is adjusted between 3 to 8 while the rapid mixing speed is in the range of 150 to 200 rpm. Fenugreek dosage is fixed at 6 g/L while okra dosage is set at 60ml/500 ml POME. The highest TSS removal efficiency from Fig. 2(d) is observed when the pH is at the minimum value while the rapid mixing speed is at both edges (either maximum or minimum value) of the graph. At maximum value (200 rpm), the TSS removal efficiency is slightly higher by 2%.

Based on Fig. 3(a), TUR removal efficiency was best when the pH is at the minimum while the rapid mixing speed is at the maximum. A removal efficiency of around 97% is obtainable at this point. As for the COD removal efficiency displayed on Fig. 3(e), high removal efficiency is achievable between the pH of 3 to 8 as long as the rapid mixing speed is more than 187.5 rpm. It is also notable that when pH is more than 7, COD removal efficiency higher than 70% can be obtained within the range of 150 to 200 rpm.

All three removal efficiencies (TSS, TUR and COD) were able to perform well at a minimum pH of 3 is because the rapid mixing speed is at a maximum value (200 rpm). This aligns with [30] that claim the rapid mix parameter is one of the important parameters in determining an optimized process. It is required to disperse the coagulant throughout the POME solution [31]. Additionally, when the POME particles are destabilized, agitation allows the POME particles to collide with each other and form flocs.

In this case, all three removal efficiencies are favorable at low pH may be due to the reduction of the electrostatic repulsion of the POME particles [32]. This is supported from the results obtained from the zetasizer whereby the zeta potential value of POME increases from -36.4 to +2.055 mV when pH is reduced from 8 to 3. Hence, varying the pH value changes the surface charges of the POME particle allowing better aggregation. Moreover, strong acidic conditions would also aggravate the POME to break the oil droplets and destabilize the suspended solids in the suspension [26]. As TSS and TUR are always caused by the same contaminants in the solution, an increase in TSS will result in a higher TUR just like the presence of small salt as a contaminant of suspended solid in water will cause the water to turn cloudy [29]. It is also notable that at high pH, COD removal efficiency can perform better at a slightly lower rapid mixing speed. A lower rapid mixing speed is able to reduce the operating cost of the process. However, it is preferable to minimize the pH and maximize the rapid mixing speed in order to achieve high removal efficiencies for all responses.

3.2.3 Interaction between Fenugreek Dosage and Okra Dosage (A-B)

The interaction between the fenugreek dosage (*A*) and okra dosage (*B*) only affects the removal efficiency of TSS and TUR. It is insignificant for the COD removal efficiency; thus, it was removed from the model equation. Fenugreek dosage is varied between 4 to 8 g/L while okra dosage is in the range of 20 to 100 ml/500 ml POME. pH is kept at 5.5 while the rapid mixing speed is constant at 175 rpm. Fig. 2(a) shows the relationship between fenugreek dosage and okra dosage in affecting the TSS removal efficiency. Okra dosage must be above 40ml/500ml POME in order to achieve a higher removal efficiency, but it is not limited by the dosage of fenugreek. The highest TSS removal efficiency is 92% when both fenugreek and okra dosage are at their maximum value of 8 g/L and 100 ml/ 500 ml POME, respectively. As for the TUR removal efficiency from Fig. 2(e), it performs well when both fenugreek and okra dosage are either at the minimum or maximum value. 95% removal efficiency is achievable at these design points.

Based on the result, fenugreek dosage does not have a large effect on the TSS and TUR removal efficiencies. This proves that fenugreek has good coagulating efficiency even at low doses which is a characteristic of using bio-coagulant. Based on Table 9, it is evident when the dosage of fenugreek is comparable with other bio-coagulants from previous studies give the same coagulating performance.

Additionally, other factors such as cost of coagulant needs to be taken into account when choosing the optimum condition of the fenugreek dosage [33]. Therefore, it is ideal to minimize the fenugreek dosage (4 mg/L) so that the overall cost of raw materials can be reduced. As for the flocculant, okra dosage must be above 40 ml/500 ml POME to ensure good flocculating efficiency as stated previously in this paper.

Table 9 Type of coagulant with their respective removal efficiencies at their optimum doses.

Type of Coagulant	Optimum dose of coagulant (g/L)	Removal efficiency (%)			Reference
		TSS	TUR	COD	
Cassia obtusifolia **seed gum**	1	87	-	55	[34]
Chitosan	0.5	95.0	-	-	[26]
Dragon fruit foliage	0.3	98.8	48.7	99.2	[33]
Moringa oleifera **seed**	6	95.0	-	52.2	[16]
Rich starch	0.74	92.5	-	30.9	[35]
Wheat germ	12	93.5	97.7	55.0	[36]

3.2.4 Interaction between Fenugreek Dosage and Mixing Speed (A-D)

This interaction has effect only on the TSS removal efficiency. Fenugreek dosage (*A*) is within the range of 4 to 8 g/L while the rapid mixing speed (*D*) is set between 150 to 200 rpm. At the okra dosage of 60 ml/500 ml POME and a pH of 5.5, a maximum TSS removal efficiency of around 98% is observed from Fig. 2(b). It is obtained when fenugreek dosage is at the maximum while rapid mixing speed is at the minimum and vice versa.

It is able to work at both design points because fenugreek is a capable coagulant. Based on the zeta potential analysis, fenugreek is also a natural anionic (polymer) polysaccharide. This is due to its zeta potential of -39.6 mV. As aggregation of particles in a solution occurs via four basic coagulation mechanisms which include (1) double-layer compression; (2) intraparticle bridging; (3) adsorption; and (4) charge neutralization [7]. It is believed that fenugreek coagulates based on adsorption and interparticle bridging. This is because double-layer compression mechanism seldom occurs in polymeric coagulants [37]. Besides, fenugreek and POME have a similar negative charge, thus, charge neutralization would not happen in this case. Similar finding was also reported by Sethu et al. [38] when using Opuntia as coagulant where it shows that bridging mechanisms occurred based on highest zeta potential condition. Fenugreek shows good coagulating performance is because it is a polysaccharide that consists of D-galactose and D-mannose in 1:1 and 1:1.2 ratios [23]. These long chain polymers adsorbed on particles can have head and tails entering several techniques into the solution, thus, destabilizing the POME particles in the process [39]. Then, intraparticle bridging occurs by connecting the POME particles to form a network, typically known as flocs [40]. Furthermore, Ramamurthy et al. [41] also proves the seed extract of fenugreek has 80 % coagulation properties.

Based on the experiments, the existing repulsive forces have no effect on the performance of POME treatment using 8 g/L of Opuntia powder at this highest zeta potential condition (-25.33 mV). This has proven that the bridging mechanism occurs in POME treatment by Opuntia coagulants.

Hence, at low fenugreek doses, it can be compensated by the high rapid mixing speed. High speed agitation increases causing intraparticle interaction followed by the formation of flocs [42]. As for the combination of high fenugreek dosage and low rapid mixing speed, it is capable to achieve high TSS removal is because a high dosage means the fenugreek polymer has more available sites to bind on the

POME particles. Therefore, increasing the rate of adsorption. For the intraparticle bridging mechanism, it is also necessary to have much space that can attach divisions of polymer chains to engross other particles. Based on the result, fenugreek dosage versus rapid mixing speed only affects the TSS removal efficiency. Hence, it is decided to operate the physico-chemical treatment at a low fenugreek dosage but high rapid mixing speed.

3.2.5 Interaction between Fenugreek Dosage and pH (A-C)

This interaction only has significance on the COD removal efficiency. The fenugreek dosage (*A*) is within 4 to 8 g/L while pH (*C*) is varied between 3 to 8. The constant factors are okra dosage and rapid mixing speed at 60ml/500ml POME and 175 rpm, respectively. Based on Fig. 3(b), the highest COD removal efficiency is observed when the fenugreek dosage and pH value are both at the maximum point and vice versa.

It can be concluded that fenugreek is less dependent on the pH. Besides, it is also pH stable. Varying the pH does not alter the stability of the coagulation active components in the solution, thus, the coagulating efficiency is also not affected [41]. Mishra and Bajpai [29] proved that fenugreek works best at neutral condition of pH7. This aligns with the result that a high COD removal efficiency can be obtained when the pH is either at the minimum or maximum value. As COD is used to assess the concentration of organic matter in the POME, an increase in COD is probably due to the contribution of the fenugreek. Its organic matter is still present in the supernatant, contributing to the final COD value [43].

As for the fenugreek dosage, it requires more at high pH. This is to ensure the fenugreek polymer has more available sites to adsorb the POME particles. At low pH, it requires less fenugreek dosage is because POME particles changes from anionic to cationic when the pH becomes acidic. In this case, charge neutralization occurs whereby the fenugreek hydrolyses and neutralizes electrical charges on the POME particles causing flocs to form through agglomeration [8]. Based on the results, it is desirable to operate the coagulation-flocculation process at a low pH to achieve high COD removal efficiency while minimizing the fenugreek dosage.

3.2.6 Interaction between Okra Dosage and Mixing Speed (B-D)

This interaction has significance on the COD removal efficiency. Factors that are varied include okra dosage (*B*) and rapid mixing speed (*D*) while fenugreek dosage and pH are kept constant at 6 g/L and pH 5.5, respectively. From Fig. 3(d), COD removal efficiency is the highest when okra dosage falls between the range of 40 to 80ml/500 ml POME while the rapid mixing speed must be above 187.5 rpm.

There may be an error in this relationship as okra is added after the rapid mixing stage. Additionally, mixing of flocculation is not critical as claimed by Han and Lawler [44] as long as the slow mixing speed is minimal to allow suspended particles to floc without breakage. Hence, this may cause a deviation between the actual and predicted COD. If so, the model term (B-D) may not be required in the calculation for the COD removal efficiency.

3.3 Optimization and Validation Experiment

After analyzing the interactions in detail, it is concluded that a low pH, low fenugreek dosage and high rapid mixing speed is preferable while the okra dosage must be within the range of 40 to 80ml/500 ml POME. These conditions would achieve a high removal efficiency for TSS, TUR and COD. The statement is aligned with the result of the optimization shown in Table 10. Optimization was carried out by maximizing the removal efficiency of TSS, TUR and COD to obtain the optimum operating conditions. One solution with the desirability of 1.0 was chosen out of the 100 solutions. The experiment at the optimum operating conditions was conducted to test the empirical models.

The actual responses were then compared with the predicted values to prove the suitability of the models. Based on Table 11, a large deviation between the actual and predicted COD removal efficiency shows an error in the model. This error may be contributed by the interaction between okra dosage and mixing speed (*B-D*). It is not possible because okra is added after the rapid mixing stage. However, TSS and TUR removal efficiencies perform well with errors less than 10%.

Table 10 Optimum operating condition for the fenugreek-okra.

Factors	Optimum Operating Condition
pH	3.2
Fenugreek dosage (g/L)	4.1
Okra dosage (ml/ 500 ml POME)	58
Rapid mixing speed (rpm)	197

Table 11 Actual and predicted responses.

Responses	Actual Responses (%)	Predicted Responses (%)	Percentage Error (%)
TSS	92.70	99.48	6.82
TUR	94.97	97.56	2.66
COD	63.11	87.01	27.48
TSS	92.70	99.48	6.82

3.4 Characterization Tests

3.4.1 FTIR

The FTIR spectrum of both fenugreek solution and okra mucilage are similar in terms of the location of the peak. The broadening of the band starting after 3000 cm^{-1} with its peak centered around 3273 cm^{-1} shows the presence of the OH functional group. Additionally, stretching of the hydroxyl group (-OH) participating in hydrogen bonding that correspond to the basic carbohydrate structure of the polysaccharides [20].

C=O is found at the peak around 1636 cm^{-1} indicates the bending of amide or stretching of the carbonyl of carboxylic acid which may be a ketone or aldehyde group [19]. Additionally, absorbance ranging between 600-500 cm^{-1} shows the presence of C-OH bonding [45]. All the aforementioned functional groups are important as they act as active sites for the attachment of POME particles [46].

3.4.2 FESEM

FESEM analysis reveals the surface morphology as images of fenugreek powder, raw POME and treated POME sludge were taken and displayed in Fig. 6, 7 and 8, respectively. The shape of fenugreek powder is irregular but has void spaces to allow adsorption of POME particles so that adsorption and intraparticle bridging can be formed. As for raw POME, it forms a fibrous cluster network structure that are believed to be micro flocs. These micro flocs are not strong and would easily disperse under shear stress. After treating the POME with fenugreek and okra, it is evident that the structure becomes more compact and have a smoother surface due to the formation of larger, denser and easier settling flocs.

3.4.3 FESEM

The elemental composition of fenugreek powder, raw POME and treated POME sludge were tabulated in Table 12. It is reported by Ramamurthy et al. [41] that a fenugreek seed contains 23-26% of protein, 6-7% of fat and 58% carbohydrates of which 25% is dietary fiber, saponins and rich in flavonoids. Based on the analysis, the most abundant elements present in fenugreek are carbon and oxygen while traces of elements like nitrogen, magnesium, sulphur, potassium and calcium are present in small amount. Pt that appears in fenugreek is a background reading that is contributed from the sputter system. The sputter system was used to increase the conductivity of the fenugreek so that peaks can be observed for a more accurate reading [41].

For raw POME, carbon and oxygen are present in abundant while the rest is magnesium, aluminium, silicon, sulphur, potassium and sulphur. As for treated POME sludge, others being the same, nitrogen, phosphorus and chlorine are present while aluminium and calcium are absent. Presence of nitrogen and phosphorus may not be favorable for the treated POME sludge to be used as a fertilizer. This is because these elements when contacted with water would cause algae to grow, contributing to eutrophication [19].

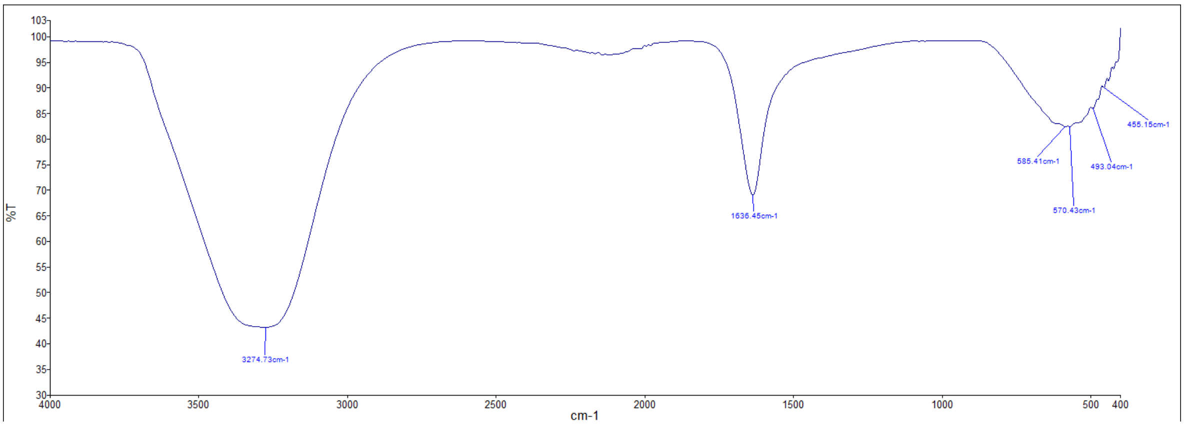

Fig. 4 FTIR spectra of fenugreek solution.

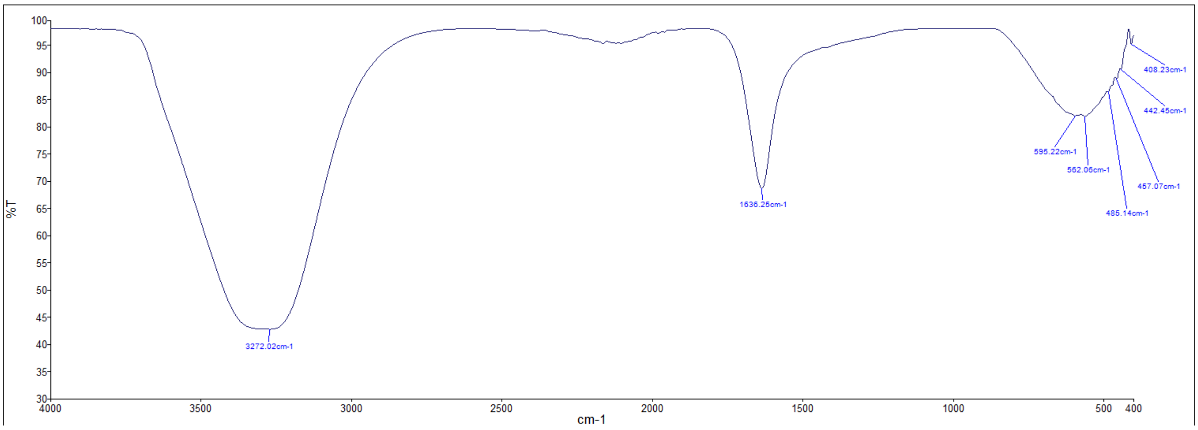

Fig. 5 FTIR spectra of okra mucilage.

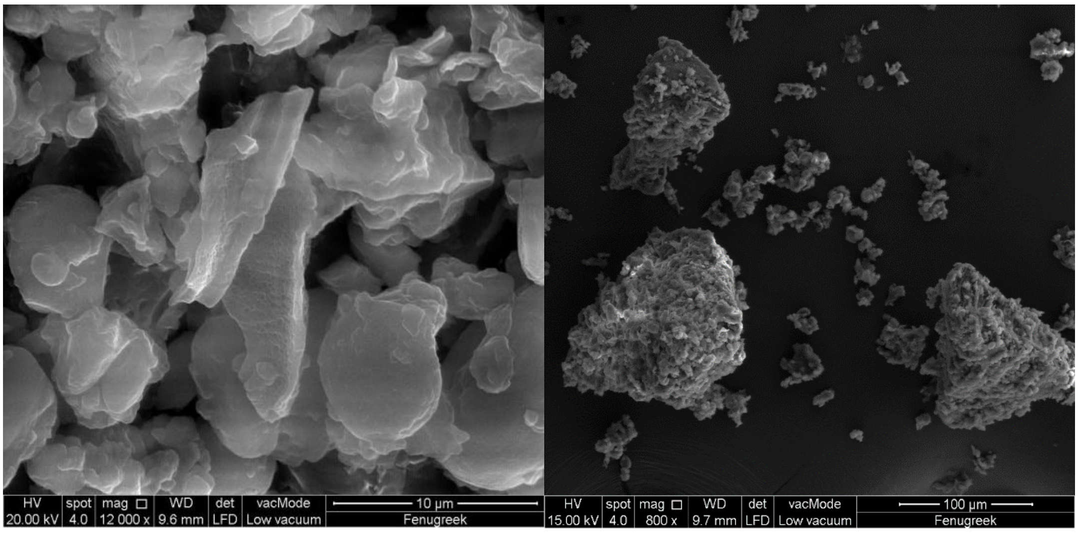

Fig. 6 FESEM images of fenugreek powder.

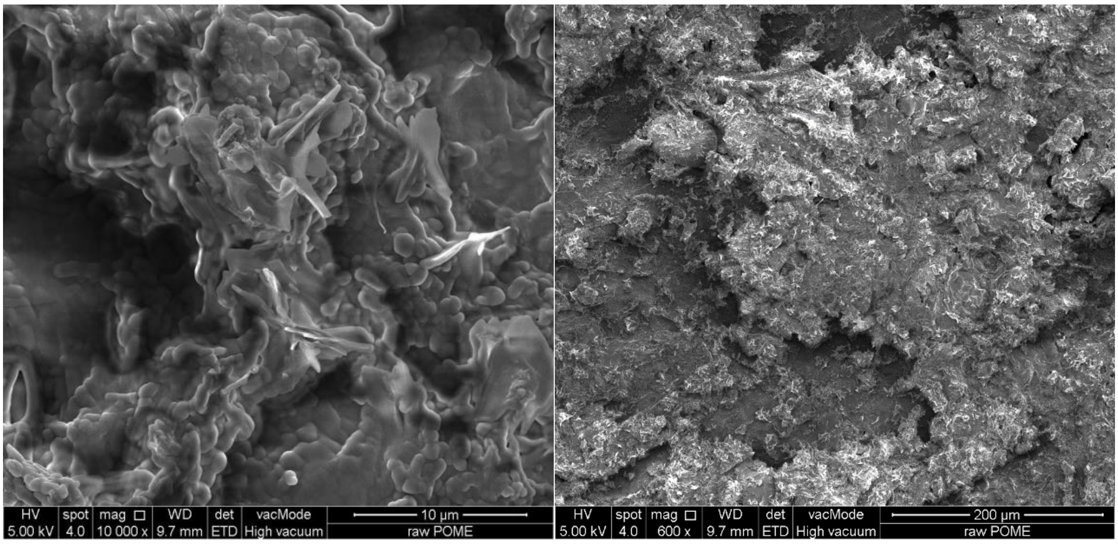

Fig. 7 FESEM images of raw POME.

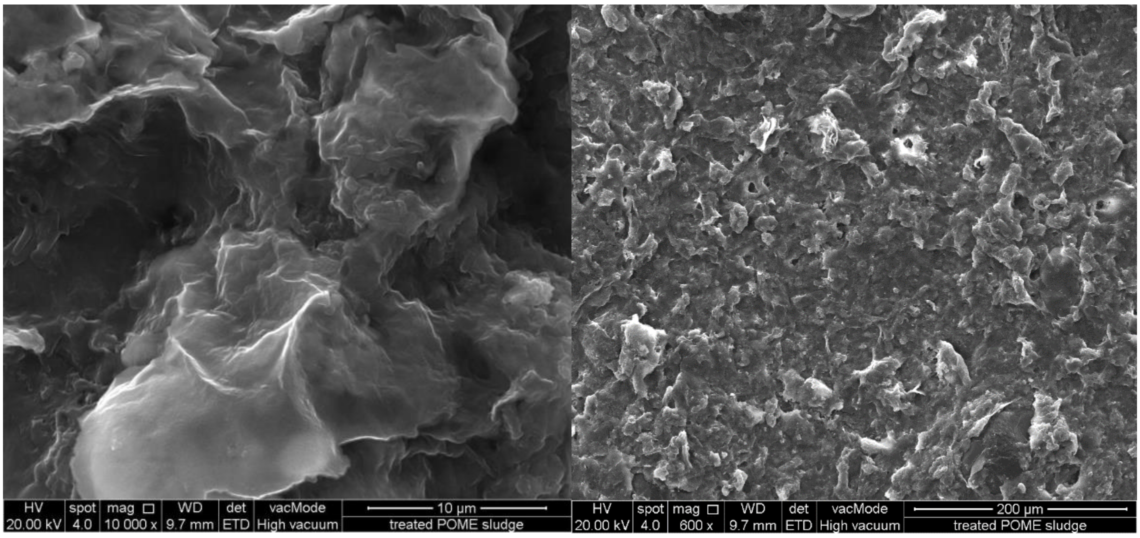

Fig. 8 FESEM images of treated POME sludge.

Table 12 Elemental composition of fenugreek, raw POME and treated POME sludge.

Element	Weight		
	Fenugreek	Raw POME	Treated POME Sludge
C	61.15	79.68	57.45
O	27.29	17.32	30.97
N	5.61	0.00	2.54
Mg	0.25	0.27	0.68
Al	0.00	0.30	0.00
Si	0.00	0.92	0.69
P	0.00	0.00	0.28
S	0.28	0.38	2.15
Cl	0.00	0.00	1.88
K	1.87	0.76	3.37
Ca	0.51	0.36	0.00
Pt	3.04	0.00	0.00

3.4.4 BET

The specific surface area of fenugreek powder was compared with Moringa oleifera seed powder whereby it is a known capable bio-coagulant and widely used in the industrial scale. Based on Table 13, their specific surface area is comparable and shows that fenugreek powder can be a good choice to act as a bio coagulant. Additionally, Choong Lek et al. [19] stated that a high specific area is advantageous as it will reduce the dosage required for treatment as there is a high concentration of adsorption sites available. Hence, increasing the dosage may also increase the removal efficiency as there is more active sites to adsorb POME particles and connecting them through intraparticle bridging forming more flocs in the process.

Table 13 Specific surface area of fenugreek powder.

Material (Powder)	BET Surface Area (m^2/g)	Reference
Fenugreek	0.2370	-
***Moringa oleifera* seed**	0.3965	[16]

The specific surface area of fenugreek powder was compared with Moringa oleifera seed powder whereby it is a known capable bio-coagulant and widely used in the industrial scale. Based on Table 14, their specific surface area is comparable and shows that fenugreek powder can be a good choice to act as a bio coagulant. Additionally, Chong Lek et al. [19] states that a high specific area is advantageous as it will reduce the dosage required for treatment as there is a high concentration of adsorption sites available. Hence, increasing the dosage may also increase the removal efficiency as there is more active sites to adsorb POME particles and connecting them through intraparticle bridging forming more flocs in the process.

3.4.5 Zeta Potential

Based on the zeta potential analysis, fenugreek solution is anionic (-39.6 mV). This means it has an electropositive property. As for the electrostatic charge of the raw POME particles, it changes from negative to positive when the pH is adjusted from the alkaline to acidic condition. At pH 3, the zeta potential of the raw POME is positive causing an attraction with the negative charge the fenugreek-okra allowing better removal due to charge neutralization, adsorption and intraparticle bridging. When zeta potential of the raw POME becomes negative, there would be a repulsion with the negative charge of the fenugreek-okra lowering the TSS and TUR removal efficiency [19].

Table 14 Electrostatic charge of the raw POME within the pH range.

pH	Value
3	+2.1
4	-23.6
5	-23.2
6	-32.6
7	-34.3
8	-36.4

3.4.6 Bomb Calorimeter

The treated POME sludge was dewatered and tested using a bomb calorimeter. It is to determine the calorific value and to assess its feasibility for use as a source of fuel. The gross heat of the treated POME sludge was found to be 23.63 MJ/kg. It is evident to have the potential of becoming a source of fuel when compared with calorific value of typical fuel. For an example, its calorific value is higher than brown coal (14.65 MJ/kg) but lower than fuel oil 39.86 MJ/kg).

4 Conclusion

The purpose of this study is to evaluate the potential of using fenugreek-okra as bio-coagulant-flocculant in the treatment of POME. It is determined based on the removal efficiencies of total suspended solids (TSS), turbidity (TUR) and chemical oxygen demand (COD) by varying the pH, fenugreek dosage, okra dosage and rapid mixing speed. Experiments were conducted using the Jar Test method while the design and optimization were performed by applying response surface methodology (RSM) using I-Optimality (Custom) design. The optimum operating parameters were pH 3.2, 4.1 g/L fenugreek dosage, 58 ml okra/500 ml POME and rapid mixing speed of 197 rpm. After the repeated experiment, the percentage error between actual vs predicted response of TSS, TUR and COD removal efficiencies were 6.82 %, 2.66 % and 27.48 %, respectively. The low percentage error for TSS and TUR removal showed reliable results while the large deviation of the COD removal efficiency required further studies. It could be due to the presence of the unwanted model term of the relationship between okra dosage and rapid mixing speed (B-D) causing an error in calculating the COD removal efficiency. However, it is notable that fenugreek-okra was still able to achieve 92.70 %, 94.97 % and 63.11 % removal efficiency for TSS, TUR and COD despite the deviation. Therefore, fenugreek-okra have the potential to become bio-coagulant-flocculant for POME treatment.

Declaration of Conflict of Interest

The authors declared that there is no conflict of interest with any other party on the publication of the current work.

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