Spray Dried Powder Biocoagulant From *Jatropha Curcas* Liquid Extract And Its Preliminary Assessment In Turbidity Removal

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This research focuses on converting liquid *J.curcas* extract into a powder coagulant using spray drying to improve the shelf life. The inlet temperature ($120-180^{\circ}C$), feed concentration ($20-50\%$) and pump flow rate ($600-4600$ ml/h) of drying was investigated and Response Surface Methodology was used for optimization, with powder yield, moisture, and protein content as responses. Spray drying optimum conditions were $180^{\circ}C$, 50% of feed concentration and 600 ml/h pump flow rate, to give a powder yield of 5.69% with 4.22% and 78.8mg/l of moisture and protein content respectively. Fourier transform infrared spectroscopy analysis revealed presence of amino and carboxyl groups in the powder. Agglomerated powder with particle size between 5.62 to 7 μm were seen with presence of carbon, oxygen and nitrogen through FESEM-EDX, which were associated with existing protein in coagulant agent in powder. The spray-dried *J.curcas* press cake liquid extract powder performance was evaluated in the coagulation process, showing optimal performance at pH 3 with the dose used of 200 mg/l. Comparison of *J. curcas* press cake liquid extract coagulant with conventional alum resulted in a comparable turbidity removal of 99.47% and 99.33% respectively. The findings proved spray-dried powder effectiveness as coagulant which marked a significant steppingstone for a viable industrial application.

Keywords: Biocoagulant, Jatropha, spray drying, coagulation, water treatment

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1. Introduction

Jatropha curcas (JC) seeds have been widely used as feedstock for the biodiesel industry. Apart from biodiesel production, this process also produces abundance of by-products, mainly seed cakes which still contain nutrients, minerals [1] and protein, a biopolymer that is proven to assist the coagulation process [2].

Previous studies showed successful lab scale application of natural coagulants including JC in water and wastewater treatment but still limited at industry level. Here, the bioactive compound (mainly consisted of protein) was extracted from JC seed and press cake (after oil removal) through mechanical blending [2]. Removing oil is necessary because oil is hydrophobic, which can coat the active compounds, reduce coagulation performance, and cause odor and cloudiness in the treated water. However, being a natural bioresource, the active coagulant components (proteins and polysaccharides) are prone to deterioration, since they biodegrade over time due to moisture and external

environmental conditions during processing (extraction and storage) [3–5]. This negatively impacts the effectiveness of the biocoagulant during the treatment process, thus necessitating a solution for prolonging its shelf life by preserving its quality. In addition, the integrity (performance) and logistical (transportation and storage) issues will significantly influence the natural coagulants' ability to be commercialized [6].

Essentially, food drying techniques had been used a long time ago in traditional approaches by sun-drying or via the blowing wind. Nowadays, common methods used to produce foods are sun drying, spray drying, drum dryer, microwave drying, freeze drying, tunnel dryer and fluidized bed drying [7]. Previous research utilized freeze drying and spray drying process in producing *M. Oleifera* in powder form for the purpose to preserve the active components in the *M. Oleifera* [8–10]. Mohammad et al. [9] focused on the effects of spray drying, storage and packaging conditions. Experiments compared *M. Oleifera* extracts prepared using distilled water, NaCl solution, and spray drying. Spray-dried extracts, produced at 115°C, showed turbidity removal rates of 90− 95.3%, requiring 50% less dosage than non-spray-dried samples. Storage at room or refrigerated temperatures, and different packaging methods, did not significantly impact coagulation performance. However, their work did not optimize the drying process, while the powder produced was not analyzed. This information is important in scaling up and also elucidating quality aspects of the coagulant for subsequent use. Therefore, this research goal aims to fill the gaps by producing powder form of JC press cake liquid extract by spray drying optimization and assess the powder properties for turbidity removal.

2. Materials and methods

2.1. Oil extraction from JC seeds

The JC seeds were obtained from Africa via BATC Development Bhd. The husk of the seeds was crushed and removed manually. The high-quality seeds were selected and ground to fine powder ($63 - 500\ \mu$m) using a food processor (Model MX-GM1011H, Panasonic). Initially, oil was removed from the seed using 96% purity hexane from R&M Chemicals (ratio seed to solvent 1:6) in Soxhlet extractor at around 65°C. After extraction, the oil and the hexane were separated from the solid residues which was known as the press cake [11] which was used in subsequent process.

2.2. Preparation of the JC press cake liquid extract as a coagulant

15 g of JC press cake was blended (Panasonic Blender, MX-GM1011) with 75 ml of distilled water for 2 min to make feed concentration of 20% [8]. The liquid mixture was then filtered using muslin cloth and the filtrate was further spray dried to produce coagulant powder. These processes were repeated for preparing filtrate with different ratio of JC press cake to water, depending on the various concentration applied in the experiment $(20 - 65\%\ (\mathrm{w/v})$. The weight of the filtered solution was recorded for the calculation of powder yield.

2.3. Spray drying process

The filtered solution was spray dried using spray dryer (Model SD-05, LabPlant). The obtained powder was collected from cyclone, kept in a sealed plastic bag and stored in a chiller at 4°C until further analysis [8, 12].

2.4. Optimization of spray drying process

The experiments were divided into two parts. The first part was the preliminary screening study, followed by further optimisation using Response surface methodology (RSM) via central composite design (CCD) and Design Expert version 8.0.6.1 as the software. The parameters involved were inlet air temperature ($120 - 180$°C), feed concentrations (20 to 50%) and pump flow rate (20 to 50 corresponded to 600 to 4600 ml/h).

18 runs defined by face-centred central composite design (F-CCD) based on 3 independent variables (factor). Each factor varied with three levels and the results were analyzed using nonlinear regression to fit the second order equation. Four replication and central points were selected to determine the lack of fit for the experimental error evaluation [13]. The three responses used are powder yield, moisture content and protein towards the 3 factors.

2.4.1. Statistical analysis

The responses obtained based on experimental work were used to create an empirical regression model by correlating the response to the investigated factors through the second-order polynomial model shown in this equation:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k-1} \beta_{ij} x_i x_j \qquad (1)$$

Where y is the response variable, either powder yield (%), moisture content (%) or protein content $(\mathrm{mg/l})$, $\beta_0, \beta_i, \beta_{ii}$, and β_{ij} are the regression coefficient for the intercept, linear, quadratic and interaction respectively, x_i and x_j are the

independent variables (factors) that affect the response values and k is the number of studied variables. The positive and negative sign for each term indicates the increasing or decreasing effects of that term towards the powder yield (the response) [14].

The importance of all factors including individual factor and the interaction were analyzed by using analysis of variance (ANOVA). In ANOVA, a few parameters such as standard deviation, adjusted R^2, and predicted R^2 were used to validate the model. The performance of the fitted model was determined via residual analysis, with a significant level[14].

2.5. Zeta potential of JC press cake liquid extract

For zeta potential measurement, 1g of JC press cake was blended with 100 ml of distilled water. The JC press cake liquid extract was divided into 12 centrifuge tubes. The pH of JC liquid extracts for all the tubes were adjusted from pH 1 to pH 12 and labelled accordingly. The zeta potential of the liquid extract was measured using Zetasizer, Model ZEN3600 Malvern Instruments.

2.6. Analysis of the properties of powder

The response of the drying process with different processing parameters was based on powder yield, moisture content, and protein content. While the powder produced from the optimum spray drying process were subjected for further characterization of functional groups, surface morphology, particle size and elemental composition.

2.6.1. *Powder yield*

The powder yield was measured based on the obtained dried powder (g) recovered from spray drying process to the mass of the JC press cake extract solution (g) as in below Eq. (2) [15]:

$$\text{Powder yield } (\%) = \frac{obtained\ dried\ powder\ (g)}{extract\ solution\ (g)} \times 100\% \tag{2}$$

2.6.2. *Moisture content*

The moisture content was measured using a moisture analyser (Moisture analyser XM 50) which consists of a weighing and heating unit. The powder was spread on the heating plate around 0.1 to 0.3 g . The temperature was set at 100°C and the reading from the digital screen was recorded when it became constant.

2.6.3. *Protein content*

The protein content in the powder produced was measured using Bradford method. Bradford is a dye-based assay used to estimate protein concentration that works by binding the Coomassie Blue G250 dye to proteins. The Bradford reagent was prepared by dissolving the 200 mg of Coomassie Blue G250 dye (Merck) in 50 ml of 95% ethanol (Merck). Then, the solution was mixed with 100 ml of concentrated (85%) phosphoric acid (Merck). The final volume of the solution was kept at 1000 ml by adding distilled water. The reagent was filtered using Whatman No. 1 filter paper and the filtrate was transferred into an amber-coloured bottle and stored at room temperature.

A 100 μl sample was added into the test tube with 5 ml of Bradford reagent. The solution was mixed homogeneously using a vortex mixture (SciLab) The solution was measured using a UV Spectrophotometer (Model HALO RB-10-5110026) at wavelength 595 nm . The calibration graph was plotted using the ovalbumin as the standard protein. The standard protein was measured to be 5 mg and dissolved in 5 ml of distilled water (1 mg/ml of concentration) and stored at -20°C.

2.6.4. *Functional groups*

Fourier Transform Infrared (FTIR) spectrophotometer (Perkin Elmer Spectrum, 100 FT-IR Spectrometer) was used to characterise the functional group of the powder. The spectra were obtained at wavelengths ranging from 4000 cm^{-1} to 650 cm^{-1}.

2.6.5. *Surface morphology and particle size*

Field emission scanning electron microscope (FESEM) (NOVA NanoSEM 230) connected to Energy dispersive X-ray (EDX) was used to study the powdered JC structure and particle size at 15.0 kV of accelerated voltage. The sample was coated with gold before being analyzed.

2.6.6. *Elemental analysis*

The elemental analysis of powdered JC was examined at 30.0 kV accelerating voltage by using the same instrument as FESEM through EDX method by using area method (5 μm^2).

2.7. Coagulation process using powder coagulant

JC press cake liquid extract powder produced from the optimised spray drying process was evaluated for coagulation performance. Synthetic kaolin wastewater served as the model water to determine the best parameters for the coagulation process including powder coagulant dosage, pH and initial concentration of the wastewater. The rapid mixing was set at 100 rpm for 4 min, the slow mixing was at 40 rpm for 25 min and the settling time was 30 min. Filtering using muslin cloth ensured clear collection of samples without sediment [2].

2.7.1. Preparation of kaolin wastewater

Synthetic wastewater was prepared using stock kaolin suspension and tap water. To create the stock kaolin suspension, 10 g of kaolin (R&M Chemicals) was added to 1 L of distilled water. The mixture was slowly homogenized at 20 rpm for 1 h using a jar test. Prior to the jar test experiment, wastewater samples were prepared in separate beakers with 500 ml of volume each, adjusting the initial turbidity to specified values. The initial pH of the wastewater was adjusted by using 1M NaOH and 1M H_2SO_4 [2]. One factor at a time (OFAT) method was adopted to investigate the effect of various parameters to the coagulation process.

2.7.2. Effect of pH

The pH was varied between pH 3 to 8 to determine the effect of pH to the performance of JC press cake liquid extract powdered coagulant. The coagulant dosage and initial turbidity were fixed at 0.5 g and 2500 NTU respectively [2]. Other than measuring the turbidity as the results, the pH after the treatment was also monitored to see the effect of adjusted pH.

2.7.3. Effect of coagulant dosage

The best pH of the synthetic wastewater from the previous experiment was used in this experiment to determine the most suitable coagulant dosage in treating the kaolin water. The coagulant dosage varied between 0.1 to 1.0 g with an interval of 0.2 g and the initial turbidity was fixed at 2500 NTU.

2.7.4. Effect of initial turbidity

The best pH and coagulant dosage from the previous experiment was used in this experiment to find the influence of initial turbidity of the wastewater to the performance of the coagulation process. The initial turbidity of the wastewater varied at 100, 500, 1000, 2000 and 2500 NTU.

2.7.5. Comparison with aluminium sulphate

The performance of the JC press cake liquid extract powder was compared with the aluminium sulphate (R&M Chemicals). Aluminium sulphate was prepared by mixing 1 g of aluminium sulphate into 100 ml of distilled water (1% of concentration). The experiment was conducted at 120 mg/l of dosage, 2500 NTU of initial turbidity, and pH 5 [2]. At pH 5 , the flocs formed were able to re-grow even after the flocs were broken without any addition of coagulant dosage [16]. Meanwhile for the JC press cake liquid extract powder, the condition of the experiment was fixed based on the best pH , coagulant dosage and initial turbidity of the previous steps.

2.7.6. Sedimentation time and sludge volume

Sedimentation time and sludge volume were also measured. After rapid and slow mixing was completed, the treated wastewater was poured into a 1 L measuring cylinder. The sedimentation time was recorded until most of the flocs settled at the bottom. The sludge volume can be quantified based on the wet sludge that is settled at the bottom after 30 min of settling. The difference between initial volume and settled volume were determined to find the sludge volume [17].

2.7.7. Turbidity measurement

The equipment used to measure the turbidity in this experiment was Hach Turbidimeter Model 2100 N. The percentage of turbidity removal was calculated as Eq. (3) [18]:

$$\% \text{ of removal } = \frac{Initial\ turbidity - Final\ turbidity}{Initial\ turbidity} \times 100\% \tag{3}$$

3. Results and discussion

3.1. Optimization of spray drying parameters

The analysis of powder yield from the RSM study on spray drying of JC press cake liquid extracts is summarized in Table 1. The quadratic model emerged as the best fit for the experimental data, chosen based on the p-values from F statistics. A p-value of less than 0.0001 indicates significant statistical relevance for powder yield, validating the model's capacity to elucidate observed variations. Key statistical metrics for the model are as follows: The R^2 value of 0.9619 (Coefficient of determination) indicates that 96.19% of the variability in powder yield is explained by the model. The Adjusted R^2 of 0.9191 , adjusting for the number of terms in the model, remains high, indicating robustness in explaining the relationship between variables and responses. The Predicted R^2 of 0.7686 demonstrates satisfactory predictive capability for new observations.

Significant factors influencing powder yield include the feed flow rate (C), deemed most significant (p-value < 0.0001), indicating its strong impact. Both inlet temperature (A) and feed concentration (B) are also significant (p-value < 0.01), highlighting their considerable influence. The quadratic term for feed flow rate (C^2) is likewise significant (p -value < 0.01), suggesting a non-linear effect. Factors with moderate influence include interaction terms like AC (inlet temperature and feed flow rate) and BC (feed concentration and feed flow rate), with p-values between 0.01 and 0.05 , indicating their combined effects. Factors considered insignificant are AB (interaction of inlet temperature and feed concentration), A^2 (quadratic term for

inlet temperature), and B^2 (quadratic term for feed concentration), with p -values > 0.05. Lack of Fit (LOF) analysis yielded an insignificant F -value of 0.63 , suggesting the model adequately fits the data, with any unexplained variation likely attributable to random error rather than systematic deficiencies.

The quadratic model for the powder yield is shown in Eq. (4):

$$\begin{aligned} \textit{Powder Yield} &= 1.43 + 0.56A + 0.50B - 1.60C + 0.15AB \\ &- 0.45AC - 0.47BC - 0.16A^2 - 0.31B^2 + 0.88C^2 \end{aligned} \tag{4}$$

The Eq. (4) involved all terms including linear, interactions and quadratic terms. In this condition, the linear terms expressed as an increase of inlet temperature (A) and feed concentration (B) and decreasing the value for the feed flow rate (C) will increase the powder yield. The quadratic terms show negative values for inlet temperature and feed concentration indicate further increase of inlet temperature and feed concentration will cause the powder yield to decrease while positive value for pump flow rate indicate exponential effect showing smaller value of pump flow rate will cause the powder yield to increase slightly. The negative notations on the two-factor interaction terms which are AC and BC represented the antagonistic effects of the inlet temperature and feed flow rate as well as the particle feed concentration and feed flow rate on the powder yield. Then, the positive notation on AB suggested that the combination of inlet temperature and feed concentration would positively affect the powder yield.

Fig. 1(a) presents both two-dimensional (2D) contour plots and three-dimensional (3D) response surface plots that illustrate the interaction between inlet temperature (A) and feed concentration (B) on powder yield, based on Eq. (4). The blue colour is referred to as the minimum response zone, green is the intermediate zone while the red shows a high response zone. The contour plot depicts how powder yield varies as both factors change, while keeping the pump flow rate (C) at a constant middle-level value. At higher levels of inlet temperature (up to 180°C) and feed concentration (up to 50%), the surface plot shows an increase in powder yield. This relationship aligns with expectations: higher feed concentration increases the availability of solids relative to water, which enhances powder production by reducing evaporation rates during spray drying. Increasing the inlet temperature also contributes to higher powder yield, as more powder can be collected with less adhering to chamber walls due to increased drying efficiency [19, 20]. However, it is important to note that the influence of inlet temperature on powder yield is contingent upon other factors, such as pump flow rate, which also affects drying kinetics and powder formation.

Fig. 1(b) illustrates the interaction effect between inlet temperature (A) and pump flow rate (C) on powder yield, as demonstrated through both the two-dimensional (2D) contour plot and the three-dimensional (3D) response surface plot. The statistical significance of this interaction is indicated by a p-value of less than 0.05 . In the 2D surface plot, it is observed that the powder yield reaches its maximum when the inlet temperature is set at the middle-level of 150°C, and the pump flow rate is maintained at the low-level of 600 ml/h, with the feed concentration held constant at 50%. This configuration suggests that decreasing the pump flow rate enhances the contact time between the feed and the drying air, thereby improving the efficiency of heat transfer during the spray drying process[20]. It is important to note that the influence of pump flow rate on powder yield is also influenced by other variables such as inlet temperature and feed concentration. These factors collectively influence the kinetics of drying and powder formation, underscoring the complexity of optimising spray drying conditions to achieve maximal powder yield from JC press cake liquid extracts.

Fig. 1(c) depicts the response surface plots as a function of feed concentration (B) and pump flow rate (C) at a fixed inlet temperature (A) of 150°C. The powder yield was maximised at the middlelevel (35%) of feed concentration (B) and low-level of pump flow rate (C) at 600 ml/h while the inlet temperature was maintained at 150°C.

Other than powder yield, the experiments also used moisture content and protein content as the responses. The moisture content was measured between 3.42 to 15.59% while the protein content was measured between 36.2 to 95.1 mg/l.

3.1.1. Optimization and model validation

The optimum operating condition to produce powder form using spray dryer was determined by Numerical optimisation model. The responses were quantified according to the combination of the levels of the factors based on the set-up goal as shown in Table 2.

The optimised conditions were determined by maximising powder yield and protein content while minimising the moisture content. During the optimisation, the inlet temperature, feed concentration and pump flow rate were kept within range. Based on the statistical analysis, the inlet temperature 180°C, feed concentration 50%, and pump flow rate 600 ml/h was found to be optimum with the desirability of 0.96. The desirability function indicates the appropriate approximation of the prediction point to acquire the desired response [21]. The verification experiment

Table 1. ANOVA for Quadratic Model (Response 1 - Powder Yield).

Source	Sum of Squares	df	Mean Square	F Value	p -value Prob > F	
Model	37.16	9	4.13	22.45	< 0.0001	significant
A-Inlet temperature	3.19	1	3.19	17.36	0.0031	
B-Feed concentration	2.52	1	2.52	13.70	0.0060	
C-Pump Flowrate	25.66	1	25.66	139.55	< 0.0001	
AB	0.17	1	0.17	0.93	0.3630	
AC	1.65	1	1.65	8.96	0.0173	
BC	1.74	1	1.74	9.46	0.0152	
A^2	0.070	1	0.070	0.38	0.5539	
B^2	0.25	1	0.25	1.38	0.2740	
C^2	2.12	1	2.12	11.52	0.0095	
Residual	1.47	8	0.18			
Lack of Fit	0.75	5	0.15	0.63	0.6987	not significant
Pure Error	0.72	3	0.24			
Cor Total	38.63	17				

$R^2 = 0.9619$; Adjusted $R^2 = 0.9191$; Predicted $R^2 = 0.7686$; Adequate Precision = 17.391; coefficient variation = 25.88%
* Values greater than 0.05 indicate the model terms are not significant

Table 2. ANOVA for Quadratic Model (Response 1 - Powder Yield).

Factor/ Response	Optimisation goal	Lower limit	Upper limit	Prediction point
Factors				
A	In range	120	180	180
B	In range	20	50	50
C Pump flow rate	In range	20	50	20
Response				
Powder yield (%)	Maximise	0.26	5.53	5.58
Moisture content (%)	Minimise	3.42	15.59	3.94
Protein content (mg/l)	Maximise	36.2	95.1	90.40

was carried out under the recommended optimum operation conditions to confirm the accuracy of the established regression model and the reliability of the optimum combination. The experiment has a powder yield of 5.69%, moisture content of 4.22% and protein content of 78.8 mg/l. This result is within the 95% prediction intervals; thus, the obtained regression model is acceptable.

The powder yield obtained from the optimised experiment (5.69%) is somewhat comparable to other reported studies (8.25%) [15]. The nature of the samples also contributed to the amount of powder yield obtained. Being a bioresources, biodegradation is inevitable. For instance, denaturation can cause losses of protein and reduce the yield. This was clearly evidenced by the formation of sticky deposition on the chamber walls which in turn made powder collection difficult. According to Kingwatee et al. [22], the powder yield can be increased by adding carrier agents such as maltodextrin and gum arabic.

Reducing moisture content in the product able to increase the shelf-life and hence biodegradation. Moisture content should be maintained less than 10% to avoid microbial growth [23, 24]. The value of moisture content in this optimised finding is 4.22% which is below the acceptance range to ensure microbial growth is under controlled. Reddy et al. [25] was using spray drying to dry Osmanabadi goat milk and they found the best moisture content of the dried process is 4.08%. In addition, the powder form makes it easier for transporting and storing [26, 27].

In terms of protein content, the amount obtained in the powder was 78.8 mg/l, which is significantly low compared to the protein content in JC press cake extract in liquid form ($>$ 1000 mg/l) [28]. Furthermore, the extracted protein in liquid form was also found to be less than the initial protein in JC press cake solid which was reported to be half of press cake weight. Apparently, the final protein content in JC powder has reduced significantly from its initial value prior extraction from press cake and also drying process. Meaning that efficiency of processing can cause loss of valuable product. In terms of spray drying, an increase in inlet temperature more than 165°C can reduce the protein content, potentially due to protein denaturation [29]. Hence, it can be said that the inlet temperature of the spray drying is responsible for the substantial reduction when transforming the liquid coagulant to powder form.

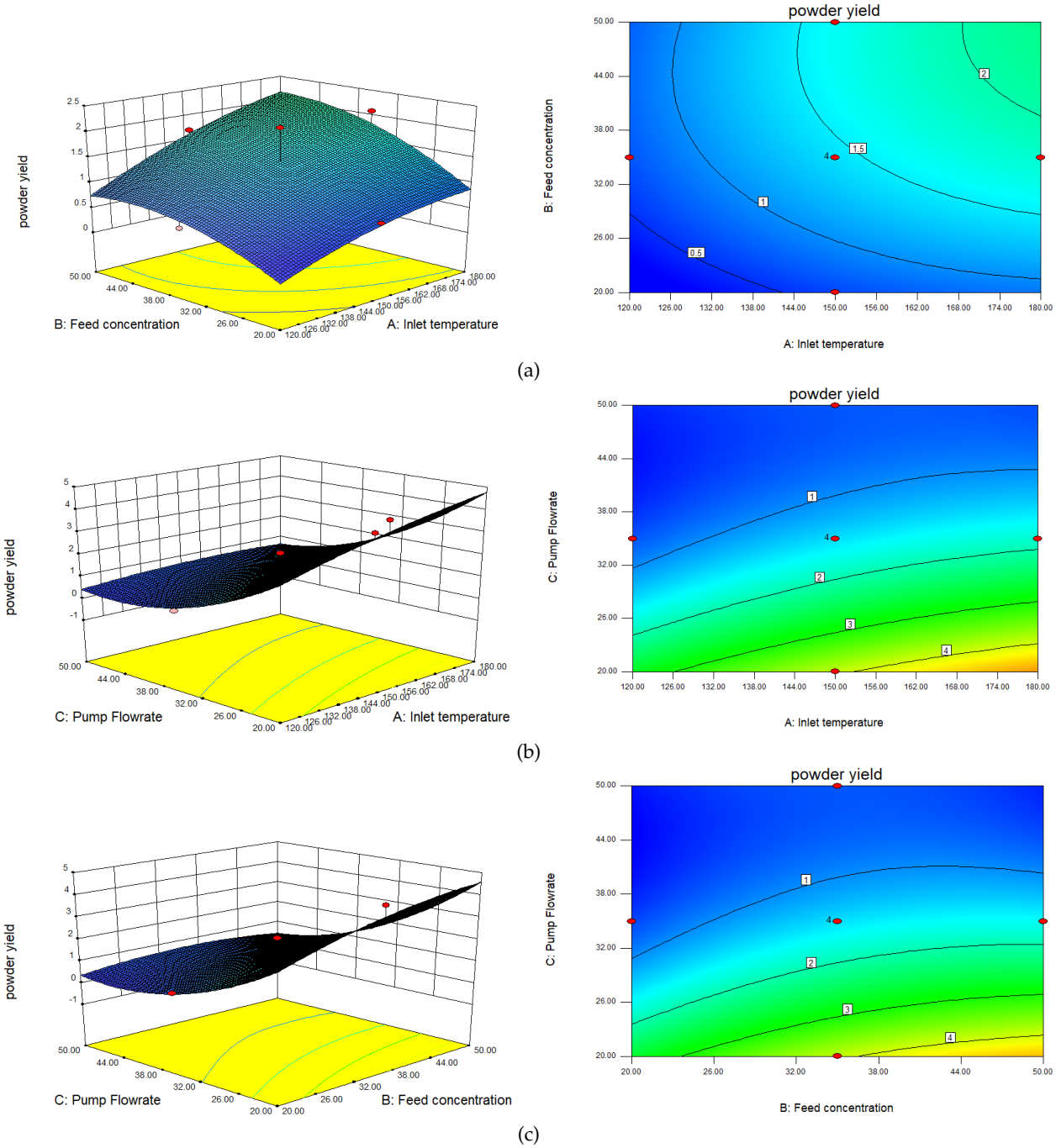


Fig. 1. Response surface plots of the interaction effects of spray dryer factors on powder yield: (a) Inlet temperature and feed concentration (AB), (b) Inlet temperature and pump flow rate (AC) and (c) Feed concentration and pump flow rate (BC).

3.2. JC press cake liquid extract powder characterization

The powder was further characterized by functional group, surface morphology, particle size and elemental composition. The functional group was measured using FTIR while for the surface morphology and elemental composition, Scanning Electron Microscope which is connected to EDX was utilised. The particle size was analyzed by ImageJ processing program.

3.2.1. Functional group

The FTIR analysis was conducted to analyse the functional group presented in the JC press cake liquid extract powder by infrared light. This analysis is important to recognize the unknown compounds, detecting impurities within a

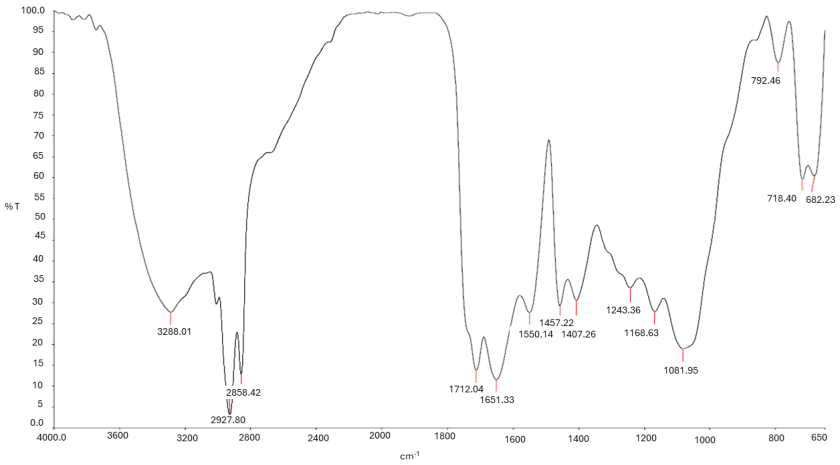


Fig. 2. FTIR spectrum of powder produced from JC press cake liquid extracts.

substance and determining the decomposition and oxidation [30]. Fig. 2 shows the FTIR spectra of JC powder produced by spray dryer. Several FTIR distinctive peaks that corresponded to specific functional groups and chemical bonds could be observed in the FTIR spectrum. Table 3 also includes the information that may be utilised to determine the presence of amino acids and other chemical constituents in the powder.

Typically, the basic components of an amino acid molecule are made up of a carboxyl group (COOH), an amino group ($-NH_2$), a hydrogen atom, and a substituent group (-R). On the other hand, majority of the functional groups in an amino acid molecule are the carboxyl and amino groups. Based on Fig. 2, $N-H, C=O, O-H$ and $C-H$ groups are often found in amino acid molecule has been identified in powdered JC press cake liquid extract powder where the wavelength was at 1651.33 cm^{-1}, 1712.04 cm^{-1}, 2927.80 cm^{-1} and 3288.01 cm^{-1}, and 2858.42 cm^{-1}, respectively.

These findings confirm the presence of amino acids in the press cake powder, with carboxyl groups comprising $C=O$ and $O-H$ bonding atoms, and the amino groups featuring $N-H$ groups. Research had been conducted earlier by Abidin et al. [28] shows that the JC press cake contains of alcohols, amines, amides, alkynes, nitriles, alkenes, and aromatics which confirm the same functional group also existed in the spray dried powder produced from *Jatropha* press cake [28].

Other than this, the powder also contained other groups such as $C-S, C-Cl, C-O-O-C, NO_2$ and $C-N$ groups. The $C-S$ group resulted in this powder probably due to cysteine and methionine being present as amino acids. Cysteine is classified as a thiol, whereas methionine is classified as a sulphide. The results justified that the JC press cake liquid extract powder is mostly composed of amino acids. The findings from this work conformed with [28, 31] who also reported the existence of cysteine and methionine among the 18 amino acids found in the coagulant agent liquid extracted from JC press cake liquid extracts.

3.2.2. *Surface morphology and particle size*

The Field Emission Scanning Electron Microscopy (FESEM) was used to observe the morphology of the JC press cake liquid extract powder across varying magnifications. FESEM provides topographical and elemental evidence at magnifications of 10 X to 300, 000X with almost unlimited depth of field [32]. To evaluate the samples in this research, the microstructure picture was captured using a NOVA NANOSEM 230. Fig. 3 shows the FESEM micrograph of JC press cake liquid extract powder at different magnification. The micrograph clearly reveals that the powder's particle morphology was mostly irregularly shaped, agglomerated, dents in the structure, and smooth on the surface. Similar findings were also reported by Fyfe et al. [33] and Dantas et al. [34]. The Milk Protein Concentrate (MPC) produced from laboratory scale dryer have large dents and smooth surface which is probably due to high protein concentration in the powder [33].

Similarly, Dantas et al. [34] describe particle agglomeration due to high levels of surface free fat or proteins, facilitated by water bridges between particles during drying. At that stage, the water droplets inside diffuse to the outer layer while other molecules diffuse in the opposite direction. Another reason that could be related to the agglomeration is because no coating materials such as maltodextrin and gum Arabic were used in preparing the feed

Table 3. The functional groups or chemical bonds in JC press cake liquid extract powder.

Wavenumber (cm^{-1})	Functional groups / Chemical bonds	Compounds
682.23	C-S stretching	Thiol
718.40	C – Cl stretching	Aliphatic chloro compound
792.46	C – Cl stretching	Aliphatic chloro compound
1081.95	C – O – O – C stretching	Peroxide
1168.63	C – N stretching	Secondary amine
1243.36	P – O – C stretching	Aromatic phosphate
1407.26	N = O bending	Nitro group
1457.22	N – H bending	Secondary amine
1550.14	NO2 asymmetric stretching	Aliphatic nitro compound
1651.33	N – H bending	Primary amine
1712.04	C = O stretching	Carboxylic acid
2858.42	C – H symmetric stretching	Methylene (CH2) group
2927.80	O – H stretching	Carboxylic acid
3288.01	O – H stretching	Hydroxy group

concentration. This in turn affects the size uniformity that resulted in the powder having a less scattered distribution. It can be seen clearly that few particles come together as a group to form a bigger particle. The addition of maltodextrin increases droplet viscosity, as highlighted by Dantas et al. [34], which could further influence particle formation and structure during drying processes.

There are other natural coagulants available in powder form, using SEM, but the structure is not able to compare due to different methods of preparation. Typically, these powders are derived directly from the respective plants. After pretreatment, the plants are heated and finely crushed to produce fine powder. The surface morphology differs significantly from that of JC press cake liquid extract powder which exhibit more fibrous textures and high level of porosity [35, 36]. Particle size has been analyzed by using FESEM micrograph image at the scale of 100 μm via imageJ, which showing the common particle size distribution is ranged between 5.6 to 7 μm.

3.2.3. *Elemental composition*

The elemental composition of this powder was identified by the Energy-Dispersive X-ray (EDX) method. The imaging capacity of the microscope is used to identify the specimens of particular interest when these systems are used in conjunction with electron microscopy instruments. The elemental composition present in the powder is carbon (C), oxygen (O), magnesium (Mg), phosphorus (P), potassium (K), sulphur (S), and chlorine (Cl). The element in JC extract powder and its composition in terms of weight, atomic and formula are shown in Table 4.

Based on Table 4, the S and Cl were very small and almost undetectable. The elemental composition results by weight percent demonstrates the presence of carbonyl, hydroxyl, amine and sulfonyl group.

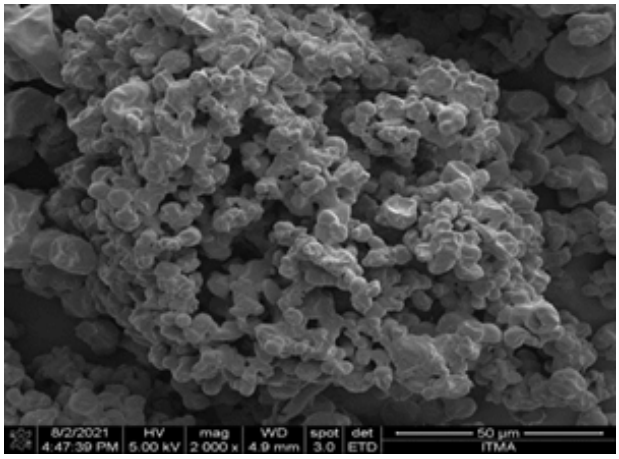
Table 4. Elemental composition of JC press cake liquid extract powder.

Element	Weight (%)	Atomic (%)	Formula
C K	43.46	52.71	$CaCO_3$
O K	47.25	43.02	SiO_2
Mg K	2.05	1.23	MgO
P K	2.97	1.40	GaP
S K	0.41	0.19	FeS_2
Cl K	0.48	0.20	KCl
K K	3.38	1.26	
Total	100.0	100.0	

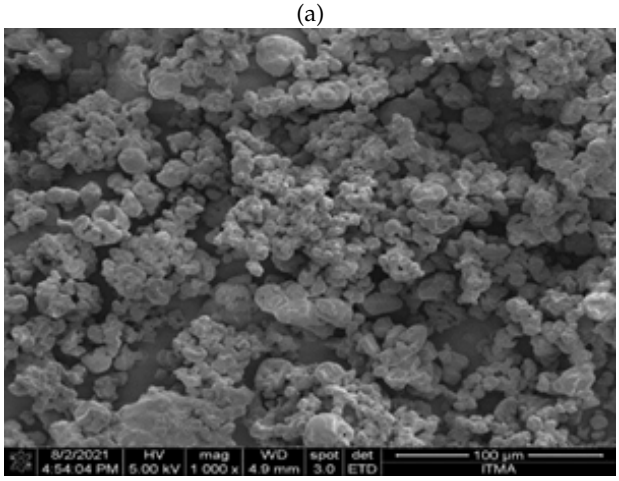
Murrieta-Pazos et al. [37] conducted a study using energy dispersive X-ray microanalysis to characterize the surface composition of milk powder particles (mostly composed of milk protein). They have identified that the powder consisted of elements such as carbon, oxygen, nitrogen, potassium, calcium, chlorine, sulphur, phosphorus, sodium and magnesium. Calcium and potassium appeared after removal of fat while magnesium and sodium corresponded to the abundance of each element in milk [37]. Their results can also be linked to our powder of JC press cake liquid extract since it is composed of protein and fats, with the fats being removed during the Soxhlet extraction. At the same time, the presence of carbon element may also help in the process of sorption, as carbon serves as a binder to create stable flocs [35, 38].

3.3. Application of powder in kaolin wastewater

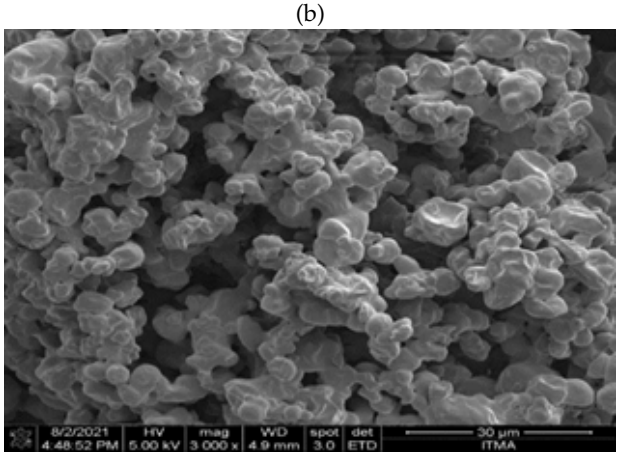
The optimized powder produced from JC press cake liquid extract using spray dryer was further applied in the coagulation process as the natural biocoagulant. The synthetic kaolin wastewater was used to study the effect of pH, coagulant dosage and initial turbidity to the efficiency of the treatment. The performance of the JC press cake liquid ex-



(a)



(b)



(c)

Fig. 3. FESEM micrograph of JC press cake liquid extract powder at the magnification of (a) 1000 X, (b) 2000 X (c) 3000 X.

tract powder was compared with conventional aluminium sulphate.

3.3.1. Effect of pH

The pH level ranged between pH 3 to pH 8 . This range was selected based on Abidin et al. [2] indicating optimal performance at acidic pH levels. 1000 mg/l of JC press cake liquid extract powder was applied to treat an initial turbidity of 2500 NTU. Fig. 4 shows the percentage of turbidity removal at different pH values. It was found that the maximum turbidity removal occurred at pH 3 with 97.8% of removal, then followed by pH 4 at 30.8% of removal. Other results were shown below than 30% of removal. Deductively, larger flocs formation occurs at pH 3 compared to small and fewer flocs were seen at other pH.

Based on the characterization conducted to the liquid extracted (which was the feed to spray dryer) from JC press cake, the isoelectric point is between pH 2 and pH 3 which justified the results of this experiment. The isoelectric point is where a molecule or surface carries no net electrical charge, with amino acids becoming positively charged below this pH. Therefore, below pH 3, the JC protein possesses a net positive charge or exists as cationic. This enables them to interact with the anionic suspended particulates of kaolin in the solutions for coagulation to occur [28]. This finding also aligned with Abidin et al. [2] who reported the best pH in treating kaolin wastewater was at pH 3. In this paper, the author used liquid extract from JC seeds and press cake. Other researchers also reported the best pH for JC seeds and press cake to treat variation of wastewater such as palm oil mill effluent and pharmaceutical wastewater was between pH 2 and pH 3 [28, 39].

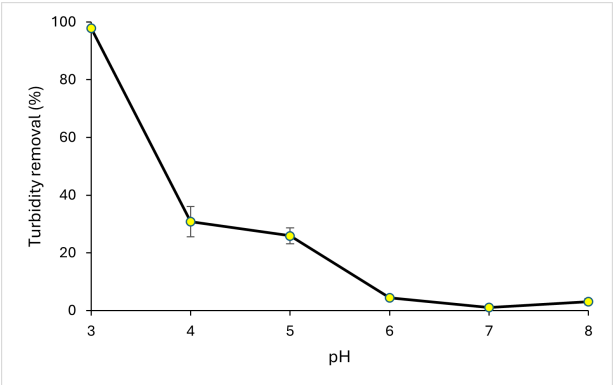


Fig. 4. Percentage of turbidity removal in synthetic kaolin wastewater against pH (bars represent standard error of the mean, n=3).

3.3.2. Effect of coagulant dosage

In this experiment, the coagulant dosage of JC press cake liquid extract powder was varied from 200 to 2000 mg/L

at intervals of 400 mg/L, while the pH was maintained at 3 based on prior optimization, and the initial turbidity of the kaolin suspension was fixed at 2500 NTU. As illustrated in Fig. 5, the highest turbidity removal efficiency (99.3%) was achieved at a dosage of 200 mg/L, indicating this to be the optimal dosage under the given conditions.

However, as the coagulant dosage increased beyond 200 mg/L, a gradual decline in turbidity removal efficiency was observed. This reduction is likely attributed to the phenomenon of charge reversal or restabilization. At optimal dosage, the coagulant effectively neutralizes the negative surface charge of suspended kaolin particles, promoting flocculation and sedimentation. When the dosage exceeds the optimal threshold, the excess cationic compounds from the JC coagulant may oversaturate the particle surface charge, resulting in a net positive charge. This leads to restabilization of the particles due to electrostatic repulsion, thereby inhibiting aggregation and reducing sedimentation efficiency [16].

Visual observation supported these findings, where complete floc formation and settling were evident at dosages of 200 mg/L and 600 mg/L. In contrast, at the highest tested dosage of 2000 mg/L, significant residual turbidity and poor floc settling were observed, further confirming the detrimental effects of over-dosing on coagulation performance.

In a previous study, 120 mg/l was recorded as the optimum dosage to treat kaolin wastewater and domestic sewage using liquid extract from JC seeds and press cake [2, 40]. This indicates that the performance of powdered coagulant is comparable to that of liquid extract, with the additional advantage of enhanced long-term preservation of quality.

Comparatively, the coagulation performance aligns with a study conducted by Mohammad et al. [9] that used *M. Oleifera* powder produced from a spray dryer. They achieved 91.05% turbidity removal with a dosage of 16 mg/l and initial turbidity of approximately 200 NTU in kaolin wastewater. The liquid extract from *M. Oleifera* seeds was prepared by using NaCl [9]. The final pH after the sedimentation process was recorded to be around pH 3 to 5 which did not alter much compared to the initial kaolin wastewater pH. This is probably due to the balancing of hydrogen ions in powder with kaolin wastewater [41]. It can be observed that the higher the coagulant dosage, the higher the final pH.

3.3.3. Effect of initial turbidity

The initial turbidity levels of the synthetic kaolin wastewater were adjusted between 100, 500, 1000, 2000 and 2500 NTU, while maintaining a fixed pH 3 and a coagulant dosage of 200 mg/l. Fig. 6 indicates the percentage removal of turbidity against the initial turbidity of the synthetic kaolin wastewater. The percentage of turbidity removal increased when the initial turbidity increased from 100 NTU to 500 NTU and gradually plateau until 2500 NTU. It shows here that the JC press cake liquid extract powder is much better in treating wastewater that contains high turbidity.

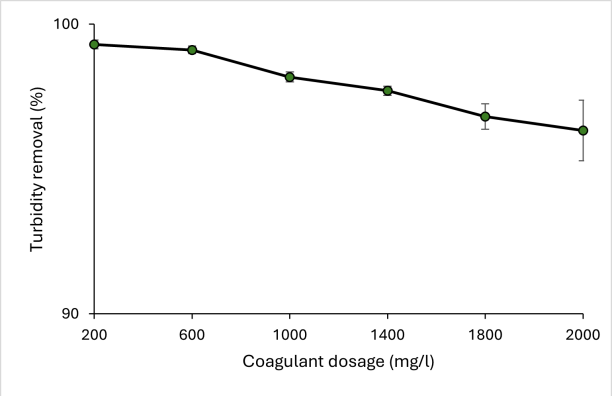


Fig. 5. Percentage of turbidity removal in synthetic kaolin wastewater against coagulant dosage (bars represent standard error of the mean, n=3).

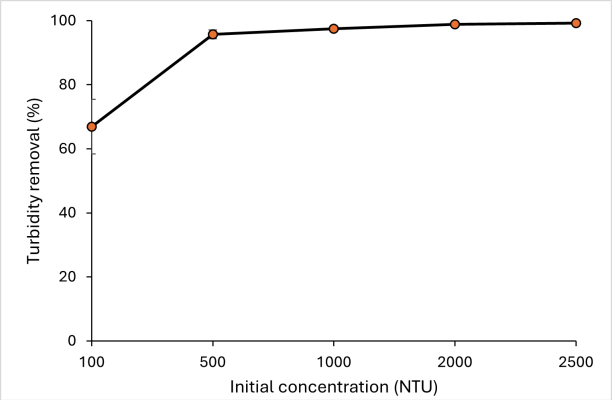


Fig. 6. Percentage of turbidity removal against initial turbidity of synthetic kaolin wastewater (bars represent standard error of the mean, n=3).

In a previous study by Abidin et al. [2], it was observed that the highest percentage removal of turbidity reached 99.5% of turbidity removal at the concentration of 500 NTU. It was also found that a slight decrease had occurred at concentrations above 500 NTU, but all values remained above 98%. This highlights the effectiveness and suitability of JC coagulant to treat different ranges of initial turbidity for water and industrial wastewater [2]. In another study using *M. Oleifera* was conducted by varying initial turbidity in the range of 100 to 500 NTU. That study showed the

highest turbidity removal was achieved at initial turbidity of 500 NTU with the percentage of turbidity removal of 96.5% [42].

3.3.4. *Comparison with aluminium sulphate in kaolin wastewater*

The coagulation capability of the powder produced was also compared with the selected conventional coagulant that is aluminium sulphate. Both coagulants were set up at the best values of the parameters accordingly.

Based on the percentage of the turbidity removal, the performance of the JC press cake liquid extract powder was 99.47% of turbidity removal which is comparable with aluminium sulphate (99.33% of turbidity removal) in treating kaolin wastewater. After treatment, the final pH of the kaolin wastewater remained at pH 3 for the JC press cake liquid extract powder and reduced to pH4 for aluminium sulphate.

In terms of sedimentation time and sludge volume, the study found that suspended kaolin particulates settled within 30 minutes, producing a sludge volume of 80 mL/L when treated with JC press cake liquid extract powder. These results are aligned with the findings from Abidin et al. [28] for the sedimentation time of JC extract from seeds and press cake, which also achieved complete settling within 30 minutes. In contrast, aluminium sulphate required 40 minutes for sedimentation and produces 100 mL/L of sludge volume. A shorter sedimentation time is preferred in industry to reduce production costs [28].

Moreover, the sludge volume produced by JC was lower compared to aluminium sulphate. This can reduce the sludge handling, dewatering and final disposal cost. Since JC was derived from a plant, the by-products are organic and biodegradable and hence offer a more environmentally friendly alternative such as composting or land application compared with other chemicals counterparts [2]. Sludge produced from chemical coagulants often requires stricter disposal protocols due to its inorganic content, which increases overall treatment costs. This result is also supported by Bhatia et al. [43] where use of chemical coagulants such as aluminium sulphate and polyaluminium chloride (PAC) are not appropriate choices in treating wastewater due to high concentration of aluminium in wastewater that may cause bad effects to human health and produce a large volume of sludge.

Table 5 presents a comparative cost analysis between aluminium sulphate and JC powder as coagulants for wastewater treatment. For a treatment 1000 litres, the required dosages are 120 mg/L for aluminium sulphate and 200 mg/L for the JC-based coagulant, corresponding to 120 g and 200 g of material, respectively. Based on the estimated market price per kilogram, aluminium sulphate is substantially more economical, with a treatment cost ranging from 0.0396 to 0.066 USD per 1000 L. In contrast, the JC powder incurs a higher treatment cost, between 0.22 and 0.44 USD per 1000 L, due primarily to the need for additional processing steps such as oil extraction and drying.

However, from a sustainability and life-cycle perspective, the JC coagulant offers several advantages. It generates lower amounts of sludge, which is also biodegradable, reducing posttreatment handling and disposal costs. Furthermore, its application avoids the release of residual aluminium $\left(Al^{3+}\right)$, which is associated with potential environmental and health concerns. Instead, the JC-based coagulant presents a biodegradable and eco-friendly alternative, aligning with global trends toward greener and circular economy practices.

From an industrial standpoint, although the initial cost of JC powder is higher, its lower environmental footprint, potential for valorization of agricultural waste (e.g., Jatropha cake), and compatibility with sustainable water treatment strategies may justify its adoption, particularly in regions prioritizing ecological compliance or pursuing green certifications. Based on experimental evaluations involving variations in pH , coagulant dosage, and a comparison with aluminium sulphate, the performance of the JC press cake liquid extract in powder form was found to be comparable to its liquid counterpart.

In addition to wastewater treatment, the JC press cake liquid extract in powder form was also evaluated for its effectiveness in treating lake water. The initial turbidity and pH of the lake water were recorded as 48 NTU and pH 5 , respectively. Two experimental setups were designed: the first employed JC press cake powder at a dosage of 200 mg/L with the pH adjusted to 3 using 1 M sulphuric acid, while the second used aluminium sulphate at a dosage of 120 mg/L without pH adjustment, as its optimal coagulation pH is naturally aligned with the lake water's initial pH of 5. Following treatment, aluminium sulphate achieved a turbidity removal efficiency of 96.74%, while JC press cake powder achieved 64.58%. The final pH of the treated water in both cases was pH 4, indicating the necessity for post-treatment pH adjustment before discharge, in order to comply with environmental regulations that require a final effluent pH between 6 and 9.

These results suggest that JC press cake powder demonstrates promising coagulation performance for moderately turbid water, though it is somewhat less efficient than aluminium sulphate under the tested conditions. Notably, natural coagulants such as *Moringa oleifera* have shown

Table 5. The functional groups or chemical bonds in JC press cake liquid extract powder.

Item	Aluminium sulphate	JC press cake liquid extract in powder form
Effective dosage (mg/L)	120	200
Coagulant required (g/1000 L)	120	200
Market price per kg (estimate)	0.33 to 0.55	1.10 to 2.20 (raw seed/ unprocessed)
Cost per 1000 L (USD)	0.0396-0.066	0.22-0.44
Additional processing needed	No	Yes (oil extraction/ drying)
Sludge production	Moderate	Lower (biodegradable)
Environmental Impact	Al^{3+} residue	Biodegradable, eco-friendly

higher turbidity removal efficiencies (approximately 60%) primarily in water with initial turbidity levels exceeding 100 NTU. However, their performance tends to decline in low-turbidity waters due to limited flocculation efficiency [44]. This trend is consistent with the observed performance of JC press cake powder in this study, highlighting the importance of matching natural coagulant application to appropriate water quality conditions.

In overall, extracting the liquid coagulant from press cake solids can significantly enhance the performance and functional properties of the resulting natural coagulant based on the presented results. This process enables the enrichment of active compounds in this case proteins, that is primarily responsible for the coagulation effect through mechanisms like charge neutralization and particle bridging. By isolating these functional constituents, the extract becomes more effective per unit mass compared to raw press cake. Furthermore, extraction helps eliminate inert or interfering materials, including insoluble fibers, fats, and other non-coagulating substances, which can otherwise dilute the activity or increase sludge production [28]. The resulting extract exhibits improved solubility and dispersion in water, leading to better interaction with suspended particles during treatment. Additionally, the liquid extract can be concentrated or dried into a more stable powder form, enhancing its shelf life, storage, and handling properties while reducing susceptibility to microbial degradation [9]. This also allows for more consistent and controlled dosing, which improves treatment reproducibility and reduces the risk of overdosing that may lead to charge reversal and particle restabilization [16]. Moreover, the extraction process can be tailored to selectively enhance binding affinity or target specific contaminants, thereby offering opportunities to further optimize the natural coagulant's functionality for various water treatment applications.

4. Conclusion

Powder form of Jatropha press cake liquid extracts was successfully produced using spray drying. The optimized spray drying conditions were at 180°C inlet temperature, 50% feed concentration, and 600 ml/h pump flow rate to yield a powder with 5.69% yield, 4.22% moisture content, and a protein content of 78.8 mg/L. Functional group analysis, FESEM, and EDX, confirmed the presence of amino and carboxyl groups, indicative of amino acids, along with carbon, oxygen, and nitrogen, which are associated with protein content. Irregular, agglomerated morphology with smooth surfaces, consisting of particle sizes ranging from 5.62 to 7 μm was revealed. Coagulation of kaolin solution with the powder was effective under acidic conditions of around pH 3 with dosage 200 mg/L, and an initial turbidity of 2500 NTU. Percentage turbidity removal of powder coagulant was comparable with alum ($>$ 98%) while a shorter sedimentation time of 30 mins was recorded. pH of the kaolin solution after treatment increased to 4, indicating effective coagulation and partial neutralization of the solution. These findings highlight superiority of powder form to its liquid extract counterparts, offering a promising approach to utilize agricultural by-products in environmental applications.

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Author contributions

Conceptualization, N.I., Z.Z.A. and F.S.T; methodology, N.I., Z.Z.A. and F.S.T; software, N.I., Z.Z.A, and N.A.M.F; validation, N.I and Z.Z.A.; formal analysis, N.I., Z.Z.A. and F.S.T.; investigation, N.I., Z.Z.A. and F.S.T.; resources, N.I., Z.Z.A. and F.S.T.; data curation, N.I, N.A.M.F, I.M.A.H and Z.Z.A.; writing—original draft preparation, N.I.; writing—review and editing, N.I. and Z.Z.A.; visualization, N.I. and Z.Z.A.; supervision, Z.Z.A and F.S.T.; project administration, Z.Z.A.; funding acquisition, Z.Z.A.

Competing interests

The authors declare that there is no conflict of financial and non-financial interest to disclose.

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