Comparative Evaluation of Avocado Pear and *Moringa Oleifera* Seeds as Sustainable Bio-Coagulants for Brewery Wastewater Treatment

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Abstract

High levels of chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), turbidity, and total dissolved solids (TDS) in brewery wastewater (BWW) pose serious environmental and public health concerns. Using avocado pear seed (PS) and *Moringa oleifera* seed (MOS) as sustainable bio-coagulants, we treated BWW sample through coagulation–flocculation experiments. The bio-coagulants were prepared by aqueous extraction and characterized through Fourier transform infrared (FTIR) spectroscopy and proximate analysis. TDS was selected as the sole response variable for process optimization. One-factor-at-a-time experiments established the operating ranges after which response surface methodology (RSM) based on central composite design was employed to optimize the effects of pH, coagulant dosage, and treatment time on TDS removal. The developed quadratic models adequately described the experimental responses and were successfully validated. FTIR analysis confirmed the presence of amine, hydroxyl, carbonyl, and aromatic functional groups associated with coagulation activity. Combined with evidence from the literature, these results suggest that pollutant removal proceeds mainly through charge neutralization, adsorption, and interparticle bridging. Under the optimized operating conditions of pH 4, dosage 4 g and treatment time 40 min, experimental TDS removals of 91.33% and 94.23% were obtained for PS and MOS, respectively. Under the same experimental treatment conditions, PS and MOS also achieved turbidity removals of 85.1% and 93.3%, COD removals of 88.9% and 77.5%, and BOD_5 removals of 89.2% and 68.8%, respectively. Comparison with previously reported plant-derived bio-coagulants further revealed that both PS and MOS exhibit competitive treatment performance despite the use of simple distilled-water extraction. These findings establish avocado pear and *Moringa oleifera* seeds as effective, low-cost, and environmentally sustainable alternatives to conventional chemical coagulants for brewery wastewater treatment and provide a basis for future evaluation of their combined application.

Keywords: avocado pear seed; bio-coagulants; brewery wastewater; coagulation-flocculation; *Moringa oleifera* seed; total dissolved solids removal; response surface methodology optimization; turbidity removal; chemical oxygen demand removal; biochemical oxygen demand removal

1. INTRODUCTION

Brewery industries are widely known to consume large volumes of water during brewing, cleaning, cooling, and packaging [1]. As a result, significant quantities of wastewater containing organic materials, nutrients, suspended solids, and by-products of fermentation are generated. Because of these characteristics, brewery wastewater (BWW) remains a critical environmental and public health concern [1,2]. According to [3], high chemical oxygen demand (COD), biochemical oxygen demand (BOD_5) and total dissolved solids (TDS) are the key pollution indicators for the design of effective wastewater treatment systems. High BOD_5 can deplete dissolved oxygen in receiving water bodies resulting from strong microbial activities [4]. Also, BOD_5 contains biodegradable organics, while COD contains both biodegradable and non-biodegradable organics. For this reason, COD has greater concentration of pollutants than BOD_5 [2,4]. Beside organic pollutants, solids also play significant roles in determining the overall degree of wastewater contamination. Total solids (TS) is a measure of the sum of the total suspended solids (TSS) and total dissolved solids (TDS). With suspended solids often being easily removed through sedimentation or filtration, total dissolved solids such as small organic molecules, salts, ions and sugars may remain even after treatment of the effluent [4]. As reported by [5], high levels of TDS in BWW can pose a serious risk to aquatic ecosystems by increasing salinity, altering conductivity, and in the long run, affect the health of aquatic organisms. The research shared by [6] reflected on the effects of wastewater with high TDS levels when applied for irrigation. It results in soil salinity build-up, reduced crop growth, and long-term soil degradation. These concerns have inspired research direction toward the development of efficient treatment strategies capable of simultaneously reducing organic pollutants (BOD_5 and COD) and dissolved solids (TDS) before been released into the environment.

Nigerian Breweries Plc., Enugu, located within the industrial zone of Ninth Mile Corner, Enugu State, Nigeria, is a large-scale brewery industry which generates wastewater streams that reflect the complex nature of modern industrial brewing [7]. According to [7,8], the Enugu facility is one of the oldest in Nigeria that produces effluents rich in organic matter, carbohydrates, proteins, spent yeast, detergents, cleaning agents, and process derived salts. These impurities represent the global brewery wastewater profiles [8]. The effluent from the Enugu facility has been reported by [8,9] to contain high levels of BOD_5, COD, TDS, and turbidity, indicating the need for treatment solutions that are adaptable and sustainable to developing regions. The wastewater investigated in this study was collected from this facility, which gives a realistic representation of industrial case study for evaluating sustainable treatment technologies.

Conventional treatment technologies such as chemical coagulation, ion exchange, membrane filtration, reverse osmosis, and other physicochemical processes have been employed for the treatment of brewery wastewater because of their ability to remove suspended and dissolved contaminants effectively [8-11]. Despite their effectiveness, these technologies are often expensive, energy-intensive, and may require advanced operational expertise or in some cases generate secondary pollution arising from chemical sludge and residual metal ions [12,13]. Such limitations hinder their widespread application, especially in underdeveloped and developing nations where financial and technological resources are generally scarce. As a result, increasing attention has been directed toward plant-derived bio-coagulants as environmentally sustainable alternatives because they are biodegradable, renewable, locally available, and capable of reducing sludge production and residual chemical contamination [14]. Developing countries, where advanced treatment facilities may be economically constrained, stand to benefit considerably from these low-cost and environmentally friendly treatment alternatives capable of achieving high pollutant removal without excessive operational costs.

Among plant-derived bio-coagulants, *Moringa oleifera* seed (MOS) is by far the most extensively investigated owing to its high protein content and the presence of positively charged water-soluble proteins that effectively destabilize negatively charged colloidal particles through charge neutralization and interparticle bridging [14-16]. Hence, MOS has been successfully applied for the removal of turbidity, suspended solids, and organic pollutants in numerous water and wastewater treatment studies [14-18]. On the other hand, avocado pear seed (PS) has received relatively little scientific attention despite the fact that it is produced in large quantities as an agricultural by-product in many tropical countries. The relatively limited investigation is mainly due to the longstanding research focus on MOS as the benchmark natural coagulant and the relatively less characterization of coagulation properties of avocado seed extracts. However, the presence of an excess of proteins, carbohydrates, polyphenolic compounds, and oxygen-containing functional groups present in PS suggests a high potential for organic pollutant removal and adsorption-assisted coagulation [17]. Moreover, avocado pear seed is an abundant agro-waste that can be used as a value-added treatment material. This promotes waste valorization and circular economy as well as reduces the environmental burden of the fruit-processing waste and the dependence on traditional chemical coagulants. Therefore, more systematic investigation is needed to establish its treatment performance and identify applications where it may complement or even outperform existing plant-derived bio-coagulants.

According to [14,18], the coagulation performance of natural seeds is determined largely by their molecular structure and functional groups. These molecular characteristics are often determined using the characterization tool – Fourier transform infrared (FTIR) spectroscopy [19]. This tool is used to identify important active sites which have been associated with coagulation activity such as amine (N–H), hydroxyl (O–H), carbonyl (C=O), alcohol (C–O), and aromatic functional groups [19-21]. These functional groups are known to support coagulation-flocculation mechanisms such as charge neutralization, adsorption, hydrogen bonding, hydrophobic interactions, and interparticle bridging, all of which facilitate particle destabilization, floc formation, and contaminant removal [14,19,21,22]. Coagulation efficiency is generally enhanced by higher protein content because proteins contain charged amino acids that promote electrostatic attraction and destabilization of colloidal particles. This is particularly true for MOS, which often demonstrates higher protein content compared to many other plant-based seeds [14-18].

Coagulation and flocculation remain central processes in water and wastewater treatment. The performance ability of bio-coagulants depends on the physicochemical mechanisms through which contaminants are destabilized, aggregated, and removed [22-25]. The procedure typically occurs by one or more of the following mechanisms: (a) Charge neutralization: Proteins carry a positive charge which neutralizes the negative charge of the colloidal particles [22]. (b) Adsorption and interparticle bridging: Long chain molecules link numerous particles and form bigger flocs [23]. (c) Sweep flocculation: High dosages of coagulant lead to the formation of precipitates which entrap particles during settling. This is a more typical process in chemical coagulants, but can also be observed in natural coagulants [24]. (d) Hydrogen bonding and hydrophobic interactions: Hydroxyl, amine and aromatic functional groups promote hydrogen bonding and hydrophobic interactions that are favorable to floc formation [25]. Several studies [14-18,22-24] have shown that coagulants derived from plant seeds such as moringa oleifera species, commonly operate through the mechanisms of charge neutralization and adsorption–interparticle bridging, due to the presence of functional groups such as amine, hydroxyl, and aromatic structures, and high protein content. The aforementioned studies further indicate that these molecular characteristics contribute to the significant removal of organic matter (BOD_5 and COD), turbidity and TDS in wastewater treatment applications using bio-coagulants.

Recent studies have investigated moringa oleifera seed as an eco-friendly coagulant and adsorbents for industrial wastewater treatment [14-18,22-26]. Comparatively, fewer studies have been conducted on avocado pear seed as an abundant agricultural by-product with promising biochemical composition [17]. Moreover, these investigations have concentrated mainly on the reduction of turbidity, dye removal, or synthetic wastewater, without integrating mechanistic interpretation and comparative performance evaluation of different plant-derived bio-coagulants within the same experimental framework. Direct comparative studies between PS and MOS for the simultaneous removal of biodegradable organic matter (COD and BOD_5), suspended solids and dissolved solids (TDS) from real brewery wastewater, particularly from Nigerian Breweries Plc., Enugu are scarce. Therefore, further studies are required to address these knowledge gaps, evaluate under-researched bio-coagulants and establish comparative performance data that can justify their practical application in sustainable wastewater treatment, especially in developing countries where cost-effective treatment technologies are urgently required.

Although avocado pear seed (PS) and *Moringa oleifera* seed (MOS) have been investigated in our previous studies for paint wastewater treatment, those studies addressed fundamentally different scientific objectives, namely process optimization and predictive modelling of TDS removal using response surface methodology (RSM) and artificial neural networks (ANN) [27], and elucidation of the adsorption-assisted coagulation–flocculation mechanism through adsorption kinetics, diffusion modelling, and equilibrium isotherm analyses for TDS removal [28]. The present study therefore comparatively evaluates avocado pear seed and *Moringa oleifera* seed as sustainable bio-coagulants for the treatment of brewery wastewater collected from Nigerian Breweries Plc., Enugu, Nigeria. Specifically, the study investigates their physicochemical characteristics, processing yields, functional groups, and coagulation–flocculation performance for the removal of TDS, turbidity, COD, and BOD_5. TDS was selected as the primary response variable for process optimization, and the effects of pH, coagulant dosage, and treatment time were investigated by one-factor-at-a-time experiments and optimized through response surface methodology. In addition, the study interprets the coagulation mechanisms of both bio-coagulants using FTIR evidence supported by established literature, validates the developed statistical models experimentally, and compares the treatment performance of PS and MOS with previously reported plant-derived bio-coagulants. The findings provide mechanistic, optimization and comparative insights into the complementary treatment behaviour of the two bio-coagulants and demonstrate their potential as low-cost, environmentally sustainable alternatives for industrial wastewater treatment, especially in developing regions where affordable treatment technologies are needed.

2. Materials and Methods

2.1 Equipment and Raw Materials

The equipment used in this study included a graduated measuring cylinder, Whatman No. 1 filter paper, beakers, electronic weighing balance, laboratory mortar and pestle, pH meter, magnetic stirrer, heating tube, drying oven, and stopwatch. The raw materials employed for bio-coagulant preparation and coagulation–flocculation experiments, together with the analytical reagents used for the physicochemical and proximate characterization of the bio-coagulants, are presented in Table 1.

Table 1. Materials and Analytical Reagents Used in this Study

Raw materials	Functions	Quantity
Distilled water	Solvent for active ingredient extraction	300ml
PS powder	Coagulant source	25g
MOS powder	Coagulant source	25g
H_2SO_4	Digestion reagent for proximate (protein) analysis	10ml
NaOH	Neutralization reagent for Kjeldahl protein determination	45g/100ml
Boric acid (H_3BO_3)	Ammonia trapping reagent for Kjeldahl protein determination	4g/100ml
Petroleum ether	Solvent for lipid extraction during proximate analysis	120ml
KOH	Digestion reagent during proximate analysis	150ml

2.2 Preparation and Extraction of Bio-coagulants

PS and MOS were obtained from Agbani, in Nkanu West Local Government Area of Enugu State, Nigeria. They were separately washed, dried and ground to fine powder using laboratory mortar and pestle. The active ingredient from each processed seed was extracted in line with standard method adopted by [26]. Five bio-coagulant extracts of different concentrations were prepared by dispersing 1, 2, 3, 4, and 5 g of finely ground PS or MOS powder separately in 100 mL of distilled water. Each suspension was stirred for 15 min at room temperature and filtered through Whatman No. 1 filter paper. The resulting filtrates were used immediately as bio-coagulants in the coagulation–flocculation experiments. The filtrate obtained from each preparation was used directly as the corresponding bio-coagulant extract during the coagulation–flocculation experiments. So, extracts prepared from 1, 2, 3, 4, and 5 g of seed powder represented the respective experimental dosages applied to each 500 mL wastewater sample. This process was done in batches for each experimental run to minimize deterioration of the active components, and the filtrates were shaken thoroughly before use to ensure homogeneity. An aqueous extraction procedure was selected because it provides a simple, environmentally benign, and low-cost method that can readily be adopted in decentralized wastewater treatment systems. Although salt extraction, particularly with NaCl, has been reported to improve the recovery of coagulating proteins from *Moringa oleifera* seeds [29], distilled water extraction was adopted in the present study to ensure a fair comparison between both bio-coagulants under identical extraction conditions while avoiding the introduction of additional dissolved salts into the treatment system. A similar approach has also been adopted for date seed bio-coagulants, where aqueous extraction was shown to produce effective coagulation performance [30]. The aqueous extracts obtained in this study were applied directly after preparation without drying or concentration. As a result, the dry weight of the extracts and the protein concentration of the liquid extracts were not determined. Instead, identical extraction conditions were maintained for both PS and MOS to enable a direct and unbiased comparison of their coagulation–flocculation performance. Processing yields based on the processed seed materials are presented in Section 3.2.

2.3 Fourier Transform Infrared (FTIR) Analysis

The functional groups present in PS and MOS powders were identified using Fourier Transform Infrared (FTIR) spectroscopy following standard analytical procedures [27]. The spectra were recorded over the mid-infrared region (4000–400 cm^{-1}), and the characteristic absorption bands were interpreted by comparison with published literature to identify the major functional groups responsible for the coagulation–flocculation properties of the bio-coagulants.

2.4 Proximate Analysis

The proximate compositions of PS and MOS, including moisture, ash, crude protein, crude fat, crude fibre, and carbohydrate contents, were determined according to standard AOAC procedures [8,9,15]. Crude protein was determined by the Kjeldahl method, crude fat by Soxhlet extraction using petroleum ether, while moisture, ash, and crude fibre were determined using standard gravimetric methods. Carbohydrate content was calculated by difference. The results are presented in Section 3.2.

2.5 Sampling Site

The brewery wastewater sample used for this study was collected from the final effluent discharge point of Nigerian Breweries Plc., Enugu, located within the industrial zone of Ninth Mile Corner, Enugu State, Nigeria. The BWW sample was collected at peak production period (February – March 2024). The collected BWW sample was carefully transferred into an acid cleaned polyethylene container that was well rinsed before use. This process ensures that the physicochemical properties of the sample are maintained before laboratory work.

2.6 Characterization of Brewery Wastewater (BWW)

The physical and chemical properties of the BWW sample was determined using standard analytical procedures in accordance with APHA methods [31]. The following parameters of the BWW sample were determined immediately after sample collection: total solids, total dissolved solids (TDS), COD, BOD_5, turbidity, conductivity, viscosity, pH, temperature, and selected metal constituents. After treatment with PS and MOS, the clarified supernatants were also characterized to evaluate changes in wastewater quality. These measurements represent the before and after treatment characteristics of the BWW and are different from the optimization experiments conducted under controlled pH conditions. In accordance with established procedures [31], TDS which is the primary focus of the study was determined using the gravimetric dry residue method. A measured volume of filtered wastewater was transferred into a pre-weighed beaker and evaporated to dryness, followed by cooling and reweighing to quantify the residual solids. TDS concentration was computed following Equation (1) [26]:

$$\mathrm{TDS} = \frac{(\mathrm{W_2} - \mathrm{W_1}) \times 1000}{\mathrm{V}} \qquad (1)$$

where W_1 is the weight of empty beaker (mg), W_2 is the weight of beaker + dried residue (mg) and V is the volume of sample used (mL)

Total solids (TS) were determined using the same approach but without any prior filtration of the sample [31]. BOD_5 and COD were determined using the standard 5-day incubation and the dichromate digestion methods, respectively [31]. Turbidity was measured using a nephelometric turbidity meter graduated with standard reference solutions, while electrical conductivity was determined using a conductivity meter standardized prior to use [26,31]. The pH, temperature and viscosity of the samples were measured in situ using a calibrated pH meter, thermometer, viscometer under controlled temperature conditions, respectively [26,31]. For metal analysis, selected metals were determined using atomic absorption spectrophotometry (AAS). All measurements were conducted following established standard methods to ensure consistency, accuracy, and reproducibility of results [26,31].

2.7 Coagulation–Flocculation (Jar test) Experiment

Jar-test experiments were performed using a standard six-paddle jar test apparatus following established procedures [26]. Each experiment was conducted in separate beakers containing 500 mL of paint wastewater (PWW). The bio-coagulant extracts prepared from 1, 2, 3, 4, and 5 g of seed powder (Section 2.2) were each applied to separate 500 mL wastewater samples, corresponding to experimental dosages of 1–5 g/500 mL, respectively. The coagulation–flocculation process consisted of rapid mixing at 100 rpm for 5 min, followed by slow mixing at 40 rpm for 25 min to promote floc formation. Thereafter, the suspension was allowed to settle for the prescribed experimental treatment time before the clarified supernatant was decanted, filtered through Whatman No. 1 filter paper, and analyzed for BOD_5, COD, and TDS. For one-factor-at-a-time (OFAT) experiments, the effects of pH (2–10), coagulant dosage (1–5 g/500 mL), and treatment time (10–50 min) on TDS removal were investigated by varying one factor while keeping the other operating conditions constant. The same experimental ranges were later employed in the response surface methodology (RSM) optimization. All coagulation–flocculation experiments were conducted in triplicate, with pH adjusted using 0.1 M H_2SO_4 and 0.1 M NaOH. Contaminant removal efficiency was calculated using Equation (2) [26]:

$$\text{Contaminant removal (\%)} = \frac{C_0 - C_t}{C_0} \times 100 \qquad (2)$$

Where:

C_0 = initial concentration of the contaminant (BOD_5, COD, or TDS)
C_t = final concentration after coagulation–flocculation treatment

2.8 Experimental Design and Statistical Analyses

2.8.1 Experimental design and optimization using RSM

Preliminary experiments were first conducted using a one-factor-at-a-time (OFAT) approach to evaluate the individual effects of pH, coagulant dosage, and treatment time on TDS removal while keeping the remaining operating variables constant. These preliminary investigations provided the experimental basis for selecting suitable factor ranges for subsequent optimization. Thereafter, Response Surface Methodology (RSM) based on Central Composite Design (CCD) generated with Design-Expert software (Version 9.0.1) was employed to investigate the combined effects and interactions among the three operating variables and to determine the optimum treatment conditions for maximum TDS removal. A face-centered CCD (α = 1) was chosen to avoid extreme axial points [26,27], and consisted of 20 experimental runs (Table 2) incorporating factorial, axial, and center points. A second-order polynomial model was fitted to the experimental data to describe the relationships between the process variables and TDS removal efficiency, evaluate interaction effects, and determine the optimum operating conditions.

Table 2. Central Composite Design Matrix for TDS Removal Experiments

Run	Dosage (g/500 mL)	pH	Time (min)	Experimental TDS Removal (%)
1	4	4	40	-
2	5	6	30	-
3	5	2	50	-
4	3	6	30	-
5	4	4	30	-
6	4	4	50	-
7	4	4	40	-
8	4	4	40	-
9	5	2	30	-
10	4	4	40	-
11	4	4	40	-
12	3	4	40	-
13	3	2	30	-
14	3	6	50	-
15	5	6	50	-
16	4	4	40	-
17	3	2	50	-
18	4	2	40	-
19	4	6	40	-
20	5	4	40	-

Note: The symbol (–) indicates that the experimental TDS removal values are not presented in this table because the design matrix is included solely to illustrate the experimental combinations employed in the RSM. The values are presented in the Results and Discussion section.

2.8.2 Statistical analysis

All coagulation–flocculation experiments were performed in triplicate under identical operating conditions, and the results are reported as the arithmetic mean of the triplicate values. The experimental responses presented in the RSM design matrix correspond to these mean values used for model development. Analysis of variance (ANOVA) was used to evaluate the statistical significance of the developed quadratic models, with differences considered significant at $p < 0.05$ and highly significant at $p < 0.0001$ [26,27]. Model adequacy was assessed using the coefficient of determination (R^2), adjusted R^2, predicted R^2, coefficient of variation (CV), and Adequate Precision. These statistical parameters were used to evaluate the goodness-of-fit, predictive capability, and reliability of the developed response surface models.

2.8.3 Model validation

The optimum operating conditions predicted by the developed RSM models were validated experimentally. Independent coagulation–flocculation experiments were conducted in triplicate under the predicted optimum conditions, and the corresponding TDS removal efficiencies were compared with the model predictions. The agreement between experimental and predicted values was utilized to assess the prediction capacity and practical usefulness of the developed models.

3. Results and Discussion

3.1 FTIR Analysis and Characterization of Functional Groups

FTIR spectroscopy was used to identify the major functional groups responsible for the coagulation activity of PS and MOS. The absorption peaks and FTIR spectra are presented in Table 3 and Figure 1 respectively. Both bio-coagulants showed oxygen and nitrogen containing functional groups previously associated with effective coagulation–flocculation processes [16,22–24,32,33]. The absorption bands at 1617.7 cm^{-1} (PS) and 1610.2 cm^{-1} (MOS) corresponding to N–H bending vibrations of amine groups together with a distinct N–H stretching band at 3693.8 cm^{-1} for MOS were observed. These protein related functional groups are recognized as the principal active sites responsible for charge neutralization of negatively charged colloidal particles that promote particle destabilization and floc formation [16,26]. The higher protein content of MOS (37.16%) compared with PS (18.96%) agrees with the stronger amine bands observed in MOS and provides molecular evidence for its superior TDS and turbidity removal. Both PS and MOS also exhibited strong C–O stretching vibrations (1151–1238 cm^{-1}), indicating the presence of alcohols and other oxygen-containing functional groups capable of promoting hydrogen bonding, adsorption and interparticle bridging [23,32]. Additionally, the C–O–C symmetric and asymmetric stretching band at 1200.2 cm^{-1} in MOS can be indicative of the presence of ether linkages that can give additional adsorption sites for dissolved pollutants [33]. The absorption bands of carbonyl groups at 1733.2 cm^{-1} (PS) and 1729.5 cm^{-1} (MOS) also confirmed the presence of aldehydes and esters. These groups are polar in nature and promotes interactions with dissolved organic contaminants by means of dipole–dipole interactions and hydrogen bonding [23,29,32]. These adsorption-assisted interactions can account for the superior COD and BOD_5 removal achieved with PS. The spectra further revealed C–H stretching vibrations of alkanes (2851–2919 cm^{-1}), C≡C stretching vibrations of alkynes (2106–2117 cm^{-1}), and aromatic C–H bending vibrations (779 cm^{-1}). Although these groups are not primary coagulating sites, they contribute to the structural integrity of the extracted biopolymers and favour floc growth through hydrophobic interactions [24]. The FTIR results obtained in this study experimentally confirmed the presence of amine, hydroxyl, carbonyl and aromatic functional groups capable of supporting the two principal coagulation mechanisms widely reported for plant-derived bio-coagulants such as charge neutralization and adsorption–interparticle bridging [22]. But the coagulation mechanisms discussed later in Section 3.4 were inferred by relating these experimentally identified functional groups to established mechanisms reported for similar plant-derived bio-coagulants in the literature. FTIR identifies the chemical functionalities present but does not directly monitor the coagulation process. Furthermore, as FTIR characterization was performed only on the bio-coagulants before been applied for BWW treatment, possible shifts in absorption bands after wastewater treatment which could provide direct evidence of contaminant binding, were not investigated. Hence, we recommend that future studies should compare FTIR spectra before and after treatment to elucidate the molecular interactions responsible for pollutant removal.

Table 3. FTIR Functional Groups Identified in PS and MOS Bio-coagulants

PS Peak (cm^{-1})	MOS Peak (cm^{-1})	Functional Group	Class of Compound
3283.8	3287.5	≡C–H stretch	Alkynes
—	3693.8	N–H stretch	Amines
2918.5	2918.5	C–H stretch	Alkanes/Alkyls
2851.4	2851.4	C–H stretch	Alkanes/Alkyls
2105.9	2117.1	C≡C stretch	Alkynes
1733.2	1729.5	C=O stretch	Aldehydes (PS), Esters (MOS)
1617.7	1610.2	N–H bend	Amines
1375.4	1367.9	CH_3 bending	Alkanes
1237.5	—	C–O stretch	Alcohol
—	1200.2	C–O–C stretch	Ethers
1151.7	1155.5	C–O stretch	Alcohol
1319.5	1315.8	C–F stretch	Alkyl halides
779.0	779.0	C–H bend	Aromatic compounds
1010.1	1028.7	C–F/C–O stretch	Alkyl halides / Alcohol
—	663.5	C–H bend	Alkynes

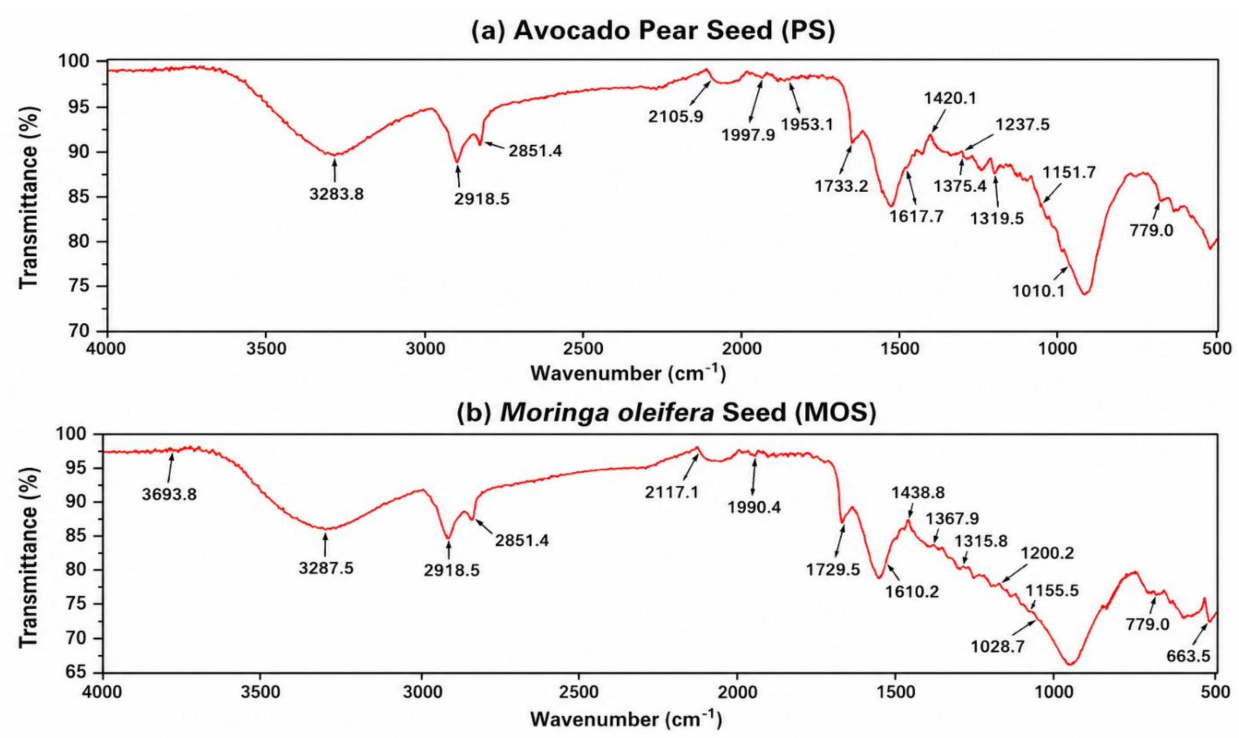

Figure 1. FTIR spectra of (a) avocado pear seed (PS) and (b) *Moringa oleifera* seed (MOS) bio-coagulants, illustrating the characteristic absorption peaks used for functional group identification.

3.2 Proximate Composition of PS and MOS

Based on the proximate compositions presented in Table 4, both PS and MOS produced processing yields above 20%, which confirms their suitability as natural coagulant precursors [15,16], with MOS showing a higher yield (30.52%) than PS (25.63%). The obtained yields for both bio-coagulants are not the dry weight of the aqueous extracts because the extracts were prepared freshly and applied directly in the coagulation experiments. The slightly lower bulk density of MOS impacts floc behavior [26]. Low moisture and ash contents for both bio-coagulants implies good storage stability [26]. Also, the higher protein content of MOS (37.16% vs 18.96%), shows its stronger potential coagulation activity over PS as proteins are the primary active agents in bio-coagulants [15]. The fat contents of both bio-coagulants were below 5%, which means minimum risk of floc destabilization [16]. In agreement with the report of [11], the higher crude fiber and carbohydrate contents of PS contribute to floc structure and supplementary adsorption mechanisms respectively.

Table 4. Proximate Composition of PS and MOS

Parameters	PS	MOS
Processing yield (%)	25.63	30.52
Bulk density (g/mL)	0.322	0.318
Moisture Content (%)	9.74	9.05
Ash content (%)	2.23	2.43
Protein content (%)	18.96	37.16
Fat content (%)	4.41	1.82
Crude Fibre (%)	11.58	4.91
Carbohydrate (%)	53.08	44.63

3.3 Comparative Assessment of PS and MOS for BWW Treatment

Figure 2 presents a comparative evaluation of four major water quality parameters, namely TDS, turbidity, COD, and BOD_5, after treatment with MOS and PS bio-coagulants. As shown, both coagulants achieved high removal efficiencies (>65%), although with varying performance patterns. MOS exhibited superior efficiency in reducing TDS (94.23%) and turbidity (93.30%) compared with PS (91.33% and 85.07%, respectively). This indicates that MOS has a greater ability to remove dissolved and suspended solids. In contrast, PS performed better than MOS in removing organic pollutants, with higher removals in COD (88.88% vs. 77.49%) and BOD_5 (89.18% vs. 68.81%). Clearly, PS was more effective for organic load reduction, whereas MOS was more effective for solids removal. The complementary performances of these bio-coagulants imply that their combined application could potentially provide a more balanced and optimized wastewater treatment method. Table 5 presents the physicochemical characteristics of the wastewater before treatment and after treatment with PS and MOS bio-coagulants. These measurements were obtained for comparative assessment of treatment performance and should not be interpreted as the operating conditions employed during the OFAT and RSM optimization experiments. As observed, the conductivity and trace metal concentrations were greatly reduced by both the coagulants confirming the removal of ionic and particulate contaminants. However, MOS showed a greater reduction in viscosity indicating more effective destabilization and aggregation of suspended and colloidal particles [14,15,16].

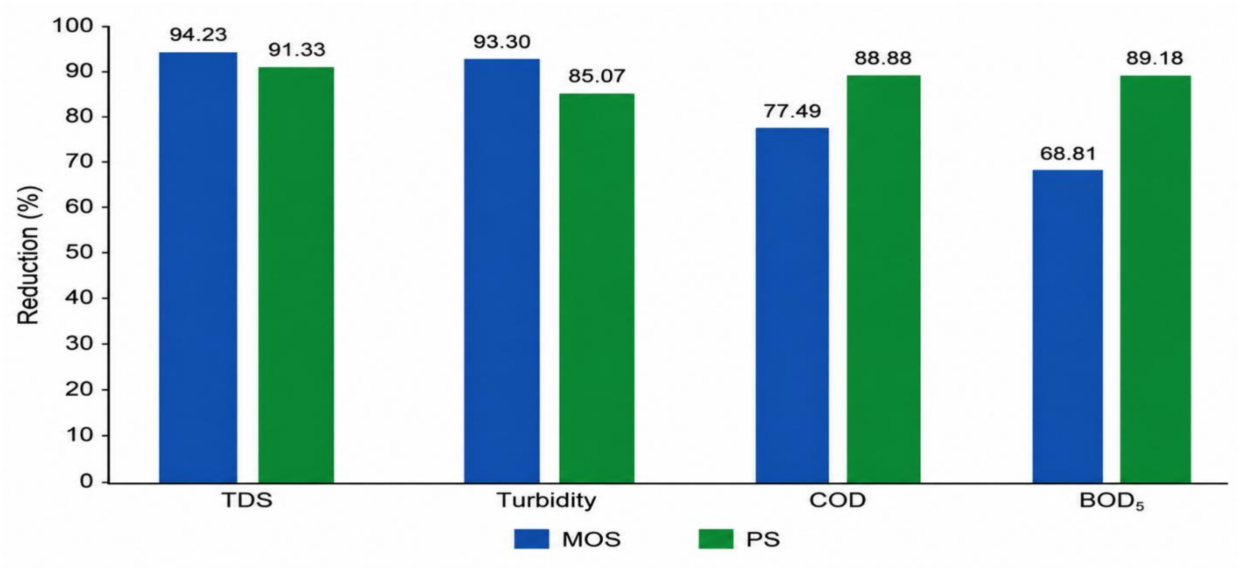

Figure 2. Comparative evaluation of the removal efficiencies of MOS and PS across four key water quality parameters: TDS, Turbidity, COD and BOD_5

Table 5. Characteristics of BWW Before and After Treatment with PS and MOS

Parameters	Unit	Before Treatment	After PS Treatment	After MOS Treatment
pH	-	6.27	6.52	6.51
Temperature	(°C)	30.13	31.34	31.32
Total solids (TS)	(mg/ L)	2043.96	178.18	120.24
TSS	(mg/ L)	59.81	4.96	4.96
TDS	(mg/ L)	1984.15	173.22	115.28
BOD_5	(mg/ L)	1202.57	130.15	375.14
COD	(mg/ L)	1880.61	209.12	423.38
Turbidity	NTU	26.26	3.921	1.76
Conductivity	µS/cm	462	88.00	94.8
Viscosity	Cp	3.04	1.35	1.153
Arsenic	(mg/L)	2.975	0.536	0.105
Chromium	(mg/ L)	0.071	0.048	0.009
Mercury	(mg/ L)	0.043	0.014	0.028
Zinc	(mg/ L)	0.352	0.071	0.227
Aluminium	(mg/ L)	3.264	0.681	0.051
Iron	(mg/ L)	1.408	0.212	0.054

3.4 Mechanistic Interpretation of Coagulation–Flocculation Pathways of MOS and PS

Figure 3 illustrates the coagulation–flocculation pathways of MOS and PS bio-coagulants. The proposed mechanisms were developed by combining the functional groups identified by FTIR presented in Section 3.1 with established coagulation mechanisms reported for protein and polysaccharide based natural coagulants [14,15,16]. Thus, FTIR confirms the presence of chemical functionalities capable of participating in coagulation, whereas the individual pathways illustrated in Figure 4 represent mechanistic interpretations supported by literature. For MOS, FTIR confirmed the presence of amine, hydroxyl, carbonyl and other oxygen-containing functional groups, while proximate analysis showed a relatively higher protein content. These characteristics favour charge neutralization of negatively charged colloidal particles through electrostatic interactions, thereby reducing repulsive forces and promoting particle aggregation. Long-chain biomolecules subsequently facilitate adsorption–interparticle bridging, leading to the formation of larger and denser flocs [14,15,16]. This mechanism explains the higher TDS and turbidity removal efficiencies obtained with MOS due to high protein content and larger abundance of cationic functional groups that promote particle–coagulant interactions. However, FTIR analysis of PS indicated the presence of several hydroxyl, carbonyl, aromatic and C–O containing functional groups associated with polyphenolic compounds and polysaccharides. The functional groups provide numerous active sites for adsorption of dissolved organic molecules through hydrogen bonding, hydrophobic interactions and van der Waals forces [14,15,16]. The continued adsorption and interparticle bridging promote formation of organic rich flocs that subsequently settle from the aqueous phase. This mechanism explains the superior performance of PS in COD and BOD_5 reduction, as its chemical composition favours interactions with dissolved and biodegradable organic matter rather than mainly suspended particles. MOS and PS have different dominant pathways, but both lead to floc formation and sedimentation. MOS thus acts mainly by charge-driven destabilization of suspended particles, while PS is more based on adsorption and bridging of dissolved organic matter. Their complementary behaviour suggests that combining both bio-coagulants could provide a synergistic approach for the simultaneous removal of suspended solids and dissolved organic pollutants in wastewater treatment. It should be noted that direct confirmation of charge neutralization mechanism proposed for MOS through zeta potential measurements was not performed in the present study. Future work should therefore combine FTIR characterization with zeta potential analysis to provide direct evidence of surface charge neutralization during the coagulation–flocculation process.

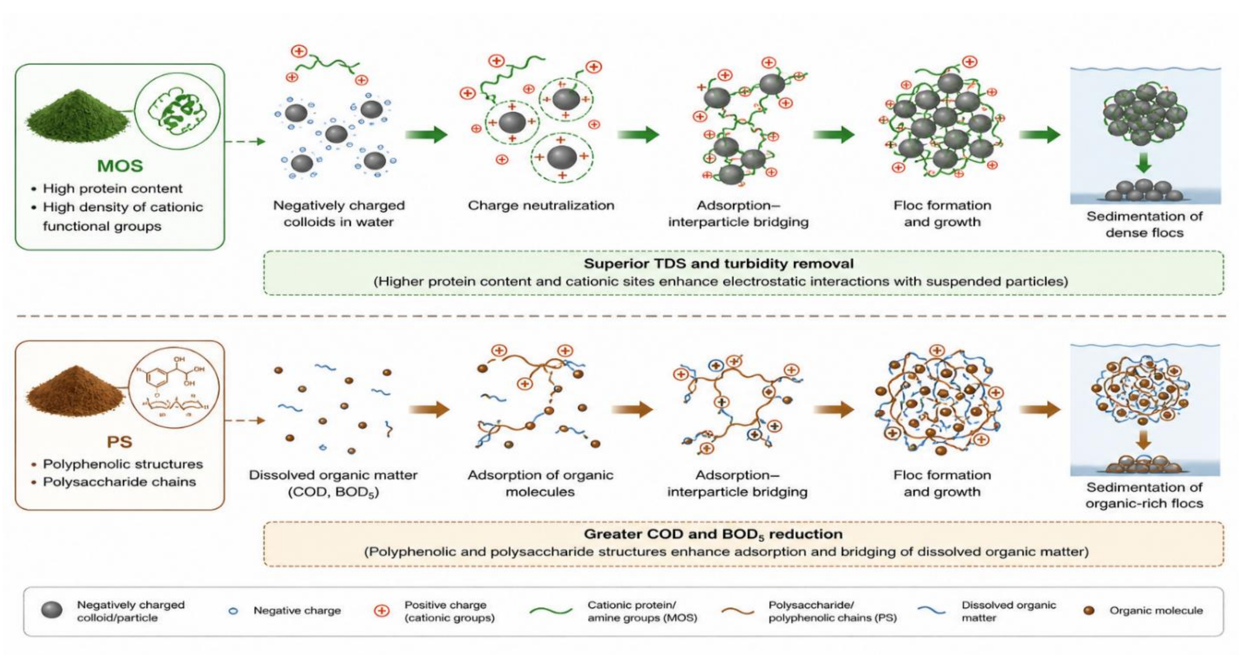

Figure 3. Coagulation–flocculation mechanism of *Moringa oleifera* seed (MOS) and avocado pear seed (PS) bio-coagulants. **Created with BioRender.com.**

3.5 Selection of TDS as the Response Variable

TDS was selected as the primary response variable for subsequent optimization because it represented the dominant pollutant in the brewery wastewater and served as an integrative indicator of treatment performance. Unlike turbidity, which reflects suspended particles, or COD and BOD_5, which quantify organic pollution, TDS encompasses dissolved inorganic salts and low molecular weight organic compounds that are more difficult to remove during conventional coagulation–flocculation [6]. This makes TDS a more sensitive parameter for evaluating the effects of operational variables. On practical water treatment objectives, focusing on TDS aligns with the reports of [6,11], where TDS is widely recognized as an important regulatory and operational control parameter. Consequently, optimizing TDS removal was considered the most stringent criterion for evaluating the performance of the bio-coagulants.

3.6 Effects of Individual Process Variables (OFAT) on TDS Removal

3.6.1 Effect of pH on TDS removal

At a constant dosage of 4 g/500 mL and a treatment time of 40 minutes, the effect of pH on TDS removal efficiency was evaluated. As shown in Figure 4, TDS removal increased with increasing pH of the bio-coagulants and reached the highest removal efficiency within the investigated range at approximately pH 4, after which efficiency declined with increasing pH. This behaviour is consistent with the pH-dependent performance characteristics of bio-coagulants, and agrees with the reports that there is enhanced removal of TDS under acidic conditions [14–16].

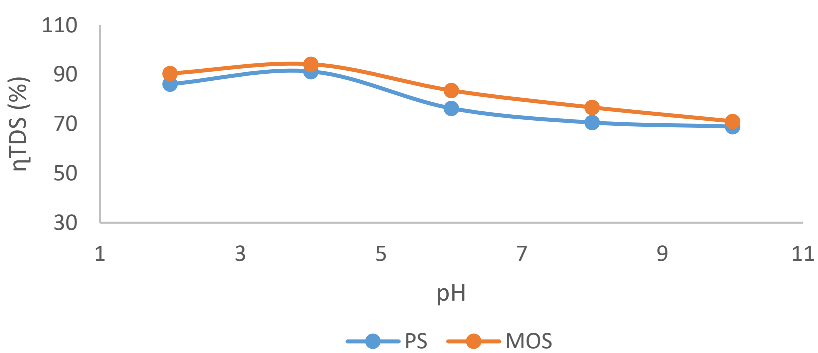

Figure 4. Effect of pH on TDS removal

3.6.2 Effect of coagulant dosage on TDS removal

The effect of coagulant dosage on TDS removal efficiency for PS and MOS (Figure 5) was evaluated at pH 4 and a treatment time of 40 minutes. This shows a non-linear trend with increasing dosage. TDS removal increased steadily up to the highest removal within the investigated dosage range of 4 g/500 mL as a result of enhanced charge neutralization and particle–coagulant interactions, but declined beyond the highest point as overdosing caused particle destabilization and the introduction of excess dissolved matter [11,12,14]. This pattern shows that the best coagulant dosing is important to achieve maximum coagulation efficiency.

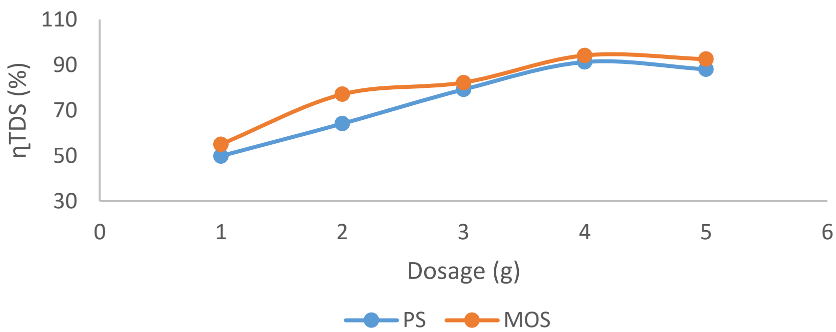

Figure 5. Effect of dosage on TDS removal

3.6.3 Effect of treatment time on TDS removal

Figure 6 shows the effect of treatment time on TDS removal efficiency using PS and MOS at constant pH 4 and a dosage of 4 g/500 mL. The removal efficiency increased steadily with time and reached a maximum at 40 minutes, after which it started decreasing gradually. This is a pattern that reflects flocculation kinetics as sufficient contact time is required for particle collision, aggregation, and settling [24]. Both bio-coagulants reaching the highest removal within the investigated treatment-time range of 40 minutes indicate that both coagulants require moderate flocculation duration to achieve maximum performance. This is in good agreement with the reported behavior of natural coagulant systems [34].

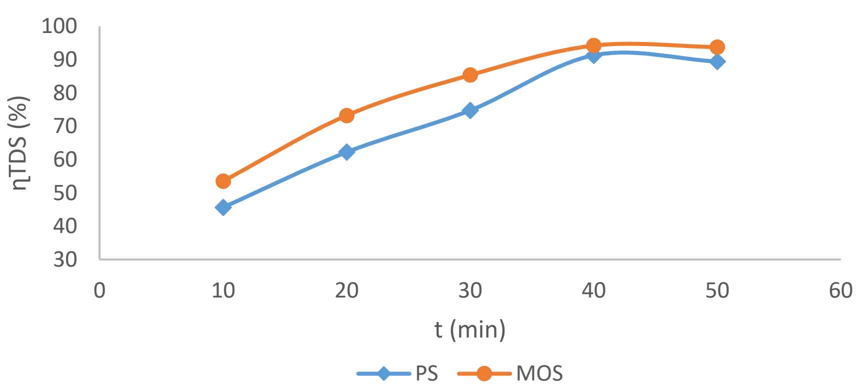

Figure 6. Effect of treatment time on TDS removal

3.7 Response Surface Methodology (RSM) Optimization of TDS Removal

Although the individual effects of pH, dosage and treatment time provide useful insight into the behaviour of the coagulation process, these operating variables may also interact simultaneously. Therefore, RSM based on CCD was employed to investigate the combined effects of the process variables, develop predictive quadratic models, and identify the optimum operating conditions for maximum TDS removal.

3.7.1 Experimental design

Table 6 presents the CCD matrix together with the experimentally observed TDS removal efficiencies for brewery wastewater treated with avocado pear seed and *Moringa oleifera* seed. The design consisting of 20 experimental runs investigated the combined effects of coagulant dosage, pH, and treatment time on TDS removal efficiency. The replicated center-point experiments (Runs: 1, 7, 8, 10, 11 and 16) produced identical TDS removal efficiencies of 91.74% for PS and 94.63% for MOS. These repeated runs demonstrate high experimental reproducibility, minimal random variation, and the reliability of both the experimental procedure and the subsequent statistical modelling [26,35]. As observed, TDS removal ranges from 55.98% to 91.74% for PS, and from 58.93% to 94.63% for MOS. These broad response ranges confirm that the experimental domain was sufficiently large to capture both favourable and unfavourable operating conditions, thus enabling reliable development of predictive response surface models [26,27,35]. The table further shows that maximum TDS removal for both bio-coagulants occurred at 4 g dosage, pH 4 and 40 min. Lower removal efficiencies were generally observed when the pH or dosage deviated from these optimum conditions. A direct comparison of the two bio-coagulants shows that MOS consistently achieved slightly higher TDS removal efficiencies than PS under corresponding conditions. This agrees with its higher protein content and supports the hypothesis that the larger concentration of positively charged coagulating proteins enhances charge neutralization and floc formation during wastewater treatment.

Table 6. Experimental Design Matrix and Observed TDS Removal Efficiencies for PS and MOS Obtained Using Central Composite Design (CCD).

Run	Dosage (g/500 mL)	pH	Time (min)	PS TDS Removal (%)	MOS TDS Removal (%)
1	4	4	40	91.74	94.63
2	5	6	30	67.21	70.16
3	5	2	50	80.15	83.14
4	3	6	30	55.98	58.93
5	4	4	30	80.63	83.59
6	4	4	50	90.58	93.51
7	4	4	40	91.74	94.63
8	4	4	40	91.74	94.63
9	5	2	30	72.16	75.09
10	4	4	40	91.74	94.63
11	4	4	40	91.74	94.63
12	3	4	40	80.28	83.23
13	3	2	30	70.01	72.98
14	3	6	50	64.03	66.98
15	5	6	50	77.42	80.39
16	4	4	40	91.74	94.63

17	3	2	50	71.15	74.09
18	4	2	40	91.01	93.96
19	4	6	40	79.68	82.61
20	5	4	40	91.08	94.00

3.7.2 Analysis of variance (ANOVA)

Table 7 presents the ANOVA results for the quadratic response surface models developed for PS and MOS bio-coagulants. The models were highly significant ($p < 0.0001$) with F-values of 133.78 and 131.74, respectively, confirming that the developed quadratic models adequately described the relationship between TDS removal and the operating variables. The three linear factors (dosage, pH and treatment time) significantly influenced TDS removal ($p < 0.0001$) for both bio-coagulants, indicating that each operating variable independently contributed to process performance [26,27]. The interaction terms (Dosage × pH, Dosage × Time and pH × Time) were also statistically significant ($p < 0.05$) indicating that the effect of one operational variable was dependent on the level of another variable [35]. More importantly, the quadratic terms ($Dosage^2$, pH^2 and $Time^2$) were all highly significant ($p < 0.0001$) for both PS and MOS. This confirms the presence of significant curvature in the response surfaces and justifies the adoption of a second-order polynomial model for process optimization rather than a simple linear model [26,27,35]. The significance of these quadratic terms also explains the existence of well-defined optimum operating conditions for TDS removal.

Table 7. Comparative ANOVA Results for Quadratic Models for TDS Removal Using PS and MOS

Model Term	**PS (p-value)**	**MOS (p-value)**
Model	<0.0001	<0.0001
Dosage	<0.0001	<0.0001
pH	<0.0001	<0.0001
Time	<0.0001	<0.0001
Dosage × pH	0.0069	0.0071
Dosage × Time	0.0470	0.0458
pH × Time	0.0446	0.0458
$Dosage^2$	<0.0001	<0.0001
pH^2	<0.0001	<0.0001
$Time^2$	<0.0001	<0.0001

3.7.3 Model Adequacy

The statistical adequacy for the developed quadratic models is presented in Table 8. The coefficients of determination ($R^2 = 0.9918$ for PS and 0.9916 for MOS) indicate that over 99% of the variability in TDS removal was explained by the developed models. The close agreement between the adjusted R^2 (0.9843 for PS and 0.9841 for MOS) and predicted R^2 (0.9257 for PS and 0.9245 for MOS) indicates excellent predictive performance with no evidence of model overfitting [35]. The low coefficients of variation (1.73% and 1.68%) demonstrate good experimental precision and reliability, while the high Adequate Precision values (36.59 and 36.31), which greatly exceed the recommended minimum value of 4, confirm an adequate signal-to-noise ratio and indicate that the models can be used to navigate the design space [27]. Furthermore, the low standard deviation (1.41) for both models reflects minimal unexplained experimental variability and further supports the robustness of the developed response surface models [35].

Table 8. Statistical Indicators of Model Adequacy for PS and MOS

Statistic	PS	MOS
Standard deviation	1.41	1.41
Mean response (%)	81.09	84.02
Coefficient of variation (%)	1.73	1.68
R^2	0.9918	0.9916
Adjusted R^2	0.9843	0.9841
Predicted R^2	0.9257	0.9245
Adequate Precision	36.59	36.31

3.7.4 Response Surface Analysis

The 3-D response surface plots in Figure 7(a–f) illustrate the combined effects of coagulant dosage, pH and treatment time on TDS removal by PS and MOS. For both bio-coagulants, TDS removal increased as the dosage approached 4 g, the pH approached 4, and the treatment time increased to approximately 40 min, beyond which further increment produced little or no improvement. This behaviour indicates the existence of optimum operating conditions, although excessive dosage, longer treatment time or higher pH reduced coagulation efficiency because of particle restabilization, saturation of active binding sites and reduced ionization of the functional groups responsible for contaminant binding [35]. The response surfaces also show that pH exerted the strongest influence on TDS removal, while the elliptical contour profiles confirm significant interactions among the operating variables which is consistent with the ANOVA results [26,27,35]. Although both bio-coagulants exhibited similar response trends, MOS consistently achieved higher predicted TDS removal than PS and displayed a broader high efficiency region, indicating greater process robustness. This superior performance agrees with the higher protein content of MOS and the greater abundance of amine containing functional groups identified by FTIR analysis, which favour charge neutralization and interparticle bridging. The response surface models predicted optimum operating conditions of approximately 4 g dosage, pH 4 and 40 min for both bio-coagulants, with maximum predicted TDS removals of 92.2325% for PS (Figure 7a–c) and 94.2731% for MOS (Figure 7d–f). The close agreement between these predicted values and the experimental validation results (Section 3.7.5) confirms the adequacy and predictive reliability of the developed quadratic models.

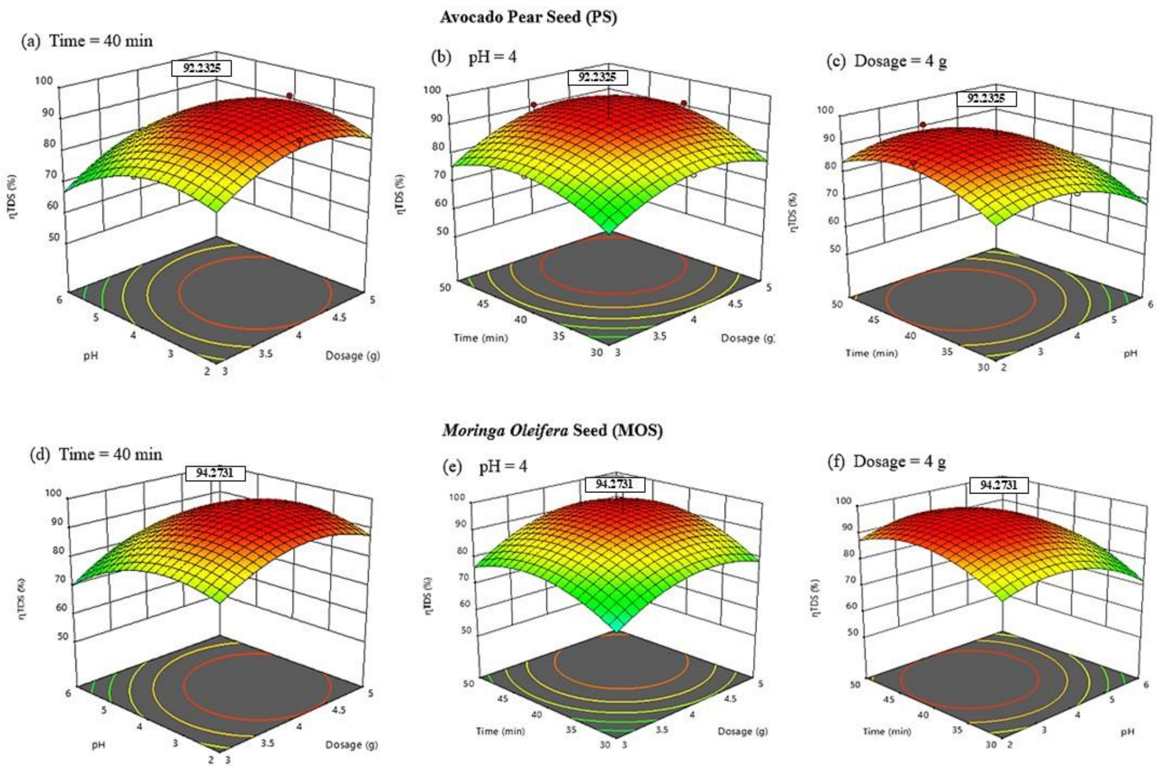

Figure 7. Response surface plots showing the interactive effects of dosage, pH, and treatment time on TDS removal using: (a–c) PS and (d–f) MOS bio-coagulants.

3.7.5 Experimental Validation

The experimental validation results are presented in Table 9. Validation experiments were conducted in triplicate under the optimum operating conditions predicted by the response surface models to verify their predictive capability. The optimum conditions consisted of a coagulant dosage of 4.0 g, solution pH of 4.0, and treatment time of 40 min for both bio-coagulants. The triplicate validation experiments yielded TDS removal values of 89.7%, 92.5% and 91.8% for avocado pear seed (PS), and 92.9%, 95.0% and 94.8% for *Moringa oleifera* seed (MOS), corresponding to mean removal efficiencies of 91.33 ± 1.46% and 94.23 ± 1.16%, respectively. These experimental values were in excellent agreement with the corresponding model-predicted TDS removals of 92.23% for PS and 94.27% for MOS, with percentage deviations of only 0.98% and 0.04%, respectively. The strong agreement between the predicted and experimental responses confirms the adequacy, robustness, and predictive reliability of the generated quadratic models. These results further illustrate that the response surface methodology is an efficient optimization tool for predicting and maximizing TDS removal during coagulation–flocculation treatment of brewery wastewater.

Table 9. Experimental Validation of the Optimized RSM Models for TDS Removal

Bio-coagulant	Optimum Dosage (g)	Optimum pH	Optimum Time (min)	Experimental TDS Removal (%) (Mean ± SD)	Predicted TDS Removal (%)	Percentage Deviation (%)
PS	4.0	4.0	40	91.33 ± 1.46	92.23	0.98
MOS	4.0	4.0	40	94.23 ± 1.16	94.27	0.04

3.7.6 Practical Implications and Industrial Considerations

Numerical optimization showed that for both PS and MOS, pH 4 was the optimum operating condition. However, adjusting large volumes of industrial wastewater to this acidity may result in increased chemical consumption and operating costs at full-scale implementation. Thus, the optimum laboratory conditions determined in this study should be considered as conditions for maximum treatment efficiency rather than fixed operating requirements. Operation at moderately acidic or near-neutral pH may provide a practical compromise between treatment efficiency and cost, depending on wastewater characteristics and existing treatment infrastructure. Compared with conventional coagulants such as alum and ferric chloride, PS and MOS offer additional sustainability advantages reported in the literature, including their renewable origin, biodegradability, lower risk of residual metal contamination, and production of more environmentally acceptable sludge [14,15,16,36]. Also, the use of avocado pear seed supports resource recovery from wastes, hence promoting circular economy principles [17]. Although treatment cost, sludge production, and residual metal concentrations were not experimentally evaluated in this study, the high pollutant removal efficiencies obtained suggest that both bio-coagulants are promising sustainable alternatives to conventional chemical coagulants. Future studies should therefore investigate their techno-economic performance under realistic operating conditions, including strategies such as partial pH adjustment and integration with existing treatment processes to minimize chemical consumption.

3.8 Comparison with Previous Studies

Table 10 compares the performance of PS and MOS with other plant-derived bio-coagulants reported for different wastewater types. Unlike most previous studies which evaluated only one or two water quality parameters, the present work simultaneously investigated TDS, turbidity, COD, and BOD_5, thereby providing a more comprehensive assessment of bio-coagulant performance. The TDS removal efficiencies achieved by MOS (94.2%) and PS (91.3%) are comparable to or higher than those reported for most natural coagulants. Although slightly higher TDS removals were reported for okra, aloe, and cactus-based coagulants, as well as watermelon seed blended with alum, these studies either did not evaluate COD and BOD_5 or involved chemical coagulants or more elaborate preparation methods. Particularly, considerable variation in coagulant preparation, ranging from simple seed powders and aqueous extracts to NaOH extraction, NaCl extraction, and defatted seed cake blended with alum were observed. Conversely, the present work used solely distilled-water extracts for PS and MOS yet obtained comparable removal efficiencies. This shows that effective bio-coagulants could be prepared by simple and eco-friendly methods without the necessity of chemical extraction or modification. Altogether, the comparison confirms that PS and MOS are among the most effective plant-based bio-coagulants reported in the literature and indicates the novelty of this study as it is one of the few studies that simultaneously assess TDS, turbidity, COD and BOD_5 removal using two plant-based bio-coagulants prepared under the same conditions.

Table 10. Comparison of TDS, Turbidity, COD and BOD_5 Removal Efficiencies of Plant-Derived Bio-Coagulants Reported in Literature

Bio-coagulant	Wastewater	TDS (%)	Turbidity (%)	COD (%)	BOD_5 (%)	Coagulant Preparation	Reference
Moringa oleifera seed	Brewery	94.2	93.3	77.5	68.8	Distilled-water extract	This study
Avocado pear seed	Brewery	91.3	85.1	88.9	89.2	Distilled-water extract	This study
Date seed	Textile	—	83.96	56.19	—	Distilled-water and NaCl extracts	[30]
Watermelon seed + alum	Surface Water	98.26	—	95.26	96.10	Defatted seed cake (n-hexane) blended with alum	[36]
Okra seed	Brewery malting	95.25	88.83	—	—	Oven-dried, ground and sieved powder	[37]
Aloe vera	Brewery malting	95.40	74.25	—	—	Oven-dried, ground and sieved powder	[37]
Opuntia cactus	Brewery malting	95.00	74.66	—	—	Oven-dried, ground and sieved powder	[37]
Blend (Okra + Aloe + Cactus)	Brewery malting	95.12	83.92	—	—	Blend of oven-dried, plant powders	[37]
Moringa oleifera seed	Mining pond	23.1*	70.6*	35.8*	—	Seed powder (direct application)	[38]
Watermelon seed	Tannery	—	86.7	50.0	55.0	Not specified	[39]
Moringa oleifera leaves	Surface Water	83.46	87.0	88.57	—	Fresh leaf aqueous extract (10% w/v)	[40]
Banana trunk peduncle	Municipal	—	90.2	94.8	—	2.5 M NaOH extract	[41]
Moringa oleifera seed cake	University dormitory	52–64	96.0	92.0	92.0	Air-dried, ground and sieved seed cake	[42]

Notes: (i) Blank entries (—) indicate that the corresponding parameter was not reported in the referenced study. (ii) The percentages marked with an asterisk (*) were calculated from the initial and final values reported in the cited study rather than being explicitly stated. Specifically, turbidity decreased from 17 to 5 NTU (≈70.6%), TDS from 13 to 10 mg L^{-1} (≈23.1%), and COD from 23.2 to 14.9 mg L^{-1} (≈35.8%).

4.0 Conclusion

This work explored the potential of avocado pear seed and Moringa oleifera seed as sustainable bio-coagulants for brewery wastewater treatment. The two bio-coagulants were efficient in reducing the principal pollutants such as total dissolved solids, turbidity, chemical oxygen demand, biological oxygen demand and other critical water quality indicators. Maximum TDS removal efficiencies of 91.33 ± 1.46% and 94.23 ± 1.16% were obtained for PS and MOS, respectively, under the optimum conditions predicted by RSM and confirmed through validation experiments. The optimum operating conditions for both bio-coagulants were pH 4, 4 g/500 mL of wastewater, and 40 min treatment time. Comparative evaluation showed that MOS was more suitable for suspended solids reduction, as reflected by its higher TDS and turbidity removal and PS was effective for organic load reduction, particularly COD and BOD_5. FTIR characterization revealed that both bio-coagulants possess functional groups that can participate in coagulation–flocculation. Based on the FTIR results and relevant literature, mechanistic interpretation revealed that MOS mainly operates through charge neutralization and interparticle bridging, while PS mainly functions through adsorption and bridging of dissolved organic matter. Although pH 4 produced the best treatment efficiencies under laboratory conditions, its practical implementation at industrial scale may require consideration of the additional costs associated with wastewater acidification. Finally, the complementary treatment behaviour observed for MOS and PS suggests that their combined application may provide enhanced simultaneous removal of suspended solids and dissolved organic pollutants. However, this hypothesis was not experimentally investigated in the present study and should therefore be viewed as a significant direction for future work.

List of Abbreviation

3-D – Three Dimension

AAS – Atomic Absorption Spectrophotometry

AOAC – Association of Official Analytical Chemists (AOAC INTERNATIONAL)

APHA – American Public Health Association

BOD_5 – Biochemical Oxygen Demand (5-day)

BWW – Brewery Wastewater

CCD – Central Composite Design

COD – Chemical Oxygen Demand

FTIR – Fourier Transform Infrared Spectroscopy

MOS – *Moringa oleifera* Seed

NTU – Nephelometric Turbidity Unit

OFAT – One-Factor-at-a-Time

PS – Avocado Pear Seed

R^2 – Coefficient of Determination

RSM – Response Surface Methodology

TDS – Total Dissolved Solids

TS – Total Solids

TSS – Total Suspended Solids

ANOVA- Analysis of Variance

SD - standard deviation

CV - Coefficient of variation

Author Contributions

Conceptualization, methodology, software: C.O.A. O.C.N., and O.O.; Validation, formal analysis, funding acquisition: C.O.A. O.C.N., and O.O; Investigation, resources, data curation, writing—original draft preparation, writing—review and editing, visualization, supervision, project administration: C.O.A. O.C.N., and O.O. All authors have read and agreed to the published version of the manuscript.

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Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. Further details, including raw experimental measurements and supporting materials, are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there is no conflicts of interests regarding the publication of this manuscript.

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AI Declaration

The authors confirm that no AI tools were used to generate any part of the manuscript text, including the research design, data collection, data analysis, interpretation of the findings, and manuscript preparation and revision.

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