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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 (BOD5), turbidity, and total dissolved solids (TDS) in brewery wastewater pose environmental and public health concerns. Avocado pear seed (PS) and *Moringa oleifera* seed (MOS) were evaluated as sustainable bio-coagulants for treating brewery wastewater through coagulation–flocculation experiments. The bio-coagulants were characterized using Fourier transform infrared spectroscopy and proximate analysis. Response surface methodology 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, suggesting pollutant removal proceeds through charge neutralization, adsorption, and interparticle bridging. Under optimized operating conditions (pH 4, dosage 4 g, treatment time 40 min), PS and MOS achieved TDS removals of 91.27% and 94.19%, respectively. Turbidity removal efficiencies were 85.1% and 93.3%, while COD removal efficiencies were 88.9% and 77.5%, and BOD5 removal efficiencies were 89.2% and 68.8% for PS and MOS, respectively. Comparison with previously reported plant-derived bio-coagulants showed both PS and MOS exhibit competitive treatment performance despite using simple distilled water extraction. These findings establish avocado pear and *Moringa oleifera* seeds as effective, low-cost, and sustainable alternatives to conventional chemical coagulants for brewery wastewater treatment.

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 recognized for their substantial water consumption during brewing, cleaning, cooling, and packaging operations [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 as a result of intense microbial activity [4]. In addition, BOD_5 contains biodegradable organics, while COD contains both biodegradable

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and non-biodegradable organics. For this reason, COD generally has a higher value than BOD_5 [2,4]. Besides 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, affecting the health of aquatic organisms. The study by [6] examined the effects of irrigating with wastewater containing high TDS levels, reporting soil salinity build-up, reduced crop growth, and long-term soil degradation. These concerns have driven research toward the development of efficient treatment strategies capable of simultaneously reducing organic pollutants (BOD_5 and COD) and dissolved solids (TDS) before being 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 that generates wastewater streams reflecting the complex nature of modern industrial brewing [7]. According to [7,8], the Enugu facility is one of the oldest in Nigeria and produces effluents rich in organic matter, carbohydrates, proteins, spent yeast, detergents, cleaning agents, and process-derived salts. These impurities are consistent with typical 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 for developing regions. The wastewater investigated in this study was collected from this facility, providing a realistic 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 to treat brewery wastewater because they can effectively remove suspended and dissolved contaminants [8–11]. Despite their effectiveness, these technologies are often expensive and energy-intensive, may require advanced operational expertise, and can 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, attention has increasingly shifted toward plant-derived bio-coagulants as environmentally sustainable alternatives because they are biodegradable, renewable, locally available, and can reduce 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 high operational costs.

Among plant-derived bio-coagulants, *Moringa oleifera* seed (MOS) is 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 to remove 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 being produced in large quantities as an agricultural by-product in many tropical countries. The limited investigation is mainly due to the longstanding research focus on MOS as the benchmark natural coagulant and the more limited characterization of the coagulation properties of avocado seed extracts. However, the abundance of proteins, carbohydrates, polyphenolic compounds, and oxygen-containing functional groups in PS suggests strong 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 principles while reducing the environmental burden of 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 characterized using 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 support coagulation-flocculation mechanisms such as charge neutralization, adsorption, hydrogen bonding, hydrophobic interactions, and interparticle bridging, which facilitate particle destabilization, floc formation, and contaminant removal [14,19,21,22]. Coagulation efficiency generally increases with higher protein content because proteins contain charged amino acids that promote electrostatic

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attraction and destabilize colloidal particles. This is particularly true for MOS, which often demonstrates higher protein content compared with many other plant-based seeds [14–18].

Coagulation and flocculation remain central processes in water and wastewater treatment. The performance of bio-coagulants depends on the physicochemical mechanisms through which contaminants are destabilized, aggregated, and removed [22–25]. The procedure typically proceeds through 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 larger flocs [23]. (c) Sweep flocculation: High coagulant dosages lead to precipitate formation that entraps particles during settling. This mechanism is more typical of 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* 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. These studies further indicate that these molecular characteristics contribute to 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 adsorbent for industrial wastewater treatment [14–18,22–26]. Comparatively, fewer studies have examined avocado pear seed as an abundant agricultural by-product with a promising biochemical composition [17]. Moreover, these investigations have concentrated mainly on turbidity reduction, dye removal, or the treatment of 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 modeling 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 modeling, 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 treating 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 removing TDS, turbidity, COD, and BOD_5. TDS was selected as the primary response variable for process optimization. The effects of pH, coagulant dosage, and treatment time were initially investigated using one-factor-at-a-time experiments and subsequently optimized using 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 behavior 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, an electronic weighing balance, a laboratory mortar and pestle, a pH meter, a magnetic stirrer, a heating tube, a drying oven, and a stopwatch. Table 1 presents the raw materials used for bio-coagulant preparation and coagulation–flocculation experiments, along with the analytical reagents used for physicochemical and proximate characterization of the bio-coagulants.

Table 1: Materials and analytical reagents used in this study.

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

2.2. Preparation and Extraction of Bio-Coagulants

PS and MOS were obtained from Agbani in Nkanu West Local Government Area, Enugu State, Nigeria. They were separately washed, dried, and ground to a fine powder using a laboratory mortar and pestle. The active ingredient from each processed seed was extracted in line with a 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, and 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. Therefore, 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 carried out in batches for each experimental run to minimize deterioration of the active components. The filtrates were thoroughly shaken 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 the two 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 used directly after preparation without drying or concentration. Consequently, 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. Section 3.2 presents processing yields based on the processed seed materials.

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 fiber, 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 fiber 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 during the peak production period (February–March 2024). The collected BWW sam-

ple was carefully transferred to an acid-cleaned polyethylene container that was well rinsed before use. This process ensured that the physicochemical properties of the sample were maintained prior to laboratory analysis.

2.6. Characterization of Brewery Wastewater (BWW)

The physical and chemical properties of the BWW sample were 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 differ 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, evaporated to dryness, then cooled and reweighed to quantify the residual solids. TDS concentration was computed following Equation (1) [26]:

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

where W_1 is the weight of the empty beaker (mg), W_2 is the weight of the beaker plus the 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 calibrated with standard reference solutions, while electrical conductivity was determined using a standardized conductivity meter before use [26,31]. The pH, temperature, and viscosity of the samples were measured in situ using a calibrated pH meter, thermometer, and viscometer, respectively, under controlled temperature conditions [26,31]. For metal analysis, selected metals were determined using atomic absorption spectrophotometry (AAS). To ensure consistency, accuracy, and reproducibility of results, all measurements were conducted following established standard methods [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 brewery wastewater (BWW). 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. Subsequently, 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. Based on the OFAT results, narrower experimental ranges were subsequently selected for 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]:

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

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 conducted using a one-factor-at-a-time (OFAT) approach to assess the individual effects of pH, coagulant dosage, and treatment time on TDS removal. During each experiment, the remaining operating variables were kept constant to isolate the effect of the variable under investigation. These preliminary investigations provided the experimental basis for selecting suitable factor ranges for subsequent optimization.

Thereafter, Response Surface Methodology (RSM) based on a 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 determine the optimum treatment conditions for maximum TDS removal. A face-centered CCD ($\alpha = 1$) 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 spec-

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tra 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 3283.8 cm^{-1} (PS) and 3287.5 cm^{-1} (MOS), corresponding to O–H/N–H stretching vibrations, together with a distinct O–H stretching band at 3693.8 cm−1 for MOS, were observed. These protein- and hydroxyl-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 presence of nitrogen-containing functionalities 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 may indicate ether linkages that provide 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 promote interactions with dissolved organic contaminants through 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 favor floc growth through hydrophobic interactions [24]. The bands at 1319.5 and 1315.8 cm^{-1} were assigned to C–N/C–O and deformation vibrations, while the bands at 1010.1 and 1028.7 cm^{-1} were assigned to C–O/C–O–C stretching associated with carbohydrate and polysaccharide structures. 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]. However, 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, because FTIR characterization was performed only on the bio-coagulants before BWW treatment, possible shifts in absorption bands after wastewater treatment, which could provide direct evidence of contaminant binding, were not investigated. Hence, it is recommended that future studies 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

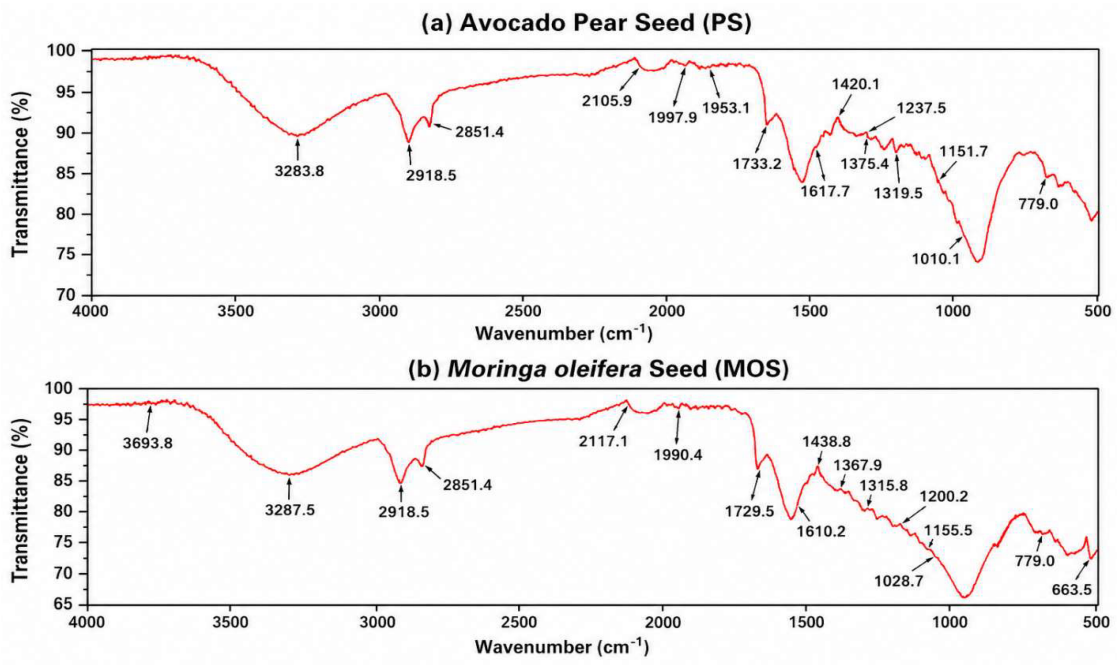


Figure 1: FTIR spectra of (**a**) avocado pear seed (PS) and (**b**) *Moringa oleifera* seed (MOS) bio-coagulants, illustrating the characteristic absorption peaks used for functional group identification.

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 freshly prepared 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 imply good storage stability [26]. The higher protein content of MOS (37.16% vs. 18.96%) also indicates greater potential coagulation activity relative to PS, as proteins are the primary active agents in bio-coagulants [15]. The fat contents of both bio-coagulants were below 5%, indicating a minimal 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 Fiber (%)	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. Both coagulants achieved high removal efficiencies (>65%), although with different performance patterns. MOS exhibited superior efficiency in reducing

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TDS (94.19%) and turbidity (93.30%) compared with PS (91.27% 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 performance of these bio-coagulants suggests that their combined application could provide a more balanced and optimized approach to wastewater treatment. 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, both coagulants greatly reduced conductivity and trace metal concentrations, 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 [14–16].

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
Aluminum	(mg/L)	3.264	0.681	0.051
Iron	(mg/L)	1.408	0.212	0.054

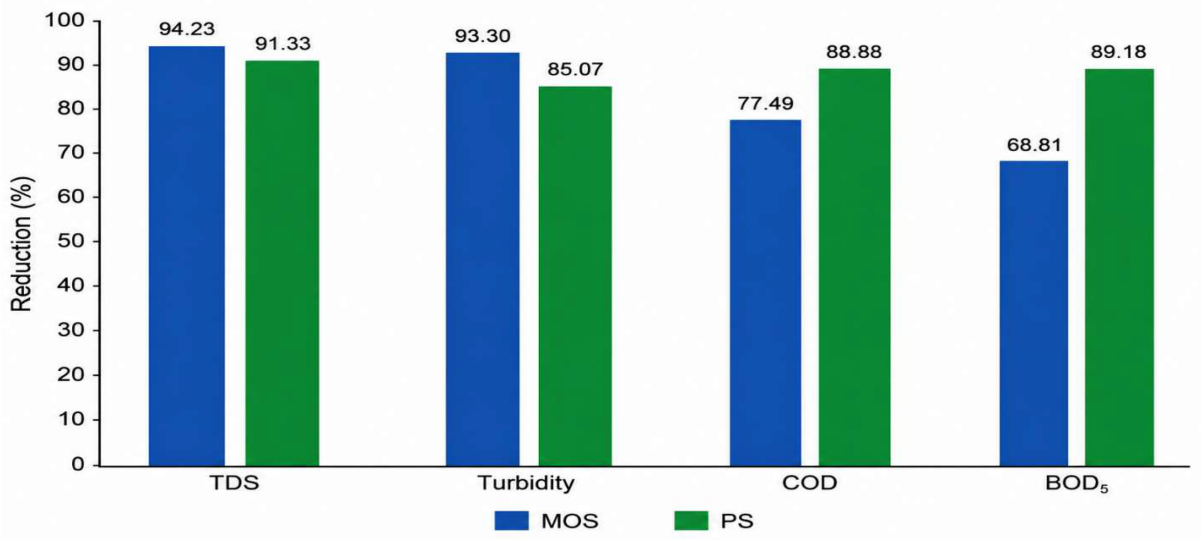


Figure 2: Comparative evaluation of the removal efficiencies of MOS and PS across four key water quality parameters: TDS, Turbidity, COD and BOD_5.

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 by FTIR in Section 3.1 with established coagulation mechanisms reported for protein- and polysaccharide-based natural coagulants [14–16]. Thus, FTIR confirms the presence of chemical functionalities capable of participating in coagulation, whereas the individual pathways illustrated in Figure 3 represent mechanistic interpretations supported by the 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 favor charge neutralization of negatively charged colloidal particles through electrostatic interactions, reducing repulsive forces and promoting particle aggregation.

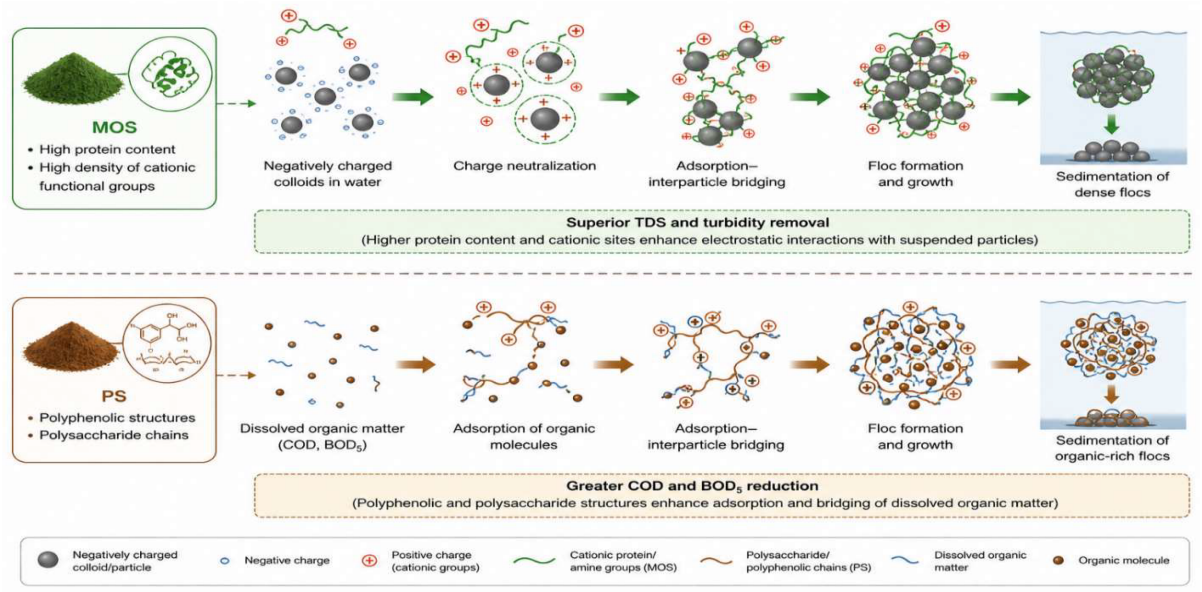


Figure 3: Coagulation–flocculation mechanism of *Moringa oleifera* seed (MOS) and avocado pear seed (PS) bio-coagulants. Created with BioRender.com.

Long-chain biomolecules subsequently facilitate adsorption–interparticle bridging, leading to the formation of larger and denser flocs [14–16]. This mechanism explains the higher TDS and turbidity removal efficiencies obtained with MOS due to its high protein content and greater 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 [14–16]. The continued adsorption and interparticle bridging promote the 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 favors interactions with dissolved and biodegradable organic matter rather than with 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 relies more on adsorption and bridging of dissolved organic matter. Their complementary behavior 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 the 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.

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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. In practical water treatment, the focus on TDS aligns with reports [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 bio-coagulant performance.

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 min, the effect of pH on TDS removal efficiency was evaluated. As shown in Figure 4, TDS removal increased with pH up to approximately pH 4, beyond which efficiency declined with further increases in pH. This behavior is consistent with the pH-dependent performance characteristics of bio-coagulants and agrees with reports of enhanced TDS removal under acidic conditions [14–16].

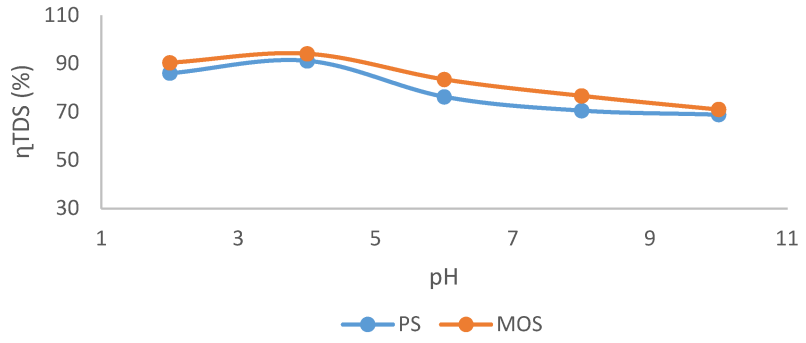


Figure 4: Effect of pH on TDS removal.

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 min. The removal efficiency showed a non-linear trend with increasing dosage. TDS removal increased steadily up to a maximum at a dosage of 4 g/500 mL, as a result of enhanced charge neutralization and particle–coagulant interactions, but declined beyond this point as overdosing caused particle destabilization and the introduction of excess dissolved matter [11,12,14]. This pattern indicates that optimal coagulant dosing is critical for achieving maximum coagulation efficiency.

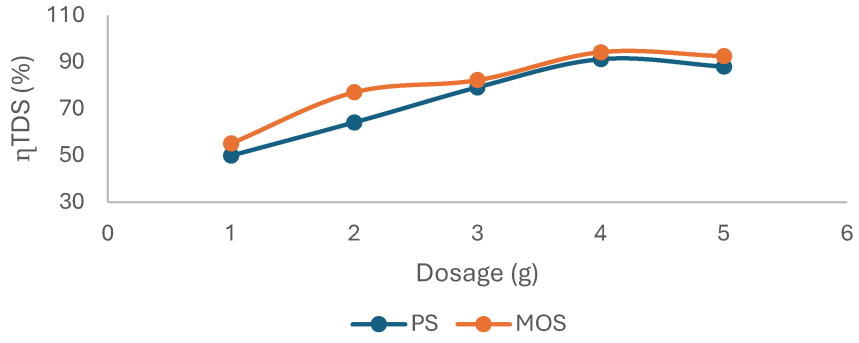


Figure 5: Effect of dosage on TDS removal.

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 min, after which it gradually decreased. This pattern reflects flocculation kinetics, as sufficient contact time is required for particle collision, aggregation, and settling [24]. The fact that both bio-coagulants achieved maximum removal within the investigated treatment-time range of 40 min indicates that both require a moderate flocculation duration to achieve maximum performance. This agrees well with the reported behavior of natural coagulant systems [34].

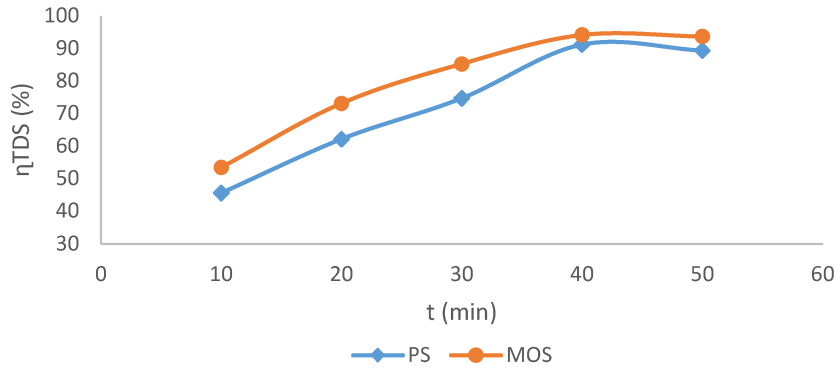


Figure 6: Effect of treatment time on TDS removal.

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 behavior 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 modeling [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 favorable and unfavorable 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 a dosage of 4 g, pH 4-, and 40-min. Lower removal efficiencies were generally observed when the pH or dosage deviated from these optimum conditions. A direct comparison 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 higher concentration of positively charged coagulating proteins enhances charge neutralization and floc formation during wastewater treatment.

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 significant curvature in the response surfaces and justifies adopting 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 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

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

Table 8 presents the statistical adequacy of the developed quadratic models. The coefficients of determination (R^2 = 0.9918 for PS and 0.9916 for MOS) indicate that the developed models explain over 99% of the variability in TDS removal. 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 ex-

perimental variability and further supports the robustness of the developed response surface models [35].

Equations (3) and (4) present the fitted second-order polynomials—including linear, interaction, and quadratic terms—generated from the CCD experimental design, serving as predictive tools for estimating TDS removal within the studied factor ranges; both final empirical models are expressed here in terms of coded factors.

$$\mathrm{TDSPS} = +92.23 + 4.66\mathrm{A} - 4.02\mathrm{B} + 3.73\mathrm{C} + 1.68\mathrm{AB} + 1.13\mathrm{AC} + 1.14\mathrm{BC} - 7.29\mathrm{A}^2 - 7.63\mathrm{B}^2 - 7.37\mathrm{C}^2 \quad (3)$$

$$\mathrm{TDSMOS} = +95.13 + 4.66\mathrm{A} - 4.02\mathrm{B} + 3.74\mathrm{C} + 1.69\mathrm{AB} + 1.14\mathrm{AC} + 1.14\mathrm{BC} - 7.28\mathrm{A}^2 - 7.61\mathrm{B}^2 - 7.34\mathrm{C}^2 \quad (4)$$

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 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 increases produced little or no improvement. This behavior indicates the existence of optimum operating conditions, although excessive dosage, longer treatment time, or higher pH reduced coagulation efficiency because of particle destabilization, 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 are 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 favor charge neutralization and interparticle bridging. The response surface models predicted optimum operating conditions of a dosage of approximately 4g, 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.

3.7.5. Experimental Validation

Table 9 presents the experimental validation results. 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 findings further demonstrate the effectiveness of response surface methodology as an optimization tool for predicting and maximizing TDS removal during the coagulation and flocculation treatment of brewery wastewater.

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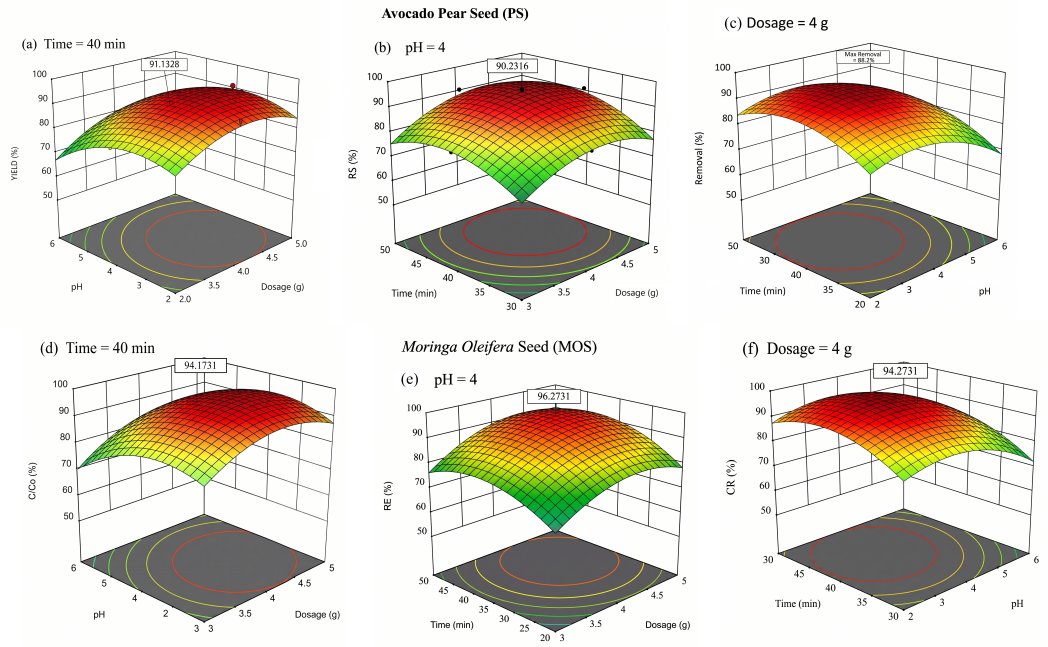


Figure 7: Response surface plots showing the interactive effects of process variables on TDS removal using PS and MOS bio-coagulants: (**a**) effect of pH and dosage at a treatment time of 40 min for PS; (**b**) effect of dosage and treatment time at pH 4 for PS; (**c**) effect of pH and treatment time at a dosage of 4 g for PS; (**d**) effect of pH and dosage at a treatment time of 40 min for MOS; (**e**) effect of dosage and treatment time at pH 4 for MOS; and (**f**) effect of pH and treatment time at a dosage of 4 g for MOS.

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 pH 4 was the optimum operating condition for both PS and MOS. However, adjusting large volumes of industrial wastewater to this acidity may increase chemical consumption and operating costs during 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 [14–16,36]. In addition, using avocado pear seed supports resource recovery from waste, promoting circular-economy principles [17]. Although this study did not experimentally evaluate treatment cost, sludge production, or residual metal concentrations, the high pollutant removal efficiencies 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 that evalu-

ated 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. In particular, 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, was observed. Conversely, the present work used only distilled-water extracts for PS and MOS yet obtained comparable removal efficiencies. This shows that effective bio-coagulants can be prepared by simple, eco-friendly methods, without the need for 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 the 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%).

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4. Conclusions

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 efficiently reduced key 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 higher TDS and turbidity removal, while 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. At the same time, 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 behavior 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 evaluated in the present study and should therefore be considered an important area for future investigation.

List of Abbreviations

3-D	Three Dimension
AAS	Atomic Absorption Spectrophotometry
ANN	Artificial Neural Networks
ANOVA	Analysis of Variance
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
CV	Coefficient of variation
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
SD	standard deviation
TDS	Total Dissolved Solids
TS	Total Solids
TSS	Total Suspended Solids

Author Contributions

Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft, writing—review and editing, visualization, supervision, and project administration: C.O.A., O.C.N., and O.O. All authors contributed equally to this work and have read and agreed to the published version of the manuscript.

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 no conflicts of interest.

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AI Declaration

The authors confirm that no AI tools were used in the research design, data collection, data analysis, interpretation of findings, or preparation and revision of the manuscript text. The authors take full responsibility for the accuracy, originality, integrity, and scientific content of the manuscript.

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