Malaysian Journal of Chemistry, 2024, Vol. 26(1), 154-167
(Special Issue: *International Conference on Natural Products, ICNP 2023*)

Potential of Fenugreek and Cowpea Seeds as Natural Coagulants for Wastewater Effluent Treatment

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Water is vital to all living organisms. However, rapid urbanization has deteriorated the water ecosystem. Therefore, emerging treatments must be implemented to enhance water quality before consumption. Thus, it is important to improve the quality of effluent in wastewater treatment plants before being discharged into the water bodies. This study comprised the potential of fenugreek and cowpea seeds as natural coagulants in the tertiary treatment to reduce the effluent pollutants. A jar test experiment was conducted with variables ratio of green substances (fenugreek and cowpea seeds) in the effluent medium. The characteristics of the effluent before and after treatment were analyzed for biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, and turbidity. The optimum concentration and pH for each bio-coagulant were evaluated. From the experimental results for the highest turbidity removal, 7.11 Nephelometric Turbidity unit (NTU) was determined for cowpea seeds of 0.2 mg/L concentration. Meanwhile, the optimum concentration for fenugreek was 0.6 mg/L, with turbidity removal rates of 7.93 NTU. Therefore, from the observation, the lowest turbidity removal was 20%, and the maximum was 41%. The Fourier Transform Infrared (FTIR) Spectroscopy indicated that the chemical structure of each polymer had changed (before and after treatment), which involved the production of C=O, O-H in fenugreek, cowpea seeds, with both coagulants and using aluminium sulfate, respectively. Moreover, isotherm analysis for the adsorption capacity of bio-coagulants has been determined. From the output evaluation, this natural coagulant can be an effective agent in the water treatment process. It can be compared with the efficiency values achieved using these green substances in contrast to the treatment of chemicals coagulant.

Keywords: Biopolymer; coagulant; cowpea seeds; fenugreek; effluent; treatment

Received: September 2023 ; Accepted: November 2023

The global trends in pollution cases have risen yearly, and this scenario greatly impacted the quality of surface water bodies. Moreover, environmental degradation has deteriorated the aquatic ecosystem [1]. In order to stipulate the Sustainable Development Goals (SDGs), Goal 6 - clean water and sanitation and Goal 14 - life below water, the treated effluent from the wastewater treatment plant (WWTP) needs to be in excellent condition [2] which need to be referred to standard guidelines [3]. The discharges standard of wastewater must follow the Environmental Quality (Industrial Effluents) Regulations addressed by the Department of Environment, Malaysia [3].

Recently, several methods have been explored to replenish the techniques in upgrading the tertiary treatment for wastewater treatment plants [4]. This is crucial to enhance the quality of the final discharge. Furthermore, preserving the water bodies from emerging contaminants (EC) substantially reduces pollution to the environmental ecosystem. Tertiary treatment emphasized several methods, and one of the techniques employed uses coagulation-flocculation-based processes [5]. Coagulant usage can be classified into inorganic coagulant (IC) and organic coagulant (OC). Each coagulant

†Paper presented at the International Conference on Natural Products 2023 (ICNP 2023)

working principle has advantages and disadvantages in removing pollutants efficiently. IC is mostly used in Europe, Asia, and other continents due to its cost-effectiveness and competence [6]. However, OC is considered highly beneficial regarding the lower dosage, less sludge produced and natural working pH [7]. Four basic removal mechanisms have been evaluated for contaminants removal, especially turbidity: sweep flocculation, charge neutralization, double layer compression and inter-particle bridging (biopolymer) [1].

The coagulation and flocculation processes happen, whereas chemicals are frequently added to water in a rapid mixing stage, boosting particle instability and resulting in floc formation. In the sedimentation processes, the formed flocs will be removed after being separated from the liquid water [8]. It can be denoted that chemical coagulants and flocculants are undoubtedly the most efficient techniques for lowering water turbidity [9]. However, their residues in treated water could bring damage to human health as well as the environment. For example, the widespread use of aluminium sulfate may result in secondary environmental contamination issues [10]. It is due to the vast amount of toxic sludge created, which is associated with health concerns, particularly Alzheimer's disease, which will be triggered by residual aluminium in the treated water. Furthermore, traces of monomers that remain in the water after the clarification process, utilized from synthetic polymers, are known to be neurotoxic and carcinogenic [11].

Nevertheless, because of public awareness and growing concerns due to the ecotoxicological effects of the chemical's coagulants, scientific research has embarked on acquiring safer substitutes [12]. Green chemistry has been applied to derivate natural coagulants from plant-based materials [13]. Even though exploiting plants for water purification are already present, a deeper investigation into the mechanism of natural coagulants towards pollutants still needs to be completed [14]. Developing a new, more efficient, economic, and ecological precursor will enable the adaptation of sustainable solutions. Indigenous and inexpensive renewable plant sources can be implemented as coagulant substances for the newly proposed treatment. These green materials can be vastly used in treating water for consumption and industrial purposes [15].

In embracing the circular economy, the approaches to using organic waste material such as seed, peel, skin, or plant leaves are aligned with the zero-waste principle. Natural coagulants (such as biopolymers) are commonly claimed to be more environmentally friendly regarding production use and reducing biological waste in landfill [4]. Therefore, this research investigated the performance of cowpea and fenugreek seed extract as biopolymers for tertiary wastewater treatment. It is to enhance the quality of effluent following the stringent quality standards before being discharged to the water bodies [3]. This study emphasized the removal efficiency of pollutants by employing several dosages of cowpea and fenugreek seeds bio-polymer. Thus, applying the biopolymer will be the substitute for the chemical coagulants. Moreover, this can tremendously reduce the amount of chemical coagulant usage and promote sustainable practices.

EXPERIMENTAL

Sample Collection

The samples have been collected from the waste-water treatment plant (WWTP) effluent in Universiti Teknologi MARA, Kampus Dengkil. The effluent samples from WWTP are stored in a bottle and kept cool in the refrigerator before being tested [16]. The testing was done in situ and in the laboratory. For laboratory testing, the experiment was conducted in the Environmental Laboratory in the School of Civil Engineering, College of Engineering, Universiti Teknologi MARA, Shah Alam, Selangor.

In-situ Parameter

The in-situ testing was done on-site using HORIBA portable instruments [17]. These include physical parameters such as dissolved oxygen (DO), pH and temperature [16].

Laboratory Testing

Laboratory assessments have been done in triplicate, and the average values are obtained for biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia-nitrogen (NH_3-N), nitrite-nitrogen, nitrate-nitrogen, and total suspended solids (TSS) using HACH spectrophotometer [18]. In addition, the microbiology testing of *E-Coli* is done using the Lovibond *E-Coli* test apparatus to measure the existence of *E-coli* bacteria in the samples [16].

Environmental Quality Regulations

The effluent samples before and after treatment were compared based on Environmental Quality (Industrial Effluents) Regulations 2009 [3].

Figure 1: Cowpea *(V. unguiculata* (L.) Walp.).

Figure 2: Fenugreek Seeds *(T. foenum-graecum*).

Preparation of the Biopolymer

All the seeds utilized in this study were cowpea (*Vigna unguiculata* (L.) Walp., Leguminosae) (see Figure 1) and fenugreek (*Trigonel foenum-graecum*, Fabaceae) (see Figure 2) purchased from a local Malaysian market. The seeds were washed with deionized water to eliminate the impurities such as stones, plant debris, and dust and then dried in a 40°C oven until completely dry. The dry seeds were ground manually into a fine powder using a laboratory mortar and pestle. The powder seed was sieved, and only powder retained in the 0.3 mm sieve sizes was utilized for the coagulant production [1].

Preparation of Crude Extract

An amount of 25 g produced powder was suspended in 500 mL of distilled water or 0.5 mol/L NaCl solution and agitated for 10 minutes with a magnetic stirrer [1]. The suspensions were then gravity filtered through a filter paper, yielding crude extracts of coagulation active components.

Preparation of Aluminium Sulfate Solution

An amount of 1 g of analytically grade pure $Al_2(SO_4)_3$. $18H_2O$ (Sigma Aldrich Chemical Co. (USA)) has been dissolved in 1 L of deionized water to make the aluminium sulfate coagulant solution. The aluminium sulfate solution was mixed and homogenized before being stored at 4°C in the refrigerator.

Jar Test Experiments

Jar tests were performed with a standard apparatus that included six 1 L beakers. This experiment was done to obtain the appropriate coagulant concentration for coagulation processes. The water samples containing 1 L each were rapidly agitated at 80 rpm for 2 minutes [1]. During the testing, varied coagulant concentration were introduced to the beakers for effective coagulant dispersion. The coagulant concentration was 0.2 mg/L, 0.4 mg/L, 0.6 mg/L, 0.8 mg/L, 1.0 mg/L and 1.2 mg/L were administered in the 1 L effluent sample of jar test.

Furthermore, a control beaker with the same amount of effluent under the same conditions but without any coagulant was also performed. The mixing speed was dropped to 30 rpm for 10 minutes at the flocculation stage. In facilitating the settlement processes, the suspension was left undisturbed for 30 minutes [13]. The extended sedimentation time was chosen to evaluate the process's effectiveness. A final treated water sample (10 mL) was collected from the top surface of the water in the beakers with a syringe [13]. Treated samples (in triplicates) were then checked following the parameters of in-situ and laboratory testing.

Fourier Transform Infrared (FTIR)

The Fourier Transform Infrared (FTIR) Spectrometer (Perkin et al./SDTA 851, USA) has been used to evaluate coagulant structural changes. The specimen was examined in the 4000-650 range [1]. This analysis was done for the sample of coagulants before and after the jar test experiment.

RESULTS AND DISCUSSION

Coagulant concentration and pH are the two important factors to consider when assessing the coagulation-flocculation processes' effectiveness. The effects on the coagulant concentration and the starting pH of the untreated sample in this jar test experiment were critically emphasized in this research. Later, this enables the evaluation of optimum values concerning the coagulation and flocculation processes.

Comparison of pH and Dosage of Coagulants

At varied pH levels, trials were conducted to examine the reaction of cowpea and fenugreek seeds powder to remove turbidity. The pH of the effluent sample was adjusted prior to the coagulation experiment with the extraction of cowpea and fenugreek seeds. The surface charge of coagulants and the stability of suspension are mostly affected by the pH of the wastewater. The results of the coagulation test using cowpea and fenugreek seeds extract are depicted in Figure 3. The pH of the solution was changed using HCl (0.1 M) or NaOH (0.1 M) solutions. As shown, the alkaline condition benefited the coagulation performance of cowpea seeds in lowering the turbidity. At pH 9, the cowpea seeds extract was shown to be the most effective coagulant. Moreover, the dual (cowpea and fenugreek seeds) can lower turbidity during the alkaline state.

The highest turbidity reduction can be obtained at pH 9, whereas 9.51 NTU for fenugreek and 6.62 NTU for cowpea. The sample looked clearer at this pH, and more flocs formed in the beaker. However, due to the increasing dosage of the fenugreek bio-polymer, the water became cloudier, with a turbidity reduction ranging from 7.93 to 13.3 NTU. Moreover, fewer flocs were discovered during the settling process. Hence, the lowest turbidity reduction was reported as the sample became more acidic at pH 6.

In the case of cowpea, the water becomes cloudier, with a turbidity reduction ranging from 7.11 to 7.72 NTU, and more flocs were discovered following the settling process due to pH changes. The lowest turbidity reduction was reported when the water became more acidic at pH 5. Dual biopolymer (cowpea and fenugreek seeds) caused the water to become cloudier, with a turbidity reduction ranging from 8.46 to 9.66 NTU as a higher dosage was introduced. The same situation was identified at pH 6 as the lowest turbidity reduction for the biopolymer. It can be denoted that aluminium sulfate caused the water to become unclear, with a turbidity reduction ranging from 7.58 to 8.31 NTU and fewer flocs were discovered during the settling process due to the increased dosage. The lowest turbidity reduction was reported at pH 8 for the aluminium sulfate.

The turbidity has reduced at 20% and 41%, respectively, in the optimum pH for cowpea and fenugreek seed coagulants. When the acidity of the effluent sample rose between pH 5 and pH 6, the coagulative behaviour of cowpea and fenugreek seeds diminished. As a result, the output findings showed that cowpea and fenugreek seeds performed well in alkaline conditions. The acidic nature of water promotes positive charges on molecules, which may impact and improve the molecules' ability to operate as coagulants. Water has an acidic condition due to the presence of hydrogen ions, which raises the positive charge of the water. As a result, this phenomenon considerably impacts the turbidity reduction of wastewater effluent [19].

According to the analysis in Figure 4, the are no significant changes in the *E-coli* and total coliform test after the treatment of coagulants. The measured MPN value for all coagulant, including the raw sample, were < 2419.6. The data has been obtained from the Colilert test using a UV lamp [16]. The results indicated that *E-coli* is present in the effluent sample. It can be denoted that the effluent sample may induce diarrhoea and may cause pneumonia, respiratory ailments, urinary tract infections and other illnesses [20]. Similar findings have been obtained for total coliform bacteria. Disinfection techniques must be imposed to reduce the amount of *E. coli* and coliform bacteria in the sample.

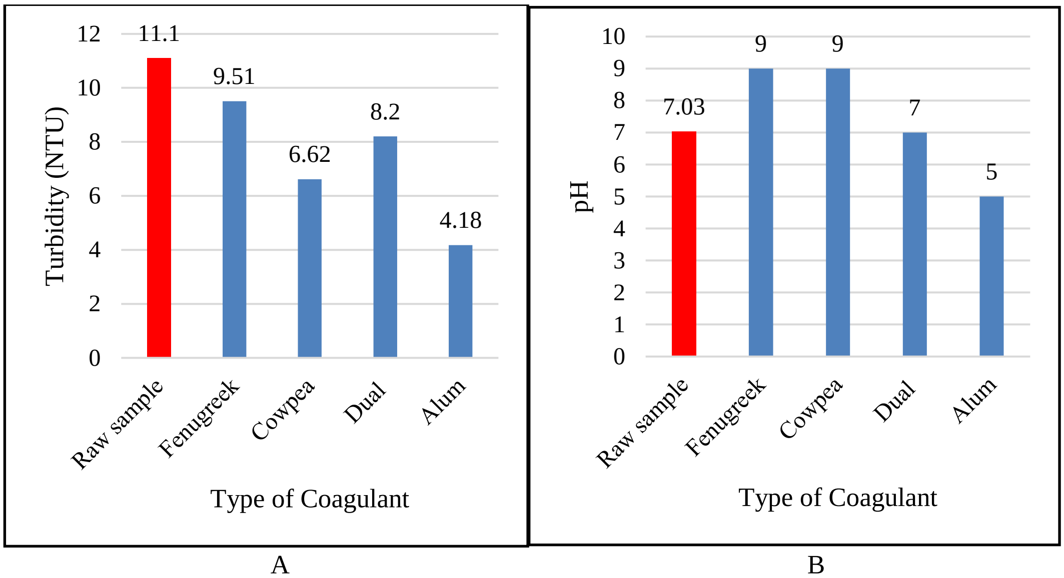


Figure 3. Comparison between A) Dosage and B) pH for the Coagulants.

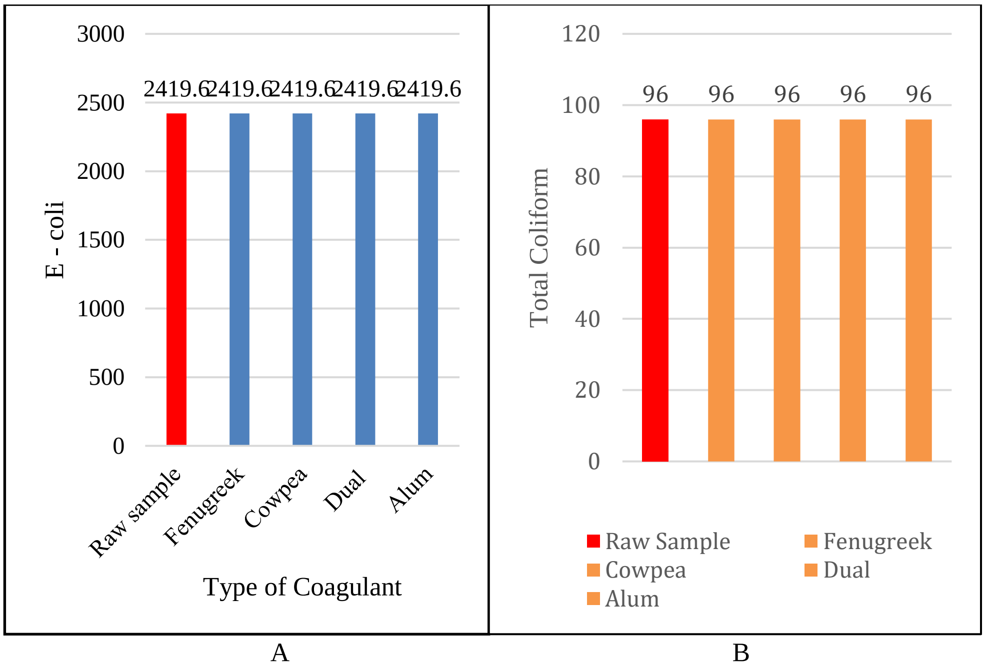


Figure 4. Comparison between A) *E-Coli* and B) Total Coliform for the Coagulants.

Figure 5 shows that the initial sample had a COD reading of 71 mg/L, lower than the Environmental Quality (Industrial Effluents) Regulations, that is, 120 mg/L [3]. After fenugreek, cowpea, and dual coagulant treatment, the values obtained were much lower than the previous concentration. Therefore, these coagulants demonstrated a promising result for reducing COD concentration. It can be considered a tertiary treatment to enhance the quality of the effluent before being discharged into the water bodies. It can be denoted that; fenugreek coagulant showed a good reduction rate for the effluent samples from the initial value. Aluminium sulfate possessed the highest drop for the effluent sample after treatment which was 22 mg/L.

Suspended solids are one of the main physical characteristics of wastewater clarification. Waters with high suspended solids concentration would trigger inorganic materials, bacteria, algae, and others to deposit in water. According to Figure 6, the initial sample had a TSS level of 10 mg/L, which did not exceed the Environmental Quality (Industrial Effluents) Regulations reading of less than 50 mg/L [3].

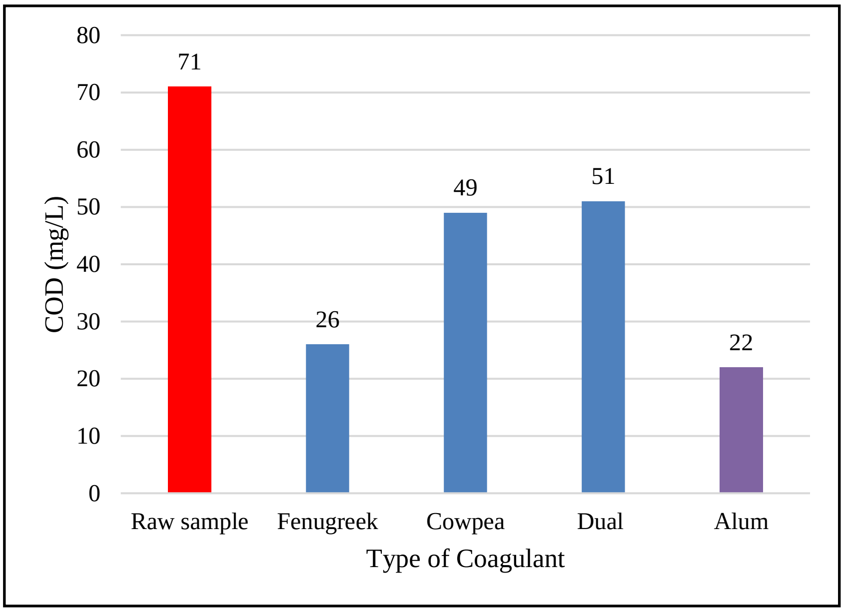


Figure 5. The concentration of Chemical Oxygen Demand (COD) for the Coagulants.

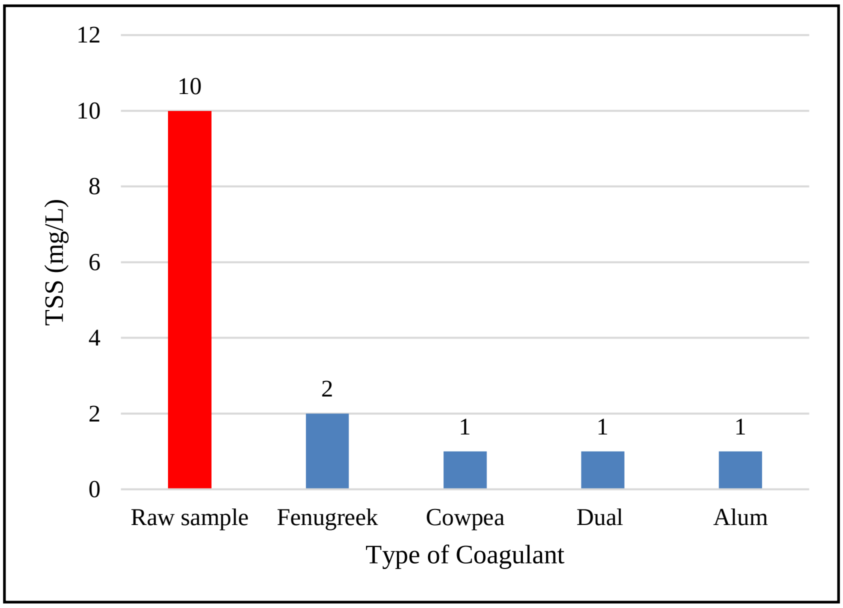


Figure 6. The concentration of Total Suspended Solids (TSS) for the Coagulants.

Meanwhile, the treatment using coagulants displays a significant decline in value from the initial concentration. All the treatments have convincedly shown an outcome concentration which is below par. The three coagulants with the highest reduction rate were aluminium, dual coagulants, and cowpea, which had the same 1 mg/L decrease. Fenugreek shows the last fall, which was 2 mg/L.

According to the findings, the BOD of the treated sample decreased more than the effluent sample after treatment. The BOD reading for dual coagulants was 5.91 mg/L. On day 5, the values of all the coagulants are lower than those of the initial effluent sample. The increased BOD value of 7.44 mg/L indicated that the effluent had the presence of bacteria. The reading for BOD did not exceed the maximum limit (20 mg/L) of Environmental Quality (Industrial Effluents) Regulations [3].

The initial samples of nitrite-nitrogen and nitrate-nitrogen measurement of 0.008 mg/L were lower than the level of Environmental Quality (Industrial Effluents) Regulations [3]. Every coagulant exhibits a decrease in value and is much lower than the limit due to the treatment imposed.

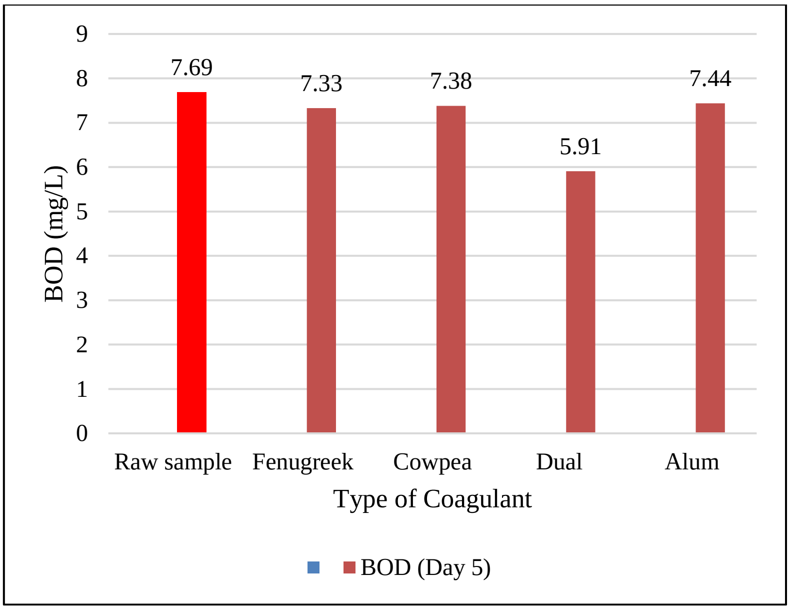


Figure 7. The concentration of Biochemical Oxygen Demand (BOD) for the Coagulants

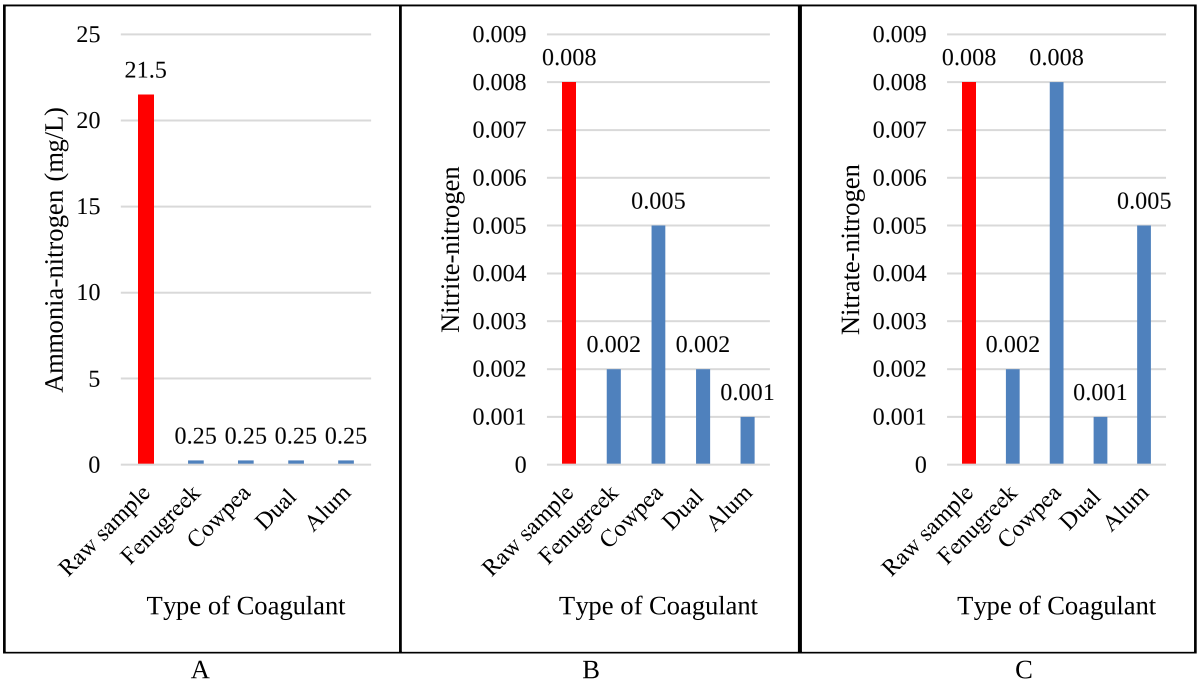


Figure 8. The concentration of A) Ammonia-nitrogen, B) Nitrite-nitrogen and C) Nitrate-nitrogen for the Coagulants.

All coagulants have nitrite-nitrogen and nitrate-nitrogen readings less than 0.1 mg/L. Dual coagulant contains the least nitrite-nitrogen and nitrate-nitrogen after treatment, with only 0.001 mg/L.

Isotherm Analysis

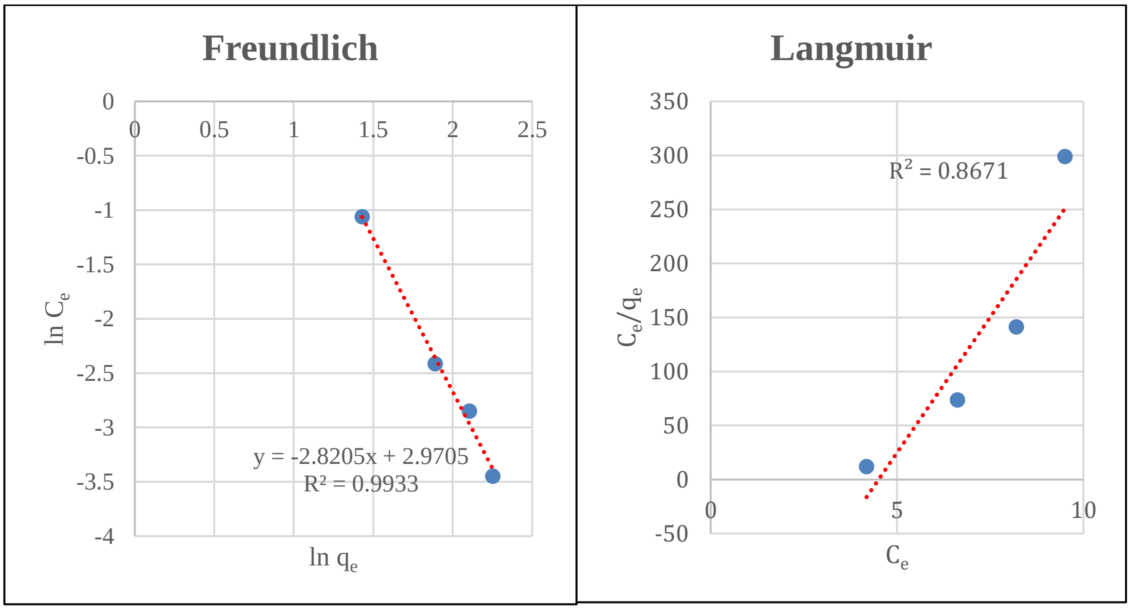
The mechanism of adsorption reactions can be discovered by analyzing the equilibrium data referred to as the adsorption capacity acquired from the experiments. Adsorption isotherms were developed to obtain the equilibrium relationship between the amounts of pollutants absorbed on the surface of a biochar-based adsorbent [21]. The Langmuir and Freundlich isotherms were employed in this study [22]. The removal behaviour is most frequently assessed using these isotherms. The experiment's findings were examined to reveal the optimal coagulant for single and mixed situations. The results of the turbidity removal from the effluent samples using fenugreek, cowpea, dual coagulant, and aluminium sulfate were displayed in Table 1.

Table 1. The percentages of removal of turbidity using coagulants.

Type of coagulant	Turbidity initial raw sample (NTU) C_0	Turbidity after removal (NTU) C_e	Percentages removal (%)
Fenugreek	11.1	9.51	14.32
Cowpea	11.1	6.62	40.36
Dual	11.1	8.20	26.13
Aluminium sulfate	11.1	4.18	62.34

Table 2. The value of C_e/q, q, C_e, ln q and ln C_e in the removal of turbidity.

Type of coagulant	C_e/q_e	C_e	q_e	ln q_e	ln C_e
Fenugreek	299.06	9.51	0.0318	-3.448	2.252
Cowpea	73.88	6.62	0.0896	-2.412	1.890
Dual	141.38	8.20	0.0580	-2.847	2.104
Aluminium sulfate	12.08	4.18	0.3460	-1.061	1.430



A B

Figure 9. Isotherm Analysis A) Freundlich B) Langmuir

The values of C_e/q_e, C_e, q_e, ln q_e, and ln C_e for creating the Langmuir and Freundlich graph are derived from the removal result in Table 1. The values for C_e/q_e, C_e, q_e, ln q_e, and ln C_e are shown in Table 2. In choosing the isotherm that best fits the data achieved, the input from Table 2 was used to construct the linear graph of Langmuir and Freundlich isotherm. Figure 9 shows a linear relationship between Langmuir and Freundlich's adsorption isotherm. The Freundlich model from Figure 9 fits the data better than the Langmuir model for both conditions, according to correlation coefficients R^2. For both cases, Freundlich shows R^2 to be greater than 0.9, which was 0.9933. However, R^2 for Langmuir is just 0.8671. Compelling results on correlations between coagulants were led by the value, demonstrating the Freundlich model's relevance and a better fit than the Langmuir model. Due to the coagulants' removal capacities, Freundlich's R^2 value was higher than the Langmuir.

From the results shown in Figure 9, it has been anticipated that higher R^2 emphasized a good correlation between the adsorption capacity and turbidity removals. Freundlich model demonstrated significant relationships on both parameters, as the R^2 obtained was 0.9933, which is nearly 1 [22].

Fourier Transform Infrared (FTIR)

From the Fourier Transform Infrared (FTIR) analysis, this experiment identified the absorbance peak of the coagulant polymer. This assessment evaluated the chemical structures that changed in confirming the degradation process. The IR spectrum table was used and referred to for the absorption peak [23].

The FTIR spectra of fenugreek before and after treatment using the jar test are shown in Figure 10. Table 3 shows the summary of the functional group's changes. Before the treatment, a functional group with the peaks of O-H at 3393.19 cm^{-1} and C=H at 1653.19 cm^{-1} existed. It can be observed that the carbonyl group adsorption will be due to hydrogen bonding, as this represents the mechanisms of coagulation for the biopolymer [1]. The peaks at 3393.22 cm^{-1} have been linked to the O-H. However, after the treatment, the rise at 1631.52 cm^{-1} for C=H was gone. It showed that coagulants can degrade the pollutants due to the creation of additional carbonyl groups.

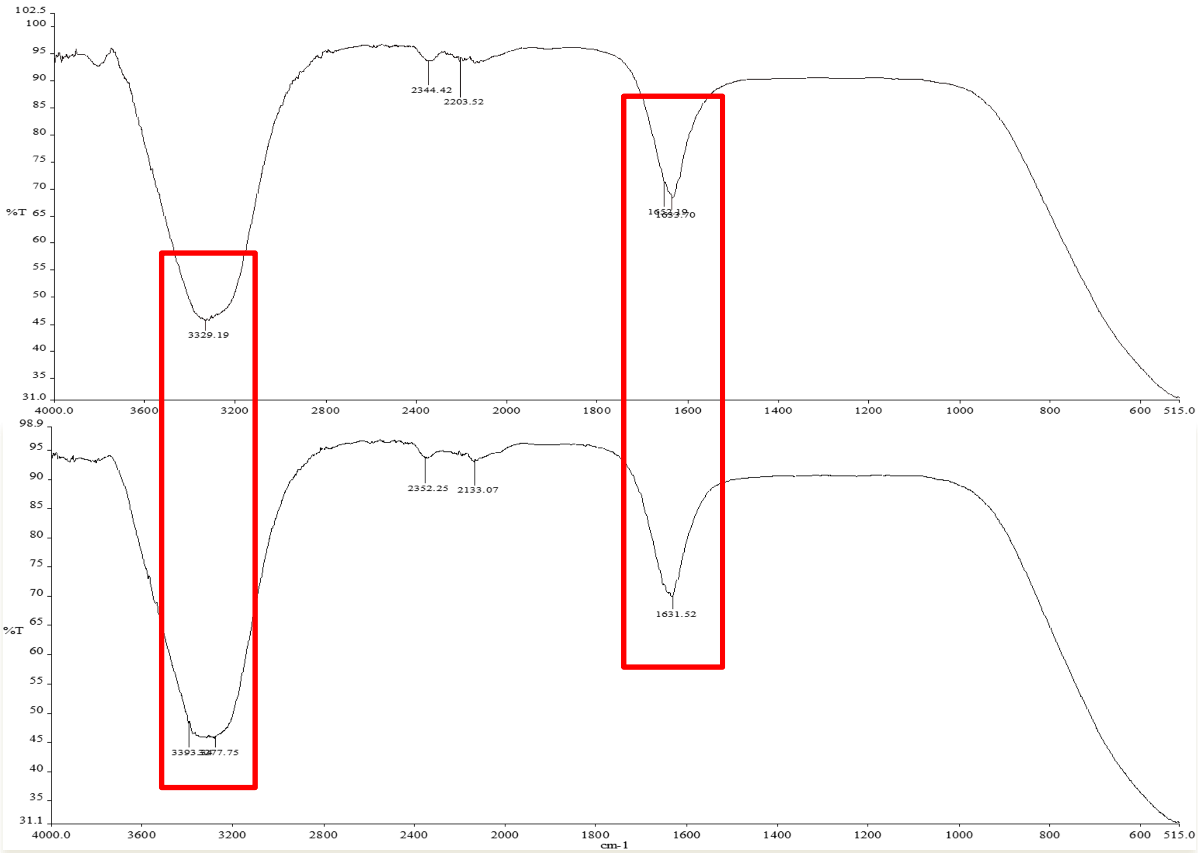


Figure 10: FTIR Analysis fenugreek

Table 3: Functional group of fenugreeks after degradation.

Coagulant	Condition	Wavenumber (cm^{-1})	Functional group	Changes
Fenugreek	Before	3393.19	O-H	Formation
		1653.19	C=H	Formation
	After	3393.22	O-H	Formation
		1631.52	C=H	Absence

Figure 11 shows the modifications of cowpea reduction's chemical composition after the experiment. Table 4 shows the summary of the functional group's changes. The emergence of C=O is related to the oxidation reactions frequently needed to enhance the hydrophilicity of the polymer by adding a functional group, such as alcohol or carbonyl groups. It can strengthen the attachment and degradation processes. The new absorption peak at 3341.30 cm^{-1} assigned to the C=O has formed before treatment in cowpea extract [1]. Next, the O-H before treatment was responsible for the absorption peak at 3341.30 cm^{-1}. The treatment eliminated the missing peaks, 1652.19 cm^{-1}, attributed to the C=O. The coagulation of the flocs during the treatment of the jar test may cause this deterioration. Therefore, the functional group of cowpea undergoes a new formation called C=O, and even a decline in the absorption peaks suggests that the cowpea has undergone degradation processes.

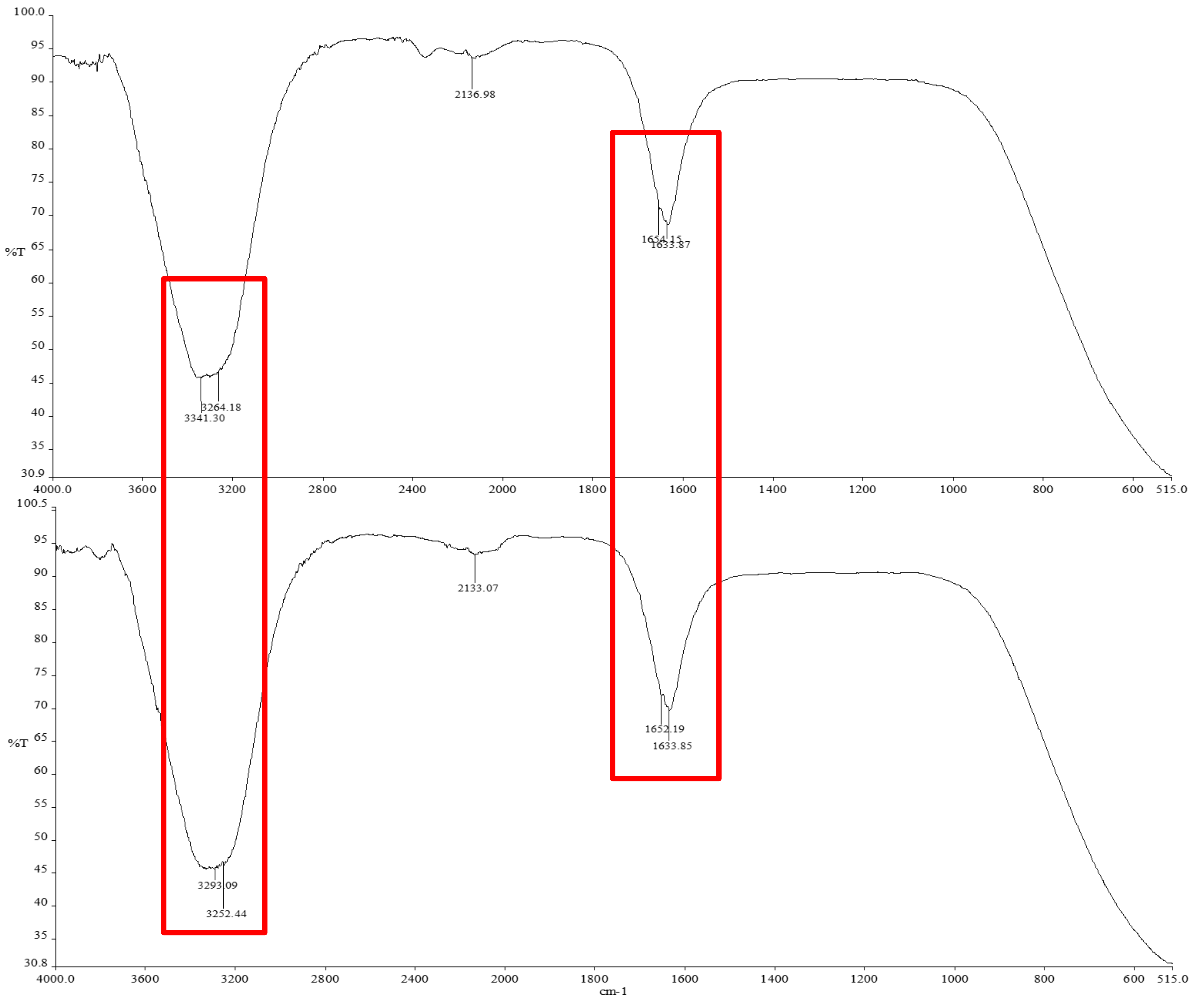


Figure 11: FTIR Analysis cowpea

Table 4: Functional group of cowpeas after degradation.

Coagulant	Condition	Wavenumber (cm^{-1})	Functional group	Changes
Cowpea	Before	3341.30	O-H	Formation
		1654.09	C=O	Formation
	After	3293.09	O-H	Formation
		1652.19	C=O	Absence

The trend of the dual coagulant's FTIR spectrum in terms of chemical structural changes is depicted in Figure 12. Table 5 contains a summary of the functional group's changes. The O-H carboxylic group was created as a dual degrader (3377.69 cm^{-1}) before and (3342.51 cm^{-1}) after degradation. The synthesis of minute fragments and the carboxylic functional group is accelerated by this procedure [24]; prior to treatment with dual extract, a new absorption peak at 1632.51 cm^{-1} ascribed to the development of C=O bond [1]. The treatment procedure has removed the 1652.19 cm^{-1} of missing peaks assigned to the C=O. This deteriorating process can be due to the flocs' coagulation during the experiment.

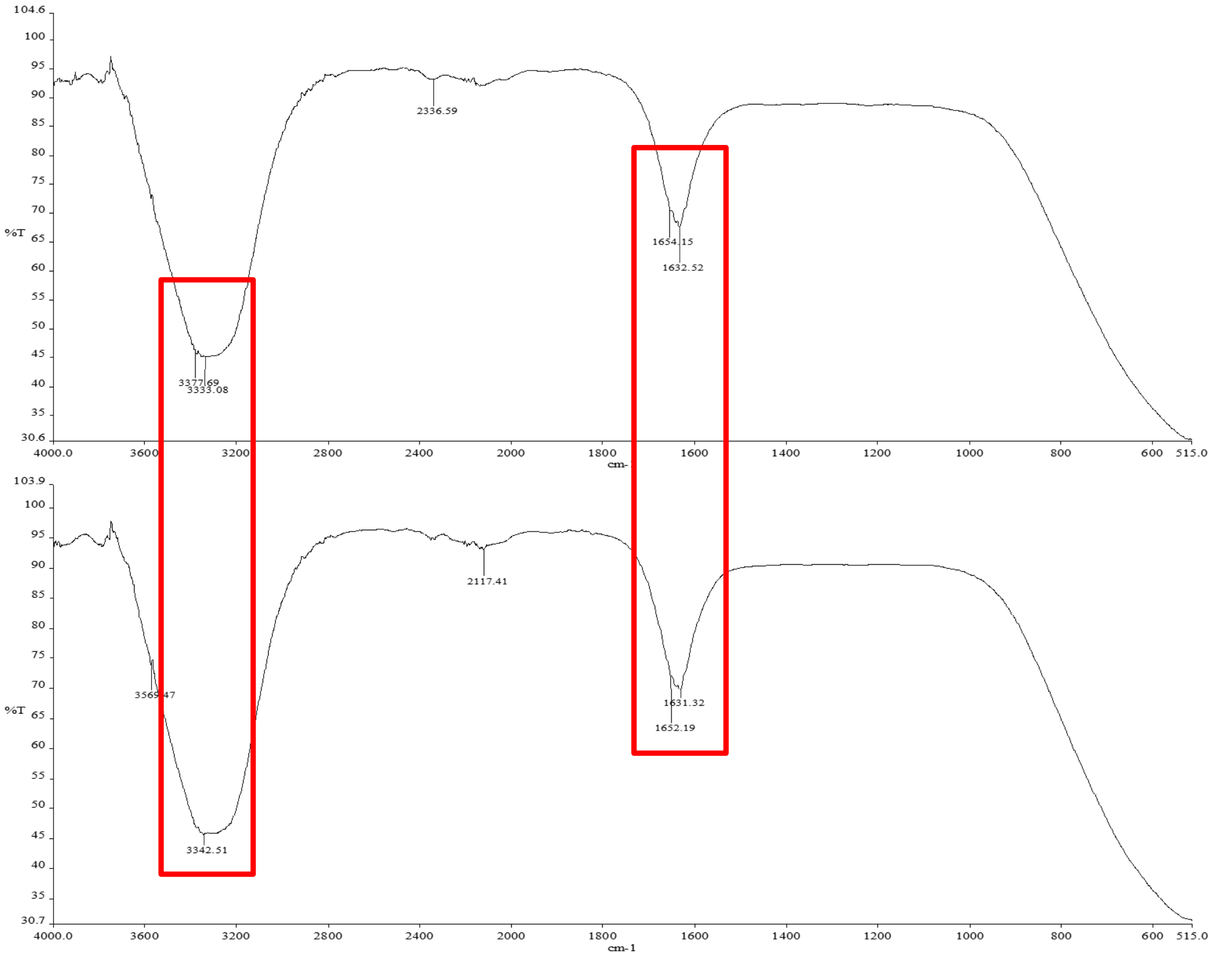


Figure 12: FTIR Analysis Dual.

Table 5: Functional group of Dual after degradation.

Coagulant	Condition	Wavenumber (cm^{-1})	Functional group	Changes
Dual	Before	3377.69	O-H	Formation
		1632.51	C=O	Formation
	After	3342.51	O-H	Formation
		1652.19	C=O	Absence

Figure 13 and Table 6 display the FTIR spectra of the substances of aluminium sulfate. The Al-OH-Al stretching and OH vibrations of H_2O could cause strong, broad bands for aluminium sulfate at 3248.52 cm^{-1}. While the peaks at 1633.65cm^{-1} may be due to the unsymmetrical stretching of H_2O molecules with aluminium sulfate, the peaks at 1631.47cm^{-1} suggest the O-H bending of water. In addition, excessive dissolved metal ion concentrations could be problematic during post-biological treatment and when releasing treated wastewater into water bodies. Raising the pH of the biologically treated wastewater to pH 7 will cause some dissolved metals to precipitate [12].

This research has investigated two different types of coagulant (cowpea and fenugreek seed) for the effectiveness of pollutant removals compared to the conventional aluminium sulfate coagulant. The biopolymer introduced in this study has advantages in reducing the waste materials that need to be discarded. Moreover, this bio coagulant has no long-term effect on the environment and may reduce pollution to the surrounding ecosystem.

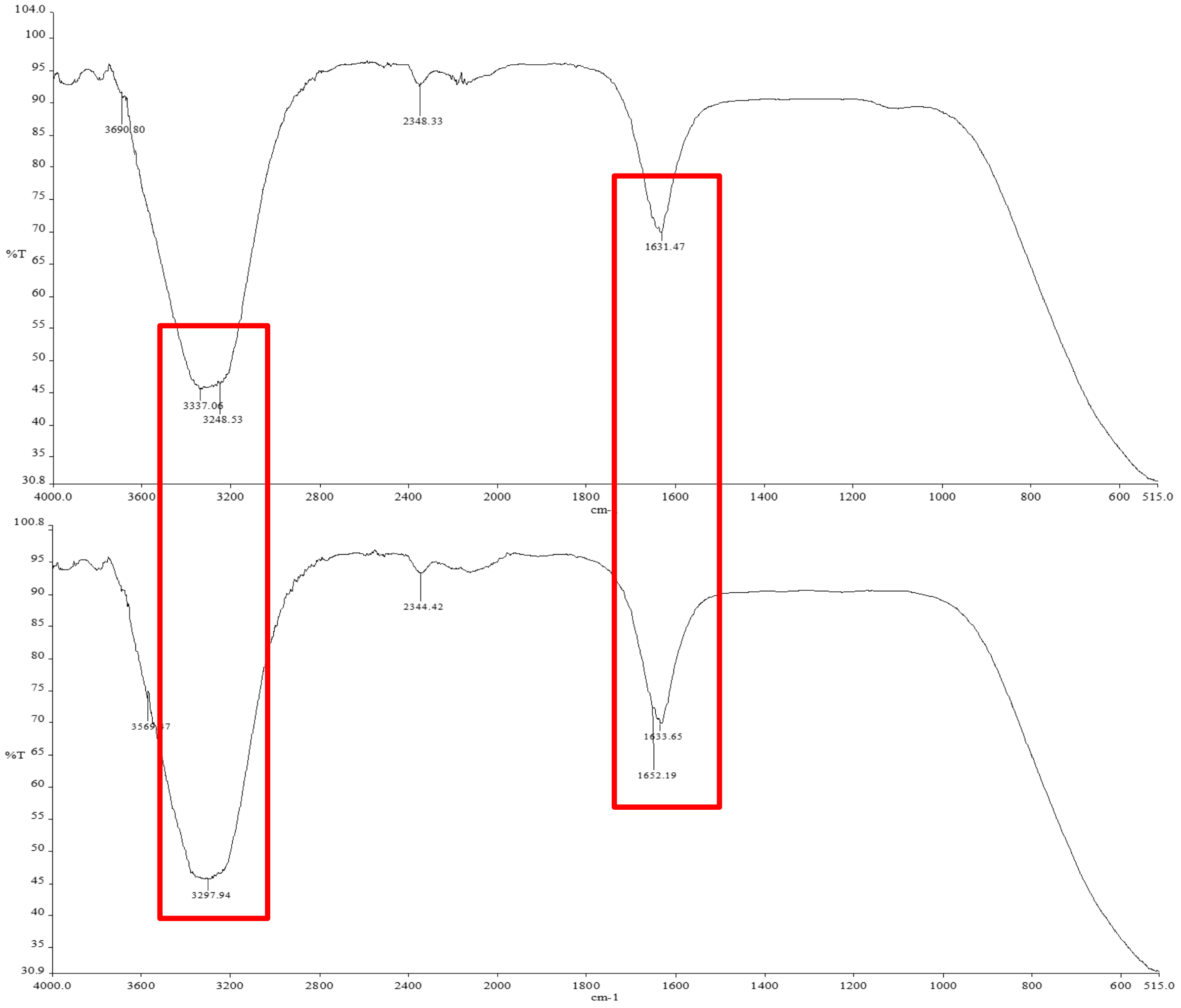


Figure 13: FTIR Analysis Aluminium sulfate.

Table 6: Functional group of aluminium sulfate after degradation.

Coagulant	Condition	Wavenumber (cm^{-1})	Functional group	Changes
Aluminium sulfate	Before	3248.53	O-H	Formation
		1631.47	O-H	Formation
	After	3297.94	O-H	Formation
		1633.65	O-H	Absence

CONCLUSION

The findings of this study indicate that cowpea and fenugreek seeds can be used as natural coagulants in removing pollutants. The removal for fenugreek reaches 20%, whereas the removal for cowpea reaches 40% for turbidity reduction. Cowpea and dual biopolymer successfully removed 1 mg/L of suspended solids. A high removal efficiency for suspended solids by cowpea and dual coagulants has demonstrated that both are superior to aluminium sulfate. Regarding BOD, the dual coagulant reading achieved 5.91 mg/L. Following the ammonia-nitrogen concentration, all types of coagulant decreased to 0.25 mg/L. For COD concentration, fenugreek coagulant can reduce the COD value to 26 mg/L, followed by alum at 22 mg/L. For the results of *E. coli* and total coliform bacteria, no changes have been detected before and after treatment using bio coagulants. Later, the modifications to each polymer's chemical structure showed the development of a new peak following the treatment intervals. The chemical bonds C=O and O-H have formed in fenugreek, cowpea, and dual, and the O-H bond has developed in aluminium sulfate. Here, efforts to improve the chemical features of these natural compounds are needed to boost the coagulation-flocculation mechanisms and increase the effectiveness of the removal processes. Coagulants from cowpea and fenugreek seeds can be promising biopolymers for tertiary treatment and are more sustainable for enhancing the quality of the effluent.

ACKNOWLEDGEMENTS

The authors sincerely thank the School of Civil Engineering, College of Engineering, Universiti Teknologi MARA, Shah Alam, for their assistance throughout the research study. The authors gratefully acknowledged Universiti Teknologi MARA, Shah Alam, for providing Strategic Research Partnership (SRP) Grant (100-RMC 5/3/SRP (090/2021)) for financially supporting this study and providing the resources.

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