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Purified natural coagulant protein from green bean and pigeon pea seed for turbidity removal

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***Abstract* –** This work studies the effect of extraction, and purification treatment of pigeon peas (PiP) and green bean (GB) seeds as a natural coagulant for turbidity removal from wastewater. NaCl was used to extract the active coagulation agents from the seed samples and was further purified with the protein isoelectric precipitation method to obtain a purified coagulant. The turbidity removal efficiency of the coagulants was evaluated, and the purified protein coagulants were characterised using the FTIR analysis. The purified protein coagulants resulted in higher turbidity removal of 97.17% and 97.03% for PiP and GB, respectively. The FTIR analysis of the seed powder samples supported the findings. It is concluded that purified protein coagulant from green beans and pigeon peas effectively removed suspended solids from water while maintaining the organic matter (COD) level in the treated water.

Keywords – Turbidity Removal; Natural Coagulant; Green Bean; Pigeon Pea; Isoelectric Protein Precipitation.

I. Introduction

The drive towards exploring potential substitutes for chemical coagulants is comparatively because of the health hazards threat to human and aquatic animals and environmental pollution caused by chemical leaching. This led to the search for environmentally friendly, non-toxic, natural, and biodegradable coagulants based on plant materials, animals, and microorganisms.

Pigeon pea (Cajanus cajan) and green bean (Phaseolus vulgaris) are leguminous seeds. Various legumes seed, including soybeans, dal seed, guar bean, hyacinth bean, peanut, horsegram, cowpea, and chickpea, has been reported to perform efficiently in turbidity removal and wastewater treatment [1]. Grain legumes are highly affordable, with high amino acid and protein content. Their protein is synthesised from simple nitrogen compounds from their roots and nitrogen-fixing bacteria primarily used by legumes [2].

Several investigations using legume-based coagulants in wastewater treatment have been recorded. For example, Lek et al. [3] successfully used chickpeas to remove turbidity, TSS, and COD from palm oil mill effluent (POME). An 86%, 87%, and 56% reduction was achieved for turbidity, TSS, and COD, respectively. Furthermore, the FTIR analysis of the chickpea powder identified the presence of OH, CH, NH, CC, CO, and CN groups, contributing to the bridging flocculation mechanism during the coagulation process [3]. Meanwhile, NaCl extraction of peanut efficiently removed 92% turbidity from the initial turbidity of 200 NTU in the work of Birima et al. [4].

Most plant-based coagulants are composed of proteins or polysaccharides, which could be anionic, cationic, or non-ionic. Proteins are the main component that aids coagulation in leguminous seeds [5]. Green beans (GB) have a protein content of 21.22 to 24.06% [2]. Pigeon Pea (PiP) is amongst the 10th most important grain legumes in Asia, Africa, and the Caribbean. It has a protein and carbohydrate content of 20-22% and 65%, respectively [6]. However, from various literature reviews, no study has been done on using green beans and pigeon peas as a coagulant in water treatment.

II. Materials and Method

The green beans and pigeon pea seeds used in this research were obtained locally in Nibong Tebal, Malaysia. The seeds were cleaned and oven-dried at 60 °C for 1 h before milling them into a fine powder with a laboratory grinder. The powder was sieved using a sieve size of 1.0 mm particle size. The extraction of active coagulant from GB and PiP seeds was done by adding 2 g of powder into 100 mL of 1.0 M NaCl solution, and the suspension was stirred for 1 h using a magnetic stirrer. The Eppendorf Centrifuge 5702 RH was used to separate the mixture at 4000 rpm for 30 min; the filtrate obtained is the active coagulation crude extract (CE).

The CE was purified to obtain the purified form of active coagulation agent using the isoelectric precipitation method. The protein from the crude extracts was precipitated by adjusting the solution's pH to 2.8-3.3. The cloudy solution was further stirred for 15 min before centrifugation at 4400 rpm for 30 min. The sediment formed was washed in cold acetone and filtered with a filter paper; the powder was air-dried and ground for further analysis and experiment [7].

The turbid water was prepared in the laboratory by mixing 1 g of kaolin powder with 1L of distilled water. The mixture was stirred on a magnetic stirrer for 1 h before allowing it to undergo complete hydration for 24 h before use. For each experimental run, the kaolin solution was diluted with distilled water to obtain initial turbidity of 250 ± 10 NTU. The turbid water was treated with crude extracts and purified coagulants obtained from GB and PiP seeds.

The functional groups responsible for coagulation efficiencies in the ground seeds powder were examined using the FT-IR Shimadzu IR affinity, using the potassium bromide (KBr) pellet method. First, the sample powders were mixed with the KBr in the proportion of 10:1 and ground, and the pellet was prepared by compressing the mixture powder with a hand compressor. The FTIR analysis recorded the spectra result between 4000 cm^{-1} and 500 cm^{-1}.

III. RESULTS

After the protein purification, the sample was left for 3 days to allow complete drying and weighed. About 3 g of green beans (GB) and pigeon pea (PiP) powdered seeds produced 7.96% and 6.53% of purified powder, respectively. The purified protein (PP) powder was used directly in solid form for the coagulation-flocculation process, as shown in Figure 1.

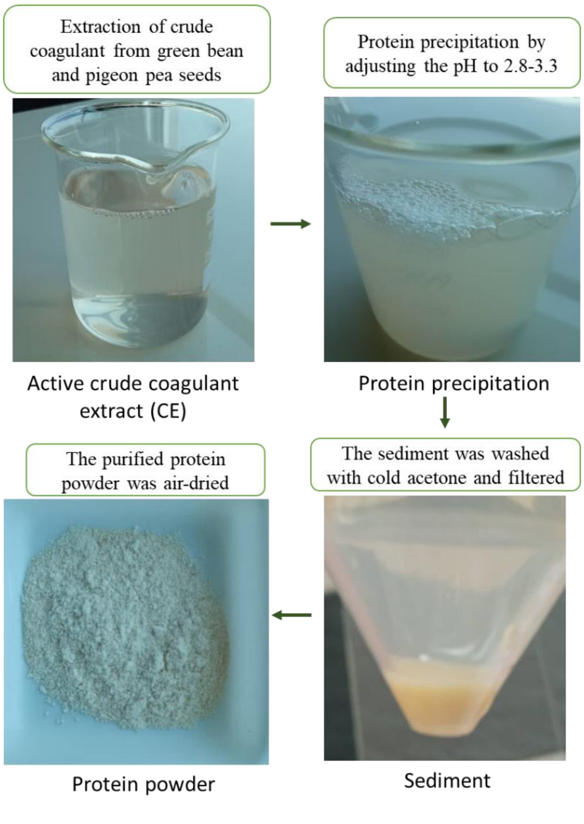

Figure 1: Coagulants obtained from isoelectric purification.

The FTIR spectra identified different functional groups for the purified proteins shown in Figure 2, with 3409.87 (GB) and 3382.40 cm-^{1} (PiP) peaks, indicating the stretching of N-H bonds of secondary amines, O-H hydroxyl group, and -COOH carboxylic acid. Small peaks at 2937.93 (GB) and 2952.91 cm^{-1} (PiP) indicated the existence of the C-H stretch of alkane. The appearance of peaks at 1655.29 cm^{-1} (GB) and 1656.12 cm^{-1} (PiP) for both samples corresponded to C=O stretching of the carbonyl group was observed.

Aromatic hydrocarbon C=C stretching or O-H bending carboxylic group indicated at 1402.12 cm^{-1} (GB), and 1423.98 cm^{-1} (PiP) appeared in the FTIR spectra. The -COOH, -OH, and N-H groups have been reported to show the presence of proteins, lipids, fatty acids, and carbohydrates, which aid excellent coagulation efficiency [8]. Other peaks within the wavelengths of 1200 to 600 cm^{-1} indicate the presence of C-N stretching, C-O stretching, and C-C bending vibrations. During coagulation, these groups act as active sites for attaching suspended particles and colloids in the water. Studies have also reported that natural coagulants are composed of proteins, carbohydrates, and lipids; also, higher protein content results in higher turbidity and suspended solids removal [3].

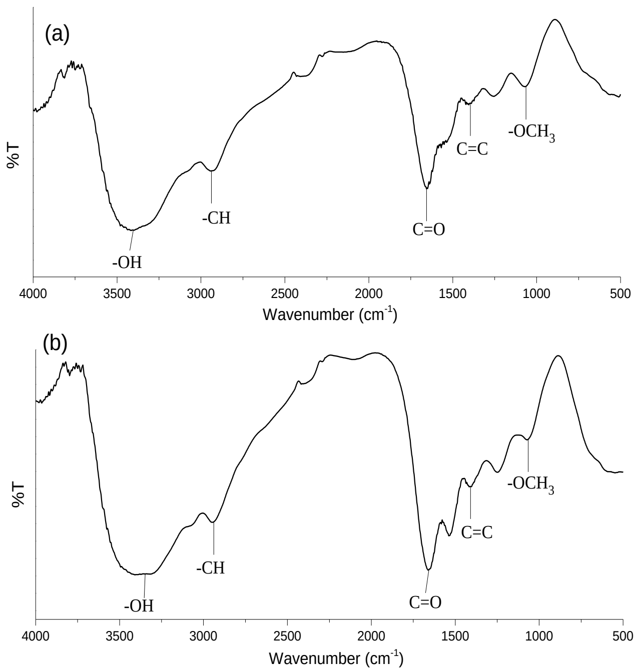

Figure 2: FTIR spectra of the purified (a) green bean (GB) and (b) pigeon pea (PiP) protein coagulants.

The turbidity removal experiment used a jar test apparatus (VELP JLT6 Flocculation Tester) using the coagulants. The CE and PP were added at a coagulant dose of 2 ml/l and 0.02 g/L, respectively, into a 500 mL beaker filled with turbid water. Fast and slow mixing was carried out at 100 and 25 rpm for 4 and 20 mins, respectively, followed by 30 mins of setting time. The turbidity and COD measurement sample was withdrawn at 3 cm below the surface using a pipette and used for further analysis.

Jar tests made with the purified protein coagulant gave a higher turbidity removal when compared with that of crude extract. PP and CE coagulants from pigeon pea seed gave a high turbidity removal of 97.17% and 91.59%, respectively. In comparison, 97.04% and 91.07% turbidity removal were achieved for the PP and CE coagulants from green beans seed.

The crude extracts obtained from natural coagulants have been reported to contain water-soluble organic and inorganic materials, which results in increased organic load and microbial activity in the water treated [9]. Therefore, the COD analysis evaluated the organic matter content in crude extract (CE) and purified protein (PP) treated water. The COD results showed negligible change in water treated with PiP and GB purified protein coagulants. However, an increase of 23.5% and 13.4% were observed for water treated with PiP and GB crude extracts, respectively. These results obtained in both turbidity removal and COD evaluation indicate the effectiveness of natural coagulants in both extracted and purified forms.

IV. CONCLUSION

Generally, both coagulants proved their efficacy in turbidity removal; however, it was especially noted that the purified protein coagulants gave higher turbidity removal while maintaining the COD level in the treated water. Therefore, since the purified proteins gave good COD results for this study, it is suggested that the protein present in the samples should be identified.

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