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Research Article
Effect of Storage Conditions on *Jatropha curcas* Performance as Biocoagulant for Treating Palm Oil Mill Effluent

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Abstract
Background and Objective: *Jatropha curcas* has been shown to be an effective bio-coagulant in turbidity removal for water and wastewater. In this work, the effect of storage time and conditions of the *Jatropha curcas* coagulant agent had been investigated and Palm Oil Mill Effluent was used as the sample wastewater. **Materials and Methods:** *Jatropha* seed and presscake was stored at room temperature and the coagulant was extracted at 1st, 3rd and 5th month for performance evaluation in coagulation. Next, the coagulant was extracted and stored in different conditions. The effect on the coagulation process was evaluated and the bio-coagulant quality was analysed using FTIR and Bradford method. **Results:** Results showed that the turbidity removal reduced from 99-92% as storage time increased. Storing at lower temperature resulted to reduce significant degradation of biocoagulant quality and able to maintain the coagulation performance. **Conclusion:** These findings were supported by the FTIR and protein content analysis. These findings suggested that storage conditions greatly affect the performance of *Jatropha curcas* as a coagulant.

Key words: *Jatropha curcas*, storage time, protein, coagulant agent, palm oil mill effluent, wastewater, coagulation performance, jatropha seed and presscake

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Coagulation research has been moving progressively towards exploring alternative environmental friendly and efficient biodegradable natural coagulant that may originate from animals, plants and even micro-organism. This is partly due to the potential health risk posed by chemical coagulants to human and living creatures residing in water bodies if the chemical unexpectedly leach into the environment[1].

Jatropha curcas seed and presscake has been reported to perform well as bio-coagulant for turbidity removal[2-4] and also wastewater treatment[5] which add further value to this multipurpose trees. *Jatropha curcas* is from Euphorbiaceae family and popular as an alternative source of biodiesel[6]. Among its uses are for making soap, plastics and synthetics fibre[7], biodiesel[8,9] and animal feedstocks. The seed or presscake after the oil extraction is reported to contain 19-27% of crude protein[5,10].

Pritchard *et al.*[3] and Yongabi[2] reported on the successful use of *Jatropha curcas* seed for the removal of turbidity in water. Yongabi[2] stated that a clear supernatant was observed with the flocs settled easily at the bottom. Pritchard *et al.*[3] meanwhile reported a reduction of turbidity to roughly 5 NTU from initial ~50 NTU (Kumponda well) which was around >90% turbidity removal efficiency. Besides that, both authors also conducted a disinfectant test and found that *Jatropha* seed was able to reduce the number of coliform in the treated water up to 60-80%. Later, Abidin *et al.*[4] investigated the effect of several parameters (pH, initial turbidity, coagulant dosage) on the removal of turbidity from kaolin water using *Jatropha curcas* seed extracts. High percentages of removal (>95%) has been recorded for high and low NTU values. About 0.5 M Sodium Hydroxide has been found to be the best solvent to extract maximum amount of coagulant[5]. *Jatropha curcas* seed extracts also capable of producing 50-60, 60 and 70-85% for COD, BOD and TSS removal, respectively.

Typical treatment of POME involves aerobic and anaerobic digestion to breakdown the complex pollutants exist in it[11]. Coagulation treatment has normally been adopted as pre-treatment of POME to reduce its heavy loading of contaminants. Among the investigations on coagulation of POME using bio-degradable source were done using chitosan[12,13], *Moringa olefeira*[14], chickpea[15] and bio-degradable polyacrylamide[16].

Most of natural coagulants are polysaccharides or proteins which can be cationic, anionic and non-ionic in nature. Abidin *et al.*[17] conducted proximate analysis, amino acids determination and FTIR analysis to establish the component of the coagulant extract from the *Jatropha curcas* seed. Protein was found to be the main component which is responsible for the coagulative ability of *Jatropha* seed. A positive zeta potential was observed for low pH (<3) values while a negative zeta potential was recorded for high pH (>3,4). Generally, protein is a natural product which is susceptible to bio-degradation and microbial attack. Previous work on *Moringa oleifera as* coagulant had shown that storage conditions and time can have an influence on the quality of the bio-products whereby coagulant kept in refrigerator performed better then coagulant stored at room temperature[18-20]. Elucidating the behaviour and performance of *Jatropha curcas* coagulant to remove turbidity at different storage conditions (time and temperature) is important since *Jatropha curcas* has been used as cheap and readily available coagulant agent to treat water in developing countries[2]. The assessment will be conducted using Palm oil mill effluent (POME) as sample wastewater.

MATERIALS AND METHODS

Preparation of Jatropha curcas seed and presscake biocoagulant: *Jatropha curcas* seed was obtained from Jatro Malacca Plantation Berhad, Melaka, Malaysia. The coat was removed manually and good quality seeds were selected. The shell was manually removed to obtain the seed kernels. Next, the kernel was grounded to a fine powder using a blender (Model BL 333, Khind Brand). The powder was used in every experiment. The coagulant was prepared by weighing 5000 mg of the powder and mixed with 100 mL of distilled water for 2 min in order to extract the active ingredients of the *Jatropha* seeds as coagulant. The resulting suspension was filtered through muslin cloth. The suspensions obtained were milky in color.

Jatropha press cake meanwhile was obtained by extracting *Jatropha* oil from seed by solvent extraction using soxhlet. Solvent extraction of *Jatropha* oil was performed at 60°C by using hexane (96%) for 8 h at a solvent to solid ratio[21] of 1:6. The solution of the *Jatropha* cake coagulant was prepared by dissolving 5000 mg of this cake in 100 mL distilled water. The mixture was blended by using a blender for 2 min to extract the active ingredients. The resulting suspension was filtered using muslin cloth and used in the coagulation experiment.

Palm oil mill effluent (POME) wastewater: Palm oil mill effluent (POME) wastewater sample was collected from Palm Oil Mill in Dengkil, Selangor, Malaysia. Fresh wastewater POME

was collected just after the milling process had finished at the temperature of 80-90°C. The wastewater sample was stored inside the cool room at temperature of 4°C.

Coagulation experiment for JC seed and JC presscake: The POME was taken out from storage and allowed to reach the room temperature before used. The concentrated wastewater was diluted by mixing the wastewater sample with distilled water at the ratio of 1:5 (initial turbidity of 3500 NTU), measured using HACH 2100N Turbiditimeter. Coagulation efficiency of *Jatropha* seed and presscake was studied by using the jar test equipment (JLT model). Coagulation test was carried out to investigate the *Jatropha* performances in turbidity removal using optimum pH and optimum dosage obtained earlier[15]. Beaker filled with 500 mL POME wastewater was adjusted to the optimum pH (2 and 3) and optimum dosage (120 mg L^{-1}) was added for the jar test. The beaker was agitated for 4 min at 100 rpm, followed by 40 rpm for 25 min. The solution was left to sediment for 30 and 60 min for *Jatropha* and alum, respectively. Finally, 20 mL treated sample was taken for final turbidity measurement.

In investigating the effect of storage time, *Jatropha curcas* seed and presscake were stored at the room temperature and tested its performance at 1, 3 and 6 months, counted from the day it was received. For studying the effect of storage conditions, *Jatropha* coagulant extract prepared by mixing 5000 mg of coagulant into 100 mL of distilled water and blended for 2 min using kitchen blender and divided into two sets. First set of coagulant extract was left in a container at room temperature of 24°C, while another set was kept inside the refrigerator at 4°C.

Coagulant characterization

Protein content: Bradford method has been applied to determine the total protein concentration in the sample by using Bovine Serum Albumin (BSA) as standard protein for standard calibration curve. About 5 µL of *Jatropha* seed extract solution was pipetted into reaction tubes and filled up to 100 µL with distilled water. About 1 mL of Bradford reagent was added to the tube and the contents mixed by vortexing. The absorbance at 595 nm was measured after 2 min and before 1 h after addition of reagent. The weight of protein was determined based on the standard curve. The procedure was repeated for *Jatropha* presscake solution.

FTIR analysis: The FTIR analysis was also conducted to identify functional groups in the coagulant extract using Fourier Transform Infrared spectroscopy, (FTIR), model (Spectrum 100 FT-IR Spectrometer, Perkin Elmer) instruments. FTIR had been reported as a method that can be employed to study properties of Amide I and Amide II groups that represents the vibrational bands of the biopolymer backbone[22-24].

RESULTS

Effect of storage time: The effect of the storage time towards the quality and thus performances of the coagulant during water treatment was conducted for 5 months. Figure 1 depicted the results on the effect of time storage for *Jatropha curcas* presscake and seed that contain the active coagulant agent. Based on the figure, *Jatropha curcas* presscake experienced reduction of turbidity removal 99-92% and *Jatropha curcas* seed exhibited reduction from 98-95% at optimum pH of 2 and 120 mg L^{-1} dosage.

Effect of storage conditions: Figure 2 showed the result on the effect of storage conditions on the performance of

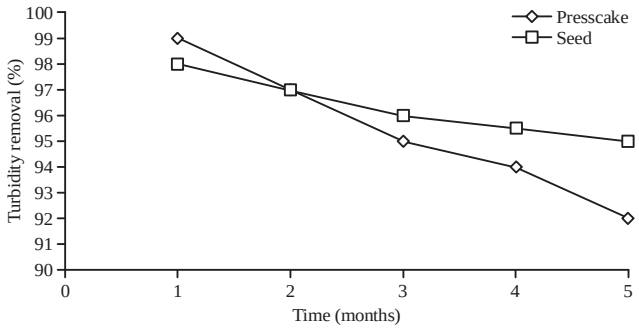

Fig. 1: Effect of storage time for the *Jatropha curcas* presscake and seed performances in POME treatment

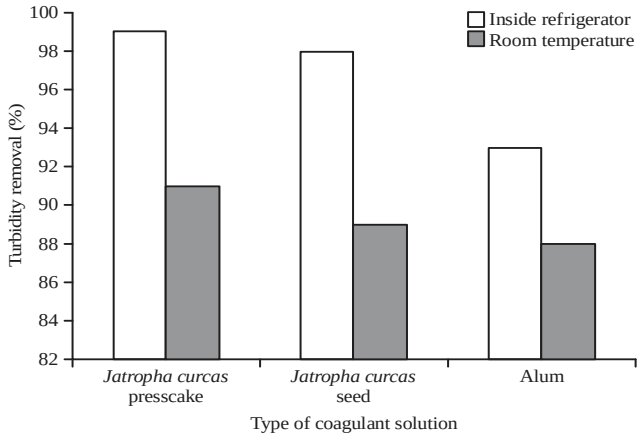

Fig. 2: Turbidity removal (%) by different type of coagulants used due to the effect of the storage condition

Table 1: Protein content of *Jatropha curcas* presscake at 0 and 7 days after storing in 4°C and room temperature

Quantification of protein contents	Final protein concentration (μg μL^{-1})
Protein content Jatropha presscake at 0 days	337.3
Protein content Jatropha presscake in refrigerator at 4°C after 7 days	257.5
Protein content Jatropha presscake at temperature room after 7 days	52.5

Table 2: Protein content of *Jatropha curcas* seed at 0 and 7 days after storing in 4°C and room temperature

Quantification of protein contents	Final protein concentration (μg μL^{-1})
Protein content Jatropha seed at 0 days	522.48
Protein content Jatropha presscake in refrigerator at 4°C after 7 days	289.80
Protein content Jatropha seed at temperature room after 7 days	83.16

Jatropha curcas seed extracts, presscake extracts and also alum after keeping these solutions for 7 days at room temperature and refrigerator.

For *Jatropha curcas* presscake and seed, the percentage turbidity removal did not show any significant changes after storing them 7 days in the refrigerator (4°C). However, the performance of the presscake dropped from 98% turbidity removal to 91% when using the extract that was kept at room temperature. The seed meanwhile also experienced a reduction of turbidity removal of 89% for extract at room temperature. The 5-10% reduction of coagulant performances from initial conditions when it was stored at room temperature for 7 days was significant.

FTIR analysis: Further analysis was done for investigating the polypeptides and protein characteristic to sample of 0 and 7 days after storing process in different environment (refrigerator and room temperature). The results were as shown in Fig. 3 (presscake), Fig. 4 (seed) and Fig. 5 (alum) storing in refrigerator at 4°C and at room temperature, respectively (Fig. 3a, b).

Figure 3a and 4a represented the coagulant extracts at day 7 for *Jatropha curcas* presscake and seed after keeping the extracts in the refrigerator at 4°C. Four peaks with different intensity was detected at different frequencies for both presscake and seed. Peak A with O-H stretch bonded with medium intensity at 3372.79 cm^{-1}, peak B with C=N stretch at 2111.77 cm^{-1}, peak C with C-O stretch at 1640.11 cm^{-1} and peak D with C-H aromatic with small intensity at 700.59 cm^{-1}. The spectral region that was most sensitive to the components of the secondary structure of proteins was in the region of 1600-1690 cm^{-1}. Amide I band was 80%, related to the C=O stretching vibration of the peptide linkage with minor contributions from the out-of-phase CN stretching vibration, CCN deformation and the NH in-plane band. Contributions from amide II (~1550 cm^{-1}) and amide III (b and 1400-1200 cm^{-1}) was not apparent. Figure 3b and 4b meanwhile showed the FTIR spectra for *Jatropha curcas* presscake and seed after 7 days storing coagulant extract at room temperature (25°C). It can be seen that FTIR spectra for *Jatropha curcas* presscake (Fig. 3b) showed a tremendous change in its functional groups and chemical structure. Many new peaks were detected compared to the freshly extract FTIR and also presscake extract stored at 4°C (Fig. 3a). Whereas, for *Jatropha curcas* seed (Fig. 4b), only a slight shifting of the peaks was observed.

Protein quantification: Quantification of the protein content in the extract of *Jatropha curcas* presscake and seed was also conducted and the results were as shown in Table 1 and 2. There was a decrease in the protein content after day 7. The solution kept in the fridge had higher protein content compared to the solution stored at room temperature. The solution in the refrigerator at 4°C still experience degradation but at a lesser extend compared to sample at room temperature. Similar trends can also be seen with *Jatropha* seed extract solution after 7 days in refrigerator.

FTIR analysis on alum coagulant was also conducted and after a week, alum also showed slight reduction in performance although it was an inorganic coagulant. This can be attributed to the changing of chemical structure due to decrease of moisture content in the container that disturbed its chemical interactions with solvents and promote dissociations to occur.

DISCUSSION

These results suggested that *Jatropha curcas* seed undergo less physical and chemical changes by natural degradation compared to *Jatropha curcas* presscake. This can be owed to the presence of the hard shell that protects the kernel inside the shell. *Jatropha curcas* presscake meanwhile, is the residue of the seed after oil has been extracted. Prior to oil extraction, the presscake undergoes grinding process to reduce it into a fine powder. This mechanical pretreatment process can result in disruption of cell wall and membrane that is supposed to serve as a protection barrier to the seed. Hence, this degradation of *Jatropha curcas* presscake was inevitable. Exposure to harsh chemical during extraction further worsen its quality. Other natural plant

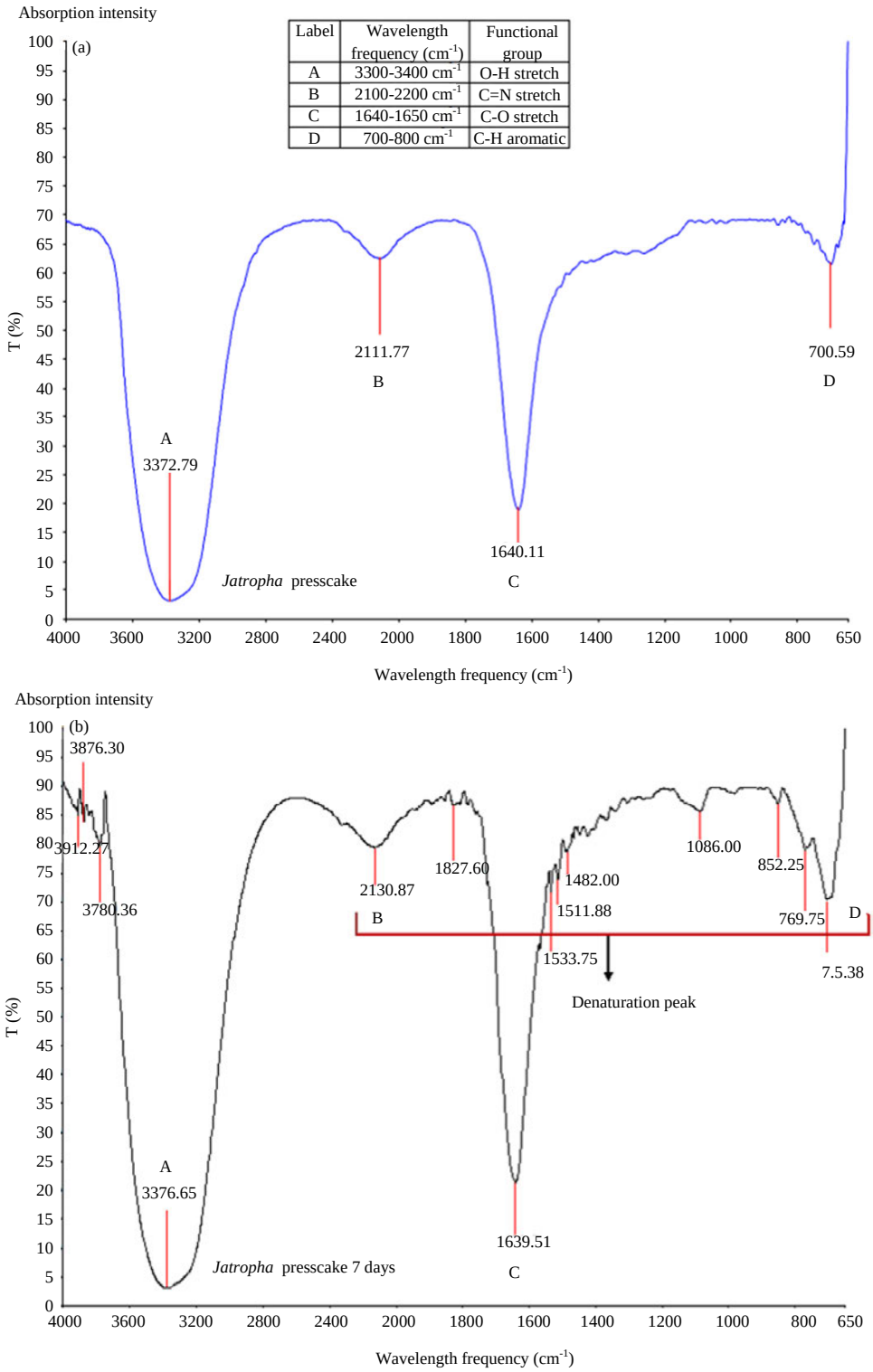

Fig. 3(a-b): (a) Infrared (IR) spectroscopy for *Jatropha curcas* presscake coagulant stored in refrigerator at 4°C and (b) Infrared (IR) spectroscopy for *Jatropha curcas* presscake coagulant stored at room temperature of 24°C

coagulant like *Moringa oleifera*, which was composed of protein[24] as well, had also been reported to show coagulation inefficiency as storage duration is increased[18]. This was in line with the nature of natural plant that decomposed with time.

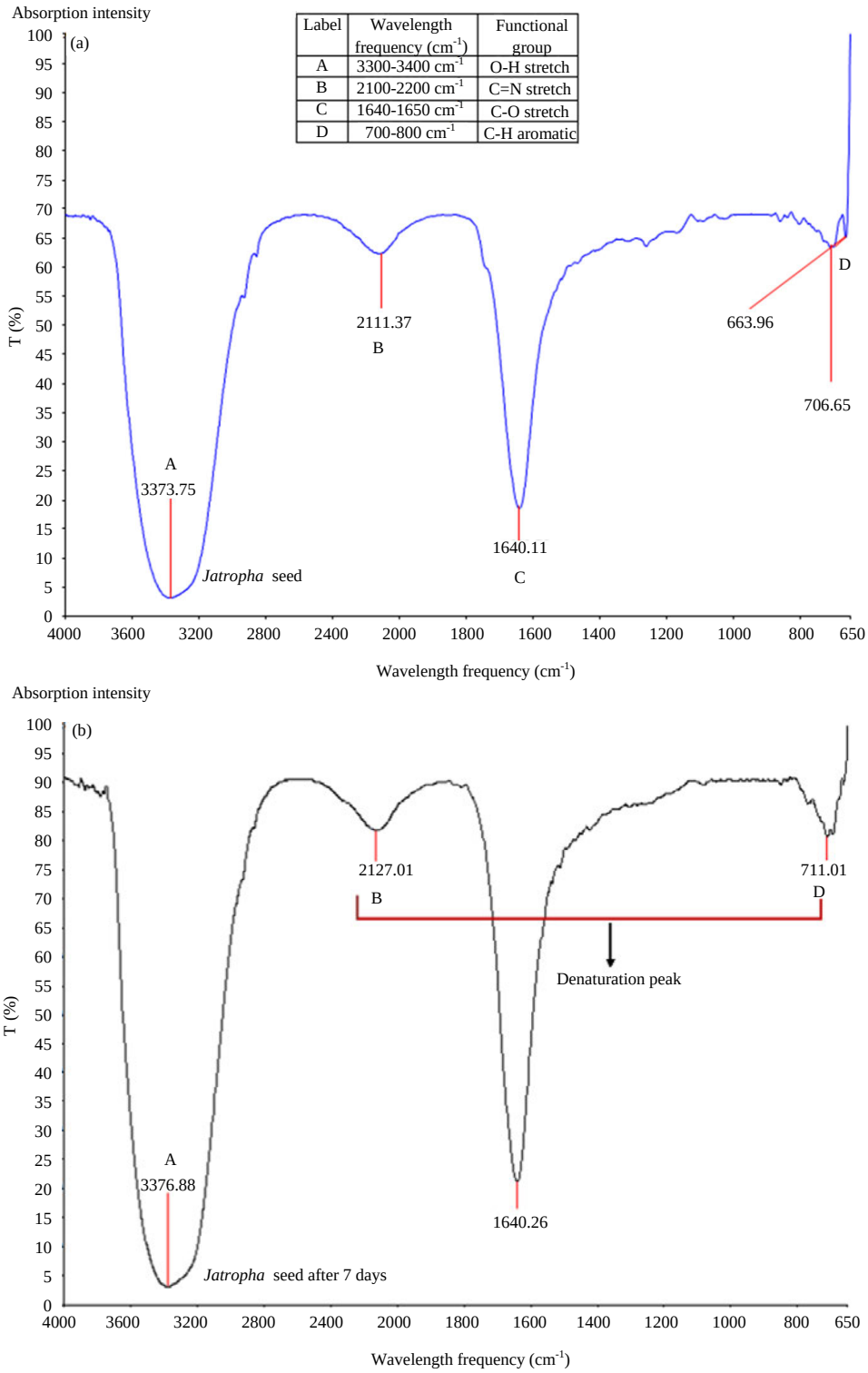

Fig. 4(a-b): (a) Infrared spectroscopy for *Jatropha curcas* seed coagulant stored in refrigerator at 4°C and (b) Infrared spectroscopy for *Jatropha curcas* seed coagulant stored at room temperature of 24°C

Degradation was the alteration of the natural existing compound including protein which is to be acting as coagulant in this case. *Jatropha* seed and presscake among other also consists a substantial amount of protein that is

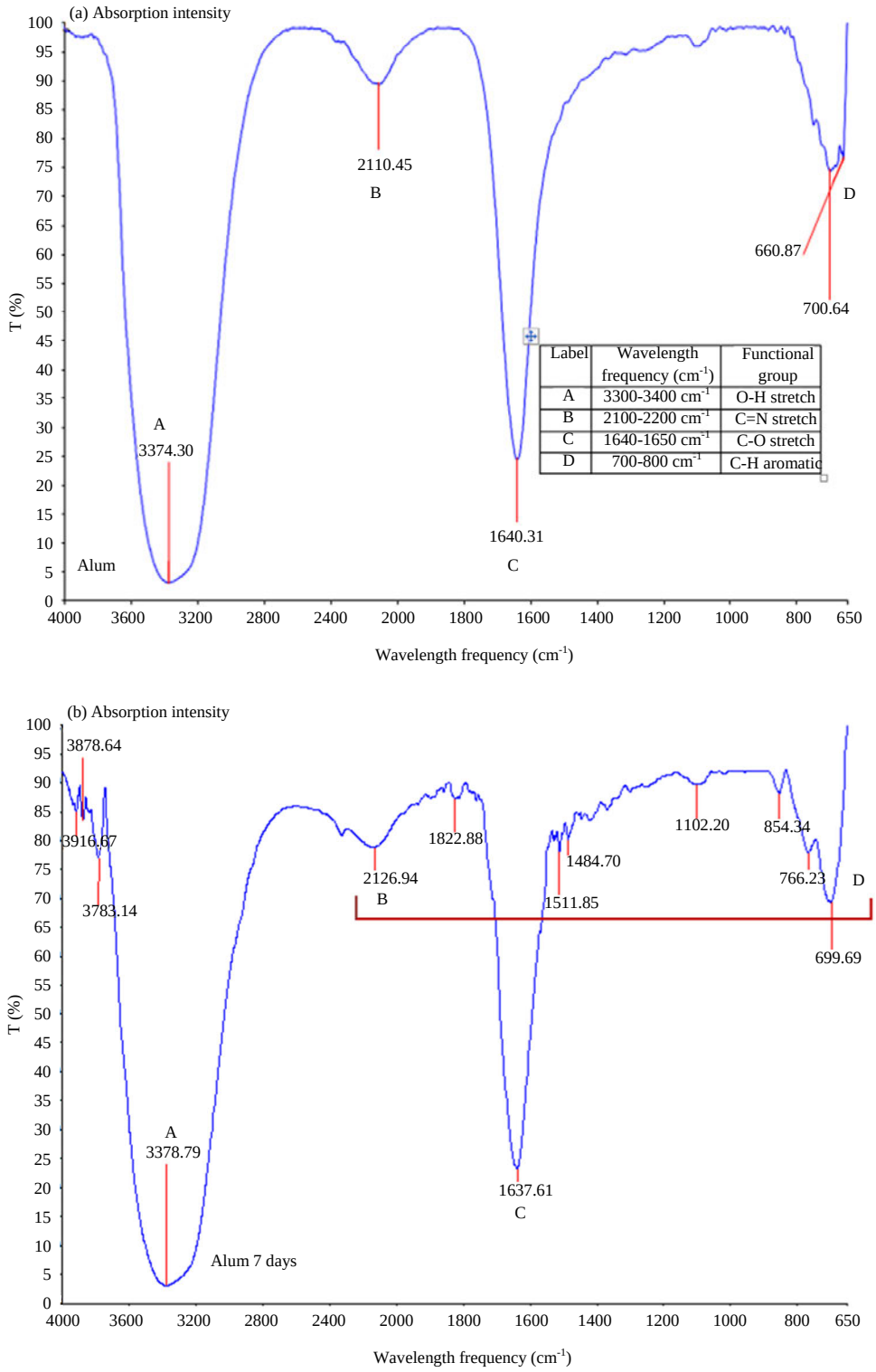

Fig. 5(a-b): (a) Infrared spectroscopy for alum coagulant stored in refrigerator at 4°C and (b) Infrared spectroscopy for alum coagulant stored at room temperature of 24°C

susceptible towards material degradation and microbial attack since it can be consumed as microbial nutrient source. Since degradation was unavoidable, hence in order to prevent any aging effects, such as change in pH, viscosity and coagulation

activity due to microbial decomposition of organic compound during storage[18,19], a fresh solution was suggested to be prepared for each sequences of the experiments to retain the quality of the biocoagulant extracted. Otherwise, the transformation of the liquid coagulant extract into a powder form would also be favourable[19,25,26]. These workers transformed liquid extractant from *Moringa oleifera* into powder by using freeze drying[26] and also spray drying methods[25]. By doing so, the deterioration in the quality and performance of the biocoagulant may be reduced significantly.

Similar FTIR spectra for *Jatropha curcas* seed and presscake at 4°C after 7 days was found to be similar to FTIR spectra for a fresh extract (day 0 in this case) from *Jatropha* presscake and seed by other researchers[5]. This means 4°C is sufficient to store samples. Results exhibited Amide I vibration which normally is not affected by the nature of the side chain[22] but depends on the secondary structure of the backbone. On the other hand, tremendous change in FTIR of *Jatropha curcas* presscake after 7 days storing at room temperature suggested protein denaturation that contributed to poor coagulative performance. Besides destruction to sample during grinding and exposure to chemicals as mentioned before, high storing temperature is another factor that increase susceptibility to microbial attack. Optimal temperature range is in the cool to moderate range (4-21°C). Deterioration of stored foods will increase as the temperature increase[27]. Respiration and metabolic rates were directly related to room temperatures within a given range. The higher the rate of respiration, the faster the produce deteriorates. Lower temperatures slow respiration rates and senescence processes, which prolongs the storage life of a bioproduct. Low temperatures also slowed the growth of pathogenic fungi which cause spoilage of fruits and vegetables in storage[28].

Katayon *et al.*[18,29,30] also reported that the biological products will be affected as all biological materials is susceptible to changes due to humidity and temperature changes that affect product quality and performance. Moisture and temperature are two critical factors in bio-products storage[30,31]. Proper storage conditions were needed to lengthen product shelf life and maintain quality. Bio-products from plants are living tissues although they are no longer associated with the plant. They continues respiration and their composition and physiology continue to change after harvesting. Cellular breakdown and death are inevitable but can be slowed with optimal storage conditions. Ideally, low moisture content is essential ~~since~~ the coagulant is in powder form. High humidity encourage growth of molds and bacteria that can lead to spoilage and degradation in quality. Furthermore, moisture content of the coagulant also affects the mobility of the ion to form agglomerates during coagulation process.

Denaturation is an inevitable composition or structural changes to biological compound that cause it to lose some or all of its functional characteristics. Extracted coagulant agent must be kept in a conducive environment to suppress microbial attack that lead to degradation. Refrigeration and freezing are an easy, cheap and efficient method to minimize this effect.

CONCLUSION

Storage time and conditions influence the performance of *Jatropha curcas* as bio-coagulant due to product denaturation and microbial attack. Presscake experienced greater denaturation than seed due to its exposure to harsh treatment during disruption and recovery that destroy its protective cell wall and membrane. Effectiveness and performance of *Jatropha* is comparable to commercial alum with many advantages like short sedimentation time and lesser sludge volume. As a natural product, some thought needs to be put into testing its feasibility for commercial used. Producing powder *Jatropha* coagulant can be an option. However, other aspects like transportation, storage and packaging can become major issues that need attention.

SIGNIFICANCE STATEMENT

This work, the effect of storage conditions of *Jatropha curcas* seed and presscake biocoagulant on coagulation performance and its quality on wastewater treatment. Palm Oil Mill was used as sample wastewater model. Up to date, there is no work yet being reported about this investigation with respect to biocoagulant except for the study of *Moringa olefiera*.

As a seed, *Jatropha curcas* is susceptible to microbial attack and hence degradation. This inturn will affect its performance as biocoagulant. This work aim to unveil the effect of storage conditions and time to Jatropha curcas coagulant. Analysis on the bio-coagulant quality were also conducted using FTIR and protein content determination. Results show that storage time of the seed and presscake resulted in a slight reduction of performance. Storing in refrigerator i.e., at lower temperature helps to suppress

degradation of biocoagulant product which originated from protein and hence insignificant changes of coagulating performance was recorded. These results are supported by FTIR and protein analysis. The findings is important to assist industrial application of this Jatropha coagulant and can be an impactful steps towards a greener approach.

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