1 **Enhanced coagulant extraction from *Jatropha curcas* in aqueous solutions and the**
2 **application in turbidity removal**
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20 **ABSTRACT**

21 In this study, the effect of the extraction medium on the properties and efficiency of bio-
22 coagulant, extracted of *Jatropha curcas* (Jc), in turbidity removal from aqueous solutions has
23 been investigated. The optimised values of NaCl concentration (*i.e.* NaCl), solution pH and
24 solution temperature were identified to improve the extraction of the coagulant. The optimised
25 conditions were associated with an optimum coagulant dosage and a maximum turbidity removal
26 from the synthetic aqueous solutions. The highest turbidity reduction was achieved with the
27 coagulant extracted at a solution pH of 10 and an extraction temperature of 60 °C (pH10/60°C-

JcPc). Under these conditions, the coagulant dosage required was reduced by 80-90%, depending on the coagulation pH. At the coagulation pH=6, the pH10/60°C-JcPc well reduced the turbidity by 85%. However, the distilled water-based extract failed to lower the turbidity. Several analytical techniques were employed to characterize the nature of the active components derived from Jc. SDS-PAGE electrophoresis showed that Jc extract was mainly made up of proteins with molecular weights between 20 and 35 kDa. The optimized extraction conditions significantly improved the efficiency of this promising bio-derived coagulant in turbidity reduction. This study demonstrates the potential employability of these enhanced bio-coagulants. This can be a step ahead in helping with the development of sustainable processes in (waste)water treatment, particularly in tropical regions *e.g.* Malaysia with an abundant access to Jc.

Keywords: Jatropha curcas; natural coagulant; water treatment; biomass; protein; turbidity removal.

1. Introduction

Today, one of the key changes in the process industries is waste minimisation in the conversion of waste to value-added end products. Furthermore, the continual depletion of available resources has led to a rising growth in research opportunities *e.g.* the use of waste biomass in energy production and water/wastewater treatments. Natural materials are known to be effective coagulants. They are of great importance especially in countries where access to conventional chemicals has proven to be difficult or not economical [1, 2]. These easily-biodegradable biomaterials are readily found in abundance as waste by-products with practically little or no secondary large-scale application [3, 4].

51 Active components derived from natural coagulant can be produced or extracted from
52 microorganisms, animals [5] and/or plants [6]. Plant-based coagulants are categorised into three
53 main groups depending on the nature of their active components. The first group comprises
54 oilseeds of which press-cake has shown a good coagulation activity with protein as their active
55 component. The next group is mucilage of the cactus family plants with a mixture of
56 polysaccharides, capable of treating water *via* floc forming. And finally, tannin, a general name
57 given to large polyphenolic compounds derived from natural materials [6].

58 Among plant-based coagulants, the active component in *Moringa oleifera* (*M. oleifera*) seeds
59 is a promising alternative for the conventional coagulants. The corresponding coagulation
60 mechanism has been thoroughly investigated in the literature [7, 8]. It was shown that the active
61 components in the water extract are the dimeric cationic polypeptides (6–17 kDa) with 10–11
62 isoelectric points [9]. Another potential source of bio-coagulant is *Jatropha curcas* (Jc),
63 sometimes referred to as the “physic nut”. The seeds are made up of about 30–35% oil. Jc has
64 been widely grown for oil extraction from its seeds [9-11]. A solid waste residue, called press
65 cake (Pc), with a good protein content is generated after the oil extraction process [10-12].

66 In comparison to *M. oleifera*, it has been reported [13] that the aqueous extracts of Jc kernel
67 demonstrate an acceptable coagulation activity when tested on river water samples. The active
68 coagulant component in the Jc is believed to be a soluble protein (Jc is an oilseed with a high
69 protein content). It has also been reported that the key factors in increasing the solubility of
70 protein in aqueous solutions are the ionic strength, solution pH and temperature [14-16].

71 The first objective of the present work is to investigate and optimize the extraction conditions
72 in order to reduce turbidity in aqueous solutions with a minimum dosage of bio-coagulant. This

73 study also seeks to specify and characterize the produced extract. To the best of our knowledge,
74 the composition, nature and properties of Jc extract has not been reported elsewhere. Elemental
75 analysis, FTIR analysis, enzymatic hydrolysis and sodium dodecyl sulphate polyacrylamide gel
76 electrophoresis (SDS-PAGE) measurements were employed in order to characterize the active
77 components.

78 **2. Materials and methods**

79 *2.1 Materials*

80 The Jc seeds were supplied by the Malaysian Agricultural Research and Development
81 Institute (MARDI). The raw plant was freshly collected from Universiti Putra Malaysia's farm.
82 Kaolin, sodium hydroxide pellets and sodium chloride were purchased from R&M Chemicals,
83 Essex, UK. Hexane from Systerm Co., Shah Alam, Malaysia, Alcalase from Novozymes A/S,
84 Baegsvard, Denmark and Hydrochloric acid (HCl, 36%) from Fisher Scientific, UK were the
85 other chemicals used in the present work.

86

87 *2.2 Characterisation of Jc*

88 A hexane-based solid–liquid extraction process was employed in order to prepare the Jc press
89 cake [17]. The results from the proximate and elemental analyses (Truspec CHNS/CHNSO
90 elemental analyzer (LECO, USA)) of the shelled Jc seeds as well as the press cake are shown in
91 Table 1. All chemicals used in the experiments were of analytical grade and were used as
92 received without any further purification. In addition, the functional groups in Jc and JcPc were
93 detected on a Spectrum 100 FTIR spectrometer (Thermo Nicolet) within a spectral range of
94 4000-400 cm^{-1}. The spectra of the kernel and the press cake were similarly recorded.

95

96 2.3 *Extraction of the coagulant's active component*

97 In order to extract the active component, two grams of the sieved JcPc (100-550 µm) was
98 mixed with 100 ml of the extraction solution. In order to determine the optimum extraction
99 conditions, the effects of three experimental parameters *i.e.* solution temperature, NaCl
100 concentration and solution pH (*i.e.* the extraction pH), were studied. To achieve this, different
101 aqueous media were used: distilled water (Favorit Water Still W4L) at neutral pH (*i.e.* pH 7) as
102 well as at pH values of 9 and 10 (adjusted by using 0.1 and 1 M NaOH) and NaCl solutions (0.1,
103 0.2, 0.3, 0.5, 1.0, 2.0 M) [11, 18]. The extraction temperature was studied at three levels: the
104 room temperature (RT), 40 °C and 60 °C [10]. All mixtures were continuously stirred for five
105 minutes on a heated hotplate, using a magnetic stirrer (Fisher Scientific Isotemp). The
106 suspension was next filtered through a muslin cloth and cooled down to room temperature. The
107 supernatant was then centrifuged (High Speed Refrigerated Centrifuge CUBOTA 6500) at 3000
108 rpm for 15 minutes. These coagulant solutions, extracted in distilled water (DW) and NaCl
109 solutions, were referred to as DWE-JcPc and SCE-JcPc (*i.e.* Distilled Water Extract/Sodium
110 Chloride Extract of JcPc), respectively. A fresh solution was prepared for each sequence of
111 experiments to prevent any change in coagulation activity due to microbial decomposition of the
112 organic compounds during storage. The coagulant extract from JcPc at optimum extraction
113 condition was labelled as pH10/60°C-JcPc indicating that the extraction solution pH and solution
114 temperature were adjusted at 10 and 60 °C, respectively.

115

116 *2.4 Isolation, partial purification and characterization of the active component*

117 In order to identify the nature of the active components in Jc, enzymatic hydrolysis was
118 performed by adding Alcalase to pH10/60°C-JcPc. The solution pH of the pH10/60°C-JcPc was
119 adjusted at 8 using HCl (0.1 M and 1 M) prior to the addition of the enzyme (pre-adjusted
120 medium pH). The mixture was then continually stirred inside a water bath regulated at 50 °C for
121 6 hr [19, 20]. During this process, the entire peptide bonds were broken down [21]. This would
122 then allow a coagulation test to potentially prove the quality of the extracted active component in
123 the solution [19].

124 In order to study the nature of the active component, three samples from the original
125 pH10/60°C-JcPc solution (30 ml, pH=8) were prepared in three volumetric flasks (100 ml).
126 Alcalase (500 μl) was next added to one of the three samples. The second sample was used as the
127 control experiment with no added Alcalase. Finally, the third sample (Alcalase free) was placed
128 and kept inside the refrigerator (4-7 °C) in order to prevent any microbial decomposition of the
129 organic compounds. An enzyme control test was also employed by adding of Alcalase (500 μl)
130 to distilled water (30 ml, pH = 8). After the completion of the hydrolysis reactions, the
131 coagulation activities of all four samples were tested *via* the jar test.

132 Active proteins were isolated and characterized using a range of techniques including
133 isoelectric precipitation, dialysis and electrophoresis. A modified version of an existing method
134 [10] was employed in order to precipitate the active proteins from the JcPc solutions. In order to
135 isolate the protein from the pH10/60°C-JcPc, the pH of the coagulant solution was adjusted to 5
136 using HCl (Fisher Scientific 36%) before being centrifuged at 3000 rpm for 20 min at room
137 temperature (High Speed Refrigerated Centrifuge CUBOTA 6500). The generated white

138 suspension was then dissolved in distilled water and was continually stirred for four hours on a
139 magnetic hotplate (Fisher Scientific Isotemp). Finally, the non-soluble residue was filtered firstly
140 through a Whatman No. 1 filter paper and then through a 0.45 and 0.22 μm nylon membrane.
141 The supernatant was dialyzed overnight at 4 °C against deionized water in a dialysis bag (UC36-
142 32-100, Viskase Sales Corp) with a molecular weight cut-off of 12-14 kDa.

143 The protein's Molecular Weight Distribution of the distilled-water extract and the purified
144 pH10/60°C-JcPc samples were monitored by SDS-PAGE (sodium dodecyl sulphate
145 polyacrylamide gel electrophoresis) on 12.5 % (w/v) polyacrilamid gel. Protein bands were
146 visualized *via* Coomassie brilliant blue R-250 staining [22].

147 The total sugar and protein contents of the natural coagulant solutions were measured *via* the
148 phenol-sulfuric acid method with glucose as the standard [23] and the Bradford method with
149 bovine serum albumin as the standard, respectively [12].

150

151 *2.5 Turbid water samples*

152 In order to identify the optimised experimental condition for the extraction of the active
153 component of Jc, synthetic kaolin turbid water was prepared and used in the subsequent turbidity
154 removal experiments. Kaolin was used to establish a desired level of turbidity. By adding 7.5 ml
155 of kaolin stock solution [24] to 500 ml of tap water and storing the solution over night to
156 maintain a constant temperature and remove any residual chlorine, a 200-NTU turbid water was
157 successfully synthesized. The turbidity was determined using a HACH Turbidimeter (Model
158 2100 N). The pH of the turbid water samples was 7.41 ± 0.07 and where indicated, the desired

159 pH (*i.e.* coagulation pH) was adjusted by adding hydrochloric acid (0.1 M and 1 M) and sodium
160 hydroxide (0.1 M and 1 M).

161 *2.6 Coagulation experiments*

162 Jar floc test, comprising a series of batch experiments, was employed in order to study the
163 coagulation process. All experimental parameters were kept constant during the tests except for
164 the target variable. The coagulation activities of the different extracts were evaluated in a jar
165 tester (VELP, model JLT6). Samples were added to beakers filled with turbid water in different
166 dosages (10-400 mgL^{-1}). The solutions were then constantly stirred (100 rpm) for 4 min,
167 followed by a slow mixing phase (40 rpm) for 25 min. After about 30 minutes of sedimentation,
168 an aliquot of the clarified sample (20 mL) was collected from the top of the beaker and the
169 residual turbidity was measured. The final (residual) turbidity of the treated water samples was
170 measured and the turbidity removal percentage was calculated by the difference between the
171 initial and the final turbidity values divided by the initial turbidity, multiplied by 100. All
172 experiments were run at room temperature (25 ± 1 °C).

173 In order to investigate the reproducibility of the results, the extraction experiments in pH 10
174 and temperature 60 °C were repeated in quintuples and in irregular intervals for two months
175 followed by the subsequent analyses of the efficiency of the pH10/60°C-JcPc in turbidity
176 removal at coagulation pH of 4. Each experiment was run in triplicates. The residual turbidity of
177 these runs *i.e.* 4.22±0.43, 4.02±0.20, 4.63±0.34, 5.48±0.45, 4.65±0.31 NTU, indicates that the
178 extracted natural coagulant could successfully reduce the turbidity of the synthetic turbid water
179 with a high degree of reproducibility.

180 **3. Results and discussions**

181 *3.1 Optimisation of the extraction conditions*

182 The coagulation activity of the DWE-JcPc was investigated in a synthetic aqueous kaolin
183 solution. Through a series of initial tests, it was concluded that acidic pH facilitates turbidity
184 reduction from synthetic water. Fig. 1 presents the effect of DWE-JcPc dosage on residual
185 turbidity of synthetic aqueous kaolin solution at coagulation pH values of 4, 5 and 6.

186 The residual turbidities of water treated with the optimum dose of the coagulating solution -
187 corresponding to the highest turbidity reduction - were found to be 5 and 33 NTU at pH 4 and 5,
188 respectively. The optimum doses of coagulant at pH 4 and 5 were 100 and 200 mgL^{-1},
189 respectively. However, at a coagulation pH of 6, DWE-JcPc could not reduce the turbidity to an
190 acceptable level (*i.e.* >30% [25]) and therefore, distilled-water extraction proved not to be
191 suitable for successful coagulation at coagulation pH of 6.

192 The elemental analysis on the JcPc (Table 1) showed an enrichment of protein in the sample:
193 8.38 wt% nitrogen (7.69 wt% carbon) as compared to the kernel, which contained only 4.98 wt%
194 nitrogen (57.05 wt% carbon). The crude protein contents of Jc's kernel and JcPc are 31.52% and
195 54.04%, respectively (Table 1). Both kernel and press cake were further analysed *via* FTIR to
196 confirm the presence of the protein. As illustrated in Fig. 2, FTIR analysis for Jc kernel and press
197 cake showed two strong absorption bands at 1635 cm^{-1} and another at 1535 cm^{-1}. Another band
198 was also recorded in the range of 1240-1246 cm^{-1}. These peaks are characteristic of carbonyl
199 C=O stretching vibrations in primary, secondary, and tertiary amides, respectively [26, 27]. This
200 suggests that the Jc seeds hold up a high percentage of protein within them. The comparison
201 between spectra of the press cake and the kernel demonstrated that the amide infrared absorbance

202 has not been remarkably altered in the press cake due to the structural changes in protein after
203 solvent extraction of lipids.

204 Therefore, the active component of Jc press cake as a coagulant might be protein molecules.
205 In both laboratory and commercial-scale extraction processes, water with sodium or potassium
206 hydroxide are used to modify the pH and to ultimately extract protein from press cake [10, 28].
207 On this ground, the same approach was adapted throughout the JcPc active component extraction
208 process.

209 With NaCl solution, it was observed that the optimum dosage of JcPc extract was halved
210 compared to when water was used as the extraction medium at coagulation pH 4. The
211 corresponding amount of SCE-JcPc was 40 mgL^{-1}. On the other hand, the lowest concentration
212 of NaCl showing the same degree of turbidity reduction was 0.3 M. Additionally, higher
213 concentrations of up to 0.5 M did not show any significant difference while elevated salt
214 concentrations (1.0 and 2.0 M NaCl) had a negative impact on turbidity reduction. The decrease
215 in coagulation performance at 1.0 and 2.0 M could be due to the "salting-out" phenomena [10].

216 In the next phase of the experiments, the effects of temperature and pH on extraction were
217 investigated. Although the temperature of the extraction solution was expected to improve the
218 extraction yield (increasing temperatures facilitated the molecular dissolution of solutes in the
219 solvents), the results indicated that solely preheating the coagulant solution would not have a
220 significant effect on the coagulating efficiency. It has been observed that in some cases, an
221 increase in temperature could decrease the protein solubility at isoelectric point (pI); however, at
222 other pH values (below and above pI), the effect of temperature on solubility of protein was
223 strongly affected by solution pH [28]. On the other hand, Saetae et al. 2011 [16] reported that Jc

224 protein solubility increases with temperatures within the range of 30 to 50 °C at an extraction pH
225 of 12. In general, it is understood that the effect of temperature on solubility is strongly
226 dependent on the pH of the extracting solution and therefore, there exists an interaction between
227 these two experimental factors. Hence, in the subsequent experiments, the effects of temperature
228 and pH of the media were studied together.

229 Fig. 3 demonstrates the effects of ionic strength, pH and temperature of the extraction solution
230 on the optimum coagulant dosage to achieve a residual turbidity of 5 NTU ≤ in synthetic water at
231 a coagulation pH of 4.

232 The optimum dosage was observed to be 100, 40 and 20 mgL^{-1} for DWE-JcPc, SCE-JcPc and
233 pH10/60°C-JcPc at a coagulation pH of 4, respectively. A notable achievement was the
234 improvement in coagulation activity of JcPc extract at coagulation pH values of 5 and 6 by
235 changing the extraction pH and the temperature of the extraction media. Fig. 4 draws a
236 comparison among various extracting solutions with respect to turbidity removal at coagulation
237 pH values of 5 and 6. The results show that the extraction of active components at an extraction
238 pH of 10 and higher temperature substantially improved the performance of coagulant at
239 coagulation pH values greater than 4.

240 The efficiency of different extracting solutions at coagulation pH=4 was understood to be the
241 same in terms of the observed residual turbidity. They all showed residual turbidity of ≤ 5 NTU.
242 However, the optimum coagulant dosages dropped by 80% and 90% for coagulation pH 4 and 5,
243 respectively. Additionally, a higher coagulation activity (by 91.6%) with the pH10/60°C-JcPc
244 was observed at coagulation pH=5 comparing with 83% when using DWE-JcPc. Therefore,
245 regarding the optimum dosage improvemnt, the highest achieved turbidity removal with
246 pH10/60°C-JcPc at coagulation pH=5 was almost 11 times higher than that of distilled water

247 extract. Interestingly, at the coagulation pH=6, and with the use of an alkaline-medium extract,
248 the coagulation activity increased by 85% despite the absence of coagulation activity with
249 distilled water extract. Therefore, it is concluded that extraction at pH 10 and at 60 °C presents
250 two major advantages: a reduction in the optimum dosage to achieve the maximum turbidity
251 removal and the improvement of coagulation activity at coagulation pH=5 & 6. Although salt
252 extraction solution showed its capability in reducing the optimum dosage at coagulation pH=4
253 and it also indicated an improvement in coagulation activity at coagulation pH=5 compared to
254 DWE-JcPc, it was still not as efficient as the solution extracted at pH 10 and at 60 °C.

255 Since the active coagulant component was now believed to be protein, the protein and
256 carbohydrate content of the extractant were subsequently analysed. The results indicate a
257 significant increase in the yield of protein extraction from JcPc through alkaline extraction.
258 Extraction at 60 °C and a pH of 10 corresponded to a higher protein content (*i.e.* 6.65 ± 0.20 gL^{-1})
259 than extraction in a water-only medium (*i.e.* 0.81 ± 0.06 gL^{-1}). Furthermore, the total amount
260 of carbohydrate in DWE-JcPc and pH10/60°C-JcPc were 1.35 ± 0.17 gL^{-1} and 1.63 ± 0.02 gL^{-1},
261 respectively. Similar findings by other researchers [10, 29] have also been reported in the
262 literature for optimising the extraction of protein from oil seed biomass.

263

264 *3.2 Isolation and identification of pH10/60°C-JcPc active component*

265 In order to characterize the extracts, several characterization techniques were employed.
266 Protein hydrolysis and SDS-PAGE electrophoresis were used to characterize the coagulation
267 active components in Jc.

The hydrolysis process is a reaction in which a substance is degraded. Enzymatic hydrolysis involves minimum side reactions and leads to a limited hydrolysis of selected bonds. Proteases hydrolysis breaks down proteins into various peptides which are further reduced into amino acids. This leads to a complete degradation of protein structure [19]. The results could support our hypothesis that the active component of Jc-derived coagulant is a protein if the hydrolysed sample does not show a significant coagulation activity. Fig. 5 compares the performance of the pH10/60°C-JcPc and the hydrolysed pH10/60°C-JcPc solutions for their turbidity removal.

Sample no. 1, 2 and 4 were maintained inside a water bath under identical Alcalase hydrolysis conditions. The hydrolysed pH10/60°C-JcPc solution did not show any coagulation activity. The turbidity removal of the treated water using the hydrolysed pH10/60°C-JcPc was measured to be 2-5% compared to the turbid water treated with sample no. 2 and 3 (*i.e.* 85-92%). This indicated that sample no. 1 completely failed to reduce the turbidity and therefore, no coagulation activity was observed. Alcalase just reacts with protein components and break downs peptide bonds, resulting in new components such as amino acids with completely different properties from proteins. In addition, the results of sample no. 4 confirmed that Alcalase did not demonstrate any coagulation activity.

The SDS-PAGE was used to determine the molecular weight profile for the isolated proteins. The SDS-PAGE patterns of pH10/60°C-JcPc (Fig. 6 (a)) contain three major bands indicating the presence of various types of proteins with different molecular weights. The highest protein intensities of pH10/60°C-JcPc are grouped into two classes of molecular weights: <25 kDa and >25-35 kDa. In general, a minimum number of 25 protein bands are present in the Jc seed protein [18]. The number of proteins extracted in distilled water was much larger than the optimum extraction conditions while the concentration of the target protein was significantly

291 higher in the pH10/60°C-JcPc solution. These results were consistent with the findings by
292 Hamarneh et al. 2010 [22]. They stated that the molecular weight of the protein in the Jc extract
293 (under isoelectric conditions) contains three major bands between 20 and 45 kDa (Fig. 6 (b)). In
294 our study, this was between 20 and 35 kDa.

295 The total protein content in pH10/60°C-JcPc after isoelectric precipitation was measured to be
296 1.5 ± 0.06 gL^{-1}. However, the total amount of carbohydrate was observed to be negligible. This
297 also confirmed that the protein isolation procedure was successful. The protein content of the
298 pH10/60°C-JcPc after isoelectric precipitation was 77% less than of the pH10/60°C-JcPc, owing
299 to the loss of proteins during the precipitation and dissolution stages.

300

301 **4. Conclusion**

302 The purpose of this study was to optimise the extraction method of the active coagulation
303 component from Jc seeds. In order to identify the extracted species, a range of characterisation
304 techniques were employed. The key findings in this study are summarised as follows:

305 1- Among various extraction media employed to extract the active components, an extraction
306 medium with a pH of 10 and a temperature of 60 °C was found to be the optimised condition for
307 both maximum turbidity removal and optimum coagulant dosage required. In the removal of
308 kaolinite turbidity at the coagulation pH 4 and 5, JcPc extracted in an alkaline media
309 demonstrated an improved coagulation activity with a five- and tenfold reductions in the required
310 coagulant dosage compared to JcPc extracted in distilled water, respectively. The experimental
311 results indicate the efficiency of the optimized extraction conditions in the active component
312 extraction from JcPc. The protein content of pH10/60°C-JcPc was 8.2 times higher than that of
313 the DWE-JcPc. The pH10/60°C-JcPc could efficiently coagulate more than 91% of the initial

200-NTU kaolin turbidity using only a dosage of 20 mgL^{-1} at a coagulation pH of 5. However, 200 mgL^{-1} of the DWE-JcPc was required to remove only 80% of the same kaolin water. With an alkaline solution as the extractant, a coagulation activity at pH 6 corresponding to more than 85% turbidity removal was observed. However, distilled water extract did not show any coagulation activity at this pH.

2- As confirmed *via* the elemental/proximate analyses as well as the FTIR spectra, the effective coagulant components were protein molecules. This was further confirmed through enzymatic hydrolysis. The water-extract solution demonstrated at least 20 protein bands in SDS-PAGE gel. Comparing the two SDS-PAGE gels, it was realised that the active proteins had a molecular weight between 20 and 35 kDa.

This study has showed that the pH10/60°C-JcPc can be a promising coagulant to be used in water treatment plants, especially in tropical countries such as Malaysia with an easy access to Jc. It was realized that the Jc seeds could be used as an efficient coagulant in water and wastewater treatment upon an initial purification of the active component. It is mentionable that the ultimate application of this coagulant in pilot and full-scale plants requires appropriate cost-benefit and a full techno-economic analyses for a viable commercial application of pH10/60°C-JcPc. Therefore, the authors would see it critical and with key benefits to perform such calculations in future works.

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416

417 **Table 1.** Elemental and proximate analyses of shelled Jc seed.

		Elemental analysis (dry wt%)				
		Nitrogen	Hydrogen	Carbon	Sulfur	Oxygen
Kernel		4.98±0.03	8.87±0.17	57.05±0.24	2.20±0.06	22.56±0.16
Press cake		8.38±0.30	6.80±0.12	47.69±0.15	0.18±0.02	33.11±0.23

	Proximate analysis (raw wt%)					
	Crude protein	Crude Lipids	Ash Content	Moisture Content	Crude Fiber	Total Carbohydrates*
Kernel	31.52±0.35	51.79±0.09	3.30±0.14	3.95±0.04	6.56±0.27	2.88±0.06
Press cake	54.04±0.43	16.19±0.02	6.03±0.03	5.20±0.02	7.64±0.13	10.90±0.12

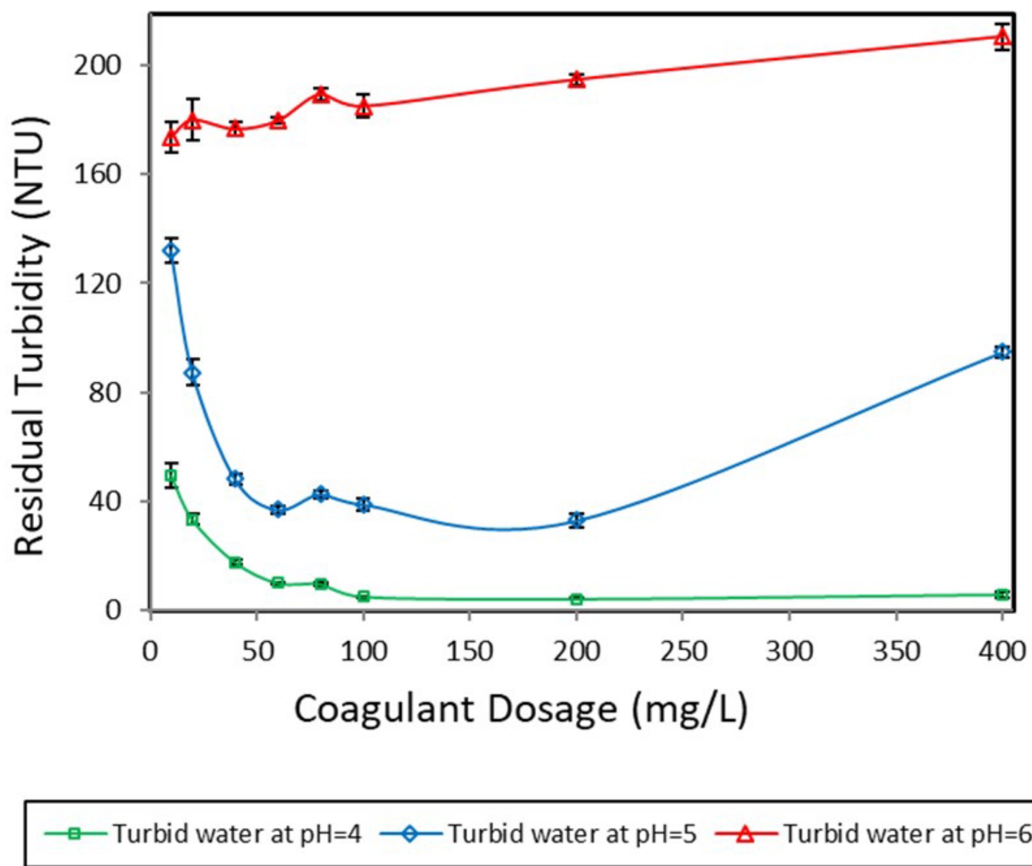
418 * Calculated from difference

419

420 **Figure captions**

421 **Fig. 1.** Effect of water-extracted coagulant (DWE-JcPc) dosage on turbidity reduction of

422 synthetic kaolin/water at coagulation pH values of 4, 5 and 6.



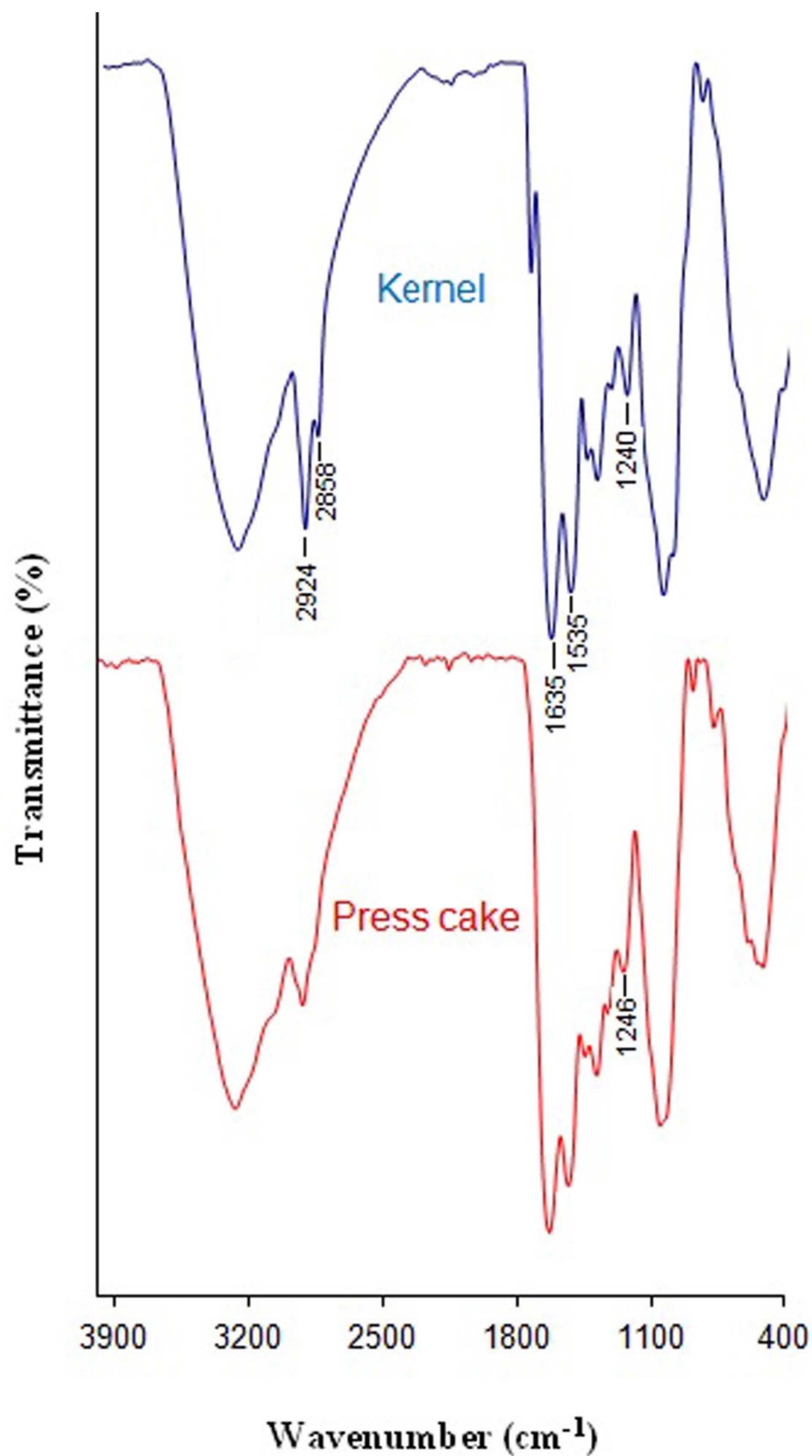
423

424 **Fig. 2.** FTIR spectra of Jc kernel and press cake.

425 The classical bands at 2924 and 2858cm^{-1} are typical features of compounds containing long

426 alkyl chains [10]. The absence of 2858 cm^{-1} band in the press cake spectrum validates the fat

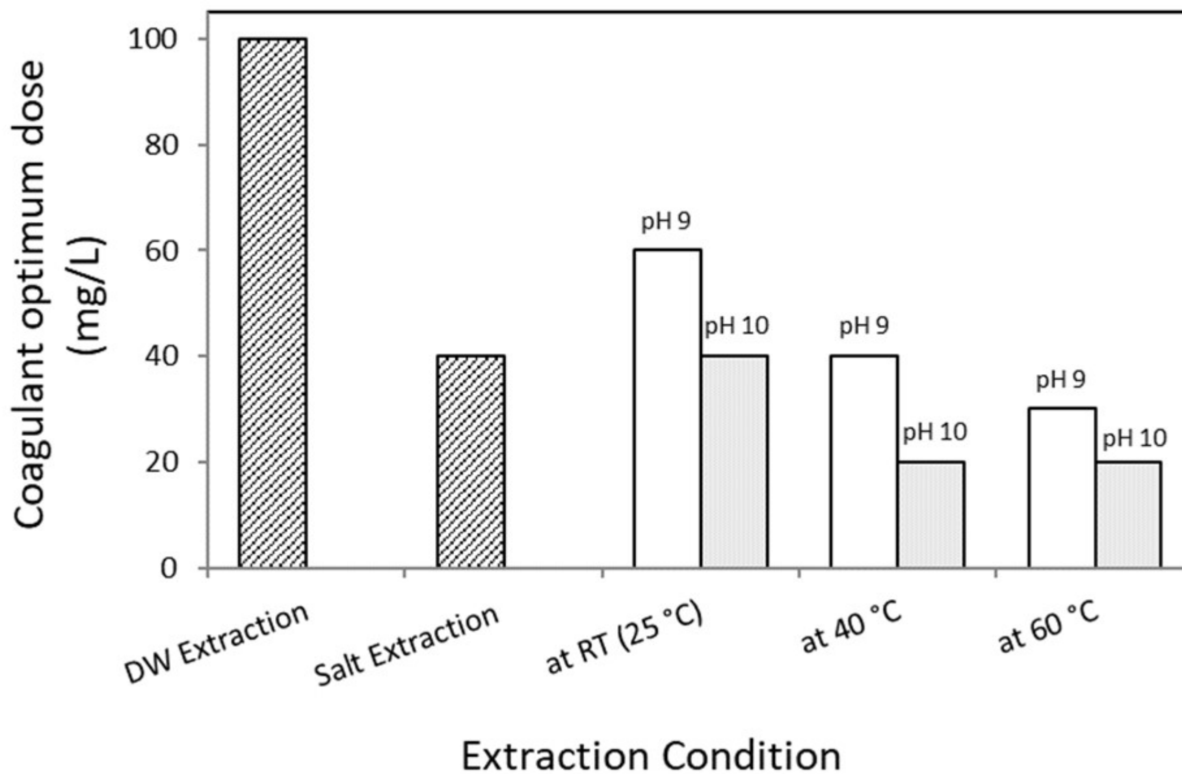
427 extraction from the Jc.



428

429 **Fig. 3.** Optimum dose corresponding to a residual turbidity of 5 NTU ≤ of water at coagulation

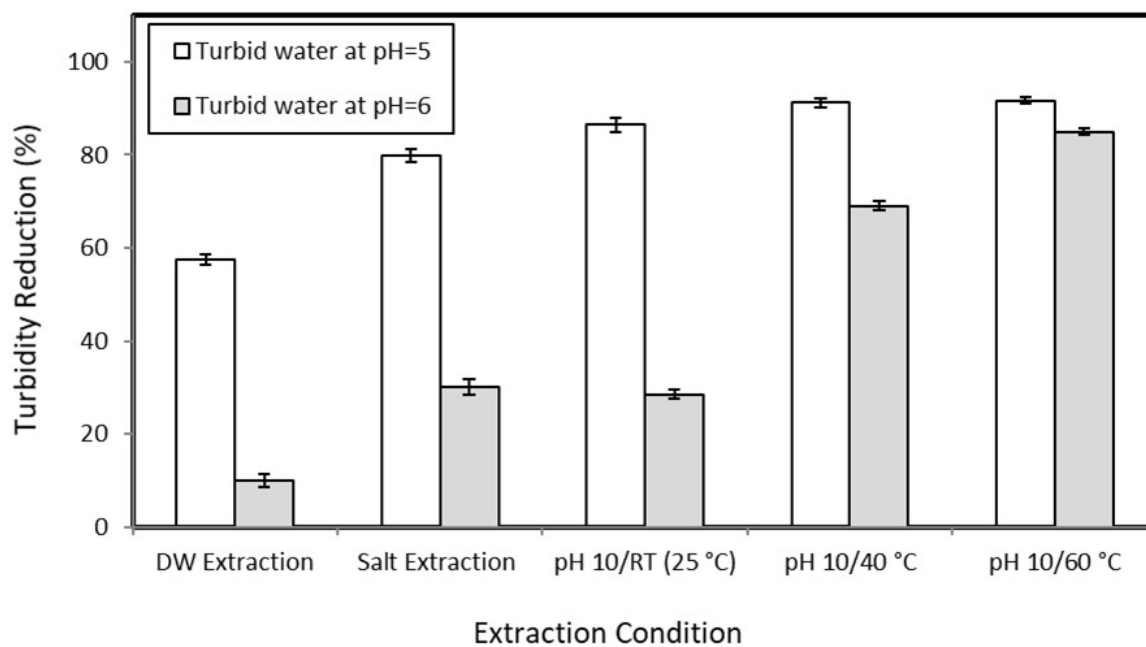
430 pH 4 using various extracting solutions.



431

432 **Fig. 4.** Turbidity reduction of synthetic water at two coagulation pH values of 5 and 6 with

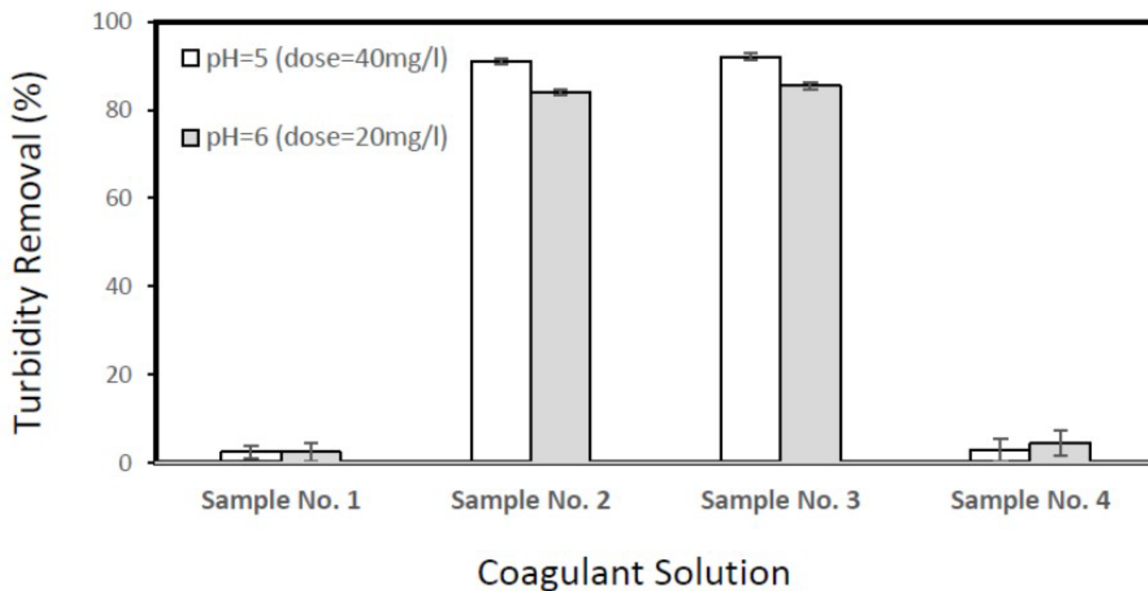
433 various extracting solutions with a constant coagulant solution dosage of 20 mgL^{-1}.



434

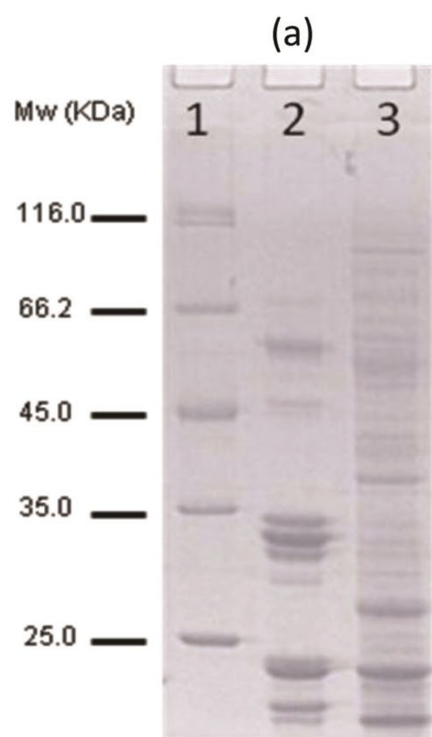
435 **Fig. 5.** Residual turbidity of kaolin water at two-coagulation pH values of 5 and 6 by using

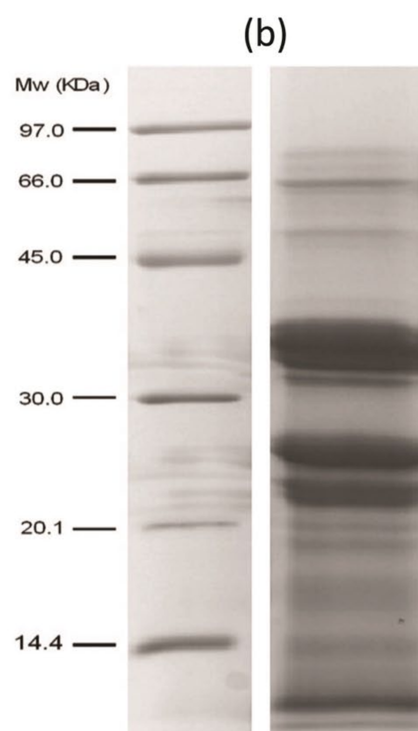
436 pH10/60°C-JcPc solution under protein hydrolysis reaction.



437

438 **Fig. 6.** Protein molecular weight distributions of JcPc extracts: (a) Isolated protein from
439 pH10/60°C-JcPc by isoelectric precipitation & dialysis (2), DWE-JcPc (3), and protein marker
440 (1) and (b) Jc proteins extracted by isoelectric precipitation [22].





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