

1 **Application, characterization and model development of *Cassia obtusifolia* seed gum in**
2 **treating raw and complex industrial effluent together with alum**

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ABSTRACT

Coagulation is effective, simple to operate, and widely used in treatment of industrial effluent. However, commonly used inorganic coagulants pose detrimental effects on human health and living organisms, and generate large amount of toxic sludge. Thus, this study investigated the optimized use of *Cassia obtusifolia* alone or combination of alum with lower dosages in treatment of industrial effluent. In this study, raw pulp and paper mill effluent was used as a model wastewater in confirming the potential use of *C. obtusifolia* seed gum as a plant-based coagulant or coagulant aid. Response surface methodology enabled the study of interactive effects between operating treatment conditions, and to determine the optimal treatment conditions. The analysis of variance results obtained in the present study reflected significant quadratic models and strong correlations between operating conditions and treatment performance. In addition, current findings suggested that the coagulation mechanism using *C. obtusifolia* seed gum was most likely based on adsorption with inter-particle bridging. Floc characterization also showed distinctive results, indicating the presence of active functional groups and the thermal stability of floc formed using *C. obtusifolia* seed gum. A significant reduction of coagulant dosages up to 67-91% was attained with the combined use of *C. obtusifolia* seed gum and alum. At natural pH of raw PPME (pH 7.2), optimal dosages of 0.17 and 0.09 g/L *C. obtusifolia* seed gum and alum, respectively were required to achieve maximum total suspended solids and chemical oxygen demand removals (91.6 and 58.3%, respectively). The present study revealed *C. obtusifolia* seed gum could be used as a potential plant-based coagulant or coagulant aid in treatment of raw and complex industrial effluent.

Keywords: Optimization; Natural coagulant; Response surface methodology; Coagulation; Alum; Wastewater treatment

13 **1. Introduction**

14 Solid-liquid separation through coagulation process is considered as an important process
15 in wastewater primary treatment (Lee et al., 2012). It is effective, simple to operate, and widely
16 used in wastewater treatment (Wang et al., 2014). Inorganic coagulants are commonly used in
17 coagulation process. However, the drawbacks associated with their prolong and excessive usage
18 include detrimental effects on human health and living organisms, ineffectiveness in low-
19 temperature water, high procurement cost, and significant generation of toxic sludge (Antov et
20 al., 2012; Yang et al., 2013).

21 On the contrary, natural coagulants are favorable due to its biodegradability, low toxicity,
22 low cost and low sludge production (Antov et al., 2012). *Cassia obtusifolia* is a plant that grows
23 1.5-2.5 m in height, and bears 8000 seeds per plant (Mackey et al., 1997). Department of
24 Agriculture, Fisheries and Forestry, Queensland, Australia (2014), reported that 2000 seeds/m²
25 of soil is harvested from local seed reserves. The present work was considered as a notable
26 advancement in introducing the use of emerging plant-based coagulant, *C. obtusifolia*, in real
27 wastewater treatment. Thus far, *C. obtusifolia* is explored mostly in the field of medicine and
28 bioscience, and not many works are associated with real wastewater treatment processes
29 (Vadivel et al., 2011; Queiroz et al., 2012; Sultana et al., 2012; Cai and Chen, 2013).

30 Among many factors, type of coagulant, coagulant dosage, effluent pH, mixing,
31 temperature, and retention time can influence the coagulation process (Wang et al., 2014).
32 Therefore, optimization of these factors is crucial in increasing the coagulation treatment
33 efficiency. Conventional one-factor-at-a-time approach is carried out by varying a single factor
34 while keeping all other factors in constant. However, this approach is time consuming, laborious,
35 and expensive because large number of experiments must be carried out (Wu et al., 2009; Teh et

36 al., 2014a). Thus, response surface methodology (RSM) was proposed in this study. RSM is a
37 combination of mathematical and statistical techniques for designing experiments, building
38 models, evaluating the effects of several factors and their interactions, and achieving optimal
39 conditions for desirable responses with a limited number of planned experiments (Srinu Naik and
40 Pydi Setty, 2014; Wang et al., 2014). Recently, this optimization technique has been applied in a
41 wide range of processes, such as absorption (Bhanarkar et al., 2014), engine performance
42 (Sivaramakrishnan and Ravikumar, 2014), biosorption (Aytar et al., 2014), electrocoagulation
43 (Sridhar et al., 2014), biodiesel production (Moradi and Mohammadi, 2014) and others.
44 However, to the best of our knowledge, the application of RSM in optimizing the combined use
45 of natural coagulant and inorganic coagulant in pulp and paper mill effluent (PPME) treatment
46 is not yet reported. Generally, appropriate combination between natural and inorganic coagulants
47 in water treatment is promising in terms of both treatment efficiency and cost (Teh and Wu,
48 2014; Teh et al., 2014a).

49 The main objective of the present work was to conduct a comprehensive optimization
50 study on an emerging natural coagulant, *C. obtusifolia* seed gum, in treatment of raw and
51 complex industrial PPME. To reduce the dosage of coagulants in PPME treatment, optimization
52 study was also conducted in investigating the potential combination between alum and *C.*
53 *obtusifolia* seed gum. A model relating the coagulation performance (in terms of both removals
54 of total suspended solids and chemical oxygen demand) to the key operating parameters
55 (coagulant dosage, effluent pH, and slow-mixing duration) was developed using RSM approach.
56 In addition, better insights into coagulation mechanism and detailed floc of combination between
57 alum and *C. obtusifolia* seed gum characterization were attained using a scanning electron

58 microscope (SEM), Fourier-transform infrared spectroscopy (FTIR), and thermogravimetric
59 analysis (TGA).

60 **2. Materials and Methods**

61 ***2.1 Materials***

62 Raw PPME was collected from Muda Paper Mills, Selangor, that generates up to 25000
63 m³/day of wastewater. The average pH, total suspended solids (TSS), and chemical oxygen
64 demand (COD) of the collected PPME was 7.2, 841 mg/L, and 1453 mg/L, respectively. The
65 collected PPME was stored at 4 °C to reduce possible biodegradation. The raw PPME was used
66 in jar-test experiments without introducing any dilution.

67 *C. obtusifolia* whole seeds were purchased locally (Fig. 1). The seeds were ground into
68 finer granules using Pulverisette 14 Variable Speed Rotor Mill (Germany) and were kept in an
69 air-tight glass container. Fresh *C. obtusifolia* seed gum stock solution of 25 g/L was prepared
70 daily. Alum (aluminium sulfate octadecahydrate) was purchased from Sigma-Aldrich with A.C.S
71 grade and used as a control without further purification.

72 ***2.2 Experimental procedure***

73 A jar-test procedure was set up at room temperature for each experimental run. Each beaker
74 contained 300 mL of raw and undiluted PPME. The effects of initial effluent pH, coagulant
75 dosage, and slow-mixing time were investigated by using *C. obtusifolia* seed gum and alum
76 separately. On the other hand, similar effects were studied for combination use of both *C.*
77 *obtusifolia* seed gum and alum but at natural pH of raw PPME.

78 The initial pH (pH 7.2) of the PPME was adjusted accordingly (pH 1.98-7.02) using 1
 79 mol/L of HCl or NaOH when required. Coagulants (0.01-3.57 g/L) were added into the PPME
 80 during flash-mixing stage (150 rpm for 5 minutes). The effluent was subsequently subjected to
 81 slow-mixing at 10 rpm. The floc was allowed to settle for 3 min. The sample for analysis was
 82 taken 2 cm below the surface level for measurement of final TSS and COD values. Each run was
 83 repeated in 3 replicates (n=3). The TSS and COD removals were calculated as shown in
 84 equations (1) and (2), respectively:

$$85 \quad \text{TSS removal, \%} = \frac{\text{TSS}_i - \text{TSS}_f}{\text{TSS}_i} \times 100\% \quad (1)$$

$$86 \quad \text{COD removal, \%} = \frac{\text{COD}_i - \text{COD}_f}{\text{COD}_i} \times 100\% \quad (2)$$

87 where TSS_i and TSS_f are initial and final TSS values (mg/L), respectively, and COD_i and
 88 COD_f are initial and final COD values (mg/L), respectively.

89

90 ***2.3 Experimental design and model development***

91 Central composite design (CCD) was selected as the standard RSM for the optimization
 92 of key operating factors (coagulant dosage, initial pH, and slow-mixing time) in the present
 93 study. In this study, CCD was recognised as an efficient design tool to fit second-order models.
 94 The range and coded levels of the selected operating factors (independent variables) for model
 95 development are shown in Table 1. Treatment efficiency in terms of TSS and COD removals
 96 were chosen as the responses (dependent output variables). Design-Expert[®] (Version 6.0.10,
 97 Stat-Ease Inc. Minneapolis, USA) was used to fit the responses using a predictive polynomial

98 quadratic model to obtain a correlation between the responses and factors (Teh et al., 2014a). A
 99 general form of second-order polynomial quadratic model used to describe the effects of
 100 different factors on the responses was represented in equation (3) (Hay et al., 2012):

$$101 \quad Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i < j} \beta_{ij} X_i X_j \quad (3)$$

102 where Y = responses (TSS and COD removals); β_0 = constant; β_i = linear coefficient; β_{ii} =
 103 quadratic coefficient; β_{ij} = interactive coefficient; X_i and X_j = actual factor level. The
 104 interactive effects between the operating factors on the responses were represented as 3-D
 105 contour plots. In addition, the TSS and COD removals were maximized to achieve the highest
 106 coagulation performance possible for each coagulant (*C. obtusifolia*, alum, and combined use of
 107 both coagulants) at optimal values of the operating factors. Experimental runs were then
 108 conducted in triplicate to serve as verification of the obtained optimal treatment conditions from
 109 the developed quadratic models through equation (3).

110 **2.4 Floc characterization**

111 Samples of the coagulant floc were analysed using special instrumentation. SEM (Hitachi
 112 S3400N-II model, Japan) was used to analyze the morphology of floc. The infrared spectra of
 113 floc was analysed using a FTIR spectrometer (Thermo Scientific Nicolet iS10, USA) from 400 to
 114 4000 cm^{-1} . Lastly, TGA of floc was determined using a thermal analyzer (TA Instrument TGA
 115 Q50, USA) under nitrogen atmosphere with a heating rate of 10 $^{\circ}\text{C}/\text{min}$ to 800 $^{\circ}\text{C}$.

116 3. Results and Discussion

117 3.1 Assessment of experimental results through RSM

118 Twenty experimental runs, including center and axial points were investigated in CCD-
119 designed experiment to visualize the effects of each operating factor on the responses. Based on
120 the experimental results, quadratic models were recommended to represent the coagulation
121 performance of each coagulant used in this study. Table 2 presents the Analysis of variance
122 (ANOVA) results of the regression parameters from the predicted quadratic models in Table 3.
123 The p value is used to estimate whether F value is large enough to indicate statistical significance
124 (Sridhar et al., 2014). Values of p less than 0.05 imply that the quadratic model terms are
125 significant whereas values greater than 0.10 indicate that the model terms are insignificant. All
126 quadratic models predicted in the present work were significant as indicated by the p values less
127 than 0.0001 (Table 2). Adequate precision ratios obtained were greater than 4 (Table 2),
128 indicating that adequate signal for all models could be used to navigate the design space
129 (Bhanarkar et al., 2014).

130 The lack-of-fit test describes the variation of the data around the fitted model (Muhamad
131 et al., 2013). If the quadratic models fit the data, the lack-of-fit test will be insignificant. All
132 models have insignificant lack of fit values (Table 2), indicating a significant model correlation
133 between the operating factors and responses (Muhamad et al., 2013). Table 2 shows that a good
134 quadratic fit was achieved for all models whereby, all R^2 values were close to 1, denoting a
135 satisfactory adjustment of the quadratic models to the experimental data. It should be noted that a
136 R^2 value greater than 0.75 indicates the aptness of the model (Srinu Naik and Pydi Setty, 2014).
137 Moreover, a closely high value of the adjusted R^2 indicates the capability of the developed model
138 to satisfactorily describe the system behaviour within the range of operating factors (Bhanarkar

139 et al., 2014). In terms of actual factors, quadratic models in reduced form of both TSS and COD
140 removals are expressed in Table 3.

141 **3.2 Optimization of operating treatment conditions**

142 Optimization of the treatment process using *C. obtusifolia* seed gum and/or alum was
143 conducted using RSM to minimize the operating cost by reducing the coagulant dosages but
144 achieving the highest TSS and COD removals from the wastewater. The highest removal
145 efficiencies were maximized using the optimal treatment conditions and the results are presented
146 in Table 4. Under the optimum conditions, the experimental TSS and COD removals were very
147 close to the predicted values, in which case, the error obtained were in the range of 0-2.2 and 0.4-
148 6.1%, respectively.

149 Overall, natural and unmodified *C. obtusifolia* seed gum demonstrated a positive and
150 comparatively high in both TSS and COD removals (Table 4). A slight difference in TSS
151 removal was noted between the use of *C. obtusifolia* seed gum and alum. However, the COD
152 removal using *C. obtusifolia* seed gum alone was 29.2 and 27.4% lower than alum and the
153 combined use of both coagulants, respectively. Since *C. obtusifolia* seed gum is an organic plant-
154 based compound, an addition of *C. obtusifolia* seed gum into coagulation process would
155 contribute to the increase of dissolved organic matter in the treated effluent, resulting in higher
156 final COD value (Teh and Wu, 2014; Teh et al., 2014a). However, the combined use between *C.*
157 *obtusifolia* seed gum and alum at natural pH of wastewater substantially reduced the optimal
158 dosage by 90.5 and 62.5%, respectively as compared to the use of *C. obtusifolia* seed gum or
159 alum alone (Table 4). Moreover, the combination of both coagulants resulted in TSS and COD
160 removals comparable to alum. Based on these findings, natural *C. obtusifolia* seed gum was
161 proven to be an effective natural coagulant in treating raw and complex industrial effluent

162 (Subramonian et al., 2014). Furthermore, *C. obtusifolia* seed gum could be used as a coagulant
163 aid by reducing the amount of alum required in coagulation process but achieving almost similar
164 treatment efficiency when higher dosage of alum was used.

165 ***3.3 Effect of operating parameters on treatment efficiency***

166 Optimization study using *C. obtusifolia* seed gum alone revealed that the increase in seed
167 gum dosage beyond optimal dosage (1.79 g/L) improved the TSS and COD removals due to re-
168 stabilization of suspended particles at higher dosages. *C. obtusifolia* seed gum also showed
169 higher removals at acidic pH due to better adsorption of hydrolysed organic pollutants
170 (Subramonian et al., 2014). Based on the optimization study of alum alone, an increase in alum
171 dosage beyond optimal value (0.24 g/L) showed insignificant improvement in removals because
172 of charge reversal might occur when aluminium species were present in excess (Jangkorn et al.
173 2011; Teh et al., 2014a). On the other hand, alum performed better at pH 6 due to better
174 adsorption of colloids onto its surface at pH near to neutral (Subramonian et al., 2014).

175 Since alum demonstrated better efficiency under natural pH, the optimization study using
176 both *C. obtusifolia* seed gum and alum was conducted under the aforementioned condition (Fig.
177 2), with optimized conditions shown in Table 4. Generally, all response surfaces (Fig. 2) were
178 convex in nature, suggesting that there was a well-defined optimum point for maximum TSS and
179 COD removals (Wu et al., 2009; Tan et al., 2013). By not adjusting the initial pH of wastewater
180 prior to treatment was not only economical but environmentally less toxic. When alum was used
181 as a coagulant, *C. obtusifolia* seed gum behaved as a coagulant aid in bridging the coagulated
182 particles (Bolto and Gregory, 2007). This phenomenon justified the high treatment efficiency of
183 raw PPME (91.6 and 58.3% of TSS and COD removals, respectively) despite the large reduction
184 in *C. obtusifolia* seed gum and alum dosages (dosage reductions of 90.5 and 62.5% of seed gum

185 and alum, respectively) when they were used in combination (Table 4). TSS and COD removals
186 were enhanced at longer mixing duration (Figs. 2b-c and 2e-f) up to optimum points because
187 slow-mixing promoted better adsorption and floc formation (Kumar et al., 2011; Zhang et al.,
188 2013). After optimum points, the improvements of TSS and COD removals seemed to be less
189 significant. However, the removals decreased at higher mixing time for both types of coagulants
190 due to the re-dispersion and re-stabilization of floc. This phenomenon was also observed by
191 Şengil (1994) when alunite ore was used as a coagulant aid.

192 **3.4 Floc morphology**

193 Evaluation of floc morphology provided a better understanding in the possible
194 coagulation mechanism using *C. obtusifolia* seed gum. Fig. 3 illustrates SEM images of floc
195 formed using different coagulants. Fig. 3a shows that the formation of floc formed using *C.*
196 *obtusifolia* seed gum alone had high fibrous-like morphology (Shak and Wu, 2014). This
197 phenomenon strengthened the possibility that the proposed coagulation mechanism of *C.*
198 *obtusifolia* seed gum involved adsorption with inter-particle bridging. On the contrary, alum floc
199 exhibited rough and irregular surface (Fig. 3b), which was due to aggregation of pollutants in the
200 wastewater, as observed by Teh et al. (2014b). According to Ni et al. (2012), the irregular
201 morphology was also attributed to the aluminum species present in the floc. The combined use of
202 both coagulants resulted in a composite morphology, consisting of irregular surface and fibrous-
203 like structure (Fig. 3c). This phenomenon indicates the functionality of both mechanisms
204 (adsorption with inter-particle bridging and charge neutralization) when both *C. obtusifolia* seed
205 gum and alum were used during coagulation of PPME.

206 **3.5 FTIR analysis**

207 The presence of active functional groups in *C. obtusifolia* seed gum and alum were
208 analyzed using FTIR. The infrared spectra of floc formation using both *C. obtusifolia* seed gum
209 and alum is shown in Fig. 4. The OH band is clearly indicated as the broad and strong bands at
210 3319 cm^{-1} (Singh et al., 2012). Similarly, peak at 2908 cm^{-1} was due to C-H linkages (Singh et
211 al., 2007). The weaker bands at 1409 cm^{-1} represented the bending vibration of CH_3 and the
212 scissor vibration of CH_2 (Ni et al., 2012). Peak positions around the region of $1010\text{-}1027\text{ cm}^{-1}$
213 were due to HOO matrix, OH stretching and C-O stretching (Singh et al., 2007; Singh et al.,
214 2012). Other than that, peak at 1628 cm^{-1} were assigned to aluminum composite formed with the
215 suspended particles (Singh et al., 2012). Peak at 2162 cm^{-1} was attributed to the formation of
216 interactions between alum and the suspended particles after binding (Singh et al., 2007). A close
217 comparison of floc spectra obtained from Subramonian et al. (2014) showed no signs of
218 additional new peaks for the combined use of *C. obtusifolia* seed gum with alum. The absence of
219 new peaks might confirm that no new complex was formed.

220 **3.6 TGA**

221 TGA provided the thermal decomposition of carbonaceous materials and thermal
222 stabilities of the floc with rising temperature (Subramonian et al., 2014). According to Fig. 5a, a
223 weight loss of 10% at temperature below $200\text{ }^\circ\text{C}$ was a result from moisture and volatile
224 compounds lost in all samples (Shak and Wu, 2014). As the temperature elevated from 200 to
225 $800\text{ }^\circ\text{C}$, significant amount of weight loss (55%) was observed due to gradual decomposition of
226 the sample. Differential thermal gravimetric (DTG) curve represented the decomposition stages
227 for the floc (Fig.5b). Four distinctive and similar mass change regions were noted. At
228 temperature less than $200\text{ }^\circ\text{C}$, the intra- and inter-molecular moisture were evaporated from the

229 flocculation (Lee et al., 2012). On the other hand, the sudden loss of mass observed after 200°C was
230 attributed to the loss of absorbed species. At temperatures beyond 300°C, a significant weight
231 loss occurred due to decomposition of organic components in the floc. Severe decomposition
232 occurred at temperatures above 600°C. The present result was concurrent with Lee et al. (2012),
233 reporting that most components in the floc decomposed at high temperatures. The weightlessness
234 at high temperatures (> 750 °C) was due to the total decomposition of floc (Subramonian et al.,
235 2014).

236 **Conclusion**

237 Since the study of *C. obtusifolia* seed gum, especially successful combination with alum
238 in wastewater treatment remains limited, a comprehensive study on its application in coagulation
239 process and floc characterization was conducted. The floc characteristics using both *C.*
240 *obtusifolia* seed gum with alum, revealed distinctive results on the presence of functional groups
241 and thermal stability of the floc. Based on the combined floc morphology, possible coagulation
242 mechanisms included adsorption with inter-particle bridging and charge neutralization. On the
243 other hand, RSM approach served as a functional tool in investigating the optimal treatment
244 conditions and the interactive effects between operating factors on the coagulation performance.
245 Based on the current findings, the combined use of *C. obtusifolia* seed gum and alum
246 tremendously reduced the optimal amount of coagulants by 90.5 and 62.5%, respectively.
247 Without adjusting the initial pH of raw PPME, optimal dosages of 0.17 g/L *C. obtusifolia* seed
248 gum and 0.09 g/L alum were required to achieve the maximum treatment performance (91.6 and
249 58.3% TSS and COD removals, respectively). The present study successfully established that *C.*
250 *obtusifolia* seed gum, as an emerging plant-based coagulant, could be potentially used as a

251 coagulant substitute or coagulant aid together with alum in treatment of raw and complex
252 industrial effluent.

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256 **References**

257 Antov, M.G., Šćiban, M.B., Prodanović, J.M., 2012. Evaluation of the efficiency of natural
258 coagulant obtained by ultrafiltration of common bean seed extract in water turbidity
259 removal. *Ecol. Eng.* 49, 48-52.

260 Aytar, P., Gedikli, S., Buruk, Y., Cabuk, A., Burnak, N., 2014. Lead and nickel biosorption with
261 a fungal biomass isolated from metal mine drainage: Box-Behnken experimental design.
262 *Int. J. Environ. Sci. Technol.* 11, 1631-1640.

263 Bhanarkar, A.D., Gupta, R.K., Biniwale, R.B., Tamhane, S.M., 2014. Nitric oxide absorption by
264 hydrogen peroxide in airlift reactor: A study using response surface methodology. *Int. J.*
265 *Environ. Sci. Technol.* 11, 1537-1548.

266 Bolto, B., Gregory, J., 2007. Organic polyelectrolytes in water treatment. *Water Res.* 41, 2301–
267 2324.

268 Cai, W., Chen, J., 2013. Chapter 33 – Herbs used in traditional Chinese medicine in treatment of
269 heart diseases, in: Watson, R.R., Preedy, P.R., *Bioactive Food as Dietary Interventions*
270 *for Cardiovascular Disease.* Elsevier Inc. pp. 551-590.

- 271 Department of Agriculture, Fisheries and Forestry, Biosecurity Queensland, 2014. Sicklepods.
272 http://www.daff.qld.gov.au/_data/assets/pdf_file/0013/51052/IPA-Sicklepod-PP18.pdf
273 (accessed 20.05.14.).
- 274 Hay, J.X.W., Wu, T.Y., Teh, C.Y., Jahim, J.M., 2012. Optimized growth of *Rhodabacter*
275 *syhaeroides* O.U.001 using reponse surface methodology (RSM). J. Sci. Ind. Res. 71,
276 149-154.
- 277 Jangkorn, S., Kuhakaew, S., Theantanoo, S., Klinla-or, H., Sriwiriyarat, T., 2011. Evaluation of
278 reusing alum sludge for the coagulation of industrial wastewater containing mixed
279 anionic surfactants. J. Environ Sci. 23, 587–594.
- 280 Kumar, P., Teng, T.T., Chand, S., Wasewar, K.L., 2011. Treatment of paper and pulp mill
281 effluent by coagulation. Int. J. Civil Environ. Eng. 3, 222-227.
- 282 Lee, K.E., Morad, N., Teng, T.T.T., Poh, B.T., 2012. Development, characterization and the
283 application of hybrid materials in coagulation/flocculation of wastewater: A review.
284 Chem. Eng. J. 203, 370-386.
- 285 Mackey, A.P., Miller, E.N., Palmer, W.A., 1997. Sicklepod (*Senna obtusifolia*) in Queensland.
286 Pest Status Reviews 1-46.
- 287 Moradi, G., Mohammadi, F., 2014. Utilization of waste coral for biodiesel production via
288 transesterification of soybean oil. Int. J. Environ. Sci. Technol. 11, 805-812.
- 289 Muhamad, M.H., Abdullah, S.R.S., Mohamad, A.B., Rahman, R.A., Kadhum, A.A.H., 2013.
290 Application of response surfave methodology (RSM) for optimisation of COD, NH₃-N
291 and 2,4-DCP removal from recycled paper wastewater in a pilot-scale granular activated

- 292 carbon sequencing batch biofilm reactor (GAC-SBBR). J. Environ. Manage. 121, 179-
293 190.
- 294 Ni, F., Peng, X., He, J., Zhao, J., Luan, Z., 2012. Preparation and characterization of composite
295 biofloculants in comparison with dual-coagulants for the treatment of kaolin suspension.
296 Chem. Eng. J. 213, 195-202.
- 297 Queiroz, G.R., Ribeiro, R.C.L., Romão, F.T.N.M.A., Flaiban, K.K.M.C., Bracarense, A.P.F.R.L.,
298 Lisbôa, J.A.N., 2012. Spontaneous *Senna obtusifolia* poisoning in cattle in the state of
299 Parana, Brazil. Presq. Vet. Bras. 32, 1263-1271.
- 300 Şengil, I.A., 1994. The utilization of alunite ore as a coagulant aid. Wat. Res. 29, 1988-1992.
- 301 Shak, K.P.Y., Wu, T.Y., 2014. Coagulation-flocculation treatment of high-strength agro-
302 industrial wastewater using natural *Cassia obtusifolia* seed gum: Treatment efficiencies
303 and flocs characterization. Chem. Eng. J. 256, 293-305.
- 304 Singh, P.K., Kumar, P., Seth, T., Rhee, H.-W., Bhattacharya, B., 2012. Preparation,
305 characterization and application of nano CdS doped with alum composite electrolyte. J.
306 Phy. Chem. Solids 73, 1159-1163.
- 307 Singh, V., Tiwari, S., Sharma, A.K., Sanghi, R., 2007. Removal of lead from aqueous solutions
308 using *Cassia grandis* seed gum-graft-poly(methylmethacrylate). J. Colloid Interf. Sci.
309 316, 224-232.
- 310 Sivaramakrishnan, K., Ravikumar, P., 2014. Optimization of operational parameters on
311 performance and emissions of a diesel engine using biodiesel. Int. J. Environ. Sci.
312 Technol. 11, 949-958.

- 313 Sridhar, R., Sivakumar, V., Prakash Maran, J., Thirugnanasambandham, K., 2014. Influence of
314 operating parameters on treatment of egg processing effluent by electrocoagulation
315 process. *Int. J. Environ. Sci. Technol.* 11, 1619-1630.
- 316 Srinu Naik, S., Pydi Setty, Y. 2014. Optimization of parameters using response surface
317 methodology and genetic algorithm for biological denitrification of wastewater. *Int. J.*
318 *Environ. Sci. Technol.* 11, 1537-1548.
- 319 Subramonian, W., Wu, T.Y., Chai, S.-P., 2014. A comprehensive study on coagulant
320 performance and floc characterization of natural *Cassia obtusifolia* seed gum in
321 treatment of raw pulp and paper mill effluent. *Ind. Crop. Prod.* 61, 317-324.
- 322 Sultana, S., Ahmad, M., Zafar, M., Khan, M.A., Arshad, M., 2012. Authentication of herbal drug
323 Senna (*Cassia angustifolia* Vahl.): A village pharmacy for Indo-Pak subcontinent. *Afr. J.*
324 *Pharm. Pharmacol.* 6, 2299-2308.
- 325 Tan, H.T., Dykes, G.A., Wu, T.Y., Siow, L.F., 2013. Enhanced xylose recovery from oil palm
326 empty fruit bunch by efficient acid hydrolysis. *Appl. Biochem. Biotechnol.* 170, 1602-
327 1613.
- 328 Teh, C.Y., Wu, T.Y., 2014. The potential use of natural coagulants and flocculants in the
329 treatment of urban waters. *Chem. Eng. Trans.* 39, 1603-1608.
- 330 Teh, C.Y., Wu, T.Y., Juan, J.C., 2014a. Optimization of agro-industrial wastewater treatment
331 using unmodified rice starch as a natural coagulant. *Ind. Crop. Prod.* 56, 17-26.

- 332 Teh, C.Y., Wu, T.Y., Juan, J.C., 2014b. Potential use of rice starch in coagulation-flocculation
333 process of agro-industrial wastewater: Treatment performance and flocs characterization.
334 Ecol. Eng. 71, 509-519.
- 335 Vadivel, W., Kunyanga, C.N., Biesalski, H.K., 2011. Antioxidant potential and type II diabetes-
336 related enzyme inhibition of *Cassia Obtusifolia* L.: Effect of indigenous processing
337 methods. Food Bioprocess Technol. 5, 2687-2696.
- 338 Wang, Y., Chen, K., Mo, L., Li, J., Xu, J., 2014. Optimization of coagulation-flocculation
339 process for papermaking-reconstituted tobacco slice wastewater treatment using response
340 surface methodology. J. Ind. Eng. Chem. 20, 391-396.
- 341 Wu, T.Y., Mohammad, A.W., Jahim, J.M., Anuar, N., 2009. Optimized reused and
342 bioconversion from retentate of pre-filtered palm oil mill effluent (POME) into microbial
343 protease by *Aspergillus terreus* using response surface methodology. J. Chem. Technol.
344 Biotechnol. 84, 1390-1396.
- 345 Yang, Z., Liu, X., Gao, B., Zhao, S., Wang, Y., Yue, Q., Li, Q., 2013. Flocculation kinetics and
346 flocculation characteristics of dye wastewater by polyferric chloride-poly-epichlorohydrin-
347 dimethylamine composite flocculant. Sep. Purif. Technol. 118, 583-590.
- 348 Zhang, Z., Liu, D., Hu, D., Li, D., Ren, X., Cheng, Y., Luan, Z., 2013. Effects of slow-mixing on
349 the coagulation performance of polyaluminium chloride (PACl). Chin. J. Chem. Eng. 21,
350 318-323.



Fig. 1. Whole seeds of *C. obtusifolia*.

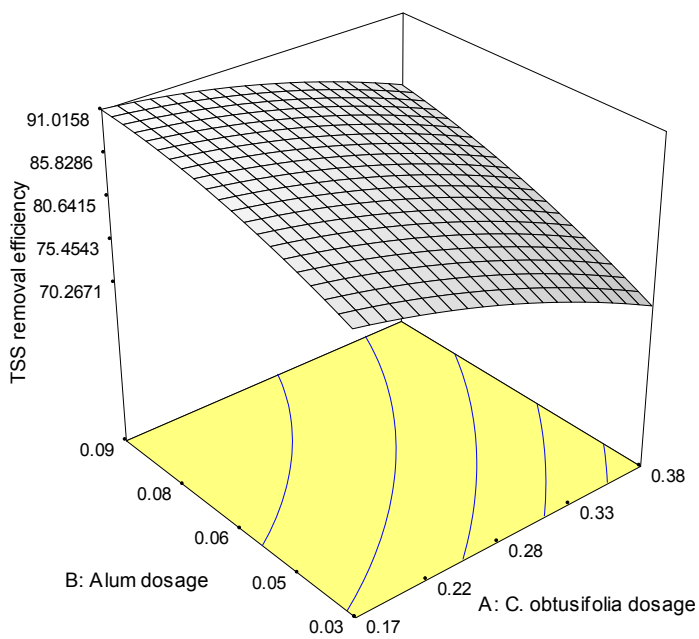


Fig. 2 (a)

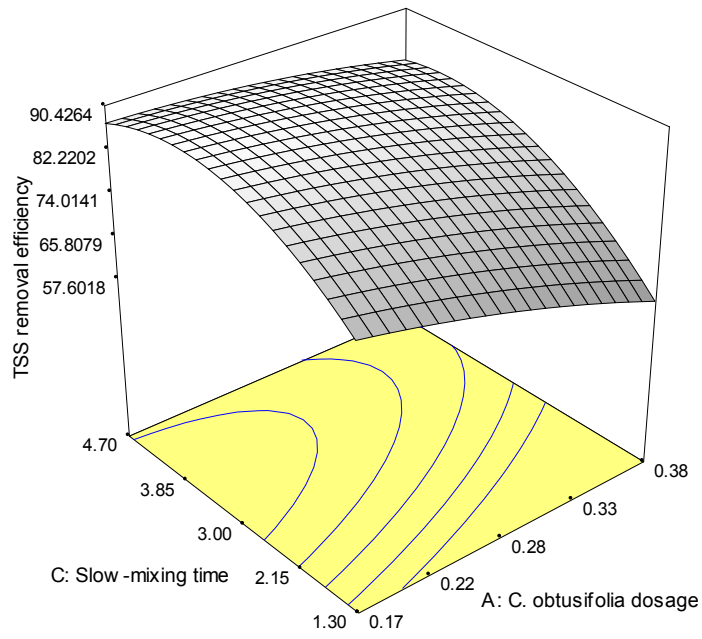


Fig. 2 (b)

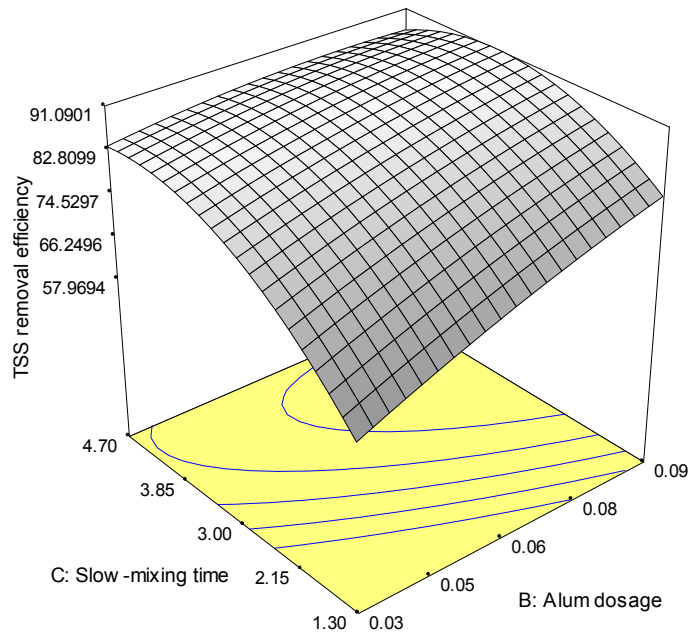


Fig. 2 (c)

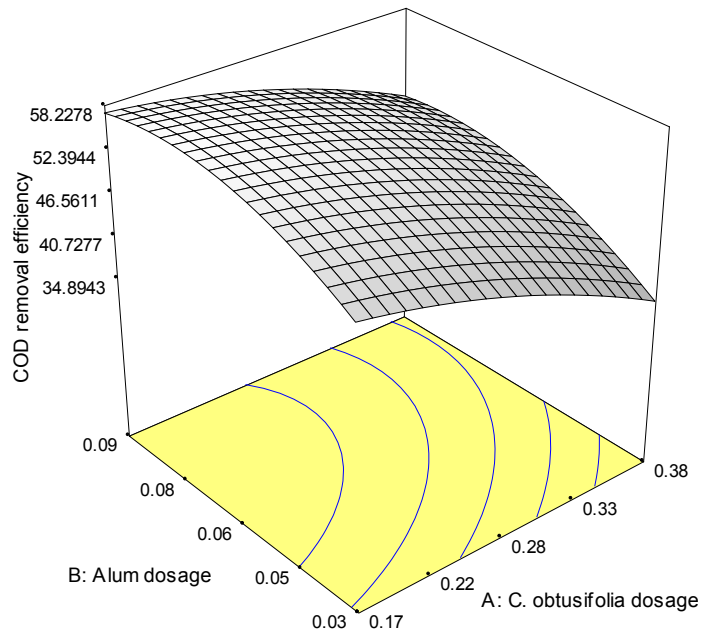


Fig. 2 (d)

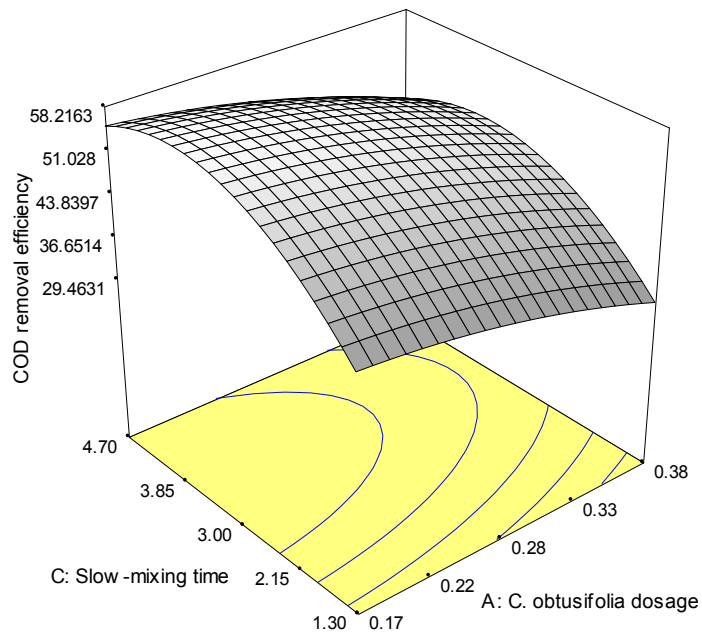


Fig. 2 (e)

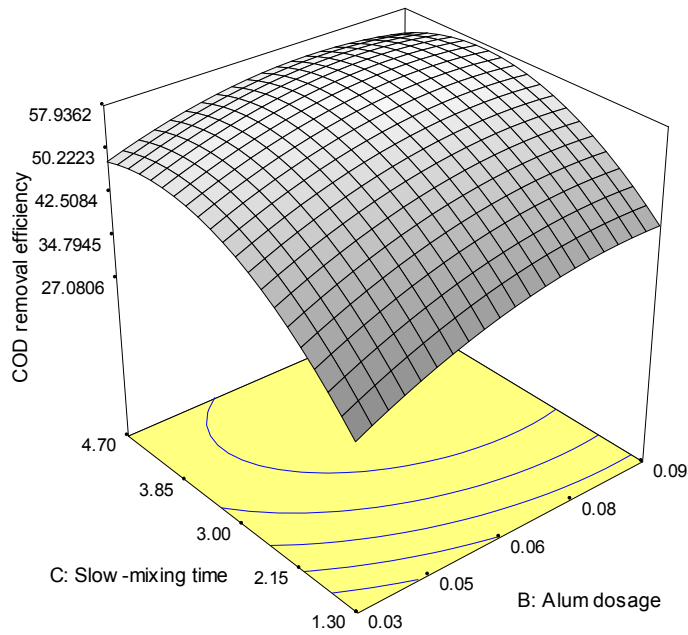


Fig. 2 (f)

Fig. 2. 3-D response surface plots showing combined use between *C. obtusifolia* seed gum and alum in TSS removal as a function of (a) *C. obtusifolia* seed gum and alum dosages, (b) *C. obtusifolia* seed gum dosage and slow-mixing time, (c) alum dosage and slow-mixing time; COD removal as a function of (d) *C. obtusifolia* seed gum and alum dosages, (e) *C. obtusifolia* seed gum dosage and slow-mixing time, (f) alum dosage and slow-mixing time. The other factors were held constant at their corresponding centre points and all combined use were done at natural pH of PPME.

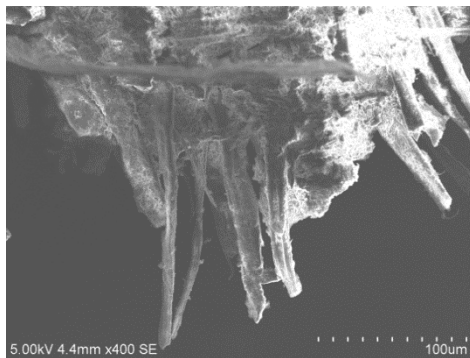


Fig. 3 (a)

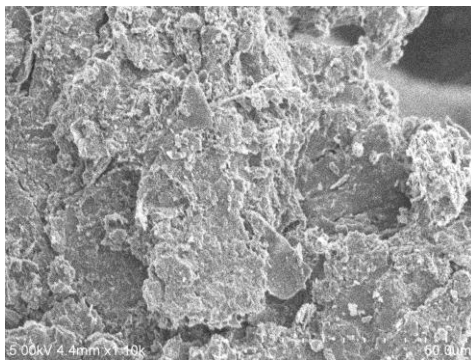


Fig. 3 (b)

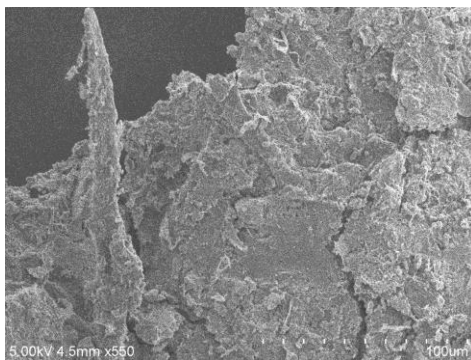


Fig. 3 (c)

Fig. 3. SEM images of floc formed using (a) *C. obtusifolia* seed gum; (b) alum; (c) *C. obtusifolia* seed gum with alum.

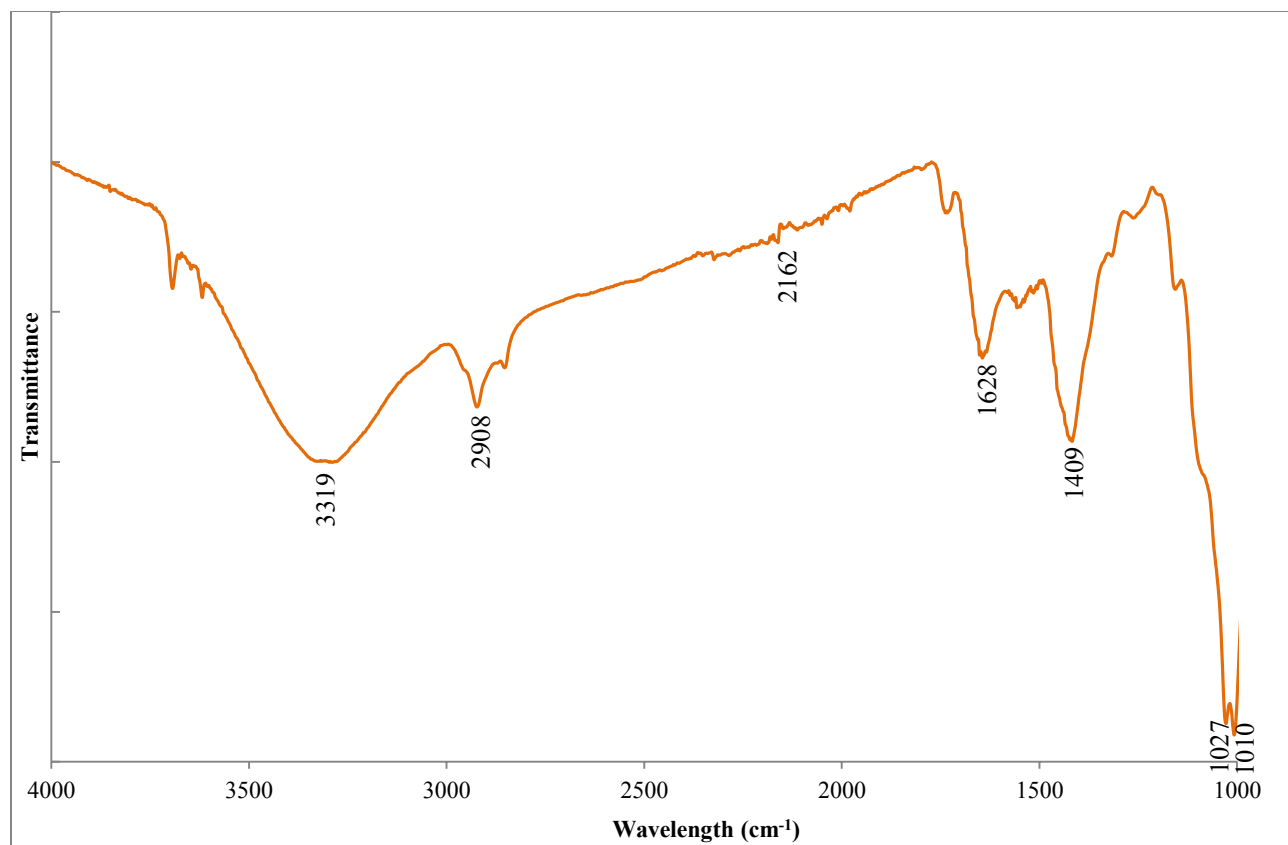


Fig. 4. FTIR spectra of floc formed using combined use of *C. obtusifolia* seed gum with alum.

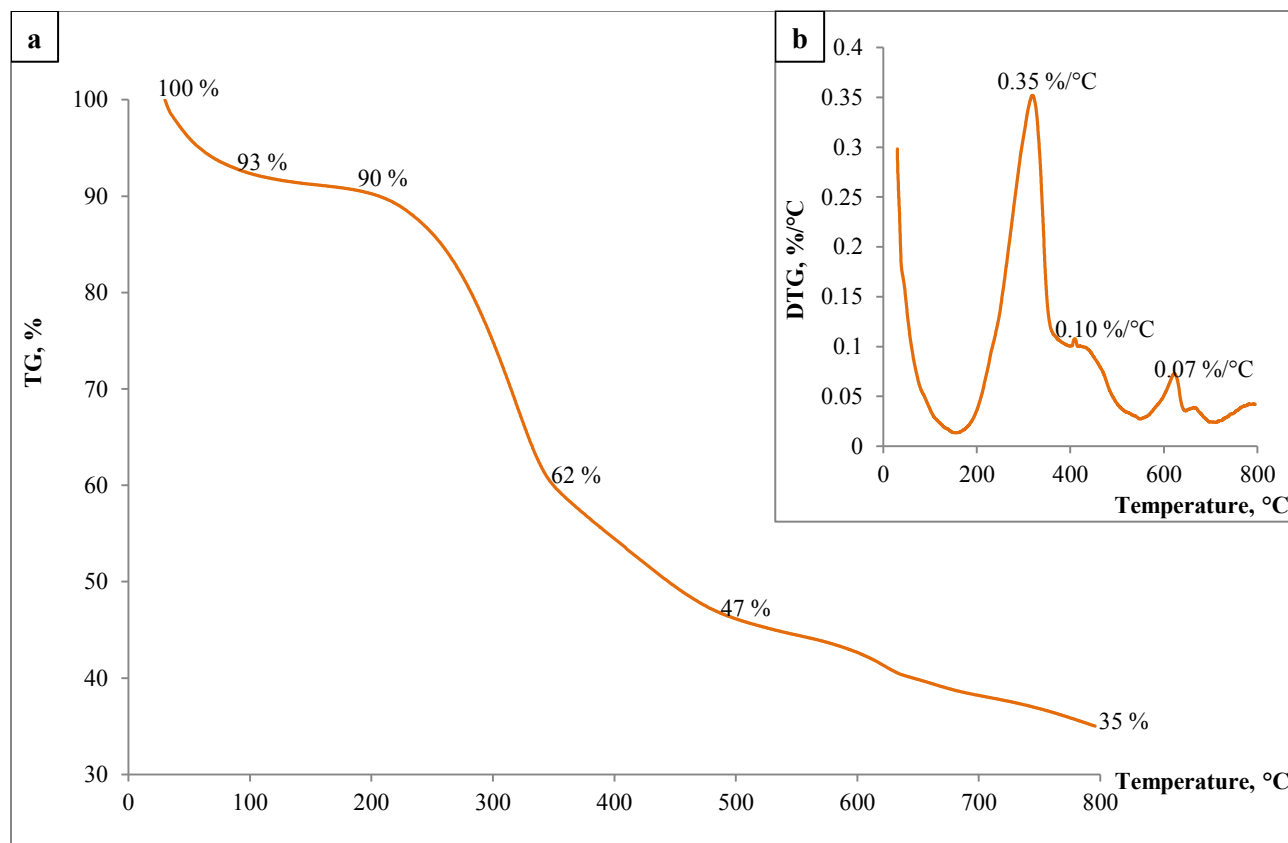


Fig. 5. (a) TG plot; (b) DTG plot of floc formed using combined use of *C. obtusifolia* seed gum with alum.

Table 1

Experimental range and coded levels of coagulant dosage, initial effluent pH, and slow-mixing time using *C. obtusifolia* seed gum and/or alum.

<u>Factors</u>	<u>Symbols</u>		<u>Coded levels</u>				
	Actual	Coded	-1.682	-1	0	+1	+1.682
<u><i>C. obtusifolia</i> seed gum</u>							
coagulant dosage, g/L	X_1	x_1	0.03	0.75	1.80	2.85	3.57
initial pH	X_2	x_2	2.45	3.00	3.85	4.60	5.15
slow-mixing time, min	X_3	x_3	0.12	2.10	5.00	7.90	9.88
<u>Alum</u>							
coagulant dosage, g/L	Y_1	y_1	0.00	0.06	0.15	0.24	0.30
initial pH	Y_2	y_2	1.98	3.00	4.50	6.00	7.02
slow-mixing time, min	Y_3	y_3	0.14	1.30	3.00	4.70	5.86
<u><i>C. obtusifolia</i> seed gum and alum</u>							
<i>C. obtusifolia</i> seed gum dosage, g/L	Z_1	z_1	0.10	0.17	0.28	0.38	0.45
Alum dosage, g/L	Z_2	z_2	0.01	0.03	0.06	0.09	0.12
slow-mixing time, min	Z_3	z_3	0.14	1.30	3.00	4.70	5.86

Table 2ANOVA of the quadratic models developed for TSS and COD removals using *C. obtusifolia* seed gum and/or alum.

TSS removal						COD removal					
Source	Sum of squares	Degree of freedom	Mean square	F value	p value	Source	Sum of squares	Degree of freedom	Mean square	F value	p value
<u><i>C. obtusifolia</i> seed gum</u>											
Model	11718.57	9	1302.06	13.19	< 0.0001*	Model	2581.23	9	286.80	39.43	< 0.0001*
x_1	1060.80	1	1060.80	10.75	0.0083*	x_1	121.86	1	121.86	16.75	0.0022*
x_2	5284.13	1	5284.13	53.53	< 0.0001*	x_2	1283.22	1	1283.22	176.40	< 0.0001*
x_3	2944.89	1	2944.89	29.83	0.0003*	x_3	563.12	1	563.12	77.41	< 0.0001*
x_1^2	994.76	1	994.76	10.08	0.0099*	x_1^2	134.08	1	134.08	18.43	0.0016*
x_2^2	54.48	1	54.48	0.55	0.4747	x_2^2	59.00	1	59.00	8.11	0.0173*
x_3^2	490.39	1	490.39	4.97	0.0499*	x_3^2	17.62	1	17.62	2.42	0.1507
$x_1 x_2$	528.13	1	528.13	5.35	0.0433*	$x_1 x_2$	154.88	1	154.88	21.29	0.0010*
$x_1 x_3$	6.13	1	6.13	0.062	0.8083	$x_1 x_3$	1.28	1	1.28	0.18	0.6837
$x_2 x_3$	528.13	1	528.13	5.35	0.0433*	$x_2 x_3$	228.98	1	228.98	31.48	0.0002*
Lack-of-fit	601.84	5	120.37	1.56	0.3183	Lack-of-fit	15.41	5	3.08	0.27	0.9122
Pure error	385.33	5	77.07			Pure error	57.33	5	11.47		
R ² = 0.9223, adjusted R ² = 0.8524, adequate precision = 12.342						R ² = 0.9726, adjusted R ² = 0.9479, adequate precision = 21.933					

Alum

Model	6101.03	9	677.89	71.38	< 0.0001*	Model	3499.17	9	388.80	1029.17	< 0.0001*
y_1	3687.44	1	3687.44	388.27	< 0.0001*	y_1	2372.41	1	2372.41	6279.92	< 0.0001*

y_2	665.27	1	665.27	70.05	< 0.0001*	y_2	382.47	1	382.47	1012.41	< 0.0001*
y_3	1021.91	1	1021.91	107.60	< 0.0001*	y_3	554.22	1	554.22	1467.06	< 0.0001*
y_1^2	266.52	1	266.52	28.06	0.0003*	y_1^2	46.91	1	46.91	124.18	< 0.0001*
y_2^2	120.05	1	120.05	12.64	0.0052*	y_2^2	2.19	1	2.19	5.80	0.0367*
y_3^2	312.15	1	312.15	32.87	0.0002*	y_3^2	90.89	1	90.89	240.60	< 0.0001*
$y_1 y_2$	2.00	1	2.00	0.21	0.6561	$y_1 y_2$	0.000	1	0.000	0.000	1.0000
$y_1 y_3$	84.85	1	84.85	8.90	0.0137*	$y_1 y_3$	32.00	1	32.00	84.71	< 0.0001*
$y_2 y_3$	50.00	1	50.00	5.26	0.0447*	$y_2 y_3$	32.00	1	32.00	84.71	< 0.0001*
Lack-of-fit	64.14	5	12.83	2.08	0.2203	Lack-of-fit	0.94	5	0.19	0.33	0.8734
Pure error	30.83	5	6.17			Pure error	2.83	5	0.57		
R ² = 0.9847, adjusted R ² = 0.9709, adequate precision = 29.656						R ² = 0.9989, adjusted R ² = 0.9980, adequate precision = 116.451					

C. obtusifolia seed gum and alum

Model	4058.49	9	450.94	80.94	< 0.0001*	Model	3195.77	9	355.09	49.71	< 0.0001*
z_1	637.64	1	637.64	114.46	< 0.0001*	z_1	571.14	1	571.14	79.96	< 0.0001*
z_2	620.37	1	620.37	111.35	< 0.0001*	z_2	407.89	1	407.89	57.10	< 0.0001*
z_3	1624.62	1	1624.62	291.62	< 0.0001*	z_3	880.87	1	880.87	123.32	< 0.0001*
z_1^2	28.84	1	28.84	5.18	0.0462*	z_1^2	51.65	1	51.65	7.23	0.0227*
z_2^2	28.84	1	28.84	5.18	0.0462*	z_2^2	212.25	1	212.25	29.71	0.0003*
z_3^2	953.03	1	953.03	171.07	< 0.0001*	z_3^2	1158.06	1	1158.06	162.13	< 0.0001*
$z_1 z_2$	8.00	1	8.00	1.44	0.2584	$z_1 z_2$	6.13	1	6.13	0.86	0.3762
$z_1 z_3$	60.50	1	60.50	10.86	0.0081*	$z_1 z_3$	1.13	1	1.13	0.16	0.6998
$z_2 z_3$	144.50	1	144.50	25.94	0.0005*	$z_2 z_3$	36.13	1	36.13	5.06	0.0483*
Lack-of-fit	26.88	5	5.38	0.93	0.5298	Lack-of-fit	22.60	5	4.52	0.46	0.7912
Pure error	28.83	5	5.77			Pure error	48.83	5	9.77		
R ² = 0.9865, adjusted R ² = 0.9743, adequate precision = 30.516						R ² = 0.9781, adjusted R ² = 0.9585, adequate precision = 23.485					

*significant terms where p values < 0.05

Table 3

Reduced quadratic models using Design-Expert[®] in treatment of raw PPME.

C. obtusifolia seed gum

$$\text{TSS removal} = -69.67 + 71.65 X_1 + 10.33 X_2 + 25.08 X_3 - 7.36 X_1^2 - 0.67 X_3^2 - 9.67 X_1 X_2 - 3.50 X_2 X_3$$

$$\text{COD removal} = 4.07 + 32.35 X_1 - 16.49 X_2 + 10.98 X_3 - 2.67 X_1^2 + 3.33 X_2^2 - 5.24 X_1 X_2 - 2.31 X_2 X_3$$

Alum

$$\text{TSS removal} = -62.40 + 411.95 Y_1 + 19.14 Y_2 + 22.38 Y_3 - 542.92 Y_1^2 - 1.28 Y_2^2 - 1.61 Y_3^2 - 21.48 Y_1 Y_3 - 0.98 Y_2 Y_3$$

$$\text{COD removal} = -42.16 + 256.08 Y_1 + 7.44 Y_2 + 14.47 Y_3 - 227.78 Y_1^2 - 0.17 Y_2^2 - 0.87 Y_3^2 - 13.22 Y_1 Y_3 - 0.78 Y_2 Y_3$$

C. obtusifolia seed gum and alum

$$\text{TSS removal} = 22.73 - 40.73 Z_1 + 644.82 Z_2 + 24.14 Z_3 - 128.30 Z_1^2 - 1471.96 Z_2^2 - 2.81 Z_3^2 - 15.41 Z_1 Z_3 - 80.65 Z_2 Z_3$$

$$\text{COD removal} = -21.88 + 32.86 Z_1 + 800.44 Z_2 + 25.88 Z_3 - 171.72 Z_1^2 - 3993.48 Z_2^2 - 40.32 Z_2 Z_3$$

Table 4Optimal treatment conditions of raw PPME using *C. obtusifolia* seed gum and/or alum.

Coagulant	<i>C. obtusifolia</i> seed gum dosage, g/L	Alum dosage, g/L	Initial pH	Slow-mixing time, min	TSS removal, %			COD removal, %		
					Predicted	Experimental	Error	Predicted	Experimental	Error
<i>C. obtusifolia</i> seed gum alone	1.79	-	3.0	7.9	87.3	87.3±1.2	0	37.9	40.2±0.7	6.1
Alum alone	-	0.24	6.0	3.6	93.7	93.1±1.0	0.6	57.0	56.8±1.0	0.4
Combination between <i>C.</i> <i>obtusifolia</i> seed gum and alum*	0.17	0.09	Natural	3.4	91.6	89.6±0.5	2.2	58.3	55.4±0.5	5.0
Reduction of coagulant dosage, %	90.5	62.5								

*The coagulation process was done at natural pH of raw PPME, which was pH 7.2

Table 5

Market prices of *C. obtusifolia* seed gum and alum and treatment cost incurred for the present study.

Coagulant	Material cost (USD/metric ton)	Coagulant dosage (g/L)	Treatment cost (USD/L)
<i>C. obtusifolia</i> seed gum alone	400	1.79	7.2×10^{-4}
Alum alone	1000	0.24	2.4×10^{-4}
<i>C. obtusifolia</i> seed gum and alum	400 1000	0.17 0.09	1.6×10^{-4}