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## **Optimisation of extraction and sludge dewatering efficiencies of bio-flocculants extracted from *Abelmoschus esculentus* (okra)**

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### **Abstract**

The production of natural biopolymers as flocculants for water treatment is highly desirable due to their inherent low toxicity and low environmental footprint. In this study, bio-flocculants were extracted from *Hibiscus/Abelmoschus esculentus* (okra) by using a water extraction method, and the extract yield and its performance in sludge dewatering were evaluated. Single factor experimental design was employed to obtain the optimum conditions for extraction temperature (25-90 °C), time (0.25-5 hours), solvent loading (0.5-5 w/w) and agitation speed (0-225 rpm). Results showed that extraction yield was affected non-linearly by all experimental variables, whilst the sludge dewatering ability was only influenced by the temperature of the extraction process. The optimum extraction conditions were obtained at 70 °C, 2 hours, solvent loading of 2.5 w/w and agitation at 200 rpm. Under the optimal conditions, the extract yield was 2.38%, which is comparable to the extraction of other polysaccharides (0.69-3.66%). The bio-flocculants displayed >98% removal of suspended solids and 68% water recovery during sludge dewatering, and were shown to be comparable with commercial polyacrylamide flocculants. This work shows that bio-flocculants could offer a feasible alternative to synthetic flocculants for water treatment and sludge dewatering applications, and can be extracted using only water as a solvent, minimising the environmental footprint of the extraction process.

Keywords: bio-flocculant; optimisation; extraction; okra; sludge dewatering

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Abbreviations	
A = agitation speed	SVI = sludge volume index
AF = aqueous bio-flocculants	t = extraction time
DF = dried bio-flocculants	T = extraction temperature
DS = dissolved solids	TS = total solids
S = solvent loading	TU = turbidity
SS = suspended solids	

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## 1. Introduction

Flocculants play a significant role in flocculation and sludge dewatering processes for the removal of solids and reduction of sludge volume in wastewater treatment (Wang et al. 2013). Inorganic flocculants (e.g. metal salts) and organic polymeric flocculants (e.g. polyacrylamide) are commonly used in the treatment of water and industrial effluents. Among various types of chemical flocculants, polyacrylamide-based flocculants with high molecular weight (typically  $>10^6$ ) have gained wide popularity due to their flocculating efficiency in the removal of pollutants (generally  $> 90\%$ ) and formation of strong and dense flocs at very low dosage (Singh et al. 2000). However, the potential problems associated with the use of chemical flocculants are their lack of biodegradability and dispersion of metal or polymer residues in the treated water that may represent a health hazard (Bae et al. 2007; Bolto and Gregory 2007; Renault et al. 2009; Sharma et al. 2006). In addition the sludge formed at water treatment plants has a limited potential for recycling due to the toxicity resulting from the use of chemical flocculants (Anastasakis et al. 2009). With the identification of acrylamide as a suspected carcinogen (Dearfield et al. 1988; Shipp et al. 2006) and the toxicity of chemical flocculants, their application in water treatment has raised public awareness regarding their use (Smith and Oehme 1991). Stringent regulations have been implemented by Japan, Switzerland and France, amongst others, to strictly control their usage in drinking water treatment and food-related processing (Bolto and Gregory 2007; Lu et al. 2014).

Under the tightened environmental regulations, researchers have started to develop natural flocculants for water purification, food and beverage clarification, and other separation processes in cosmetic, biotechnology and pharmaceutical industries (Bratskaya et al. 2004). Natural plant-based bio-flocculants with polysaccharides as the main components have emerged as a potential alternative to polymeric flocculants. Their application in wastewater

treatment has increased on account of their widespread abundance in nature and biodegradability. These bio-flocculants are derived from *Abelmoschus esculentus*, *Malva sylvestris*, *Plantago psyllium*, *Plantago ovata*, *Tamarindus indica*, and *Trigonella foenum-graecum*, and have shown promising results with respect to the treatment of biological effluent, landfill leachate, dye-containing wastewater, textile wastewater, tannery effluent, and sewage effluent (Al-Hamadani et al. 2011; Anastasakis et al. 2009; Mishra et al. 2002a; Mishra et al. 2003; Mishra and Bajpai 2005, 2006; Mishra et al. 2002b; Mishra et al. 2004).

Work to date has shown that bio-flocculants are needed in larger dosage compared with synthetic flocculants (Mishra et al. 2012; Sengkhampan et al. 2009). Conventionally, bio-flocculants are prepared using drying and extraction processes (Mishra and Bajpai 2005; Sharma et al. 2006), which are both time-consuming and require significant amounts of energy. These drawbacks have restricted the subsequent application within industry. It is known that functional properties of mucilage are sensitive to the extraction methods and conditions (Jaya and Durance 2009), however, the effects of extraction parameters on the extract yield, and flocculating and sludge dewatering abilities of bio-flocculants have not, as yet, been investigated. There is therefore a need for research to systematically determine the optimal extraction conditions in order to evaluate the potential value of bio-flocculants to industry, and to identify strategies to reduce extraction times and energy consumption.

Among the plant-based bio-flocculants that have been studied thus far, okra has attracted considerable attention mainly because it is inexpensive and readily available in tropical countries all year round. Its flocculating property in removal of solids and turbidity has been proven in clarification of kaolin solution and biologically-treated effluent (Anastasakis et al. 2009), tannery (Agarwal 2003) and sewage wastewaters (Agarwal et al. 2001). However, its capability in sludge dewatering has not been reported to date.

Conventional extraction by using solvent coupled with the use of heat and/or agitation is commonly employed to extract the bio-flocculants from plant materials (Mishra and Bajpai 2005; Sharma et al. 2006). Even though conventional extraction is time-consuming, it has been used for decades in extraction of a wide range of active components from plants, and still remains as the primary reference for preliminary or fundamental studies related to extraction and for evaluating the performance of extraction efficiency (Wang and Weller 2006).

In this work, a water extraction process was studied using shorter extraction times than existing extraction methods combined with process parameters to enhance mass transfer. The objective of this study was to optimise the extraction temperature, time, solvent loading and

agitation conditions to produce bio-flocculant with optimal extract yield and sludge dewatering ability comparable to commercial flocculants.

## **2. Material and methods**

### **2.1 Materials**

Fresh okras were sourced from the Selangor region in Malaysia. Nylon cloth (500  $\mu\text{m}$ ) was used for filtration. Kaolin slurry without chemical treatments and purification was purchased from Kaolin Malaysia Sdn. Bhd and stored at room temperature in plastic containers. The cationic (FO 4400 SH) and anionic (AN 934 SH) polyacrylamides with 30% charge density and  $1.5 \times 10^7$  g/mol were purchased from SNF Floerger dealer (KemPro Sdn. Bhd.) in Malaysia.

### **2.2 Preparation of kaolin sample**

Kaolin samples were prepared for evaluation using a Jar Test (Bratby 2006). The slurry was mixed at 1200 rpm for 10 minutes and sampled during stirring to ensure homogeneity. The pH of the slurry was measured, and suspended solids (SS) and turbidity (TU) measurements were taken. All the measurements of SS and TU in this study were conducted with colorimeter (DR/890, HACH, USA) according to Standard Methods 8006 and 8237 respectively (USEPA 2012). The characteristics of the kaolin slurry are listed in Supplementary Table S1.

### **2.3 Bio-flocculant extraction**

The okras were washed and then rinsed with deionised water. The upper crown head and the seeds inside the pods were then removed, and the pods sliced into 5-10 mm cubes. The sliced pods were ground and mixed with deionised water with a predetermined solvent loading (w/w) in conical flasks. The flasks were sealed and placed in a shaker bath at a constant temperature and agitation speed (Supplementary Figure S1). The evaporation rate was verified to be less than 0.05% over the duration of the extraction. After the predetermined extraction time, the flasks were kept aside at room temperature for 1 hour for complete release of the mucilage into water (Ameena et al. 2010).

The marc was separated from the extract by filtration, and the filtered marc subjected to centrifugation at 7000 rpm for 20 minutes to recover the remainder of the extract. The aqueous bio-flocculant (AF) was kept at 4 °C before further testing on its flocculating property. A sample of AF was dried at 40 °C until a constant weight of the sample was

obtained, then the dried bio-flocculant (DF) was stored in a desiccator at room temperature prior to use. In order to obtain the intrinsic extract yield, the dried sample of each experimental run was further dried at 105 °C for 24 hours to obtain the dry mass. The percentage of intrinsic extract yield was determined by Equation 1:

$$\% \text{ Intrinsic extract yield} = \frac{\text{mass of dried extract}}{\text{mass of plant materials}} \times 100\% \quad (1)$$

## **2.4 Experimental design**

Single factor experimental design was used to optimise the extraction conditions and study the effects of the extraction temperature, time, solvent loading and agitation speed on the extract yield and sludge dewatering ability of the bio-flocculants. Six sets of experiments were carried out to establish the most significant factors affecting the extraction of bio-flocculant. The parameters studied are presented in Supplementary Table S2.

## **2.5 Jar Test procedures on kaolin clarification**

Jar Tests were used to evaluate the flocculating efficiency of the bio-flocculants in clarification of kaolin slurry and were conducted with a commercially available Jar Tester (ET 720, Lovibond, Germany) at room temperature. The kaolin suspension was used as a representative colloidal material because its surface characteristics are well-understood and is commonly used to evaluate the characteristics of newly developed flocculants (Lee et al. 2010; Yang et al. 2009).

The bio-flocculant was injected into kaolin slurry whilst stirring at 700 rpm. The flocculation process consisted of stirring at 700 rpm for 1 minute, followed by stirring at 350 rpm for 1 minute and settling for 30 minutes. The settled sludge volume was taken for calculation of the sludge volume index (SVI) according to Method 2710D (Rice et al. 2012). The supernatant was used for measurement of the SS and TU. The treated solution was then subjected to a filtration test to analyse the sludge dewatering ability.

## **2.6 Sludge dewatering study on kaolin sludge quality**

The filtration test was performed to analyse the floc strength and the water recovery capability (Chong 2009). A Buchner funnel with 500 µm nylon filter cloth was assembled above a graduated cylinder (Lo et al. 2001; Lu et al. 2014; Wang et al. 2013). The treated solution after Jar Tests was poured into the Buchner funnel. The water recovery (filtrate volume) collected at 10s was recorded and the SS in the filtrate was measured.

### **3. Results and Discussion**

#### **3.1 Flocculating ability in removal of suspended solids and turbidity**

Prior to evaluation of sludge dewatering ability, the flocculating efficiencies of bio-flocculants extracted under different extraction conditions in SS and TU removal were examined. Before Jar Tests, the pH values of kaolin slurry were 4.5 to 5 while the pH values of bio-flocculants were 6 to 6.5. After the flocculation process the results showed that AF and DF exhibited remarkable efficiencies in SS and TU removal (>99%). Most of the SVI values were maintained lower than 10 mg/L, indicating good settling capability. All measured values were found to have a standard deviation below 3%, indicating the results were reproducible. These findings confirm that bio-flocculants extracted from okra are naturally neutral and effective for kaolin clarification under acidic conditions without pH alteration.

##### **3.1.1 Comparison of flocculating ability of extracted bio-flocculant with literature results**

The flocculating ability of the okra bio-flocculant extracted in this study was compared with other work that studied the application of okra bio-flocculants on different types of wastewater treatment. The comparison is displayed in Supplementary Table S3.

In other studies it was noted that the treatment process may require the addition of coagulant or pH adjustment, and require long treatment times in order to obtain good flocculating performance. For treatment of solutions low in SS or TU the negative colloidal particles are generally well dispersed, and hence a coagulant is required. In many cases, pH adjustment is needed to achieve the isoelectric point for formation of flocs (Chong 2012).

In this work very efficient flocculating performance was achievable directly, without addition of coagulant and pH alteration. The treatment time was significant shorter compared to other studies. There is no requirement of pH adjustment due to the near neutral behavior of the bio-flocculant. It is postulated that the bio-polymer extracted has long and branched chains which adsorb on the surface of colloidal particles and bring them together. This indicates that the extracted bio-flocculant exhibits the ability to form big and densely packed flocs which settle rapidly during treatment of solutions high in SS.

#### **3.2 Intrinsic extract yield and sludge dewatering ability**

##### **3.2.1 Effect of extraction temperature**

In order to study the effect of extraction temperature on the extract yield and sludge dewatering ability, the extraction process was carried out at 25, 40, 50, 60, 70, 80 and 90 °C while other extraction conditions as shown in Supplementary Table S2 (Sets 1 and 2) were

constant. Figure 1 shows the variation of extraction temperature on the extract yield at 100 and 200 rpm.

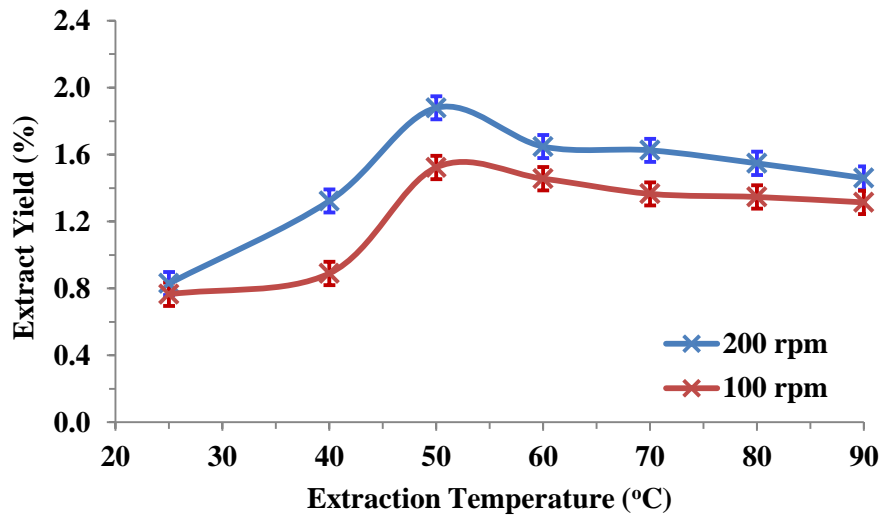


Fig. 1. Effect of extraction temperature on the extract yield (Constant conditions:  $t = 2$  hours and  $S = 1$  w/w).

The extract yield increased with temperature and achieved a maximum at 50 °C. The initial increase is most likely due to the reduction in viscosity and surface tension with temperature, and thus increases solubility and diffusion from the cell wall to the liquid phase (Carr et al. 2011; Chemat and Cravotto 2013; Samavati 2013; Ye and Jiang 2011). With further increase in temperature the extract yield declined from the peak value at 50 °C. This may be due to degradation of thermolabile components caused by prolonged exposure at these higher temperatures. A similar trend was also observed in other studies (Cai et al. 2008; Li et al. 2013; Qian 2014; Ying et al. 2011; Zheng et al. 2011). It has been reported that most biodegradable natural biopolymers contain hydrolysable groups along with the main chain, which can cause biodegradation to happen via hydrolysis, oxidation, methylation, isomerization or other degradation reactions that depend on the extraction temperature and the exposure time (Carr et al. 2011; Singh et al. 2000). To further affirm the trend the effect of extraction temperature on the extract yield was determined at a higher agitation speed (200 rpm). The extraction trend was observed to be similar, with a peak at 50 °C, but higher yields were obtained at all temperatures using the higher agitation speed.

The effect of extraction temperature on the sludge dewatering ability for extractions carried out at 200 rpm is presented in Figure 2.



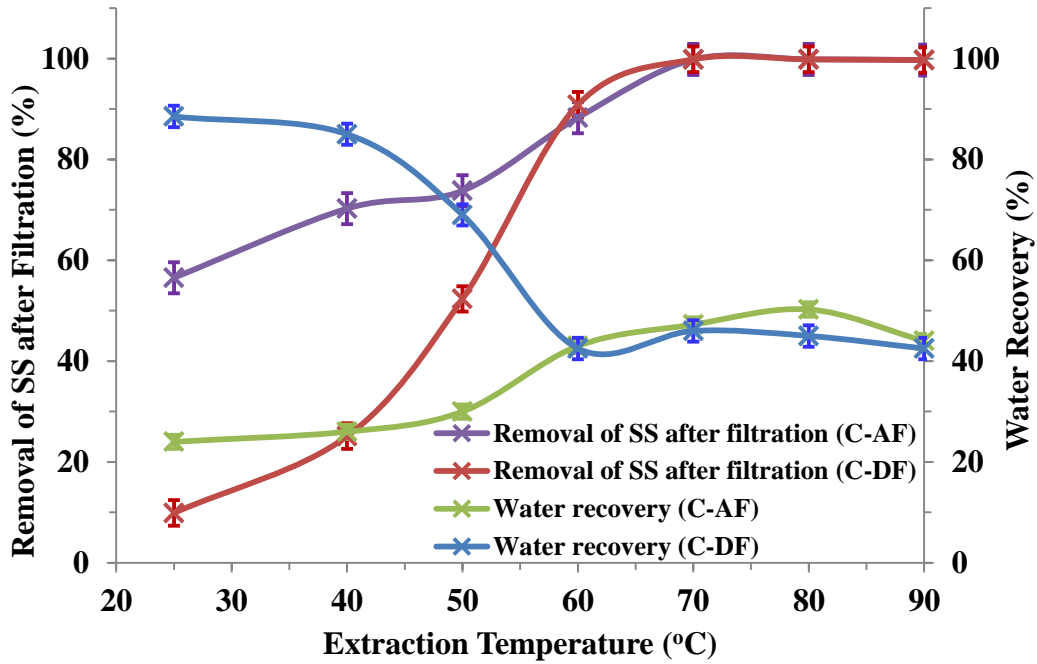


Fig. 2. Effect of extraction temperature on the sludge dewatering ability (Constant conditions:  $t = 2$  hours,  $S = 1$  w/w and  $A = 200$  rpm).

With increased extraction temperature the removal of SS after filtration for AF and DF increased and reached the maximum removal at 70 °C, then remained constant to 90 °C. Between 25 °C to 50 °C, the SS removal for AF was higher than the DF; in contrast, lower water recovery was observed for AF compared with DF. The flocs formed by DF were very fine and passed through the pores of the filter cloth during filtration whilst leading to high water recovery and low SS removal. On the other hand, the flocs formed by AF were bigger but fluffy (not dense flocs) and were retained by the filter cloth and hindered the passage of water which led to lower water recovery. As the flocs were loosely packed, some of it were easily broken into fine particles during filtration and passed through the filter cloth and thus lower the solid removal efficiency. The observed phenomena for both AF and DF indicated weak floc formation and low flocculating ability. Both observations are undesirable if a high sludge dewatering efficiency needs to be achieved.

These results reveal that even though extraction at 50 °C gives the maximum yield, it may not reflect the optimal extraction conditions. One possible reason for this is the extracted active components may not be those responsible for efficient flocculating and dewatering properties. One study states that the extraction temperature affects the polarity of water, which defines the type of compounds that are extracted (Carr et al. 2011). For temperatures between 60 and 90 °C, the bio-flocculants exhibited more desirable dewatering properties. The water recovery for AF and DF were almost constant at 45-50% and the SS removal was more than 90%. This indicates that the produced sludge flocs were stronger and more

compact than the ones at lower extraction temperatures, thus promoting the dewaterability of the flocs with high removal of SS. Despite this benefit it is noted that increasing temperature will also lead to an increase in energy consumption and hence cost of the extraction process, thus 70 °C was adopted as the optimal extraction temperature for the remainder of the parametric study.

### 3.2.2 Effect of extraction time

Figure 3 shows the effect of extraction time on the yield at 50 and 70 °C.

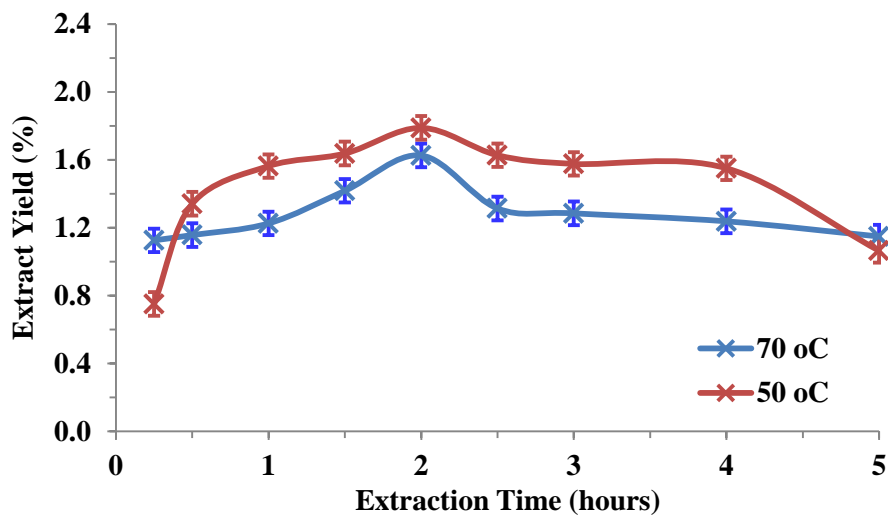


Fig. 3. Effect of extraction time on the extract yield (Constant conditions: S = 1 w/w and A = 200 rpm).

An increasing trend of extract yield with time was observed from 0.25 to 2 hours. This is expected given that sufficient extraction time is needed for the liquid to penetrate into the plant material and dissolve the active components, and for the dissolved active components diffuse out from the plant materials (Samavati 2013; Ye and Jiang 2011). The maximum yield was observed at 2 hours, and decreased from 2 to 5 hours. This supports the observations and hypothesis derived from Figure 1, whereby prolonged exposure at high temperatures may cause decomposition or hydrolysis of the target compounds and thus decrease the extract yield (Chemat and Cravotto 2013).

The influence of extraction time on the sludge dewatering efficiency of AF and DF at 70 °C is presented in Figure 4.

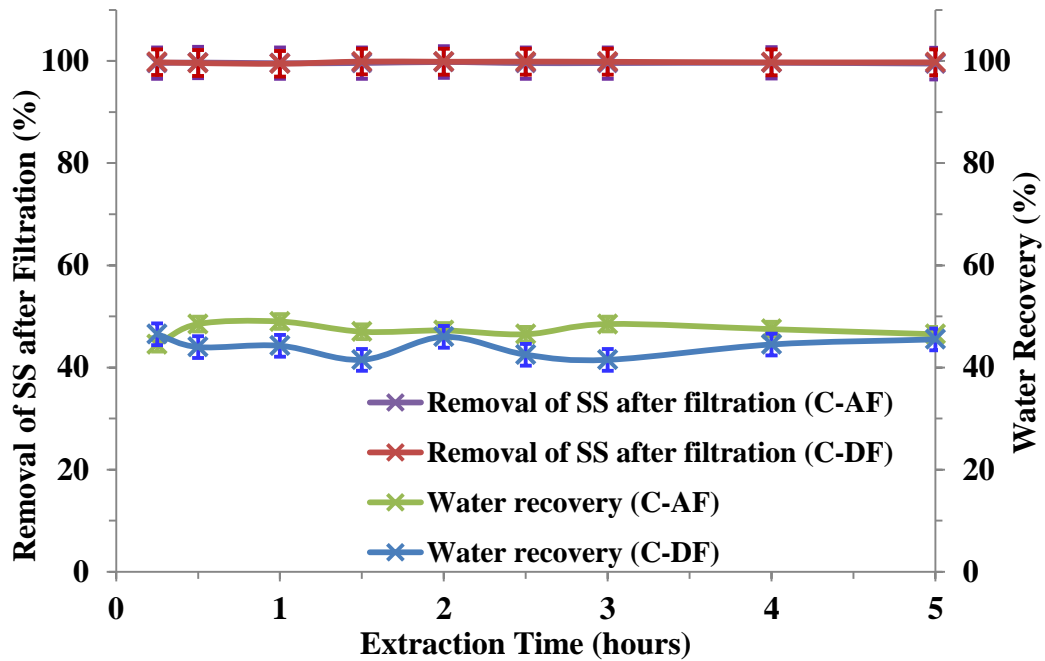


Fig. 4. Effect of extraction time on the sludge dewatering ability (Constant conditions:  $T = 70\text{ }^{\circ}\text{C}$ ,  $S = 1\text{ w/w}$  and  $A = 200\text{ rpm}$ ).

The variation in extraction time did not lead to any obvious changes in the sludge dewatering properties. Dewatering ability was stable, and is considered to be effective at all extraction times with more than 99.5% removal of SS after filtration and 45-50% water recovery obtained. This indicates that the extraction time does not affect the principal components responsible for flocculating and sludge dewatering at  $70\text{ }^{\circ}\text{C}$ . Since time did not have significant effect on the dewatering ability, the extract yields shown in Figure 3 were used to determine that the optimum extraction time was 2 hours.

### 3.2.3 Effect of solvent loading (solvent/plant ratio)

The effect of solvent loading on the extract yield is shown in Figure 5.

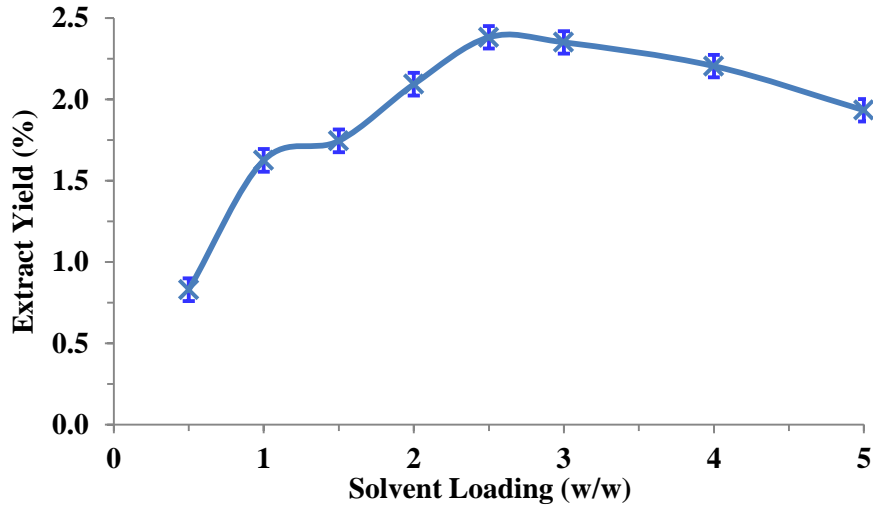


Fig. 5. Effect of solvent loading on the extract yield ( $T = 70\text{ }^{\circ}\text{C}$ ,  $t = 2\text{ hours}$  and  $A = 200\text{ rpm}$ ).

When the solvent loading increased from 0.5 to 2.5, the extract yield was enhanced, and reached a maximum value at 2.5. A larger solvent loading implies greater concentration difference between the interior plant cells and the exterior solvent, and thus increases the driving force for mass transfer of extractable components into solvent (Qiao et al. 2009; Yang et al. 2013; Ying et al. 2011). However, the extract yield indicated a decreasing tendency when the solvent loading was further increased from 2.5 to 5. This observation was not expected, and indicates that the mass transfer regime in the apparatus used for this study may vary with the solvent loading.

As shown in Figure 6, solvent loading did not have a significant effect on the sludge dewatering efficiencies of AF and DF. More than 99.5% removal of SS after filtration and water recoveries of 45-50% were obtained under all conditions. A solvent loading of 2.5 was identified as the optimum condition based on the maximum extraction yield obtained at this condition.

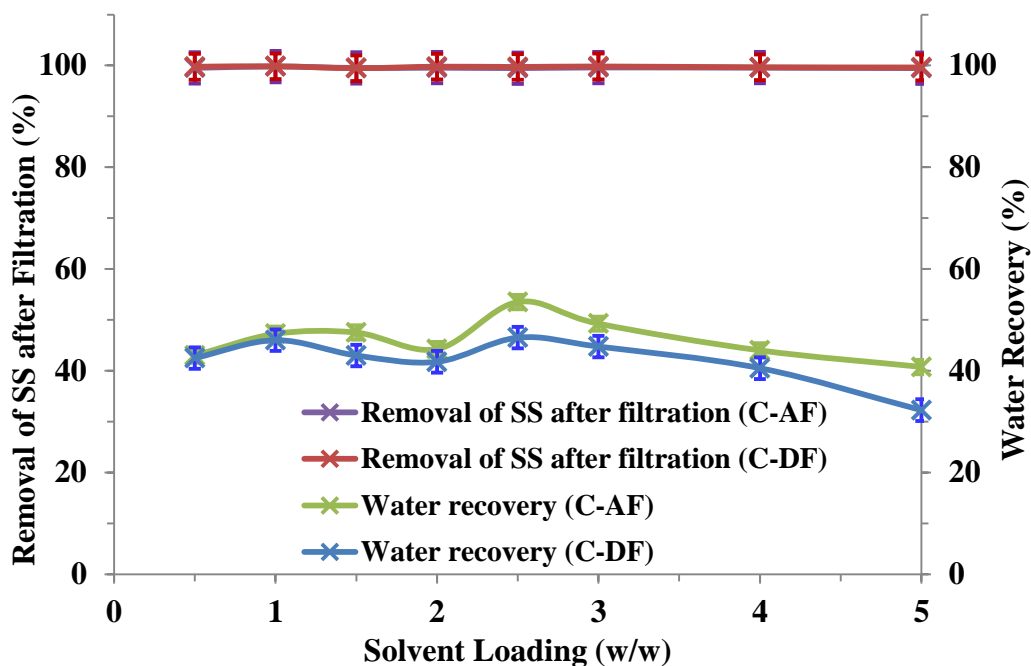


Fig. 6. Effect of solvent loading on the sludge dewatering ability ( $T = 70\text{ }^{\circ}\text{C}$ ,  $t = 2\text{ hours}$  and  $A = 200\text{ rpm}$ ).

### 3.2.4 Effect of agitation speed

The effect of agitation speed on the extract yield is presented in Supplementary Figure S2 according to the conditions listed in Supplementary Table S2 (Set 6).

The extract yield increased progressively from 0 to 200 rpm. Within this range the data indicates that the extraction process is limited by diffusion into the bulk liquid phase, and the extraction is enhanced by promoting turbulence within the solvent (Veggi et al. 2013). The yield decreased markedly when the agitation speed is increased from 200 to 250 rpm. The reasons for this decrease are not clear, however it is likely that the hydrodynamic conditions changed above 200 rpm, i.e. by the creation of vortices and regions of laminar flow rather than turbulence. This hypothesis is also supported by the non-linear behaviour with solvent loading shown in Figure 5.

Supplementary Figure S3 shows that agitation speed did not have a significant effect on the sludge dewatering properties of the extracted bio-flocculants. Efficient dewatering ability with high solids removal (96-99%) and water recovery (45-53.5%) were obtained at all agitation speeds, however the decrease in water recovery from 200 to 250 rpm is noted and correlates with the decrease in yield shown in Supplementary Figure S2. Agitation at 200 rpm was chosen as the optimum speed based on the yield and water recovery obtained at this condition.

### **3.3 Magnitude of extraction yield**

The optimised extraction conditions were identified as 70 °C, 2 hours, a solvent loading of 2.5 and agitation speed of 200 rpm. At these conditions the bio-flocculants exhibited the highest extract yield at  $2.38 \pm 0.07\%$ . Whilst this seems a low value, it is comparable to the yield obtained for extraction of polysaccharides from other studies, as shown in Supplementary Table S4. The yield obtained in this work is within the range identified by other studies (0.69-3.66%).

### **3.4 Comparison of sludge dewatering abilities of bio-flocculants with commercial flocculants**

The sludge dewatering abilities of aqueous and dried bio-flocculants extracted at optimised conditions were compared with commercial cationic and anionic polyacrylamides at various dosages, and the results are displayed in Supplementary Figure S4.

The commercial flocculants exhibit their optimum sludge dewatering and water recovery at around 50-70 g/L, whereas the bio-flocculants require a much higher dose (150 g/L for dried bio-flocculant and 175 g/L for aqueous bio-flocculant) to achieve a comparable performance. At dosages below 175 mg/L, it was found that DF exhibited higher sludge dewatering ability than AF, indicating that the drying process improves the flocculating performance by reducing the degradation that occurs with time in the aqueous phase. There is clearly an economic drawback in using either form of bio-flocculant due to the increased dosage required. Yet, the major importance of their applications in food and other industries, and the value of its substantial benefit to environment and human health may offset this extra cost in circumstances where the use of polymeric flocculants is undesirable or prohibited (Lee et al. 2014). In addition, the sludge or flocs formed are biodegradable and safely to be landfilled without further treatment and would reduce the overall treatment cost. To enable more widespread use of bio-flocculants it is therefore necessary to improve the extraction efficiency and the bio-flocculant quality such that the extract yield is enhanced and lower dosages of the resultant extract can be used, and further work by the authors will focus on the use of advanced extraction methods (e.g. microwave assisted extraction) for these purposes.

## **4. Conclusion**

This study has shown that a bio-flocculant can be successfully extracted from okra using water as a solvent. The bio-flocculant could be used without pH adjustment or addition of coagulant, and exhibited high removal of suspended solids and turbidity and water recovery.

The sludge dewatering performance was verified to be comparable to commercial flocculants, albeit at 2-3 times the dosage. A single-parameter experimental approach identified optimum extraction temperature to be 70 °C, extraction time of 2 hours, solvent loading of 2.5 and agitation at 200 rpm. At the optimised conditions, the extract yield was 2.38%, >98% solids removal and 68% water recoveries were attained. The extraction process was shown to be mass-transfer limited, specifically by diffusion into the bulk liquid phase. Further gains in extraction yield may be possible using apparatus that can achieve high Reynolds numbers, and this will be the focus of future work.

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## Supplementary Materials

Table S1. Characteristics of kaolin slurry.

Parameter (unit)	Mean $\pm$ Standard deviation
pH	4.82 $\pm$ 0.26
Suspended solids (g/L)	80 $\pm$ 0.78
Turbidity (NTU)	81000 $\pm$ 0.93

Table S2. Parameters studied and the range used in single factor experimental design.

Set of experiments	Extraction temperature	Time	Solvent loading	Agitation speed
	T (°C)	t (hrs)	S (w/w)	A (rpm)
1	25, 40, 50, 60, 70, 80, 90	2	1	200
2	25, 40, 50, 60, 70, 80, 90	2	1	100
3	70	0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5	1	200
4	50	0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5	1	200
5	70	2	0.5, 1, 1.5, 2, 2.5, 3, 4, 5	200
6	70	2	2.5	0, 50, 100, 150, 200, 225

Table S3. Comparison of flocculating abilities of okra bio-flocculants from different literature studies.

Treated solution	Characteristics of treated solution	Addition of coagulant	pH adjustment	Flocculant dosage (mg/L)	Treatment time (hours)	% removal	Reference
Kaolin slurry	SS: 81 g/L, TU: 81000 NTU, pH: 4.5-5	No	No	150-175	0.5	SS: 99.8%, TU: 99.9%	This work
Synthetic kaolin solution	TU: 63-73 NTU, pH: 5.9	Aluminium sulfate	No	5	0.75	TU: 93-97.3%	(Anastasakis et al. 2009)
Biologically-treated effluent	TU: 55 NTU, pH: 6.9			2.5		TU: 70-74%	
Tannery effluent	TS: 9.94 g/L, SS: 2.21 g/L, pH: 8.27	No	Yes	0.04	1-5	SS: 95%, DS: 69%	(Agarwal 2003)
Sewage wastewater	TS: 1.87 g/L, SS: 0.17 g/L, pH: 7.63	No	Yes	0.12	5	SS: 86%, DS: 95%	(Agarwal et al. 2001)

Table S4. Comparison of extraction yield of okra bio-flocculant with other literature.

Extracted material	Extraction conditions			Extract yield (%)	Reference
	Temperature (°C)	Time (hours)	Solvent loading (w/w)		
<i>Abelmoschus esculentus</i> (okra pods)	70	2	2.5	2.38	This work
<i>Myrtus communis</i> L. (myrtle fruit)	80	24	40	3	(Chidouh et al. 2014)
<i>Opuntia milpa alta</i> (cactus)	86.1	3.61	3.72	0.69	(Cai et al. 2008)
<i>Hyriopsis cumingii</i> (pearl mussels)	80	4.5	8	3.66	(Qiao et al. 2009)
<i>Glycyrrhiza glabra</i> (shrub)	-	4.3	35	3.6	(Liang 2008)

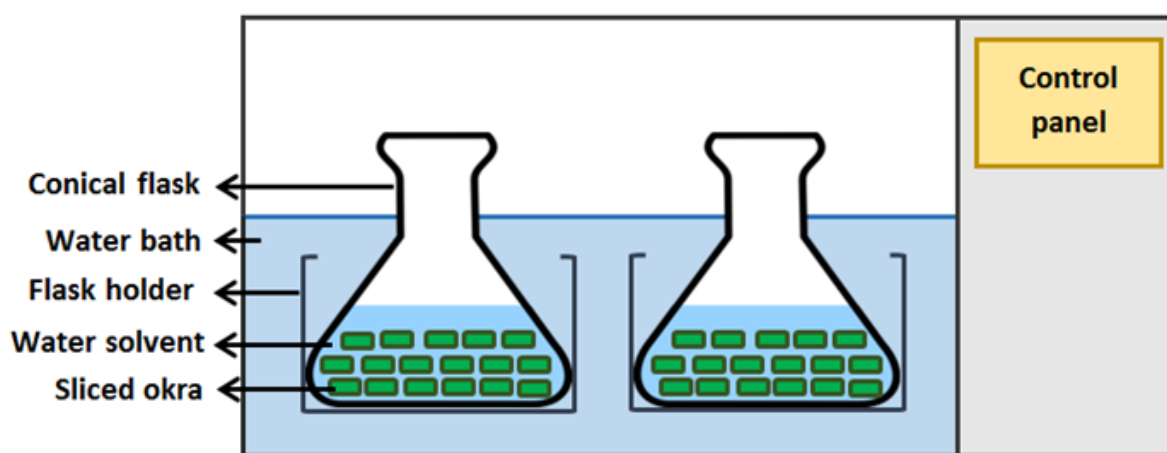


Fig. S1. Schematic diagram of conventional extraction process.

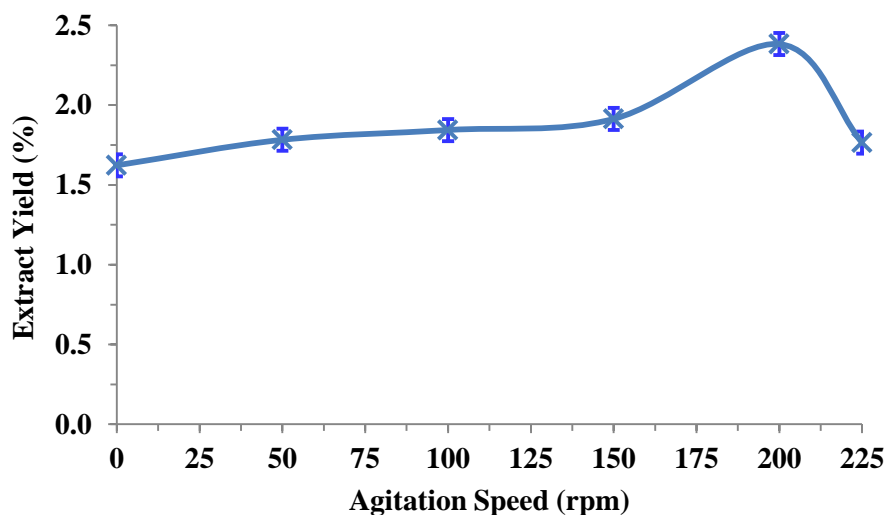


Fig. S2. Effect of agitation speed on the extract yield ( $T = 70\text{ }^{\circ}\text{C}$ ,  $t = 2\text{ hours}$ ,  $S = 2.5\text{ w/w}$ ).

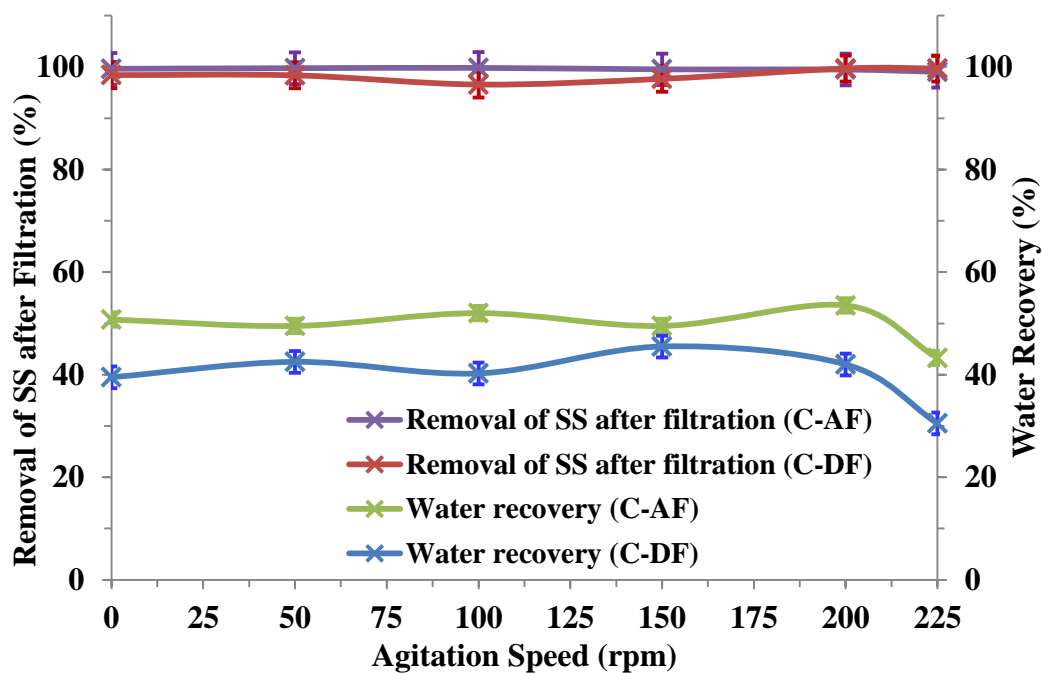
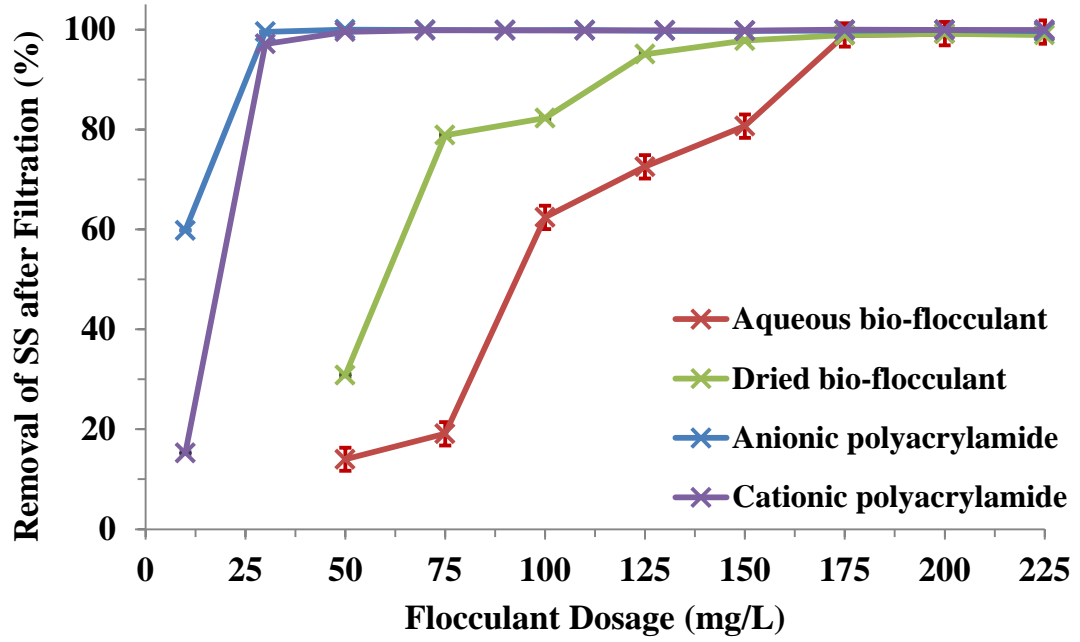
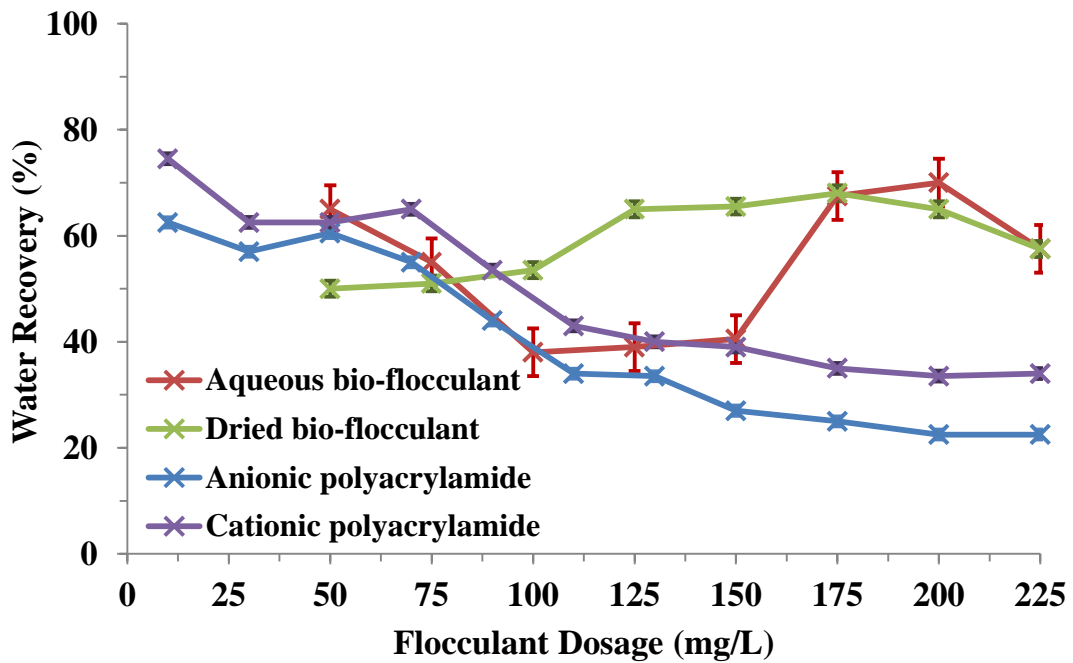


Fig. S3. Effect of agitation speed on the sludge dewatering ability ( $T = 70\text{ }^{\circ}\text{C}$ ,  $t = 2\text{ hours}$ ,  $S = 2.5\text{ w/w}$ ).



(a)



(b)

Fig. S4. Comparison of sludge dewatering abilities of different flocculants in (a) % removal of SS after filtration and (b) % water recovery.