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Preliminary Study on Extraction of Bio-flocculants from Okra and Chinese Yam

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Natural bio-flocculants were extracted from okra and Chinese yam using water extraction method, and the extract yield and their flocculating abilities were evaluated. Results showed that extraction of okra with seed removal and incubation followed by freeze drying enhanced the extract yield by 91% and improved the flocculating ability greatly by achieving solids removal of above 99% when compared with extraction without incubation and followed by oven drying. The effect of an incubation step was further investigated by using Chinese yam. With incubation, a higher extract yield of 2.95% was obtained compared with the extraction without incubation at 2.13% and high flocculating ability was achieved at 99.5% solids removal. To further investigate the application of bio-flocculants, the samples with the highest extract yield and flocculating ability were selected for a case study focusing on treatment of oleochemical wastewater. Yam bio-flocculant showed its flocculating activity with 80% solids removal when it was coupled with coagulant without pH alteration. However, pH adjustment was required for okra bio-flocculant. In conclusion, highly efficient okra and yam bio-flocculants were successfully extracted and their applicability to wastewater treatment was proven.

Keywords : Okra; Chinese yam; bio-flocculant; extraction; flocculation; wastewater treatment.

INTRODUCTION

Flocculation is one of the most important industrial processes for water and wastewater treatment because of its high efficiency and facile operation (Yang *et al*. 2012). At present, inorganic metal-based and synthetic polymeric flocculants are commonly used in flocculation process. However, the presence of metal ions or noxious monomers in treated water may have unwelcome implications to both related ecosystems and human health (Wang *et al*. 2013, Yang *et al*. 2013).

Due to higher requirements on discharged water quality and environmental protection, synthesis of natural bio-flocculants is urgently required.

In recent years, bio-polymer flocculants extracted from plant materials have received substantial attention in industrial wastewater treatment on account of their biodegradability, environmental friendliness and widespread abundance in nature (Renault *et al*. 2009). Plant-based bio-flocculants derived from *Hibiscus/Abelmoschus esculentus* (okra), *Malva sylvestris* (Mallow), *Plantago psyllium* (Psyllium), *Plantago ovata* (Isabgol), *Tamarindus indica* (Tamarind), and *Trigonella foenum-graecum* (Fenugreek) exhibit efficient flocculating performance in removal of environmental-concerned parameters (e.g. suspended solids (SS), chemical oxygen demand, dye and colour) in treatment of various types of wastewater (A. Mishra 2002, Al-Hamadani *et al*. 2011, Anastasakis *et al*. 2009, Mishra *et al*. 2003, Mishra *et al*. 2005, Mishra *et al*. 2006, Mishra *et al*. 2002, Mishra *et al*. 2004). However, the flocculating abilities of bio-flocculants were tested in treatment of wastewater low in solids concentration (<1 g/L). The flocculating performance of bio-flocculants in treatment of solution high in SS (> 10 g/L) has not been reported thus far. Therefore, the flocculating efficiency of okra bio-flocculant in the clarification of kaolin slurry with high SS concentration (59.8 g/L) was investigated in this study. In addition, the flocculating activity of a new plant-based bio-flocculant extracted from *Dioscorea opposita* (Chinese yam) was reported for the first time.

Flocculating properties are closely related to processing and drying conditions; however no published work has addressed this topic. Thus, the main objective of this work was to investigate the preparation of eco-friendly okra bio-flocculant by using water extraction method with special attention on the effects of seed removal, incubation step during extraction and different drying methods (oven and freeze) on the extraction and flocculating efficiency in clarification of kaolin slurry. The effect of incubation was further evaluated in extraction of Chinese yam bio-flocculant. With the aim to further explore the applicability of these bio-flocculants, the flocculating ability in solids removal from oleo-chemical wastewater was investigated.

MATERIALS AND METHODS

Materials

Okra and Chinese yam were sourced from Selangor region in Malaysia. Kaolin slurry was purchased from Kaolin Malaysia Sdn. Bhd and stored at room temperature (RT). Oleo-chemical effluent was collected freshly from a wastewater treatment plant in Palm Oleo Sdn. Bhd, Malaysia. Sodium hydroxide (NaOH, ≥98%, anhydrous) was purchased from Sigma-Aldrich Co., Malaysia. The polyaluminum chloride (PAC), cationic (FO 4400 SH) and anionic (AN 934 SH) polyacrylamides with 30% charge density and 1.5×10^7 g/mol molecular weight were purchased from SNF Floerger dealer (KemPro Sdn. Bhd.) in Malaysia.

Table 1. Characteristics of kaolin slurry and oleo-chemical wastewater

Parameters (unit)	Mean ± Standard deviation	
	Kaolin slurry	Oleo-chemical wastewater
pH	4.6 ± 0.3	6.0 ± 0.4
Suspended solids (mg/L)	59760 ± 0.9	197 ± 0.9

Preparation of Kaolin and Oleo-Chemical Wastewater Samples

Kaolin slurry and oleo-chemical wastewater were freshly prepared for each Jar test. The solution was steadily mixed at RT for re-suspension of sedimented solids in water and it was sampled during stirring to ensure homogeneity. The pH was measured and SS measurement were taken with colorimeter (DR/890, HACH, USA) according to Standard Method 8006 (USEPA September 2012).

Bio-Flocculants Extraction

The fresh plant materials were washed with deionised water (ddH_2O). The upper crown head and the seeds inside the okra pods were removed, and the skin of Chinese yam was peeled off. Afterward, 100 g of okra pods and the yam fruits were sliced into 5-10 mm cubes. The sliced plant materials were ground and mixed with 100 ml ddH_2O in conical flasks. The flasks were sealed and extracted together in a shaker bath at 300 rpm for 3 hours at RT. Then, the extracts were incubated at 90 ^{o}C in the shaker bath for half hour. After extraction, the flasks were kept aside at RT 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

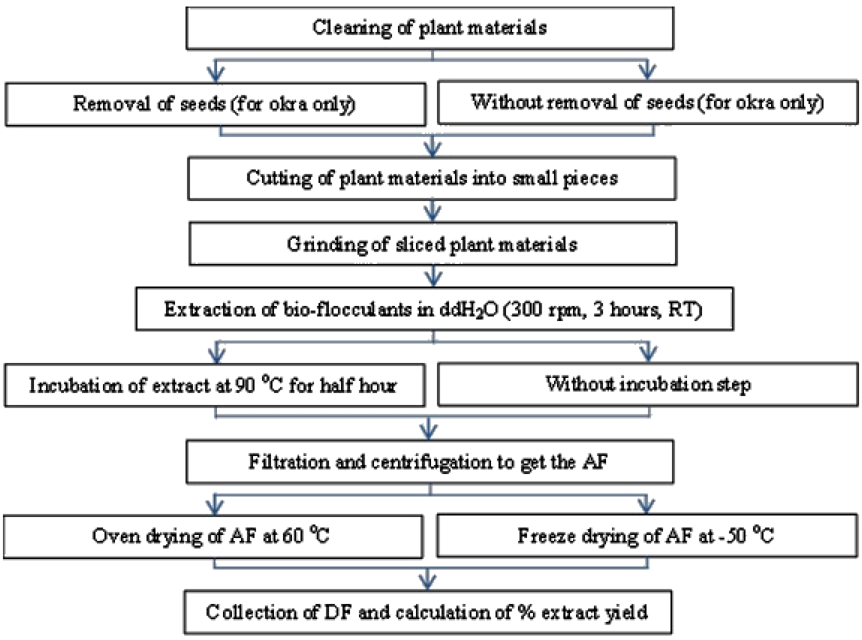

Figure 1. Schematic view of overall extraction process of bio-flocculants

subjected to centrifugation at 7000 rpm for 20 minutes to recover the remainder of the extract. A sample of aqueous bio-flocculant (AF) was sent for oven or freeze drying while the rest was kept at 4 °C before further testing on its flocculating property. A schematic of overall extraction procedures of bio-flocculants is presented in figure 1.

Drying of Bio-Flocculants

For oven drying, a sample of AF was dried in a hot air oven at 60 °C (UNB 500, Memmert, Germany) until a constant weight was obtained. The predetermined volume of AF was pre-frozen at -20 °C for 24 hours before freeze drying. Then, the samples were dried in vacuum condition in a freeze dryer (Alpha 1-2 LD_{Plus}, Christ, Germany) at -50 °C for 30 hours. After drying, the dried bio-flocculants (DF) were stored in a dessicator at RT. The extract yield was calculated using equation 1.

$$\% \, extract\ yield = \frac{mass\ of\ dried\ extract}{mass\ of\ plant\ materials}\ x\ 100\% \qquad (1)$$

Experimental Design

The effects of seed removal, an incubation step during extraction and drying method on the extract yield and flocculating ability were studied by using single factor experimental design method. The design of the experimental work with the results of % extract yield, the dosage of bio-flocculants used in clarification of kaolin slurry and the obtained SVI values are summarised in Table 2.

Flocculation Analysis

Jar tests were used to evaluate the flocculating abilities of bio-flocculants in flocculation of kaolin slurry and oleo-

Table 2. Experimental design with the results of extraction and flocculation of kaolin slurry

Plant materials	Experiment set	Seeds removal	Incubation	Drying method	% extract yield	Bio-flocculant dose (mg/L)	SVI (ml/g)
Okra	OAF 1-1	No	Yes	No	-	290	0.03
	ODF 1-2	No	Yes	Oven	0.86	1000	0.85
	ODF 1-3	No	Yes	Freeze	2.82	290	0.03
	OAF 2-1	Yes	Yes	No	-	290	0.04
	ODF 2-2	Yes	Yes	Oven	0.97	1000	0.82
	ODF 2-3	Yes	Yes	Freeze	2.92	290	0.03
	OAF 3-1	Yes	No	No	-	1450	0.78
	ODF 3-2	Yes	No	Oven	0.25	1560	0.98
Chinese yam	YAF 4-1	-	No	No	-	3600	0.02
	YDF 4-2	-	No	Freeze	2.13	3600	0.01
	YAF 5-1	-	Yes	No	-	2950	0.01
	YDF 5-2	-	Yes	Freeze	2.95	2950	0.01

chemical wastewater at RT with a Jar Tester (ET720, Lovibond, Germany). Coagulant was injected into the solution followed by pH adjustment with NaOH if necessary followed by addition of flocculant at stirring of 200 rpm. The flocculation process consisted of stirring at 200 rpm for 1 minute, followed by stirring at 100 rpm for 2 minutes and settling for 30 minutes. The settled flocs volume was taken for calculation of sludge volume index (SVI) according to Method 2710D (ASN Jan 1999) and the SS of supernatant were measured.

RESULT AND DISCUSSIONS

Extraction Efficiency

The effects of seed removal, an incubation step and drying methods on the extract yield are shown in Table 2 and the standard deviation was controlled within 10%. The drying method showed the most apparent impact on the extract yield, followed by the incubation step and finally seed removal for okra bio-flocculant extraction.

Extraction of okra without seeds produced slightly higher extract yield compared to extraction with seeds; the increment of extract yield were 11.3% and 3.4% for oven and freeze drying respectively. A study (Sanjeet Kumar 2010) has reported that mucilaginous substances in the okra extract are usually concentrated in the pod walls and not in the seeds. Therefore, it can be concluded that the presence of seeds in okra extraction has no contribution to the extraction efficiency of okra bio-flocculant.

The extract yield was enhanced by 74.2% and 27.8% for okra and Chinese yam separately with addition of an incubation step at 90 ^{o}C for half hour after extraction at RT. Extraction at the elevated temperature would reduce the viscosity of the solvent and enhance the penetration of solvent into the plant matrix and thus resulted in faster mass transfer of water-soluble active components from the cell wall into the extract (Amid *et al*. 2012, Samavati 2013).

The highest extract yield of okra was obtained with freeze drying compared to oven drying; the yield was increased by 66.8% and 69.5% for extraction without seeds and extraction with seeds respectively. The low temperature maintained in freeze drying may minimise the destructive forces of heat during drying and thus prevent the degradation of heat-sensitive components (Ghasemi Pirbalouti *et al*. 2013). However, oven drying at 60 ^{o}C may cause degradation of heat-sensitive volatile constituents (Rahimmalek *et al*. 2013). Other studies showed identical findings where higher essential oil yield was obtained through freeze drying compared to oven drying above 50 ^{o}C (Ghasemi Pirbalouti *et al*. 2013, RahimmalekGoli 2013).

In summary, the highest efficiency of okra extraction was obtained at processing conditions of seed removal and addition of an incubation step at 90 ^{o}C in extraction followed by freeze drying. Meantime, addition of an incubation step and use of freeze drying were preferable for higher extraction efficiency of yam bio-flocculant.

Flocculation Efficiency

Clarification of Kaolin Slurry

The flocculating abilities of bio-flocculants prepared under different processing conditions was evaluated based on the quantity of flocculant dosage used to achieve nearly 100% SS removal and the results are presented in Table 2. Direct flocculation process was employed in clarification of kaolin slurry where no coagulant and pH adjustment were required.

It was seen that seed removal in okra extraction did not exert an obvious effect on the solid removal because bio-flocculants OAF 1-1 with 2-1 and ODF 1-3 with 2-3 exhibited similar flocculating efficiency in removal of nearly 100% of SS at dosage of 290 mg/L. Addition of an incubation step during extraction was verified to be significant to enhance the flocculating abilities of okra and yam bio-flocculants. In order to attain high solid removal efficiency, much higher flocculant dosage was needed for both bio-flocculants extracted without incubation step (OAF 3-1 and YAF 4-1) compared with bio-flocculants extracted with incubation step (OAF 2-1 and YAF 5-1). Thus, it was postulated that some active ingredients with flocculating property could be extracted at high temperature, which was 90 ^{o}C in this case.

Bio-flocculants were found to be sensitive to oven drying at 60 ^{o}C, lower flocculating efficiency was obtained after drying where higher dosage was needed for ODF 1-2, 2-2 and 3-2 compared with bio-flocculants without drying (OAF 1-1, 2-1 and 3-1). Functional properties of hydrocolloid gums could be significantly altered by the drying processes (Jaya *et al*. 2009), which is consistent with the findings in this work. Drying at high temperature for long period is not recommended in bio-flocculants production because it may degrade the high molecular weight hydrophilic bio-polymers and thus affect the formation of strong bridging flocculation.

The DF obtained with freeze drying (ODF 2-3 and YAF 5-2) was found to exhibit the flocculating abilities as efficient as AF (OAF 2-1 and YAF 5-1). Freeze drying conducted at very low temperature prevents the degradation or deterioration of the active ingredients high in flocculating ability (Ghasemi Pirbalouti *et al*. 2013, Ratti 2001). For all experiments, the SVI values as shown in Table 2 were well below 100 ml/g which was desirable because it indicated good settling characteristics of the flocs formed (Adrianus van Haandel 2012).

Figure 2 shows the comparison pictures of kaolin slurry before treatment and after treatment with okra bio-flocculant (OAF 2-1). After treatment, the supernatant looked clear with a low content of SS of 4 mg/L relative to the initial kaolin suspension of 59.76 g/L. These results indicated that okra bio-flocculant is very efficient in solids removal from solution high in suspended solids.

The flocculating performance of the bio-flocculants was compared with PAC and polyacrylamides, the results are displayed in figure 3. The increment order of flocculant dosage for different types of flocculants was polyacrylamides < okra bio-flocculant < PAC < yam bio-flocculant.

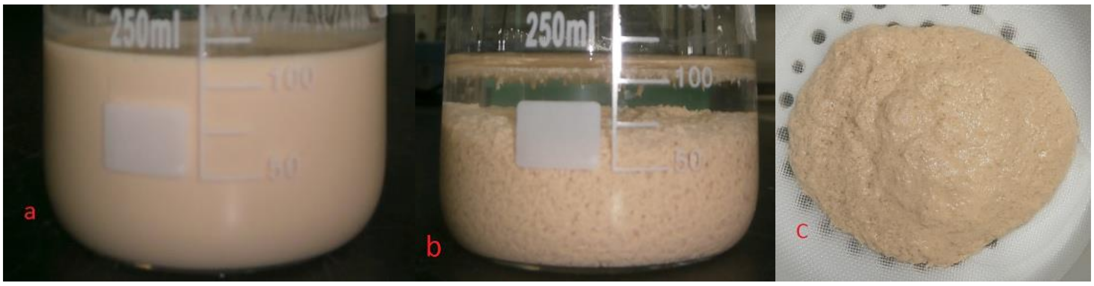

Fig.2 : Flocculation effects of okra bio-flocculant in clarification of kaolin slurry (a) before treatment, (b) after treatment, and (c) the flocs formed

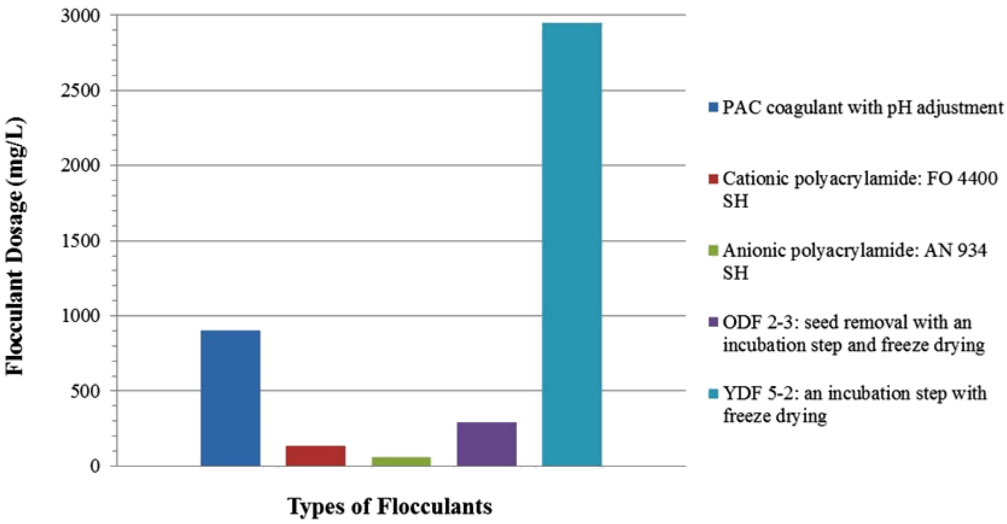

Fig.3 : Comparison of flocculant dosage of different types of flocculants in clarification of kaolin slurry

Okra bio-flocculant was as efficient as polyacrylamides in solids removal if higher dosage was used in treatment, but it exhibited higher flocculating performance than PAC where lower dosage and no pH adjustment were needed for bio-flocculation. For yam bio-flocculant, its flocculating activity was proven but a considerably higher dosage was required to achieve the same level of flocculating efficiency as commercial flocculants.

The most efficient bio-flocculants (OAF 2-1 and YAF 5-1) identified in clarification of kaolin slurry were selected to explore their potential feasibility in treatment of real oleo-chemical wastewater.

Flocculation of Oleo – Chemical Wastewater

The flocculating results of bio-flocculants,PAC and polyacrylamides in treatment of oleo-chemical wastewater are shown in Table 3 and figure 4.

PAC with high dosage coupled with pH adjustment to neutral value was required in order to achive high removal of SS. Application of metal salts without flocculant in wastewater treatment is usually associated with flocculation mechanism of charge neutralisation where

Table 3. Flocculating abilities of bio-flocculants and commercial flocculants in treatment of oleo-chemical wastewater

PAC dose (mg/L)	pH adjustment	Type of flocculant	Flocculant dose (mg/L)	Contents of SS (mg/L)	% removal of SS	SVI (ml/g)
900	Adjust to pH 7	-	-	7	96.45%	2.54
3600	Adjust to pH 7	-	-	1	99.5%	2.54
-	-	AN 934 SH	20		No floc is formed.	
0.18	-		10	4	97.97%	0.25
-	-	FO 4400 SH	165		No floc is formed.	
0.18	-		165		No floc is formed.	
3600	Adjust to pH 7		165	20	89.85%	4.31
-	-	OAF 2-1	580		No floc is formed.	
0.18	-		580		No floc is formed.	
3600	Adjust to pH 7		1740	23	88.3%	2.2
-	-	YAF 5-1	1180		No floc is formed.	
0.18	-		590	22	88.83%	0.25
-	-	YDF 5-2	1180		No floc is formed.	
0.18	-		590	49	75.13%	0.25
0.18	-		1180	37	81.22%	0.25

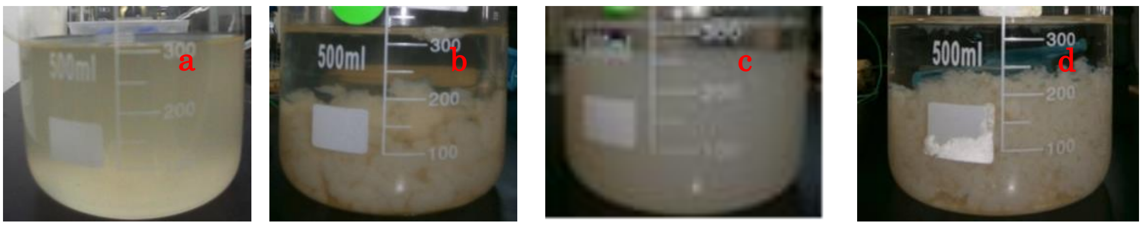

Fig.4 : Flocculation effects of different flocculants in treatment of oleo-chemical wastewater: (a) before treatment, (b) PAC coupled with yam bio-flocculant, (c) PAC with pH adjustment, (d) PAC coupled with AN 934 SH

the micro-flocs formed are weaker and settled slowly (Figure 4c) (Ahmad *et al.* 2008), thus high dosage was required in this case study.

The results showed that polyacrylamides and bio-flocculants did not show flocculating ability in direct flocculation process, there was no formation of flocs. Direct flocculation is limited to the application in organic-based wastewater with high concentration of SS (Chong 2012, Lee *et al.*), which was contradicted to treatment of oleo-chemical wastewater low in SS. Coagulation-flocculation process is commonly preferable in treatment of wastewater low in SS (Chong 2012).

Anionic polyacrylamide displayed high flocculating efficiency if it was used with small amount of PAC (0.18 mg/L) in wastewater treatment (Figure 4d). Yam bio-flocculant (YAF 5-1) exhibited the similar flocculating property as anionic polyacrylamide but higher dosage was requied. After the bio-flocculation, the particles clustered to flocs and precipitated while the supernatant looked clean (Figure 4b). These results further demonstrated that the extracted bio-flocculants played a key role in the removal of SS, which met the requirements for an efficient flocculant.

Meanwhile,cationic polyacrylamide and okra bio-flocculant demonstrated their flocculating effects if high dosage of PAC followed by pH adjustment was performed before addition of flocculant. At the neutral pH, the suspended sludge particles aggregate easily into micro flocs due to the formation of cationic species followed by surface charge neutralisation (Suopajärvi *et al*. 2013). Flocculant helps to agglomerate the slow settling micro-flocs to form larger and denser flocs (Lee *et al*. 2012).

CONCLUSIONS

Efficient and eco-friendly okra and Chinese yam bio-flocculants were successfully extracted with a simple and green extraction method. Seed removal and addition of an incubation step during extraction followed by freeze drying was recommended for prepation of okra bio-flocculant. Meanwhile, addition of incubation step was further verified to be important in extraction of yam bio-flocculant. Okra bio-flocculant exhibited higher flocculating ability than PAC due to lower dosage and no pH alteration were required in bio-flocculation. However, higher dosage of okra bio-flocculant (290 mg/L) were required to have comparable performance to the polyacrylamides (133 mg/L) in solid removal. Hence, optimisation of processing methods and conditions is recommended to further enhance the flocculating abilities of bio-flocculants. As the last conclusion, the high suspended solids removal to the kaolin slurry and oleo-chemical wastewater as well as the biodegradable and bio-safe characteristics suggest bio-flocculants as efficient flocculants to replace conventional flocculants in treatment of water and industrial wastewater.

Abbreviations

AF	:	Aqueous bio-flocculant
DF	:	Dried bio-flocculant
ddH_2O	:	Deionised water
NaOH	:	Sodium hydroxide
OAF	:	Okra aqueous bio-flocculant
ODF	:	Okra dried bio-flocculant
PAC	:	Polyaluminium chloride
RT	:	Room temperature
SS	:	Suspended solids
SVI	:	Sludge volume index
YAF	:	Yam aqueous bio-flocculant
YDF	:	Yam dried bio-flocculant

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