EXTRACTION OF BIO-FLOCCULANT FROM OKRA USING HYDROTHERMAL AND MICROWAVE EXTRACTION METHODS COMBINED WITH A TECHNO-ECONOMIC ASSESSMENT

LEE CHAI SIAH

B.E. (Chem. Eng., UM), MSc (Chem. Eng., NTU)

THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMICAL AND ENVIRONMENTAL ENGINEERING
FACULTY OF ENGINEERING
UNIVERSITY OF NOTTINGHAM

JUNE 2017

ABSTRACT

Since the usage of chemical flocculants especially polyacrylamides is closely related with environmental pollution and health hazards, synthesis of environmental friendly and economic viable bio-flocculants that exhibit high flocculating efficiency is highly desirable. The production of natural bio-flocculants extracted from plant is urgently needed as an alternative to chemical flocculants due to their inherent low toxicity, biodegradability and low environmental footprint.

In this study, a plant-based bio-flocculant was extracted from Hibiscus/Abelmoschus esculentus (okra) with conventional hydrothermal extraction (CHE) and microwave assisted extraction (MAE) processes by using water as solvent. The aims of this work were to produce the bio-flocculant through environmentally friendly and economically feasible process, optimise its extraction yield, optimise its flocculating and sludge dewatering properties, and also minimise its production cost in order to be comparable to chemical flocculants (polyacrylamides) from perspectives of quality and cost. The extraction efficiencies of bio-flocculants were justified by the optimised yields, the flocculating abilities were evaluated by the removal of suspended solids (SS) and turbidity after flocculation process and sludge volume index (SVI) whereas the sludge dewatering abilities were assessed by SS removal after filtration and water recovery.

Single factor experimental design was employed to study the effects of extraction temperature, time, solvent loading, and agitation speed and particle size on yield, flocculating and dewatering properties of bio-flocculants. The influence of extraction parameters to yield and the reliability of the experimental data were verified by analysing the single factor experimental results with response surface methodology. Results showed that extraction yields were significantly affected by extraction temperature and time and solvent loading. Prolonged extraction (few hours) at high temperature (60-90 ˚C) decreased the yields of bio-flocculants extracted with CHE method. Conversely, extraction at high temperature was favourable for MAE method to raise the yield due to short extraction time in minutes.

Flocculating abilities of bio-flocculants were not significantly affected by the extraction conditions whilst the sludge dewatering abilities were mainly influenced by the temperature and particle size. High extraction temperature at ≥ 70 ˚C and the smallest okra particle size at 1mm were the crucial conditions for extraction of bio-flocculants with high dewatering abilities which showed >95% of SS removal after filtration and ≥75% of water recovery.

The optimised yield of CHE at 25.9% was obtained at 50 ˚C, 2 hours, solvent loading of 2.5 w/w and agitation at 200 rpm. On the other hand, microwave-extracted bio-flocculants were best extracted at 90 ˚C, 10 minutes, and solvent loading of 3.5 w/w and okra particle size at 1mm to get the optimised yield at 48.7%. These results revealed that the extraction yield has been enhanced by 87.8% by using MAE method and the extraction time was markedly reduced from hours to minutes.

This study uses a combination of empirical observations and an analysis of mass transfer behaviour to yield new insights into the mechanism of MAE. Enhancements in extraction rate and yield achieved by microwave extraction were observed experimentally compared with hydrothermal extraction at temperatures in excess of 50 ˚C, however at lower temperatures there was no observable difference between the two processes. A step-change in extraction yield between microwave and hydrothermal processes was shown to be caused by selective heating. A temperature gradient of the order of 1 ˚C is sufficient to reduce the water chemical potential within the plant cell structure, which causes diffusion of water solvent into plant cell such that internal plant cell pressures can increase to the point where disruption occurs. These findings demonstrate the need to operate microwave extraction processes at a temperature that enables selective heating, and a newly-proposed mass transfer phenomenon that could have wider positive implications for extraction and leaching processes.

The bio-flocculants extracted with both methods were applied in flocculation and sludge dewatering without pH alteration and addition of coagulant. Efficient flocculating abilities were attained with >99% of SS and turbidity removal and <10 mg/L of SVI. However, microwave extracted bio-flocculants were shown to exhibit higher dewatering abilities than hydrothermal extracted bio-flocculants. The bio-flocculants displayed the results of >95% SS removal after filtration and 75% water recovery during sludge dewatering at dosage of 30 mg/L, and was shown to be comparable to or even better than polyacrylamides due to achievement of higher water recovery at the same dosage. Dried bio-flocculants were found to have higher dewatering abilities than aqueous bio-flocculants, probably because drying at low temperature (40 ˚C) has minimised the moisture content and extended the shelf life of bio-flocculants. Biopolymer bridging was preliminary predicted as the plausible bio-flocculation mechanism.

The economic feasibility of production of industrial scale bio-flocculant was investigated and the production process was modelled by using SuperPro Designer v9.0 simulation software. The MAE process in continuous mode was verified to be more economically viable than CHE process either in batch or continuous mode if the production scale was beyond 15 tonne/year. Sensitivity analysis for continuous microwave process was conducted and the results indicated the strong influences of annual production and extraction yield followed by raw material okra price on the unit production cost. Continuous microwave process with extraction conditions at 90 ˚C, 10 minutes and solvent loading of 3.5 w/w was identified as the optimised scheme for production of industrial scale bio-flocculant at the lowest production cost. The estimated selling price of aqueous and dried bio-flocculants for an annual production of 220 tonne/year were 41 and 52 $/kg respectively, and was shown to be lower than food grade bio-flocculants but higher than polyacrylamides.

This work has clearly showed that okra bio-flocculant could offer a feasible and sustainable alternative to synthetic flocculants for water treatment and sludge dewatering applications due to its high efficiency in flocculating and dewatering, and can be extracted using only water as a solvent, minimising the environmental footprint of the extraction process.

ACKNOWLEDGEMENTS

To me, PhD study is a long journey full of challenges. However, I feel fortunate that I have full support and care from my husband and family, supervisors and friends along the way.

Firstly, I would like to thank my husband for encouraging me to pursue PhD study after my Master graduation. Without him, I won't have the courage to take the plunge. Throughout these three years plus, we experienced long distance relationship, the birth of our lovely daughter, and many others. I feel gratitude to him for always showing his concern and support to me.

Secondly, my family especially my mum always gives me her support and concern regardless of any decision I made. I would like to express my love and gratitude to her great love and understanding.

Apart from family, supervisors play the important role to assist and guide me to complete this study. The success of this project depends largely on the advice, guidance, support, knowledge and experience sharing from all the supervisors. I would like to show my greatest appreciation to my supervisors; Dr. John Robinson, Dr. Eleanor Binner, Dr. Chong Mei Fong, and Dr. Rachel L. Gomes for their kind encouragement and guidance.

I also wish to thank my best friends such as Siew Fong, Hazren, Zaid, Maggie, Patrick, Jay and others that showed their help and concern to me. In addition, I want to show my gratitude to the laboratory supervisors and technicians for their assistance and co-operation. Furthermore, I also highly appreciate the kind advice from Roger Freestone (a design consultant in the University of Nottingham) on the industrial scale design of bio-flocculant production plant.

Last but not least, I sincerely express many thanks and appreciation to my family, supervisors, friends and others for all their love, support, guidance and advice. Without all of you, I can't achieve this milestone successfully.

CONTENTS

Part 2: Extraction methods for plant materials 29

Chapter 6: Economic feasibility study 102

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A	Agitation speed
AD	Anaerobic digestion
AF	Aqueous bio-flocculant
BBD	Box-Behnken design
BOD_5	5 days biochemical oxygen demand
BPE	Boiling point elevation
CCD	Central composite design
CEPCI	Chemical Engineering Plant Cost Index
CHE	Conventional hydrothermal extraction
CIP	Cleaning in place
COD	Chemical oxygen demand
DF	Dried bio-flocculant
DFC	Direct fixed capital
DoF	Degree of freedom
DS	Dissolved solids
EM	Electromagnetic
H-AF	Hydrothermal extracted aqueous bio-flocculant
H-DF	Hydrothermal extracted dried bio-flocculant
IM	Installed multiplier
IRR	Internal rate of return
ISM	Industrial, scientific and medical
MAATPE	Microwave-assisted aqueous two-phase extraction
MAE	Microwave assisted extraction
M-AF	Microwave extracted aqueous bio-flocculant
M-DF	Microwave extracted dried bio-flocculant
MoC	Material of construction
MS	Mean squares
NPV	Net present value
P	Pressure
PBT	Payback time
PC	Purchase cost
PS	Particle size
PACl	Poly-aluminium chloride
ROI	Return on investment
RP	Reactive phosphorus
RSM	Response surface methodology
S	Solvent loading

SEM	Scanning electron microscope
SIP	Sterilisation in place
SoS	Sum of squares
SS	Suspended solids
SVI	Sludge volume index
SWE	Subcritical water extraction
t	Extraction time
T	Extraction temperature
$TOC_{soluble}$	Soluble total organic carbon
TP	Total phosphorus
TU	Turbidity
UAE	Ultrasound assisted extraction
UMSE	Ultrasonic-microwave synergistic extraction

LIST OF SYMBOLS

a_w	water activity at T
a_{wc}	water activity within the plant cell
a_{ws}	water activity outside the plant cell
α	temperature coefficient
f_w	fugacity of pure water at T
f_w^0	fugacity of pure water at T and the standard pressure
f_w^*	fugacity of water at saturation
$\hat{f}_w$	fugacity of water within the mixture at T
p_w^*	saturated vapour pressure at T
P_c	pressure within the plant cell
P_s	external pressure within the solvent
μ_s	chemical potential of solute
μ_w	chemical potential of water
$\hat{\mu}_{wc}$	chemical potential of water within a plant cell
$\hat{\mu}_{ws}$	chemical potential of water outside the plant cell
μ_w^0	chemical potential of pure water at temperature T and a standard pressure
s	entropy
s^o	standard entropy
v_w	molar volume of water at T
R^2	coefficient of determination

LIST OF PUBLICATIONS AND PRESENTATIONS

Master thesis: Lee, C.S., Chen, Y.P., (2010) Study of the RESS (Rapid Expansion of Supercritical Solution) process for micronization of Antipyrine, Benzocaine and 4-Dimethylaminoantipyrine, National Taiwan University.

Journal publications:

1. Lee, C.S., Robinson, J., Chong, M.F., (2014) A review on application of flocculants in wastewater treatment, Process Safety and Environmental Protection 92, p. 489–508.
2. Lee, C.S., Chong, M.F., Robinson, J., Binner, E., (2014) A review on development and application of plant-based bioflocculants and grafted bioflocculants, Industrial and Engineering Chemistry Research 53, p. 18357–18369.
3. Lee, C.S., Chong, M.F., Robinson, J., Binner, E., (2015) Preliminary study on extraction of bio-flocculants from okra and Chinese yam, Asean Journal of Chemical Engineering 15 (1), p. 41-51.
4. Lee, C.S., Chong, M.F., Robinson, J., Binner, E., (2015) Optimisation of extraction and sludge dewatering efficiencies of bio-flocculants extracted from *Abelmoschus esculentus* (okra), Journal of Environmental Management 157, p. 320-325.
5. Lee, C.S., Chong, M.F., Robinson, J., Binner, E., Winkworth-Smith, C., John, R., Gomes, R.L., (2016) Enhancing natural product extraction and mass transfer using selective microwave heating, Chemical Engineering Science 149, p. 97-103.

Conference presentations:

1. Poster presentation: Lee, C.S., Chong, M.F., Thielemans, W., (2013) A short review on flocculants in wastewater treatment, In: Postgraduate Colloquium for Environmental Research 2013 (POCER 2013), Pahang, Malaysia 28-29 June 2013.

2. Poster presentation: Lee, C.S., Chong, M.F., Thielemans, W., (2013) Preliminary feasibility study of plant-based bio-flocculant, In: Postgraduate Research Week 2013 (PGR 2013), Selangor, Malaysia 30 Sept – 4 Oct 2013.
3. Oral presentation: Lee, C.S., Chong, M.F., Robinson, J., Binner, E., (2014) Preliminary study on extraction of bio-flocculants from okra and Chinese yam, In: 27th Symposium of Malaysian Chemical Engineers (SOMChe 2014), Selangor, Malaysia 29-30 October 2014.
4. Oral presentation: Lee, C.S., Chong, M.F., Robinson, J., Binner, E., Gomes, R.L., (2015) Extraction of a plant-based bio-flocculant from okra with application in sludge dewatering, In: 6th International Congress of Energy and Environment Engineering and Management (CIIEM15), Paris 22-24 July 2015.

CHAPTER 1: INTRODUCTION

Wastewater effluents produced from different industrial sectors normally contain suspended, dissolved and colloidal solids; which comprise of inorganic and organic and other impurities. Coagulation/flocculation followed by dewatering are important solid-liquid separation processes that are extensively employed in wastewater treatment typically for the removal of colloidal and suspended matters (Renault, 2009b).

Operating as either individual processes or combined, coagulation involves the destabilisation and initial coalescing of colloidal particles and flocculation (and sometimes a flocculant) provides the conditions to promote agglomeration of destabilised smaller particles into compact, fast settleable larger particles or flocs (Sharma, 2006). The resulting water matrix is then separated either by gravity settling, centrifugation, air flotation or filtered (dewatered) to separate the flocs from the clarified water.

As flocculants play the major role in flocculation and sludge dewatering process, the search for high efficient and cost-effective flocculants has always become the challenge in many studies (Lee, 2014b). The main process variables that are commonly measured to justify the flocculating and dewatering performance include settling rate of flocs, sediment volume (sludge volume index, SVI), percent solids settled, turbidity or supernatant clarity, percentage of pollutants removal or water recovery depending on the industrial application (Dobias, 2005). All these output variables are actually manifestations of the floc or aggregate size distribution and the shape and structure of flocs produced during the flocculation process. Flocculants that exhibit the capability to form floc of particles that have the density to achieve good separation between the water phase and solid floc phase are preferable for efficient gravity settling followed by dewatering process.

1.1 Coagulation-flocculation

Coagulation-flocculation is the conventional treatment method where the inorganic metal salts with cationic charge are commonly used as coagulants and long chains non-ionic or anionic polymers are usually employed as flocculants (Chong, 2012). It is generally known that most of the colloidal particles in wastewater carry negative charge in aqueous medium. After addition of cationic inorganic coagulant and pH adjustment with acidic or alkaline solution, metal salts will hydrolyse rapidly in wastewater at isoelectric point to form cationic species, which are adsorbed by negatively charged colloidal particles, resulting in simultaneous surface charge reduction and formation of micro-flocs (Suopajärvi, 2013). However, the coagulation process is not always perfect as it may result in small flocs when coagulation takes place at low temperature or produce fragile flocs which settle slowly and easily break up when subjected to physical forces (Lee, 2014b).

In order to conquer those problems and also to improve the process, anionic/non-ionic polymeric flocculants (or coagulant aids) are widely used to bring together and agglomerate the slow-settling micro-flocs formed by the coagulant to form larger and denser flocs, thereby facilitating their removal in subsequent settling and dewatering stages (Lee, 2012). The general steps in coagulation-flocculation process are illustrated in Figure 1.1.

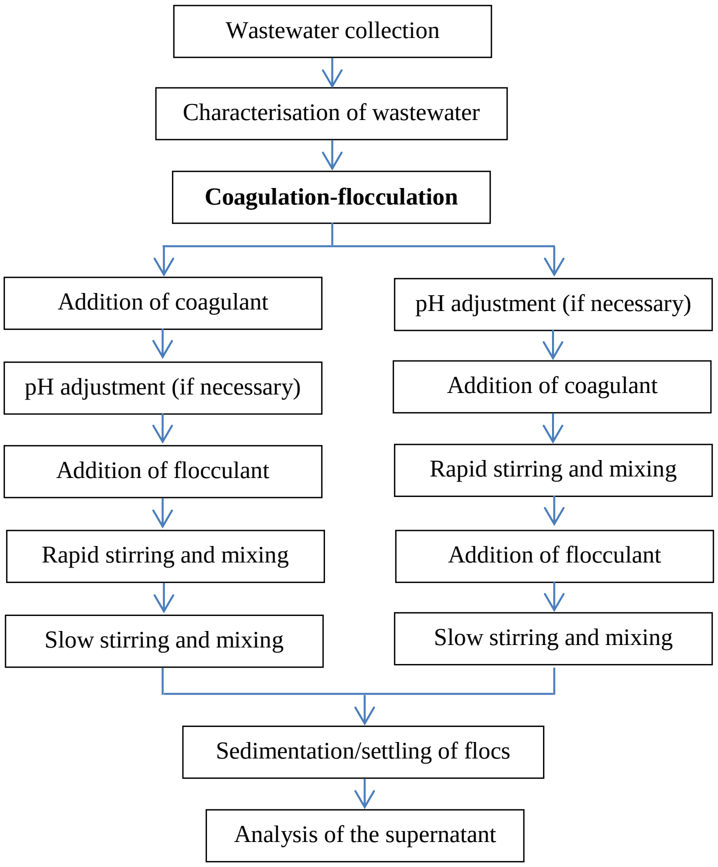


Figure 1.1: General steps in coagulation-flocculation process (Martín, 2011; Razali, 2011; Sher, 2013; Yue, 2008)

The use of flocculants not only increase the density and the solidity of the flocs formed which gives the advantage of reduction of sludge volume, but also has the advantage to reduce the consumption of coagulants. A study has reported that the combined use of coagulant (ferric chloride) and non-ionic polyacrylamide resulted in the production of sludge volume with reduction of 60% of the amount produced when coagulant was solely used for the treatment of beverage industrial wastewater (Amuda, 2007).

As shown in Table 1.1, many studies demonstrate that the addition of a polymeric flocculant to an inorganic coagulant deliver superior removal of water quality parameters including chemical oxygen demand (COD), suspended solids (SS), turbidity and colour compared to wastewater treated with coagulant only. For instance, the turbidity reduction efficiency in treatment of pulp and paper mill wastewater was improved from 80% to 96% when flocculant was added (Teng, 2014). Furthermore, addition of flocculant has the advantage of reducing the amount of coagulant used and thus reduced the cost of the coagulation/flocculation process (Ahmad, 2008; Amuda, 2006; Martín, 2011; Sher, 2013; Teresa, 2007; Yang, 2010).

Table 1.1: Application of coagulation-flocculation process with chemical coagulant(s) and flocculant(s) in wastewater treatment

Coagulant(s)	Flocculant(s)	Type of wastewater	Optimum results	
Lime, ferrous sulphate (Ginos, 2006)	Four cationic (FO-4700-SH, FO-4490-SH, FO-4350-SHU and FO-4190-SH) and two anionic (AN 934-SH and FLOCAN 23) polyelectrolytes	Olive mill effluent	SS TP COD	30-95% removal 30-80% removal 10-40% removal
Alum, ferric chloride and ferric sulfate (Amuda, 2006)	Anionic polyacrylamide	Abattoir wastewater	COD SS TP	94% removal 94% removal 97% removal
Commercial coagulant: T-1 (Teresa, 2007)	Commercial flocculants: Ecofloc 6260, Ecofloc 6700, Ecofloc 6705, Ecofloc 5400, Ecofloc 6708	Coffee wastewater	COD	55-60% removal
Ferric chloride (Amuda, 2007)	Non-ionic polyacrylamide	Beverage industrial wastewater	COD TP SS	91% removal 99% removal 97% removal
Alum/ferric salt (Joo, 2007)	Synthetic cyanoguanidine-formaldehyde based polymer	Synthetic reactive dyes wastewater	Colour	About 100% removal
		Real reactive dye wastewater	Colour	62% removal

Alum and polyaluminium chloride (PACl) (Ahmad, 2008)	Cationic (Organopol 5415) and anionic (Chemfloc 430A) polyacrylamides	Pulp and paper mill wastewater	Turbidity	99.7% removal
			SS	99.5% removal
			COD	95.6% removal
			SVI	38 ml/g
			Settling time	12s
Palm oil mill boiler (POMB) – adsorbent (Chong, 2009b)	Cationic polymer (KP 1200B) and anionic polymer (AP 120C)	Ceramic industry wastewater	Boron	15mg/L to 3mg/L
			SS	2g/L to 5mg/L
Mixture of ferric chloride and polyaluminium chloride (Yang, 2010)	Cationic, anionic and non-ionic polyacrylamides	High-phosphorus hematite flotation wastewater	Turbidity	13530NTU to 12NTU
Aluminium polychloride (Martín, 2011)	Anionic polyacrylamide (Actipol A-401)	Wastewater from sauce manufacturing plant	COD	82% removal
			Turbidity	72% removal
			$\text{TOC}_{\text{soluble}}$	13% removal
Aluminium sulphate (Sher, 2013)	Anionic polyacrylamide (Magnafloc 155)	Industrial polymer effluent	COD	98% removal
			SS	91% removal
			Turbidity	99% removal
Polyaluminium chloride (Liang, 2014)	Polydiallyldimethyl ammonium chloride (cationic)	Dyes (acid, basic and reactive) wastewater	Dye	90% removal
Polyaluminium chloride (Wang, 2014)	Cationic polyacrylamide	Papermaking-reconstituted tobacco slice wastewater	COD	67.8% removal
			Colour	77.7% removal

In order to save treatment cost and time, flocculant is used as a coagulant in some cases and pH adjustment is not needed. As presented in Table 1.2, cationic polymers with medium charge density and high molecular weight are normally acted as a coagulant in treatment of organic-based wastewater such as paper and pulp and textile effluents. A case study has shown that coagulation-flocculation generated larger volume of sludge which resulted to higher total treatment cost as compared to using flocculant as coagulant (no inorganic coagulant was used) in treatment of palm oil mill effluent (Chong, 2012).

Table 1.2: Application of flocculants as coagulants in wastewater treatment

Flocculant(s)	**Type of wastewater and its pH value**	**Optimum results**	
Cationic (FO-4700-SH and FO-4490-SH) polyelectrolytes (Ginos, 2006)	Olive mill effluent, 5.1-5.3	SS	97-99% removal
		TP	50-56% removal
		COD	17-35% removal
Polyacrylamide-based polymers (anionic: Praestol 2515, Praestol 2540, non-ionic: Magnofloc 351, cationic: Praestol 857 BS) (Sabah, 2006)	Coal waste slurry, 8.3	Turbidity	25NTU to 6.8NTU
Cationic (Organopol 5415, Organopol 5020, Organopol 5470, Organopol 5450, Chemfloc 1515C) and anionic (Organopol 5540, Chemfloc 430A, AN 913, AN 913SH) polyacrylamides (Wong, 2006)	Pulp and paper mill wastewater, 7.3-8.3	Turbidity	95% removal
		SS	98% removal
		COD	93% removal
		SVI	14 ml/g

		Water recovery	91%
Cationic polydiallyldimethylammonium chloride (PDADMAC) (Kang, 2007a)	Simulated reactive dye wastewater, 7	Colour	>90% removal
Cationic polyamine (Yue, 2008)	Simulated dye liquor wastewater, 6.63-7.89	Colour	96% removal
	Actual printing and dyeing wastewater, 11.2	Colour	90% removal
		COD	89% removal
Cationic polydiallyldimethylammonium chloride (polyDADMAC) (Razali, 2011)	Pulp and paper mill wastewater, 7	Turbidity	91% removal
		SS	Nearly 100% removal
		COD	98% removal

1.2 Sludge dewatering

Large amount of waste sludge are produced annually in municipal and wastewater treatment. For instance, pulp and paper industry produces more than ten million tons of primary sludge, waste activated sludge and de-inking sludge (Lauri, 2011). Sludge management is a major challenge in wastewater treatment and has been a focus of research activity for decades due to its large production, potential for environmental pollution and high cost in terms of effective management and disposal (Yu, 2008; Zhang, 2010). Operating cost for sludge treatment can amount to approximately 50% of the total operating cost of the whole wastewater treatment plant (Boráň, 2010). In addition to the economic aspects, wastewater treatment plants are also facing the pressure of sludge management in a sustainable way because of ever increasingly stringent environmental laws and regulations (Lu, 2014).

Since the moisture content represents 60 to 85% weight of the sludge matrix, a feasible strategy is to decrease the water content through removal of the moisture from sludge (sludge dewatering) (Lu, 2014). It allows a reduction in sludge volume which can result in less sludge to manage and consequently reduce the cost of sludge processing and disposal as low as possible.

Apart from the free water that could be readily removed by gravity separation, bound water involving the interaction between water and solid surface requires more energy for water to be released from the sludge (Yu, 2008). To solve this problem, chemical flocculation and dewatering are often applied and considered efficient because the flocculant could adsorb onto the particles and bridge the dispersed particles together, increasing the effective particle size of the solid phase. The stability of the suspension is broken and the liquid phase is released by flocculating the sludge particles with flocculants, and then improve the mechanical dewaterability of the waste sludge (Lu, 2014).

At present, the cationic derivatives of polyacrylamides with high molecular weight are the most commonly used flocculants in sludge dewatering. It has ample binding sites (hydroxyl, carboxyl and amino groups) for adsorption onto sludge particles and strong van der Waals forces with the sludge particles which promote an increase in floc size, floc density and dewaterability of the sludge (Guo, 2015; Zhang, 2010). In certain cases, anionic and nonionic polymers are used depending on the characteristics of the sludge to be flocculated (Lauri, 2011). A recent study demonstrated that the combination use of soy with cationic polyacrylamide in dewatering of fibrous sludge is cheaper and greener when compared to a full polyacrylamide dose (Banerjee, 2014).

1.3 Chemical coagulants and flocculants

The conventional chemicals that are widely applied in industrial wastewater treatment can be classified into two major groups depending on the chemical compositions: inorganic mineral additives/metal salts which are used as coagulants and organic polymeric materials that are employed as flocculants.

1.3.1 Inorganic coagulants

Inorganic salts of multivalent metals such as iron and aluminium salts have been widely used for decades as coagulant and are still being used nowadays (Joo, 2007). It is mainly because of its advantage of low cost, where their market price is very much lower compared to the chemical polymeric flocculants as shown in Table 1.3.

Table 1.3: Market prices for bulk sales of chemical coagulants and flocculants in Greece in 2004 (Sarika, 2005)

Chemical materials	**Coagulants**		**Cationic flocculants**		**Anionic flocculants**	
	Lime	$FeCl_3$	FO-4700	FO-4490	FLOCAN	AN 934
Price, €/ton	130	450	2980	2800	2500	2550

However, the application of inorganic coagulants in wastewater is limited nowadays and has been reduced due to numerous disadvantages. As reported in many studies, its usage would cause production of large volumes of metal hydroxide (toxic) sludge. It will create disposal problem and an increase in metal (e.g. aluminium) concentration in the treated water which may have human health implications (Ranade, 2014). Aluminium is a widely recognised neurotoxin and causes various adverse effects in plants, animals and humans. The link between the neurotoxicity of aluminium and the pathogenesis of Alzheimer's disease has been the subject of scientific debate for several decades (Masahiro, 2011).

Other drawbacks of inorganic coagulants include inefficient towards colloidal particles and large amount is required for efficient flocculation if polymeric flocculant is not used together, and easily affected by pH of the solution (Sharma, 2006). In addition, the flocs produced are generally small and less compact, resulting in low sedimentation velocities. Again the flocs are weak and can be broken down under high shear conditions (Ranade, 2014). In order to minimise the drawbacks of inorganic coagulants, many factors have been taken into consideration to find the alternative and reduce the dosage of the harmful inorganic coagulants. Application of organic polymeric flocculant after coagulant is commonly employed to tackle the problems faced by using coagulant and also to enable the formation of denser and bigger flocs that can settle faster and withstand the shear force during filtration. The details are presented in next section.

1.3.2 Organic synthetic flocculants

In recent years, many synthetic polymers have been used as flocculants (coagulant aids) which could enhance the coagulation and flocculation efficiency with promising results have been reported (Ahmad, 2008; Kang, 2007b). Commercial organic flocculants are mostly linear water soluble polymers which are based on repeating units of various monomers such as acrylamide and acrylic acid. In most cases, they are derived from oil-based and non-renewable raw materials (Suopajärvi, 2013). Polymeric acrylamide, or polyacrylamides, are the flocculants that are commercially produced in the largest volume (Lu, 2014).

As shown in Table 1.4, polymers can vary in molecular weight, structure (linear versus branched), amount of charge, charge type and composition but generally, the synthetic polymers are classified into four forms: cationic (positively charged), anionic (negatively charged), amphoteric (contains both cationic and anionic groups) and non-ionic (close to neutral). Strictly, ionic polymers are addressed as polyelectrolytes. The nature of the charges, molecular weight and charge density are the main parameters that will have significant effect on the efficiency of flocculation process.

Table 1.4: The main characteristics of synthetic polymeric flocculants

Characteristics	**Categorisation**	
Nature of charges	Amphoteric / anionic / cationic / non-ionic	
Molecular weight	Low	1 to 3 millions
	Medium	3 to 6 millions
	Standard	6 to 10 millions
	High	10 to 15 millions
	Very high	Greater than 15 millions
Charge density	Low	1 to 10%
	Medium	10 to 40%
	High	40 to 80%
	Very high	80 to 100%

As presented in Tables 1.1 and 1.2, it is obvious that the role of polymeric flocculants especially polyacrylamides in wastewater treatment is very well established, where it has successfully removed colloidal particles and contaminants (pollutants) from various types of wastewater. The effectiveness of the flocculation was normally measured based on the reduction of turbidity, SS, COD and colour.

The extensive use of polyacrylamides as a flocculant is due to their distinct characteristic attributes and the ability to accelerate the flocculation process or strengthen the floc making subsequent separation more attainable. The polymers are convenient to use, immediately soluble in aqueous systems, no influence on the pH of the medium and highly efficient with little quantities (e.g. few milligrams per litre). Normally, an appropriate polyelectrolyte can increase floc size, and thus form strong and dense floc of regular shape which has good settling characteristics (Razali, 2011). The use of polymers in this way results in a substantial reduction of coagulant dose required with a 40-60% reduction is expected. Hence, the volume of sludge, the ionic load of the waste water (especially the level of aluminium), and the overall costs can be reduced (Bolto, 2007).

1.4 Environmental effects of synthetic polymers and their regulatory control

The use of synthetic polymers is debatable because its application may cause environmental consequences and health hazards (Lee, 2014b). Bolto and Gregory (Bolto, 2007) reported that commonly used anionic and non-ionic polymers are generally of lower toxicity, but cationic polyelectrolytes are more toxic, especially to aquatic organisms.

Contaminants of synthetic polymers used in water and wastewater treatment generally arise from residual unreacted monomers (such as acrylamide, ethyleneimine), unreacted chemicals used to produce the monomer units (such as epichlorohydrin, formaldehyde and dimethylamine) and reaction by-products of the polymers in water (Hesami, 2014). Acrylamide which is the monomer impurity of polyacrylamide is identified as a suspected carcinogen and extremely toxic causing severe neurotoxic effects. For instance, the United States Environmental Protection Agency has regulations on residual acrylamide concentration in polyacrylamide which is 0.05% by weight and also the polyacrylamide dosage at 1 mg/L to avoid the possible accumulation of acrylamide in the water stream (USEPA, 2012a).

From technical point of view, a reliable control on monomer residue (e.g. acrylamide) is challenging in industrial manufacturing, especially for the powder type products. Since acrylamide has a relatively high boiling point of 125 ˚C/25 mmHg, it is not feasible to remove the monomer either by reduced vacuum or solvent washing. Currently the residual monomer is diminished by adding sufficient amount of radical initiator before quenching in emulsion polymerization (Lu, 2014). However it is technically difficult to eliminate monomer residue in a bulk solid state efficiently. In addition, it is the biggest technical barrier for many small polyacrylamide manufacturers to control the monomer residue to a satisfactory level (Lu, 2014).

Also, the majority of commercial polymers are derived from petroleum-based raw materials using processing chemistry that is not always sustainable. Moreover, most synthetic polymer structures are resistant to biodegradation (Bolto, 2007; Brostow, 2009) and their degradation products are considered hazardous because of the release of monomers that could enter in the food chain and may cause carcinogenic effect (Sharma, 2006). Due to the poor biodegradability of synthetic polymers, the sludge treated with polyacrylamides is excluded from application on areas under cultivation starting from the end of 2013 in Germany (Lauri, 2011). Some countries such as Japan, Switzerland and France have started to strictly control the polyacrylamide usage in drinking water treatment and food-related processing (Bolto, 2007; Lu, 2014).

1.5 Emergence of plant-based bio-flocculants

With increasing awareness of the potential harm and environmental pollution caused by chemical flocculants and implementation of more stringent environmental regulations, research is increasingly focused towards discovering high efficient and eco-friendly bio-flocculants from natural sources with the aim to replace the conventional flocculants.

Compared with conventional chemical flocculants, natural organic flocculants are made of polysaccharides or natural polymers so that they have the advantages of biodegradability, non-toxicity and are more readily available from renewable agricultural resources (Bolto, 2007; Renault, 2009a). In addition, as biopolymers are biodegradable, the sludge they form can potentially be more efficiently degraded by microorganisms (Renault, 2009a) and used for bioenergy generation through anaerobic digestion (Appels, 2008).

For the past ten years, plant-based bio-flocculants have been studied for their flocculating efficiencies in the treatment of different types of wastewater (Al-Hamadani, 2011; Anastasakis, 2009; Belbahloul, 2015; Mishra, 2006a; Shak, 2015). The literature review concerning the background and application of these bio-flocculants in wastewater treatment is presented in Chapter 2.

1.6 Problem Statement

Most of the studies have proven that plant-based bio-flocculants are effective for treatment of various types of wastewaters at laboratory scale. However, its flocculating ability for the treatment of wastewater high in suspended solids (SS) with concentration more than 10 g/L is rarely investigated and the dewatering efficiency of bio-flocculant have not been explored yet. In addition, there are other problems that restrict its development and application in different industries including sensitivity of bio-products to preparation and extraction process, fast degradation with time and processing conditions, inefficient extraction process with moderate flocculating efficiency, and higher production cost compared to commercial flocculants (Lee, 2014a).

The functional properties of the hydrocolloid mucilage are sensitive to the preparation methods and could be altered by the extraction and drying processes to a great extent (Jaya, 2009). In addition, the chemical composition and molecular structure of hydrocolloid mucilage that are responsible for its functional properties often depend on the source, extraction methods and conditions, and any further processing conditions (Mirhosseini, 2012). For instance, the rheological properties and viscoelastic behaviour of natural plant gums depend on the method and condition of extraction, purification, drying, and further modification processes (Amid, 2012). Thus, more investigation concerning the processing methods and conditions (particularly extraction) of bio-flocculants is strongly important because it will determine the quality and stability of bio-flocculants.

Biopolymers face degradation of products with time and processing parameters or conditions (Jani, 2009). Fresh mucilage in aqueous form is susceptible to microbial attack due to its high water activity and composition, and reducing its shelf life to a few days at room temperature (León-Martínez, 2011). In addition, the flocs tend to lose stability and strength with time because of their biodegradability (Pal, 2009). Materials with reduced moisture content will resist germination under favourable conditions, thus prolonging the storage life. In addition, extreme extraction conditions such as high temperature and long extraction time are reported to cause degradation or decomposition of bio-polymer structure during extraction (Veggi, 2013). Therefore, the optimisation of extraction conditions and drying process are of vital importance to produce high quality bio-flocculants in which the desired active constituents with flocculating properties could be well preserved and the storage period could be extended.

Conventionally, bio-flocculants are prepared using drying and water bath extraction processes, which are both time-consuming and require significant amounts of energy (Lee, 2015). In addition, the extracted bio-flocculants are moderately effective and are needed in huge dosage compared to synthetic flocculants (Mishra, 2012). Most of the application of bio-flocculants reported in literature was focused in the treatment of wastewater low in SS (<10 g/L) (Al-Hamadani, 2011). For some applications, a cationic coagulant was required for charge neutralisation before the bio-flocculant could bridge the micro-flocs together and the dosage required to achieve efficient flocculation is higher than polyacrylamides (Lee, 2014b). Hence, the selection of appropriate extraction method with optimisation of its extraction conditions is highly important to minimise the extraction time required in production of high efficient bio-flocculants for application in treatment of solution high in SS (>10 g/L).

It is anticipated that another significant drawback of using bio-flocculants is the processing and production cost which is higher than conventional flocculants (e.g. polyacrylamides). Yet, this drawback can be overcome because of the major importance of their applications in food and other industries, allowing for a price premium product, and its substantial benefit to environment and human health (Lee, 2014b). However, the primary step is to design and model an economical production process of bio-flocculants coupled with mass and energy balance, sizing and costing analysis in order to calculate the production cost of bio-flocculants. Afterwards, the process could be optimised to minimise the production cost of bio-products in order to be competitive to the commonly-used polyacrylamides from aspects of quality and price.

Nonetheless, the future development of cost-effective and environmental-friendly plant based bio-flocculants for applications in food and beverage, cosmetic, pharmaceutical, and other industries is highly possible and urgently needed. Intensive research efforts related to bio-flocculants should continue and be in-line with environment and health protection.

1.7 Aim and Objectives

The aim of this study is to produce a plant-based bio-flocculant from okra through environmentally friendly and economically viable extraction process where its quality (flocculating and sludge dewatering abilities) and production cost are comparable to commercial flocculants (polyacrylamides). The research objectives associated with the aim are as follows:

1. To establish the feasibility of applying a more efficient hydrothermal (water bath) extraction method and microwave assisted extraction method than existing extraction methods by using water as a green solvent in production of bio-flocculant with maximal yield and quality.
2. To investigate the impact of extraction parameters (extraction temperature, extraction time, solvent loading, agitation speed and particle size of okra) on the extraction yield and bio-flocculant quality to determine the most efficient appropriate extraction conditions for hydrothermal and microwave extractions.
3. To compare the extraction efficiencies of the hydrothermal and microwave methods and then investigate the underlying extraction mechanism and mass transfer phenomenon of both methods that affects the extraction efficiency.
4. To establish the potential application of bio-flocculants in treatment of solution with high concentration of SS (>10 g/L) and its dewatering efficiency without addition of coagulant and pH adjustment.
5. To define the quality of extracted bio-flocculants in aqueous and dried forms by comparing its efficiency with polyacrylamides in terms of dosage, flocculating and dewatering abilities.
6. To evaluate the economic viability of scaling up the production of bio-flocculant to industrial scale with hydrothermal and microwave extraction methods, minimise the production cost through parameter sensitivity analysis and finally propose the most cost effective plant design of bio-flocculant production.

1.8 Research Scope

This thesis focuses on an investigation of an environmentally-benign and economically feasible plant-based bio-flocculant extracted from okra. It is expected that the extracted bio-flocculant is comparable to the commercial flocculants (polyacrylamides) from aspects of quality and production cost. After preliminary study, the main processes that are involved in the production of bio-flocculant are identified as following:

a) washing and size reduction of plant materials,
b) hydrothermal or microwave extraction process,
c) separation of aqueous extract from biomass to get aqueous bio-flocculant,
d) and drying process to obtain dried bio-flocculant.

Conventionally, bio-flocculants are extracted from plant materials through time-consuming water bath extraction method (Belbahloul, 2015; Mishra, 2006b, 2008). With the purposes to understand the extraction behaviour of bio-flocculant and to compare the extraction efficiencies between hydrothermal and advanced extraction methods, a more efficient conventional hydrothermal extraction (CHE) method with shorter extraction time than current method in literature was designed to extract bio-flocculant from okra. In order to evaluate the possibility to further reduce the extraction time, increase the extraction yield and enhance the bio-flocculant quality; an advanced method which was microwave assisted extraction (MAE) was designed and employed to extract bio-flocculant from okra. The application of MAE to extract bio-flocculant is reported in literature for the first time.

The effects of several important extraction parameters (extraction temperature, extraction time, solvent loading, and agitation speed and particle size of okra) on the extraction yield and bio-flocculant quality (flocculating and dewatering abilities) were investigated and the extraction conditions were optimised for both extraction methods. The extraction efficiencies of bio-flocculants were justified by the yield. The influences of extraction parameters on yield were further evaluated by analysing the experimental data with response surface methodology.

The bio-flocculant quality in treatment of solution that contains high SS content (>10 g/L) without addition of coagulant and pH adjustment and its dewatering efficiency were investigated in this study by using kaolin colloidal solution as representative colloidal material. After flocculation process, the flocculating abilities of the extracted bio-flocculants were examined based on its performance in removal of SS and turbidity and also sludge volume index.

On the other hand, the dewatering efficiencies of bio-flocculants were evaluated with reference to SS removal after filtration and reduction of sludge volume which was represented by water recovery after dewatering process. Water recovery is inversely proportional to sludge volume; which means that higher water recovery denotes lower sludge volume.

The flocculating and dewatering abilities of aqueous and dried bio-flocculants extracted with both methods were evaluated and the most efficient bio-flocculant was compared with commercial flocculants (cationic and anionic polyacrylamides). The effects of bio-flocculant dosage and drying process on the sludge dewatering efficiencies of bio-flocculants extracted with both methods were investigated as well. Preliminary investigation of the plausible bio-flocculation mechanism was discussed with reference to the zeta potential analysis.

The extraction efficiencies of both methods in terms of optimised extraction conditions, optimised yield and the bio-flocculant quality were compared. The hypothesis for process acceleration and higher extraction efficiency observed in microwave extraction method was proposed, investigated and discussed with reference to the difference of heating mechanism and mass transfer phenomenon between hydrothermal and microwave processes. The interaction of the solvent and plant material with microwave heating was investigated through measurement of its dielectric properties. These results were used to support the argument of selective heating of okra with microwave energy which contributes to speedy and high extraction yield.

The economic feasibility study for industrial scale production of bio-flocculant with CHE and MAE processes were conducted using SuperPro Designer v9.0 simulation software. The simulation of each process involved steady-state mass and heat balance, sizing and costing which allowed calculation of unit production cost. The economic performance of batch versus continuous modes and hydrothermal versus microwave processes was studied and compared. Parameter sensitivity analysis was also conducted to study the effects of annual production, extraction yield and raw material okra price on the production cost. In order to judge the viability of the plant design, assessment of profitability was conducted and the estimated bio-flocculant selling price was compared with other commercial flocculants.

CHAPTER 2: LITERATURE REVIEW

This chapter is divided into two main sections, which are (part 1) plant-based bio-flocculants and (part 2) extraction methods for plant materials. The first section provides an overview of the development and flocculating efficiencies of plant-based bio-flocculants. Furthermore, the processing methods and bio-flocculation mechanism were discussed. All the reported studies about plant-derived bio-flocculants are conducted under lab-scale conditions in wastewater treatment. Hence, the possibility to apply natural bio-flocculants in food and beverage, mineral, paper and pulp, and oleo-chemical and biodiesel industries was discussed and evaluated.

In this study, the extraction of bio-flocculant from okra by using conventional hydrothermal extraction (CHE) and microwave assisted extraction (MAE) methods was investigated. Thence, the review in second section provides an insight into CHE and MAE processes and the application of these techniques in the extraction of polysaccharides from renewable plant sources. The reported heating phenomena and mechanisms of both methods which lead to disruption of cell wall were highlighted. The extraction efficiencies of both methods were compared with each other and the influences of important parameters on the extraction efficiency were discussed. The importance of dielectric properties in microwave heating was elucidated as well.

Part 1: Plant-based Bio-flocculants

Natural plant-based bio-flocculants emerge as an attractive alternative to polymeric flocculants, and their application in wastewater treatment has become increasingly essential in light of their biodegradability, nontoxicity, wide availability from renewable resources, and limited impact to the environment. The applications of plant-derived bio-flocculants for treatment of various types of wastewater in 10 years ago (2006-2016) are reviewed and discussed.

2.1 Plant materials and bio-flocculants preparation methods

As depicted in Table 2.1 below, plant-based bio-flocculants derived from some plant species (*Cassia obtusifolia, Hibiscus/Abelmoschus esculentus, Malva sylvestris, Plantago ovata, Tamarindus indica, and Trigonella foenum-graecum*) have shown promising results with respect to the treatment of biological effluent (Anastasakis, 2009), palm oil mill effluent (Shak, 2015), landfill leachate (Al-Hamadani, 2011), dye-containing wastewater (Mishra, 2006a) and textile wastewater (Freitas, 2015).

Table 2.1: Bio-flocculants extracted from plants (Note: N/A = not available)

Plant scientific name (common name)	**Charge**	**pH**	**Active ingredients**	**Processing method**	**Extracted plant part**	**References**
Cassia obtusifolia (Sicklepod)	Non-ionic	N/A	D -mannopyranose and D -galactopyranose	Solvent extraction	Seeds	(Shak, 2014, 2015)
Hibiscus / Abelmoschus esculentus (Okra / lady finger)	Anionic	5.2 to 8	L-rhamnose, D-galactose and L-galacturonic acid	Solvent extraction and precipitation, drying and grinding	Seedpods	(Ameena, 2010; Anastasakis, 2009; Mishra, 2008; Sengkhamparn, 2009)
Malva sylvestris (Mallow)	N/A	6.5 to 7	N/A	Drying and grinding	Seedpods and lobs	(Anastasakis, 2009)
Plantago ovata (Isabgol)	Anionic	N/A	N/A	Drying and grinding	Seed husk	(Al-Hamadani, 2011)
Tamarindus indica (Tamarind)	N/A	N/A	D-galactose, D-glucose and D-xylose	Solvent extraction and precipitation	Seeds	(Mishra, 2006a)
Trigonella foenum-graecum (Fenugreek)	N/A	7.73 to 8.62	D-galactose and D-mannose	Solvent extraction and precipitation	Seeds	(Mishra, 2008)

It is discovered that all plants being studied for bio-flocculant production have one similarity, which is a mucilaginous texture with polysaccharides as the main component, and a neutral pH. Mucilage is plant hydrocolloids that have viscous colloidal dispersion properties in water. They are heterogeneous in composition and are typically polysaccharide complexes formed from the sugars of different monosaccharides, including galactose, glucose, mannose, xylose, rhamnose, and uronic acid units (Jani, 2009; Mirhosseini, 2012). Natural polysaccharides are reported to exhibit excellent binding selectivity toward aromatic compounds and metals, thus are efficient in the removal of pollutants from wastewater (Crini, 2005). Therefore, extraction becomes the essential step to isolate the active components that exhibit the flocculating activity from the plants.

As stated in Table 2.1, there are two methods for the production of plant-derived bio-flocculants which have been reported thus far: (i) solvent extraction and precipitation and (ii) drying and grinding. The detailed procedures for each method are illustrated in Figure 2.1.

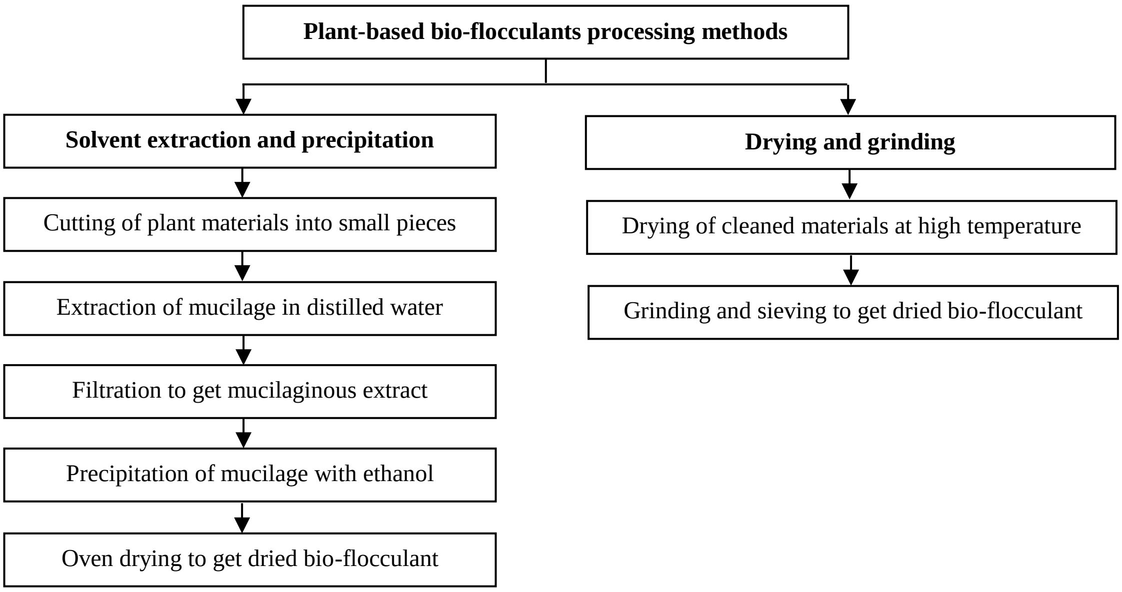


Figure 2.1: Processing methods of plant-based bio-flocculants

The solvent extraction and precipitation method has been applied for extraction of bio-flocculants from sicklepod, okra, tamarind, and fenugreek. The cleaned plant materials were extracted with distilled water and the filtered mucilaginous extract was then precipitated using ethanol. The drying and grinding method was used for preparation of bio-flocculants from mallow, okra, and isabgol. The cleaned materials were dried at high temperature, and then ground and sieved to obtain the bio-flocculants. It was discovered that the bio-flocculants obtained with drying and grinding method had lower flocculating activities than the bio-flocculants obtained with extraction method (Al-Hamadani, 2011; Anastasakis, 2009). In addition, it is only effective if a coagulant was added before bio-flocculant in a coagulation−flocculation process.

On the other hand, the bio-flocculants that are prepared with solvent extraction and precipitation displayed higher flocculating abilities than the bio-flocculants prepared with drying method. When these bio-flocculants are applied in the treatment of wastewater, flocculation can happen without addition of inorganic coagulant and pH adjustment. The flocculating efficiencies for the bio-flocculants prepared with these two different methods are presented in Table 2.2 in next section with more detailed discussion. This finding indicates that the extraction step exhibits notable impact to the flocculating efficiencies of bio-flocculants and plays the major role in the extraction of the active constituents with high flocculating activity from the plant materials.

To date, there is no published study that investigates the relationship between extraction and flocculation. It is very important to relate the extraction methods and conditions with the flocculating activity, evaluate the extraction parameters that may degrade the flocculating efficiency of the products, and optimise the extraction conditions in order to produce the most efficient bio-flocculant that is comparable to or better than commercial flocculants in terms of cost and flocculating efficiency.

2.2 Flocculation efficiencies of bio-flocculants

The jar test is used to evaluate the flocculating abilities of plant-based bio-flocculants and to optimise the flocculation process in most studies (Ramavandi, 2014). The identified usage of natural flocculants and their technical viability for industrial wastewater treatment are currently limited to academic research, with restricted examples within industry. The flocculating performance of bio-flocculants reported in recent literature (10 years ago) has been compiled and summarised in Table 2.2.

Table 2.2: Flocculating efficiencies of plant-based bio-flocculants

Plant-based bio-flocculants (processing method)	**Treated wastewater (SS concentration in g/L or turbidity in NTU)**	**Sedimentation period (minutes)**	**Optimum pH**	**Optimum coagulant dose (mg/L)**	**Optimum bio-flocculant dose (mg/L)**	**Flocculation efficiency**	
						Types of removal	**% removal**
Sicklepod (extraction)	Palm oil mill effluent (7.5 g/L) (Shak, 2014)	45	3	-	1000	SS	93.2
						COD	56.7
						SVI	63ml/g
	Palm oil mill effluent (30 g/L) (Shak, 2015)	35	7	1150	2470	SS	81.6
						COD	48.2
Tamarind (extraction and precipitation)	Golden yellow dye and direct fast scarlet dye (Mishra, 2006a)	60	7	-	10 and 15	Dye	60 and 25
	Azo dyes, basic dyes and reactive dyes (Mishra, 2006b)	10	6, 9.2 and 1	-	10, 10 and 15	Dye	36.4, 39 and 52.1
Okra (extraction and precipitation)	Textile (Freitas, 2015)	60	6	88	3.2	Turbidity	97.2
						COD	85.7
						Colour	93.6
Okra and Fenugreek (extraction and precipitation)	Textile (0.35 g/L) (Mishra, 2008)	60	9.2	-	0.8 and 0.04	SS	98 and 94.2
						DS	31.6 and 43.7
						Colour	53.5 and 6

Isabgol (drying and grinding)	Semi-aerobic landfill leachate (0.38 g/L) (Al-Hamadani, 2011)	120	6.5 to 7.5	7200	400	COD Colour SS	64 90 96
Mallow and okra (drying and grinding)	Kaolin synthetic (68 NTU) (Anastasakis, 2009)	10, 20, 30	6.2 and 6	8.6	12 and 5	Turbidity	97.4 and 97.3
	Biologically-treated effluent (55 NTU) (Anastasakis, 2009)		-	171.1	62.5 and 2.5	Turbidity	67 and 74

As reported in the previous section, bio-flocculants obtained with drying and grinding exhibit lower flocculating efficiencies and must be coupled with the use of coagulant in a coagulation−flocculation process. Table 2.2 shows that Isabgol husk prepared with drying and grinding was effective as a coagulant aid for the treatment of landfill leachate with poly-aluminium chloride (PACl) as the coagulant (Al-Hamadani, 2011). However, low removal efficiency of COD, colour, and SS at 17, 27, and 41%, respectively, was reported when they were used as flocculant without addition of coagulant in the process. Another recent study showed that the dried and ground mallow and okra bio-flocculants were efficient in removing turbidity from a synthetic kaolin suspension and biologically treated effluent when aluminium sulfate was used as the coagulant (Anastasakis, 2009).

Alternately, bio-flocculants extracted with water as the solvent display remarkable flocculating performance when it is compared with the bio-flocculants obtained with drying method. In some cases, coagulant was not required for flocculation to happen (Mishra, 2008; Shak, 2014). High removal efficiency either in suspended solids (80% above), dye (36-60%), turbidity (97%), COD (48-86%) and colour (53-93%) was achieved by using a low concentration of extracted bio-flocculant.

The findings presented in Table 2.2 indicated a good flocculating potential of bio-flocculants with removal of suspended solids and turbidity up to 80% for various types of industrial wastewater treatment. However, a long sedimentation period (60 and 120 mins) was reported in some literature. It is postulated that the flocs formed are weak and small in size and thus a long settling time is required. In the study of *Tamarindus indica* as bio-flocculant, it was not suggested as an effective flocculant for the removal of vat (golden yellow) and direct (direct fast scarlet) dyes from textile wastewater because of unsatisfactory dye removal after a long period of contact time (Mishra, 2006a). In most of the reported studies, the suitable pH range was neutral for maximum flocculating efficiencies of bio-flocculants. Some were reported to be workable in acidic or alkali conditions depending on the type and characteristics of treated wastewater.

2.3 Bio-flocculation mechanisms

The most common mechanisms of flocculation include charge neutralisation and polymer bridging (Bolto, 2007). The flocculation mechanism of charge neutralisation is only applicable when the colloidal or suspended particles and the added flocculants are of opposite charge (Bolto, 2007). In many cases the impurity particles are negatively charged. As shown in Table 2.1, the ionic charges of bio-flocculants are anionic for okra and isabgol, non-ionic for sicklepod and unknown for others. Since most of the bio-flocculants are verified to be either anionic or non-ionic, there is high probability that charge neutralisation is not the responsible mechanism.

Thus, the most probable mechanism for plant-derived bio-flocculants with particulate matter in effluent is polymer bridging in which the biopolymers (polysaccharides) serve as a bridge based on particle−polymer−particle complex formation. The bridging mechanism involved in bio-flocculation as shown in Figure 2.2 has been reported in a study about production of bioflocculant ETH-2 with high flocculating activity from strain *Enterobacter* sp. isolated from activated sludge (Tang, 2014).

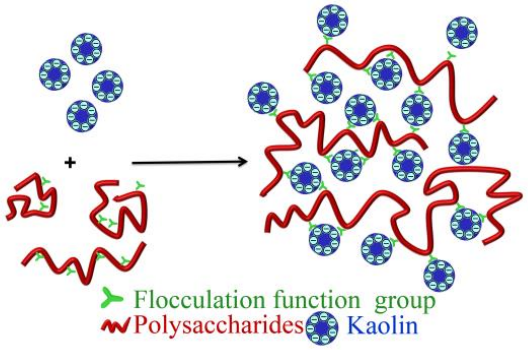


Figure 2.2: Bridging mechanism in bio-flocculation of ETH-2 with kaolin particles, reproduced with permission from PLoS ONE (Tang, 2014)

To be effective in destabilisation, a biopolymer molecule must contain chemical functional groups (e.g. free hydroxyl, carboxyl and amino groups), which comprise the possible binding sites that can interact with sites on the surface of the colloidal or suspended particles (Guo, 2015; Zhang, 2010). When a biopolymer molecule comes into contact with a colloidal particle, some of these groups adsorb at the particle surface, leaving the remainder of the molecule extending out into the solution. If a second particle with some vacant adsorption sites contacts with these extended segments, attachment will occur. A particle–polymer–particle complex is thus formed in which the biopolymer serves as a bridge (Mishra, 2006b).

Polymer bridging has been proposed as the mechanism for flocculation behaviour in treatment of textile wastewater with *Tamarindus indica* mucilage (Mishra, 2006a). For other bio-flocculants where the surface charge is unidentified, its flocculation mechanism is difficult to be predicted and has not been discussed with the experimental findings.

2.4 Application of bio-flocculants in different industries

To date, there is no detailed study about the applicability or feasibility of plant-derived bio-flocculants in other industries apart from wastewater treatment. However, there are many processing industries utilising a clarification or flocculation process in the manufacturing of certain products (Table 2.3).

Table 2.3: Clarification/flocculation process in different industries

Application area		**Separated components**	**Clarification/flocculation method/agent**
Beverage industry	Clarification of wine (Catarino, 2007; Lambri, 2012)	Iron compounds, suspended solids, proteins, dead yeast cells	Activated carbon, gelatin, albumen, bentonite
	Clarification of fruit juices (Domingues, 2012; Martín-Diana, 2009; Turfan, 2011)	Suspended particles, proteins, polyphenols, carbohydrates	Gelatin, bentonite, kieselsol, silica sol, polyvinylpolypyrrolidone (PVPP), chitosan
	Clarification of dates extract (Nasehi, 2012)	Non-soluble matter, colouring matter and semi-soluble (e.g. pectin) material	Activated carbon, bentonite, PVPP
	Clarification of sugar cane juice (Chen, 2007; Fabio, 2009; Laksameethanasana, 2012; Thai, 2012)	Organic and inorganic constituents, suspended solids	Lime, bentonite, activated carbon, polyacrylamide
Food industry	Recovery of muscle proteins from fish processing by-products (Chen, 2007; Taskaya, 2009)	Bones, scales, skin, fats	Isoelectric solubilisation/precipitation, polymeric flocculants (anionic, non-ionic and cationic)
	Recovery of muscle proteins from meat processing by-products (Tahergorabi, 2011)	Bones, scales, skin, fats	Isoelectric solubilisation/precipitation, mechanical deboning
Mineral industry	Clarification of kaolin slurry (Kogel, 2006; Nongkhran, 2008)	Discolouring contaminants (e.g. titanium, iron), organic and inorganic carbon, impurity clay minerals	Anionic polymeric flocculants (e.g. polyvinyl alcohol, polyacrylamide)

Paper industry	Retention of fine matters during papermaking (Lauri, 2011; Raluca, 2013; Rasteiro, 2007; Zakrajšek, 2009)	Cellulose fines, fibre fines and fillers	Polyelectrolytes flocculants (e.g. polyacrylamides, poly-aluminium chloride, polyethylene oxide), modified starches, chitosan
Oleo-chemical or biodiesel industry	Clarification of crude glycerol (Mah, 2012; Sdrula, 2010; Singhabhandhu, 2010; Wagner, 2011; Wu, 2007)	Methanol, inorganic salt (catalyst residue), free fatty acids, lipids, unreacted mono- or di- or triglycerides, non-glycerol organic matters	Chemical treatments, physical treatments (gravitational settling, centrifugation, evaporation, distillation), micro and ultra-filtration

Many of the conventional clarification and flocculation methods listed in Table 2.3 have their own drawbacks, and it has become necessary to study new clarification or flocculation methods and agents in order to achieve efficient clarification and also to maintain the quality of the end products. In this respect, plant-based bio-flocculants may emerge as an attractive option. The background and detailed explanation for natural bio-flocculants that could be a suitable alternative to the current flocculation or clarification methods in the food and beverage, mineral, paper-making, oleo-chemical, and biodiesel industries are explained below.

2.4.1 Beverage industry

Clarification is a routine part of the wine manufacturing process to reduce the turbidity, total solids in suspension and various iron compounds which are largely responsible for the clouding in wines (Catarino, 2007; Lambri, 2012). Clarification is also used to remove colloidal and suspended particles and turbidity from the fruit juices (Lee, 2007; Turfan, 2011). In addition, clarification and discoloration of date extract is one of the most important steps to free the extracted raw juice from non-soluble matter, soluble (e.g. colouring matter) and semi-soluble (e.g. pectin) materials in the production of products such as syrup, jam, date-jelly and date butter (Nasehi, 2012). In the processing of sugar from sugar cane juice, clarification is essential to remove the organic and inorganic constituents in soluble, suspended and colloidal form and minimise colour formation (Fabio, 2009).

As reported in Table 2.3, clarifying agents such as activated carbon, gelatin, albumen and bentonite are commonly used as clarification agents in beverage and juice processing. However, they have generally been found to be unsatisfactory in terms of the cost involved and inconsistent efficiency (Santos, 2014). Inadequate or improper use of these materials generally results in a re-clouding of the clarified product. In sugar industry, the use of lime and synthetic polymers as clarifying agents have been reported but it is not recommended due to the hidden toxicity effect to human health (Fabio, 2009; Thai, 2012). In addition, the use of lime and chemical polymers will generate solid waste which may contain residual chemicals that is harmful to the environment.

Plant-based bio-flocculants are suggested to be used as clarifying agents in aiding the separation of colloidal or suspended particles and turbidity from beverage, fruit juice or fruit extracts. Further, it is biodegradable, derived and extracted from natural sources, easily dissolved in water at room temperature; the treated solution and the sludge is not anticipated to have any significant environmental impact when compared to conventional flocculating agents.

2.4.2 Food industry

Flocculation processes could be used in the seafood or meat processing industries to recover the muscle proteins from the by-products (meat left over on bones, head, skin, etc.). Processing of the by-products would enable the industry to diversify its product offerings and offer another source of proteins for human or animal consumption and reducing the environmental impact of their processing wastes (Chen, 2007).

As shown in Table 2.3, isoelectric solubilisation or precipitation has been applied to remove impurities such as bones, scales, skin, etc., from by-products and resulting in improved protein recovery yields (Chen, 2007; Tahergorabi, 2011; Taskaya, 2009). However, the last step of this process is dewatering of the precipitated protein, which is inefficient and requires the use of centrifuges and long timescales compared to the rest of the process. Therefore, scale up and industrial take-up has so far been limited as short centrifugation times are required in order to maintain the economic feasibility of the process (Taskaya, 2009).

A study has been conducted to determine the separation efficiency of fish proteins recovered from fish processing by-products by means of isoelectric solubilisation/precipitation enhanced with a wide range of polymeric flocculants (anionic, non-ionic and cationic) (Taskaya, 2009). The results proved that this method could potentially be used to recover muscle proteins from the by-products. However, the safety concerns of proteins recovered from fish with the aid of the synthetic flocculants on human and animal health has limited its usage. In this respect, the use of plant-based bio-flocculants that exhibit the similar flocculating properties as polymeric flocculants is proposed to aid in dewatering of the precipitated protein after isoelectric solubilisation treatment. The dewatering efficiency can be enhanced, hence reduce the energy consumption and centrifugation time. Additionally, the safety concerns of the products recovered using bio-flocculants can be significantly reduced.

2.4.3 Mineral industry

Naturally occurring kaolin clays vary considerably in their colour properties. Selective flocculation is one of the widely used techniques to remove the discolouring contaminants (e.g. titanium and iron minerals, and other clay impurities), thereby improving the brightness and making the kaolin acceptable for pigment, coatings, cosmetic and pharmaceutical applications (López-Galindo, 2007).

Anionic flocculants such as polymers of sodium acrylate, acrylamide and also sulphonate polymers are widely used for flocculating mineral suspensions (Kogel, 2006). However, conventional flocculants are non-biodegradable and dispersion of monomers or residual polymers in the kaolin slurry may represent a health hazard (Bae, 2007; Bolto, 2007; Renault, 2009a). In addition, contamination of the clay products by the presence of the flocculating agent may require additional processes to remove the flocculant in order to meet the safety requirement of the products.

The flocculating abilities of plant-based bio-flocculants in synthetic wastewater (kaolin suspension) have been verified in literature (Anastasakis, 2009; Lee, 2015). Therefore, bio-flocculants may be applied to flocculate the kaolin particles while leaving the undesirable impurities in the solution. The kaolin flocs would settle at the bottom of the solution. After filtration or dewatering process, purified kaolin clays which contain a minimum amount of undesirable impurities could be obtained.

2.4.4 Papermaking industry

In papermaking process polyelectrolytes are generally used to promote flocculation by aggregating fine particles and fillers to form flocs that are large enough to be retained within the fibre network, which otherwise would be lost or accumulated in the white waters (Lauri, 2011; Zakrajšek, 2009).

Modified starch, modified celluloses and chitosan have been studied as papermaking retention and dewatering aids because they have the abilities to bind fillers and pigments as well as to retain the cellulose fibres (Lauri, 2011; Raluca, 2013). However, there are safety and environmental concerns with modified flocculants due to the chemical methods and the harsh conditions used in the modification process (Kaur, 2012). The application of chitosan in papermaking retention was proven but the flocs formed were smaller and weaker than polyelectrolytes (Raluca, 2013).

Stricter governmental requirements for controlling the contaminants from papermaking effluents and the chemicals used in the synthesis process of modified flocculants or polyelectrolytes has led the paper industry to search for bio-flocculants as retention aids. Bio-flocculants are easily extracted with green solvent (water), readily dissolved in water and can be added directly to the papermaking process, which is in contrast to the complexity of synthesis and preparation of modified flocculants.

2.4.5 Oleo-chemical and biodiesel industries

In oleo-chemical and biodiesel plants chemical pre-treatment is generally conducted in batches to purify crude glycerol (sweetwater). In this process, a coagulant is added to the sweetwater and the pH is adjusted to coagulate the residual fatty acids before it is further purified with a distillation process (Mah, 2012). However, chemical pre-treatment has the disadvantages of high chemical consumption and production of toxic effluent (Mah, 2012; Wagner, 2011).

Membrane technology such as micro and ultra-filtration has been introduced to replace conventional purification methods but fouling is a major problem in membrane operations which can cause significant flux decline by several different mechanisms (e.g. adsorption, pore blockage and solute aggregation on the membrane surface) (Wu, 2007).

To overcome the problems of chemical treatment and membrane filtration, bio-flocculants can be used as a pre-treatment option to remove the unreacted oil or fat, unreacted catalyst and other impurities from crude glycerol. The capability of bio-flocculants to remove organic contaminants from wastewater without the requirement of pH alteration has been examined (Mishra, 2008). Strong and dense flocs could be formed in a short time and are easily separated through sedimentation.

The use of bio-flocculants is postulated to have the relative advantage of being a simple process with reduced number of operations, lower purification time, lower treatment cost, and mild conditions of treatment with low temperature and pressure. Since no chemicals are used in the bioflocculant-based purification process, purified glycerol could be applied safely for a range of application in pharmaceutical or cosmetic industries.

2.5 Selection of plant and solvent for extraction and colloidal material for flocculation in this study

All of the plants previously studied for flocculation produce mucilage with polysaccharides as the main components. These plants are native to the tropical Asian region. Among these plants, okra has attracted considerable attention mainly because it is inexpensive and widely available all year round, especially in tropical countries.

Okra extract is a natural polysaccharide made up of D-galactose, L-rhamnose and L-galacturonic acid. These water-soluble polysaccharides are reported to exhibit the ability to destabilise colloidal suspensions and to flocculate small particles (Freitas, 2015). Its flocculating property in removal of solids and turbidity has been proven in the clarification of kaolin solution and biologically-treated effluent (Anastasakis, 2009). However, its capability in sludge dewatering has not been reported to date.

Okra was chosen as the model bio-flocculant for this study given that it has been widely characterised in previous studies, and is available all year round. The previous studies using okra act as a benchmark on which to compare the different extraction techniques used within this study. Water was chosen as the solvent given that the flocculation applications are based on aqueous systems, and alternative solvents are likely to require extra separation processes before the extracted bio-flocculant could be used. In addition, the polysaccharides which are the main components in the extract are a class of macromolecular compounds containing many hydroxyl groups that can easily soluble in water due to formation of hydrogen bonds with the water molecules (Wang, 2015).

A kaolin colloidal solution was used as a representative colloidal material in flocculation process because its surface characteristics are well-understood and is commonly used to evaluate the characteristics of newly developed flocculants (Lee, 2010; Yang, 2009). Most literature investigation of flocculating properties of flocculants involves treatment of real wastewater or synthetic wastewater (kaolin colloidal solution), with concentration of SS below 1 g/L (Lee, 2014a).

However, in real cases, some of the sludge from industrial wastewater or sewage treatment processes contains high concentration of SS. The colloidal or SS content of sludge water (water treatment residuals) from domestic water treatment plants is generally about 1 g/L and sometimes even above 10 g/L (Sun, 2015). The concentration of SS of alkaline-thermal pre-treated sludge was reported to be 25 g/L (Guo, 2015). In other examples, the biological sludge collected from sludge digestion tanks of the Tai Po sewage treatment plant in Hong Kong and the waste sludge collected from the secondary settling tank of a municipal wastewater treatment plant in Shanghai, China were reported to contain SS in the concentrations of 18 g/L (Lo, 2001) and 55 g/L (Zhang, 2010) respectively. In addition, palm oil mill effluent with SS concentration as high as 30 g/L renders treatment by membrane technology or adsorption difficult and therefore flocculation is suggested as a useful pre-treatment step to reduce the content of SS (Shak, 2015).

The sludge from water and wastewater treatment plants should be flocculated and dewatered before final disposal and reuse to reduce transportation cost (Sun, 2015). Up to date, most of the studies only paid attention to the treatment of sludge low in SS with flocculants but there is limited focus on the investigation of flocculant efficiency in treatment of sludge high in SS. Therefore, the attention of this study was focused on the exploration of the flocculating efficiency of bio-flocculant in treatment of kaolin colloidal solution high in SS concentration.

Part 2: Extraction Methods for Plant Materials

2.6 Fundamental of extraction of solutes from plant materials

Solid-liquid extraction of plants is a multicomponent, multiphase, unsteady state mass transfer operation, which involves transfer of more than one chemical species (the solute) from a solid to a solvent (Jose, 2003). It is a unit operation essential for isolation of valuable natural compounds from plants, and proper optimisation is required to optimise the extraction conditions and maximise the product recovery (Chemat, 2012). During extraction, the concentration of solute inside the solid varies leading to the nonstationary or unsteady condition. A series of mass transfer process as shown in Figures 2.3 and 2.4 have to occur during the period of interaction between the solute-containing particle and the solvent.

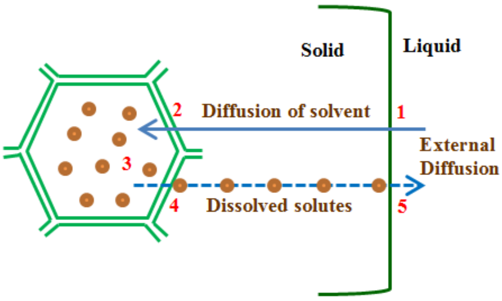


Figure 2.3: Scheme of the main steps involved in solvent extraction, redrawn with reference to (Jose, 2003)

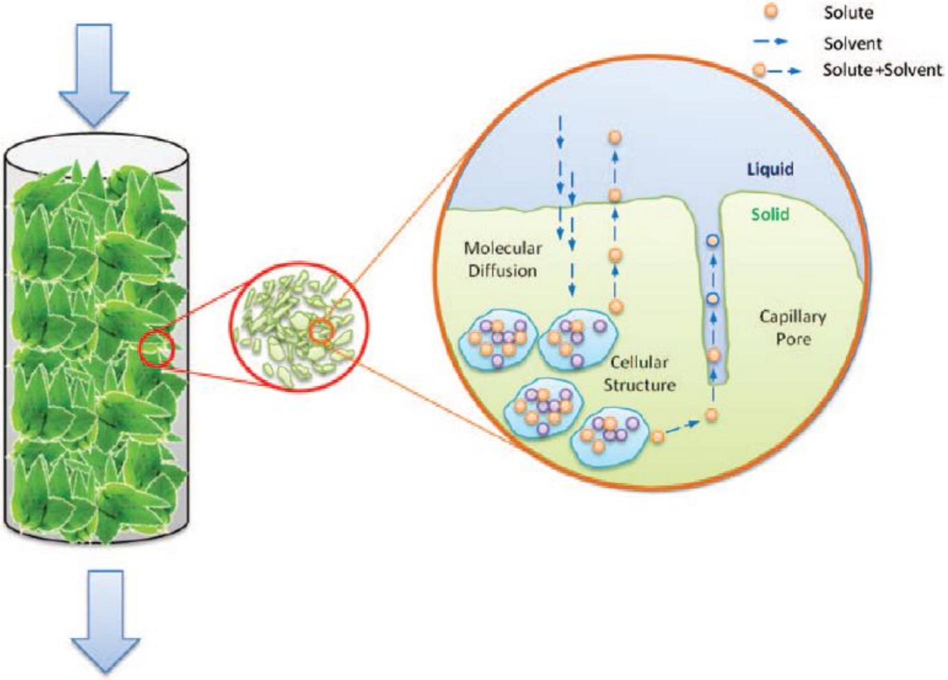


Figure 2.4: Schematic representative of the solvent extraction mechanism, reproduced by permission of The Royal Society of Chemistry (Palma, 2013)

As shown in Figures 2.3 and 2.4, extraction of solutes from plant materials involves (Palma, 2013; Veggi, 2013): (1) entrance of the solvent into the external part of solid by molecular diffusion, (2) transport of solvent into the interior of solid matrix, (3) solubilisation and/or breakdown of components into solvent, (4) transport of the dissolved solutes to the exterior of solid matrix by molecular diffusion and (5) migration of dissolved solutes to the bulk solvent by natural or forced convection. The desired solute in the tissue may be present inter- or intracellularly. Hence, intact cell walls and adhering membranes constitute a major resistance to diffusion (Jose, 2003). Disruption of the plant cells renders the cell walls more permeable for transport of solvent through the solid matrix and is a crucial step in solid-liquid extraction (Flórez, 2015). It has been reported that the fastest extraction is achieved in the situation of full rupture of the plant cell complex, and the slowest for intact plant cells (Jose, 2003).

2.7 Extraction methods

Aqueous based extraction methods are particularly promising and commonly used in extraction of plant components since water is the greenest solvent, being nontoxic, non-corrosive, non-flammable, environmentally benign, naturally abundant, and available at low cost (Flórez, 2015). In addition, many plant compounds have high solubility in water solvent.

Conventional hydrothermal extraction (CHE) in water bath coupled with heat and agitation is commonly employed to extract the bio-flocculants with polysaccharides as the main components from plants by using water as solvent (Sharma, 2006). CHE method is simple and safe and has been used for decades as the primary reference for preliminary or fundamental studies to evaluate the performance of extraction efficiency of bioactive components from plant materials.

Nevertheless, this technique has numerous drawbacks such as high extraction temperature which is near to the boiling point of solvent, long extraction time in several hours, large quantity of solvent consumption, and low extraction yield (Flórez, 2015). In addition, high temperature with long extraction time incorporates the risk of thermal degradation of the phytoconstituents and decrease of its associated physicochemical activities or properties (Flórez, 2015). Due to those disadvantages, this method is unfavourable from commercial perspective nowadays.

Over the past decade, advanced extraction methods such as ultrasound assisted extraction (UAE) (Xie, 2010; Ying, 2011), microwave assisted extraction (MAE) (Wang, 2010; Wei, 2010; Xie, 2010) and subcritical water extraction (SWE) (Yang, 2013) are gaining attention in academic and industrial sectors and have been applied to extract polysaccharides from different plant materials. MAE in particular has drawn significant attention due to its special heating mechanism that will be discussed in Chapter 5. This offers the potential to achieve higher extraction rate with shorter extraction time when compared with CHE in extraction of polysaccharides from tea flower, *Potentilla anserine* L. and *Cyclocarya paliurus* (Batal.) Iljinskaja (Wang, 2010; Wei, 2010; Xie, 2010). Furthermore, MAE method was reported to use shorter extraction time if compared with UAE in extraction of polysaccharides from *Cyclocarya paliurus* (Batal.) Iljinskaja (Xie, 2010) and in extraction of pectin from grapefruit (Bagherian, 2011). A review article pointed out that MAE has the advantages of time-saving and energy-saving with mild extraction conditions when compared with SWE (Heng, 2013).

Since the principal constituents in the bio-flocculants extracted from plants are polysaccharides (Belbahloul, 2015; Chakrabarti, 2008), the published literature on the extraction of polysaccharides with hydrothemal and microwave methods were reviewed and discussed in this chapter. The details about UAE and SWE are not covered in this review because these two methods are not investigated in this study. However, the overview of UAE and SWE in extraction of polysaccharides from different plant materials has been reported in some literature (Ebringerová, 2010; Zakaria, 2016).

Botanical polysaccharides exist as structural constituents of plant cell wall and middle lamellae of plant tissues (Mato Chain, 2016). As a result the basic theory of extraction of polysaccharides from botanical materials is to disrupt or break the cell wall under certain conditions such as heating (Zhang, 2014). Therefore, the selection of an effective extraction method plays the key role to extract the desirable active constituents from plants.

2.8 Conventional hydrothermal extraction (CHE)

2.8.1 Fundamental of heating mechanism of CHE

The whole process of conventional heating is time-consuming and energy intensive due to long extraction time at high temperature (Charoensiddhi, 2015). As illustrated in Figure 2.5, heat is transferred inwards from high temperature to low temperature. The heat is initially transferred from the heating medium (e.g. water bath) to the extraction vessel wall, and then to the solvent and plant matrix in the presence of thermal gradient via conduction and convection heat transfer (Robinson, 2010a).

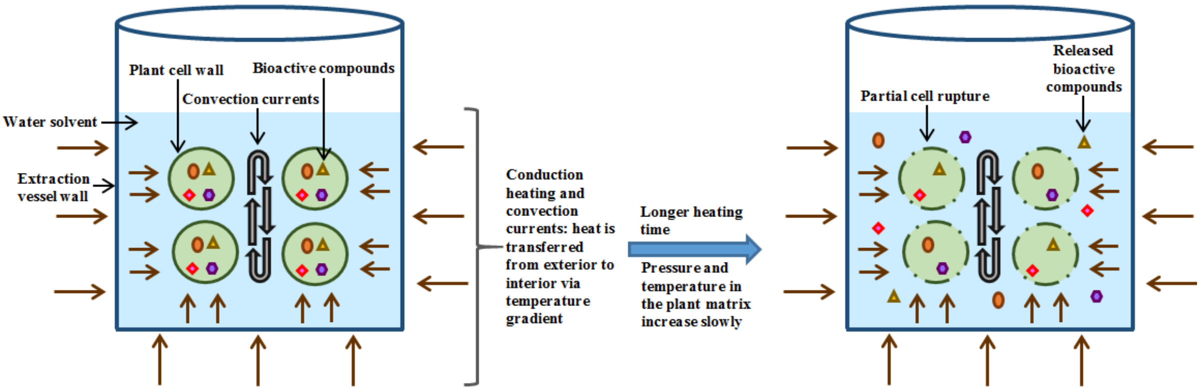


Figure 2.5: Heat and mass transfer phenomena of conventional extraction

A certain amount of heat transferred to the sample volume is firstly utilised to increase the temperature of the vessel surface which is called "wall heating" before the sample is heated and some energy is lost to the environment through conduction and convection currents (Gude, 2013). It is followed by heating of the sample that is in contact with the vessel wall and a temperature gradient within the sample is developed (Figure 2.6).

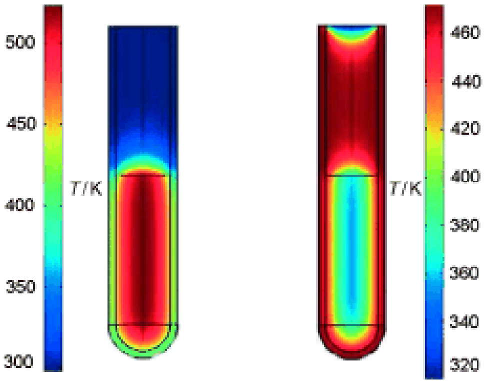


Figure 2.6: Thermal behaviour in conventional (right) and microwave (left) heating methods (temperature scales in Kelvin), reproduced with permission from Wiley (Kappe, 2005)

Although high temperature in the plant matrix produces some internal (within plant) evaporation but the rising of internal pressure is slow (Stuart, 2001). Partial cell wall rupture has been reported (McMillan, 2013), along with the hypothesis that it is caused by thermally induced pressure leading to partial cell wall rupture when the cell wall fails to support the elevated internal pressure. The heated solvent diffuses through the cell walls of plant matrix, and then the solutes are diffused into the solvent due to concentration gradient and the diffused solutes are transported out from the interior of the solid matrix (Flórez, 2015; McMillan, 2013; Philip, 2012).

2.8.2 Application of CHE in extraction of crude polysaccharides from plants

Due to the unique bioactivities and chemical structures of polysaccharides, a great deal of attention has been paid to extraction of water-soluble polysaccharides from plants by using CHE method with water as the solvent (Jia, 2014; Zhang, 2014). An abundance of literature have been published regarding discovery and evaluation of new polysaccharides from different plants, optimisation of the extraction conditions, characterisation of extracted polysaccharides and investigation of its bioactivities. Some examples of polysaccharide extraction with CHE method are summarised in Table 2.4.

Table 2.4: Extraction of polysaccharides with CHE method

Name of plant	**Optimised extraction conditions**				**Optimisation method(s)**	**Extraction yield (dry basis, %)**
	Temperature (˚C)	**Time (mins)**	**Solvent / material ratio (v/w)**	**Number of extractions**		
Abelmoschus esculentus (okra) (Samavati, 2013)	95	296	22	4	Single factor + CCD	16.9
Bruguiera gymnorrhiza (Li, 2013a)	71	31	42	-	Single factor + BBD	16.4
Cicer arietinum L. (chickpea) (Ye, 2016)	99	168	24	-	Single factor + BBD	5.4
Cyclocarya paliurus (Batal.) Iljinskaja (sweet tea tree) (Xie, 2010)	100	180	20	-	Single factor + CCD	3.9
Fructus Jujubae (Cui, 2014)	90	194	33	3	Single factor + BBD	6.5
Litsea coreana Lévl. var. lanuginose (Hawk tea) (Jia, 2014)	89	128	11	-	BBD	12.7
Lycium barbarum (Yin, 2008)	100	330	31	5	Single factor + BBD	23.1
Opuntia milpa alta (Cai, 2008)	86	217	3.7	-	Single factor + BBD	0.7 (wet basis)
Plantago asiatica L. (Ye, 2011)	91	234	24	4	Single factor + CCD	4.4
Rubus chingii Hu (Zhang, 2015)	88	185	31	-	Single factor + BBD	9.6
Semen cassia (Liu, 2014)	80	210	30	-	Single factor	5.5 (wet basis)

Tetrastigma hemsleyanum Diels et Gilg (Shao, 2011)	83	93	29.5	-	Single factor + BBD	5.2
Zanthoxylum bungeanum (Li, 2015)	89	180	29	2	Single factor + BBD	14

As shown in Table 2.4, the important parameters that significantly affected the process efficiency of CHE include extraction temperature, extraction time, solvent/material ratio and number of extractions. Most studies showed that the yield of polysaccharide was raised significantly with increasing of these extraction parameters until achieved a peak value. The investigation of individual and interactive effects of process variables and the optimisation of extraction conditions are frequently studied by varying each factor independently (single factor experiments) to determine the proper range for each parameter, followed by using response surface methodology based on central composite or Box-Behnken experimental designs (CCD/BBD) with the extraction yield of crude or purified polysaccharides as the response.

From Table 2.4, it is obvious that optimised extraction temperature in the range of 80 to 100 ˚C, which is close to the boiling point of water solvent and long extraction time in few hours were the necessary prerequisite to decrease the viscosity and surface tension of water, hence improving the solvent diffusion into the plant matrix and its ability to solubilise solutes (Samavati, 2013; Ye, 2011). Nonetheless, for most of the studies, the high temperature and long extraction time beyond the optimised value led to reduction of yield which was probably due to thermal degradation and damage to the structure of polysaccharides under these conditions (Cai, 2008; Li, 2015; Zhang, 2015).

High solvent/material ratio with optimised value in the range of 20 to 30 v/w were reported in many papers because the use of large volume of solvent increased the diffusivity of the solvent into and out of the plant cell and thus enhanced the extraction recovery (Samavati, 2013; Zhang, 2015). For most of the cases, low extraction yield below 10% was obtained if only one extraction cycle was conducted. In some studies, the samples were extracted more than one time to maximise the product recovery (Cui, 2014; Li, 2015; Samavati, 2013; Ye, 2011; Yin, 2008). However, it involved larger amount of solvent and longer extraction time and more energy consumption was required to concentrate the aqueous extract.

Since extraction temperature and time and solvent loading were found to exhibit strong effect to the yield, therefore the influences of these parameters to bio-flocculant yield were investigated in this work. It is clear that the control of extraction conditions of CHE method is essential to limit the degradation rate of polysaccharide and preserve the extract quality (Flórez, 2015). CHE was reported with downsides of low yield and long extraction time at high temperature; hence MAE was introduced as an alternative technique in this study. MAE was reported (Veggi, 2013) to greatly shorten the extraction time and was suited for thermolabile constituents enabling cost-effective production of high quality extracts.

2.9 Microwave assisted extraction (MAE)

Microwaves have received increased attention among researchers and microwave energy has been successfully adopted in laboratories and industries for the extraction of natural compounds from plants (Veggi, 2013). MAE is based on heating the solvent and sample by application of microwave energy and its effect is strictly related to the conversion of electromagnetic energy to heat in controlled systems. The benefits of microwave in extraction processes are reported to be in relation to volumetric and selective heating which enable rapid and uniform heating process in minutes (Flórez, 2015).

2.9.1 Microwave characteristics

Microwaves are electromagnetic waves that consist of electric field and magnetic field that are oscillating perpendicularly to each other in frequency range of 0.3 to 300 GHz (Kappe, 2005; Meredith, 1998). Commercial microwave ovens approved for domestic applications and all dedicated microwave reactors for chemical synthesis operate at a frequency of 2450 MHz in order to avoid interference with telecommunication and cellular phone frequencies (Kappe, 2005). Most of the reported microwave related experiments are conducted at 2450 MHz because the microwave energy absorption by liquid water is maximal near to this frequency and the availability of microwave equipment at this frequency (Gude, 2013). There are other frequency allocations for microwave heating applications, which is ISM frequencies (Table 2.5) for industrial and scientific and medical use (Kappe, 2005). Interaction of dielectric materials with microwaves at molecular level without altering the molecular structure leads to volumetric and selective heating (Refaat, 2009) and the underlying mechanisms will be discussed further.

Table 2.5: ISM microwave frequencies (Kappe, 2005)

Frequency (MHz)	Wavelength (cm)
433.9	69.1
915	32.8
2450	12.2
5800	5.2
24125	1.4

2.9.2 Heating mechanism of MAE

Microwaves are transmitted as waves and have the ability to penetrate biomaterials. Microwaves will predominantly interact with molecules that have a dipole moment such as the polar and ionic molecules in the solvent, free water molecules and ionic molecules in the plant matrix by two mechanisms which are dipolar polarisation and ionic conduction (Figure 2.7) to generate heating energy (Gude, 2013; Kappe, 2005).

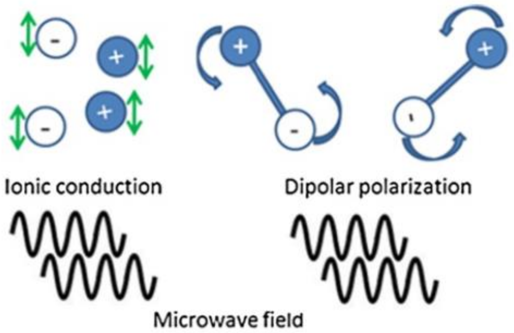


Figure 2.7: Ionic conduction and dipolar polarisation under microwave conditions, redrawn with reference to (Gude, 2013)

When the solvent and the plant matrix are subjected to microwave heating, the oscillating electromagnetic field would cause dipole rotation of the polar molecules to align with the applied electric field. Since the electric field is oscillating, the dipoles constantly try to realign to follow this movement. The continual reorientation of the molecules results in molecular friction, oscillation and collision which generates and dissipates energy in the form of heat to the surrounding (mechanism of dipolar polarisation) (Kappe, 2005; Soria, 2015).

If a molecule is charged, then the electric field component of the microwave moves the ions back and forth through the sample following the electric field. The direction of the ions changes as many times as the field changes its sign. The resistance of the solution to this flow of ions and the collision of ions with each other results in friction that generates heat (mechanism of ionic conduction) (Kappe, 2005; Soria, 2015).

The penetration of microwaves into the material and the interactions of the material with the microwaves, effects volumetric heating, whereby all the components constituting the volume of solvent and plant matrix are heated instantaneously (Figure 2.8) (Meredith, 1998). Volumetric heating reduces the thermal gradients between internal and external and between solvent and plant matrix, which may lead to inversion of heat flow (outwards) which is the inverse of the heat flow of conventional heating (inwards).

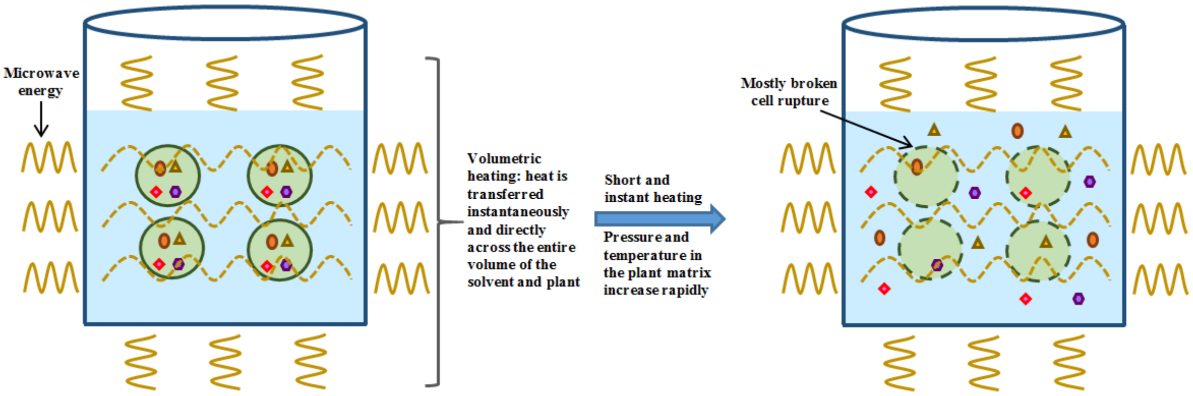


Figure 2.8: Heat and mass transfer phenomena of microwave extraction

It is reported that this causes sudden increase of temperature (Figure 2.6) and moisture evaporation within the plant matrix which generates pressure effects that produces stress on the cell wall and results in the swelling and bursting of the plant cells (Destandau, 2013; Wahidin, 2014). It is postulated that the plant cell wall could support a certain pressure limit. Once this critical point is crossed by the elevated stress generated within the cell wall, plant cell starts to lyse (Ali, 2015). This mechanism is termed as physical cell wall disruption in this study. The cell wall disruption can shorten the diffusion path and facilitate the mass transfer of the solvent into the plant materials and hence allowing release of intracellular components into the solvent (Destandau, 2013). Up to now, no study has been reported to verify the microwave heating mechanism mentioned above. In this work, the correlation of dielectric properties of okra and water solvent with microwave heating mechanism was investigated and then the physical cell wall disruption mechanism was justified.

Some studies employed scanning electron microscope (SEM) to show the destructive changes in the plant tissue after microwave extraction which explained the observation of considerable increase in the extract yield (Chen, 2016a; Cheng, 2015). For example, microwave extraction of pectin polysaccharide from tangerine peel was reported and the authors used SEM images to show the difference between slight damage and significant damage on the cell walls of tissues caused by conventional and microwave extraction respectively (Chen, 2016a). However, for most of the cases, it is difficult on close examination to see any definite difference in the images obtained from microwave and conventionally treated samples.

Another specific microwave effect is the selective heating of microwave absorbing solvent or the components in the plant material. Selective heating generally means that in a sample containing more than one component, the individual components heat at different rates according to a characteristic known as loss tangent (see Section 2.9.3). Any non-absorbing (microwave transparent) components are thus not heated directly, but only by heat transfer from the heated component (Kappe, 2005). As plant materials contain mixtures of different components, selective heating would occur based on the relative loss tangent of the individual components (Robinson, 2010a). It has been reported that plant components such as water, monosaccharides and ions have high dielectric activity (Sahin, 2006). For the case where the components in the plant material are strong microwave absorbers (selectively heated), it can be assumed that the temperature within the plant matrix is higher than the bulk temperature of the solvent (direction of heat flow is outwards), in particular when a solvent with a lower capability in absorbing microwaves than plant material is chosen (Kappe, 2005; Soria, 2015). In this work, the dielectric properties of okra and water solvent at different temperatures were measured to find out when the selective heating of okra would take place.

2.9.3 Dielectric properties of materials

The response of a given material to electromagnetic waves is defined by dielectric properties. The understanding of dielectric properties of substances is of primary importance because it dictates the interaction of the solvent and plant matrix with the microwave energy and the correlated heating efficiency. The mobile ions, water and the molecules with permanent dipole moments are the dominant components governing the dielectric properties and behaviour of materials (Stuart, 2001).

Dielectric properties include dielectric constant (ε'), which measures the ability of a material to absorb microwave energy; dielectric loss/loss factor (ε''), which indicates the efficiency of converting microwave energy into heat, and loss tangent/dissipation factor ($\tan \delta = \varepsilon'' / \varepsilon'$) which measures the ability of the sample to absorb microwave energy and dissipate heat to the surrounding molecules (Chan, 2011). Solvents or substances with a high dielectric constant and a high dissipation factor facilitate absorption and dispersion of heat throughout the matrix and thus increase the extraction yield of solutes (Veggi, 2013).

As microwaves move through the slab at any point, the rate of heat generated per unit volume decreases. For materials having a high loss factor, the rate of heat generated decreases rapidly and microwave energy does not penetrate deeply. A parameter which is called penetration depth indicates the distance that microwaves will penetrate into the material before it is reduced to a certain fraction of its initial value. Penetration depth (d_p) is a function of both ε' and ε'' and is defined as the depth into the material at which the microwave power flux has fallen to *l/e* (=36.8%) of its surface value (Meredith, 1998) (Figure 2.9).

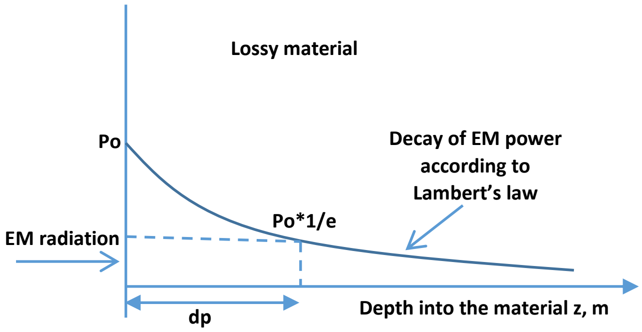


Figure 2.9: Typical penetration depth inside a large sized material (larger than the wavelength), EM = electromagnetic, redrawn with reference to Wiley (Komarov, 2005)

Penetration depth gives an immediate first-order indication of the heat distribution within the material. It can be calculated following equation 2.1(a) (Meredith, 1998) where λ_0 is the free space microwave wavelength (3 x 10^8 m/s) (Sosa-Morales, 2010).

$$d_p = \frac{\lambda_0}{2\pi\sqrt{(2\varepsilon')}} \cdot \frac{1}{\left[\sqrt{\left\{1+\left(\frac{\varepsilon''}{\varepsilon'}\right)^2\right\}^{0.5} - 1}\right]} \qquad (2.1(a))$$

When $\varepsilon'' \leq \varepsilon'$, equation 2.1(a) could be simplified to equation 2.1(b), with an error up to 10% (Meredith, 1998).

$$d_p = \frac{\lambda_0\sqrt{\varepsilon'}}{2\pi\varepsilon''} \qquad (2.1(b))$$

Up to now, the dielectric data for water and aqueous solutions are well documented and interpreted. The water solvent consists of polar molecules that strongly absorb microwave energy because of its high ε' (80.4 at ambient temperature and 2.45 GHz) (Veggi, 2013). However, it has lower tan δ (0.12 at 20˚C (Freitas, 2015)) than other conventional solvents such as ethanol and acetone. It indicates that the rate of microwave energy absorption by water is higher than the dissipation of this energy into heat compared with ethanol and acetone.

On the other hand, dielectric data for plant materials are scarce and markedly affected by the water content in the material, which can vary during measurement process (Navarrete, 2011). Water can exist in either the free or bound state in food systems. Free water is found in capillaries or cavities but bound water is chemically combined to other molecules or physically adsorbed to the surface of dry material (Sahin, 2006). Moist food or fresh plant materials are dielectric materials which exhibit the ability to store and dissipate electric energy when subjected to an electromagnetic field (Sosa-Morales, 2010; Veggi, 2013). Figure 2.10 shows the variation of dielectric loss factor with moisture content in food.

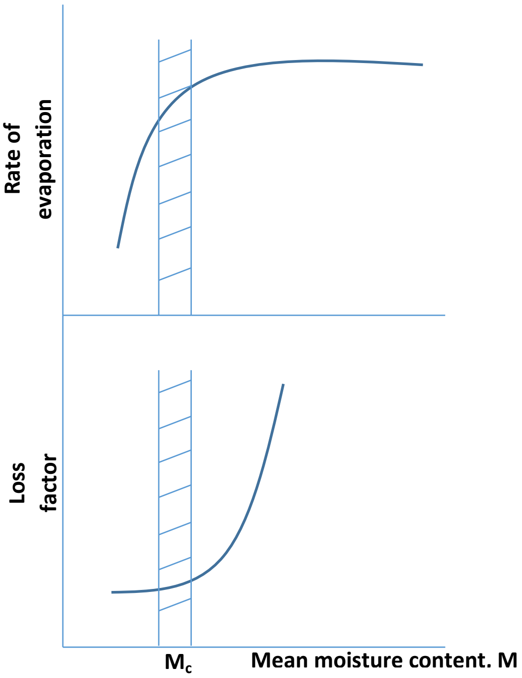


Figure 2.10: Rate of evaporation and dielectric loss factor as affected by food moisture content , redrawn with reference to Wiley (Komarov, 2005)

In general, the higher the moisture content, the larger the dielectric constant and loss factor of the materials (Komarov, 2005) due to increase in polarisation (Sahin, 2006). At low moisture contents, variation of dielectric properties with moisture content is small. As shown in Figure 2.10, dielectric properties of biomaterials decrease rapidly with decreasing moisture content to a critical moisture level (M_c). Below this moisture level, the reduction in loss factor is less significant because of the bound water (Komarov, 2005). It indicates that the effect of bound water on dielectric properties is negligible. For food materials having high moisture contents, bound water does not play a significant role and the dielectric properties are affected by dissolved constituents as well as free water content (Sahin, 2006).

The temperature of a material has a significant effect on the dielectric properties. Generally, the dielectric constant of polar material decreases with increasing temperature because the average alignment of the dipoles decreases with the field (DoITPoMS, 2004-2015). Figure 2.11 shows the variation of loss factor components with temperature.

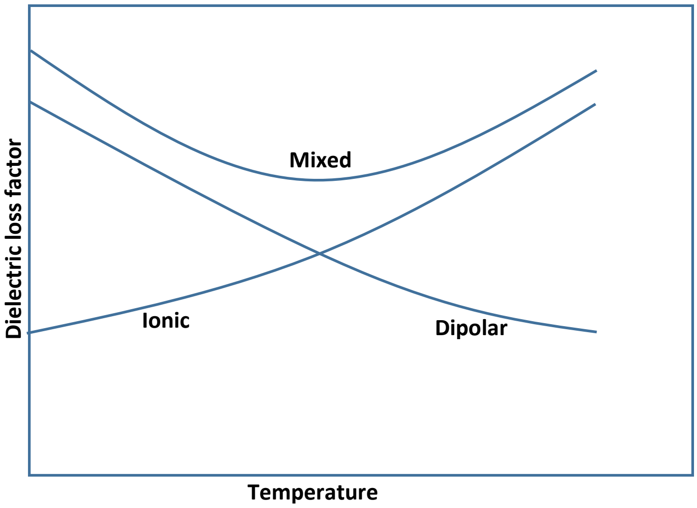


Figure 2.11: Variation of loss factor components with temperature, redrawn with reference to Springer (Sahin, 2006)

As shown in Figure 2.11, the ionic loss and dipolar loss are the dominant components of total loss due to the aqueous phase of foods. Dipolar loss decreases with temperature at frequencies used in microwave processing because the polar molecules can more easily follow the alternating electric field and consequently the molecular friction decreases (i.e. as the viscosity decreases) and less energy is dissipated as heat (Robinson, 2010a). In contrast to dipolar loss, loss factor from ionic conduction increases with temperature owing to the decreased viscosity of the liquid and increased mobility of the ions. At higher temperatures, ions become more mobile and not tightly bound to water, and thus the loss factor from ionic loss component increases with temperature (Sahin, 2006). For materials containing both dipolar and ionic components, the total loss of food products decreases initially with increasing temperature when dipolar loss is the dominant component, but then increases with increasing temperature when ionic loss becomes the dominant component (Stuart, 2001).

2.9.4 Application of MAE in extraction of crude polysaccharides from plants

As presented in Table 2.6, the applications of MAE to extract polysaccharides from different plant materials have attracted great interest among researchers due to the aforementioned potential benefits of MAE. Some of the studies were purely concentrated on the investigation of the efficiency of MAE process in extraction of polysaccharides from plants and the optimisation of the extraction conditions (Maran, 2015; Zeng, 2015; Zhou, 2014). Other reports focused on the characterisation and investigation of therapeutic bioactivities (Thirugnanasambandham, 2015; Xie, 2010), antitumor activity (Zhao, 2015), and antioxidant activity (Chen, 2016a; Wang, 2009) of the polysaccharides extracted with MAE under optimised conditions.

Table 2.6: Extraction of polysaccharides with MAE method

Name of plant	**Extraction method**	**Optimised extraction conditions**						**Optimisation method(s)**	**Extraction yield (dry basis, %)**
		Extraction solvent	**Temperature (˚C)**	**Time (minutes)**	**Solvent / material ratio (v/w)**	**Microwave power (W)**	**Others**		
Artemisia sphaerocephala	MAE (Wang, 2009)	Water	60	70	32.8	523	-	BBD	31.8
Chuanminshen violaceum	MAE (Dong, 2016)	Water	64.5	15	40	466	-	Single factor + BBD	34.6
Cornus officinalis	UMSE (Yin, 2016)	Water	-	31.5	28.2	99.4	-	BBD	11.4
Cucurbita pepo L. (pumpkin)	MAE (Zheng, 2011)	Aqueous ethanol	79	29	22	-	-	Single factor	16.8
Cyclocarya paliurus (Batal.) Iljinskaja	MAE (Xie, 2010)	Water	100.9	20.8	22.1	-	Grinding degree: 40 screen mesh Extraction steps: 2	Single factor + CCD	5.1 (wet basis)
Fortunella margarita (Lour.) Swingle	UMSE (Zeng, 2015)	Water	-	2	62	105	Ultrasonic power: 131W	Single factor + CCD	9.2
Lilium davidii var. unicolor Salisb	MAE (Zhao, 2013)	Water	50	60	65	597	-	BBD	36.6
Mulberry	MAE (Thirugnanasambandham, 2015)	Water	-	10	-	170	Sample weight: 20g	BBD	9.4
Opuntia ficus indica Cladodes	MAE (Felkai-Haddache, 2016)	Water	-	5.2	4.8	700	pH: 11	Single factor + BBD	25.6
Pericarpium granati	UMSE (Zhou, 2014)	Polyethylene glycol (PEG) aqueous solution	90	10	20	365	Ultrasonic power: 240W, concentration of PEG: 30%	BBD	7.9
Potentilla anserina L.	MAE (Wang, 2010)	Water	63.3	76.8	14.5	369	-	BBD	13.3
Psidium guajava L.	MAE (Arasi, 2016)	Water	-	20	3	200	-	CCD	6.8 (wet basis)
Semen Cassiae	MAATPE (Chen, 2016b)	Ethanol/ammonium sulfate system	80	20	60	-	25% ethanol and 22% ammonium sulfate	Single factor	13.3
Tangerine peels	MAE (Chen, 2016a)	Water	52.2	41.8	30	704	-	Single factor + BBD	19.9
Taxus chinensis var. mairei	UMSE (Zhao, 2015)	Water	-	10	33	560	Fixed ultrasonic power: 50W	Single factor + BBD	4.3
Vigna radiate (mung bean)	MAE (Zhong, 2012)	Water	-	1.2	17	700	-	BBD	6
Waste mango peel	MAE (Maran, 2015)	Water	-	2.2	18	413	pH: 2.7	BBD	28.9
Ziziphus jujube	MAE (Rostami, 2016)	Water	75	60	30	400	-	Single factor + CCD	9

As shown in Table 2.6, microwave extraction technique has been modified and improved to enhance its performance. For instance, the application of ultrasonic-microwave synergistic extraction (UMSE) in extraction of polysaccharides from plants have been reported (Yin, 2016; Zeng, 2015; Zhao, 2015; Zhou, 2014). It was postulated that the combination of microwave and ultrasonic waves provides high momentum and energy to rupture the plant cell and hence intensifies the mass transfer of active compounds to the extraction solvent (Chan, 2011).

Selection of solvent is an important consideration in microwave extraction. Solvent choice is not only dictated by the solubility of the extracts of interest, but also by the microwave absorbing properties of the solvent because microwaves are effective on solvents with high dielectric properties. Most of the studies listed in Table 2.6 used water as the solvent because it is a generally recognised as safe (GRAS) solvent and also possesses the ability to strongly absorbs microwave energy. Due to these reasons, water was selected as the solvent in microwave extraction of bio-flocculant.

Once a suitable solvent has been selected, its ratio to the plant feedstock has to be determined as it affects the extraction yield in most cases (Dong, 2016; Wang, 2010; Yin, 2016; Zhao, 2013). It is vital to have sufficient solvent volume to immerse the entire sample in the solvent and to accommodate the swelling effect throughout the entire extraction process (Veggi, 2013). An optimum ratio of solvent to solid provides homogeneous and effective heating. However, excessive solvent may reduce energy efficiency because the microwaves would be absorbed by the solvent and thus additional power is required (Mandal, 2010). On the other hand, less solvent may promote mass transfer barrier as the distribution of active compounds is concentrated in certain regions which limits the solubilisation and diffusion of the compounds out from the plant cell (Mandal, 2010). The optimised ratio of solvent to solid presented in Table 2.6 varies according to plant and is influenced by its ability to absorb microwave power and dissipate heat (Chan, 2011), and is probably dependent on other extraction conditions.

Extraction temperature and microwave power are interrelated as high microwave power could raise the temperature of the system. The microwave power employed ranged from 100 to 700W to provide the impact energy to heat and maintain the extraction temperature throughout the entire process (Chan, 2011). Generally, the extraction yield increases with increasing of microwave power and temperature up to a limit before the yield becomes insignificant or decline (Chen, 2016a; Dong, 2016; Zhao, 2013). With increasing of temperatures the solvent power increases because of a drop in viscosity and surface tension, facilitating the solvent to solubilise solutes (Veggi, 2013). As shown in Table 2.6, the extraction temperatures for extraction of polysaccharides were confined below or near to the boiling point of solvent. It is because the high microwave power and extraction temperature beyond the optimised values may cause thermal degradation of active constituents and hence reduce the extraction yield (Chan, 2011; Veggi, 2013). Whilst trying to achieve high extraction yield, the control of these parameters is significant to maintain the stability of bioactive compounds during extraction process and limit the hydrolytic cleavage of polysaccharide chains (Flórez, 2015).

Normally, longer extraction time tends to increase the extraction yield. However, prolonged microwave heating (up to hour) exhibits the risk of thermal degradation which lead to decomposition of thermolabile compounds (Veggi, 2013). Attributed to microwave heating mechanism, the optimum extraction time can be as short as 1.2 minutes (Zhong, 2012) up to an hour (Wang, 2009) for polysaccharide extraction. The time is at least few times less than the time used by CHE, and thus minimises the degradation of target components and maintains the physicochemical characteristics of crude polysaccharides. A research work compared the extraction efficiency of MAE with CHE and UAE in extraction of polysaccharides from *Cyclocarya paliurus* (Batal.) Iljinskaja (Xie, 2010). The results showed that the extraction time of MAE (20 minutes) was the shortest and the highest yield (5.07%) was obtained while the extraction times of UAE and CHE were 60 and 180 minutes respectively and both methods gave lower yields.

In this study, the effects of extraction temperature and time and solvent/material ratio to the bio-flocculant yield were examined because the studies presented in Table 2.6 revealed that these parameters showed notable effect to yield. The influence of microwave power to yield was not investigated because this factor is closely related to extraction temperature.

The extraction method efficiency is judged based on the highest recovery. By comparing the extraction yields of CHE and MAE presented in Tables 2.4 and 2.6, higher yield in the range of 5-35% was observed with MAE method. Even though this comparison is based on the extraction of polysaccharides from different plants under different extraction conditions, MAE is shown to be advantageous over CHE with regard to the yield and extraction time. The summary of comparisons between CHE and MAE methods in terms of different parameters are listed in Table 2.7 below.

Table 2.7: Comparison between CHE and MAE processes

Parameter	**CHE**	**MAE**
Form of energy transfer (Flórez, 2015)	Conduction and convection currents	Electromagnetic waves
Extraction time	Long extraction time in few hours	Short extraction time in minutes
Extraction temperature	Close to boiling point of water solvent	Close to boiling point of water solvent
Extraction pressure	Atmospheric pressure	Atmospheric pressure
Ratio of solvent to plant material	High	Inconsistent and varies
Extraction yield	Low, below 10% generally	High, >10% mostly
Energy consumption (Veera, 2015)	High	Moderate to low

2.9.5 Challenges and future perspectives of MAE process

So far microwave technology has been mainly used in lab scale experiments and proved itself useful for extraction of bioactive compounds from different plant materials (Périno, 2016). Recently, it has been upgraded to pilot scale production such as in the extraction of polyphenols from leaf salad (Périno, 2016) and extraction of oil from soy flour and rice bran (Terigar, 2011).

Up to the present, the comparison of microwave efficiency with other methods such as CHE under the same extraction conditions and variables is very limited. In addition, there is lacking of fundamental understanding and knowledge in dielectric properties of materials. These gaps in knowledge need to be filled in before the microwave technology could be extended further to larger scale.

With consideration of the problems abovementioned, the dielectric properties of okra and water were measured in this study and the results were used to understand and develop the microwave heating mechanism. Apart from that, the extraction efficiencies in terms of yield and bio-flocculant quality between

CHE and MAE methods were compared and the interrelationship between extraction efficiency with different heating mechanisms was investigated.

Two major disadvantages which restrict the development of MAE to industrial scale are reported: (i) high investment cost of the microwave generator and (ii) higher cost of electricity than fuels (Flórez, 2015). This leads to the viewpoint that microwave extraction will be more costly than conventional extraction. To address this perspective, the economic feasibility study for production of bio-flocculant with CHE and MAE methods was conducted in this study and the production costs of bio-flocculant with both methods were compared with each other. Another drawback is application of microwaves on nonpolar compounds or solvents is discouraged as they are poor absorbents for microwave heating (Chan, 2011). There is difficulty in selecting solvent for MAE when the solubility of extract of interest is higher in nonpolar solvent.

Despite there are challenges in scaling up MAE process to industrial scale, but approaches to scale up is still feasible based on its potential advantages of improvement in extraction yield, enhancement of product quality and increase of production efficiency with short extraction time. In addition, from the view of long term running operation, MAE process has a promising future and could potentially offer economic benefits compared with conventional processes due to lower time required to attain similar yields, quality and properties.

CHAPTER 3: RESEARCH METHODOLOGY

This chapter describes the methodologies of bio-flocculant extraction with conventional hydrothermal and microwave assisted methods. The experimental design used to optimise the extraction conditions is also presented. The experimental steps for the flocculation process followed by sludge dewatering are explained in detail. Lastly, the measurements of dielectric properties of okra and water, and analysis of zeta potential are introduced.

3.1 Materials

Fresh okras were sourced from local wet market and used as a model feedstock for this study. Nylon filter cloth with 35 mesh size was used for filtration in sludge dewatering process. The cationic (FO 4400 SH) and anionic (AN 934 SH) polyacrylamides with 30% charge density (medium category) and 1.5×10^7 g/mol (high molecular weight) were obtained from SNF Floerger.

For the first year of study based in Malaysia, the kaolin slurry without chemical treatment and purification was purchased from Kaolin Malaysia Sdn. Bhd and used directly for the Jar Test without dilution (concentration of suspended solids is ±80 g/L). For the second and third years of research work in U.K., the kaolin sample (Polwhite B China Clay) in powder form was purchased from Richard Baker Harrison Ltd. The kaolin colloidal solution was prepared by adding the kaolin powder into water and the mixture was stirred for uniform dispersion. The kaolin samples from different destinations were verified to have the same pH (4.82), same concentration of suspended solid (SS) at 80 g/L and turbidity at 81000 NTU. All the kaolin samples were stored at room temperature in plastic containers before sample preparation.

3.2 Preparation of okra sample

Before processing, 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 uniform size with a food processor (FP730, Kenwood, UK).

A sample of fresh okra feedstock was dried at 105 ˚C until a constant mass was obtained to calculate the moisture content in the fresh okra, which was 90.93% ± 0.11 on wet basis.

3.3 Preparation of kaolin sample

Kaolin samples were prepared for evaluation of flocculating efficiencies of bio-flocculants using a Jar Test (Bratby, 2016). Kaolin colloidal solution was prepared at 700 rpm for 10 minutes and sampled during stirring to ensure homogeneity. The pH of the kaolin colloidal solution was measured to evaluate the pH for the flocculation process, and then the SS and turbidity measurements were taken. All measurements of SS in this study were conducted according to the Standard APHA Method 2540D (Rice, 2012). The measurements of turbidity was conducted with colorimeter (DR/890, HACH, USA) according to Standard Method 8237 (USEPA, 2012b). The characteristic results of the kaolin colloidal solution are listed in Table 3.1.

Table 3.1: Characteristic results of kaolin colloidal solution

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

3.4 Bio-flocculant extraction

The extraction procedures were developed by referring to the procedures described in some studies with few modifications (Chan, 2011; Maran, 2015; Wang, 2009, 2010).

3.4.1 Conventional hydrothermal extraction (CHE)

Before starting extraction, a shaker bath (Memmert, France) was filled with distilled water and heated to the desired extraction temperature. The sliced pods were mixed with deionised water with a predetermined solvent loading (solvent/plant ratio) in conical flasks. The flasks were sealed and placed in the shaker bath at a constant extraction temperature and agitation speed (Figure 3.1). Water level in the shaker was regularly monitored to ensure that it was higher than the sample height. The cover of the shaker was closed during extraction to minimise evaporation, and the evaporation rate was verified to be less than 0.05% over the duration of the extraction process.

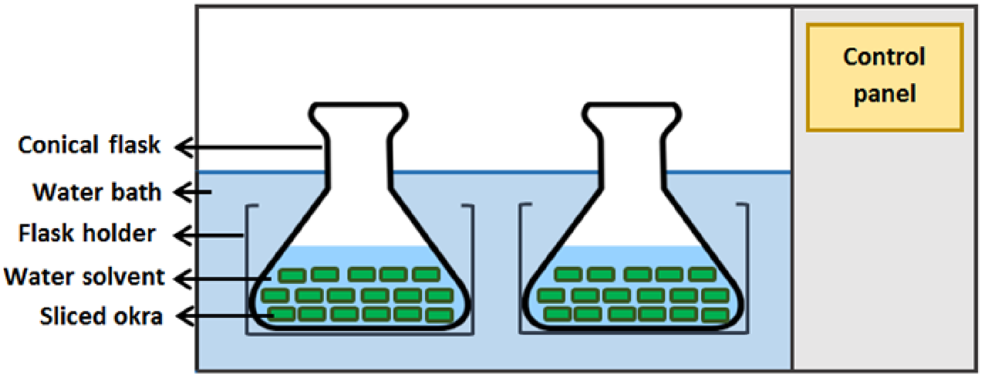


Figure 3.1: Schematic diagram of conventional hydrothermal extraction process

3.4.2 Microwave assisted extraction (MAE)

A Miniflow 200SS (Sairem, France) microwave heating system (Figure 3.2) was used for okra extraction. It consists of three major components, which are the source, the transmission lines or waveguides and the applicator. The magnetron is the microwave source that generates the electromagnetic power. The transmission cable or waveguides delivers the electromagnetic energy from the source to the applicator. In the single mode cavity (applicator), the energy is either absorbed or reflected by the material. Before starting the extraction, the reflected power was measured and controlled to a minimal value to ensure efficient microwave heating.

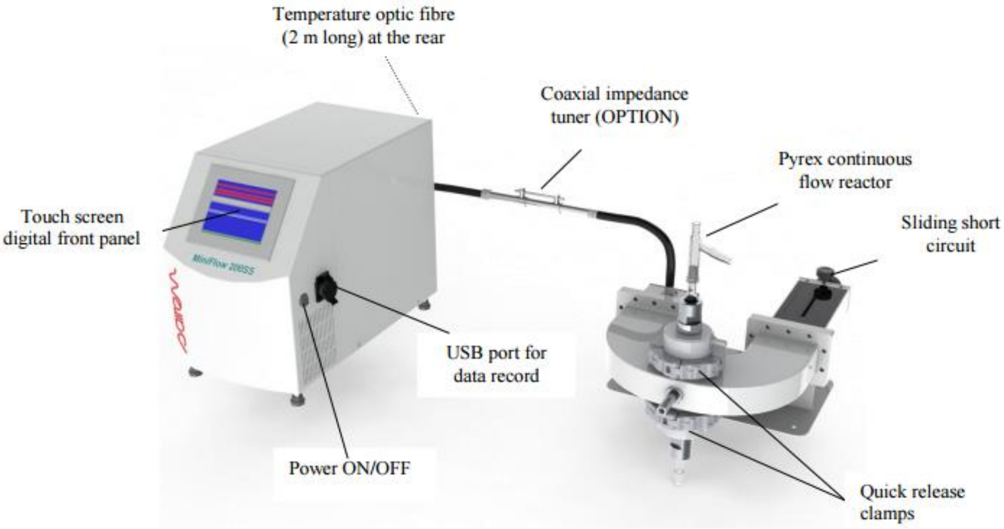


Figure 3.2: Miniflow 200SS microwave heating system

A mixture of sliced okra sample and deionised water with predetermined solvent loading was loaded into a Pyrex extraction vessel and sealed with a plastic lid. The extraction vessel with sample was placed in the Miniflow 200SS and secured in place with a clamp (Figure 3.3). A fibre-optic temperature probe was inserted into the flask to monitor the bulk temperature of the solvent (water). Note that it was not possible to measure the temperature of the okra during treatment. Microwave extraction was performed at a working frequency of 2450 MHz and the power applied to the system was varied to achieve a desired temperature set-point. A magnetic stirrer was placed below the microwave heating system to control the agitation speed during the extraction.

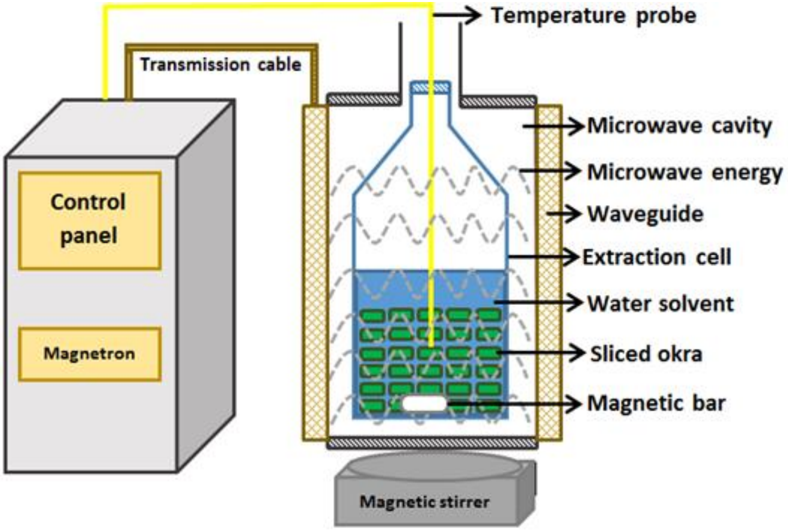


Figure 3.3: Schematic diagram of microwave assisted extraction process

3.4.3 Post-extraction

After the predetermined extraction time, the extracts were kept aside at room temperature for cooling and complete release of the extractable solutes into water (Ameena, 2010; Zhang, 2014). The marc was separated from the aqueous extract by centrifugation at 4000 rpm for 30 minutes. The recovered extract, which was termed as aqueous bio-flocculant (AF), was kept at 4 ˚C before further testing of its flocculating and dewatering properties. 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 dessicator 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 of extract. The percentage of extract yield on dry basis was determined by Equation 3.1 listed as below.

$$\%\, Extract\ yield\ (dry\ basis) = \frac{dry\ mass\ of\ extract}{dry\ mass\ of\ plant\ feedstock} \times 100\% \qquad (3.1)$$

The dry mass of plant feedstock was obtained after deducting the moisture content contained in the fresh okra.

3.5 Experimental design

Single factor experimental design was performed to optimise (maximise) the extract yield and bio-flocculant quality in terms of flocculating and dewatering abilities. Single factor experiments are easy to control and can be used to find whether one variety is superior to another (Hoshmand, 2006). Since the purpose was to study the effects of each factor on the response (yield and bio-flocculant quality), hence separate experiment in which each deals with a single factor was conducted.

The effects of the extraction temperature, time, solvent loading (ratio of solvent to plant), and particle size of okra and agitation speed on the extract yield, flocculating and sludge dewatering abilities of the bio-flocculants were investigated individually by single factor experiments. For each experiment set, only one factor was varied while the other extraction variables were held constant. After conducting the experiments, the experimental results were analysed and the extraction conditions to achieve the optimised (maximum) yield and bio-flocculant quality were identified.

The ranges of the studied parameters for CHE and MAE processes listed in Tables 3.2 and 3.3 were designed with two purposes. The primary purpose is to examine changes in extraction behaviour with the variation of extraction conditions, and the second is to identify the extraction conditions which resulted in increased yield and product quality. All experiments were performed in triplicate for the mean and standard deviation calculation.

Table 3.2: The range of studied parameters for CHE process

Experiment sets	**Extraction temperature T (^{o}C)**	**Time t (hrs)**	**Solvent loading S (w/w)**	**Agitation speed A (rpm)**
H1	70	0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5	1	200
H2	25, 40, 50, 60, 70, 80, 90	2	1	200
H3	70	2	0.5, 1, 1.5, 2, 2.5, 3, 4, 5	200
H4	70	2	2.5	0, 50, 100, 150, 200, 225

Table 3.3: The range of the studied parameters for MAE process

Experiment sets	Extraction temperature T (°C)	Time t (minutes)	Solvent loading S (w/w)	Agitation speed A (rpm)	Particle size PS (mm)
M1	70	2.5, 5, 10, 15, 20, 25	3.5	500	5-10
M2	25, 40, 50, 60, 70, 80, 90	10	3.5	500	5-10
M3	70	10	2, 2.5, 3, 3.5, 4, 4.5, 5	500	5-10
M4	70	10	3.5	0, 250, 500, 750, 1000	5-10
M5	70	10	3.5	0	1, 2, 2-5, 5-10

In order to further investigate the effects of multiple parameters (alone or in combination) on the response (yield), the results obtained from single factor experiments were analysed by using response surface methodology (RSM) with Minitab17 software. In RSM, custom response surface design was selected because the results obtained from single factor experiments were used for this analysis and no additional experiment was carried out. The range of the independent variables (studied parameters) for CHE and MAE are listed in Tables 3.2 and 3.3 above. Multiple regression analysis was applied to the experimental data to construct second order polynomial model which exhibits the relationship between independent variables and response (yield).

3.6 Jar Test procedures on flocculation of kaolin colloidal solution

Jar Tests were used to evaluate the flocculating efficiencies of the bio-flocculants in flocculation of kaolin colloidal solution and were conducted with a commercially available Jar Tester (ET 720, Lovibond, Germany) at room temperature (Bratby, 2016). A classical flocculation process consists of three separate steps:

i) Rapid mixing: The suitable flocculant (pH adjustment may be required) is added to the colloidal solution, which is stirred and intensively mixed at high speed.
ii) Slow mixing: The solution is only moderately stirred in order to form large flocs, which are easily settled out.
iii) Sedimentation: The flocs formed during flocculation are allowed to settle out and separated from the treated/clarified water.

In this study, the bio-flocculant was injected into kaolin colloidal solution whilst stirring at 700 rpm (82 *g*). The flocculation process consisted of stirring at 700 rpm (82 *g*) for 1 min, followed by stirring at 350 rpm (21 *g*) for 1 min and settling for 30 min. The settled sludge volume was taken for calculation of the sludge volume index according to Standard APHA Method 2710D (Rice, 2012). A small volume of the supernatant was taken for measurement of the SS and turbidity. The treated solution was then subjected to a filtration test to analyse the sludge dewatering abilities of bio-flocculants.

3.7 Sludge dewatering study on kaolin flocs quality

The filtration test was performed to analyse the floc strength and the water recovery capability (Chong, 2009a). A Buchner funnel with nylon filter cloth was assembled above a graduated cylinder (Figure 3.4) (Lu, 2014; Wang, 2013). The treated solution obtained after Jar Tests was poured into the Buchner funnel. The filtrate was collected in a graduated cylinder while the kaolin flocs were trapped in the funnel. The water recovery (filtrate volume) collected at 10s was recorded and the SS content in the filtrate was measured in order to calculate the results of SS removal after filtration.

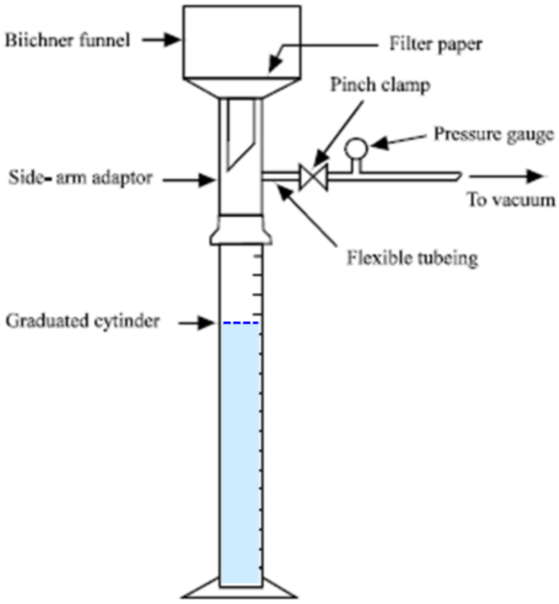


Figure 3.4: Schematic diagram of sludge dewatering setup

The water recovery and the SS content in the filtrate indicate the compactness and density of the flocs formed during flocculation process. High density flocs enable good separation between flocs and clarified water allowing reduction of sludge volume (water recovery is increased) and minimisation of the floc breakage during filtration which diminishes the SS content in the filtrate.

3.8 Dielectric property measurement

Dielectric property measurements were carried out independently of the microwave extraction measurements.

3.8.1 Okra

A resonant cavity perturbation method was used to measure the dielectric properties of okra from 20 to 100 ˚C. This technique is frequently used for measuring dielectric properties of solid food materials because it is suitable for measuring small and bulk samples across a range of temperatures with high accuracy (Shyam, 2010).

As shown in Figure 3.5 it consists of a cylindrical copper cavity connected to a vector network analyser, which measures the frequency shift and change in quality factor relative to the empty resonating cavity when a sample is introduced. The changes in reflected power of the cavity and the frequency of resonance are used to compute the dielectric properties of the sample (Sosa-Morales, 2010). Samples were loaded into a quartz tube, and held in a conventionally heated furnace above the cavity until the temperature set-point was reached. The tube was then moved into the cavity, and the properties determined at 2470 MHz, which is within 20 MHz of the microwave heating equipment used in this study.

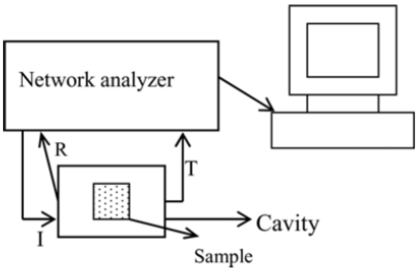


Figure 3.5: Schematic diagram of the cavity perturbation method (I = microwave signal input, R = reflected signal, T = transmitted signal), redrawn with reference to (Sahin, 2006)

3.8.2 Deionised water

The dielectric properties of deionised water were measured using an Agilent 8753 ES Vector Network Analyser and Coaxial Probe. The probe method is suitable for liquids and semisolids, does not require a definite sample size and can be used for a wide frequency range (Sahin, 2006). It uses a coaxial line connected to a tip which measures the signal reflected by the material that is in contact with the probe (Shyam, 2010; Sosa-Morales, 2010). As shown in Figure 3.6, the tip is brought into contact with the deionised water by immersing it into water, and the reflected signal is used to calculate the dielectric properties of the water.

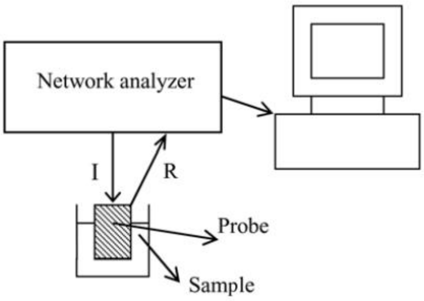


Figure 3.6: Schematic diagram of the coaxial probe method, redrawn with reference to (Sahin, 2006)

3.9 Zeta potential measurement

For preliminary assessment of the bio-flocculation mechanism, the zeta potential of the kaolin colloidal solution and bio-flocculant was measured at 25 ˚C using a Malvern Instrument Zetasizer NanoZS (Malvern Instruments Ltd, Worcestershire, UK). The measurement principle is based on electrophoretic light scattering (Zetasizer, 2016). The samples were firstly diluted with deionised water. The diluted sample was loaded into the measurement cell and allowed to equilibrate for 5 minutes prior to measurement to eliminate fluid convection. Zeta potential measurements reported are an average of 10 individual measurements.

CHAPTER 4: EXPERIMENTAL RESULTS

This chapter presents the experimental results obtained from the optimisation study of extraction parameters in conventional hydrothermal and microwave assisted extraction processes. The target of the optimisation study is to produce bio-floculant with optimal yield and sludge dewatering abilities. The investigated extraction parameters included temperature, time, solvent loading, and agitation speed and particle size of okra. The optimised yield and optimised dewatering efficiency were used as the indicators of process performance and for comparison between CHE and MAE methods.

This chapter also discusses the effect of each extraction parameter on the yield and sludge dewatering abilities of bio-flocculants. The influence of extraction variables on the yield was further evaluated by analysing the experimental results with response surface methodology. The dewatering activities of aqueous and dried bio-flocculants were compared with each other and the effect of drying on the dewatering performance was evaluated. The dewatering abilities of bio-flocculants extracted under optimised conditions were subsequently compared with commercial flocculants (polyacrylamides).

4.1 Effects of extraction parameters on the extraction yields: Single factor experiments

4.1.1 Extraction time

Figure 4.1 shows the effects of extraction time on the yields of CHE (Experiment set H1) and MAE (Experiment set M1) processes.

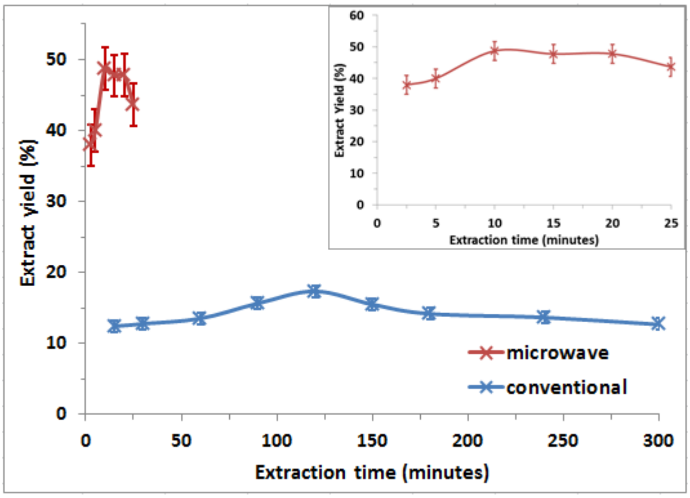


Figure 4.1: Effect of extraction time on the extract yield

For CHE, an increasing trend of extract yield with time was observed from 15 minutes to 2 hours. As reported in other studies, this is expected given that sufficient extraction time is needed for the solvent to be heated via conduction and convection heat transfer (Robinson, 2010a), penetrate into the plant material and dissolve the solutes, and for the dissolved solutes diffuse out into the bulk solvent (Samavati, 2013; Ye, 2011). The maximum yield of 17% was observed at 2 hours, and declined to 13% after 5 hours of processing. Prolonged exposure at high temperatures (70 ˚C for this case) for several hours may cause thermal decomposition or hydrolysis of the target compounds, and thus decrease the extract yield. Similar phenomenon was reported in extraction of polysaccharide from *Bruguiera gymnorrhiza* at extraction temperature of 70 ˚C, whereby the yield reached the maximum value at 30 minutes of extraction time and started to decrease as the extraction time prolonged (Li, 2013a).

For the case of MAE, the yield was at first increased with increasing extraction time, reaching a peak of 49% after just 10 minutes. However, after 20 minutes of exposure to microwave heating the yield declined, which is likely due to degradation of the extracted compounds when they are sustained in the processing environment at 70 ˚C (Bagherian, 2011).

The key findings from Figure 4.1 are that microwave extraction exhibits a much higher yield and extraction kinetic than hydrothermal method and MAE could be accomplished in few minutes when compared to CHE in hours. It indicates that a fundamentally different extraction mechanism takes place as a result, and this is discussed in next chapter. The following study of extraction temperature was conducted by maintaining the processing time consistent with the maximum yield values (2 hours for CHE and 10 minutes for MAE) identified in Figure 4.1.

4.1.2 Extraction temperature

The effects of temperature on extract yields of hydrothermal (Experiment set H2) and microwave (Experiment set M2) extractions were studied from 25 to 90 ˚C, with the results shown in Figure 4.2.

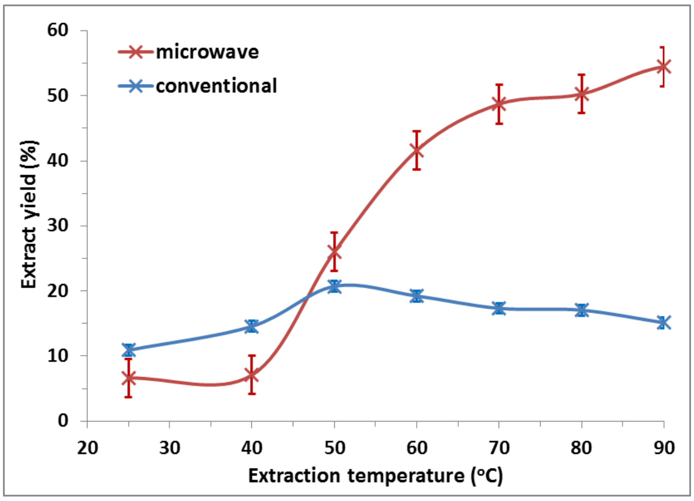


Figure 4.2: Effect of extraction temperature on the extract yield

The trend of extract yield with temperature for hydrothermal extraction is markedly different to microwave extraction. Below 50 ˚C, the yield is comparable or slightly higher with hydrothermal extraction. Above 50 ˚C, the yield obtained with microwave extraction is significantly higher, rising to 53% at 90 ˚C compared with just 15% with hydrothermal heating. No decline in yield was observed for microwave heating because the short extraction time in MAE (10 minutes) possibly avoids the thermal degradation and oxidation of biopolymers (Veggi, 2013).

In contrast, the extract yield of CHE increased with temperature and achieved a peak yield (21%) at 50 ˚C. With reference to other studies, the initial increase is most likely due to the reduction in viscosity and surface tension of solvent with temperature, and thus increases solubility and diffusion of solutes from the cell wall to the solvent (Carr, 2011; Samavati, 2013; Veggi, 2013; Ye, 2011). It has been reported that the solubility of water-soluble polysaccharides (e.g. xylans and pectins) in water is increasing with higher temperature and longer treatment time (Willför, 2003).

With further increase in temperature the extract yield of CHE declined from the peak value at 50 ˚C. This may be due to degradation of thermolabile components caused by prolonged exposure (2 hours) at these higher temperatures. A similar trend was also observed in other studies (Cai, 2008; Li, 2013a). It has been reported that thermal depolymerisation of natural biopolymers is associated with decreased of intrinsic viscosity and average molecular weight during thermal process and are affected by extraction temperature and the exposure time (Valdir, 2012).

There is a transition point at 50 ˚C, at which the yield is comparable between the two extraction techniques. The increase in yield for microwave extraction was not apparent below 50 ˚C but occurred throughout the temperature range of 50 to 90 ˚C. By interpreting the findings from Figures 4.1 and 4.2, microwave extraction appears to be governed by a different mechanism at temperatures above 50 ˚C, but the mechanism appears to be similar for both hydrothermal and microwave heating below 50 ˚C. This behaviour is further discussed in next chapter with the results of dielectric properties of okra and water across this temperature range.

4.1.3 Solvent loading (solvent/plant ratio)

The variation of solvent loading on the extract yields of CHE (Experiment set H3) and MAE (Experiment set M3) was studied and the results are presented in Figure 4.3.

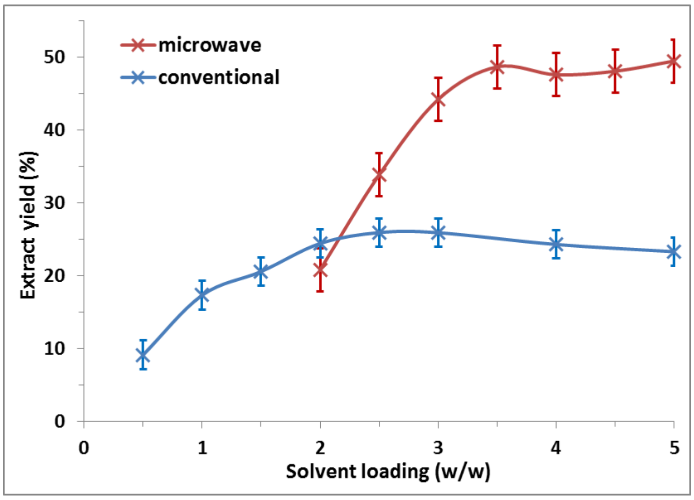


Figure 4.3: Effect of solvent loading on the extract yield

The extract yield of MAE increased with solvent loading and achieved the highest yield at 3.5 w/w, with no further increases observed at higher loadings. A larger solvent loading implies a 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 the solvent (Qiao, 2009; Yang, 2013; Ying, 2011). Similar extraction behaviour was observed on CHE of bio-flocculants with the peak value achieved at 2.5 w/w.

4.1.4 Agitation speed

The effects of agitation speeds on the extract yields of hydrothermal (Experiment set H4) and microwave (Experiment set M4) extractions are shown in Figure 4.4.

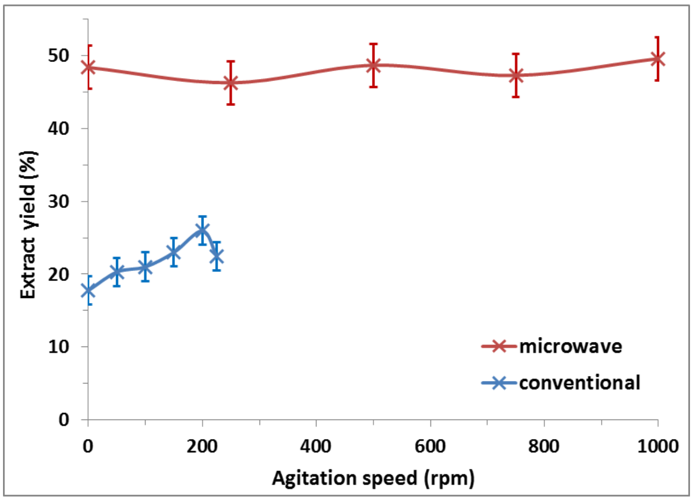


Figure 4.4: Effect of agitation speed on the extract yield

The extract yields of CHE increased progressively from 0 to 200 rpm. Increasing of agitation speed favours the convective movement in the solvent bulk and promotes turbulence within the solvent. This facilitates the heat transfer from solvent into plant and the diffusion of heated solvent through the plant cell wall, and subsequently accelerates the dissolution of solutes into solvent and diffusion of dissolved solutes from plant to solvent, and consequently the extraction rate was increased (Veggi, 2013). The results also showed that agitation speed from 200 to 225 rpm has no significant effect on yield. Similar finding has been reported in extraction of baicalein and pinostrobin from *Scutellaria violacea*, whereby the yield remained moderately constant with agitation speed above 300 rpm after achieved the maximum yield at 300 rpm (Subramaniam, 2015). A possible explanation was suggested by Raquel, R.O. (Raquel, 2009), who suggested that the hydrodynamic condition above 200 rpm causes significant sample heating and results in thermal degradation of bioactive compounds.

Even though agitation speed has been reported to exhibit an effect on MAE (Veggi, 2013), evidence of this effect is rarely explored and only limited findings have been reported (Chan, 2011; Robinson, 2010a). In this study, the variation of agitation speed did not accelerate or decelerate the extraction speed of MAE, which is likely due to the selective heating effect of microwaves which is discussed in next chapter.

4.1.5 Particle size of okra

The investigation of the effect of particle size of feedstock on MAE (Chupin, 2015; Wang, 2015) is limited and hence the significance of this parameter was studied in this work. In Fick's Law, particle size affects the mass transfer kinetics because of its direct relationship with the length of diffusive pathways. In this respect, decreasing the particle size increases the collective amount of surface area with which the solvent is in contact and reduces the obstacles to diffusion of solvent into the particle, and of solute and solvent out of the particle pores (Martinez, 2013).

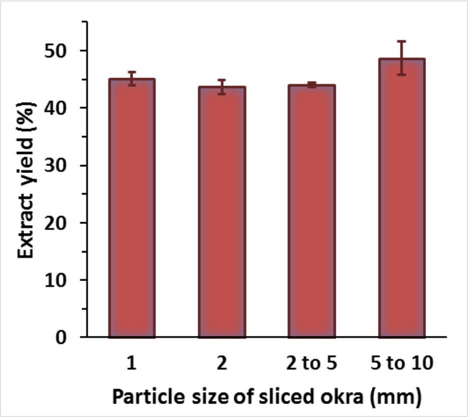


Figure 4.5: Effect of particle size of sliced okra on the extract yields obtained with MAE method

As shown in Figure 4.5, the variation of particle size of sliced raw okra showed no significant effect on the extract yields of bio-flocculants extracted with

MAE method. The experimental results reveal that reduction in surface area did not further enhance the diffusivity of the extraction medium and the mass transfer of solutes into the solvent. A study suggested that it is probably because of reduced pore sizes in finer particle sizes which causes resistance to mass transfer (Shi, 2007).

4.2 Effects of multiple variables on extraction yield: Response surface methodology (RSM)

Based on single factor experiments, the conditions to achieve optimised (maximum) yield for CHE were 50 ˚C, 2 hours, and solvent loading at 2.5 w/w and agitation speed of 200 rpm. On the other hand, the optimised yield for MAE was obtained at 90 ˚C, 10 minutes, and solvent loading at 3.5 w/w with negligible effects of agitation speed and particle size. The effects of extraction variables on the yield were further evaluated by analysing the results with RSM.

4.2.1 Statistical analysis and model fitting

The linear and quadratic effects of the independent variables on the response (yield) were obtained by multiple regression analysis. A second order polynomial mathematical equation including linear and quadratic terms has been developed for response from the observed data which represent the relationship between the independent variables and response. The developed equations for CHE and MAE could be expressed by Equations 4.1 and 4.2 respectively.

$$Yield\ (\%) = -19.68 + 11.20S + 0.47T + 0.07t + 0.06A - 1.63S^2 - 0.004T^2 - 0.0002t^2 - 0.00A^2 \quad (4.1)$$

$$Yield\ (\%) = -140.8 + 43.41S + 1.69T + 1.95t - 0.009A - 0.73PS - 5.0S^2 - 0.007T^2 - 0.06t^2 + 0.00A^2 + 0.083(PS)^2 \quad (4.2)$$

As single factor experimental data was used to run the model, hence the interaction terms could not be estimated by the model. Analysis of variance (ANOVA) was used to analyse the model for significance and to identify the significant factors, and a statistical summary was given in Table 4.1.

Table 4.1: ANOVA for the response surface quadratic model of yield (Notes: DoF = degree of freedom, MS = mean squares, R^2 = coefficient of determination)

Process	**CHE**				**MAE**			
Source	**DoF**	**MS**	**F-value**	**p-value**	**DoF**	**MS**	**F-value**	**p-value**
Model	8	75.7	22.3	0.00	10	379.9	25.4	0.00
Linear	4	44.7	13.3	0.00	5	661.1	44.3	0.00
S	1	117.7	34.6	0.00	1	495.5	33.2	0.00
T	1	2.7	0.8	0.38	1	275.2	184.3	0.00
t	1	0.7	0.2	0.65	1	31.2	2.1	0.17
A	1	61.1	18.0	0.00	1	1.2	0.1	0.78
PS	-	-	-	-	1	3.2	0.2	0.65
Square	4	52.8	15.5	0.00	5	75.2	5.0	0.01
S^2	1	119.7	35.2	0.00	1	233.1	15.6	0.00
T^2	1	22.2	6.5	0.02	1	72.8	4.9	0.04
t^2	1	45.5	13.4	0.00	1	89.3	6.0	0.03
A^2	1	1.4	0.4	0.53	1	11.8	0.8	0.39
$(PS)^2$	-	-	-	-	1	2.1	0.1	0.71
	R^2 = 89.5%, R^2 (adj) = 85.5%, R^2 (pred) = 66.2%				R^2 = 93.4%, R^2 (adj) = 89.7%, R^2 (pred) = 59.9%			

The F-value and p-value were used to compute the significance of the coefficients of the model. If the absolute F-value becomes higher and the corresponding p-value becomes lesser, it indicates the corresponding terms are more significant (Maran, 2016). The terms that are not significant ($p>0.05$) were highlighted in the table. As depicted, the model F-value of more than 20 with zero p-value indicated the high significance of the models for both processes.

The data also shows that the order of factors influencing the yield of CHE was as follows: solvent loading > agitation speed > temperature > time. In addition, the linear coefficients of temperature and time and the quadratic term of A^2 were not significant ($p>0.05$) while the other coefficients were significant ($p<0.05$). These results reveal that all the factors showed influence to the yield because either the linear or quadratic coefficient of each factor (S, A, T^2, t^2) was significant ($p<0.05$).

The variables influencing the yield of MAE were in the order of: temperature > solvent loading > time > particle size > agitation speed. The linear and quadratic effects of agitation speed and particle size on the yield were insignificant ($p>0.05$), however other terms were significant. These data indicates that the yield was affected by solvent loading and temperature and time, confirming the results from single factor experiments.

For the model fitting, the coefficients of determination (R^2), adjusted R^2 and predicted R^2 show the proportion of variability in the data explained by or accounted for by the model and the goodness of fit of the model (Ma, 2016). High values of R^2 at 89.5% and 93.4% were obtained for CHE and MAE respectively.

4.2.2 Adequacy of developed model

Residual plots such as normal probability plot and residual versus order plot (Figures 4.6 and 4.7) have been constructed from the observed and predicted experimental data and are used to examine the fit of the developed models in regression and ANOVA for CHE and MAE processes.

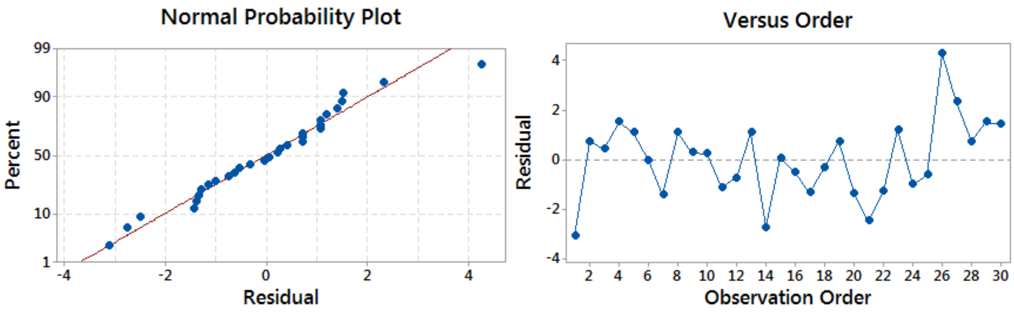


Figure 4.6: Reliability of developed model for CHE process

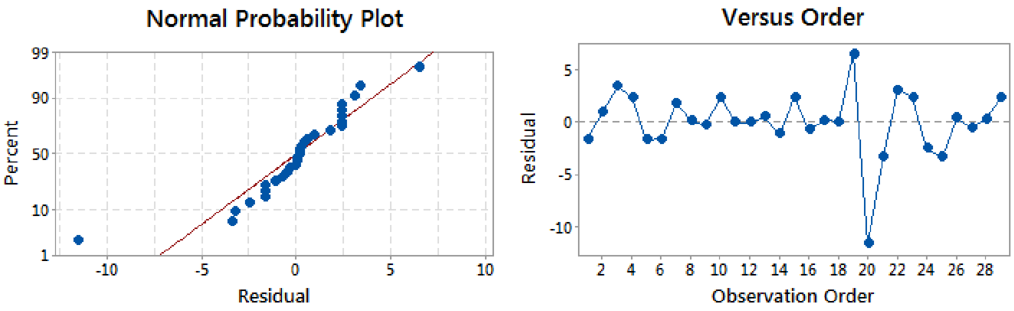


Figure 4.7: Reliability of developed model for MAE process

The developed models were found to be well fitted and have the capability to predict the experimental data in precise manner because the points in normal probability plot approximated along a straight line and the residual plots displayed a random pattern of residuals on both sides of 0 (Maran, 2016).

4.2.3 Response surface plots

The 3D response surface curves in the optimisation of two variables (Figures 4.8 and 4.9) were provided as a graphical interpretation of the regression equation to visualise the influence of process variables on the response when the other factors were hold at the optimised values which were determined from single factor experiments.

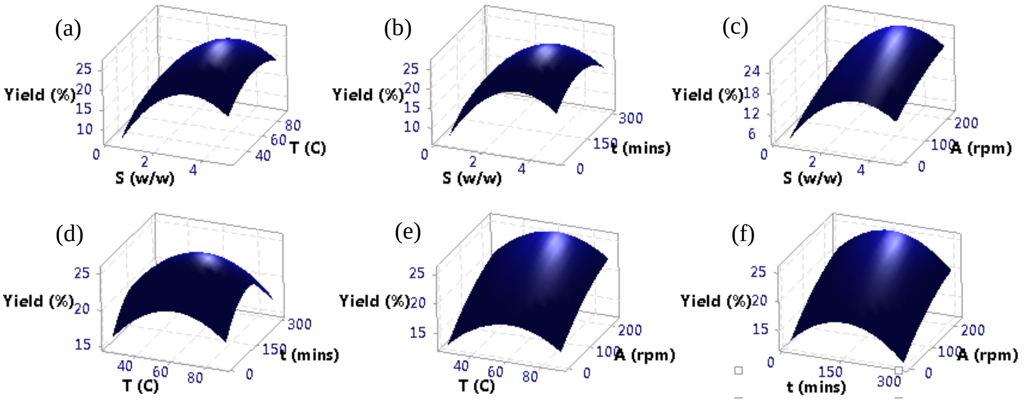


Figure 4.8: Response surface curves of (a) to (f) showing the yield response with respect to solvent loading (S), temperature (T), time (t) and agitation speed (A) on the yield in CHE process (hold values: S = 2.5 w/w, T = 50 ˚C, t = 2 hours, A = 200 rpm)

The curves in Figure 4.8 show that all the studied variables imposed significant effects on the yield of CHE. The yield increased significantly with rising of temperature, time and solvent loading within a certain range and then declined gradually after achieved the peak value. For agitation speed, the yield increased at first and then presented a smooth trend afterwards. These phenomena were correlated with the extraction trends from single factor experiments. As shown in Figure 4.8 (a-c), the effects of temperature and time and agitation speed on yield were more pronounced at solvent loading lower than 2.5 w/w.

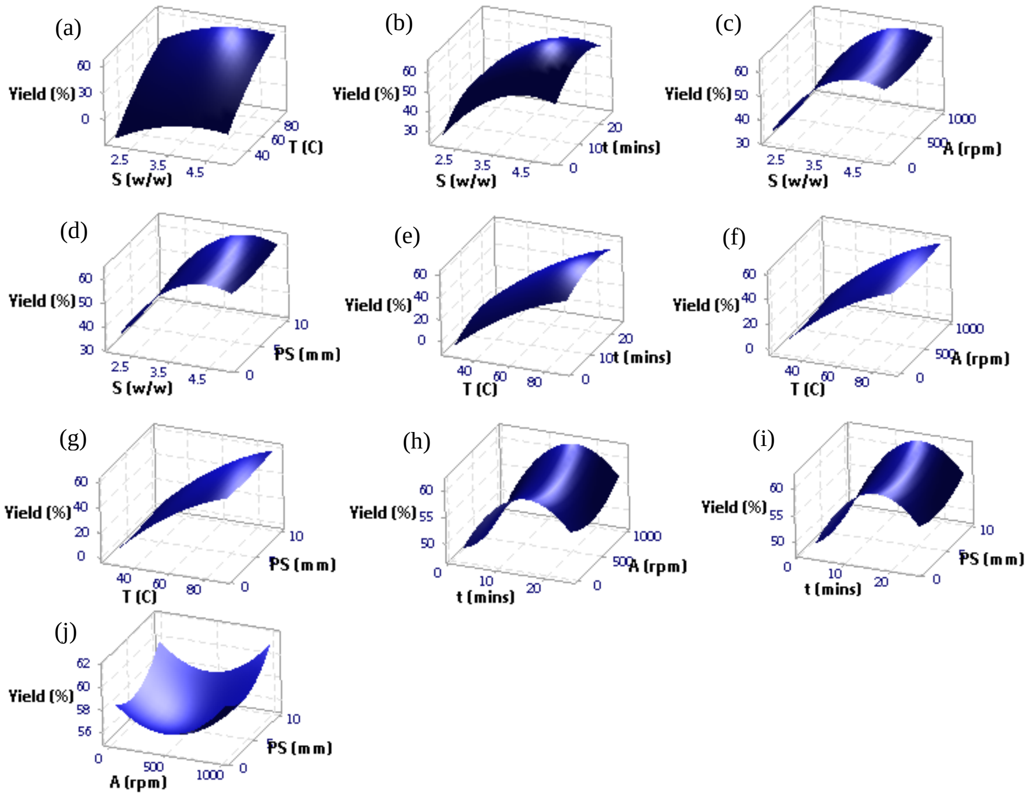


Figure 4.9: Response surface curves of (a) to (j) showing the yield response with respect to solvent loading (S), temperature (T), time (t), agitation speed (A) and particle size (PS) on the yield in MAE process (hold values: S = 3.5 w/w, T = 90 ˚C, t = 10 mins, A = 0 rpm, PS = 1mm)

As represented by Figure 4.9 (a, b and e), the curves obviously showed that the yield was impacted significantly by temperature and solvent loading followed by moderate effect by time. In surfaces shown in Figure 4.9 (c, d, h and i), there was a plateau in relation to agitation speed and particle size, indicating that the variation of these parameters does not affect the yield. As shown in Figure 4.9 (b), the yield increased with rising of solvent loading and time and then declined after achieved the peak. Figure 4.9 (e-g) showed that the yield increased with rising of temperature and subsequently attained the peak at 90 ˚C.

The above results have clearly shown the effect and sensitivity of each extraction parameter to the yield and the ANOVA analysis has verified the reliability of the experimental data due to good fitness of data to the developed model. However, the investigation of interaction effects of multiple extraction variables to yield was limited because the results from single factor experiments were used for RSM analysis. For future work, either Box-Behnken design (BBD) or central composite design (CCD) could be employed to design the experiments in order to further improve the optimisation of yield and investigate the interaction effects of multiple variables on the response. The response optimiser function in RSM could be used to optimise the independent variables corresponding to maximum yield and subsequently the experiment at the predicted optimised conditions could be carried out to validate the predicted optimised conditions obtained from response optimiser.

4.3 Effects of extraction parameters on the bio-flocculant quality

4.3.1 Flocculating abilities of bio-flocculants

Prior to evaluation of the sludge dewatering ability, the flocculating efficiencies of bio-flocculants extracted under different extraction conditions were examined. Suspended solid (SS) and turbidity removal after flocculation, and the sludge volume index (SVI) were measured. SVI is the volume in millilitres occupied by one gram of flocs, and is an indicator of the settling quality of the flocs (Spellman, 2009). As the SVI increases, the flocs are less compact and settle more slowly, and are likely to result in an increase in supernatant suspended solids. As the SVI decreases, the flocs become denser, and settling is more rapid (Spellman, 2009) .

Before flocculation tests, the pH of the kaolin colloidal solution was 4.5-5 while the pH of the bio-flocculants were 6-6.5. After the flocculation process, the results showed that aqueous and dried bio-flocculants exhibited remarkable efficiencies with >99% of SS and turbidity removal. The flocculation effect of bio-flocculant in clarification of kaolin colloidal solution was evidently demonstrated in Figure 4.10 below. After treatment with bio-flocculant, the supernatant looked clear with a low content of SS (less than 10 mg/L) relative to the initial kaolin colloidal solution of 80 g/L. These results indicated that okra bio-flocculant is very efficient in solid removal from solution high in suspended solids or turbidity.

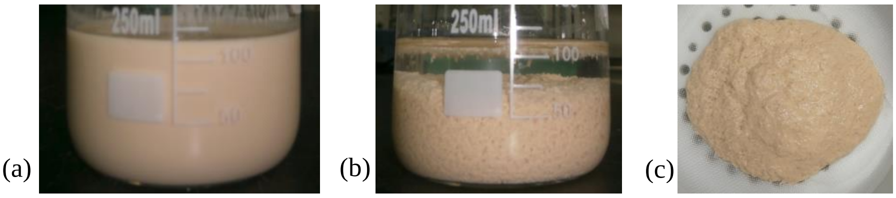


Figure 4.10: The flocculation effect of bio-flocculant in clarification of kaolin colloidal solution (a) before treatment, (b) after bio-flocculation, (c) the filtered flocs after dewatering

All the results of SVI values were maintained lower than 10 ml/g, indicating good settling capability of the flocs (Spellman, 2009). All measured values were found to have a standard deviation below 5%, denoting the results were reproducible. The experimental results show that the flocculating abilities of bio-flocculants were not significantly affected by the extraction conditions, and hence the effect of extraction parameters on flocculating ability was not discussed. The extracted bio-flocculants are naturally neutral (pH = 6-6.5) and proved to be efficient in kaolin clarification under acidic conditions without pH alteration and addition of coagulant.

4.3.1.1 Comparison of flocculating ability of extracted bio-flocculant with literature results

The flocculating ability of the okra bio-flocculant extracted under optimised conditions in this study was compared with other work that investigated the application of okra bio-flocculants in different types of wastewater. The environmental parameters investigated in these studies were suspended solids (SS) and turbidity (TU). The comparison is displayed in Table 4.2.

Table 4.2: 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**
Kaolin colloidal solution (This work)	SS: 80 g/L, TU: 81000 NTU, pH: 4.5-5	No	No	30	0.5	SS: 99.8%, TU: 99.9%
Synthetic kaolin solution (Anastasakis, 2009)	TU: 63-73 NTU, pH: 5.9	Aluminium sulfate	No	5	0.75	TU: 93-97.3%
Biologically-treated effluent (Anastasakis, 2009)	TU: 55 NTU, pH: 6.9			2.5		TU: 70-74%

In other studies (Anastasakis, 2009) it was noted that the treatment process requires the addition of inorganic coagulant (aluminium sulfate), and demand longer treatment times in order to obtain good flocculating performance with removal of turbidity more than 70%. It is probably the okra bio-flocculants were tested in treatment of solutions that were low in turbidity whereby the negative colloidal particles are generally well dispersed, and hence a coagulant is generally required to neutralise (destabilise) the charges of particles and encourage the colloidal particles to coagulate together (Anastasakis, 2009).

In this work very efficient flocculating performance with 99.8% of SS and TU removal was achieved directly, without addition of coagulant and pH alteration. The treatment time was only half an hour, which is significantly shorter, compared to other studies. There is no requirement of pH adjustment, possibly due to the near neutral behaviour of the bio-flocculant. It is postulated that the extracted bio-flocculant has long and branched biopolymer chains containing functional groups that can adsorb on the surface of colloidal particles and then bridge them together (Okaiyeto, 2016). The detail of bio-flocculation mechanism is discussed in next chapter.

4.3.2 Sludge dewatering abilities of bio-flocculants

The effects of different extraction parameters on the dewatering activities of dried bio-flocculants were investigated at a constant bio-flocculant dosage of 100 mg/L.

4.3.2.1 Extraction time

The influences of extraction time on the sludge dewatering efficiencies of dried bio-flocculants for hydrothermal and microwave extractions carried out at 70 ˚C are presented in Figure 4.11.

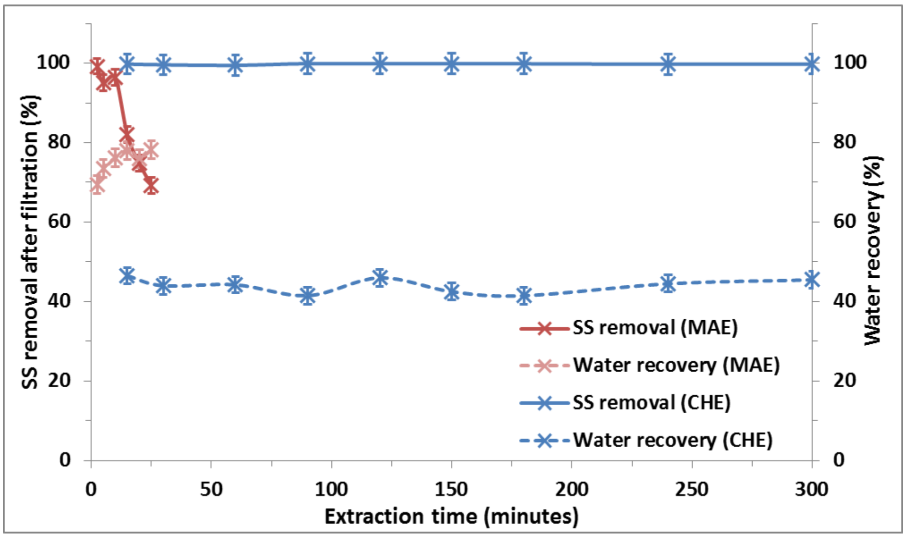


Figure 4.11: Effect of extraction time on the sludge dewatering abilities of dried bio-flocculants

The variation in extraction time did not lead to any obvious changes in the sludge dewatering properties of hydrothermal extracted bio-flocculants. The results showed that the removal of SS after filtration (>99.5%) and water recovery (45-50%) were stable at all extraction times. This indicates that the extraction time does not affect the principal components responsible for flocculating and sludge dewatering abilities when the bio-flocculant extraction was conducted at 70 ˚C.

For MAE of bio-flocculants, the water recovery was nearly constant at 70-80% with increasing extraction time, but the SS removal started to decline rapidly after 10 minutes. It is thought that the extracted components are prone to thermal degradation and oxidation which degrade the SS removal activities of bio-flocculants for extraction time longer than 10 minutes. The mechanism of degradation is presumed to be hydrolytic cleavage of polysaccharide chains as well as the breaking intermolecular hydrogen bonds (Wang, 2010). This causes a reduction in intrinsic viscosity and molecular weight of bio-polymers (Valdir, 2012) which negatively affects the bridging flocculation mechanism, and leads to formation of weaker flocs. Therefore, the control of extraction time becomes an essential step in the MAE to maintain the stability of desired active compounds and to avoid possible thermal degradation of active components. Hence, the obtained results suggested that the optimal dewatering activities of microwave extracted bio-flocculants were achieved at extraction time of 10 minutes.

4.3.2.2 Extraction temperature

The effects of extraction temperature on the sludge dewatering abilities of dried bio-flocculants are shown in Figure 4.12.

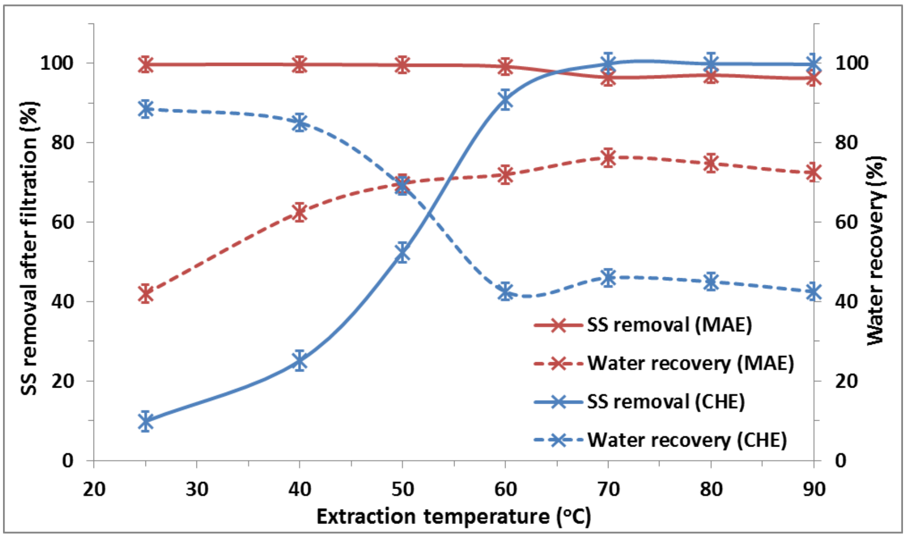


Figure 4.12: Effect of extraction temperature on the sludge dewatering abilities of dried bio-flocculants

For hydrothermal extracted bio-flocculants, high water recovery (69-89%) and low SS removal (10-52%) were observed between 25 and 50 ˚C. This phenomenon indicates weak floc formation whereby the flocs formed are very fine and would break-up during filtration process and pass through the pores of the filter cloth to flow into supernatant. It consequently increases the concentration of suspended solids in the supernatant and the recovery of supernatant volume after filtration. Therefore, the results of high water recovery and low solid removal efficiency were obtained.

On the other hand, for microwave extracted bio-flocculants, high SS removal (>95%) and moderate water recovery (40-70%) were observed between 25 and 50 ˚C. These results reveal that big and fluffy flocs are obtained at these conditions which retain water and particles in the flocs and resulted to less water recovery and high SS removal after filtration.

With increasing temperature, the water recovery for MAE and the removal of SS after filtration for CHE increased accordingly and achieved a maximum at

70˚C and then remained constant to 90 ˚C. For temperatures between 70 and 90 ˚C, the SS removal was more than 95%, and the water recoveries were almost constant at 45-50% and 70-76% for CHE and MAE of bio-flocculants respectively. It denotes that the produced sludge flocs are stronger and more compact than the ones at lower extraction temperatures, thus promoting the dewaterability of the flocs during filtration and resulted to high water recovery and high removal of SS. These findings revealed that the active constituents with high dewatering properties were best extracted at temperatures ≥ 70˚C for CHE and MAE processes.

4.3.2.3 Solvent loading

The effects of solvent loading on the sludge dewatering abilities of hydrothermal and microwave extracted dried bio-flocculants are presented in Figure 4.13.

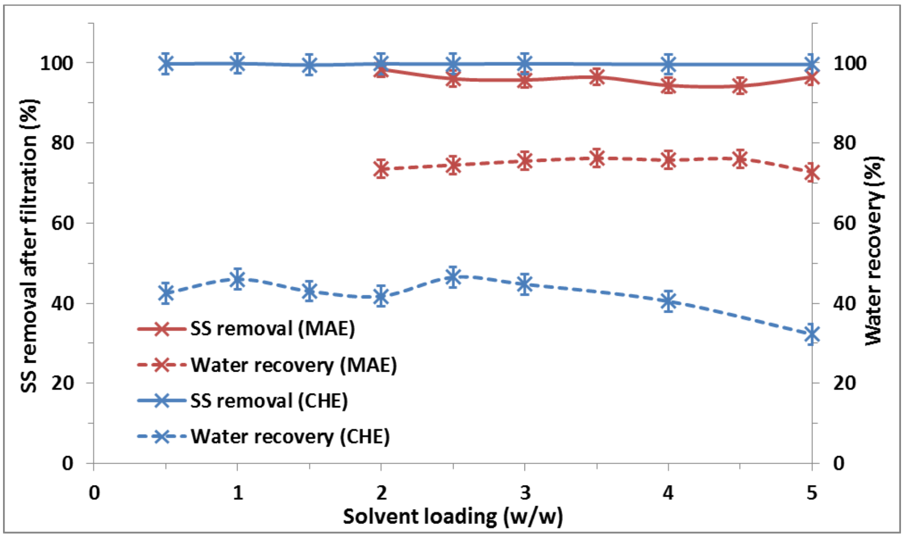


Figure 4.13: Effect of solvent loading on the sludge dewatering abilities of dried bio-flocculants

As shown in Figure 4.13, solvent loading did not significantly affect the sludge dewatering efficiencies of hydrothermal and microwave extracted bio-flocculants. More than 95% removal of SS after filtration and water recoveries of 35-45% and 70-75% were obtained for CHE and MAE respectively under all conditions.

4.3.2.4 Agitation speed

The influence of agitation speed on the sludge dewatering abilities of dried bio-flocculants extracted with CHE and MAE methods is shown in Figure 4.14.

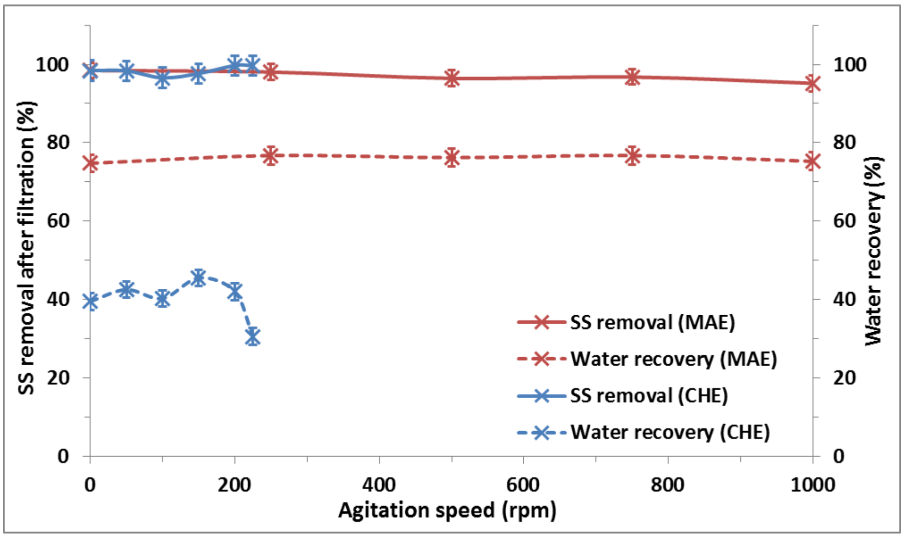


Figure 4.14: Effect of agitation speed on the sludge dewatering abilities of dried bio-flocculants

Variation in agitation speed showed negligible effect on the dewatering activities of bio-flocculants extracted with both methods. High solid removal at approximately 95% and water recoveries at 40-45% and 75% were obtained for CHE and MAE of bio-flocculants respectively. The decrease in water recovery from 200 to 225 rpm for CHE is noted and correlates with the decrease in yield shown in Figure 4.4. Therefore, agitation at 200 rpm was chosen as the optimum speed for CHE based on the highest yield and water recovery obtained at this condition. Conversely, agitation could be neglected in microwave extraction of bio-flocculant as the yield and dewatering ability were not affected by this parameter.

4.3.2.5 Particle size of okra

The influence of particle size of okra on the dewatering properties of microwave extracted dried bio-flocculants is presented in Figure 4.15. The studied particle sizes of sliced okra included 1mm, 2mm, 2-5mm and 5-10mm.

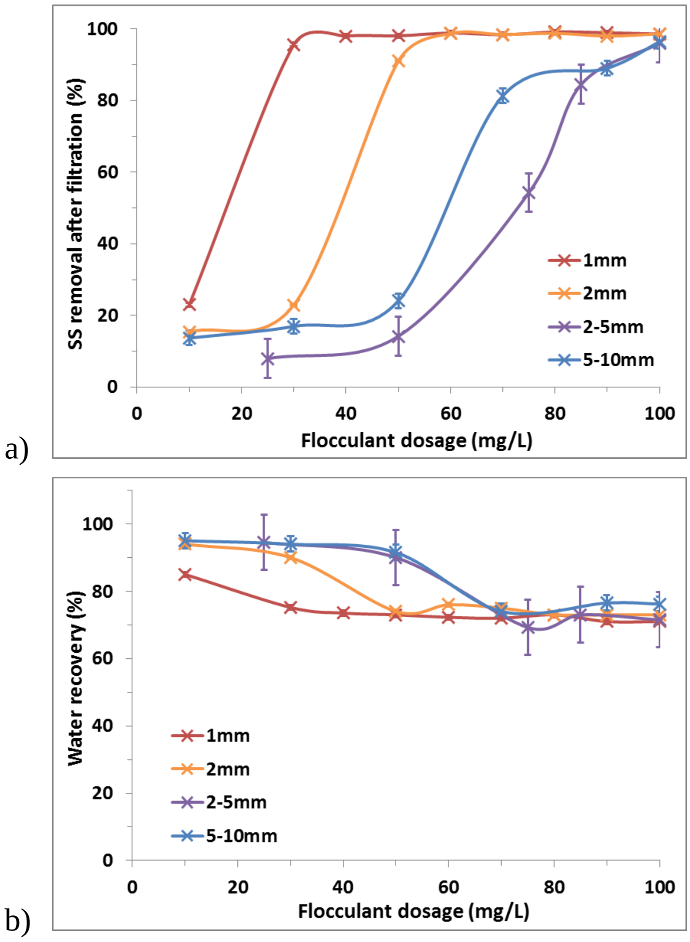


Figure 4.15: Effect of particle size on the (a) SS removal after filtration and (b) water recoveries of microwave extracted dried bio-flocculants

Variation of particle size of okra showed a notable effect on the dewatering abilities of bio-flocculants with regard to the SS removal after filtration. The results showed that the bio-flocculants extracted with 1mm sliced okra required the smallest dosage (30 mg/L) to achieve high SS removal (≥95%) and high water recovery (≥75%) when compared to others. Finer and smaller particles may increase the contact surface area between the plant matrix and the solvent (Veggi, 2013) which leads to extraction of active compounds high in flocculating and dewatering activity. Hence, it could be concluded that the bio-flocculant with high dewatering performance could be extracted by using the smallest size of okra (1mm) that can be practically achieved by the food processor.

4.4 The effects of bio-flocculant dosage and drying on the sludge dewatering efficiency

4.4.1 Hydrothermal extracted bio-flocculants

The sludge dewatering abilities of hydrothermal extracted aqueous and dried bio-flocculants (H-AF and H-DF) at different dosages is presented in Figure 4.16.

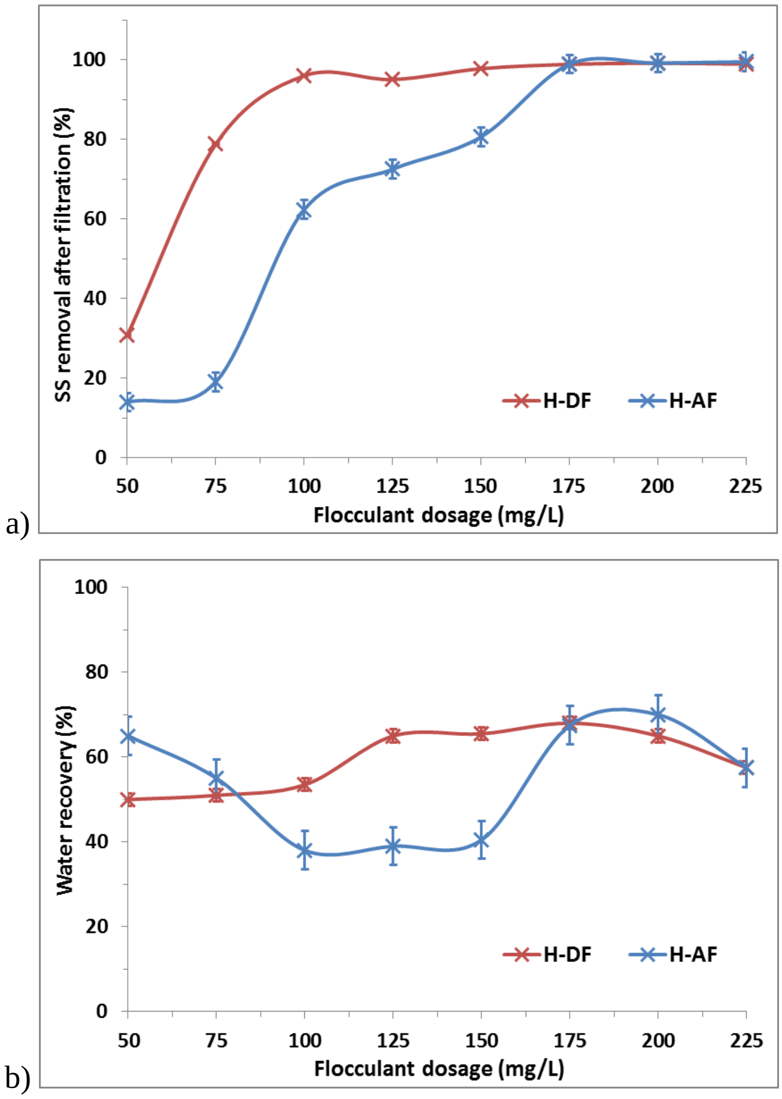


Figure 4.16: The effect of bio-flocculant dosage on the (a) SS removal after filtration and (b) water recoveries of hydrothermal extracted bio-flocculants

For H-DF, the SS removal and water recovery increased with flocculant dosage and reached the maximum removal at 95% and recovery at 65% at 125 mg/L. Adversely, overdose of bio-flocculant may affect the flocculation kinetics and cause a decrease in water recovery for dosages >175 mg/L. As reported in other literature, this is because the competition between biopolymer adsorption blocks some of the surface sites for the subsequent adsorption of the long chain biopolymers, which affects floc properties. (Blanco, 2005).

Different dewatering phenomenon was obtained for H-AF between 50 to 100 mg/L. It is postulated that the flocs formed are very fine and passed through the pores of the filter cloth during filtration, leading to the observation of high water recovery and low SS removal. For dosages between 100 to 150 mg/L higher SS removal was achieved but the water recovery was lowered which is likely due to formation of large but fluffy flocs which hinder the passage of water during filtration. Optimum SS removal at 99% and water recovery at 67.5% were attained at 175 mg/L.

The above results showed that H-DF exhibits higher dewatering abilities than H-AF. The dewatering activities of H-AF were examined on the second day after extraction; hence the active constituents with dewatering properties are likely to be degraded during the storage time. Due to high water content (>80%) in the extract, H-AF are more susceptible to microbial attack and the extracted biopolymers are more accessible to biodegradation by microorganisms (León-Martínez, 2011). The processes of biodegradation described in the literature are associated with the hydrolysis caused by the microorganisms and followed by changes in molecular weight, viscosity and morphology (Valdir, 2012). Conversely, fresh H-AF were sent for drying to obtain H-DF and some studies revealed that removal of moisture through the low temperature drying process (<60˚C) minimised the degradation that occurs with time in the aqueous phase and thus preserved the active ingredients in the dried products (Harbourne, 2009; Pirbalouti, 2013). This hypothesis was verified by using microwave-extracted bio-flocculants, and the results are presented in next section.

4.4.2 Microwave extracted bio-flocculants

Three different samples of microwave-extracted dried bio-flocculants marked as M-DF (Day 0) and M-DF (Day 1) and M-DF (Day 2) were obtained after drying fresh microwave-extracted aqueous bio-flocculant (M-AF), and M-AF that had been stored for 1 day and 2 days.

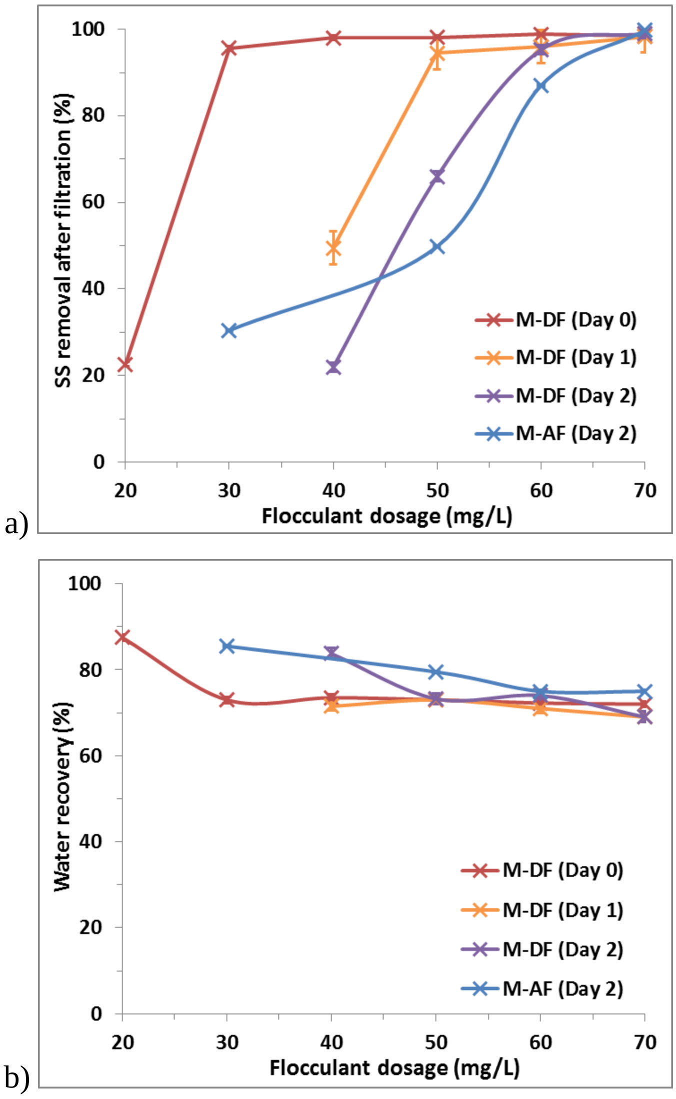


Figure 4.17: The effect of bio-flocculant dosage on the (a) SS removal after filtration and (b) water recoveries of microwave extracted bio-flocculants

The dewatering abilities of microwave-extracted bio-flocculants in terms of SS removal after filtration were greatly affected by flocculant dosage, whereas no significant effect on water recovery was observed. Samples of M-DF (Day 0), M-DF (Day 1) and M-DF (Day 2) achieved the optimum SS removal (≥95%) and water recovery (≥75%) at dosages of 30, 50 and 60 mg/L respectively. In addition, M-DF (Day 2) was shown to exhibit comparable dewatering efficiency as M-AF that has been stored for 2 days (marked as M-AF (Day 2)). These results further affirmed that the dewatering activities of aqueous bio-flocculants decrease with storage time, and drying of bio-flocculant at low temperature (40˚C) may conserve the dewatering quality by decelerating the degradation process (León-Martínez, 2011), thus extending the shelf life of product.

It has been reported that drying is by far the most widely used preservation method because the dried products obtained after drying have minimal moisture content. This may slow down the microbial growth rate and also forestall certain biochemical changes within the plant tissue that might cause degradation of their functional properties (Pirbalouti, 2013). The drying temperature must be controlled and preferably kept low to minimise the destructive forces of heat during drying and to protect the active components against thermal degradation (Harbourne, 2009; Pirbalouti, 2013).

4.5 Comparison of extraction efficiencies between CHE and MAE

With reference to the experimental results presented above, the conditions to obtain the optimised yield and optimised dewatering ability for CHE and MAE processes are summarised in Table 4.3. Meanwhile, the comparison of extraction efficiencies between CHE and MAE with regard to the results of optimised yield and sludge dewatering ability are presented in Table 4.4.

Table 4.3: Extraction conditions to obtain optimised yield and dewatering ability

Extraction parameters	**Optimised yield**		**Optimised dewatering ability**	
	CHE	**MAE**	**CHE**	**MAE**
Temperature, T (˚C)	50	90	≥ 70	≥ 70
Time, t (minutes)	120	10	Negligible	10
Solvent loading, S (w/w)	2.5	3.5	Negligible	Negligible
Agitation speed, A (rpm)	200	Negligible	200	Negligible
Particle size, PS (mm)	-	Negligible	-	1

It is apparent that CHE required 2 hours to achieve optimised yield, however MAE only needed 10 minutes to attain it. This is clearly a remarkable advantage of microwave extraction. The table above also clearly showed that microwave method enables extraction at high temperature (90 ˚C) that resulted to higher yield than hydrothermal method. It is probably the short extraction time (minutes) in MAE has avoided the thermal degradation of bioactive compounds.

The dewatering ability of hydrothermal extracted bio-flocculant was affected by temperature and agitation speed. On the other hand, the dewatering activity of microwave extracted bio-flocculant was influenced by temperature, time and particle size of okra. It is interesting to discover that extraction temperature at least 70˚C is a crucial condition to extract bio-flocculant with desirable dewatering property for both extraction processes. Another observation is microwave extraction time longer than 10 minutes is unfavourable because it not only decreases the yield but also degrades the dewatering ability of extracted bio-flocculant.

Table 4.4: Comparison of extraction efficiencies between CHE and MAE

Comparison criteria		**CHE**	**MAE**
Optimised yields (%)		25.94 ± 1.95	48.71 ± 2.96
Optimised dewatering abilities	Bio-flocculant dosage (mg/L)	125 (H-DF), 175 (H-AF)	30 (M-DF), 70 (M-AF)
	SS removal after filtration (%)	95.1 ± 0.2	95.6 ± 0.5
	Water recovery (%)	65 ± 1.5	75 ± 0.7

As shown in Table 4.4, it is apparent that MAE improved the quantitative (extraction yield) and qualitative (sludge dewatering ability) characteristics of extracted bio-flocculant compared to CHE. MAE method enhanced the extract yield by 87.8% when compared to CHE. It indicates that MAE may exhibit the capability to extract more compounds of interest from okra. The enhancement of extract yield with MAE is correlated with selective heating and is discussed in detail in next chapter.

Most importantly, bio-flocculants extracted with MAE required a small dosage (30 mg/L) to attain high SS removal (≥95%) and high water recovery (≥75%). It is likely that microwave extraction enhances the release, diffusion and solubility of high molecular weight biopolymers with dewatering properties into the solvent, and accelerates the mass transfer of biopolymers from the interior of plant cell to the solvent phase (Wei, 2010). In contrast, hydrothermal extracted bio-flocculants required at least four times the dosage (125 mg/L) in order to achieve comparable performance. In addition, higher water recovery (75%) was achieved by microwave extracted bio-flocculant which indicates a lower sludge volume when compared to water recovery of hydrothermal extracted bio-flocculant (65%). Reduction of sludge volume is favourable from an industrial perspective because it could reduce the time and cost in sludge transportation and management.

4.5.1 Comparison of extraction efficiency with literature results

The extraction efficiency of okra in this work was compared with the results of okra extraction from other literature studies in Table 4.5 with water as the solvent.

Table 4.5: Comparison of extraction efficiencies of okra from different literature studies

Extraction method	**Optimised extraction conditions**				**Extract yield on dry basis (%)**	**Reference**
	Temperature (˚C)	**Time**	**Solvent loading (ml/g)**	**Others**		
Hydrothermal (water bath) extraction	70	2 hours	2.5	Agitation speed: 200 rpm	25.9	This work (Lee, 2015)
Water bath extraction	90	2 hours	None	None	20	(Emeje, 2011)
Water bath extraction	95	5 hours	21.7	Extraction times: 4	16.9	(Samavati, 2013)
Ultrasound assisted extraction	63	10 minutes	47.4	None	6.4	(Zhai, 2015)
Microwave assisted extraction	70	10 minutes	3.5	Particle size: 1mm	48.7	This work (Lee, 2016)

Conventional water bath extraction method has been applied to extract okra mucilage at high temperatures (≥ 90 ˚C), and the extraction was found to take several hours to obtain yields of 20% (Emeje, 2011; Samavati, 2013). In this work, hydrothermal extraction could be conducted at lower temperatures at 70 ˚C and yields higher than 20% were obtained after 2 hours of extraction. Even though the hydrothermal extraction method established in this study has shorten the extraction time and improved the yield compared to other literature (Emeje, 2011; Samavati, 2013), the lengthy extraction time is energy consuming and unfavourable from an industrial point of view.

In order to further reduce the extraction time and enhance the yield, advanced extraction methods were used to extract okra mucilage. The efficiency of ultrasound assisted extraction method in okra extraction has been investigated and reported (Zhai, 2015). The extraction was carried out at lower temperature (63 ˚C) and the extraction time was significantly decreased to 10 minutes but lower yield (< 10%) was attained. Microwave assisted extraction method developed in this study was shown to be effective compared to other extraction methods because the yield has been doubled, with short extraction time in 10 minutes. The reasons for higher extraction kinetics observed with the microwave method are discussed in next chapter.

4.6 Comparison of dewatering abilities of bio-flocculants with polyacrylamides

The dewatering abilities of microwave extracted aqueous and dried bio-flocculants were further evaluated through comparison with commercial cationic and anionic polyacrylamides at various dosages, and the results are displayed in Figure 4.18.

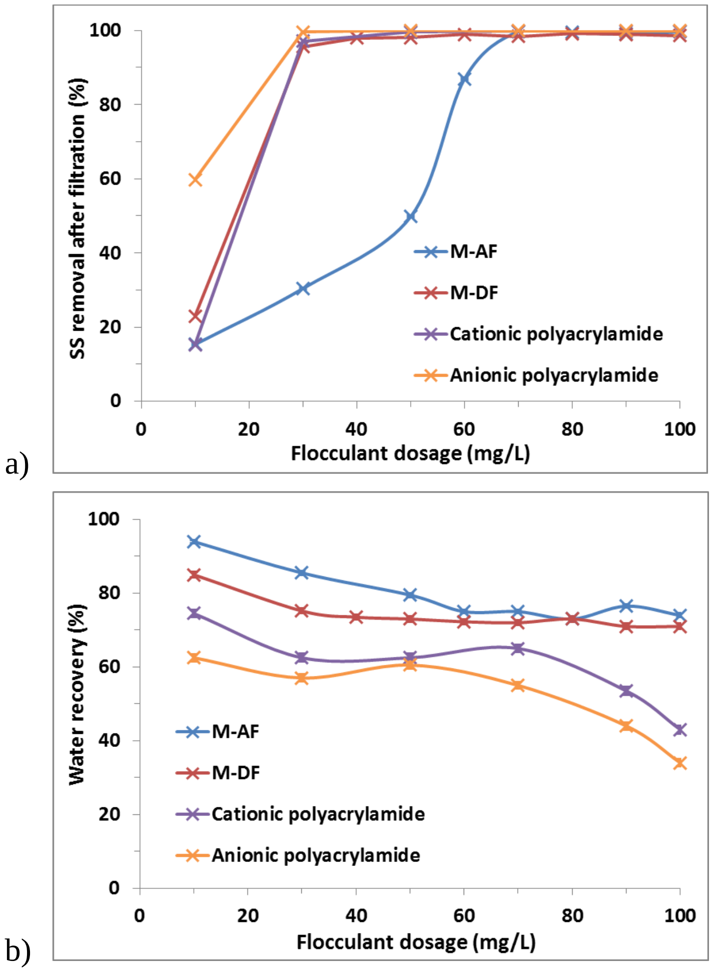


Figure 4.18: Comparison of dewatering abilities of different flocculants in terms of (a) SS removal after filtration and (b) water recovery

The required dosage to achieve optimum SS removal after filtration (≥95%) for anionic and cationic polyacrylamides and M-DF was at 30 mg/L. In addition, M-DF displayed higher water recoveries than polyacrylamides at all dosages, which indicates that lower sludge volume is achieved by using M-DF. This is a great advantage of bio-flocculant because reduction in sludge volume facilitates the sludge handling and transportation and most importantly decreases the sludge management cost. These results indicate that M-DF exhibits comparable or even higher dewatering performance than polyacrylamides.

Even though M-AF required a slightly higher dosage (70 mg/L) to achieve similar SS removal efficiency (≥95%) as polyacrylamides, however it showed higher water recoveries than polyacrylamides at all dosages. This is clearly a remarkable advantage in using either form of bio-flocculant in industrial separation process due to its superior flocculating and dewatering performance than polyacrylamides. To enable more widespread use of bio-flocculants it is therefore necessary to conduct an economic feasibility study to check the viability of producing bio-flocculant on industrial scale. The detail of economic feasibility study is introduced in Chapter 6.

CHAPTER 5: DISCUSSION

The major focus of this chapter is to investigate and understand the extraction mechanisms in solvent-based systems by comparing microwave and hydrothermal extraction methods, analyse the empirical observations against the dielectric properties of the system components, and comprehend the fundamental mass transfer properties of the system by analysing the effect of thermal gradients on chemical potential. The mechanism of solvent-based microwave selective heating was developed in this study and a quantitative model to estimate the equilibrium pressure within the plant cell was derived to evaluate the plausibility of cell wall rupture due to selective heating. The bio-flocculation mechanism was also discussed.

5.1 Dielectric properties

Dielectric properties of a plant material quantify the interaction between the electromagnetic field (microwave energy) and the plant material during microwave heating (Sosa-Morales, 2010). Measurements of dielectric properties at certain desired frequencies and temperatures are essential because these data can be used to identify and understand selective heating within heterogeneous plant mixtures (Nelson, 2006). When a component is selectively heated, it can be maintained at a higher temperature than other components within a heterogeneous system.

For practical application, the dielectric property of interest is the loss tangent ($\tan\delta$), which is the ratio of the dielectric loss factor (ε'') to dielectric constant (ε'). It defines the relative ability of a substance to be heated at a particular frequency and temperature. Substances with high $\tan\delta$ values will be heated more than those with low $\tan\delta$ values, and thus $\tan\delta$ can be used to indicate whether selective heating will occur within a given system.

The measurement of ε' and ε'' of okra and the deionised water as a function of temperatures are measured at 2470 MHz and the results are displayed in Figure 5.1. The variation of loss tangent with temperature for okra and the deionised water used in this study is shown in Figure 5.2, with these measurements carried out independently of the microwave assisted extraction tests.

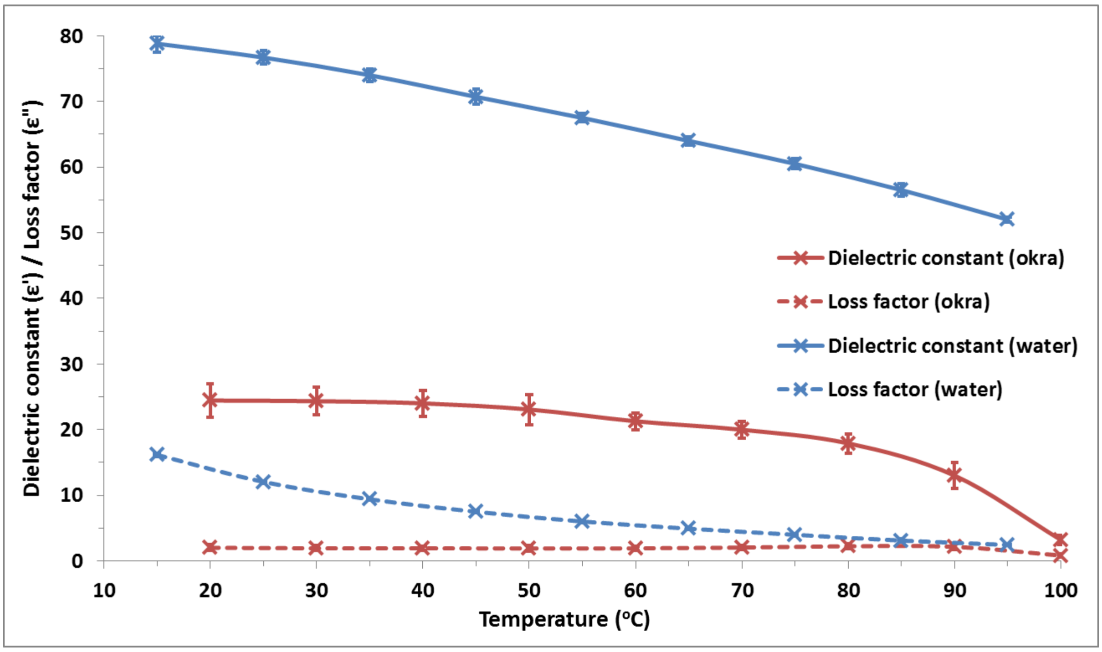


Figure 5.1: Dielectric constant (ε') and loss factor (ε'') of okra and deionised water

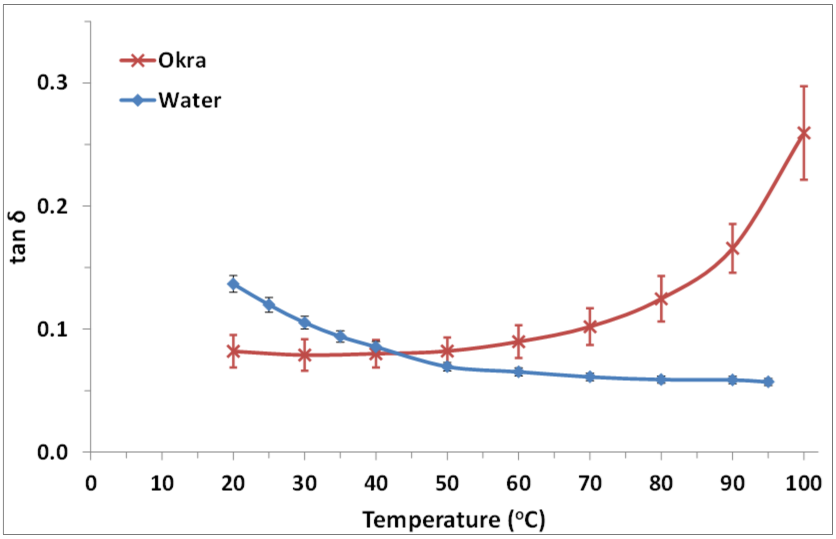


Figure 5.2: Loss tangent (tan δ) of okra and deionised water

As shown in Figure 5.1, dielectric constant as high as 25 could be obtained at room temperature because of the high water content in okra (90.93% ± 0.11 w/w on wet basis) which can easily absorb microwave energy based on the mechanism of dipolar rotation (Sahin, 2006). This indicates that okra is a good absorber of microwave energy. It was observed that the dielectric constant of okra (25) at room temperature was lower than the dielectric constant of water (77). This is probably due to limited free water contained within the okra when compared to water. It has been reported that dielectric constant in the microwave region is mainly contributed by the free water whereas bound water contributes less to the dielectric constant because the irrotationally bound water is unable to respond to the alternating electric field at such frequencies (Stuart, 2001). It can be noted that the dielectric constant of okra decreased with temperature, and the rate of decrease was found to be much higher from 50 to 90 ˚C.

The loss factor of okra in the range of 2 to 2.5 verify that okra can be classified as a dielectric material which possesses the ability to convert microwave frequencies into heat (Sosa-Morales, 2010). As tan $\delta = \varepsilon''/\varepsilon'$, hence tan δ has an inverse relationship with ε'. Figure 5.2 shows that tan δ of okra increased gradually with increasing temperature due to the decreasing effect of ε' with temperature shown in Figure 5.1. It implies that the ability of okra to absorb and convert microwave energy and dissipate heat to surrounding molecules was enhanced with increasing temperature (Veggi, 2013). This phenomenon suggests that selective heating of okra can occur at temperatures above 45 ˚C, and correlates with the observation of increasing of extract yield with rising of extraction temperature (Figure 4.2). The correlation of tan δ of okra with selective heating is discussed further in section 5.1.2.

5.1.1 Penetration depth

After obtaining the dielectric properties, the penetration depth of the electric field within okra can be calculated according to Equation 2.1. The penetration depth (d_p) is usually defined as the depth into a sample where the microwave power has dropped to 36.8% of its transmitted value (Meredith, 1998; Sosa-Morales, 2010). It is important to note that there is heating at the depth exceeding d_p. The heat dissipated in the layer bounded by the surface and the plane at depth d_p is 63.2% of the total, the balance being dissipated in the material at depth greater than d_p (Meredith, 1998).

The penetration depths of okra at different temperatures are calculated following Equation 2.1(a) and the results are presented in Figure 5.3.

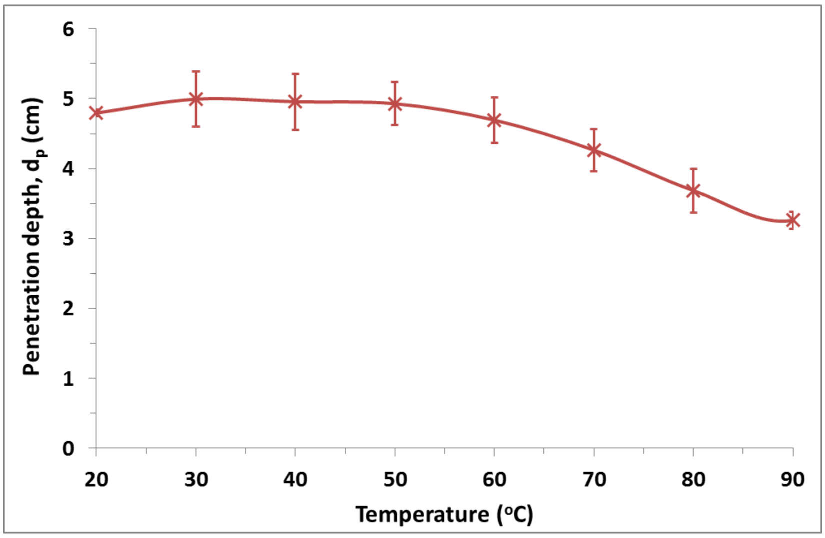


Figure 5.3: Penetration depth of okra as a function of temperature

It was found that the penetration depth of okra showed a decreasing trend for temperatures beyond 50 ˚C. By referring to Equation 2.1(b), the d_p is directly proportional to ε' but inversely proportional to ε''. Therefore, if the ε' of okra started to decrease after 50 ˚C whereas ε'' has no significant change in value (Figure 5.1), which in turn, directly influences the penetration depth (Meredith, 1998).

Figure 5.3 evidently shows that the penetration depths of okra at various temperatures from 20 to 90 ˚C are in the range of 3.3 to 5 cm. According to other studies, the penetration of microwaves at 915 and 2450 MHz in foods with high moisture content at room temperature is typically between 0.3 and 7 cm, depending on the salt content and frequency (Sosa-Morales, 2010). In the economic feasibility study (Chapter 6), the diameter of the microwave extractor was designed to be within 10cm to enable efficient penetration of microwave energy into okra during the heating process.

5.1.2 Correlation of dielectric properties with selective heating

The dielectric property data presented in Figure 5.2 shows the same transition point at around 40 to 50 ˚C that was observed in Figure 4.2. Below 45 ˚C water (the solvent) has a higher $\tan\delta$ value than okra. In this case water will be heated selectively during microwave heating, so the water solvent will be at a higher temperature than the okra during the heating process. Above 45 ˚C the $\tan\delta$ of okra is higher than water, which implies that okra will be heated preferentially within this temperature range and will therefore be at a higher temperature than water during microwave heating.

The findings from dielectric measurement suggest that at temperatures below 45 ˚C the microwave extraction process will behave in the same manner as a hydrothermal extraction process, the only difference being the technique used to heat the water. For hydrothermal and microwave processes below 45 ˚C okra will be heated primarily due to heat transfer from the surrounding water (Figure 5.4 on left), with little direct heating of the okra taking place due to interactions with the electric field component of the microwave. On this foundation it would be expected that the extraction mechanisms would be the same for both hydrothermal and microwave methods, and this is supported by the data of extract yield shown in Figure 4.2 at temperatures below 50 ˚C.

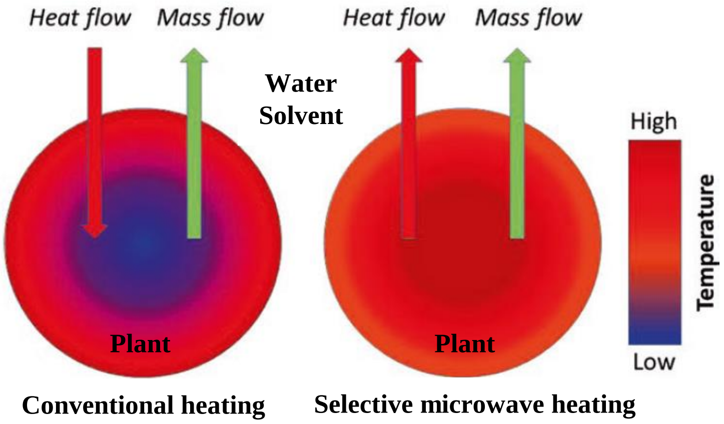


Figure 5.4: Schematic illustration of heat and mass flows in cross-section of plant subjected to conventional hydrothermal and selective microwave heating, reproduced with permission from Springer (Chen, 2015)

Above 45 ˚C okra will be heated selectively, meaning that it will be at a higher temperature than the surrounding water and heat transfer taking place in the opposite direction (from plant to the solvent, Figure 5.4 on right) compared to the case below 45 ˚C. The dielectric property measurements shown in Figure 5.2 propose that it is highly likely that the enhancements in yield with microwave heating at 50 ˚C and above are due to selective heating of okra, and that a different extraction mechanism subsequently occurs due to this phenomenon.

In the previous chapter it was shown that variation of agitation speeds at extraction temperature of 70 ˚C did not present any impact on the yield (Figure

4.4). It is now thought that selective heating of okra occurs at this temperature, and heat subsequently transfers from the interior of the plant to the solvent due to the temperature gradient. Hence, stirring of the solvent did not lead to any significant improvement in heat transfer.

5.2 Extraction mechanism due to selective heating

5.2.1 Evaluation of the possibility of physical cell wall disruption

Enhancements in the rate and yield of extraction processes when microwave heating was used have been reported by several previous studies (Farhat, 2011; Spigno, 2009; Wang, 2010). It was reported that the increased yield and kinetics are due to elution and dissolution of the active compounds within solvent once the plant cell walls are disintegrated or ruptured (Choi, 2006). Whilst the rupture or disintegration of cell walls is the most likely explanation for the empirical observations, it is still unknown how selective heating using microwaves can lead to this phenomenon.

A physical mechanism has been suggested by several authors to explain the observed cell wall disruption using microwave heating (Flórez, 2015; Rodriguez-Jasso, 2011; Yeoh, 2008). They proposed that sudden internal superheating by microwaves causes liquid vaporisation and thus steam is created within the cellular structures during the heating process. The induced vaporisation of water and creation of steam increase the pressure within the cell wall, and the pressure build-up could exceed the capacity for cellular expansion (Ferhat, 2007). This could subsequently cause the cell wall to crumble or rupture more readily, consequently leading to cell wall swelling and rupture. Once the plant cell structure is broken down, the capillary-porous structure of the tissues increases, facilitating faster diffusion of the solutes into the surrounding solvent. Microscopy images are frequently used to qualitatively support this theory (Choi, 2006; Farhat, 2011); however, it is typically difficult on close examination to see any definite difference between the images obtained from microwave and conventionally treated samples.

To date, there has been no attempt to determine quantitatively how microwave heating can lead to enhanced cell wall rupture. The aforementioned physical mechanism was verified in this study. It is possible to estimate the pressures required causing cell wall rupture, and consequently the temperatures needed to raise steam at this pressure. From a theoretical standpoint it is known that the elastic modulus for epidermal, subsidiary, and mesophyll cells are in the range of 40 to 240 bar, 30 to 200 bar, and 6 to 14 bar, respectively (Zimmermann, 1980).

The yield stress, i.e. the pressure at which cell wall rupture occurs, can be estimated as the point at which 30% strain deformation of the cell wall occurs due to the increase in pressure (Pieczywek, 2014). For epidermal cells this equates to a pressure of around 12 to 72 bar for cell wall rupture to occur. If physical disruption was to occur then the vapour pressure of water within the cell wall would need to approach the pressure value in the range of 12 to 72 bar, which would require an internal temperature (in the cell wall) in the range of 188 to 288 ˚C. A similar analysis applied to subsidiary and mesophyll cells gives temperatures in the range of 175 to 275 ˚C and 117 to 145 ˚C respectively. It is highly unlikely that these temperatures could be achieved by selective heating, as heat transfer from the plant cell to the surrounding solvent would limit the internal temperature that could be achieved.

The analysis of physical cell wall disruption suggests that cell wall rupture due to vapour pressure elevation is highly improbable to occur given the large temperature difference needed between the heating temperature and the internal temperature within the plant cell. An alternative mechanism must therefore exist, which will result in pressures high enough to cause cell wall disruption but at much lower internal temperatures. The alternative microwave heating mechanism was investigated and developed in this study, and the detail of this mechanism was presented in next section.

5.2.2 Mass transfer characteristics during selective heating

Typically, the driving force for mass transfer to occur is due to a difference in chemical potential (μ), and substances move along a chemical potential gradient (Christodoulou, 2003; Pushpavanam, 2012), and in the case of aqueous extraction processes both the solvent (water) and solutes can move across cell-wall boundaries. Osmosis and Osmotic Potential describe this phenomenon under conventional conditions where the water can flow across the plant wall membrane when an osmotic pressure difference exists between two sides of the membrane and temperatures are equal between the plant cell and surrounding water (Christodoulou, 2003).

However, the thermodynamic basis of osmosis is not valid when a temperature gradient exists, as is the case during selective heating. In this case chemical potential, from which osmotic pressure calculations are derived, is the fundamental parameter that governs water and solute equilibria as it can be applied for variable pressure, temperature and water activity (i.e. composition) (Quant, 2008). There are numerous studies that present mass transfer models for pressure-driven transport through cellular structures using chemical potential as a basis (Elmoazzen, 2009; Gekas, 2002); however the effect of a temperature gradient on mass transfer has not been reported.

When selective heating takes place the plant material is hotter than the surrounding solvent phase. In this case the water and solute within the plant material are at a higher temperature than the water and solute in the solvent phase. Chemical potential decreases with increasing temperature (Atkins, 2014), so a temperature difference between the plant material and surrounding water will impact on the chemical potential gradient and hence water and solute transfer. A qualitative illustration of this principle was developed in this study and is shown in Figure 5.5 with consideration of three different stages during microwave heating.

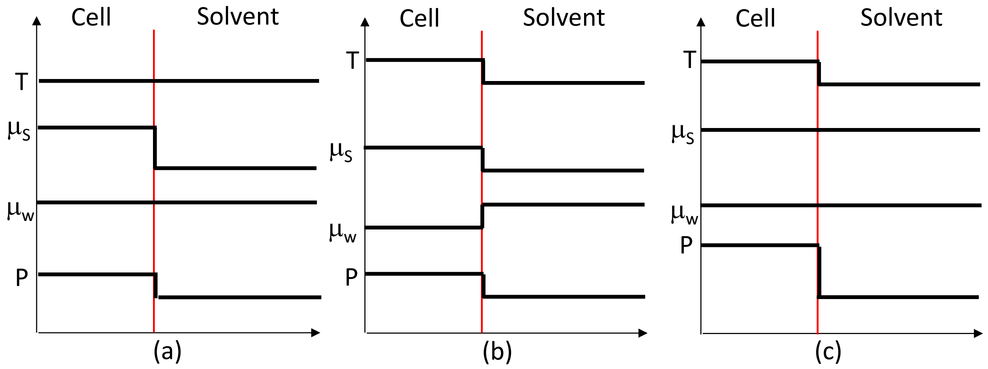


Figure 5.5: Illustration of pressure (P), temperature (T) and chemical potential (μ_s for solute and μ_w for water) across a cell wall boundary for microwave heating mechanism developed in this study. (a) Constant temperature with no selective heating, (b) Selective microwave heating, (c) Equilibrium during selective microwave heating

In case (a) selective heating of okra has not occurred and the temperature is constant between the plant material and surrounding solvent. A chemical potential gradient exists for the solute due to a difference in concentration between the plant cell and solvent phase, which leads to diffusion of the solute into the solvent as observed in hydrothermal extraction processes. The chemical potential for water is equal between the plant cell and solvent phases as a result of diffusion of water molecules from solvent phase (higher water concentration) to plant cell (lower water concentration) due to osmosis. The transport of water into the plant cell structure raises the pressure within the plant cell and balances the chemical potential of water between each phase. If case (a) in Figure 5.5 proceeds to equilibrium, then the solute chemical potentials will also be equal in each phase because the solute concentration in the solvent will increase μ_s.

In case (b) the plant cell is heated selectively during microwave processing, this causes the chemical potential of both water and solute to decrease within the plant cell. A chemical potential gradient exists for water between the plant cell and surrounding solvent, which causes more water to diffuse into the plant cell. As water transfers into the plant cell the pressure within the plant cell increases, which acts to increase the chemical potential of water within the plant cell. The solute chemical potential in the plant cell also decreases due to the increase in temperature, leading to a smaller chemical potential gradient than the constant temperature case.

Case (c) shows the result of the selective microwave heating process proceeding until equilibrium is reached when the chemical potentials of solute and water are the same in the plant cell and solvent phases (IUPAC, 2014). A higher pressure is achieved within the plant cell due to diffusion of water into the plant cell to increase the cell-side chemical potential of water such that it equals that in the solvent phase. When the chemical potential of water in both phases is the same and that of solute in both phases are the same, then the process stops and equilibrium stage is attained (De Nevers, 2012).

Figure 5.5 shows how selective heating can influence solute and water transport during an extraction process. The implications are that selective heating results in a lower driving force for solute mass transfer from plant cell to solvent phase, which is contrary to the experimental findings in Figure 4.2. However, selective heating influences water transport significantly, and it is this phenomenon that leads to a big difference of pressures within the plant cell compared to hydrothemal extraction processes. If the pressure within the plant cell is high enough then cell wall rupture/disruption could be induced under selective heating conditions, which would decrease the resistance to mass transfer of solutes and therefore explain the observations in Figure 4.2. The magnitude of the equilibrium pressure in case (c) can be estimated by developing a quantitative analysis of the mass transfer process, and can subsequently be compared to known pressure limitations within plant cells to identify the likelihood of cell wall rupture/disruption due to selective heating.

5.2.3 Quantitative model for equilibrium pressure

Equilibrium stage is attained when the chemical potential of water within a plant cell ($\hat{\mu}_{wc}$) equals that of water outside the plant cell ($\hat{\mu}_{ws}$) (De Nevers, 2012):

$$\hat{\mu}_{wc} = \hat{\mu}_{ws} \tag{5.1}$$

The ˆ notation denotes that water is within a mixture, i.e. solutes are present. Subscript c relates to the plant cell, and subscript s relates to the surrounding solvent. The chemical potential of water within a generic mixture depends on the temperature, pressure and composition according to Equation 5.2 (IUPAC, 2014):

$$\hat{\mu}_{w} = \mu_{w}^{0} + RT\ln\left(\frac{\hat{f}_{w}}{f_{w}^{0}}\right) = \mu_{w}^{0} + RT\ln\left(\frac{a_{w} f_{w}}{f_{w}^{0}}\right) \tag{5.2}$$

where μ_{w}^{0} is the chemical potential of pure water at temperature T and a standard pressure (usually taken as 1 bar), $\hat{f}_{w}$ is the fugacity of water within the mixture at T, f_w is the fugacity of pure water at T, a_w is the water activity at T and f_{w}^{0} is the fugacity of pure water at T and the standard pressure. If the water outside the plant cell is assumed to be solute free then $a_{ws}=1$, and combining Equations 5.1 and 5.2 yields:

$$\mu_{wc}^{0} + RT\ln\left(\frac{a_{wc} f_{wc}}{f_{w}^{0}}\right) = \mu_{ws}^{0} + RT\ln\left(\frac{f_{ws}}{f_{w}^{0}}\right) \tag{5.3}$$

The fugacity of liquid water at temperature T is related to pressure according to Equation 5.4 (Narayanan, 2013):

$$\ln f_w = \ln f_w^* + \frac{v_w}{RT}\left(P - p_w^*\right) \tag{5.4}$$

f_w^* is the fugacity of water at saturation, v_w the molar volume of water at T and p_w^* the saturated vapour pressure at T. Combining Equations 5.3 and 5.4 gives:

$$\mu_{ws}^0 - \mu_{wc}^0 = \left(P_c - P_s\right)v_w - RT\ln a_{wc}$$

(5.5)

where P_c is the pressure within the plant cell and P_s is the external pressure within the solvent. When the temperature is constant between plant cell and solvent and an ideal mixture exists then $\mu_{ws}^0 = \mu_{wc}^0$ and $a_{wc} = x_{wc}$, and Equation 5.5 reverts to the classical definition for osmotic pressure (Bergethon, 2010). However, when selective heating takes place the assumption of constant temperature cannot be made, so a different approach is required. In this case there are two temperature values, T_c and T_s, μ_{ws}^0 and μ_{wc}^0 will have different values based on the temperature of the solvent and plant cell respectively and Equation 5.5 could be extended as displayed below.

$$\mu_{ws}^0 - \mu_{wc}^0 = RT_c\left[\ln a_{wc} + \ln f_w^{*T_c} + \frac{v_w^{T_c}}{RT_c}\left(P_c - p_w^{*T_c}\right) - \ln f_w^{0T_c}\right] - RT_s\left[\ln f_w^{*T_s} + \frac{v_w^{T_s}}{RT_s}\left(P_s - p_w^{*T_s}\right) - \ln f_w^{0T_s}\right]$$

Extension of Equation (5.5)

If the change in chemical potential with temperature for pure water is quantified then Equation 5.5 can be adapted to estimate the equilibrium pressure within the plant cell for a variable temperature case, provided that a_{wc} is known or can be estimated.

At the standard pressure of 1 bar $f_w^0 = f_w^*$ as water is liquid under these conditions. Fugacities at saturation can be approximated to the saturated vapour pressure (Narayanan, 2013), which gives the final result shown by Equation 5.6:

$$P_c = p_w^{*T_c} + P_s - p_w^{*T_s} + \frac{\mu_{ws}^0 - \mu_{wc}^0}{v_w} - \frac{RT_c}{v_w}\ln a_{wc} \tag{5.6}$$

Equation 5.6 can be solved for P_c for fixed values of T_s and T_c based on widely available thermodynamic data for v_w and p^* (Lide, 2010). The water activity within the plant cell, a_{wc}, is dependent on the solutes present within the plant cell and cannot be measured directly; however it can be estimated from osmotic pressure measurements at constant temperature. The water activities for high moisture plant materials were reported to be in the range of 0.95 to 1 (Maltini, 2003). The change in chemical potential with temperature can be related according to Equation 5.7 below (Job, 2006).

$$\Delta\mu = \alpha\Delta T \tag{5.7}$$

The parameter α is a temperature coefficient, which is numerically equivalent to $-s$, where s is the entropy over the temperature range. For liquid water the standard state entropy is 80 J/mol K at 70 ˚C (Allison, 2016; Lide, 2010), the detail of calculation is shown in Appendix. For μ_{ws}^{0}, it can be calculated from the bulk water temperature and μ_{wc}^{0} can be estimated based on an assumed plant cell temperature. When $T_s = T_c$ then $\mu_{ws}^{0} = \mu_{wc}^{0}$ and Equation 5.6 reverts to the standard expression for osmotic pressure, and a_{wc} values can subsequently be calculated from osmotic pressure measurements.

T_s was chosen to be 70 °C because the bio-flocculant with solutes high in dewatering activity was best extracted at temperature $\geq$ 70 ˚C (Figure 4.12). A range of water activities were chosen such that the equilibrium pressure within the plant cell at constant temperature gave values within the range of known osmotic/Turgor pressures for plant materials. The osmotic pressure of plant cells exhibits a very wide range. Among higher plants (plants of relatively complex or advanced characteristics) the osmotic pressure rarely falls below 3.5 bar while at the other end of the range an osmotic pressure as high as 153 bar has been recorded (Stiles, 2006). However, osmotic pressures of the cells of land plants are usually from about 10 to 20 bar (Stiles, 2006). The lowest value for a_{wc} was chosen to be 0.96, which corresponds to an osmotic pressure of 64 bar. The equilibrium pressure as a function of temperature difference between plant cell and solvent for a range of water activities within the plant is shown in Figure 5.6.

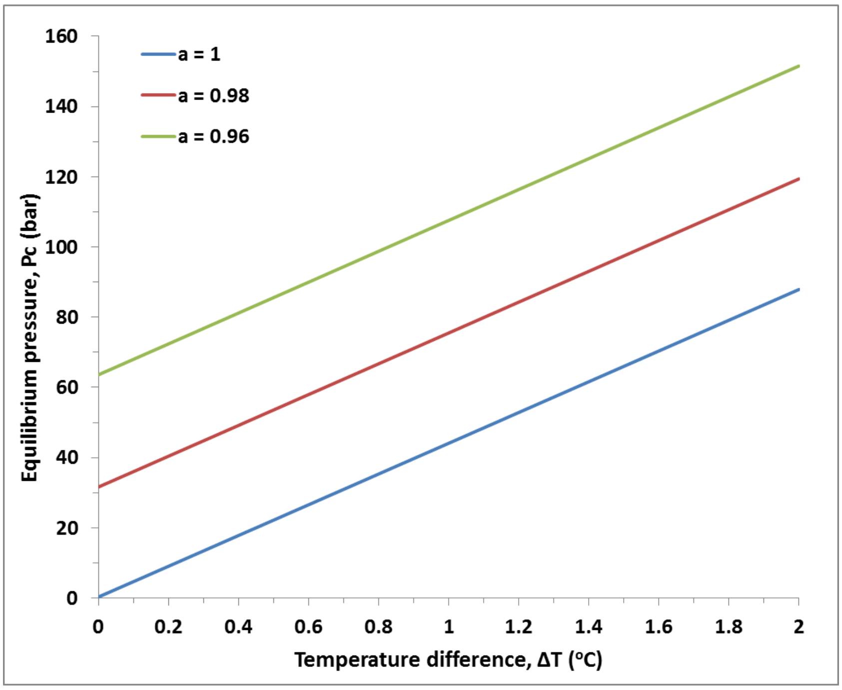


Figure 5.6: Theoretical equilibrium pressure as a function of temperature difference between plant and solvent for varying water activity within the plant material. Baseline solvent temperature = 70 ˚C

The predicted equilibrium pressure within the plant material varies linearly with the temperature difference between the plant cell and surrounding water. The *y*-intercepts shown in Figure 5.6 represent the osmotic pressure (absolute) at 70 ˚C for the range of water activities investigated. A solute free plant system ($a_{wc} = 1$) has an osmotic pressure of 1 bar, by definition, but shows that an equilibrium pressure of around 44 bar would be achieved if the plant material was 1 ˚C hotter than the surrounding water. When solutes are present within the plant system then the equilibrium pressures are higher still. When a_{wc} = 0.96, a solute concentration equivalent to an osmotic pressure of 64 bar, the equilibrium pressure exceeds 100 bar with a temperature difference of just 1 ˚C.

Whilst water activity within the plant cell is not known for the okra/water system used in this study, it is apparent from Figure 5.6 that a temperature difference of a few degrees will be sufficient to induce an equilibrium pressure that will exceed the yield stress of most cellular structures, irrespective of the water activity within the plant material (Pieczywek, 2014). When the yield stress is reached then cell wall disruption is likely to occur, which reduces the resistance to solute mass transfer into solvent and results in a step-change in the extraction kinetics.

The theoretical predictions shown in Figure 5.5 imply that cell wall disruption due to increased water uptake is the most likely mechanism that takes place during MAE when selective heating occurs, and it is this mechanism that accounts for the difference between microwave and hydrothermal heating. With hydrothermal heating the plant material and solvent are at the same temperature, so there is no net chemical potential gradient for water transport into the plant cell.

When microwave heating is applied at temperatures below 50 ˚C there is no selective heating of the okra, hence no chemical potential gradient for water and the process behaves in the same manner as conventional hydrothermal extraction. For extraction above 50 ˚C the okra heats selectively due to its tan δ value being higher than water, and under these conditions the okra is at a higher temperature than the surrounding water. A temperature difference of the order of 1 ˚C is sufficient to induce an equilibrium pressure within the plant cell of around 100 bar, which will exceed the yield stress and lead to disruption of the cell wall structures.

The previously-proposed physical disruption mechanism based on vapour pressure of water requires a temperature difference between plant cell and solvent of the order of at least 100 ˚C before pressures are sufficient to overcome the yield stress of the plant cell. It is therefore more likely that cell wall disruption occurs due to a gradient in water chemical potential, which subsequently results in the yield stress being exceeded when the temperature difference is of the order of 1 ˚C.

The implications of this newly-proposed mechanism could be far-reaching in the field of microwave-assisted extraction, and other mass-transfer limited processes. Whilst the exact extraction mechanism is yet to be fully-proven and is likely to be dependent on the nature and location of the target extract, this study has categorically shown that significant enhancements in yield and extraction rate can be achieved provided that selective heating occurs.

Measurement of the dielectric properties of the system components across the process temperature range is essential in order to develop this understanding. The cell wall disruption mechanism based on chemical potential is likely to apply for a wide-range of systems that use other solvents for extraction and/or reaction purposes where mass-transfer is the rate-determining step, and consequently this work could lead to an enhanced understanding and improvement in process performance where MAE is utilised.

The scalability of microwave processing for industrial use has already been demonstrated for extraction (Filly, 2014; Petigny, 2014) and hydrocarbon removal processes (Buttress, 2016; Robinson, 2010b). The findings of this work can be used to improve both yield and efficiency of these processes by identifying an optimum temperature based on the dielectric properties of the feedstock to ensure that selective heating is achieved. Selective heating could also provide a rationale for the choice of solvent to be used within the process, and could also enable the extraction of thermally-sensitive compounds by creating a small thermal gradient, thereby offering a route to process materials which would be degraded if current processing technologies are used.

5.3 Postulation of bio-flocculation mechanism

In contrast to the well-known mechanisms of flocculation in colloidal systems, the study of the mechanisms of bio-flocculation is lacking and has not been investigated extensively. As the chemistry of coagulation and flocculation primarily depends on the electrical properties, analysis of zeta potential is an essential first step to measure the magnitude of electrical charge surrounding the colloidal particles and bio-flocculant (Al-Hamadani, 2011). This information is needed to predict the plausible bio-flocculation mechanism.

The kaolin suspended particles are colloidal particles which remain stable indefinitely in water and show no tendency to aggregate because of their electrical charge. Destabilisation of colloidal suspension must happen to induce the flocculation process, which is the agglomeration of destabilised particles to form flocs. Commonly, destabilisation could be achieved by two main mechanisms including charge neutralisation and particle bridging, or a combination of both mechanisms.

Charge neutralisation followed by electrostatic attraction occurs if a bio-flocculant that carries a charge opposite to the net surface charge of the colloidal particles is added into the kaolin colloidal solution. Due to adsorption of highly charged bio-flocculant on oppositely charged particles (Figure 5.7), the surface charge will be neutralised and the repulsion between particles will be eliminated, allowing the particles to aggregate (Dobias, 2005).

The zeta potentials (magnitude of charge at the shear surface) of okra bio-flocculant and kaolin suspension were −25.3 ± 3.5 mV and −24.4 ± 5.8 mV, respectively. If charge neutralisation was the main mechanism for the flocculation, flocculation should occur when the zeta potential of the particles is sufficiently low to eliminate repulsion between them. However, the zeta potential of the mixture of bio-flocculant and kaolin suspension retains a large negative value, −49.7 mV, suggesting that bridging, instead of the charge neutralisation, is more likely to be the dominant mechanism.

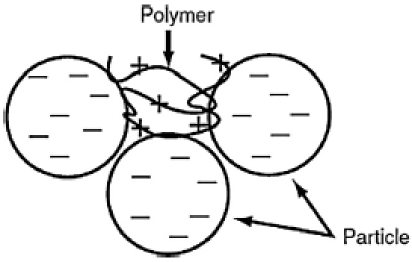


Figure 5.7: Schematic view of charge neutralisation mechanism, redrawn with reference to (Dobias, 2005)

Bridging is usually associated with the use of high molecular weight flocculants with cationic or anionic charge. The effectiveness of the bridging mechanism largely depends on the molecular weight of the biopolymers (Okaiyeto, 2016). The biopolymers extracted from okra pods are pectin-like random coil polysaccharides made up of galactose, rhamnose, and galacturonic acid units with some fractions of glucose, mannose, arabinose, and xylose (Thakur, 2015). Even though its molecular weight is not measured in this study, high molecular weight polysaccharides extracted from okra pods in the range of 1.2 to 5.6 MDa has been reported in the literature (Vera, 2009).

When long biopolymer molecules are adsorbed on the surface of kaolin particles, they tend to form long loops and tails that extend or stretch into solution far beyond the electrical double layer (Figure 5.8(a)). These 'dangling' loops and ends may come into contact with, and attach to the available sites on the adjacent particles, forming 'bridging' between particles (Figure 5.8(b)) (Lee, 2014b). It is believed that this is the bridging mode of bio-flocculation which leads to formation of dense and strong flocs that shows high sludge dewatering performance.

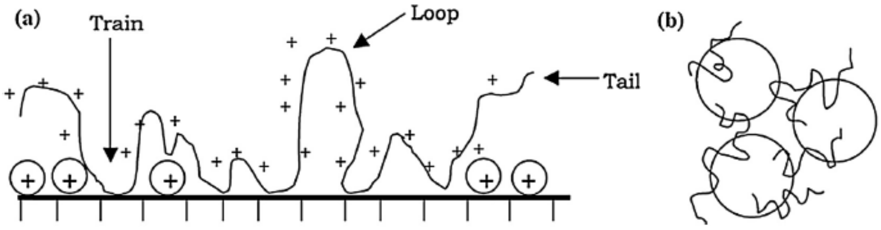


Figure 5.8: Adsorption of biopolymers and formation of loops available for binding, (b) polymer bridging between particles (aggregation), with permission from Springer (Sharma, 2006)

Polymer bridging has been proposed as the bio-flocculation mechanism in some bio-flocculant studies and the polysaccharides that are contained in the bio-flocculants are believed to be the main ingredients that exhibit the flocculating property (Mishra, 2006a; Zhang, 2013) . However, the active ingredients of the bio-flocculants and their mechanisms during bio-flocculation process were not entirely clear and have not been scientifically proven as yet. Consequently, identification of the active ingredients of bio-flocculants combined with determination of the molecular weight of biopolymers is a crucial future work to elucidate the underlying bio-flocculation mechanism.

5.4 Safety of okra bio-flocculant and its applications

Okra extract is reported to be non-toxic, and it degrades in the colon due to activity of anaerobic microbes (Choudhary, 2014). It has been investigated as a hydrophilic polymer in the pharmaceutical industry. The high viscosity characteristic of okra extract is advantageous for its retarding effect when used in sustained release formulations, as the higher the viscosity, the slower the drug release (Thakur, 2015). Use of okra gum as a binder for tablet formulations resulted in enhanced hardness, friability, and drug release profiles outperforming sodium alginate in a research study (Zaharuddin, 2014a). In African and Asian countries, okra extract has long been used in food industry to impart a slimy consistency to soups and stews (Stephen, 2006). It has been applied in clarification of sugar cane juice in India, however its clarification quality was less superior compared to other clarification agents (Whistler, 1993). Based on abovementioned literature evidence, the okra bio-flocculant should possess no risk or harm to environment and human and could be used directly after extraction.

In this study, the flocculating and sludge dewatering properties of okra extract were verified and proved to be as efficient as or even more effective than polyacrylamides. Therefore, okra bio-flocculant serves as an attractive alternative to replace polyacrylamides or other flocculation agents in water and wastewater treatment or flocculation/clarification process in other industries. Possible applications of plant-based bio-flocculant in different industries such as food and beverage, mineral, paper-making, oleo-chemical and biodiesel have been reviewed in Chapter 2. Hence, the future work on exploration of application boundaries to new areas of industrial interest is highly important to increase the application value and market demand of okra bio-flocculant.

CHAPTER 6: ECONOMIC FEASIBILITY STUDY

Taking new processes to industry requires not only technical feasibility, but also economic attractiveness. It is necessary to have reliable assessment of the potential benefits associated with its use in large scale operations. The economic feasibility of using conventional hydrothermal and microwave assisted extraction methods for production of industrial scale okra bio-flocculant was evaluated and compared in this chapter. A detailed simulation was performed to model the batch and continuous production and to assess its economic performance. The total capital and operating investment, total production cost and profitability of the production process were estimated. Sensitivity analysis was also conducted to investigate the effect of annual production, extraction yield and raw material okra price on the production cost of bio-flocculant. Last but not least, an optimised and economically viable production scheme was identified and its estimated selling price was compared with polyacrylamides and food grade bio-flocculants.

6.1 Background of economic analysis

Economic evaluation using simulation software is used as a valuable tool to determine the feasibility of using conventional hydrothermal and microwave assisted extraction (CHE & MAE) processes for production of industrial scale bio-flocculant. The objective of this work is to model and design an economically viable bio-flocculant production process at the lowest production cost by using okra as the biomass feedstock via the use of SuperPro Designer v9.0 simulation software. SuperPro Designer is widely used for modelling biochemical, food, pharmaceutical, speciality chemical, as well as other continuous and batch manufacturing processes (Athimulam, 2006). In order to lower the production cost at large scale production, several optimisation strategies were investigated including (i) batch versus continuous modes, (ii) microwave versus hydrothermal extraction processes, (iii) investigation of the effects of annual production, extraction yield and raw material okra price on the production cost through sensitivity analysis.

6.2 Process description

The production of bio-flocculant consists of 4 main steps including size reduction of fresh plant materials, extraction of bio-flocculant from the plant, separation of aqueous extract from biomass residue and finally evaporation and drying to produce the final dried bio-flocculant (DF). Aqueous bio-flocculant (AF) is another alternative product that is obtained directly after separation of biomass residue from the extract. AF gives the advantage of cost-saving and lower production cost due to elimination of evaporation and drying steps when compared to DF. However, as stated in Chapter 4, AF is exposed to degradation of functional properties with storage time. With all those considerations, AF is suggested for on-site utilisation (e.g. in water treatment site) or application to other industries within short distance or limited boundary. The equipment that is suitable for each processing step as listed in Figure 6.1 is selected based on the following criteria:

a) Suitable for processing of heat-sensitive plant materials
b) Similar function to the equipment used in laboratory studies
c) Suitable for handling viscous solutions

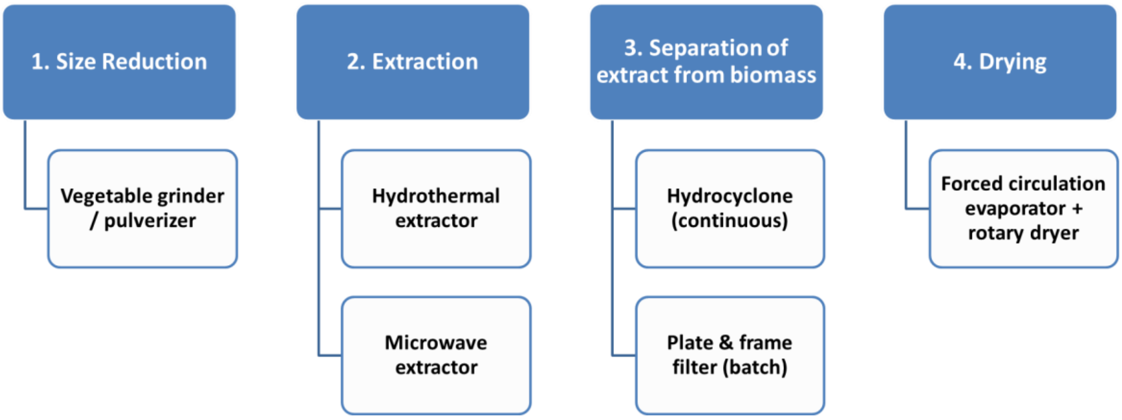


Figure 6.1: Proposed equipment options for each processing step in production of bio-flocculant

The simulation includes a mathematical model that performs material and energy balance calculations for every operation within a unit procedure (single equipment icon). On the basis of the material balances, it performs equipment sizing calculations (Papavasileiou, 2013). The lab scale optimised extraction and operating conditions are used to simulate CHE and MAE processes, and the design specification of equipment was set by referring to literature and design handbook. The basis of the full scale process assumes that the process will have identical performance with respect to the extraction yield as observed at laboratory scale if the same conditions are used.

The process plant is designed to be based in United Kingdom and the operating lifetime is assumed as 15 years. The annual operating time is fixed at 8000 hours (Heinzle, 2006) and the UK regulations are followed in the whole design process. The surplus time (760 hours annually) is the downtime reserved for inspection, maintenance, repairing and engineering work of all the equipment and the whole process plant. As with many other processes in chemical and food engineering, batch and continuous modes are considered and compared within this study. Not all the components registered in the design process are available in the software component database, and hence are approximated using reference components with similar physical/chemical properties (Table 6.1).

Table 6.1: All the components used in simulation flowsheet

Components	Nitrogen	Oxygen	Water	Fresh okra	Okra pods	Okra seeds	Okra extract	Extract waste
Reference component	None	None	None	Biomass	Biomass	Biomass	Cellulose	Biomass

The equipment arrangement for the base case extraction process is proposed as: vegetable washer → vegetable seed remover → vegetable slicer → hydrothermal or microwave extractor → plate & frame filter (batch process) or hydrocyclone (continuous process) → forced circulation evaporator → rotary dryer. An example of the process flowsheet for bio-flocculant production is illustrated in Figure B1 in the Appendix.

The supply of fresh okra is firstly washed with water in a vegetable washer. Afterwards, the washed okra is sent to a vegetable seed remover to separate seeds from the okra pods. The okra pods are then sent to a vegetable slicer to produce sliced okra with approximate width value of 0.1cm prior to the extraction step. The sliced okra is then transferred to an extractor (hydrothermal or microwave) for heterogeneous solid (sliced okra) and liquid (water solvent) extraction to obtain okra extract. For the hydrothermal extractor a turbine impeller was chosen as the mechanical agitator because it is excellent for providing circulation and effective for fluids with high viscosity (Coulson, 1999). The detailed calculation of the agitation speed and the required power is presented in the Appendix.

For microwave extraction, a microwave generator is required to generate electromagnetic energy which is delivered by a transmission line to the microwave extractor for heating the process materials. The following step is the separation of aqueous extract from the biomass residue with a plate & frame filter (batch operation) or hydrocyclone (continuous operation) with the assumption that 5% of the aqueous extract is lost and discharged as cake with extract residue. The aqueous extract could be collected at this point and sold as aqueous bio-flocculant. The calculation of the filtration period is explained in the Appendix.

The thermal efficiency of evaporators for removing water is much higher (e.g., 90%), compared to the efficiency of dryers (e.g., 60%), and in many cases evaporators and dryers are used in-line (Saravacos, 2011). This is because in an evaporator the heating medium is steam and the heat exchanged by steam when changing its state into liquid water is much higher than the one exchanged by hot air, which is the standard heating medium for dryers (Saravacos, 2016). For this reason, forced circulation evaporator is used to remove as much water as possible to concentrate the product prior to drying (Saravacos, 2011). This evaporator is the most commonly used within the food industry and suitable for processing viscous and heat-sensitive materials (Fellows, 2009) with much higher heat transfer coefficients than in any other type of evaporators for the same conditions (Sinnott, 2005). It is operated at low pressure to reduce the partial pressure of water vapour within the gas phase and increase the driving force for mass transfer without having to heat up the process material to its boiling point. It causes the liquid to evaporate at lower temperature than normal, and thus avoiding the possibility of decomposition or alteration of the heat-sensitive materials at elevated temperatures (Wang, 2007). The typical heat transfer coefficient of $3 kW/m^2.K$ is assumed for condensing steam which is a common heating medium (Seader, 2011). The boiling point elevation (BPE) of liquid food is relatively low, and high molecular weight of food or plant components dissolved or dispersed in water, such as starch, pectin and proteins give negligible BPE (Saravacos, 2011). Therefore, BPE in this case is assumed to be negligible in heat transfer calculations for the evaporator.

Finally, the concentrated bio-flocculant is dried in a rotary dryer to get a solid end product termed as dried bio-flocculant. This dryer is suitable for large scale drying of heat-sensitive materials by using hot air as the drying medium. The materials are conveyed along the inside of a rotating, inclined cylinder. When the hot gases that are flowing through the cylinder come into immediate contact with the wet material, the gas temperature is lowered rapidly and the materials are heated and its moisture content is reduced (Richardson, 2002). The evaporative capacity is assumed as 28.8 kg.m^3/h (Richardson, 2002) and the power consumption of this dryer is estimated as 0.16 kW/m^2 (Green, 2007). The calculation of the drying time is explained within the Appendix.

6.2.1 Cleaning in place (CIP)

Fouling on process plant surfaces and the subsequent cleaning needed is significant to guarantee the product quality, safety and production efficiency (Goode, 2013). CIP means that cleaning takes place without dismantling the system and is cleaned in the same set-up as it was used during production. CIP is necessary to remove any fouling materials (proteins, organic and inorganic materials and etc.) and any microbes that remain in the process line from production (Goode, 2013). Without CIP, it could impact on heat transfer efficiency and result in microbiological contamination/spoilage of the product due to a build-up of bacteria in the system or general contamination of product.

After production using processing equipment, the walls of the equipment and pipelines are more or less fouled with the biomass constituents. Generally, the higher the temperature the more fouling is deposited on the surfaces of equipment and the more burnt and hard the fouling gets. Production time is also a factor of importance regarding fouling, where long production times lead to more fouling and burnt-on fouling (Pak, 2016). The fouling matter generated in the process is the matrix of the constituents in the okra which include protein and polysaccharides. Organic foulant is most often dissolved by alkaline detergents such as sodium hydroxide (Pak, 2016).

Foulant is held on the surface by adhesive forces. Three main parameters including mechanical force, chemical force and the time the forces act are required to overcome the adhesive forces and get the foulant to leave the surface (Pak, 2016). For removal of organic foulant, 1 wt% pure sodium hydroxide is circulated through pipe work and into equipment in a cleaning circuit at the same temperature as it has been processing the products. The flow of the detergent is at least 1.5 m/s in order to create a turbulent shear force. In last step, water is used to rinse the equipment to purge the dissolved foulant and remove any residues of detergent.

Sterilisation in place (SIP) is then applied before production starts in order to kill microorganisms. The typical CIP times are 20 minutes for application of detergent and 10 minutes for rinsing and finally 10 minutes for SIP (Pak, 2016). On average, a food and beverage process in batch mode will spend about 15 to 20% of each day on CIP (about 4 to 5 hours each day) (Jude, 2013). For other industries, the frequency of CIP varies depending on the process but daily cleaning is recommended (Parker, 2007).

For this work, CIP and SIP are taken into consideration and conducted daily by assuming the cleaning cost (including the cost of cleaning solution, equipment for storage of detergent and waste, cleaning circuit, energy, and waste treatment) is directly related to the loss of production. Hence, 222 operation hours per annum are allocated for cleaning and sterilisation and the process is fully automated. Recovery CIP is performed where the cleaning detergent can be reused. By recovering the detergent, less cleaning solution will be consumed, as well as less water and energy (Pak, 2016). The aqueous waste from vegetable washer could be treated and reused as water for rinsing of equipment after applying the detergent. It is assumed that the treatment cost for aqueous waste is offset from saving in water cost.

6.3 Economic evaluation and analysis

The economic evaluation and analysis carried out for each scheme using the economic evaluation functions in the software are explained in this section. Prior to economic evaluation, the various inlet and outlet streams of the process were classified as raw material, revenue, waste or emission and then specified with their associated economic values (Alshekhli, 2011). This corresponds to the purchase cost of all the raw materials, the selling price of product for revenue and the treatment cost of generated waste or emission. The preliminary economic evaluation for manufacturing a bio-product usually involves the estimation of capital investment, estimation of operating costs and an analysis of profitability (Harrison, 2015). These provide the information needed to assess its profitability and attractiveness from an investment point of view.

6.3.1 Capital cost estimation

The total capital investment for a new process is made up of three main items: direct fixed capital (DFC), working capital and start-up and validation cost. The DFC is the total cost of the process ready for start-up. It is the cost paid to the contractors and a once only cost that is not recovered at the end of the project life (Towler, 2013). The various items of DFC are estimated based on the total equipment purchase cost (PC) using several multipliers sometimes called “Lang Factors” (Harrison, 2015). The PC is the sum of the listed equipment purchase cost and the unlisted equipment purchase cost (Heinzle, 2006). The unlisted equipment (e.g., storage silo for okra raw material, tank for storage of water from main supply, pumps, valves, transportation conveyor, condenser and vacuum pump for evaporator and so on) purchase cost is assumed to be 20% of the major equipment cost (Athimulam, 2006). Table 6.2 provides the estimated values of multipliers for various items of DFC (Harrison, 2015) considered in the Simulator.

Table 6.2: Estimated values of multipliers for various items of DFC (Harrison, 2015)

Cost item	**Average multiplier**
Total process direct cost (TPDC)	
Equipment purchase cost (PC)	
Process piping	0.40 x PC
Instrumentation	0.35 x PC
Insulation	0.03 x PC
Electrical facilities	0.15 x PC
Buildings	0.45 x PC
Yard improvement	0.15 x PC

Auxiliary facilities	0.50 x PC
Unlisted equipment installation cost	0.5 x unlisted equipment purchase cost
Total process indirect cost (TPIC)	
Engineering	0.25 x TPDC
Construction	0.35 x TPDC
Total process cost (TPC)	TPDC + TPIC
Contractor's fee	0.05 x TPC
Contingency	0.10 x TPC
Direct fixed capital (DFC)	TPC + contractor's fee and contingency

Some of the equipment purchase cost was obtained from equipment vendors and published cost data that collects the price of equipment in the form of correlating equations (Couper, 2012). The purchased equipment cost for some other equipment was calculated by referring to the cost curves with correlation in the form (Towler, 2013) of Equation 6.1 listed below.

$$C_e = a + bS^n \tag{6.1}$$

C_e = purchased equipment cost on a U.S. Gulf Coast basis, January 2010 with Chemical Engineering Plant Cost Index (CEPCI) = 532.9,
a, b = cost constants,
S = size parameter,
and n = exponent for that type of equipment.

The information about the price of the equipment is commonly not from an up-to-date source. The price of equipment changes with time owing to inflation and other market conditions. In this case, it is necessary to adjust the values considering inflation. The annual average of CEPCI is used here (Harrison, 2015) to update all the equipment cost data to March 2016 with CEPCI = 556.8 (Jenkins, 2016; Leland Blank 2011) according to the following Equation 6.2:

$$Cost\ in\ year\ A = Cost\ in\ year\ B \times \left(\frac{Cost\ index\ in\ year\ A}{Cost\ index\ in\ year\ B}\right) \tag{6.2}$$

The selection of materials of construction is made before the capital cost can be estimated as it is a major factor that will affect the purchased price of equipment. Stainless steel is commonly used as the material of construction for food and beverage processing equipment, therefore stainless steel type 304 is chosen as the corrosion resistant material for most of the equipment in this work. Polypropylene with maximum operating temperature up to 120 ˚C (Towler, 2013) is chosen as the corrosion resistant material for the microwave extractor. Thermoplastic tanks which are fabricated in black pigmented HDPE (High Density Polyethylene) material - PE100 grade and copolymer polypropylene are chosen as the waste storage tank due to its corrosion resistance, robustness and weatherable characteristics (Forbes, 2016). Material cost factors for different materials of construction for prices relative to carbon steel are considered in the calculation of equipment cost and listed in Table 6.3.

Table 6.3: Material cost factors (Sinnott, 2005; Towler, 2013)

Material of construction	**Material cost factors**
Polypropylene	0.85
Thermoplastic	0.85
Carbon steel	1.00
Stainless steel, type 304	2.25

In addition to the purchase cost of the equipment, investment is required to install the equipment. Installation costs involve costs for the freight from the factory, the unloading and handling, foundations, supports, physically putting the equipment in place and securing it, and connecting it so that it will run, piping and valves, control system, and other factors directly related to the erection of purchased equipment (Smith, 2005). The installed cost of each item of equipment are considered in the Simulator and is in the form of (Couper, 2012) Equation 6.3.

$$Installed\ cost = (purchase\ price) \times (installation\ multiplier) \tag{6.3}$$

The installation multiplier and the installed cost for each equipment are listed in Table B1 within the Appendix.

Working capital is the additional investment needed to start the process up and operate it to the point where income is earned. It accounts for cash that must be available for investments in on-going expenses and consumable materials. Most of the working capital is recovered at the end of the project. The required amount of working capital for a process is usually in the range of 10 to 20% of the DFC (Silla, 2003). In this work, the expenses of working capital to be included comprise of raw materials and labour and utilities for 4 months, negligible waste treatment and other miscellaneous expenses (Heinzle, 2006), which gives the amount of 18% of DFC. Start-up and validation costs are another significant capital investment for a process and are the costs of making the transition from construction to operation (Neil, 2005). A common value of 20% of DFC for start-up costs is considered in this case study (Harrison, 2015).

6.3.2 Operating cost estimation

An estimate of the operating cost, the cost of producing the product, is needed to judge the viability of a project. The operating cost to run a process is the sum of all on-going expenses and can be divided into two categories which are variable and fixed cost. The various components of the operating costs that are included in the Simulator with their estimated value are summarised in Table 6.4.

Table 6.4: Estimated value for various components of operating cost

Components of operating cost	**Estimated value**
Variable costs (Pereira, 2013)	
1. Raw materials	1 $/kg for fresh okra (wholesale price) (21 June 2016), 1.1 $/m^3 for water (2015/2016)
2. Utilities	0.1 $/kW.h for electricity (DECC, 2016), 12 $/MT for steam (Harrison, 2015), 0.13 $/kW.h for generation of microwave energy
3. Operating labour	30$/hour
4. Waste treatment (justification is presented afterwards)	Negligible
5. Shipping and packaging (discussion is presented in future work)	Negligible
Fixed costs (Harrison, 2015; HMRC, 16 March 2016; Sinnott, 2005)	
1. Maintenance of labour and materials (including equipment spares)	5% of DFC
2. Depreciation of fixed assets	10% of DFC
3. Laboratory, quality control and quality assurance cost	20% of operating labour
4. Supervision	20% of operating labour
5. Insurance	1% of DFC
6. Local (property) taxes	2% of DFC
7. Factory expenses (e.g., accounting, payroll, fire protection, security and cafeteria)	5% of DFC
8. Miscellaneous expenses such as R&D and other	Negligible

6.3.2.1 Variable costs

Variable costs are composed of the expenses that vary with the production rate, where this type of cost increases when the production rate is high and decreases with low production (Pereira, 2013). Each component of variable costs is explained as following:

1. Raw materials

The raw materials used in bio-flocculant production consist of okra, water solvent and the water used for washing of okra. The current wholesale price of okra at $1/kg was obtained from a trading supplier in the year 2016 (21 June 2016). There is another possible scenario where the raw material cost of okra could be eliminated if the process receives 'waste' okra materials for free and it could be used as the feedstock for extraction of bio-flocculant. The cost of the water could be reduced if the aqueous waste from vegetable washer is recycled and reused for washing of okra or for rinsing of equipment in CIP operation (Pereira, 2013). However, this step need to be properly designed and the aqueous waste must be treated before reused so that the water does not carry contaminants from one batch to the next.

2. Utilities

Utility cost was estimated based on the mass and energy balance from the computer simulation which determined the quantities of each type of utility required (Lam, 2014). The utility costs consist of electricity and steam costs. Electricity is used to run the equipment and other electrical devices, and also to generate the microwave energy. The electricity cost to generate microwave energy is 0.1 $/kWh. However, 30% of the microwave energy is consumed by the auxiliary equipment associated with the microwave unit and is taken into account. In other words, 130 kWh of electricity is required to get 100 kWh of microwave energy used for heating (Robinson, Personal communication (20 June 2016)). The steam is used as the heating medium in the evaporator and also for heating of the extraction vessel and the solvent to the desired temperature in hydrothermal extraction process. It is assumed that the steam is available and purchased for direct application.

3. Operating labour

This represents the costs related to the operators directly responsible for the processing. The operator cost is usually higher for batch process due to low levels of automation. Continuous process usually requires automated systems which imply a reduced number of direct operators (Pereira, 2013). The number of operators per shift for each unit in batch and continuous processes is shown in Table 6.5 while an example of estimation of operator requirement for batch and continuous processes is summarised in Table 6.6 below.

Table 6.5: Typical operator requirement for each equipment (Green, 2007)

	Batch process	**Continuous process**
Operators per unit per shift	Vegetable washer: 0.1 Seed removal: 0.1 Vegetable grinder: 0.1 Batch extractor: 1 Plate & frame filter: 1 Evaporator: 0.25 Rotary dryer: 0.5 Aqueous waste storage tank: 0.2 Solid waste storage tank: 0.2 Auxiliary equipment: 0.2	Vegetable washer: 0.1 Seed removal: 0.1 Vegetable grinder: 0.1 Continuous extractor: 0.5 Hydrocyclone: 0.25 Evaporator: 0.25 Rotary dryer: 0.5 Aqueous waste storage tank: 0.2 Solid waste storage tank: 0.2 Auxiliary equipment: 0.2
Total operators per shift	3.65 rounded up to 4	2.4 rounded up to 3

Table 6.6: Estimation of operator requirement (Green, 2007)

	Subject	**Calculated results**	**Remarks**
General	Annual operating hours	8736	52 weeks in a year
	Annual working hours / operator	2080	An operator is working for 40 hours weekly
	Annual labour cost ($) / operator	62400	Labour cost is $30 per hour.
	Number of operators / year	4.2 rounded up to 5	Assumption: 1 operator per shift
Batch process	Number of operators / year	20	4 operators per shift
	Annual operator cost ($)	1248000	20*62400
Continuous process	Number of operators / year	15	3 operators per shift
	Annual operator cost ($)	936000	15*62400

4. Waste handling and treatment

The cost associated with waste treatment can represent a significant proportion of the overall operating cost if it is not recovered. The order of priority in the waste hierarchy is recycling followed by energy recovery/incineration and lastly landfill.

In this case, aqueous waste is generated after the water was used to wash the fresh okra and it may contain some soil and microorganism. Water is generally recognised as safe solvent. The treatment and discharge cost for aqueous waste could be eliminated if it could be recycled and reused, however it must be treated before it is reused for washing of okra or for rinsing of equipment in

CIP operation (Pereira, 2013). There is treatment cost associated with treatment of aqueous waste but it is possible to redeem this cost through reduction of water cost. CIP operation also generates aqueous waste containing the detergent solution and water used for rinsing and this issue is addressed previously and further discussed in future work.

Furthermore, the solid waste generated in the process including okra seeds and wet biomass residue which is essentially organic materials and biodegradable, and thus can be sold or given to agricultural production sector to be used as bio-fertiliser. A popular example of such use is the application of bio-solids to soil to supply nutrients essential for plant growth such as nitrogen and phosphorus and replenish soil organic matter. The nutrients in the bio-solids offer several advantages over those in inorganic fertilisers because they are organic and are released slowly to growing plants (Wang, 2007). Okra seeds could be used for production of edible oil and okra seed flour (Kumar, 2010).

There is another sustainable option for the biomass waste to be applied in anaerobic digestion (AD) whereby the bacteria breaks down the organic materials in the absence of air, yielding a biogas containing methane and nitrogen rich bio-fertiliser (DEFRA, 22 January 2013). The biogas can be used for heating or electricity generation while the bio-fertiliser can be applied on farmland in place of fossil fuel derived fertilisers. A recent UK bioenergy statistics in 2013 reported that plant biomass (21.6%) was the primary source for generation of bioenergy compared to other such as animal biomass, domestic and industrial wood (Prime, 2013).

For these reasons, the cost of waste treatment in this work was taken to be negligible (Pereira, 2013) and it is assumed that the waste produced is the same for each processing scenario. The wastes generated are stored with the base assumption of at least 24 hours in thermoplastic tanks prior to transportation to another site for reuse or for other application. If the abovementioned options are inappropriate, the least desirable option is to send the biomass waste to landfill and create landfill methane emissions (DEFRA, 22 January 2013). The landfill costs are at least £80 per tonne and likely to increase in line with landfill tax increases (DEFRA, April 2008; HMRC, 16 March 2016). However, a constructive planning of waste utilisation and handling and disposal is imperative to be looked at within future work.

6.3.2.2 Fixed costs (Pereira, 2013)

The fixed costs do not depend directly on the production rate, and must be considered even if the process is stopped. Maintenance costs consist of labour and material components and vary with rate of operation but not linearly (Neil, 2005). An allowance of 5% of DFC is assumed for maintenance costs in this work. The depreciation cost is the cost or expense arising from natural wear or obsolescence of fixed assets (property and equipment) used in the production. Usually in economic evaluations, the depreciation time of the equipment is stipulated as 10 years, representing a cost of 10% of DFC. The taxes depend on local legislation and are about 2% of DFC. The insurance is associated with the costs to protect the company and represent 1% of DFC.

6.3.3 Profitability analysis

Estimation of the product revenues and costs of production is a key step in determining the profitability of a process (Towler, 2013). The revenues are from sales of main products, which is either aqueous or dried bio-flocculant. Dividing the annual operating cost by the annual production rate yields the unit production cost of bio-flocculant in dollars per kilogram (Harrison, 2015). The economic measures used to assess the profitability include gross margin, return on investment (ROI), payback time (PBT), internal rate of return (IRR) and net present value (NPV) (Harrison, 2015).

The sum of product revenues minus the raw material costs is known as the gross margin and expressed as a percentage (Towler, 2013). It represents the proportion of each dollar of revenue that the company retains as gross profit (Vučurović, 2012). The ROI in percentage basis is defined as annual operating income after tax divided by total initial capital (fixed capital plus working capital plus start-up costs) and a figure of 20 to 30% is expected (Athimulam, 2006; Sinnott, 2005). ROI measures how effectively the company uses its invested capital to generate profit (Heinzle, 2006). The PBT is the time required after the start of the project to pay off the initial investment from income (Sinnott, 2005). It is estimated by dividing the total initial capital by the average annual income where the taxes and depreciation are neglected (Towler, 2013). Typically, a PBT of 2 to 5 years is expected (Sinnott, 2005).

The profitability measures presented above do not consider the time value of money. However, a dollar that is earned in 5 years' time has a lower value than a dollar earned today (Heinzle, 2006). NPV considers this time-value of the earned money. NPV is the sum of the present values of the future cash flows (Heinzle, 2006). The following Equation 6.4 is the formula for calculating NPV (Towler, 2013):

$$\mathrm{NPV} = \sum_{n=1}^{n=t} \frac{CF_n}{(1+i)^n} \tag{6.4}$$

where CF_n = cash flows in year n, t = project life in years, and i = discount interest rate.

If the NPV of a prospective project is positive with assuming a discount interest of 7%, it indicates that the projected earnings generated by a project (in present dollars) exceed the anticipated costs (also in present dollars) and this investment will be profitable (Vučurović, 2012). The IRR is a discount interest rate often used in capital budgeting that makes the NPV of all cash flows from a particular project equal to zero. It is a measure of the maximum interest rate that the project could pay and still break even by the end of the project life (Towler, 2013). In other words the higher a project's IRR, the more desirable it is to undertake the project (Vučurović, 2012).

6.4 Batch versus continuous operation

In a batch extraction, a piece of equipment goes through a cycle of operations (Papavasileiou, 2013). Both solvent and substrate are fed into the extractor and the mixture is stirred to increase heat and mass transfer. After a defined extraction time, the mixture is removed and the aqueous extract is separated from the solid residue. Batch extractions generally have their used limited to processes operating at smaller scales (Martinez, 2013). In continuous processes, each piece of equipment performs the same action all the time. The solid substrate and the solvent flows continuously through the extractor for a defined time, with fixed flow rate, leaving the extractor with solute to be separated by a further step (Martinez, 2013).

Batch versus continuous operation options for hydrothermal and microwave extraction of bio-flocculants is compared from an economic point of view on the basis of the same annual production. The annual production in the range of

5 to 50 tonne/year is progressively increased to study its effect on the unit production cost of bio-flocculant and the results are presented in Figure 6.2.

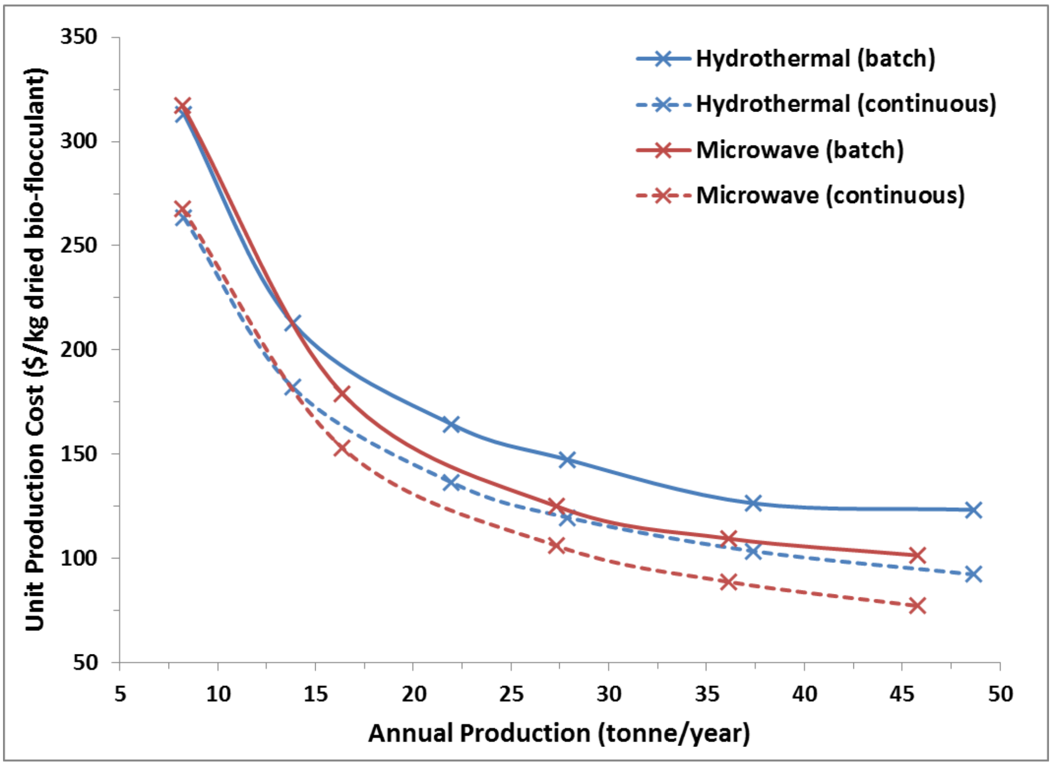


Figure 6.2: Batch versus continuous operation of hydrothermal and microwave extraction of bio-flocculants

The maximum power generated by an industrial microwave generator is 100 kW (Robinson, Personal communication (22 June 2016)). As the result, the batch extractions with microwave method is restricted to smaller scale with annual production less than 50 tonne/year due to the limitation of power generated by the microwave generator for heating of mixture of okra and solvent. Due to this reason, the comparison study between batch and continuous processes was conducted up to 50 tonne/year of annual production.

The results in Figure 6.2 show that the unit production cost for continuous operation was significantly lower than batch operation for both hydrothermal and microwave extraction processes. It indicates that continuous process that operates throughout the year tends to be more economically beneficial and profitable. To have a better idea on significant cost components that contributed to the difference in production cost between batch and continuous process, an example of three major cost components (DFC and facility dependent and operating labour costs) for batch and continuous microwave processes on the basis of similar annual production at 45.8 tonne/year are shown in Figure 6.3.

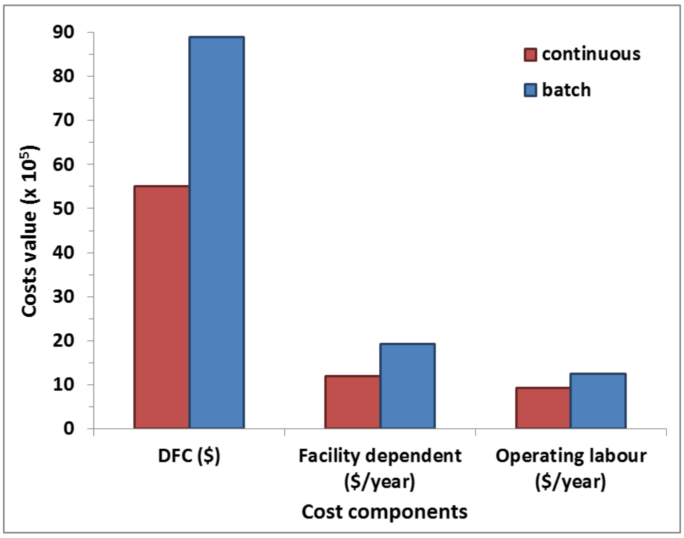


Figure 6.3: Comparison of DFC, facility dependent and labour costs for batch and continuous microwave processes

For production of similar annual production at 45.8 tonne/year, the flow rate of okra feedstock is 700kg per batch and 170 kg/h for continuous microwave process. It is clear that batch processes require larger equipment for processing of larger amounts of material. The requirement for larger equipment in batch processes increases the equipment cost, which directly contributes to the increase in DFC. In addition, the increase in DFC raises the facility dependent costs which comprise of maintenance, depreciation, taxes, insurance and factory expenses.

As presented in Figure 6.3, the DFC and facility dependent costs of batch process are 38% higher than continuous process. Furthermore, the operating labour cost for continuous process was found to be 25% less than batch process. It is because the operation of continuous process is fully automated which reduces the operator requirement and the operating labour cost. All these results revealed that continuous process exhibits the advantage of lowering the production cost and hence, the further study was conducted on the basis of continuous mode.

6.5 Hydrothermal versus microwave extraction

The economic feasibility of hydrothermal versus microwave extraction of bio-flocculants on continuous basis is compared by observing the effect of increasing annual production on the unit production cost and the results are displayed in Figure 6.4.

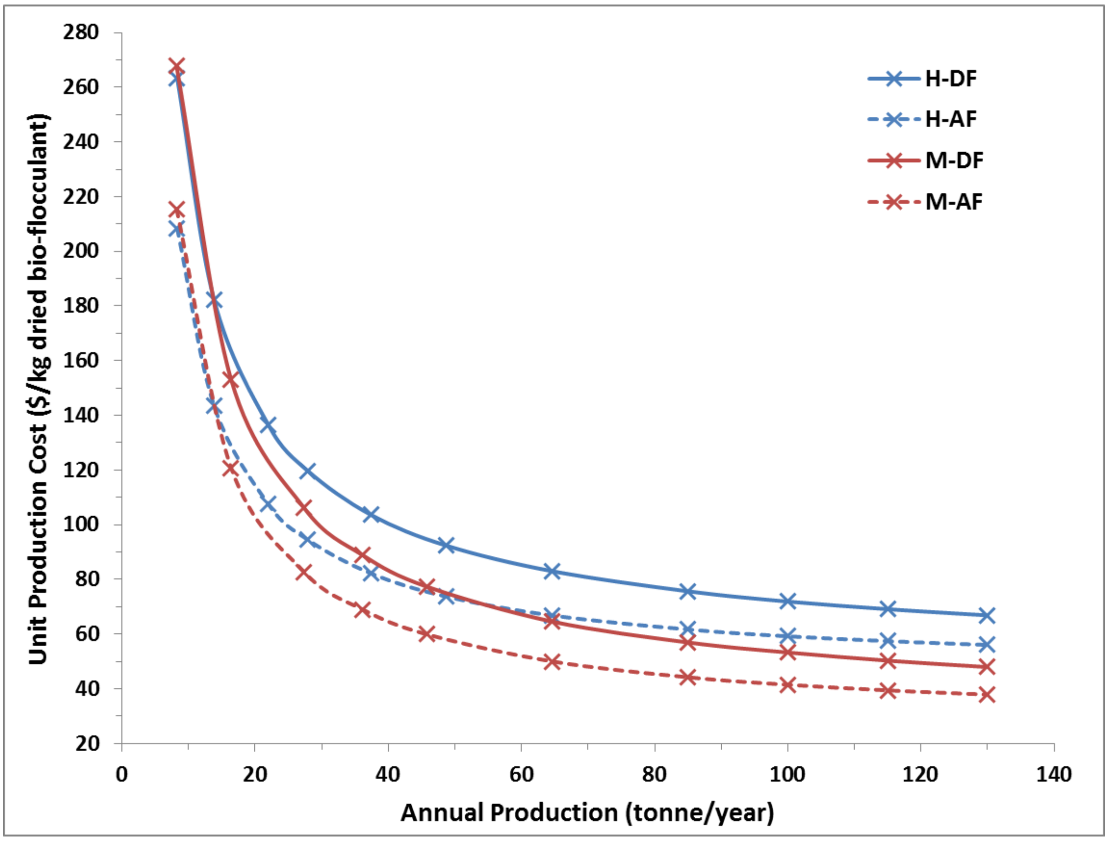


Figure 6.4: Hydrothermal versus microwave extraction of bio-flocculants (H-DF: hydrothermal extracted dried bio-flocculants, H-AF: hydrothermal extracted aqueous bio-flocculant, M-DF: microwave extracted dried bio-flocculant, M-AF: microwave extracted aqueous bio-flocculant)

For small scale production which was less than 15 tonne/year, microwave extraction process showed similar unit production cost as hydrothermal extraction process due to high capital investment of microwave generator. Conversely, for larger production capacity, microwave extraction process displayed its economic attractiveness as the unit production cost was notably lowered than hydrothermal process. Although the initial investment of microwave generator is high, however the operational costs of microwave process are lower than the hydrothermal process, and therefore it is possible to redeem the investment in a relatively short time.

Figure 6.4 also clearly demonstrated that the unit production cost was decreased with increasing annual production for both the hydrothermal and microwave processes, representing an advantage for the investment on a large industrial scale. The plausible reason is the revenue gained from high annual production could offset the annual operating cost.

In addition, it is obvious that the production costs of aqueous bio-flocculants for both hydrothermal and microwave processes were 15 to 25% less than dried bio-flocculants due to the elimination of investment cost in evaporation and drying steps. For the case in water treatment whereby the flocculation process is practically always applied subsequently before a physical separation, investment in an onsite production process of aqueous bio-flocculant is worth considering and possibly viable. It is because the cost of aqueous bio-flocculant is less than dried bio-flocculant. The aqueous bio-flocculant without undergoing evaporation and drying steps could be transferred to nearby treatment sites and applied directly and the risk of degradation of bio-flocculant quality is minimised. Even though initial investment cost is required for construction of onsite aqueous bio-flocculant process, but this amount could be counterbalanced from long term perspective through significant saving from purchasing dried bio-flocculant.

6.6 Effects of extract yield and okra price on the unit production cost

The effect of annual production on the unit production cost has been presented in Figure 6.4 and discussed in previous section. In this section, the effects of extract yields at different extraction conditions and raw material okra price on the unit production cost were investigated on the basis of 100 tonne/year of annual production. The purpose of this investigation is to identify the impact of these parameters on project viability over the expected range of variation of the parameters (Towler, 2013) and to explore possible scenarios to lower the production cost.

Each parameter is varied individually while all other variables were kept constant. The results were presented as plots of capital cost, operating cost and unit production cost versus the parameter studied. As the extraction parameters including temperature and time and solvent loading exhibit significant effect on yield, therefore the impacts of extract yields with respect to variations of extraction temperature and time and solvent loading on the unit production cost were investigated.

By referring to the operating cost breakdown shown in Figure 6.5 below, the costs of the total raw materials are found to be the dominant factor that manipulates the overall operating cost. The okra cost is the largest contributor to the raw material cost due to the low amount of the desired compounds in the plant material. The okra cost can fluctuate significantly depending upon the weather condition, supply capacity and the market demand. Hence, the influence of okra cost on the production cost was examined.

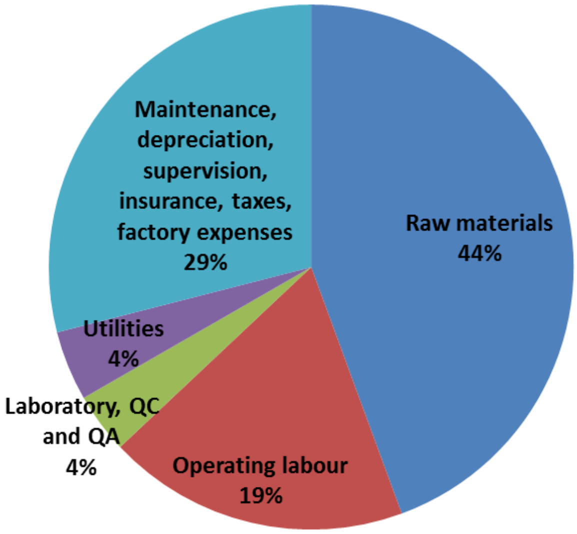


Figure 6.5: Operating cost breakdown for continuous microwave extraction process with annual production of bio-flocculant at 100 tonne/year

6.6.1 Effect of extract yield

The optimised extraction yield is essential to define the viability of the process and also to minimise the capital and operating costs (Pereira, 2013). The results for the effects of extract yields obtained at different extraction temperature and time and solvent loading on the capital, operating and production costs were presented as below.

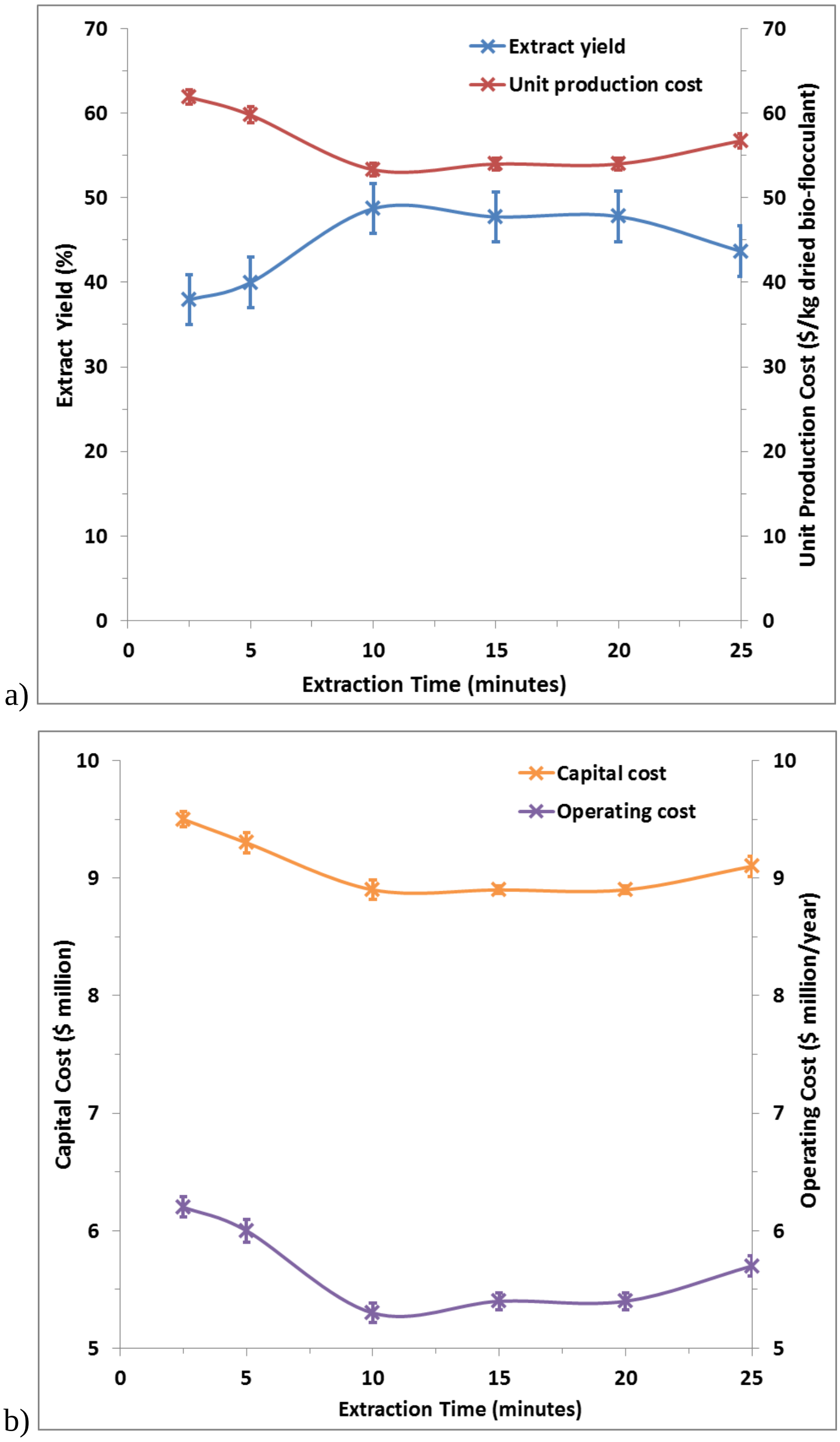


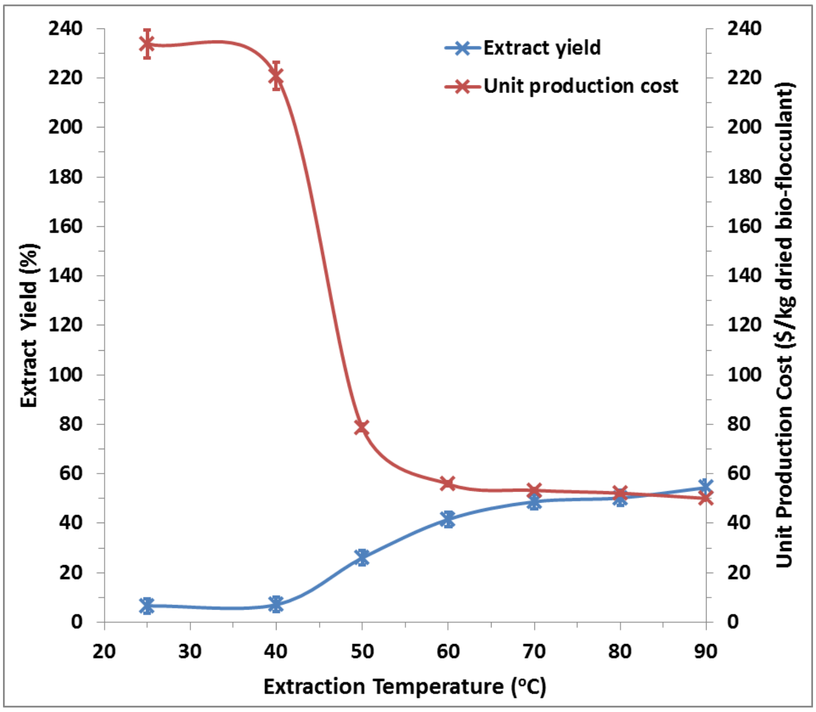
Figure 6.6: The effects of extract yield with variation in extraction time to (a) unit production cost, and (b) capital and operating costs

As shown in Figure 6.6(a), the extract yield was increased while the production cost was decreased with increasing of extraction time from 2.5 to 10 minutes. No significant change of production cost was observed between 10 to 20 minutes due to very minor change of extract yield within this extraction period. After 20 minutes of extraction time, the decrease of yield caused the rising of production cost. These results denoted the inverse relationship between extract yield and production cost.

In the light of the same annual production rate, increase of extract yield directly reduces the requirement of raw material amount and utilities and man power and also decreases the equipment size. As the result, the capital and operating costs are reduced and thereupon decrease the production cost. Figure 6.6(b) shows that the capital and operating costs exhibited similar behaviour as production cost and were reciprocally affected by the extract yield.

Figures 6.7 and 6.8 presented below demonstrate that the changes of extract yield with temperature and solvent loading also presented contrary effect on the capital and operating and production costs. Based on these results, it can be concluded that the lowest production cost (£50/kg of microwave extracted dried bio-flocculant for annual production rate of 100 tonne/year) was obtained at optimised yield with extraction conditions of 90˚C, 10 minutes and 3.5 w/w of solvent loading.

a)



b)

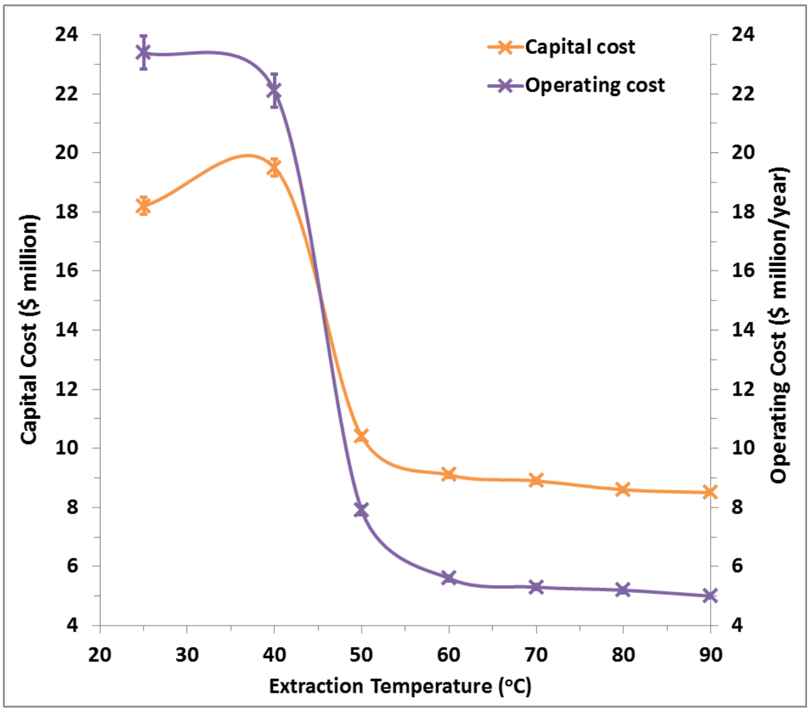
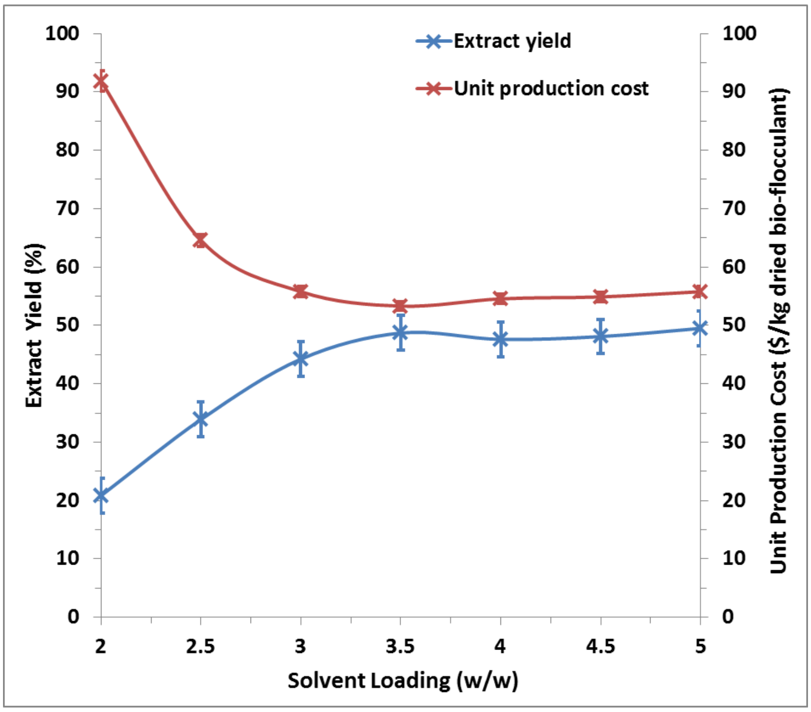


Figure 6.7: The effects of extract yield with variation in extraction temperature to (a) unit production cost, and (b) capital and operating costs

a)



b)

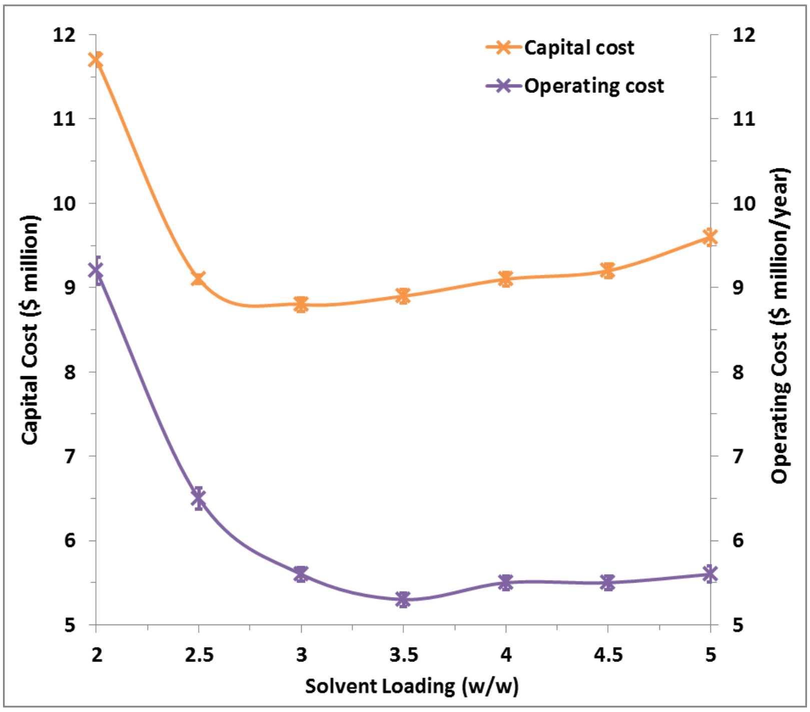


Figure 6.8: The effects of extract yield with variation in solvent loading to (a) unit production cost, and (b) capital and operating costs

6.6.2 Effect of raw material okra price

The current okra price used in the simulation was $1/kg. Another two possible case scenarios were taken into consideration where the waste okra from the market was used as the raw material with cost close to zero (Pereira, 2013) or the market price of fresh okra was doubled ($2/kg) and the results are shown in Figure 6.9.

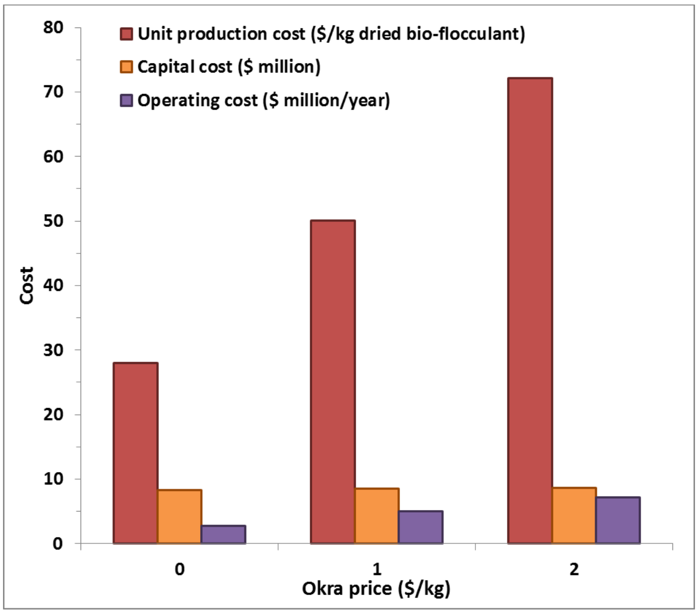


Figure 6.9: The effect of okra price on the capital, operating and unit production cost

It can be seen that okra price revealed high impact on the expenses of producing bio-flocculant, the operating and unit production cost were increased linearly with rising of okra price whilst the capital cost remained constant. From the standpoint of okra price at $1/kg, the operating and production costs were decreased by 44% if the okra was obtained for free and raised by 44% if the okra price was increased to $2/kg. Even though the extraction yield and the bio-flocculant quality by using waste okra from market as raw material is not verified in this study, but the bio-flocculant production from waste okra presents a sustainable option to reduce the raw material cost and to resolve the problem of waste okra disposal.

6.7 Sensitivity analysis

Based on the results presented above, it was found that the annual production and extract yield show inverse relationship with unit production cost. On the other side, the okra price demonstrates proportional relationship with the production cost. As fluctuations in annual production and extract yield and okra price showed impact on the production cost, thus the sensitivities of unit production costs to the annual production and extract yield and okra price were examined in this section, and the results are presented in tornado chart (Figure 6.10).

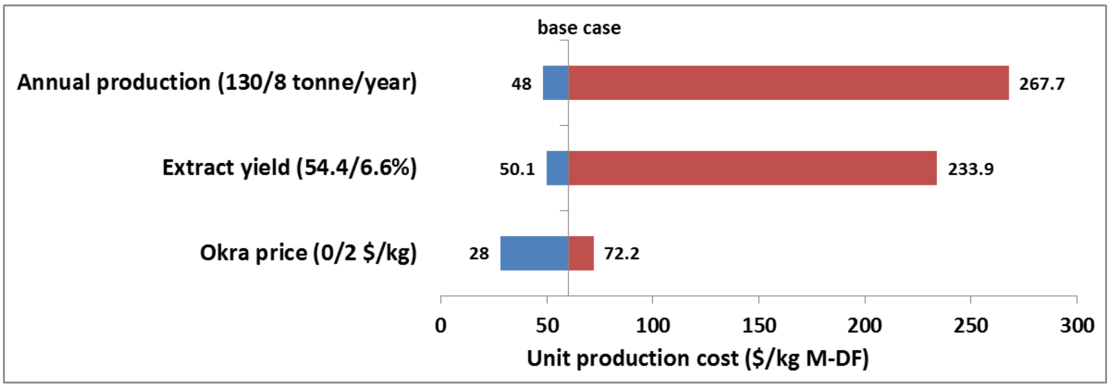


Figure 6.10: Sensitivity chart of unit production cost (The numbers in brackets on y-axis are the low and high values for each parameter.)

The range of each studied parameter from a low point (e.g. 6.6% for yield) to a high point (e.g. 54.4% for yield) was selected by referring to the results presented previously. The unit production costs that are corresponded to the low and high points are shown. For instances, the production costs at 50.1 $/kg and 72.2 $/kg are correlated to 54.5% of extract yield and 2 $/kg of okra price respectively.

Based on the sensitivity chart, it was clear that the uncertainty in annual production and extract yield caused wide range of unit production cost. The unit production cost was most sensible to annual production, followed by extract yield and least sensible to okra price. It is because the annual production and extract yield would directly affect the revenue of the production plant. By increasing the production capacity, more bio-flocculants are produced and the revenues gained from selling of products could compensate the total capital and operating costs and give profit. Enhancement of extract yield would reduce the requirement of raw material of okra and leads to reduction of raw material cost followed by lowering of operating cost. Hence, increasing the production capacity and investigation of modified extraction method/conditions that can increase the extract yield are the primary prerequisite to lower the production cost.

6.8 Optimised and economically feasible production scheme

Based on different studies conducted above, it could be concluded that continuous microwave process with extraction conditions of temperature at 90˚C, time at 10 minutes and solvent loading of 3.5 w/w was identified as the optimised scheme for production of industrial scale bio-flocculant at the lowest unit production cost. The design specification and the purchase cost of each equipment for this proposed process at the annual production of 100 tonne/year is summarised in Table B1 presented under Appendix. In addition, it should be emphasised that increasing of production capacity and extraction yield, and also decreasing of okra price would help to reduce the production cost.

Since okra bio-flocculant is a new product in the market and there is no similar product as a reference, the selling price was regulated to be approximate 30 to 40% more than the unit production cost and the annual production has been scaled up to 220 tonne/year (about 0.03% of worldwide production of polyacrylamides (Liu, 2012)) in order to achieve profitable requirement. The results of production and economic measures for the optimised scheme of bio-flocculant production at 220 tonne/year are listed in Table 6.7.

Table 6.7: The results of production and economic measures for the optimised scheme of bio-flocculant production

Process parameters		**Dried bio-flocculant**	**Aqueous bio-flocculant**
Production measures	Unit production cost ($/kg)	37.3	31.4
	Estimated selling price ($/kg)	52.3	40.9
Economic measures	Gross margin (%)	28.6	23.1
	ROI (%)	22.2	25.1
	Payback time (years)	4.5	4.0
	IRR (after tax, %)	18.2	20.4
	NPV at 7% ($)	8.8 x 10^6	5.7 x 10^6

By referring to Table 6.7, the gross margins are positive and desirable which are 29 and 23% for dried and aqueous bio-flocculants respectively. It means that the company would retain $0.29/0.23 from each dollar of revenue generated as gross profit (Vučurović, 2012). In addition, the obtained ROI values are in the expected range of 20 to 30%. Furthermore, the payback time is less than 5 years which is acceptable. Moreover, most importantly positive IRR and NPV are obtained which again showed that this proposed scheme is realisable and economically viable.

The selling price of okra bio-flocculant is compared with the price of commonly used chemical flocculants (polyacrylamides), food grade bio-flocculants and grafted starch-based flocculant in Table 6.8.

Table 6.8: Price list for different types of flocculants

Types of flocculants		**Price ($/kg)**
Chemical flocculants	Cationic polyacrylamide (such as FO 4400 SH)	4.25 (Long, 23 June 2016)
	Anionic polyacrylamide (such as AN 934 SH)	4.08 (Long, 23 June 2016)
	Non-ionic polyacrylamide (such as FA 920 SH)	4.08 (Long, 23 June 2016)
	Amphoteric polyacrylamide (such as FAM 4802)	5.54 (Long, 23 June 2016)
Food grade bio-flocculants	Coconut shell activated carbon	52 (Zhao, 4 July 2016)
	Chitosan	50 (4 July 2016)
	Sodium alginate	62.5 (2015)
Grafted starch-based flocculant	Amphoteric amylopectin	191 (2016)
Plant-based bio-flocculant from okra	Aqueous bio-flocculant	40.9
	Dried bio-flocculant	52.3

The price of okra bio-flocculant is about 10 times higher than polyacrylamides. Yet, the downside of extra production cost could be balanced out by its considerable benefits to environment and human health in circumstances where the use of polymeric flocculants is undesirable or prohibited. Most of the polyacrylamides are non-biodegradable or poorly biodegradable. Concerns have been raised that polyacrylamide employed in agriculture, food or beverage, and water treatment sectors may contaminate the treated water or food products with acrylamide, a known neurotoxin. While polyacrylamide itself is relatively non-toxic, however it is known that commercially available polyacrylamide contains minute residual amounts of acrylamide remaining from its production (Woodrow, 2008). Acrylamide has been reported to possess negative impact to the environment because it is neurotoxic to animals and is a rodent carcinogen (Shipp, 2006). As explained in Section 1.4, the sludge treated with polyacrylamides is prohibited from application in cultivation (Lauri, 2011) and the usage of polyacrylamides in drinking water treatment and food industry is strictly controlled (Bolto, 2007; Lu, 2014) due to the negative effects of polyacrylamides.

On the other hand, green bio-flocculant which is extracted from renewable resources is non-toxic and biodegradable. Hence, bio-flocculant poses minimum risk to human health and minimum negative impact to environment when compared to polyacrylamides. Due to these characteristics, bio-flocculants are safer to be consumed and could be served as an alternative to application of polyacrylamides in drinking water treatment, food and beverage, cosmetic and pharmaceutical industries. The possible applications of bio-flocculant in different industry sectors have been discussed in Chapter 2 and the safety of using extracted bio-flocculant directly has been evaluated in section 5.4. It is worth to highlight that okra extract has been employed in pharmaceutical industry as a binder for tablet formulation (Zaharuddin, 2014a) and used as a thickener in food industry (Stephen, 2006) and as a clarifier in juice clarification (Whistler, 1993).

In terms of flocculating and dewatering abilities, the results presented in Chapter 4 have proved that the bio-flocculant quality is comparable to or even better than polyacrylamides because bio-flocculant could recover more water which indicates less sludge volume at the same dosage as polyacrylamides. In addition, its price is shown to be lower than grafted starch-based flocculant and food grade bio-flocculants which are widely used in beverage processing (Lee, 2014a). For the sake of ecology and human health, okra bio-flocculant is still an attractive and economically viable option to replace chemical flocculants in water treatment industry and food-related or beverage-related processing sectors owing to its sustainability and nature friendly features.

6.9 Future recommendations

Few approaches are suggested in this section to find the possibility to further reduce the unit production cost and also to improve the viability of the whole process.

6.9.1 Reduction of raw material cost of okra

Since the operating cost is dominantly contributed from raw material cost of okra, hence replacement of fresh okra with waste okra as the feedstock for extraction of bio-flocculant is an attractive way to reduce the operating cost and to solve the problem of waste okra disposal. To verify the feasibility of this suggestion, experimental investigation is necessary to examine the yield and the flocculating properties of the bio-flocculants extracted from waste okra.

6.9.2 Possibility of eliminating the seed removing step

It has been reported that okra mucilage is extracted from fruit pods and the seeds do not contain mucilage (Zaharuddin, 2014b). The presence of seeds in the extraction step may affect the yield and the bio-flocculant quality but experimental work is required to verify this presumption. If there are no adverse effects, the seed removing step may be eliminated which can shorten the overall processing time and improve the production rate, and also reduce the overall capital and operating cost.

6.9.3 Design of CIP system

An entire CIP system consists of a CIP station, CIP distribution lines and the objects to be cleaned should be properly designed (Pak, 2016) and thence the frequency of cleaning and the overall cleaning cost could be estimated more accurately and considered in calculation of production cost. Although recovery

CIP method by recovering the cleaning solution is suggested, but future work is necessary to validate whether there is a possible enrichment of undesired substances in the recycling loop or whether hygiene problems may arise (Heinzle, 2006). The type and the amount of waste generated from CIP operation should be identified, for further planning on the appropriate treatment and disposal methods and the estimation of correlated treatment and disposal cost.

6.9.4 Waste handling and treatment/disposal

Many countries such as UK are obliged to manage the waste in order of environmental preference and the first priority is waste reduction followed by waste recycling/reuse. Sustainable management methods are suggested in previous section to handle and reuse the waste generated from this process, however future investigation is required to ensure the proposed solutions comply with regulations concerning environmental protection, duty of care, health and safety, waste handling and planning permission (DEFRA, 22 January 2013).

6.9.5 Packaging and transportation

Even though the costs associated with packaging of end products and transportation of both the raw material and the products are neglected in this work, nonetheless these costs cannot be disregarded in real case because it has impact on the unit production cost and should be considered in future work. These costs are proportional to the plant output or operation rate, and comprise of costs of drums, bags, tankers, freight charges, and etc. (Towler, 2013). Ingenious packaging method with sterilisation should be planned for the considerations of extending transportation distances locally or oversea and the shelf life of product. For quality control purposes, the products are packed directly into the final packaging and then boxed for delivery to wholesalers (Towler, 2013). Solid products are commonly packed in bags or drums or sacks or cartons and then shipped in bulk in railroad hopper cars and bulk ship containers (Towler, 2013). These costs could be overlooked in production of aqueous bio-flocculant because it is located onsite with water treatment plant and the product is applied within short distance and short period of time.

6.9.6 Steam generation

Generally, the steam used in the industries is generated using boilers and knowing the correct cost of steam is important to properly evaluate the economics of proposed project and to serve as a basis for optimising the steam generation system to minimise the utility cost. Hence, the inclusion of estimation of true cost of steam generation and delivery in future work is important because it would affect the overall calculation of operating cost.

6.9.7 Another alternative plant

As presented in Table 2.1, other plants that exhibit flocculating properties have been reported in literature. However, the research study of these plants is only at preliminary stage, their extraction efficiency and flocculating performance have not been investigated in depth. If another alternative plant with higher extraction and flocculating efficiency than okra could be identified and it is available at lower cost, then there is high possibility that the unit production cost of bio-flocculant could be reduced.

6.9.8 Additional value of okra extract

Okra extract possess various functional properties and has the potential for use as food, non-food products and medicine. It has been used as a thickener in food industry to thicken the soups and as a protectant to reduce friction in pipe-flow and also exhibits binder potential for application in tablet formulations (Ameena, 2010). In addition, it contains high amounts of dietary fibre and several valuable nutrients (Kumar, 2010). The okra extract produced in this process has the potentiality to be applied in diverse areas or industries; this could improve the market demand and the economic potential of this product.

CHAPTER 7: CONCLUSIONS AND FUTURE RECOMMENDATIONS

This chapter summarises the significant research findings in accordance with the objectives set in this study. The current challenges of bio-flocculant development and recommendations for future work were also presented.

7.1 Conclusions

This study has shown that a plant-based bio-flocculant was successfully extracted from okra by using CHE and MAE methods with water as a solvent. The extracted bio-flocculant is anionic (zeta potential is −25.3±3.5 mV) and neutral in nature (pH value is 6 to 6.5). A single parameter experimental approach identified that the optimised yield of CHE at 25.9% was obtained at extraction conditions of 50 ˚C, 2 hour, 200 rpm agitation and 2.5 w/w solvent loading. Meanwhile, the optimised yield of MAE at 48.7% was achieved at 90 ˚C, 10 minutes, 3.5 w/w solvent loading and okra particle size at 1mm.

By comparing microwave and hydrothermal extractions, it was obvious that the yield was significantly increased by 87.8% with MAE and the extraction time was notably reduced from 2 hours to 10 minutes. Extraction temperature and time were the two major parameters that influenced the extraction efficiency. Long extraction time in few hours at high temperature (60 to 90 ˚C) with CHE method was shown to decrease the extraction yield. On the contrary, extraction at high temperature with MAE method increased the yield due to short extraction time in minutes.

Microwave extraction presented a different heating mechanism when the extraction temperatures were above 50 ˚C which led to significantly higher yield than hydrothermal extraction. The mechanism for microwave heating seems to be similar as hydrothermal heating if the temperatures were below 50 ˚C. This study shows for the first time that enhancements in extraction rate and yield with MAE method can be achieved at extraction temperatures above 50 ˚C when the loss tangent of the feedstock (okra for this case) is higher than the solvent (water) and the okra is selectively heated. The extraction mechanisms and the mass transfer phenomena that explained how the microwave selective heating could lead to cell wall rupture and higher yield were developed in this work. During selective heating of okra when the extraction temperature is above 50 ˚C, the plant material is hotter than solvent phase. This causes the chemical potentials of both solute and water to decrease within the plant cells. The chemical potential gradient of water causes more water to diffuse into the plant cell which leads to increase of pressure within the plant cell. A temperature difference of the order of 1 ˚C is sufficient to induce an equilibrium pressure of over 100 bar within the plant cell structures, leading to cell wall disruption and hence improved extraction efficiency.

The flocculating abilities of bio-flocculants extracted with both methods were not apparently affected by the extraction conditions. The dewatering ability of bio-flocculant extracted with CHE was influenced by extraction temperature and agitation speed. On the other hand, the dewatering activity of microwave extracted bio-flocculant was impacted by extraction temperature and time and okra particle size. Extraction temperature ≥ 70 ˚C is a crucial condition to extract bio-flocculant with desirable dewatering property for both extraction methods.

The extracted bio-flocculants could be used for flocculation and sludge dewatering without pH adjustment and addition of coagulant. High flocculating efficiencies with >99% of SS and turbidity removal and <10 ml/g of SVI were obtained. Bio-flocculants also showed efficient dewatering abilities which were denoted by >95% of SS removal after filtration and ≥75% of water recovery. Hydrothermal extracted bio-flocculants were shown to have lower dewatering abilities than microwave extracted bio-flocculants because it required higher dosage (125 mg/L versus 30 mg/L) to achieve comparable performance.

The sludge dewatering performance of microwave extracted bio-flocculant was verified to be comparable to or even better than commercial polyacrylamide flocculants, because higher water recoveries were achieved by bio-flocculant at the same dosage as polyacrylamides. Dried bio-flocculants showed higher dewatering abilities than aqueous bio-flocculants because drying at low temperature (40 ˚C) has minimised the moisture content and consequently extended the shelf life and dewatering quality of bio-flocculants. Biopolymer bridging was preliminary identified as the plausible bio-flocculation mechanism.

For production of industrial scale bio-flocculant, MAE process in continuous mode with extraction conditions of temperature at 90˚C, time at 10 minutes and solvent loading at 3.5 w/w was the optimised scheme that showed the lowest unit production cost. Continuous mode microwave process was found to be more economically feasible than hydrothermal process either in batch or continuous mode for production scale larger than 15 tonne/year. The unit production cost was strongly impacted by annual production, extraction yield and raw material okra price. The selling price of aqueous and dried bio-flocculants for industrial unit with capacity of 220 tonne/year were estimated as 41 $/kg and 52 $/kg respectively, which was lower than food grade bio-flocculants but higher than polyacrylamides.

For the sake of ecology and human health, okra bio-flocculant still represents a sustainable and economically viable alternative to chemical flocculants due to its high efficiency in sludge dewatering and environmentally friendly characteristic in terms of production and application.

7.2 Current challenges and recommendations of future work

Up to date, the development of bio-flocculants is still in its infancy. There are some uncertainties which prevent the wider commercialisation and development of bio-flocculants in many applications and these challenges should be addressed in the coming years.

The flocculating and dewatering abilities of okra bio-flocculant in the treatment of kaolin colloidal solution high in SS (80 g/L for this work) has been established in this study. However, its bio-flocculant quality in other concentrations of SS is still unclear and requires future investigation. It is noteworthy to check whether the bio-flocculant is workable under different concentrations of SS and also to assess how the variation of SS concentrations affects the required dose of bio-flocculant in order to produce optimum flocculation and dewatering abilities.

Even though high efficiency of bio-flocculant in removal of SS and turbidity has been verified in this study, it is important to explore its ability in reduction or removal of other environmental concerned parameters such as chemical oxygen demand, colour and heavy metals. The potential application of bio-flocculant in synthetic wastewater (kaolin colloidal solution) has been proven in laboratory scale but there is very limited work on the pilot or industrial scale. Therefore, future work should concentrate on industrial trials or practices for evaluating the feasibility of applying bio-flocculant in different separation processes in diverse industry sectors.

This study has shown that the dewatering abilities of aqueous bio-flocculants would decrease with storage time, and drying of bio-flocculants at low temperature could preserve the dewatering efficiency for at least three days. In order to find out the effect of storage time on the dewatering quality and the shelf life of aqueous and dried bio-flocculants, the flocculating and dewatering efficiencies of bio-flocculants after the products are stored for a week, a month, half year, and a year and longer should be investigated in future.

Although polysaccharides were reported to be the biopolymers that exhibit the flocculating properties (Crini, 2005; Freitas, 2015) and biopolymer bridging was described as the probable bio-flocculation mechanism (Mishra, 2006a), no experimental study has been done yet to verify this hypothesis. Hence, future research is needed to identify the bioactive components that exist in the extract and detect the active constituent(s) which are responsible for the flocculating and dewatering abilities. High molecular weight of biopolymers (up to $2\text{x}10^7$ Daltons by using polyacrylamides as reference) is the prerequisite to enable the adsorption of biopolymer chains onto the particle surface and to bridge other dispersed particles together effectively in order to form high density and strong flocs (Nasser, 2006). Thus, it is significant to conduct future research to measure the molecular weight of bioactive polymers and look into how the molecular weight affects the bio-flocculant performance.

This work has demonstrated that okra bio-flocculant could be successfully extracted with MAE method in laboratory scale. Hence, the next step is the design of larger scale continuous microwave extraction system for upgrading of extraction of bio-flocculant to pilot scale or industrial scale. It has been shown that microwave selective heating can be enhanced by understanding the dielectric property variation with temperature, and this means that design of industrial microwave extractor will require further investigation/optimisation of the delivery of microwave power into the system to ensure the highest level of uniformity and efficiency of selective heating. In addition, overradiation and hidden dangers of equipment are essential parameters to consider in the design of industrial microwave extractor in order to guarantee the safety of products and operators (Li, 2013b). As part of developing an efficient microwave extraction system for industrial scale bio-flocculant production, the viability of continuous microwave production of bio-flocculant from laboratory scale to pilot scale should be investigated with respect to flow rate and residence time.

If okra is used as the raw material for bio-flocculant production, it is expected that there will be feedstock competition as global demand for food and energy increases over time (Babu, 2013). Therefore, it is necessary to search for other non-food source plant or use biomass waste as the feedstock for bio-flocculant extraction. Another significant challenge is the production cost of bio-flocculant which is higher than polyacrylamides. The recommendations to further reduce the production cost have been suggested in Chapter 7 such as using waste okra as the feedstock for bio-flocculant extraction and also utilisation of biomass waste generated in the process for generation of bioenergy through anaerobic digestion.

In addition, building large scale bio-flocculant production plant can be difficult due to the lack of experience in technologies of bio-products and estimation of supply/demand balance. In order to make these technologies economically viable, it is very important to develop (1) logistics for biomass feedstocks, (2) manufacturing routes that give high yields and low production cost, (3) efficient downstream processing methods for recovery of solvent used in extraction (Babu, 2013).

With consideration of the negative impacts possibly caused by acrylamide (monomer impurity of polyacrylamides) to the environment, it is imperative to conduct more qualitative and quantitative research to tackle the aforementioned challenges and to establish the applications of plant-derived bio-flocculants in real industries.

REFERENCES

4 July 2016. Quotation for chitosan. G.T.C. Bio Corporation, China.

21 June 2016. Frozen IQF okra. Crystal Rainbow Trading, South Africa.

2015. Quotation for sodium alginate. Modernist Pantry, U.S.

2015/2016. Scheme of charges. Severn Trent Water, U.K.

2016. Quotation for amylopectin from maize. Sigma-Aldrich, U.K.

Ahmad, A.L., Wong, S.S., Teng, T.T., Zuhairi, A., 2008. Improvement of alum and PACl coagulation by polyacrylamides (PAMs) for the treatment of pulp and paper mill wastewater. Chemical Engineering Journal 137, 510-517.

Al-Hamadani, Y.A.J., Yusoff, M.S., Umar, M., Bashir, M.J.K., Adlan, M.N., 2011. Application of psyllium husk as coagulant and coagulant aid in semi-aerobic landfill leachate treatment. Journal of Hazardous Materials 190, 582-587.

Ali, M., Watson, I.A., 2015. Microwave treatment of wet algal paste for enhanced solvent extraction of lipids for biodiesel production. Renewable Energy 76, 470-477.

Alice, 22 June 2016. Quatation for vegetable seed separation machine. Henan Langpu Trading Co. Ltd., China.

Allison, T., 2016. NIST-JANAF thermochemical tables. National Institute of Standards and Technology, USA.

Alshekhli, O., Foo, D.C.Y., Hii, C.L., Law, C.L., 2011. Process simulation and debottlenecking for an industrial cocoa manufacturing process. Food and Bioproducts Processing 89, 528-536.

Ameena, K., Dilip, C., Saraswathi, R., Krishnan, P.N., Sankar, C., Simi, S.P., 2010. Isolation of the mucilages from Hibiscus rosasinensis linn. and Okra (Abelmoschus esculentus linn.) and studies of the binding effects of the mucilages. Asian Pacific Journal of Tropical Medicine 3, 539-543.

Amid, B.T., Mirhosseini, H., 2012. Influence of different purification and drying methods on rheological properties and viscoelastic behaviour of durian seed gum. Carbohydrate Polymers 90, 452-461.

Amuda, O.S., Alade, A., 2006. Coagulation/flocculation process in the treatment of abattoir wastewater. Desalination 196, 22-31.

Amuda, O.S., Amoo, I.A., 2007. Coagulation/flocculation process and sludge conditioning in beverage industrial wastewater treatment. Journal of Hazardous Materials 141, 778-783.

Anastasakis, K., Kalderis, D., Diamadopoulos, E., 2009. Flocculation behavior of mallow and okra mucilage in treating wastewater. Desalination 249, 786-791.

Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science 34, 755-781.

Arasi, M.A.S.A.G., Rao, M.G., Bagyalakshmi, J., 2016. Optimization of microwave-assisted extraction of polysaccharide from Psidium guajava L fruits. International Journal of Biological Macromolecules 91, 227-232.

Athimulam, A., Kumaresan, S., Foo, D.C.Y., Sarmidi, M.R., Aziz, R.A., 2006. Modelling and optimization of Eurycoma longifolia water extract production. Food and Bioproducts Processing 84, 139-149.

Atkins, P., de Paula, J., 2014. Atkins' physical chemistry, 10th ed. Oxford University Press, U.K., p. 1005.

Babu, R.P., O'Connor, K., Seeram, R., 2013. Current progress on bio-based polymers and their future trends. Progress in Biomaterials 2, 1-16.

Bae, Y.H., Kim, H.J., Lee, E.J., Sung, N.C., Lee, S.S., Kim, Y.H., 2007. Potable water treatment by polyacrylamide base flocculants, coupled with an inorganic coagulant. Environmental Engineering Research 12, 21-29.

Bagherian, H., Zokaee Ashtiani, F., Fouladitajar, A., Mohtashamy, M., 2011. Comparisons between conventional, microwave- and ultrasound-assisted methods for extraction of pectin from grapefruit. Chemical Engineering and Processing: Process Intensification 50, 1237-1243.

Banerjee, S., 2014. Dewatering fibrous sludge with soy protein. Process Biochemistry 49, 120-123.

Belbahloul, M., Zouhri, A., Anouar, A., 2015. Bioflocculants extraction from Cactaceae and their application in treatment of water and wastewater. Journal of Water Process Engineering 7, 306-313.

Bergethon, P.R., 2010. The physical basis of biochemistry: The foundations of molecular biophysics, 2nd ed. Springer Science+Business Media, U.S.A., p. 923.

Blanco, A., Negro, C., Fuente, E., Tijero, J., 2005. Effect of shearing forces and flocculant overdose on filler flocculation mechanisms and floc properties. Industrial & Engineering Chemistry Research 44, 9105-9112.

Bolto, B., Gregory, J., 2007. Organic polyelectrolytes in water treatment. Water Research 41, 2301-2324.

Boráň, J., Houdková, L., Elsäßer, T., 2010. Processing of sewage sludge: Dependence of sludge dewatering efficiency on amount of flocculant. Resources, Conservation and Recycling 54, 278-282.

Bratby, J., 2016. Coagulation and flocculation in water and wastewater treatment, 3rd ed. IWA Publishing, United Kingdom.

Brostow, W., Lobland, H.E.H., Sagar, P., Singh, R.P., 2009. Polymeric flocculants for wastewater and industrial effluent treatment Journal of Materials Education 31, 157-166.

Buttress, A.J., Binner, E., Yi, C., Palade, P., Robinson, J.P., Kingman, S.W., 2016. Development and evaluation of a continuous microwave processing system for hydrocarbon removal from solids. Chemical Engineering Journal 283, 215-222.

Cai, W.R., Gu, X.H., Tang, J., 2008. Extraction, purification, and characterization of the polysaccharides from Opuntia milpa alta. Carbohydrate Polymers 71, 403-410.

Carr, A.G., Mammucari, R., Foster, N.R., 2011. A review of subcritical water as a solvent and its utilisation for the processing of hydrophobic organic compounds. Chemical Engineering Journal 172, 1-17.

Catarino, S., Madeira, M., Monteiro, F., Rocha, F., Curvelo-Garcia, A.S., de Sousa, R.B., 2007. Effect of bentonite characteristics on the elemental composition of wine. Journal of Agricultural and Food Chemistry 56, 158-165.

Chakrabarti, S., Banerjee, S., Chaudhuri, B., Bhattacharjee, S., Dutta, B.K., 2008. Application of biodegradable natural polymers for flocculated sedimentation of clay slurry. Bioresource Technology 99, 3313-3317.

Chan, C.H., Yusoff, R., Ngoh, G.C., Kung, F.W.L., 2011. Microwave-assisted extractions of active ingredients from plants. Journal of Chromatography A 1218, 6213-6225.

Charoensiddhi, S., Franco, C., Su, P., Zhang, W., 2015. Improved antioxidant activities of brown seaweed Ecklonia radiata extracts prepared by microwave-assisted enzymatic extraction. Journal of Applied Phycology 27, 2049-2058.

Chemat, F., Vian, M.A., Cravotto, G., 2012. Green extraction of natural products: Concept and principles. International Journal of Molecular Sciences 13, 8615-8627.

Chen, P., Xie, Q.L., Du, Z.Y., Borges, F.C., Peng, P., Cheng, Y.L., Wan, Y.Q., Lin, X.Y., Liu, Y.H., Ruan, R., 2015. Chapter 5: Microwave-assisted thermochemical conversion of biomass for biofuel production in: Fang, Z., Smith, R.L., Qi, X.H. (Ed.), Production of biofuels and chemicals with microwave. Springer Science+Business Media, Dordrecht.

Chen, R.Z., Jin, C.G., Tong, Z.G., Lu, J., Tan, L., Tian, L., Chang, Q.Q., 2016a. Optimization extraction, characterization and antioxidant activities of pectic polysaccharide from tangerine peels. Carbohydrate Polymers 136, 187-197.

Chen, Y.C., Jaczynski, J., 2007. Protein recovery from rainbow trout (Oncorhynchus mykiss) processing byproducts via isoelectric solubilization/precipitation and its gelation properties as affected by functional additives. Journal of Agricultural and Food Chemistry 55, 9079-9088.

Chen, Z., Zhang, W., Tang, X.Y., Fan, H.J., Xie, X.J., Wan, Q., Wu, X.H., Tang, J.Z., 2016b. Extraction and characterization of polysaccharides from Semen Cassiae by microwave-assisted aqueous two-phase extraction coupled with spectroscopy and HPLC. Carbohydrate Polymers 144, 263-270.

Cheng, Z.Y., Song, H.Y., Yang, Y.J., Liu, Y., Liu, Z.G., Hu, H.B., Zhang, Y., 2015. Optimization of microwave-assisted enzymatic extraction of polysaccharides from the fruit of Schisandra chinensis Baill. International Journal of Biological Macromolecules 76, 161-168.

Choi, I.L., Choi, S.J., Chun, J.K., Moon, T.W., 2006. Extraction yield of soluble protein and microstructure of soybean affected by microwave heating. Journal of Food Processing and Preservation 30, 407-419.

Chong, M.F., 2012. Direct flocculation process for wastewater treatment, in: Sharma, S.K., Sanghi, R. (Ed.), Advances in water treatment and pollution prevention. Springer, Netherlands, pp. 201-230.

Chong, M.F., 2009a. Membrane based palm oil mill effluent (POME) treatment. LAP LAMBERT Academic Publishing AG & Co. KG, Germany.

Chong, M.F., Lee, K.P., Chieng, H.J., Syazwani Binti Ramli, I.I., 2009b. Removal of boron from ceramic industry wastewater by adsorption–flocculation mechanism using palm oil mill boiler (POMB) bottom ash and polymer. Water Research 43, 3326-3334.

Choudhary, P.D., Pawar, H.A., 2014. Recently investigated natural gums and mucilages as pharmaceutical excipients: An overview. Journal of Pharmaceutics, 204849, 204849 pages.

Christodoulou, P., 2003. Chapter 8: Sugars and carbohydrates, in: Tzia, C., Liadakis, G. (Ed.), Extraction optimization in food engineering. Marcel Dekker, Inc., United States of America, pp. 248-316.

Chupin, L., Maunu, S.L., Reynaud, S., Pizzi, A., Charrier, B., Charrier-El Bouhtoury, F., 2015. Microwave assisted extraction of maritime pine (Pinus pinaster) bark: Impact of particle size and characterization. Industrial Crops and Products 65, 142-149.

Coulson, J.M., Richardson, J.F., Backhurst, J.R., Harker, J.H., 1999. Chemical engineering: Fluid flow, heat transfer and mass transfer, 6th ed. Butterworth Heinemann, Oxford, p. 908.

Couper, J.R., Penney, W.R., Fair, J.R., Walas, S.M., 2012. Chemical process equipment: Selection and design, 3rd ed. Butterworth-Heinemann, Oxford, p. 801.

Crini, G., 2005. Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment. Progress in Polymer Science 30, 38-70.

Cui, G.T., Zhang, W.X., Wang, Q.J., Zhang, A.M., Mu, H.B., Bai, H.J., Duan, J.Y., 2014. Extraction optimization, characterization and immunity activity of polysaccharides from Fructus Jujubae. Carbohydrate Polymers 111, 245-255.

De Nevers, N., 2012. Physical and chemical equilibrium for chemical engineers, 2nd ed. John Wiley & Sons, Inc., New Jersey, p. 361.

DECC, 2016. Statistical data set: International industrial energy prices. Department of Energy & Climate Change, United Kingdom.

DEFRA, April 2008. Waste wood as a biomass fuel. Department for Environment, Food and Rural Affairs United Kingdom.

DEFRA, D., 22 January 2013. Generating energy from waste, including anaerobic digestion. Department for Environment, Food & Rural Affairs and Department of Energy & Climate Change, U.K.

Destandau, E., Michel, T., Elfakir, C., 2013. Chapter 4: Microwave-assisted extraction, in: Mauricio, A.R., Juliana, M.P. (Ed.), Natural product extraction: Principles and applications. The Royal Society of Chemistry, UK, pp. 113-156.

Dobias, B., Stechemesser, H., 2005. Coagulation and flocculation, 2nd ed. Taylor & Francis Group, LLC, U.S.A.

DoITPoMS, 2004-2015. Effect of temperature on the dielectric constant, Dielectric materials. University of Cambridge, U.K.

Domingues, R.C.C., Faria Junior, S.B., Silva, R.B., Cardoso, V.L., Reis, M.H.M., 2012. Clarification of passion fruit juice with chitosan: Effects of coagulation process variables and comparison with centrifugation and enzymatic treatments. Process Biochemistry 47, 467-471.

Dong, H.M., Zhang, Q., Li, Y., Li, L., Lan, W.J., He, J.L., Li, H.Y., Xiong, Y.B., Qin, W., 2016. Extraction, characterization and antioxidant activities of polysaccharides of Chuanminshen violaceum. International Journal of Biological Macromolecules 86, 224-232.

Ebringerová, A., Hromádková, Z., 2010. An overview on the application of ultrasound in extraction, separation and purification of plant polysaccharides. Central European Journal of Chemistry 8, 243-257.

Elmoazzen, H.Y., Elliott, J.A.W., McGann, L.E., 2009. Osmotic transport across cell membranes in nondilute solutions: A new nondilute solute transport equation. Biophysical Journal 96, 2559-2571.

Emeje, M., Isimi, C., Byrn, S., Fortunak, J., Kunle, O., Ofoefule, S., 2011. Extraction and physicochemical characterization of a new polysaccharide obtained from the fresh fruits of Abelmoschus Esculentus. Iranian Journal of Pharmaceutical Research 10, 237–246.

Fabio, A.R.D., Rafael, J.C., 2009. Sugar cane juice clarification process, U.S. Patent, US 7338562 B2.

Farhat, A., Fabiano-Tixier, A.S., Maataoui, M.E., Maingonnat, J.F., Romdhane, M., Chemat, F., 2011. Microwave steam diffusion for extraction of essential oil from orange peel: Kinetic data, extract's global yield and mechanism. Food Chemistry 125, 255-261.

Felkai-Haddache, L., Dahmoune, F., Remini, H., Lefsih, K., Mouni, L., Madani, K., 2016. Microwave optimization of mucilage extraction from Opuntia ficus indica Cladodes. International Journal of Biological Macromolecules 84, 24-30.

Fellows, P.J., 2009. Food processing technology: Principles and practice, 3rd ed. Woodhead Publishing Limited, U.K., p. 893.

Ferhat, M.A., Meklati, B.Y., Chemat, F., 2007. Comparison of different isolation methods of essential oil from Citrus fruits: cold pressing, hydrodistillation and microwave 'dry' distillation. Flavour and Fragrance Journal 22, 494-504.

Filly, A., Fernandez, X., Minuti, M., Visinoni, F., Cravotto, G., Chemat, F., 2014. Solvent-free microwave extraction of essential oil from aromatic herbs: From laboratory to pilot and industrial scale. Food Chemistry 150, 193-198.

Flórez, N., Conde, E., Domínguez, H., 2015. Microwave assisted water extraction of plant compounds. Journal of Chemical Technology & Biotechnology 90, 590-607.

Forbes, 2016. Large thermoplastic tanks and bunds. Plastic Tanks and Environmental Technologies, U.K.

Freitas, T.K.F.S., Oliveira, V.M., de Souza, M.T.F., Geraldino, H.C.L., Almeida, V.C., Fávaro, S.L., Garcia, J.C., 2015. Optimization of coagulation-flocculation process for treatment of industrial textile wastewater using okra (A. esculentus) mucilage as natural coagulant. Industrial Crops and Products 76, 538-544.

Gekas, V., Oliveira, F.A.R., Crapiste, G.H., 2002. Chapter 13: Non-fickian mass transfer in fruit tissue, in: Welti-Chanes, J., Aguilera, J.M., Barbosa-Canovas, G.V. (Ed.), Engineering and food for the 21st century, 1st ed. CRC Press LLC, Boca Raton, pp. 211-233.

Ginos, A., Manios, T., Mantzavinos, D., 2006. Treatment of olive mill effluents by coagulation–flocculation–hydrogen peroxide oxidation and effect on phytotoxicity. Journal of Hazardous Materials 133, 135-142.

Goode, K.R., Asteriadou, K., Robbins, P.T., Fryer, P.J., 2013. Fouling and cleaning studies in the food and beverage industry classified by cleaning type. Comprehensive Reviews in Food Science and Food Safety 12, 121-143.

Green, D.W., Perry, R.H., 2007. Perry's chemical engineers' handbook, 8th ed. McGraw-Hill Professional, p. 2703.

Gude, V.G., Patil, P., Martinez-Guerra, E., Deng, S.G., Nirmalakhandan, N., 2013. Microwave energy potential for biodiesel production. sustain chem process 1, 1-31.

Guo, J.Y., Ma, J., 2015. Bioflocculant from pre-treated sludge and its applications in sludge dewatering and swine wastewater pretreatment. Bioresource Technology 196, 736-740.

Harbourne, N., Marete, E., Jacquier, J.C., O'Riordan, D., 2009. Effect of drying methods on the phenolic constituents of meadowsweet (Filipendula ulmaria) and willow (Salix alba). LWT - Food Science and Technology 42, 1468-1473.

Harrison, R.G., 2015. Bioseparations science and engineering, in: Harrison, R.G., Todd, P.W., Rudge, S.R., Petrides, D.P. (Ed.), 2nd ed. Oxford University Press, U.S., p. 577.

Heinzle, E., 2006. Chapter 4: Sustainability assessment, in: Heinzle, E., Biwer, A.P., Cooney, C.L. (Ed.), Development of sustainable bioprocesses: Modeling and assessment. John Wiley & Sons, Ltd, U.K., pp. 81-117.

Heng, M.Y., Tan, S.N., Yong, J.W.H., Ong, E.S., 2013. Emerging green technologies for the chemical standardization of botanicals and herbal preparations. Trends in Analytical chemistry 50, 1–10.

Hesami, F., Bina, B., Ebrahimi, A., 2014. The effectiveness of chitosan as coagulant aid in turbidity removal from water. International Journal of Environmental Health Engineering 2, 46-51.

HMRC, 16 March 2016. Landfill tax: Increase in rates. HM Revenue & Customs, U.K.

Hoshmand, A.R., 2006. Chapter 5: Two-factor experimental designs, Design of experiments for agriculture and the natural sciences, 2nd ed. Chapman & Hall/CRC, Boca Raton, pp. 119-158.

IUPAC, 2014. Compendium of chemical terminology (Gold book), 2nd ed.

Jani, G.K., Shah, D.P., Prajapatia, V.D., Jain, V.C., 2009. Gums and mucilages: Versatile excipients for pharmaceutical formulations. Asian Journal of Pharmaceutical Sciences 4, 309-323.

Jaya, S., Durance, T.D., 2009. Compressive characteristics of cellular solids produced using vacuum-microwave, freeze, vacuum and hot air dehydration methods. Journal of Porous Materials 16, 47-58.

Jenkins, S., 2016. Current economic trends - March 2016, Chemical Engineering: Essentials for the CPI Professionals

Jia, X.J., Ding, C.B., Yuan, S., Zhang, Z.W., Chen, Y.E., Du, L., Yuan, M., 2014. Extraction, purification and characterization of polysaccharides from Hawk tea. Carbohydrate Polymers 99, 319-324.

Job, G., Herrmann, F., 2006. Chemical potential—a quantity in search of recognition. European Journal of Physics 27, 353-371.

Joo, D.J., Shin, W.S., Choi, J.H., Choi, S.J., Kim, M.C., Han, M.H., Ha, T.W., Kim, Y.H., 2007. Decolorization of reactive dyes using inorganic coagulants and synthetic polymer. Dyes and Pigments 73, 59-64.

Jose, M.A., 2003. Chapter 2: Solid-liquid extraction, in: Tzia, C., Liadakis, G. (Ed.), Extraction optimization in food engineering. Marcel Dekker, Inc., New York.

Jude, B., Lemaire, E., 2013. How to optimize clean-in-place (CIP) processes in food and beverage operations. Schneider Electric, France.

Kang, Q., 2007a. Residual color profiles of simulated reactive dyes wastewater in flocculation processes by polydiallyldimethylammoniumchloride. Separation and Purification Technology 57, 356-365.

Kang, Q., Gao, B.Y., Yue, Q.Y., Zhou, W.Z., Shen, D.Z., 2007b. Residual color profiles of reactive dyes mixture during a chemical flocculation process. Colloids and Surfaces A: Physicochemical and Engineering Aspects 299, 45-53.

Kappe, C.O., Stadler, A., 2005. Chapter 2: Microwave theory, in: Mannhold, R., Kubinyi, H., Folkers, G. (Ed.), Microwaves in organic and medicinal chemistry. Wiley-VCH, Weinheim, pp. 9-28.

Kaur, B., Ariffin, F., Bhat, R., Karim, A.A., 2012. Progress in starch modification in the last decade. Food Hydrocolloids 26, 398-404.

Kogel, J.E., Trivedi, N.C., Barker, J.M., Krukowski, S.T., 2006. Indsutrial minerals & rocks: Commodities, markets and uses, 7th ed. Society for Mining, Metallurgy,and Exploration, Inc. (SME), United States of America.

Komarov, V., Wang, S., Tang, J., 2005. Permittivity and measurements, in: Chang, K. (Ed.), Encyclopedia of RF and Microwave Engineering. John Wiley & Sons, Inc., Washington State University, pp. 3693-3711.

Kumar, S., Dagnoko, S., Haougui, A., Ratnadass, A., Pasternak, D., Kouame, C., 2010. Okra (Abelmoschus spp.) in West and Central Africa: Potential and progress on its improvement. African Journal of Agricultural Research 5, 3590-3598.

Laksameethanasana, P., Somla, N., Janprem, S., Phochuen, N., 2012. Clarification of sugarcane juice for syrup production. Procedia Engineering 32, 141-147.

Lam, K.F., Leung, C.C.J., Lei, H.M., Lin, C.S.K., 2014. Economic feasibility of a pilot-scale fermentative succinic acid production from bakery wastes. Food and Bioproducts Processing 92, 282-290.

Lambri, M., Dordoni, R., Silva, A., De Faveri, D.M., 2012. Comparing the impact of bentonite addition for both must clarification and wine fining on the chemical profile of wine from Chambave Muscat grapes. International Journal of Food Science & Technology 47, 1-12.

Lauri, K., Sanna, H., Sari, H., Hannu, M., Riikka, K., Soili, P., Tapani, S., Hanna, K., 2011. Properties and flocculation efficiency of cationized biopolymers and their applicability in papermaking and in conditioning of pulp and paper sludge. BioResources 6, 2836-2850.

Lee, C.S., Binner, E., Winkworth-Smith, C., John, R., Gomes, R., Robinson, J., 2016. Enhancing natural product extraction and mass transfer using selective microwave heating. Chemical Engineering Science 149, 97-103.

Lee, C.S., Chong, M.F., Robinson, J., Binner, E., 2014a. A review on development and application of plant-based bioflocculants and grafted bioflocculants. Industrial & Engineering Chemistry Research 53, 18357-18369.

Lee, C.S., Chong, M.F., Robinson, J., Binner, E., 2015. Optimisation of extraction and sludge dewatering efficiencies of bio-flocculants extracted from Abelmoschus esculentus (okra). Journal of Environmental Management 157, 320-325.

Lee, C.S., Robinson, J., Chong, M.F., 2014b. A review on application of flocculants in wastewater treatment. Process Safety and Environmental Protection 92, 489-508.

Lee, K.E., Morad, N.H., Teng, T.T., Poh, B.T., 2012. Development, characterization and the application of hybrid materials in coagulation/flocculation of wastewater: A review. Chemical Engineering Journal 203, 370-386.

Lee, K.E., Teng, T.T., Morad, N., Poh, B.T., Hong, Y.F., 2010. Flocculation of kaolin in water using novel calcium chloride-polyacrylamide ($CaCl_2$-PAM) hybrid polymer. Separation and Purification Technology 75, 346-351.

Lee, W.C., Yusof, S., Hamid, N.S.A., Baharin, B.S., 2007. Effects of fining treatment and storage temperature on the quality of clarified banana juice. LWT - Food Science and Technology 40, 1755-1764.

Leland Blank , P.E., Anthony Tarquin , P.E., 2011. Engineering economy, 7th ed. McGraw-Hill, New York, p. 642.

León-Martínez, F.M., Rodríguez-Ramírez, J., Medina-Torres, L.L., Méndez Lagunas, L.L., Bernad-Bernad, M.J., 2011. Effects of drying conditions on the rheological properties of reconstituted mucilage solutions (Opuntia ficus-indica). Carbohydrate Polymers 84, 439-445.

Li, P.Q., Zhou, L.G., Mou, Y., Mao, Z.L., 2015. Extraction optimization of polysaccharide from Zanthoxylum bungeanum using RSM and its antioxidant activity. International Journal of Biological Macromolecules 72, 19-27.

Li, Q., Yu, N.W., Wang, Y.P., Sun, Y.P., Lu, K.P., Guan, W.L., 2013a. Extraction optimization of Bruguiera gymnorrhiza polysaccharides with radical scavenging activities. Carbohydrate Polymers 96, 148-155.

Li, Y., Radoiu, M., Fabiano-Tixier, A.S., Chemat, F., 2013b. Chapter 8: From laboratory to industry: Scale-up, quality, and safety consideration for microwave-assisted extraction, in: Gravotto, G., Chemat, F. (Ed.), Microwave-assisted extraction for bioactive compounds: Theory and practice. Springer Science+Business Media, New York, pp. 207-230.

Liang, C.Z., Sun, S.P., Li, F.Y., Ong, Y.K., Chung, T.S., 2014. Treatment of highly concentrated wastewater containing multiple synthetic dyes by a combined process of coagulation/flocculation and nanofiltration. Journal of Membrane Science 469, 306-315.

Lide, D.R., Haynes, W.M., 2010. CRC handbook of chemistry and physics, 90th ed. CRC Press/Taylor and Francis, Boca Raton.

Liu, C.J., Liu, Q., Sun, J.D., Jiang, B., Yan, J.F., 2014. Extraction of water-soluble polysaccharide and the antioxidant activity from Semen cassiae. Journal of Food and Drug Analysis 22, 492-499.

Liu, X.J., 2012. Annual output of 50,000 tons of polyacrylamide project of Jilin city. Government of JiLin Province, China.

Lo, I.M.C., Lai, K.C., Chen, G.H., 2001. Salinity effect on mechanical dewatering of sludge with and without chemical conditioning. Environmental Science & Technology 35, 4691-4696.

Long, R., 23 June 2016. Quotation for polyacrylamides. SNF (UK) Limited, U.K.

López-Galindo, A., Viseras, C., Cerezo, P., 2007. Compositional, technical and safety specifications of clays to be used as pharmaceutical and cosmetic products. Applied Clay Science 36, 51-63.

Lu, L.H., Pan, Z.D., Hao, N., Peng, W.Q., 2014. A novel acrylamide-free flocculant and its application for sludge dewatering. Water Research 57, 304-312.

Ma, T.T., Sun, X.Y., Tian, C.R., Luo, J.Y., Zheng, C.P., Zhan, J.C., 2016. Polysaccharide extraction from Sphallerocarpus gracilis roots by response surface methodology. International Journal of Biological Macromolecules 88, 162-170.

Mah, S.K., Leo, C.P., Wu, T.Y., Chai, S.P., 2012. A feasibility investigation on ultrafiltration of palm oil and oleic acid removal from glycerin solutions: Flux decline, fouling pattern, rejection and membrane characterizations. Journal of Membrane Science 389, 245-256.

Mai, J., 22 June 2016. Quotation for FC-301 multifunction vegetable cutter. Zhaoqing Fengxiang Food Machinery Co. Ltd., China.

Maltini, E., Torreggiani, D., Venir, E., Bertolo, G., 2003. Water activity and the preservation of plant foods. Food Chemistry 82, 79-86.

Mandal, V., Mandal, S.C., 2010. Design and performance evaluation of a microwave based low carbon yielding extraction technique for naturally occurring bioactive triterpenoid: Oleanolic acid. Biochemical Engineering Journal 50, 63-70.

Maran, J.P., Nivetha, C.V., Priya, B., Al-Dhabi, N.A., Ponmurugan, K., Manoj, J.J.B., 2016. Modeling of polysaccharide extraction from Gossypium arboreum L. seed using central composite rotatable design. International Journal of Biological Macromolecules 86, 857-864.

Maran, J.P., Swathi, K., Jeevitha, P., Jayalakshmi, J., Ashvini, G., 2015. Microwave-assisted extraction of pectic polysaccharide from waste mango peel. Carbohydrate Polymers 123, 67-71.

Martín-Diana, A.B., Rico, D., Barat, J.M., Barry-Ryan, C., 2009. Orange juices enriched with chitosan: Optimisation for extending the shelf-life. Innovative Food Science & Emerging Technologies 10, 590-600.

Martín, M.A., González, I., Berrios, M., Siles, J.A., Martín, A., 2011. Optimization of coagulation–flocculation process for wastewater derived from sauce manufacturing using factorial design of experiments. Chemical Engineering Journal 172, 771-782.

Martinez, J., Silva, L.P.S., 2013. Chapter 10: Scale-up of extraction processes, in: Rostagno, M.A., Prado, J.M. (Ed.), Natural product extraction: Principles and applications. The Royal Society of Chemistry, United Kingdom.

Masahiro, K., Midori, K.N., 2011. Link between aluminum and the pathogenesis of Alzheimer's disease: The Integration of the aluminum and amyloid cascade hypotheses. International Journal of Alzheimer's Disease 2011, 276393.

Mato Chain, R.B., Monzo-Cabrera, J., Solyom, K., 2016. Chapter 2: Microwave-assisted plant extraction processes, in: Stefanidis, G., Stankiewicz, A. (Ed.), Alternative energy sources for green chemistry. The Royal Society of Chemistry, UK, pp. 34-63.

McMillan, J.R., Watson, I.A., Ali, M., Jaafar, W., 2013. Evaluation and comparison of algal cell disruption methods: Microwave, waterbath, blender, ultrasonic and laser treatment. Applied Energy 103, 128-134.

Meredith, R.J., 1998. Engineers' handbook of industrial microwave heating. The Institution of Engineering and Technology, London, U.K., p. 377.

Mirhosseini, H., Amid, B.T., 2012. A review study on chemical composition and molecular structure of newly plant gum exudates and seed gums. Food Research International 46, 387-398.

Mishra, A., Bajpai, M., 2006a. The flocculation performance of Tamarindus mucilage in relation to removal of vat and direct dyes. Bioresource Technology 97, 1055-1059.

Mishra, A., Bajpai, M., Pal, S., Agrawal, M., Pandey, S., 2006b. Tamarindus indica mucilage and its acrylamide-grafted copolymer as flocculants for removal of dyes. Colloid and Polymer Science 285, 161-168.

Mishra, A., Srinivasan, R., 2008. Okra (Hibiscus Esculentus) and Fenugreek (Trigonella Foenum Graceum) mucilage: Characterization and application as flocculants for textile effluent treatments. Chinese Journal of Polymer Science 26, 679-687.

Mishra, S., Rani, G.U, Sen, G., 2012. Microwave initiated synthesis and application of polyacrylic acid grafted carboxymethyl cellulose. Carbohydrate Polymers 87, 2255-2262.

Mohamed, M.A., Gamea, G.R., Keshek, M.H., 2010. Drying characteristics of okra by different solar dryers. Misr Journal of Agricultural Engineering 27, 294-312.

Narayanan, K.V., 2013. A textbook of chemical engineering thermodynamics, 2nd ed. PHI Learning Private Limited, Delhi, p. 552.

Nasehi, S.M., Ansari, S., Sarshar, M., 2012. Removal of dark colored compounds from date syrup using activated carbon: A kinetic study. Journal of Food Engineering 111, 490-495.

Nasser, M.S., James, A.E., 2006. The effect of polyacrylamide charge density and molecular weight on the flocculation and sedimentation behaviour of kaolinite suspensions. Separation and Purification Technology 52, 241-252.

Navarrete, A., Mato, R.B., Dimitrakis, G., Lester, E..Robinson, J.R., Cocero, M.J., Kingman, S., 2011. Measurement and estimation of aromatic plant dielectric properties. Application to low moisture rosemary. Industrial Crops and Products 33, 697-703.

Neil, J.M., 2005. Project and cost engineers' handbook, in: Humphreys, K.K. (Ed.), 4th ed. Marcel Dekker, New York, p. 338.

Nelson, S.O., 2006. Agricultural applications of dielectric measurements. IEEE Transactions on Dielectrics and Electrical Insulation 13, 688-702.

Nongkhran, C.W., Apinon, N., 2008. Influence of pH, electrolytes and polymers on flocculation of kaolin particle. Chiang Mai Journal of Science 35, 11-16.

Okaiyeto, K., Nwodo, U.U., Okoli, S.A., Mabinya, L.V., Okoh, A.I., 2016. Implications for public health demands alternatives to inorganic and synthetic flocculants: bioflocculants as important candidates. MicrobiologyOpen 5, 177-211.

Pak, T., 2016. Cleaning in place-A guide to cleaning technology in the food processing industry. Tetra Pak International S.A., Sweden.

Pal, S., Ghosh, S., Sen, G., Jha, U., Singh, R.P., 2009. Cationic tamarind kernel polysaccharide (Cat TKP): A novel polymeric flocculant for the treatment of textile industry wastewater. International Journal of Biological Macromolecules 45, 518-523.

Palma, M., Barbero, G.F., Pineiro, Z., Liazid, A., Barroso, C.G., Rostagno, M.A., Prado, J.M., Meireles, M.A.A, 2013. Chapter 2: Extraction of natural products-Principles and fundamental aspects, in: Mauricio, A.R., Juliana, M.P. (Ed.), Natural product extraction: Principles and applications. The Royal Society of Chemistry, UK.

Papavasileiou, V., Siletti, C., Koulouris, A., Petrides, D., 2013. Chapter 1: Bioprocess design, computer-aided, in: Flickinger, M.C. (Ed.), Downstream industrial biotechnology: Recovery and purification. John Wiley & Sons, Inc., New Jersey, pp. 5-24.

Parker, A., 2007. Section 9: Effective cleaning and sanitizing procedures, JIFSAN Good Aquacultural Practices Manual Joint Institute of Foos Safety and Applied Nutrition, USA, p. 8.

Pereira, C.G., Prado, J.M., Meireles, M.A.A., 2013. Chapter 12: Economic evaluation of natural product extraction processes, in: Rostagno, M.A., Prado, J.M. (Ed.), Natural product extraction: Principles and applications. Royal Society of Chemistry, U.K., pp. 442-471.

Périno, S., Pierson, J.T., Ruiz, K., Cravotto, G., Chemat, F., 2016. Laboratory to pilot scale: Microwave extraction for polyphenols lettuce. Food Chemistry 204, 108-114.

Petigny, L., Périno, S., Minuti, M., Visinoni, F., Wajsman, J., Chemat, F., 2014. Simultaneous microwave extraction and separation of volatile and non-volatile organic compounds of Boldo leaves. From lab to industrial scale. International Journal of Molecular Sciences 15, 7183-7198.

Philip, J.L., Jessy, V.W., 2012. Chapter 1: Introduction to extraction in food processing, in: Nikolai, L., Eugene, V., Farid, C. (Ed.), Enhancing extraction processes in the food industry. CRC Press, Boca Raton, pp. 1-24.

Pieczywek, P.M., Zdunek, A., 2014. Finite element modelling of the mechanical behaviour of onion epidermis with incorporation of nonlinear properties of cell walls and real tissue geometry. Journal of Food Engineering 123, 50-59.

Pirbalouti, A.G., Mahdad, E., Craker, L., 2013. Effects of drying methods on qualitative and quantitative properties of essential oil of two basil landraces. Food Chemistry 141, 2440-2449.

Prime, J., 2013. Bioenergy statistics – UK overview. Department of Energy & Climate Change, U.K.

Pushpavanam, S., 2012. Introduction to chemical engineering. PHI Learning Private Limited, New Delhi, p. 173.

Qiao, D.L., Hu, B., Gan, D., Sun, Y., Ye, H., Zeng, X.X., 2009. Extraction optimized by using response surface methodology, purification and preliminary characterization of polysaccharides from Hyriopsis cumingii. Carbohydrate Polymers 76, 422-429.

Quant, C.A., Marla, K.T., Meredith, J.C., 2008. Osmotic pressure and chemical potential of silica nanoparticles in aqueous poly(ethyleneoxide) solution. Colloids and Surfaces A: Physicochemical and Engineering Aspects 317, 129-135.

Raluca, N., Elena, B., Ruben, M., Angeles, B., 2013. Flocculation efficiency of chitosan for papermaking applications. BioResources 8, 768-784.

Ramavandi, B., 2014. Treatment of water turbidity and bacteria by using a coagulant extracted from Plantago ovata. Water Resources and Industry 6, 36-50.

Ranade, V.V., Bhandari, V.M., 2014. Chapter 1: Industrial wastewater treatment, recycling, and reuse: An overview, Industrial wastewater treatment, recycling and reuse. Butterworth-Heinemann, Oxford, pp. 1-80.

Raquel, R.O., 2009. Chapter 3: Ultrasound assisted extraction for the analysis of organic compounds by chromatographic techniques, in: Jose-Luis, C.M. (Ed.), Ultrasound in Chemistry: Analytical applications. Wiley-VCH, Germany, pp. 55-80.

Rasteiro, M.G., Garcia, F.A.P., del Mar Pérez, M., 2007. Applying LDS to monitor flocculation in papermaking. Particulate Science and Technology 25, 303-308.

Razali, M.A.A., Ahmad, Z., Ahmad, M.S.B., Ariffin, A., 2011. Treatment of pulp and paper mill wastewater with various molecular weight of polyDADMAC induced flocculation. Chemical Engineering Journal 166, 529-535.

Refaat, A.A., 2009. Different techniques for the production of biodiesel from waste vegetable oil. International Journal of Environmental Science & Technology 7, 183-213.

Renault, F., Sancey, B., Badot, P.M., Crini, G., 2009a. Chitosan for coagulation/flocculation processes – An eco-friendly approach. European Polymer Journal 45, 1337-1348.

Renault, F., Sancey, B., Charles, J., Morin-Crini, N., Badot, P.-M., Winterton, P., Crini, G., 2009b. Chitosan flocculation of cardboard-mill secondary biological wastewater. Chemical Engineering Journal 155, 775-783.

Rice, E.W., Baird, R.B., Eaton, A.D., Clesceri, L.S. , 2012. Standard methods for the examination of water and wastewater, 22 ed. American Public Health Association, AWWA (American Water Works Association) and Water Environment Federation, Washington, p. 1496.

Richardson, J.F., Harker, J.H., Backhurst, J.R., 2002. Chemical engineering: Particle technology and separation processes, 5th ed. Butterworth-Heinemann, Oxford, p. 1208.

Robinson, J., Personal communication (20 June 2016). Utility cost associated with generation of microwave energy, in: Lee, C.S. (Ed.). Microwave Processing Engineering Research Group, The University of Nottingham, U.K.

Robinson, J., Personal communication (22 June 2016). Price for microwave generator (maximum power generation is 100 kW), in: Lee, C.S. (Ed.). Microwave Processing Engineering Research Group, The University of Nottingham, U.K.

Robinson, J., Kingman, S., Irvine, D., Licence, P., Smith, A., Dimitrakis, G., Obermayer, D., Kappe, C.O., 2010a. Understanding microwave heating effects in single mode type cavities-theory and experiment. Physical Chemistry Chemical Physics 12, 4750-4758.

Robinson, J.P., Kingman, S.W., Snape, C.E., Bradshaw, S.M., Bradley, M.S.A., Shang, H., Barranco, R., 2010b. Scale-up and design of a continuous microwave treatment system for the processing of oil-contaminated drill cuttings. Chemical Engineering Research and Design 88, 146-154.

Rodriguez-Jasso, R.M., Mussatto, S.I., Pastrana, L., Aguilar, C.N., Teixeira, J.A., 2011. Microwave-assisted extraction of sulfated polysaccharides (fucoidan) from brown seaweed. Carbohydrate Polymers 86, 1137-1144.

Rostami, H., Gharibzahedi, S.M.T., 2016. Microwave-assisted extraction of jujube polysaccharide: Optimization, purification and functional characterization. Carbohydrate Polymers 143, 100-107.

Rushton, A., 1996. Solid-liquid filtration and separation technology, in: Rushton, A., Ward, A.S., Holdich, R.G. (Ed.), 1st ed. Wiley-VCH, Weinheim, p. 538.

Sabah, E., Erkan, Z.E., 2006. Interaction mechanism of flocculants with coal waste slurry. Fuel 85, 350-359.

Sahin, S., Sumnu, S.G., 2006. Chapter 4: Electromagnetic properties, in: Heldman, D.R. (Ed.), Physical properties of foods. Springer Science+Business Media, USA, pp. 157-192.

Samavati, V., 2013. Polysaccharide extraction from Abelmoschus esculentus: Optimization by response surface methodology. Carbohydrate Polymers 95, 588-597.

Santos, L.S., Amalraj, J., Gleisner, V.F.L., 2014. Clarification and selective binding of phenolic compounds from liquid foodstuff or beverages using smart polymers. Google Patents.

Saravacos, G., Kostaropoulos, A.E., 2016. Handbook of food processing equipment, in: Barbosa-Canovas, G.V. (Ed.), 2nd ed. Springer International Publishing, Switzerland, p. 781.

Saravacos, G.D., Maroulis, Z.B., 2011. Food process engineering operations, in: Sun, D.W. (Ed.). Taylor & Francis Group, LLC, Boca Raton, p. 586.

Sarika, R., Kalogerakis, N., Mantzavinos, D., 2005. Treatment of olive mill effluents: Part II. Complete removal of solids by direct flocculation with poly-electrolytes. Environment International 31, 297-304.

Sdrula, N., 2010. A study using classical or membrane separation in the biodiesel process. Desalination 250, 1070-1072.

Seader, J.D., 2011. Separation process principles: Chemical and biochemical operations, in: Seader, J.D., Henley, E.J., Roper, D.K. (Ed.), 3rd ed. John Wiley & Sons, Inc., U.S., p. 849.

Sengkhamparn, N., Verhoef, R., Schols, H.A., Sajjaanantakul, T., Voragen, A.G.J., 2009. Characterisation of cell wall polysaccharides from okra (Abelmoschus esculentus (L.) Moench). Carbohydrate Research 344, 1824-1832.

Shak, K.P.Y., Wu, T.Y., 2014. Coagulation–flocculation treatment of high-strength agro-industrial wastewater using natural Cassia obtusifolia seed gum: Treatment efficiencies and flocs characterization. Chemical Engineering Journal 256, 293-305.

Shak, K.P.Y., Wu, T.Y., 2015. Optimized use of alum together with unmodified Cassia obtusifolia seed gum as a coagulant aid in treatment of palm oil mill effluent under natural pH of wastewater. Industrial Crops and Products 76, 1169-1178.

Shao, Q.S., Deng, Y.M., Shen, H.J., Fang, H.L., Zhao, X.F., 2011. Optimization of polysaccharides extraction from Tetrastigma hemsleyanum Diels et Gilg using response surface methodology. International Journal of Biological Macromolecules 49, 958-962.

Sharma, B.R., Dhuldhoya, N.C., Merchant, U.C., 2006. Flocculants-An ecofriendly approach. Journal of Polymers and the Environment 14, 195-202.

Sher, F., Malik, A.,Liu, H., 2013. Industrial polymer effluent treatment by chemical coagulation and flocculation. Journal of Environmental Chemical Engineering 1, 684-689.

Shi, J., Kassama, L.S., Kakuda, Y., 2007. Chapter 1: Supercritical fluid technology for extraction of bioactive components, in: Shi, J. (Ed.), Functional food ingredients and nutraceuticals: Processing technologies. CRC Press, Boca Raton, pp. 3-44.

Shipp, A., Lawrence, G., Gentry, R., McDonald, T., Bartow, H., Bounds, J., Macdonald, N., Clewell, H., Allen, B., Landingham, C.V., 2006. Acrylamide: Review of toxicity data and dose-response analyses for cancer and noncancer effects. Critical Review in Toxicology 36, 481-608.

Shyam, N.J., 2010. Chapter 8: Miscellaneous techniques, in: Shyam, N.J. (Ed.), Nondestructive evaluation of food quality: Theory and practice. Springer-Verlag Berlin Heidelberg, pp. 235-283.

Silla, H., 2003. Chemical process engineering: Design and economics. Taylor & Francis Group LLC, U.S., p. 483.

Singhabhandhu, A., Tezuka, T., 2010. A perspective on incorporation of glycerin purification process in biodiesel plants using waste cooking oil as feedstock. Energy 35, 2493-2504.

Sinnott, R.K., 2005. Chemical engineering design, 4th ed. Elsevier Butterworth-Heinemann, Oxford, p. 1055.

Smith, R., 2005. Chemical process design and integration, in: Smith, R. (Ed.). John Wiley & Sons Ltd, U.K., p. 713.

Soria, A.C., Ruiz-Aceituno, L., Ramos, L., Sanz, L.M., 2015. Chapter 34: Microwave-assisted extraction of polysaccharides, in: Ramawat, G.K., Mérillon, J.M. (Ed.), Polysaccharides: Bioactivity and biotechnology. Springer International Publishing, Switzerland, pp. 987-1008.

Sosa-Morales, M.E., Valerio-Junco, L., López-Malo, A., García, H.S., 2010. Dielectric properties of foods: Reported data in the 21st century and their potential applications. LWT - Food Science and Technology 43, 1169-1179.

Spellman, F.R., 2009. Handbook of water and wastewater treatment plant operations, in: Spellman, F.R. (Ed.), 2nd ed. Taylor & Francis Group, LLC, Boca Raton, p. 872.

Spigno, G., De Faveri, D.M., 2009. Microwave-assisted extraction of tea phenols: A phenomenological study. Journal of Food Engineering 93, 210-217.

Stephen, A.M., Phillips, G.O., 2006. Food polysaccharides and their applications, 2nd ed. CRC Press.

Stiles, W., 2006. Principles of plant physiology. Discovery Publishing House, New Delhi, p. 585.

Stuart, O.N., Ashim, K.D., 2001. Chapter 3: Dielectric properties of food materials and electric field interactions, in: Ramaswamy, C.A., Ashim K.D. (Ed.), Handbook of microwave technology for food applications. Marcel Dekker, United States of America, pp. 69-114.

Subramaniam, S., Raju, R., Palanisamy, A., Sivasubramanian, A., 2015. Development and extraction optimization of baicalein and pinostrobin from Scutellaria violacea through response surface methodology. Pharmacognosy Magazine 11, S127-S138.

Sun, Y.J., Fan, W., Zheng, H.L., Zhang, Y.X., Li, F.T., Chen, W., 2015. Evaluation of dewatering performance and fractal characteristics of alum sludge. PLoS ONE 10, e0130683.

Suopajärvi, T., Liimatainen, H., Hormi, O., Niinimäki, J., 2013. Coagulation–flocculation treatment of municipal wastewater based on anionized nanocelluloses. Chemical Engineering Journal 231, 59-67.

Tahergorabi, R., Beamer, S.K., Matak, K.E., Jaczynski, J., 2011. Effect of isoelectric solubilization/precipitation and titanium dioxide on whitening and texture of proteins recovered from dark chicken-meat processing by-products. LWT - Food Science and Technology 44, 896-903.

Tang, W., Song, L., Li, D., Qiao, J., Zhao, T., Zhao, H., 2014. Production, characterization, and flocculation mechanism of cation independent, pH tolerant, and thermally stable bioflocculant from Enterobacter sp. ETH-2. PLoS ONE 9, e114591.

Tao, V., 21 June 2016. Quotation for water bubble fruit vegetables washing machine. Zheng Zhou Azeus Machinery Co. Ltd, China.

Taskaya, L., Jaczynski, J., 2009. Flocculation-enhanced protein recovery from fish processing by-products by isoelectric solubilization/precipitation. LWT - Food Science and Technology 42, 570-575.

Teng, T.T., Wong, S.S., Low, L.W., 2014. Chapter 10: Coagulation–flocculation method for the treatment of pulp and paper mill wastewater, in: Fanun, M. (Ed.), The role of colloidal systems in environmental protection. Elsevier, Amsterdam, pp. 239-259.

Teresa, Z.P., Gunther, G., Fernando, H., 2007. Chemical oxygen demand reduction in coffee wastewater through chemical flocculation and advanced oxidation processes. Journal of Environmental Sciences 19, 300-305.

Terigar, B.G., Balasubramanian, S., Sabliov, C.M., Lima, M., Boldor, D., 2011. Soybean and rice bran oil extraction in a continuous microwave system: From laboratory to pilot-scale. Journal of Food Engineering 104, 208-217.

Thai, C.C.D., Bakir, H., Doherty, W.O.S., 2012. Insights to the clarification of sugar cane juice expressed from sugar cane stalk and trash. Journal of Agricultural and Food Chemistry 60, 2916-2923.

Thakur, V.K., Thakur, M.K., 2015. Handbook of polymers for pharmaceutical technologies. Scrivener Publishing LLC. and John Wiley & Sons, Inc. , Canada, p. 552.

Thirugnanasambandham, K., Sivakumar, V., Maran, J.P., 2015. Microwave-assisted extraction of polysaccharides from mulberry leaves. International Journal of Biological Macromolecules 72, 1-5.

Towler, G., 2013. Chemical engineering design: Principles, practise and economics of plant and process design, in: Towler, G., Sinnott, R. (Ed.), 2nd ed. Butterworth-Heinemann, U.K., p. 1269.

Turfan, Ö., Türkyılmaz, M., Yemiş, O., Özkan, M., 2011. Anthocyanin and colour changes during processing of pomegranate (Punica granatum L., cv. Hicaznar) juice from sacs and whole fruit. Food Chemistry 129, 1644-1651.

USEPA, 2012a. EPA 822-S-12-001: Edition of the Drinking Water Standards and Health Advisories. United States Environmental Protection Agency, Washington.

USEPA, 2012b. FWPCA methods for chemical analysis of water and wastes. BiblioGov, United States of America, p. 268.

Valdir, S., 2012. Chapter 14: Stability and degradation of polysaccharides, in: Severian, D. (Ed.), Polysaccharides: Structural diversity and functional versatility, 2nd ed. Marcel Dekker, New York, pp. 395-410.

Veera, G.G., Edith, M.G., 2015. Chapter 11: Green chemistry of microwave-enhanced biodiesel production, in: Zhen, F., Richard, L.S., Qi, X.H. (Ed.), Production of biofuels and chemicals with microwave. Springer Science+Business Media, Dordrecht, pp. 225-250.

Veggi, P.C., Martinez, J., Meireles, M.A.A., 2013. Chapter 2: Fundamentals of microwave extraction, in: Chemat, F., Cravotto, G. (Ed.), Microwave-assisted extraction for bioactive compounds: Theory and practice. Springer US, Boston, pp. 15-52.

Vera, J.C., Heiwall, A., Ogbonnaya, D., 2009. Pectic polysaccharides isolated from fruit pods of okra. Google Patents US 20090170809 A1.

Vučurović, D.G., Dodić, S.N., Popov, S.D., Dodić, J.M., Grahovac, J.A., 2012. Process model and economic analysis of ethanol production from sugar beet raw juice as part of the cleaner production concept. Bioresource Technology 104, 367-372.

Wagner, C.F.L., Richard, M., Jose, E.C., 2011. Process for the purification of crude glycerol, European Patent.

Wahidin, S., Idris, A., Shaleh, S.R.M., 2014. Rapid biodiesel production using wet microalgae via microwave irradiation. Energy Conversion and Management 84, 227-233.

Wang, J.L., Zhang, J., Wang, X.F., Zhao, B.T., Wu, Y.Q., Yao, J., 2009. A comparison study on microwave-assisted extraction of Artemisia sphaerocephala polysaccharides with conventional method: Molecule structure and antioxidant activities evaluation. International Journal of Biological Macromolecules 45, 483-492.

Wang, J.L., Zhang, J., Zhao, B.T., Wang, X.F., Wu, Y.Q., Yao, J., 2010. A comparison study on microwave-assisted extraction of Potentilla anserina L. polysaccharides with conventional method: Molecule weight and antioxidant activities evaluation. Carbohydrate Polymers 80, 84-93.

Wang, J.P., Yuan, S.J., Wang, Y., Yu, H.Q., 2013. Synthesis, characterization and application of a novel starch-based flocculant with high flocculation and dewatering properties. Water Research 47, 2643-2648.

Wang, L.K., Shammas, N.K., Hung, Williford, C., Chen, W.Y., Sakellaropoulos, G.P., 2007. Chapter 19: Evaporation processes, in: Wang, L.K., Shammas, N.K., Hung, Y.T. (Ed.), Biosolids treatment processes. Humana Press, New Jersey, pp. 583-612.

Wang, Y.F., Chen, K.F., Mo, L.H., Li, J., Xu, J., 2014. Optimization of coagulation–flocculation process for papermaking-reconstituted tobacco slice wastewater treatment using response surface methodology. Journal of Industrial and Engineering Chemistry 20, 391-396.

Wang, Y.H., Wu, F.H., Guo, Z.H., Peng, M.H., Zhang, Y.N., Ling, P.Z., Du, M.H., Zhang,C.Y., Liang, Z., 2015. Optimization of extraction process for polysaccharide in Salvia Miltiorrhiza Bunge using response surface methodology. The Open Biomedical Engineering Journal 9, 347-353.

Wankhade, P.K., Sapkal, R.S., Sapkal, V.S., 2013. Drying characteristics of okra slices on drying in hot air dryer. Procedia Engineering 51, 371-374.

Wei, X.L., Chen, M.A., Xiao, J.B., Liu, Y., Yu, L., Zhang, H., Wang, Y.F., 2010. Composition and bioactivity of tea flower polysaccharides obtained by different methods. Carbohydrate Polymers 79, 418-422.

Whistler, R.L., Bemiller, J.N., 1993. Industrial gums: Polysaccharides and its derivatives, 3rd ed. Academic Press Limited, California, p. 657.

Willför, S., Sjöholm, R., Laine, C., Roslund, M., Hemming, J., Holmbom, B., 2003. Characterisation of water-soluble galactoglucomannans from Norway spruce wood and thermomechanical pulp. Carbohydrate Polymers 52, 175-187.

Wong, S.S., Teng, T.T., Ahmad, A.L., Zuhairi, A., Najafpour, G., 2006. Treatment of pulp and paper mill wastewater by polyacrylamide (PAM) in polymer induced flocculation. Journal of Hazardous Materials 135, 378-388.

Woodrow, J.E., Seiber, J.N., Miller, G.C., 2008. Acrylamide release resulting from sunlight irradiation of aqueous polyacrylamide/iron mixtures. Journal of Agricultural and Food Chemistry 56, 2773-2779.

Woolfe, M.L., Chaplin, M.F., Otchere, G., 1977. Studies on the mucilages extracted from okra fruits (Hibiscus esculentus L.) and baobab leaves (Adansonia digitata L.). Journal of the Science of Food and Agriculture 28, 519-529.

Wu, T.Y., Mohammad, A.W., Md. Jahim, J., Anuar, N., 2007. Palm oil mill effluent (POME) treatment and bioresources recovery using ultrafiltration membrane: Effect of pressure on membrane fouling. Biochemical Engineering Journal 35, 309-317.

Xie, J.H., Xie, M.Y., Shen, M.Y., Nie, S.P., Li, C., Wang, Y.X., 2010. Optimisation of microwave-assisted extraction of polysaccharides from Cyclocarya paliurus (Batal.) Iljinskaja using response surface methodology. Journal of the Science of Food and Agriculture 90, 1353-1360.

Yang, L.Q., Qu, H.Y., Mao, G.H., Zhao, T., Li, F., Zhu, B., Zhang, B.T., Wu, X.Y., 2013. Optimization of subcritical water extraction of polysaccharides from Grifola frondosa using response surface methodology. Pharmacognosy Magazine 9, 120-129.

Yang, Y., Li, Y., Zhang, Y.M., Liang, D.W., 2010. Applying hybrid coagulants and polyacrylamide flocculants in the treatment of high-phosphorus hematite flotation wastewater (HHFW): Optimization through response surface methodology. Separation and Purification Technology 76, 72-78.

Yang, Z.H., Huang, J., Zeng, G.M., Ruan, M., Zhou, C.S., Li, L., Rong, Z.G., 2009. Optimization of flocculation conditions for kaolin suspension using the composite flocculant of MBFGA1 and PAC by response surface methodology. Bioresource Technology 100, 4233-4239.

Ye, C.L., Jiang, C.J., 2011. Optimization of extraction process of crude polysaccharides from Plantago asiatica L. by response surface methodology. Carbohydrate Polymers 84, 495-502.

Ye, Z.P., Wang, W., Yuan, Q.X., Ye, H., Sun, Y., Zhang, H.C., Zeng, X.X., 2016. Box–Behnken design for extraction optimization, characterization and in vitro antioxidant activity of Cicer arietinum L. hull polysaccharides. Carbohydrate Polymers 147, 354-364.

Yeoh, S., Shi, J., Langrish, T.A.G., 2008. Comparisons between different techniques for water-based extraction of pectin from orange peels. Desalination 218, 229-237.

Yin, G.H., Dang, Y.L., 2008. Optimization of extraction technology of the Lycium barbarum polysaccharides by Box–Behnken statistical design. Carbohydrate Polymers 74, 603-610.

Yin, X.L., You, Q.H., Jiang, Z.H., Zhou, X.H., 2016. Optimization for ultrasonic-microwave synergistic extraction of polysaccharides from Cornus officinalis and characterization of polysaccharides. International Journal of Biological Macromolecules 83, 226-232.

Ying, Z., Han, X.X., Li, J.R., 2011. Ultrasound-assisted extraction of polysaccharides from mulberry leaves. Food Chemistry 127, 1273-1279.

Yu, G.H., He, P.J., Shao, L.M., He, P.P., 2008. Stratification structure of sludge flocs with implications to dewaterability. Environmental Science & Technology 42, 7944-7949.

Yue, Q.Y., Gao, B.Y., Wang, Y., Zhang, H., Sun, X., Wang, S.G., Gu, R.R., 2008. Synthesis of polyamine flocculants and their potential use in treating dye wastewater. Journal of Hazardous Materials 152, 221-227.

Zaharuddin, N.D., Noordin, M.I., Kadivar, A., 2014a. The use of Hibiscus esculentus (okra) gum in sustaining the release of propranolol hydrochloride in a solid oral dosage form. BioMed Research International, 735891, 735898 pages.

Zaharuddin, N.D., Noordin, M.I., Kadivar, A., 2014b. The use of Hibiscus esculentus (okra) gum in sustaining the release of propranolol hydrochloride in a solid oral dosage form. BioMed Research International 2014, 8.

Zakaria, S.M., Kamal, S.M.M., 2016. Subcritical water extraction of bioactive compounds from plants and algae: Applications in pharmaceutical and food ingredients. Food Engineering Reviews 8, 23-34.

Zakrajšek, N., Fuente, E., Blanco, A., Golob, J., 2009. Influence of cationic starch adsorption on fiber flocculation. Chemical Engineering & Technology 32, 1259-1265.

Zeng, H.L., Zhang, Y., Lin, S., Jian, Y.Y., Miao, S., Zheng, B.D., 2015. Ultrasonic–microwave synergistic extraction (UMSE) and molecular weight distribution of polysaccharides from Fortunella margarita (Lour.) Swingle. Separation and Purification Technology 144, 97-106.

Zetasizer, 2016. Zetasizer nano series. Malvern Instruments Limited, Worcestershire in U.K.

Zhai, Z.L., Tang, L., Liu, X.H., Gao, X., Jia, J.J., 2015. Ultrasound-assisted extraction process of polysaccharides from okra assisted by response surface methodology, International Conference on Advanced Manufacturing and Industrial Application (ICAMIA 2015) Atlantis Press pp. 149-152.

Zhang, B.B., Su, H.Q., Gu, X.J., Huang, X.L., Wang, H.M., 2013. Effect of structure and charge of polysaccharide flocculants on their flocculation performance for bentonite suspensions. Colloids and Surfaces A: Physicochemical and Engineering Aspects 436, 443-449.

Zhang, L., Reddy, N., Koyyalamudi, S.R., 2014. Chapter 5 - Isolation, characterization, and biological activities of polysaccharides from medicinal plants and mushrooms, in: Atta ur, R. (Ed.), Studies in natural products chemistry. Elsevier, pp. 117-151.

Zhang, T.T., Lu, C.L., Jiang, J.C., Wang, M., Wang, D.M., Zhu, W., 2015. Bioactivities and extraction optimization of crude polysaccharides from the fruits and leaves of Rubus chingii Hu. Carbohydrate Polymers 130, 307-315.

Zhang, Z.Q., Xia, S.Q., Zhang, J., 2010. Enhanced dewatering of waste sludge with microbial flocculant TJ-F1 as a novel conditioner. Water Research 44, 3087-3092.

Zhao, B.T., Zhang, J., Guo, X., Wang, J.L., 2013. Microwave-assisted extraction, chemical characterization of polysaccharides from Lilium davidii var. unicolor Salisb and its antioxidant activities evaluation. Food Hydrocolloids 31, 346-356.

Zhao, C.J., Li, Z., Li, C.Y., Yang, L., Yao, L.P., Fu, Y.J., He, X., Shi, K.M., Lu, Z.C., 2015. Optimized extraction of polysaccharides from Taxus chinensis var. mairei fruits and its antitumor activity. International Journal of Biological Macromolecules 75, 192-198.

Zhao, T., 4 July 2016. Quotation for activated carbon. Zhengzhou Wade Water Treatment Material Co., Ltd., China.

Zheng, X.Z., Yin, F.P., Liu, C.H., Xu, X.W., 2011. Effect of process parameters of microwave assisted extraction (MAE) on polysaccharides yield from pumpkin. Journal of Northeast Agricultural University 18, 79-86.

Zhong, K., Lin, W.J., Wang, Q., Zhou, S.M., 2012. Extraction and radicals scavenging activity of polysaccharides with microwave extraction from mung bean hulls. International Journal of Biological Macromolecules 51, 612-617.

Zhou, X.Y., Liu, R.L., Ma, X., Zhang, Z.Q., 2014. Polyethylene glycol as a novel solvent for extraction of crude polysaccharides from pericarpium granati. Carbohydrate Polymers 101, 886-889.

Zimmermann, U., Hüsken, D., Schulze, E.D., 1980. Direct turgor pressure measurements in individual leaf cells of Tradescantia virginiana. Planta 149, 445-453.

APPENDIX

Appendix A: Standard state molar entropy for liquid water

The standard state molar entropy for liquid water is calculated by using Equation A.1 (Allison, 2016; Lide, 2010):

$$s^o = A \ln t + Bt + \frac{Ct^2}{2} + \frac{Dt^3}{3} - \frac{E}{2t^2} + G \tag{A.1}$$

where s^o = standard entropy (J/mol.K) and t = temperature (K) / 1000. The values for other parameters in the equation are listed as below.

Table A1: Parameter values for calculation of standard entropy

Parameter	**Value**
A	-203.606
B	1523.29
C	-3196.413
D	2474.455
E	3.855326
G	-488.7163

Appendix B: Design process flowsheet and equipment specification

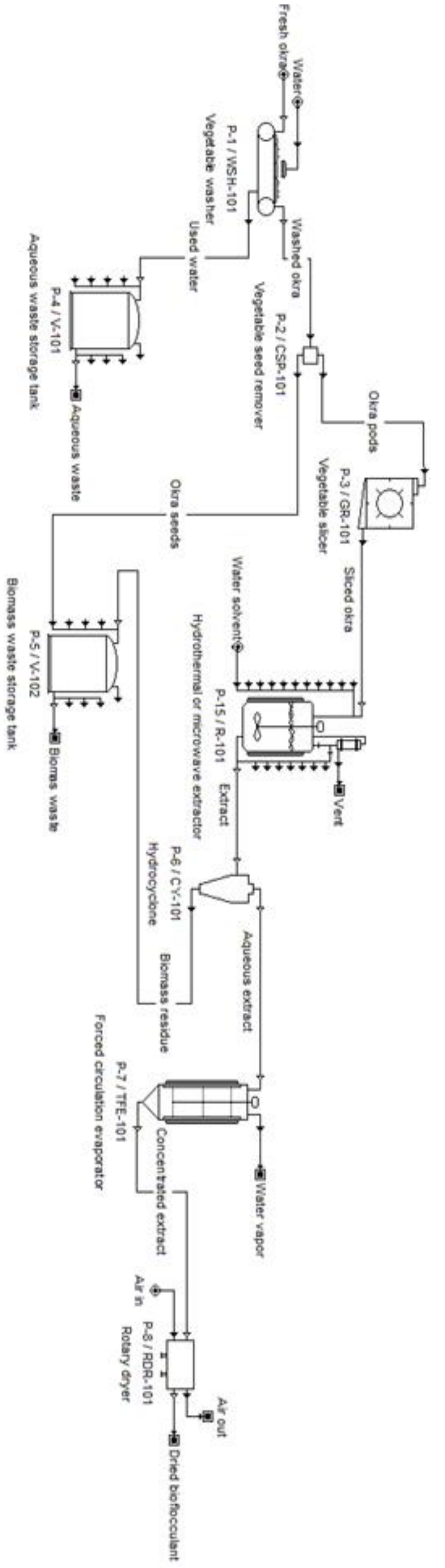


Figure B1: Extraction process simulation flowsheet

Table B1: Equipment specification and its purchase price for continuous microwave extraction of bio-flocculant at annual production of 100 tonne/year

Equipment	**Operation**	**Operating temperature (˚C)**	**Operating pressure (kPa)**	**Power (kW)**	**Capacity**	**Dimensions**	**Other specification**	**Material of construction (MoC) and its factor (Towler, 2013)**	**Base estimate equipment cost ($/unit)**	**Purchase cost (PC) = base cost x MoC ($/unit)**	**Installed multiplier (IM) (Couper, 2012)**	**Installed cost = PC x IM ($/unit)**
Vegetable washer	Bulk washing	ambient	atmospheric	1.87 (Tao, 21 June 2016)	332.9 kg/h	3m x 1m x 1.4m (Tao, 21 June 2016)	None	SS 304, 2.25	6142 (Tao, 21 June 2016)	13820	0.3*	4146
Vegetable seed remover	Component splitting	ambient	atmospheric	2.2 (Alice, 22 June 2016)	332.9 kg/h	1.2m x 0.8m x 1.3m (Alice, 22 June 2016)	None	SS 304, 2.25	4800 (Alice, 22 June 2016)	10800	0.3*	3240
Vegetable slicer	Slicing	ambient	atmospheric	1.86 (Mai, 22 June 2016)	266.35 kg/h	1.2m x 0.6m x 1.3m (Mai, 22 June 2016)	None	SS 304, 2.25	3500 (Mai, 22 June 2016)	7875	0.3*	2363
Microwave extractor	Continuous extraction	90	atmospheric	90.4	220.5L	Length: 28m, diameter: 0.1m (Robinson, Personal communication (22 June 2016))	Extraction time: 10 minutes, mass coefficient for extraction: 0.05	Polypropylene (Robinson, Personal communication (22 June 2016)), 0.85	160935 (Robinson, Personal communication (22 June 2016); Towler, 2013)	136795	0.9	123116
Hydrocyclone	Hydrocycloning	ambient	atmospheric	None	1210 L/h	None	Pressure drop: 170 kPa (Rushton, 1996), 95% recovery of product	SS 304, 2.25	4682 (Couper, 2012)	10535	0.4	4214

Forced circulation evaporator	Evaporation	50	12.3	319.3	968.4 L/h	Heat transfer area: 15.6m^2	Heat transfer coefficient: 3kW/m^2.K (Seader, 2011)	SS 304, 2.25	162580 (Towler, 2013)	365805	0.9	329225
Rotary dryer	Rotary drying	40	atmospheric	0.16 kW/m^2 (Couper, 2012)	606 kg/h	Diameter: 1.9m, length: 5.7m (Couper, 2012), area: 34 m^2	5kg air / kg evaporated water (Couper, 2012), evaporation rate: 28.8 kg.m^3/h (Couper, 2012)	SS 304, 2.25	278233 (Towler, 2013)	626024	0.6	375614
Aqueous waste storage tank	Storage	ambient	atmospheric	None	9m^3	Height: 2.9m, diameter: 2m	None	Thermoplastic (Forbes, 2016), 0.85	13770 (Towler, 2013)	11705	0.5	5853
Biomass waste storage tank	Storage	ambient	atmospheric	None	8.1m^3	Height: 2.9m, diameter: 1.9m	None	Thermoplastic (Forbes, 2016), 0.85	13308 (Towler, 2013)	11312	0.5	5656

* The installed multiplier of blender, classifier and crusher is used as a reference for installed multiplier of vegetable washer, vegetable seed removing machine and vegetable slicer respectively (Couper, 2012).

Appendix C: Hydrothermal extractor

For vertical tank mixer, the ratio of height (H) to diameter (D) is in the range of 1.5 to 2 (Coulson, 1999). Hence, H/D of 1.5 was selected in the sizing of hydrothermal extractor. The viscosity of the extract was estimated at 0.081 N.s/m^2 (Woolfe, 1977) while the calculated density of the extract was 1000 kg/m^3. In order to achieve turbulent mixing ($Re \geq 10{,}000$) during the extraction, the below Equation C1 (Coulson, 1999; Sinnott, 2005) was used for calculation of the agitation speed required for the scale-up extractor.

$$Re = \frac{D^2 N \rho}{\mu} \tag{C1}$$

where Re = Reynolds number, D = agitator diameter (m), N = agitator speed (rps), ρ = fluid density (kg/m^3), μ = fluid viscosity (N.s/m^2).

The agitator diameter was estimated as 0.6 of the tank diameter (Sinnott, 2005). The required agitation power (P in kW/m^3) was calculated from Equation C2 (Coulson, 1999; Sinnott, 2005) below.

$$P = N_p D^5 N^3 \rho \tag{C2}$$

where N_p = power number = 5 (Sinnott, 2005).

Appendix D: Rotary dryer

The total time of drying for chosen dryers was calculated with Equation D1 below (Richardson, 2002):

$$t = \frac{1}{mA}\left[\frac{(f_1 - f_c)}{f_c} + \ln\left(\frac{f_c}{f}\right)\right] \tag{D1}$$

where $f_1 = w_1 - w_e$, $f_c = w_c - w_e$, $f = w - w_e$, w_1: initial moisture content (0.9kg/kg), w: final moisture content (0.05kg/kg), w_c: critical moisture content (0.6kg/kg) (Mohamed, 2010) and w_e: equilibrium moisture content (0.01kg/kg) (Wankhade, 2013), m: evaporation rate in kg/m^2.s, A: drying area in m^2.

Appendix E: Plate and frame filter

The filtration period was calculated by referring to a plot of filtrate volume against time for this type of filtration (Rushton, 1996) shown in Figure E1.

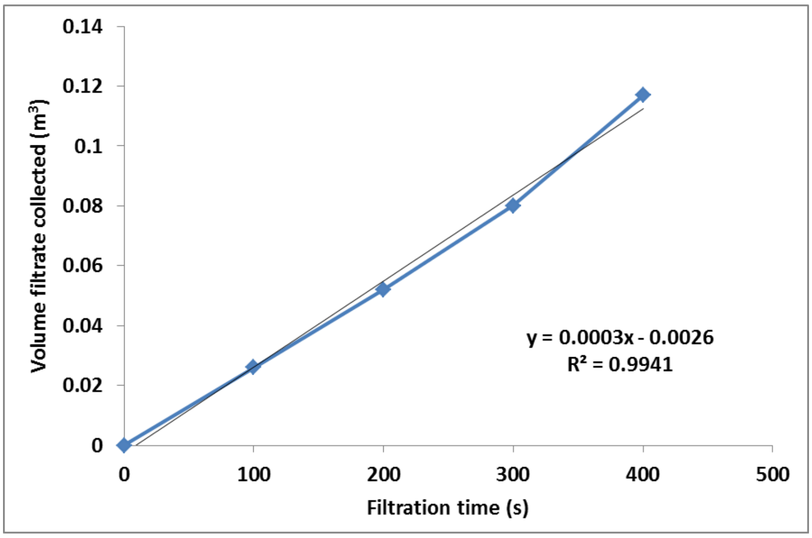
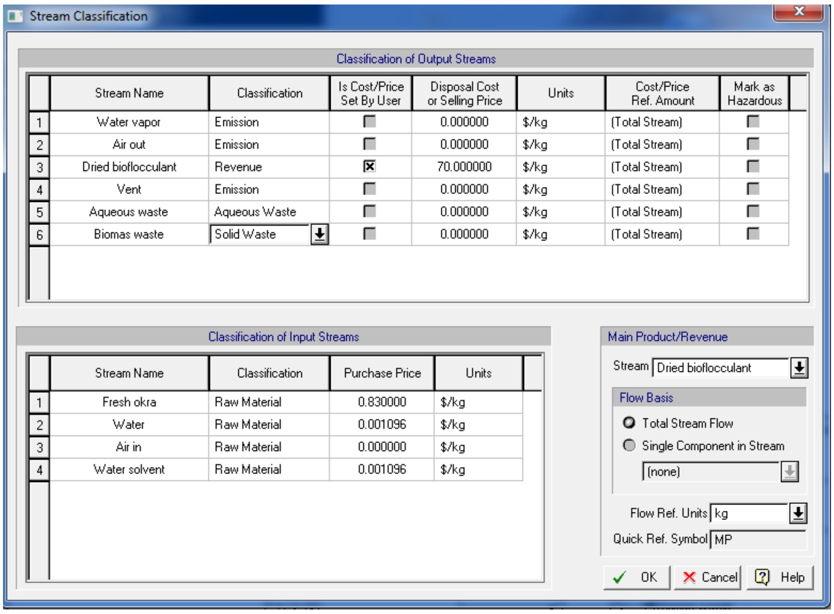


Figure E1: Filtrate volume versus time of plate and frame filter

Appendix F: Detailed explanation for simulation of bio-flocculant plant design with Superpro Designer

For first step in plant design using Superpro Designer, the mode of operation was set and all the components used in simulation flowsheet (Table 6.1) were registered and then a process flowsheet as shown in Figure B1 was drawn. Afterwards, the design parameters for all the equipment were specified by using the values listed in Table B1. Subsequently, all the inlet and outlet streams of the process were classified as shown in Figure F1 below and the mass and energy balances were solved.



Classification of Output Streams

	Stream Name	Classification	Is Cost/Price Set By User	Disposal Cost or Selling Price	Units	Cost/Price Ref. Amount	Mark as Hazardous
1	Water vapor	Emission	☐	0.000000	$/kg	(Total Stream)	☐
2	Air out	Emission	☐	0.000000	$/kg	(Total Stream)	☐
3	Dried bioflocculant	Revenue	☒	70.000000	$/kg	(Total Stream)	☐
4	Vent	Emission	☐	0.000000	$/kg	(Total Stream)	☐
5	Aqueous waste	Aqueous Waste	☐	0.000000	$/kg	(Total Stream)	☐
6	Biomas waste	Solid Waste	☐	0.000000	$/kg	(Total Stream)	☐

Classification of Input Streams

	Stream Name	Classification	Purchase Price	Units
1	Fresh okra	Raw Material	0.830000	$/kg
2	Water	Raw Material	0.001096	$/kg
3	Air in	Raw Material	0.000000	$/kg
4	Water solvent	Raw Material	0.001096	$/kg

Main Product/Revenue

Stream: Dried bioflocculant

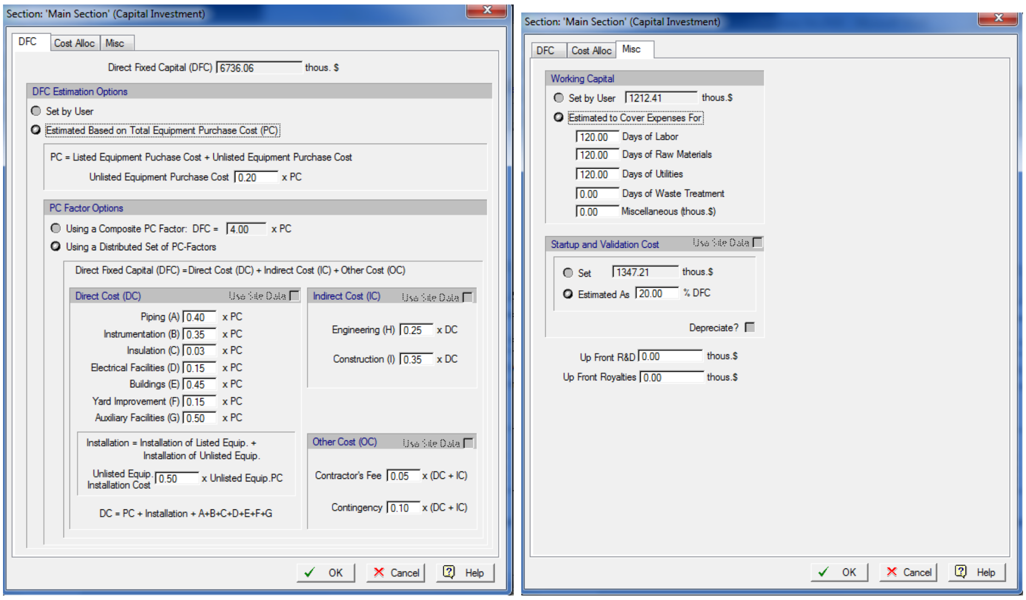
Flow Basis

● Total Stream Flow
○ Single Component in Stream
(none)

Flow Ref. Units: kg
Quick Ref. Symbol: MP

Figure F1: Classification of inlet and outlet streams

For capital cost estimation, all the items involved in DFC as shown in Table 6.2 were defined, and it was followed by specification of working capital and start-up and validation costs. An example is presented in Figure F2 below.



Direct Fixed Capital (DFC) 6736.06 thous. $

DFC Estimation Options
○ Set by User
● Estimated Based on Total Equipment Purchase Cost (PC)

PC = Listed Equipment Puchase Cost + Unlisted Equipment Purchase Cost
Unlisted Equipment Purchase Cost 0.20 x PC

PC Factor Options
○ Using a Composite PC Factor: DFC = 4.00 x PC
● Using a Distributed Set of PC-Factors

Direct Fixed Capital (DFC) =Direct Cost (DC) + Indirect Cost (IC) + Other Cost (OC)

Direct Cost (DC) ☐
Piping (A) 0.40 x PC
Instrumentation (B) 0.35 x PC
Insulation (C) 0.03 x PC
Electrical Facilities (D) 0.15 x PC
Buildings (E) 0.45 x PC
Yard Improvement (F) 0.15 x PC
Auxiliary Facilities (G) 0.50 x PC

Installation = Installation of Listed Equip. + Installation of Unlisted Equip.
Unlisted Equip. Installation Cost 0.50 x Unlisted Equip.PC

DC = PC + Installation + A+B+C+D+E+F+G

Indirect Cost (IC) ☐
Engineering (H) 0.25 x DC
Construction (I) 0.35 x DC

Other Cost (OC) ☐
Contractor's Fee 0.05 x (DC + IC)
Contingency 0.10 x (DC + IC)

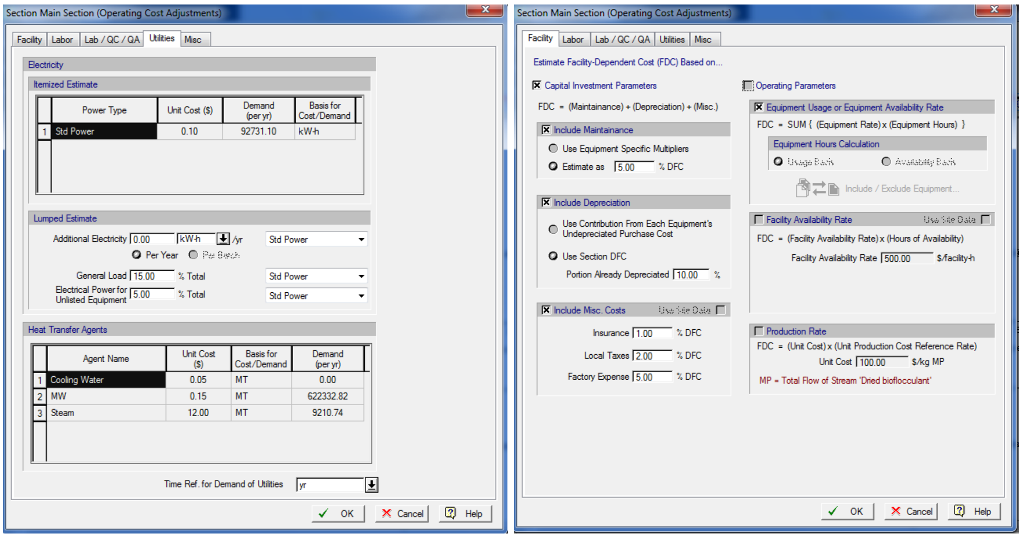
Working Capital
○ Set by User 1212.41 thous.$
● Estimated to Cover Expenses For
120.00 Days of Labor
120.00 Days of Raw Materials
120.00 Days of Utilities
0.00 Days of Waste Treatment
0.00 Miscellaneous (thous.$)

Startup and Validation Cost ☐
○ Set 1347.21 thous.$
● Estimated As 20.00 % DFC
Depreciate? ☐

Up Front R&D 0.00 thous.$
Up Front Royalties 0.00 thous.$

Figure F2: Specification of the components in DFC (left), and specification of working capital and start-up and validation costs (right)

For operating cost estimation, the price for each raw material was specified during registration of components at the beginning; meanwhile the prices for utilities and the fixed costs were specified as shown in Figure F3 below.



Electricity

Itemized Estimate

	Power Type	Unit Cost ($)	Demand (per yr)	Basis for Cost/Demand
1	Std Power	0.10	92731.10	kW-h

Lumped Estimate
Additional Electricity 0.00 kW-h /yr Std Power
● Per Year ○ Per Batch
General Load 15.00 % Total Std Power
Electrical Power for Unlisted Equipment 5.00 % Total Std Power

Heat Transfer Agents

	Agent Name	Unit Cost ($)	Basis for Cost/Demand	Demand (per yr)
1	Cooling Water	0.05	MT	0.00
2	MW	0.15	MT	622332.82
3	Steam	12.00	MT	9210.74

Time Ref. for Demand of Utilities yr

Estimate Facility-Dependent Cost (FDC) Based on...

☒ Capital Investment Parameters
FDC = (Maintainance) + (Depreciation) + (Misc.)

☒ Include Maintainance
○ Use Equipment Specific Multipliers
● Estimate as 5.00 % DFC

☒ Include Depreciation
○ Use Contribution From Each Equipment's Undepreciated Purchase Cost
● Use Section DFC
Portion Already Depreciated 10.00 %

☒ Include Misc. Costs ☐
Insurance 1.00 % DFC
Local Taxes 2.00 % DFC
Factory Expense 5.00 % DFC

☐ Operating Parameters

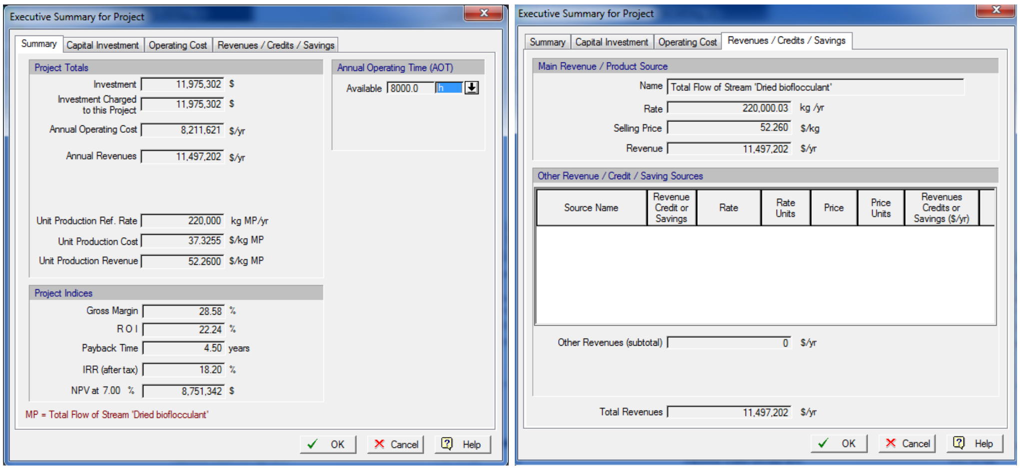
☒ Equipment Usage or Equipment Availability Rate
FDC = SUM { (Equipment Rate) x (Equipment Hours) }
Equipment Hours Calculation
● Usage Basis ○ Availability Basis

☐ Facility Availability Rate ☐
FDC = (Facility Availability Rate) x (Hours of Availability)
Facility Availability Rate 500.00 $/facility-h

☐ Production Rate
FDC = (Unit Cost) x (Unit Production Cost Reference Rate)
Unit Cost 100.00 $/kg MP
MP = Total Flow of Stream 'Dried bioflocculant'

Figure F3: Specification of prices for utilities (left), and specification of fixed costs (right)

Finally, economic analysis was performed and the results of profitability analysis (gross margin, return on investment, payback time, internal rate of return and net present value), unit production cost, revenues, capital investment and operating cost were obtained. An example of these results is presented in Figures F4 and F5 below.



Project Totals

Investment 11,975,302 $
Investment Charged to this Project 11,975,302 $
Annual Operating Cost 8,211,621 $/yr
Annual Revenues 11,497,202 $/yr

Unit Production Ref. Rate 220,000 kg MP/yr
Unit Production Cost 37.3255 $/kg MP
Unit Production Revenue 52.2600 $/kg MP

Annual Operating Time (AOT)

Available 8000.0 h

Project Indices

Gross Margin 28.58 %
R O I 22.24 %
Payback Time 4.50 years
IRR (after tax) 18.20 %
NPV at 7.00 % 8,751,342 $

MP = Total Flow of Stream 'Dried bioflocculant'

Main Revenue / Product Source

Name Total Flow of Stream 'Dried bioflocculant'
Rate 220,000.03 kg /yr
Selling Price 52.260 $/kg
Revenue 11,497,202 $/yr

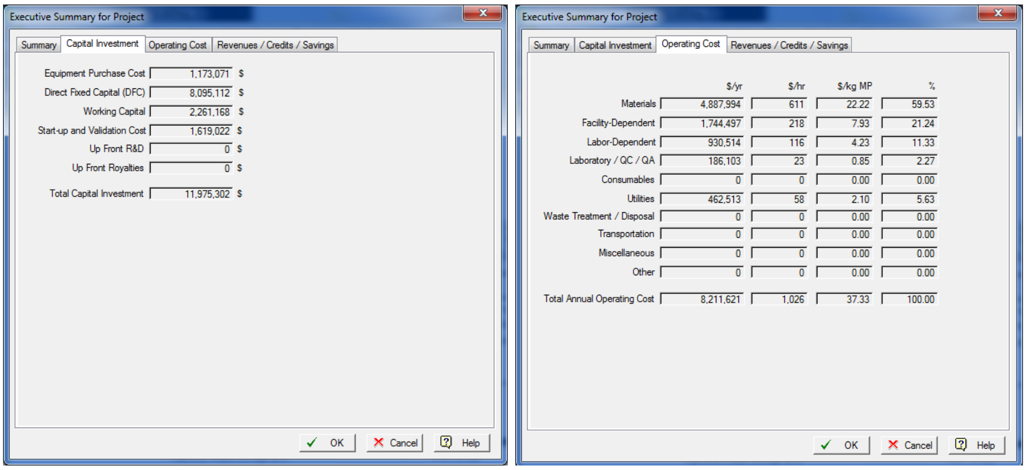
Other Revenue / Credit / Saving Sources

Source Name	Revenue Credit or Savings	Rate	Rate Units	Price	Price Units	Revenues Credits or Savings ($/yr)

Other Revenues (subtotal) 0 $/yr

Total Revenues 11,497,202 $/yr

Figure F4: Results of profitability analysis and unit production cost (left), and results of revenues (right)



Equipment Purchase Cost 1,173,071 $
Direct Fixed Capital (DFC) 8,095,112 $
Working Capital 2,261,168 $
Start-up and Validation Cost 1,619,022 $
Up Front R&D 0 $
Up Front Royalties 0 $

Total Capital Investment 11,975,302 $

	$/yr	$/hr	$/kg MP	%
Materials	4,887,994	611	22.22	59.53
Facility-Dependent	1,744,497	218	7.93	21.24
Labor-Dependent	930,514	116	4.23	11.33
Laboratory / QC / QA	186,103	23	0.85	2.27
Consumables	0	0	0.00	0.00
Utilities	462,513	58	2.10	5.63
Waste Treatment / Disposal	0	0	0.00	0.00
Transportation	0	0	0.00	0.00
Miscellaneous	0	0	0.00	0.00
Other	0	0	0.00	0.00
Total Annual Operating Cost	8,211,621	1,026	37.33	100.00

Figure F5: Results of capital investment (left) and operating cost (right)