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EXTRACTION AND CHARACTERIZATION OF JATROPHA CURCAS L. AS NATURAL COAGULANT AND ITS APPLICATION FOR TURBIDITY REMOVAL

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EXTRACTION AND CHARACTERIZATION OF *JATROPHA CURCAS* L. AS NATURAL COAGULANT AND ITS APPLICATION FOR TURBIDITY REMOVAL

By

NASRIN KHODAPANAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

February 2014

DEDICATION

To my Father and Sister

And

In memory of my Mother

Abstract of the thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

EXTRACTION AND CHARACTERIZATION OF *JATROPHA CURCAS L.* AS NATURAL COAGULANT AND ITS APPLICATION FOR TURBIDITY REMOVAL

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February 2014

Chairman: Professor Robiah Bt Yunus, Ph.D.
Faculty: Engineering

Coagulation is a critical step in water production process not only because it removes colloidal particles but it also gets rid of microorganisms that are often attached to the particles. The use of aluminium sulphate and other synthetic chemicals as commonly-used coagulants, has caused some environmental and health problems. Additionally, the cost of the imported chemicals and the disposal of scheduled sludge in hard currency have made to consider the natural coagulants as a promising alternative.

In the present study, the potentials of natural coagulants extracted from various parts of plants and animal tissue which are indigenous and abundantly available in Southeast Asia were investigated. The most promising natural coagulant from the preliminary screening study was the seeds from *Jatropha curcas*. In terms of feasibility study, *Jatropha* seed is potential candidate as coagulant due to its availability in Malaysia and the low price of raw material. Additionally, *Jatropha* press cake is a byproduct of biodiesel production which so far has not had any widely special application in Malaysia. Dragon fruit foliage was removed from potential natural coagulant list due to lack of access; currently there is no available supplier in Malaysia and Southeast Asia that can supply the dragon fruit foliages for water treatment purpose. *Moringa* seed copes with the problems of availability, sustainability and high price in Malaysia; there is no available plantation in Malaysia for *Moringa* seeds. A main problem of application of chitosan for turbidity removal was smelling an unpleasant fishy odor of treated water by chitosan. In addition, water treatment plants are confronted by operation problems due to water solubility and overdosing complications of using chitosan.

After removal of oil from *J. curcas* kernel, several experiments were conducted on press cake to identify the parameters with significant effects on the turbidity reduction. To minimize the turbidity at pH 5 and 6 of synthetic turbid water by improvement of the extraction condition, the experiments were carried out using jar tests and a full factorial central composite design and response surface methodology (RSM) were applied to model the active agent extraction. The optimum condition for extraction of the active ingredient from *J. curcas* press cake was by using 13 mM NaOH and 0.1 M NaCl at an extraction temperature of 52°C and an extraction time of 5 min to achieve a minimum turbidity at pH 5 and 6 of turbid water. Chemical analysis of two-coagulant solutions, distilled water extract (DWE-JcPc) and optimum condition extract (OCE-JcPc) of *J. curcas* press cake, showed the protein recoveries at 7.49±0.62% and 61.55±2.34% from the total solid dissolved, respectively.

The active component isolation stage was then carried out using isoelectric precipitation followed by the dialysis. The active ingredient of *J. curcas* -extract coagulant was a protein with molecular weight 30-35 kDa. In a synthetic turbid water using kaolin particles at pH 5, OCE-JcPc coagulant reduced turbidity by at least 90% for a wide range of initial turbidities. It was discovered that the coagulant performance was sensitive to the initial pH of the water.

The zeta potential of OCE-JcPc was found to be negative at its original pH, confirming that the negative charges are predominant in the crude coagulant solution. On the other hand, Ca^{2+} ions improved kaolin coagulation by OCE-JcPc. The coagulation mechanism of OCE-JcPc was postulated to be an enmeshment of kaolin with the net-like structure. In the case of Ca^{2+} ion (bivalent cations), at least 0.2 mM was necessary for coagulation at 10 mg/L dose of OCE-JcPc.

The treatment of river water by OCE-JcPc in comparison with aluminum sulphate (Alum) showed that OCE-JcPc in the presence of bivalent cations performed better than alum in turbidity reduction. Water with final turbidity of less than 5 NTU, total solids 5 mg/L and chemical oxygen demand 43.75 mg/L was achieved when 80 mg/L of OCE-JcPc natural coagulant and 2.5 mM of $CaCl_2$ were used to treat the river water at initial pH level of 6.87. In another study, OCE-JcPc also reduced the turbidity of acidic river water by 4.6 NTU with total solids at 6.7 mg/L and chemical oxygen demand at 137 mg/L when 260 mg/L of OCE-JcPc natural coagulant and 0.5 mM of $CaCl_2$ were used. Additionally, the combination of 192 mg/L alum + 30 mg/l OCE-JcPc gave the lowest residual turbidity for the acidic river water sample corresponds to 40% savings in alum usage.

This study concluded that natural coagulant solution extracted from *J. curcas* press cake has good coagulation properties for a wide range of conditions and would be a promising alternative coagulant to alum.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENYARIAN DAN PENCIRIAN *JATROPHA CURCAS* L. SEBAGAI KOAGULAN ASLI DAN PENGGUNAANNYA DALAM PENGHILANGAN KEKERUHAN

Oleh

NASRIN KHODAPANAH

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Koagulasi adalah satu langkah kritikal dalam proses penghasilan air, bukan sahaja kerana ia menghilangkan zalah koloid, tetapi ia juga menyahkan mikroorganisma yang biasanya melekat dengan zarah. Penggunaan aluminium sulfat dan kimia sintetik lain sebagai koagulan yang biasa digunakan, telah menyebabkan beberapa masalah alam sekitar dan kesihatan. Tambahan pula, kos bahan kimia yang diimport dan pelupusan enapcemar berjadual telah menyebabkan perlunya pertimbangan untuk menggunakan koagulan asli sebagai alternatif.

Di dalam kajian ini, potensi koagulan asli yang diekstrak dari beberapa bahagian tumbuhan dan tisu haiwan yang asli dan didapati dengan banyaknya di Asia Tenggara telah dikaji. Koagulan semula jadi yang berpotensi daripada kajian pemeriksaan awal adalahbenih dari jataropha curcas. Dari segi kajian kemungkinan, benih jatropha adalah calon yang berpotensi sebagai koagulan berdasarkan ketersediaannya di Malaysia dan juga harga bahan mentah yang rendah. Tambahan pula, kek jatropha ialah hasil sampingan daripada penghasilan biodiesel yang belum lagi mempunyai kegunaan tersendiri di Malaysia. Dedaunan buah naga telah dikeluarkan daripada senarai koagulan asli berpotensi kerana kekurangan akses; tiada pembekal di Malaysia dan Asia Tenggara pada masa kini yang boleh membekalkan dedaunan buah naga untuk kegunaan rawatan air. Benih moringa pula bermasalah dengan ketersediaan, kemampanan dan harga yang mahal di Malaysia; tiada ladang untuk benih moringa di Malaysia. Masalah utama menggunakan chitosan untuk menghilangkan kekeruhan ialah bau seperti ikan yang tidak enak pada air yang dirawat oleh chitosan. Tambahan lagi, loji rawatan air menghadapi masalah operasi disebabkan kebolehlarutan air dan komplikasi terlebih dos dengan penggunaan chitosan.

Selepas penyingkiran minyak dari isirong J. Curcas, pelbagai eksperimen telah dijalankan pada kek untuk mengenalpasti parameter dengan kesan signifikan pada penurunan kekeruhan. Untuk meminimumkan kekeruhan pada pH 5 dan 6 air keruh sintetik dengan penambahbaikan kondisi penyarian, eksperimen telah dijalankan dengan menggunakan ujian balang dan reka bentukfaktorial kompositberpusatpenuh dansambutanmetodologipermukaan (RSM) telah digunakanuntuk membina modelpengekstrakanejenaktif.Keadaan optimum untuk pengekstrakan bahan aktif dari kek benih Jatropha curcas adalah dengan menggunakan 13 mM NaOH dan 0.1 M NaCl pada suhu pengekstrakan 52 °C dan masa pengekstrakan 5 min bagi mencapai kekeruhan minimum pada pH5 dan 6 air keruh. Analisis kimia kedua-dua larutan koagulan, ekstrak air suling (DWE-JcPc) dan ekstrak keadaan optimum (OCE-JcPc) kek J. curcas, menunjukkan pemulihan protein adalah 7.49 ± 0.62% dan 61.55 ± 2.34% daripada jumlah pepejal terlarut, masing-masing.

Peringkat pengasingan komponen aktif kemudiannya dijalankan dengan menggunakan presipitasi isoelektrik diikuti oleh dialisis. Bahan aktif koagulan J. curcas ekstrak adalah protein dengan berat molekul 30-35 kDa. Dalam model air keruh menggunakan zarah tanah liat kaolin pada pH 5, ekstrak alkali-garam koagulan mentah mengurangkan kekeruhan sekurang-kurangnya 90% bagi pelbagai kekeruhan awal. Didapati bahawa prestasi koagulan adalah sensitif kepada pH awal air.

Potensi zeta OCE-JcPc didapati negatif pada pH asal, mengesahkan bahawa caj-caj negatif adalah utama dalam larutan koagulan mentah. Sebaliknya, ion-ion Ca^{2+}perbaiki pengenapan kaolin oleh OCE-JcPc. Mekanisme pengenapan Oce-JcPc diandaikan merupakanjaringankaolin dengan struktur seperti jaring. Dalam kes ionCa^{2+} (kation bivalent), sekurang-kurangnya 0.2mM adalah perlu bagi pengenapan pada 10 mg/L dos OCE-JcPc.

Rawatan air sungai oleh OCE-JcPc berbanding dengan aluminium klorida (tawas) menunjukkan bahawa OCE-JcPc dengan kehadiran kation bivalent berfungsi sama dengan alum dalam mengurangkan kekeruhan. Air dengan kekeruhan akhir kira-kira kurang daripada 5 NTU, jumlah pepejal 5 mg/L dan permintaan oksigen kimia 43.75 mg/L boleh dicapai apabila 80 mg/L OCE-JcPc dan 2.5 mM $CaCl_2$ telah digunakan untuk merawat air sungai dengan tahap pH awal 6.87. Dalam kajian yang lain, OCE-JcPc juga boleh mengurangkan kekeruhan air sungai berasid sebanyak 4.6 NTU dengan jumlah pepejal 6.7 mg/L dan permintaan oksigen kimia 137 mg/L apabila 260 mg/L koagulan semula jadi OCE-JcPc dan 0.5 mM $CaCl_2$ telah digunakan. Selain itu, gabungan 192 mg/L alum + 30 mg/l OCE-JcPc memberi kekeruhan baki terendah sungai berasid sampel air sepadan dengan penjimatan 40% dalam penggunaan tawas.

Kajian ini menyimpulkan bahawa larutan koagulan semulajadi yang diekstrak daripada kek Jatropha curcas mempunyai ciri-ciri yang baik pada koagulasi pelbagai keadaan dan merupakan koagulan alternatif yang memberangsangkan.

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I certify that a Thesis Examination Committee has met on 20 February 2014 to conduct the final examination of Nasrin Khodapanah on her thesis entitled "Extraction and Characterization of *Jatropha Curcas* L. as Natural Coagulant and its Application for Turbidity Removal" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

Acid OCE-JcPc	Optimum Condition Extract of *J. Curcas* Press Cake acidified to isoelectric point
Adj R^2	Adjusted correlationn coefficient
Alum	Alominium solphate
ANOVA	Analysis of variance
CA	Coagulation activity
$CaCl_2$	Calcium chloride
CCD	Central Composite Design
CD	Surface Charge
COD	Chemical Oxygen Demand
CV	Coefficient of variation
Da	Dalton
D. F. foliage	Dragon fruit foliage
DMSO	Dimethyl sulfoxide
DWE-JcPc	Distilled Water Extract of *J. Curcas* Press Cake
$FeCl_3$	Ferric chloride
FTIR	Fourier transform infrared spectroscopy
F value	Fisher variation ration
h	Hour
MW	Molecular Weight
MWCO	Molecular weight cutoff
NaCl	Sodium chloride
NaOH	Sodium Hydroxide
NTU	Nephelometric Turbidity Units
OCE-JcPc	Optimum Condition Extract of *J. Curcas* Press Cake
OVAT	One variable at a time
PAA	Polyacrylamide
PACI	Polyaluminium chloride
PFS	Poly ferric sulfate
pI	Isoelectric Point
ppt	parts per thousand
Pt-Co	Platinum-Cobalt Scale
R^2	Correlation coefficient
rpm	Revolutions per minute
RSM	Response Surface Methodology

RT	Room temperature
SCE-JcPc	Sodium Chloride Extract of *J. Curcas* Press Cake
SDS-PAGE	Sodium dodecyl sulfate-Polyacrylamide gel electrophoresis
SEM	Scanning Electron Microscope
SS	Suspended Solid
TDS	Total Dissolved Solid
TSS	Total Suspended Solid
X_1	NaOH concentration (mM)
X_2	Temperature (°C)
X_3	NaCl concentration (M)
Y_1	Turbidity reduction at pH 5 (%)
Y_2	Turbidity reduction at pH 6 (%)
% (s/l)	g solid per 100 ml of liquid

CHAPTER 1

INTRODUCTION

1.1 Background

Producing tap water that is pure enough for drinking water is a challenge to many water treatment plants (WTP) in Malaysia. Due to heavy rains and variations in ground water characteristics, the turbidity of raw untreated water vary significantly, causing difficulties to WTP operators to maintain consistent tap water quality. Turbidity removal is one of the important steps in a water treatment process, which is generally achieved using coagulants. Many coagulants are widely used in conventional water treatment processes for tap water production. These coagulants can be inorganic coagulants (e.g. aluminum sulfate and polyaluminum chloride), synthetic organic polymers (e.g. polyacrylamide derivatives and polyethylene imine) or naturally occurring coagulants (e.g. chitosan and microbial coagulants). These coagulants are used for various purposes depending on their chemical characteristics. An inorganic salt, alum (aluminum sulfate), is the most widely used coagulant in water treatment because of its proven capability and lower cost. Some synthetic organic polymers are also equally effective and relatively inexpensive. Recently, significant amounts of synthetic organic polymers are widely used for water treatment.

Polymers have been utilized in coagulation/flocculation processes for water purification for at least four decades (Bolto & Gregory, 2007). In comparison with alum, some of the advantages of using polymers in water treatment are:

- lower coagulant dose requirements,
- smaller volume of sludge,
- smaller increase in the ionic load of the treated water,
- reduced level of aluminum in treated water,
- cost savings of up to 25–30% (Bolto & Gregory, 2007).

Polymers are especially beneficial in coping with the problems of slow-settling flocs in low-temperature coagulation or in treating soft colored waters, where they improve settlebility and increase the toughness of flocs. The capacity of a treatment facility may be more than doubled with the formation of larger and stronger flocs, the rate of solid and water phase separation can be significantly increased, and the dosage of other chemicals lowered. Also, the range of raw waters that can be treated is wider. There are disadvantages of course, with higher costs in particular situations and environmental factors being the main concern. There is also greater sensitivity to incorrect dosage, with turbidity and natural organics removal less efficient in some instances. All polymers used as flocculants in water treatment must be water soluble (Bolto & Gregory, 2007).

Some studies have reported that aluminum which is the major component of alum and polyaluminum chloride, may induce Alzheimer's disease. It was also reported that monomers of some synthetic organic polymers such as acrylamide have neurotoxicity and strong carcinogenic properties (Okuda *et al.*, 1999). On the other hand, naturally occurring coagulants are biodegradable and are presumed safe for human health. Some studies on natural coagulants have been carried out and various natural coagulants were produced or extracted from microorganisms, animals or plants.

Natural coagulants of vegetable and mineral origin were in use in water and wastewater treatment before the advent of synthetic chemicals like aluminum and ferric salts. Previous studies however, have not determined whether such natural coagulants are economically and environmentally more acceptable than chemical coagulants. Recently there has been more interest in the subject of natural coagulants, especially to alleviate problems of water and wastewater treatment in developing countries and to reuse some by-products (Ndabigengesere *et al.*, 1995).

1.2 Problem Statement

Conventional coagulants in water treatment are alum ($Al_2(SO_4)_3 \cdot 14H_2O$), ferric chloride ($FeCl_3 \cdot 6H_2O$), sodium aluminate, aluminum chloride and ferric sulfate. Conventional coagulants are basically salts of a strong acid (e.g., HCl or H_2SO_4) and a weak base (Al_2 $(OH)_3$ or $Fe(OH)_3$); thus they are a mixture of a cation (from a base) and an anion (from an acid). However recent studies have pointed out several serious drawbacks of using aluminum salts, such as Alzheimer's disease (Miller *et al.* 1984; McLachlan, 1995). Aluminum has also been indicated to be a causative agent in neurological disease like '*per-senile dementia*' (Muyibi & Alfugara, 2003). There is also problem with alum reactivity towards natural alkalinity present in the water leading to a reduction of pH (Prasad, 2009). Voluminous sludge production by using aluminum salts is also another problem which has not been resolved (Fujita *et al.*, 2000). Compared with alum, ferric chloride coagulates effectively over a broader pH range, forms a stronger, heavier floc, and, of course, does not contribute to aluminum residuals in the finished effluent. However, liquid ferric chloride is an acidic, corrosive, dark brown solution which causes staining and necessitates special materials of construction (Stephenson and Duff, 1996).

Currently, more effective trivalent aluminum coagulants, such as poly aluminum chloride (PACI), poly aluminum silico sulphate and poly ferric sulfate (PFS) have been developed (Fujita *et al.*, 2000; Prasad, 2009). Polymer coagulants have many advantages over monomeric forms, such as a wide range of pH applications, lower sensitivity to water temperature and lower residual aluminum/iron concentrations (Zhao *et al.* 2011). Although these new coagulants have improved the coagulation process considerably, they have not corrected all the drawbacks mentioned earlier. Polyacrylamide (PAA) derivatives, for instance, a major group of organic synthetic polymers, are also used widely are not easily biodegraded in the natural environment and the monomers derived from them have both neurotoxicity and carcinogenicity (Vanhorick and Moens, 1983). Nonetheless, the main drawback of coagulation using these conventional coagulants in treating water is voluminous sludge production and problems associated with dewatering and disposing of generated sludge. Disposal of chemical sludge into landfills can cause secondary contamination such as landfill leachate and soil contamination. Therefore, a biodegradable and safe, i.e.; environmentally friendly, coagulant is required to be developed as an alternative to existing synthetic coagulants such as PAC and PAA. Natural coagulants of vegetable and mineral origin like *Moringa oleifera*, *Prosopis juliflora* and *Cactus latifaria* were used in water treatment before the advent of chemical salts, but have succumbed progressively under modernization and survived only in remote areas of some developing countries (Diaz *et al.*, 1999).

The number of bio materials which are indigenous to Malaysia and has the potential as coagulant is high but the easy available and physical sustainable materials are a point of discussion. Natural coagulants regard as more economical alternative for developing countries in where the biomaterial is available. To this purpose, the biomaterials should be carefully considered in terms of feasibility study after exhibiting the acceptable coagulative effect of them. Among studied biomaterials in present study, *Jatropha curcas*, in principle, is a potentially superior alternative for the existing natural polymers, e. g. *Moringa* and Chitosan in terms of feasibility, particularly due to availability. Currently, *J. curcas* press cake is a waste of oil extraction industries in Malaysia. In place of sending to landfill, a better application for press cake could be used in water clarification.

Several main reasons emphasize the necessity for extracting and isolating coagulation active component from crude suspension. First of all, the crude extract of the coagulant results in higher residual dissolved organic content (DOC) of finished water (Sánchez-Martín *et al.*, 2010). For instance, in terms of water treatment applications, extracted and purified forms of *Moringa oleifera*, , have been proved to be effective at removing suspended material, generate reduced sludge volumes in comparison to alum, soften hard waters and act as an effective adsorber of heavy metals (Bhatti *et al.*, 2007; Kumari *et al.*, 2006), surfactants (Beltrán-Heredia & Sánchez-Martín, 2009) and dyes (Prasad, 2009). These applications have been, and continue to be, important areas for researchers working primarily at laboratory level although some successful pilot and full scale water treatment trials have been reported (Muyibi & Alfugara, 2003). However, this technology has not yet been adopted on any treatment plant at any scale: thus far, research activity has not led to sustained use. The major concern in the use of seed extracts for water treatment applications is the residual organic seed material that will be present in the finished water. Interactions with disinfectants such as chlorine and subsequent potential risks due to trihalomethane formation are possible. Residuals may also act as substrate for subsequent microbial growth (Bhuptawat *et al.*, 2007).

Furthermore, all laboratory studies that have been done so far reported that even though natural seeds possess effective coagulation properties, they have different characteristics and properties, therefore leading to a belief that a number of active coagulant components were present in these seeds (Bhuptawat *et al.*, 2007). Hence, the importance of isolation of active component and investigation of structure and characterization are important to provide further insights.

Finally, in spite of numerous reports about the application of natural coagulant, the technical and economic details are not fully presented by the authors. Naturally derived coagulant materials must compete against proprietary coagulants (aluminum sulphate, ferric salts, poly electrolytes, etc.) on technical and economic terms since proprietary coagulants are now sold in relatively high volumes at relatively low unit costs and the market is highly competitive and conservative. Thus, it is inevitable that an efficient technology be developed to produce an alternative natural coagulant. Therefore, a coagulation activity test in fine detail as well as feasibility study on potential biomaterials are unavoidable and first step of introducing new alternative as coagulant. The aim of the research is to find a new method to extract active agent from seeds for water treatment plant.

1.3 Research Objectives

The main objective is to conduct an investigation to find a new method to extract active agent from seeds for water treatment.

The specific objectives of this research are as follows:
1. To identify effective and physically sustainable coagulant among selected biomaterials indigenous to Malaysia.

2. To model and optimize the extraction of active ingredient from selected natural coagulant based on its coagulation activity using Response Surface Methodology.

3. To characterize the active ingredient from selected natural coagulant and its mechanism of actions.

4. To investigate the performance of selected natural coagulant in river water turbidity removal.

1.4 Importance and Significance of the Study

The importance and significance, which may be gained from this study, are:

1. The contribution in the investigation of the potential of *Jatropha curcas* seed as a new plant-based coagulant source and its application in turbidity removal.

2. The attempt to replace the common chemical coagulants by using new plant-based coagulant in turbidity reduction to avoid toxic chemical sludge, may contribute towards green and easily-available material.

1.5 Scope of Research

1. To identify the coagulation property of plant-based materials by comparing with selected natural coagulants indigenous to Malaysia; Four natural coagulants, *Moringa oleifera, Jatropha curcas,* Chitosan, and Dragon fruit foliage, have been used to initially evaluate turbidity removal of water by coagulating process. The influence of pH on turbidity reduction across various dosages of coagulant was mainly investigated. Extracted coagulating agents were applied as coagulants in various dosages in two kinds of water; 200 NTU and 400 NTU synthetic water at different pH values and turbid river water. After evaluating coagulation properties of natural materials, conducting a feasibility study was essential to improve the decision about selected natural coagulant. The appropriate natural coagulant for further study would be selected based on several criteria such as the cost of coagulant preparation and raw material, the availability, the sustainability, the storage, and the toxicity of the natural materials.

2. To model and optimize the extraction of active ingredient based on coagulation activity by parametric study– studies will be done on improving the coagulation efficiency, possibly by improving the extraction method using Response Surface Methodology. A preliminary study was required to identify operating parameters and their effective range in extraction and their effects on turbidity reduction. Given that the active agents of *J. curcas* solution were protein, key variables that affect the solubility of a protein include pH, ionic strength, and temperature, were examined. The results from the preliminary study results were applied to design a new series of tests to find the optimum conditions for that extraction method. The classical optimization method (One-Variable-At-a-Time method (OVAT)) is not suitable to assess the combined interaction between operating parameters. Hence, response surface methodology (RSM) was applied to model the effective parameters in extraction process and possibly predict the optimal area . Ionic strength in the form of sodium chloride concentration, pH in the form of molarity of sodium hydroxide, and temperature of extractant solution were chosen as independent parameters and turbidity reductions of treated water at pH value 5 and 6 of 200-NTU kaolin water were responses. The coagulation property of active component from salt-alkaline extract was evaluated by testing various kaolin turbid waters. This part consisted of the required dose of optimized coagulant solution to produce optimum coagulation in different initial turbidities (50-800 NTU) of synthesis water and at various pH (4-9) values.

3. To characterize the active ingredient; including isolation and characterization of active agent by determination of molecular weight, FTIR, total protein, and total sugar content and its mechanism of actions. Identification of coagulation active component was carried out by applying a wide range of experiments. Enzymatic hydrolysis to identify the nature of active agent was conducted followed by active agent isolation. The active component was isolated from the crude extract through a sequence of steps that included using the principle of isoelectric precipitation, salting-out by dialysis and filtering. The coagulation mechanism of natural coagulant was investigated by measuring zeta potential of coagulant solution, kaolin turbid water and treated water, using SEM images and evaluating the effects of inorganic salt $CaCl_2$ on coagulation activity.

4. Performance evaluation of coagulant, *Jatropha curcas*, in river water turbidity removal. Samples of river waters collected from Sungai Langat and Sungai Sireh, Selangor, Malaysia were treated by natural coagulant and alum individually as well as a mixture of natural coagulant and alum at different concentrations of both coagulants. The turbidity, total suspended solids, pH and COD of treated water were measured to evaluate the coagulating efficiency for both coagulants.

1.6 Thesis Layout

This thesis is divided into seven chapters. Following this chapter is chapter two, which contains a general introduction to coagulants and types of impurities in the water as well as a review of relevant literature on natural coagulant extraction. The third chapter covers materials and methods, whereby the experimental work is described and discussed in detail. Chapter four presents the results and discussion of the coagulation performance of two new agro-based materials comparing with other two fully-expanded natural coagulants and selection of the best between them and a feasibility study to assess the economic viability of the proposed biomaterials. Chapter five addresses the effective parameters on active component extraction in order to model and optimize various variables to achieve maximum turbidity reduction. In order to assess the efficiency of optimized extract a parametric study was undertaken in chapter six followed by characterization of active coagulant agents. Additionally the application of natural coagulant in turbidity removal is presented in this chapter. Chapter seven includes the conclusion from the present study and some recommendations of using this method in water treatment and future studies.

REFERENCES

Abas Wani, A., Sogi, D., Grover, L.,Saxena, D. (2006). Effect of temperature, alkali concentration, mixing time and meal/solvent ratio on the extraction of watermelon seed proteins—a response surface approach. *Biosystems engineering, 94*(1), 67-73.

Abdulkarim, S., Long, K., Lai, O., Muhammad, S.,Ghazali, H. (2005). Some physico-chemical properties of *moringa oleifera* seed oil extracted using solvent and aqueous enzymatic methods. *Food Chemistry, 93*(2), 253-263.

Abidin, Z. Z., Ismail, N., Yunus, R., Ahamad, I.,Idris, A. (2011). A preliminary study on jatropha curcas as coagulant in wastewater treatment. *Environmental technology, 32*(9), 971-977.

Achten, W., Verchot, L., Franken, Y. J., Mathijs, E., Singh, V. P., Aerts, R.,Muys, B. (2008). *Jatropha* bio-diesel production and use. *Biomass and bioenergy, 32*(12), 1063-1084.

Aderibigbe, A., Johnson, C., Makkar, H., Becker, K.,Foidl, N. (1997). Chemical composition and effect of heat on organic matter-and nitrogen-degradability and some antinutritional components of jatropha meal. *Animal feed science and technology, 67*(2), 223-243.

Ahmad, A., Sumathi, S.,Hameed, B. (2006). Coagulation of residue oil and suspended solid in palm oil mill effluent by chitosan, alum and pac. *Chemical Engineering Journal, 118*(1), 99-105.

Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., ... & Walter, P. (2013). *Essential cell biology*. Garland Science.

Ali, G. H., Hegazy, B. E., Fouad, H. A.,Rehab, M. (2008). Comparative study on natural products used for pollutants removal from water. *Journal of Applied Sciences Research, 5*(8), 1020-1029.

Altaher, H. (2012). The use of chitosan as a coagulant in the pre-treatment of turbid sea water. *Journal of hazardous materials, 233*, 97-102.

Aregheore, E. M., Makkar, H. P.,Becker, K. (1998). Assessment of lectin activity in a toxic and a non-toxic variety of jatropha curcas using latex agglutination and haemagglutination methods and inactivation of lectin by heat treatments. *Journal of the Science of Food and Agriculture, 77*(3), 349-352.

Aririatu, L.,Gwadia, T. (1999). Comparative study of the effects of natural and chemical coagulants on the treatment of domestic effluents. *Environment Protection Engineering, 25*(4), 5-10.

Arnal, J., García-Fayos, B., Sancho, M., Lora, J.,Verdú, G. (2006). Water potabilization in developing countries: Membrane technology and natural coagulants. *Desalination, 200*(1), 325-326.

Ayotunde, E. O., Fagbenro, O. A., Adebayo, O. T., & Amoo, A. I. (2004). Toxicity of Aqueous Extracts of Drumstick, Moringa oleifera, Seeds to Nile tilapia, Oreochromis niloticus, Fingerlings and Adults. *Technical Session: Health Management and Disease, ISTA, Manila, Phillippines*, 200-208.

Basha, S., Francis, G., Makkar, H., Becker, K.,Sujatha, M. (2009). A comparative study of biochemical traits and molecular markers for assessment of genetic relationships between *Jatropha curcas* L. Germplasm from different countries. *Plant Science, 176*(6), 812-823.

Beltrán-Heredia, J.,Sánchez-Martín, J. (2009). Removal of sodium lauryl sulphate by coagulation/flocculation with *Moringa oleifera* seed extract. *Journal of Hazardous Materials, 164*(2), 713-719.

Beltrán-Heredia, J., Sánchez-Martín, J.,Barrado-Moreno, M. (2012a). Long-chain anionic surfactants in aqueous solution. Removal by *Moringa oleifera* coagulant. *Chemical Engineering Journal, 180*, 128-136.

Beltrán-Heredia, J., Sánchez-Martín, J., Munoz-Serrano, A.,Peres, J. (2012b). Towards overcoming toc increase in wastewater treated with *Moringa oleifera* seed extract. *Chemical Engineering Journal, 188*, 40-46.

Bergamasco, R., Bouchard, C., da Silva, F. V., Reis, M. H. M.,Fagundes-Klen, M. R. (2009). An application of chitosan as a coagulant/flocculant in a microfiltration process of natural water. *Desalination, 245*(1), 205-213.

Bhatia, S., Othman, Z.,Ahmad, A. L. (2007). Coagulation–flocculation process for pome treatment using *Moringa oleifera* seeds extract: Optimization studies. *Chemical Engineering Journal, 133*(1), 205-212.

Bhatti, H. N., Mumtaz, B., Hanif, M. A.,Nadeem, R. (2007). Removal of zn (ii) ions from aqueous solution using *Moringa oleifera* lam.(horseradish tree) biomass. *Process Biochemistry, 42*(4), 547-553.

Bhuptawat, H., Folkard, G.,Chaudhari, S. (2007). Innovative physico-chemical treatment of wastewater incorporating *Moringa oleifera* seed coagulant. *Journal of hazardous materials, 142*(1), 477-482.

Bina, B. (1991). *Investigations into the use of natural plant coagulants in the removal of bacteria and bacteriophage from turbid waters.* University of Newcastle upon Tyne.

Bolto, B.,Gregory, J. (2007). Organic polyelectrolytes in water treatment. *Water research, 41*(11), 2301-2324.

Bouaziz, A., Horchani, H., Salem, N. B., Chaari, A., Chaabouni, M., Gargouri, Y.,Sayari, A. (2010). Enzymatic propyl gallate synthesis in solvent-free system: Optimization by response surface methodology. *Journal of Molecular Catalysis B: Enzymatic, 67*(3), 242-250.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry, 72*(1), 248-254.

Bratby, J. (2006). *Coagulation and flocculation in water and wastewater treatment*: IWA publishing.

Broin, M., Santaella, C., Cuine, S., Kokou, K., Peltier, G.,Joet, T. (2002). Flocculent activity of a recombinant protein from *Moringa oleifera* lam. Seeds. *Applied microbiology and biotechnology, 60*(1-2), 114-119.

Caceres, A., Cabrera, O., Morales, O., Mollinedo, P.,Mendia, P. (1991). Pharmacological properties of *Moringa oleifera*. I: Preliminary screening for antimicrobial activity. *Journal of Ethnopharmacology, 33*(3), 213-216.

Carlson, R.,Carlson, J. E. (2005). *Design and optimization in organic synthesis* (Vol. 24): Elsevier.

Casey, T. J.,Casey, T. (1997). *Unit treatment processes in water and wastewater engineering*: Wiley West Sussex, England.

Chatterjee, T., Chatterjee, S.,Woo, S. H. (2009). Enhanced coagulation of bentonite particles in water by a modified chitosan biopolymer. *Chemical Engineering Journal, 148*(2), 414-419.

Cheah, L. S., & Zulkarnain, W. M. (2008, October). Status of pitaya cultivation in Malaysia. In *Seminar on Pitaya Production, Market and Export-Challenges and Prospects, Oct* (Vol. 20).

Chee, K., Ling, H.,Ayob, M. (2012). Optimization of trypsin-assisted extraction, physico-chemical characterization, nutritional qualities and functionalities of palm kernel cake protein. *LWT-Food Science and Technology, 46*(2), 419-427.

Cheftel, J. C. (1986). Nutritional effects of extrusion-cooking. *Food chemistry, 20*(4), 263-283.

Chenite, A., Gori, S., Shive, M., Desrosiers, E.,Buschmann, M. (2006). Monolithic gelation of chitosan solutions via enzymatic hydrolysis of urea. *Carbohydrate polymers, 64*(3), 419-424.

Chi, E. Y., Krishnan, S., Randolph, T. W.,Carpenter, J. F. (2003). Physical stability of proteins in aqueous solution: Mechanism and driving forces in nonnative protein aggregation. *Pharmaceutical research, 20*(9), 1325-1336.

Clesceri, S. L., Greenberg, E. A.,Eaton, D. A. (1999). *Standard methods for the examination of water and wastewater*: American Public Health Association, Washington, DC.

Corbett, P. (2003). It’s time for an oil change! Opportunities for high-oleic vegetable oils. *Inform, 14*, 480-481.

Davis. (2007). Pitahaya (Dragon Fruit) Research & Production in California UC Small Farm Program 2007 Specialty Crops Conference Davis, CA.

Devappa, R. K.,Swamylingappa, B. (2008). Biochemical and nutritional evaluation of jatropha protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors. *Journal of the Science of Food and Agriculture, 88*(5), 911-919.

Diaz, A., Rincon, N., Escorihuela, A., Fernandez, N., Chacin, E.,Forster, C. (1999). A preliminary evaluation of turbidity removal by natural coagulants indigenous to venezuela. *Process Biochemistry, 35*(3), 391-395.

Dill, K. A., Fiebig, K. M.,Chan, H. S. (1993). Cooperativity in protein-folding kinetics. *Proceedings of the National Academy of Sciences, 90*(5), 1942-1946.

Divakaran, R.,Pillai, V. (2004). Mechanism of kaolinite and titanium dioxide flocculation using chitosan—assistance by fulvic acids? *Water Research, 38*(8), 2135-2143.

Divakaran, R.,Sivasankara Pillai, V. (2002). Flocculation of river silt using chitosan. *Water Research, 36*(9), 2414-2418.

Domard, A., Rinaudo, M.,Terrassin, C. (1989). Adsorption of chitosan and a quaternized derivative on kaolin. *Journal of applied polymer science, 38*(10), 1799-1806.

Duan, J.,Gregory, J. (2003). Coagulation by hydrolysing metal salts. *Advances in Colloid and Interface Science, 100*, 475-502.

Duke, J. A. (1983). Handbook of energy crops.

Ebrahimpour, A., Rahman, R. N., Ch'ng, D. H. E., Basri, M.,Salleh, A. B. (2008). A modeling study by response surface methodology and artificial neural network on culture parameters optimization for thermostable lipase production from a newly isolated thermophilic geobacillus sp. Strain arm. *BMC biotechnology, 8*(1), 96.

Eilert, U., Wolters, B.,Nahrstedt, A. (1981). The antibiotic principle of seeds of moringa oleifera and moringa stenopetala. *Planta medica, 42*(05), 55-61.

Fatombi, J., Lartiges, B., Aminou, T., Barres, O.,Caillet, C. (2013). A natural coagulant protein from copra (cocos nucifera): Isolation, characterization, and potential for water purification. *Separation and Purification Technology*. 116: 35-40.

Felt, O., Buri, P.,Gurny, R. (1998). Chitosan: A unique polysaccharide for drug delivery. *Drug Development and Industrial Pharmacy, 24*(11), 979-993.

Fujita, M., Ike, M., Tachibana, S., Kitada, G., Kim, S. M.,Inoue, Z. (2000). Characterization of a bioflocculant produced by *Citrobacter* sp. Tkf04 from acetic and propionic acids. *Journal of bioscience and bioengineering, 89*(1), 40-46.

Gassen, H., Gassenschmidt, U., Jany, K., Tauscher, B.,Wolf, S. (1990). *Isolation and chemical characterization of flocculant-active proteins from moringa-oleifera lam. Biological Chemistry Hoppe-Seyler. 371*(9), 760-760.

Gassenschmidt, U., Jany, K.,Tauscher, B. (1991). Chemical Properties of Flocculant-Active Proteins from Moringa Oleifera Lam, *Biological Chemistry Hoppe-Seyler*, 372, 659.

Gassenschmidt, U., Jany, K. D., Bernhard, T.,Niebergall, H. (1995). Isolation and characterization of a flocculating protein from *Moringa oleifera* lam. *Biochimica et Biophysica Acta (BBA)-General Subjects, 1243*(3), 477-481.

George, T., Franklin, L.,Stensel, H. (2003). Wastewater engineering: Treatment and reuse. *Edition 4th. Metcalf and Eddi Inc.*

Ghebremichael, K. A. (2004). *Moringa Seed and Pumice as Alternative Natural Materials for Drinking Water Treatment.* Unpublished doctoral dissertation, KTH Royal Institute of Technology, Sweden.

Ghebremichael, K. A., Gunaratna, K., Henriksson, H., Brumer, H.,Dalhammar, G. (2005). A simple purification and activity assay of the coagulant protein from *Moringa oleifera* seed. *Water research, 39*(11), 2338-2344.

Goel, G., Makkar, H. P., Francis, G., & Becker, K. (2007). Phorbol esters: structure, biological activity, and toxicity in animals. *International Journal of Toxicology*, *26*(4), 279-288.

Goetz, J.,Koehler, P. (2005). Study of the thermal denaturation of selected proteins of whey and egg by low resolution nmr. *LWT-Food science and technology, 38*(5), 501-512.

Gregory, J.,Duan, J. (2001). Hydrolyzing metal salts as coagulants. *Pure and Applied Chemistry, 73*(12), 2017-2026.

Gübitz, G. M., Mittelbach, M.,Trabi, M. (1999). Exploitation of the tropical oil seed plant *Jatropha curcas* L. *Bioresource Technology, 67*(1), 73-82.

Guibal, E.,Roussy, J. (2007). Coagulation and flocculation of dye-containing solutions using a biopolymer (chitosan). *Reactive and functional polymers, 67*(1), 33-42.

Guzman, P. D. (2010). Seed characteristics, storage behavior and viability equation of *Jatropha curcas* L. Science and Technology Laboratory, College of Agriculture, University of Philippines, Los Banos., http://www.scribd.com/doc/53321811/Seed-characteristics-storage-behavior-and-viability-equation-of-Jatropha-curcas, Apr 19, 2011.

Haas, W., & Mittelbach, M. (2000). Detoxification experiments with the seed oil from *Jatropha curcas* L. *Industrial crops and products*, *12*(2), 111-118.

Habbu, P. V., Joshi, H.,Patil, B. (2007). Potential wound healers from plant origin. *Pharmacognosy Reviews*, *1*(2), 271.

Hall, G. M. (Ed.). (1996). *Methods of Testing Protein Functionality*. London: Springer.

Hamarneh, A., Heeres, H., Broekhuis, A.,Picchioni, F. (2010). Extraction of *Jatropha curcas* proteins and application in polyketone-based wood adhesives. *International Journal of Adhesion and Adhesives, 30*(7), 615-625.

Harrich, D., Garcia, J., Mitsuyasu, R.,Gaynor, R. (1990). Tar independent activation of the human immunodeficiency virus in phorbol ester stimulated t lymphocytes. *The EMBO journal*, *9*(13), 4417.

He, P., Davis, S. S.,Illum, L. (1998). In vitro evaluation of the mucoadhesive properties of chitosan microspheres. *International Journal of Pharmaceutics*, *166*(1), 75-88.

Hongve, D.,Åkesson, G. (1996). Spectrophotometric determination of water colour in hazen units. *Water Research, 30*(11), 2771-2775.

Hu, C.-Y., Lo, S.-L., Chang, C.-L., Chen, F.-L., Wu, Y.-D.,Ma, J.-l. (2012). Treatment of highly turbid water using chitosan and aluminum salts. *Separation and Purification Technology*. *104*, 322-326.

Huang, C., Chen, S.,Ruhsing Pan, J. (2000). Optimal condition for modification of chitosan: A biopolymer for coagulation of colloidal particles. *Water Research, 34*(3), 1057-1062.

Hayes, M. (2012). *Marine Bioactive Compounds*. Springer. New York Dordrecht Heidelberg London, Chapter 4. page 115-128.

Idris, J., Md Som, A., Musa, M., Ku Hamid, K. H., Husen, R.,Muhd Rodhi, M. N. (2012). Dragon fruit foliage plant-based coagulant for treatment of concentrated latex effluent: Comparison of treatment with ferric sulfate. *Journal of Chemistry, 2013*.

Incorporation, Z. M. (2005). Zeta potential: A complete course in 5 minutes. *Zeta-Meter, Inc, 765*.

Isawumi, M. (1978). 9 nigerian chewing sticks. *Nigerian field*, *43*, 50-58.
Jaafar, R. A., Rahman, B. A., Ridhwan, A., Mahmod, N. Z. C., & Vasudevan, R. (2009). Proximate Analysis of Dragon Fruit (Hylecereus polyhizus). *American Journal of Applied Sciences*, *6*(7). 1341-1346.

Jahn, A.,Al, S. (1988). Using moringa seeds as coagulants in developing countries. *Journal of the American Water Works Association, 80*(6), 43-50.

Janick, J.,Paull, R. E. (2008). *The encyclopedia of fruits and nuts*: CABI.

Johnson, E. L., Peniston, Q. P. (1980). Process for the manufacture of chitosan. *U.S. Patent No. 4,195,175*. Washington, DC: U.S. Patent and Trademark Office.

Jasmani, R. Berita Transformasi Pertanian, Bil 12/2007 Edisi DISEMBER, http://www.lpp.gov.my/ms/c/document_library/get_file?uuid=a87acb89-b09b-45fd-ab37-eeab5068195a&groupId=10171.

Joubert, P. H., Brown, J. M., Hay, I. T., & Sebata, P. D. (1984). Acute poisoning with Jatropha curcas (purging nut tree) in children. *South African medical journal= Suid-Afrikaanse tydskrif vir geneeskunde*, *65*(18), 729-730.

Kalogo, Y., Rosillon, F., Hammes, F.,Verstraete, W. (2000). Effect of a water extract of moringa oleifera seeds on the hydrolytic microbial species diversity of a uasb reactor treating domestic wastewater. *Letters in applied microbiology, 31*(3), 259-264.

Kasim, M. S., Che Haron, C. H., Ghani, J. A., & Sulaiman, M. A. (2013). Prediction Surface Roughness in High-Speed Milling of Inconel 718 under Mql Using Rsm Method. *Middle East Journal of Scientific Research*, *13*(3).

Karazhiyan, H., Razavi, S.,Phillips, G. O. (2011). Extraction optimization of a hydrocolloid extract from cress seed (*lepidium sativum*) using response surface methodology. *Food Hydrocolloids*, *25*(5), 915-920.

Katayon, S., Megat Mohd Noor, M. J., Kien Tat, W., Abdul Halim, G., Thamer, A.,Badronisa, Y. (2007). Effect of natural coagulant application on microfiltration performance in treatment of secondary oxidation pond effluent. *Desalination, 204*(1), 204-212.

Katayon, S., Ng, S., Johari, M. M.,Ghani, L. A. (2006a). Preservation of coagulation efficiency ofmoringa oleifera, a natural coagulant. *Biotechnology and Bioprocess Engineering, 11*(6), 489-495.

Katayon, S., Noor, M., Asma, M., Ghani, L., Thamer, A., Azni, I., Ahmad, J., Khor, B.,Suleyman, A. (2006b). Effects of storage conditions of *Moringa oleifera* seeds on its performance in coagulation. *Bioresource Technology, 97*(13), 1455-1460.

Katayon, S., Noor, M. M. M., Asma, M., Thamer, A., Abdullah, A. L., Idris, A., Suleyman, A., Aminuddin, M.,Khor, B. (2004). Effects of storage duration and temperature of *Moringa oleifera* stock solution on its performance in coagulation. *Int. J. Eng. Technol, 1*(2), 146-151.

Khodapanah, N., Idris, A., Yunus, R., Ahamad, I. S.,Wei, P. K. (2011). *Toward understanding the efficacy and mechanism of hylocereus costaricensis as a natural coagulant for potential application in water treatment*. Paper presented at the The 8th IWA Leading-Edge Conference on Water and Wastewater Technologies, Amsterdam, The Netherlands.

Kumar, A.,Sharma, S. (2008). An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): A review. *Industrial crops and products, 28*(1), 1-10.

Kumari, P., Sharma, P., Srivastava, S.,Srivastava, M. (2006). Biosorption studies on shelled *Moringa oleifera* lamarck seed powder: Removal and recovery of arsenic from aqueous system. *International Journal of Mineral Processing, 78*(3), 131-139.

Kwaambwa, H.,Maikokera, R. (2007). A fluorescence spectroscopic study of a coagulating protein extracted from *Moringa oleifera* seeds. *Colloids and surfaces B: Biointerfaces, 60*(2), 213-220.

Kwaambwa, H.,Maikokera, R. (2008). Infrared and circular dichroism spectroscopic characterisation of secondary structure components of a water treatment coagulant protein extracted from *Moringa oleifera* seeds. *Colloids and Surfaces B: Biointerfaces*, *64*(1), 118-125.

Konrad, E., Lang, G., & Wendel, H. (1985). *U.S. Patent No. 4,528,283*. Washington, DC: U.S. Patent and Trademark Office.

Lestari, D., Mulder, W.,Sanders, J. (2010). Improving *Jatropha curcas* seed protein recovery by using counter current multistage extraction. *Biochemical Engineering Journal*, *50*(1), 16-23.

Lestari, D., Mulder, W. J.,Sanders, J. P. (2011). *Jatropha* seed protein functional properties for technical applications. *Biochemical Engineering Journal, 53*(3), 297-304.

Li, Z.-Y.,Youravong, W. (2010). Protein hydrolysis by protease isolated from tuna spleen by membrane filtration: A comparative study with commercial proteases. *LWT-Food Science and Technology, 43*(1), 166-172.

Liew, A., Noor, M., Muyibi, S., Fugara, A., Muhammed, T.,Iyuke, S. (2006). Surface water clarification using *M. Oleifera* seeds. *International journal of environmental studies, 63*(2), 211-219.

Madsen, M., Schlundt, J.,Omer, E. (1987). Effect of water coagulation by seeds of *Moringa oleifera* on bacterial concentrations. *The Journal of tropical medicine and hygiene*, *90*(3), 101.

Mahattanatawee, K., Manthey, J. A., Luzio, G., Talcott, S. T., Goodner, K.,Baldwin, E. A. (2006). Total antioxidant activity and fiber content of select florida-grown tropical fruits. *Journal of Agricultural and Food Chemistry, 54*(19), 7355-7363.

Makkar, H. P. S., Aderibigbe, A. O., & Becker, K. (1998). Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. *Food chemistry*, *62*(2), 207-215.

Makkar, H. P. S., & Becker, K. (2009). *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts. *European Journal of Lipid Science and Technology*, *111*(8), 773-787.

Makkar, H. P. S., Becker, K., Sporer, F.,Wink, M. (1997). Studies on nutritive potential and toxic constituents of different provenances of jatropha curcas. *Journal of Agricultural and Food Chemistry*, *45*(8), 3152-3157.

Makkar, H. P. S., Francis, G.,Becker, K. (2008). Protein concentrate from jatropha curcas screw-pressed seed cake and toxic and antinutritional factors in protein concentrate. *Journal of the Science of Food and Agriculture, 88*(9), 1542-1548.

Makkar, H. P. S., Kumar, V., Oyeleye, O. O., Akinleye, A. O., Angulo-Escalante, M. A., & Becker, K. (2011). *Jatropha platyphylla*, a new non-toxic *Jatropha* species: Physical properties and chemical constituents including toxic and antinutritional factors of seeds. *Food chemistry*, *125*(1), 63-71.

Marrufo-Estrada, D. M., Segura-Campos, M. R., Chel-Guerrero, L. A.,Betancur-Ancona, D. A. (2013). Defatted *Jatropha curcas* flour and protein isolate as materials for protein hydrolysates with biological activity. *Food chemistry*. *138*(1), 77-83.

Martinez-Herrera, J., Siddhuraju, P., Francis, G., Davila-Ortiz, G.,Becker, K. (2006). Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. From mexico. *Food chemistry, 96*(1), 80-89.

Martyn, C., Osmond, C., Edwardson, J., Barker, D., Harris, E.,Lacey, R. (1989). Geographical relation between alzheimer's disease and aluminium in drinking water. *The Lancet, 333*(8629), 61-62.

Matilainen, A., Vepsäläinen, M., & Sillanpää, M. (2010). Natural organic matter removal by coagulation during drinking water treatment: A review. *Advances in Colloid and Interface Scienc*e, 159(2), 189-197.

Mccollister, D., Oyen, F.,Rowe, V. (1964). Toxicology of acrylamide. Tox*icology and Applied Pharmacology*, *6*(2), 172-181.

McKenzie, H.,Ralston, G. B. (1971). The denaturation of proteins: Two state? Reversible or irreversible? *Experientia*, *27*(6), 617-624.

McLachlan, D. (1995). Aluminium and the risk for alzheimer's disease. *Environmetrics*, *6*(3), 233-275.

McLaughlin, J.,Hostettmann, K. (1991). Methods in plant biochemistry. *Methods in plant biochemistry, 6*.

Miller, S. M., Fugate, E. J., Craver, V. O., Smith, J. A.,Zimmerman, J. B. (2008). Toward understanding the efficacy and mechanism of opuntia spp. As a natural coagulant for potential application in water treatment. *Environmental science & technology*, *42*(12), 4274-4279.

Miller, R. G., & Stober, J. A. (1984). Occurrence of aluminum in drinking water. *Journal of the American Water Works Association*, *76*(1), 84-91.

Mofijur, M., Masjuki, H. H., Kalam, M. A., Hazrat, M. A., Liaquat, A. M., Shahabuddin, M., & Varman, M. (2012). Prospects of biodiesel from *Jatropha* in Malaysia. *Renewable and Sustainable Energy Reviews*, *16*(7), 5007-5020.

Moure, A., Franco, D., Sineiro, J., Domínguez, H.,Núñez, M. J. (2003). Simulation of multistage extraction of antioxidants from chilean hazelnut (gevuina avellana) hulls. *Journal of the American Oil Chemists' Society, 80*(4), 389-396.

Muyibi, S.,Alfugara, A. (2003). Treatment of surface water with moringa oleifera seed extract and alum–a comparative study using a pilot scale water treatment plant. *Intern. J. Environ. Studies*, *60*(6), 617-626.

Muyibi, S. A.,Evison, L. M. (1995). *Moringa oleifera* seeds for softening hardwater. *Water Research*, *29*(4), 1099-1104.

Muyibi, S. A.,Evison, L. M. (1996). Coagulation of turbid water and softening of hardwater with moringa oleifera seeds. *International journal of environmental studies*, *49*(3), 247-259.

Muyibi, S. A., Mohd. Noor, M. J. M., Leong, T. K.,Loon, L. H. (2002). Effects of oil extraction from moringa oleifera seeds on coagulation of turbid water. *International journal of environmental studies*, *59*(2), 243-254.

Muyibi, S. A., Noor, M. J. M. M., Ong, D. T.,Kai, K. W. (2001). Moringa oleifera seeds as a flocculant in waste sludge treatment. *International journal of environmental studies*, *58*(2), 185-195.

Namuli, A., Abdullah, N., Sieo, C. C., Zuhainis, S. W., & Oskoueian, E. (2011). Phytochemical compounds and antibacterial activity of Jatropha curcas Linn. extracts. *Journal of Medicinal Plants Research*, *5*(16), 3982-3990.

Narasiah, K., Vogel, A.,Kramadhati, N. (2002). Coagulation of turbid waters using moringa oleifera seeds from two distinct sources. *Water supply, 2*(5-6), 83-88.

Ndabigengesere, A.,Narasiah, K. (1996). Influence of operating parameters on turbidity removal by coagulation with moringa oleifera seeds. *Environmental technology, 17*(10), 1103-1112.

Ndabigengesere, A., Narasiah, K. S.,Talbot, B. G. (1995). Active agents and mechanism of coagulation of turbid waters using *Moringa oleifera*. *Water Research, 29*(2), 703-710.

Ndabigengesere, A.,Subba Narasiah, K. (1998). Quality of water treated by coagulation using moringa oleifera seeds. *Water Research, 32*(3), 781-791.

Nkhata, D. (2001). *Moringa as an alternative to aluminium sulphate.* Paper presented at the WEDC CONFERENCE.

Nkurunziza, T., Nduwayezu, J., Banadda, E.,Nhapi, I. (2009). The effect of turbidity levels and *Moringa oleifera* concentration on the effectiveness of coagulation in water treatment. *Water Science & Technology, 59*(8).

Öhman, J. (2011). Cultivation and Management of *Jatropha curcas* L. by Smallholder Farmers in the Kenyan Districts Baringo and Koibatek. Yrkeshögskolan Novia.

Okonko, I.,Shittu, O. (2007). Bioremediation of wastewater and municipal water treatment using latex exudate from calotropis procera (sodom apple). *Electronic journal of Environmental, Agricultural and food chemistry, 6*(3), 1890-1904.

Okuda, T., Baes, A. U., Nishijima, W.,Okada, M. (1999). Improvement of extraction method of coagulation active components from *Moringa oleifera* seed. *Water Research, 33*(15), 3373-3378.

Okuda, T., Baes, A. U., Nishijima, W.,Okada, M. (2001a). Coagulation mechanism of salt solution-extracted active component in *Moringa oleifera* seeds. *Water Research, 35*(3), 830-834.

Okuda, T., Baes, A. U., Nishijima, W.,Okada, M. (2001b). Isolation and characterization of coagulant extracted from *Moringa oleifera* seed by salt solution. *Water Research, 35*(2), 405-410.

Olsen, A. (1987). Low technology water purification by bentonite clay and *Moringa oleifera* seed flocculation as performed in sudanese villages: Effects on *Schistosoma mansoni* cercariae. *Water Research*, *21*(5), 517-522.

Oluduro, A. O., & Aderiye, B. I. (2009). Effect of Moringa oleifera seed extract on vital organs and tissue enzymes activities of male albino rats. *Afr J Microbiol Res, 3,* 537-540.

Özacar, M.,Şengil, İ. A. (2003). Evaluation of tannin biopolymer as a coagulant aid for coagulation of colloidal particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *229*(1), 85-96.

Palka, K., Sikorski, Z. E.,Rakowska, M. (1985). The recovery and nutritional evaluation of alkali extracted protein coagulates from crushed bone residues. *Food chemistry*, *18*(4), 291-299.

Pelegrine, D.,Gasparetto, C. (2005). Whey proteins solubility as function of temperature and ph. *LWT-Food Science and Technology*, *38*(1), 77-80.

Pillai, C., Paul, W.,Sharma, C. P. (2009). Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Progress in Polymer Science*, *34*(7), 641-678.

Poumaye, N., Mabingui, J., Lutgen, P.,Bigan, M. (2012). Contribution to the clarification of surface water from the *Moringa oleifera*: Case M'Poko River to Bangui, Central African Republic. *Chemical Engineering Research and Design*. *90*(12): 2346-2352.

Prasad, R. K. (2009). Color removal from distillery spent wash through coagulation using *Moringa oleifera* seeds: Use of optimum response surface methodology. *Journal of hazardous materials, 165*(1), 804-811.

Prasuna, C., Chakradhar, R., Rao, J.,Gopal, N. (2009). Epr and ir spectral investigations on some leafy vegetables of indian origin. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 74*(1), 140-147.

Prihandana, R., & Hendroko, R. (2006). *Petunjuk budi daya jarak pagar*. AgroMedia.

Pritchard, M., Craven, T., Mkandawire, T., Edmondson, A.,O'Neill, J. (2010). A study of the parameters affecting the effectiveness of *Moringa oleifera* in drinking water purification. *Physics and Chemistry of the Earth, Parts A/B/C, 35*(13), 791-797.

Pritchard, M., Mkandawire, T., Edmondson, A., O'neill, J.,Kululanga, G. (2009). Potential of using plant extracts for purification of shallow well water in malawi. *Physics and Chemistry of the Earth, Parts A/B/C, 34*(13), 799-805.

Pushpakumara, D., Gunasena, H.,Kariayawasm, M. (2005). Flowering and fruiting phenology, pollination vectors and breeding system of dragon fruit (hylocereus spp.). *Sri Lankan Journal of Agricultural Science, 42,* 81-91.

Rajangam, J., Azahakia Manavalan, R. S., Thangaraj, T., Vijayakumar, A., & Muthukrishan, N. (2001, October). Status of production and utilisation of

Moringa in Southern India. In *International workshop development potential of moringa products, Tanzania* (Vol. 29).

Ramachandran, C., Peter, K.,Gopalakrishnan, P. (1980). Drumstick (*Moringa oleifera*): A multipurpose indian vegetable. *Economic Botany, 34*(3), 276-283.

Reddy, D., Ramana, D., Seshaiah, K.,Reddy, A. (2011). Biosorption of Ni (ii) from aqueous phase by *Moringa oleifera* bark, a low cost biosorbent. *Desalination, 268*(1), 150-157.

Reddy, D., Seshaiah, K., Reddy, A., Rao, M. M.,Wang, M. (2010). Biosorption of pb^{2+} from aqueous solutions by *Moringa oleifera* bark: Equilibrium and kinetic studies. *Journal of Hazardous Materials, 174*(1), 831-838.

Renault, F., Sancey, B., Badot, P.-M.,Crini, G. (2009). Chitosan for coagulation/flocculation processes–an eco-friendly approach. *European Polymer Journal, 45*(5), 1337-1348.

Rha, C. K., Rodriguez-Sanchez, D., & Kienzle-Sterzer, C. (1984). Novel applications of chitosan. *Biotechnology of Marine Polysaccharides. Hemisphere: Washington, DC*, 284-311.

Rizzo, L., Di Gennaro, A., Gallo, M.,Belgiorno, V. (2008a). Coagulation/chlorination of surface water: A comparison between chitosan and metal salts. *Separation and Purification Technology, 62*(1), 79-85.

Rizzo, L., Lofrano, G., Grassi, M.,Belgiorno, V. (2008b). Pre-treatment of olive mill wastewater by chitosan coagulation and advanced oxidation processes. *Separation and Purification Technology, 63*(3), 648-653.

Roberts, G. A. (1992). Structure of chitin and chitosan. *Chitin chemistry*, 1-100.

Ruhsing Pan, J., Huang, C., Chen, S.,Chung, Y.-C. (1999). Evaluation of a modified chitosan biopolymer for coagulation of colloidal particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 147*(3), 359-364.

Saetae, D., Kleekayai, T., Jayasena, V.,Suntornsuk, W. (2011). Functional properties of protein isolate obtained from physic nut (J*atropha curcas* L.) seed cake. *Food Science and Biotechnology, 20*(1), 29-37.

Sánchez-Martín, J., Ghebremichael, K.,Beltrán-Heredia, J. (2010). Comparison of single-step and two-step purified coagulants from *Moringa oleifera* seed for turbidity and doc removal. *Bioresource technology, 101*(15), 6259-6261.

Sarkar, M.,Majumdar, P. (2011). Application of response surface methodology for optimization of heavy metal biosorption using surfactant modified chitosan bead. *Chemical Engineering Journal, 175*, 376-387.

Sayyar, S., Abidin, Z. Z., Yunus, R.,Muhammad, A. (2009). Extraction of oil from jatropha seeds-optimization and kinetics. *American Journal of Applied Sciences, 6*(7), 1390.

Scherr, G. (2006a). *U. S. Patent Application 11/400, 822.*

Scherr, G. H. (2006b). *U.S. Patent No. 7,128,929*. Washington, DC: U.S. Patent and Trademark Office.

Schwarz, D. (2001). Water clarification using moringa olifera. *GATE-ESCHBORN-*(1), 17-20.

Šćiban, M., Klašnja, M., Antov, M.,Škrbić, B. (2009). Removal of water turbidity by natural coagulants obtained from chestnut and acorn. *Bioresource technology, 100*(24), 6639-6643.

Sivasubramanian, K., & Thiagarajan, C. P. (1997). Storage potential of Moringa seeds. *Madras Agricultural Journal*, *84*, 618-619.

Stephenson, R. J., & Duff, S. J. (1996). Coagulation and precipitation of a mechanical pulping effluent- I. Removal of carbon, colour and turbidity. *Water Research*, *30*(4), 781-792.

Sutherland, J., Folkard, G.,Grant, W. (1990). Natural coagulants for appropriate water treatment: A novel approach. *Waterlines, 8*(4), 30-32.

Sutherland, J., Folkard, G., Mtawali, M.,Grant, W. (1994). *Moringa oleifera as a natural coagulant.* Paper presented at the Proceedings of the 20th WEDC Conference Affordable Water Supply and Sanitation.

Szyguła, A., Guibal, E., Ruiz, M.,Sastre, A. M. (2008). The removal of sulphonated azo-dyes by coagulation with chitosan. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 330*(2), 219-226.

Taylor, K. A. (1995). A modification of the phenol/sulfuric acid assay for total carbohydrates giving more comparable absorbances. *Applied Biochemistry and Biotechnology, 53*(3), 207-214.

Tongpoothorn, W., Sriuttha, M., Homchan, P., Chanthai, S.,Ruangviriyachai, C. (2011). Preparation of activated carbon derived from *Jatropha curcas* fruit shell by simple thermo-chemical activation and characterization of their physico-chemical properties. *Chemical engineering research and design, 89*(3), 335-340.

Vanhorick, M., & Moens, W. (1983). Carcinogen-mediated induction of SV40 DNA amplification is enhanced by acrylamide in Chinese hamster CO60 cells. *Carcinogenesis*, *4*(11), 1459-1463.

Wani, A. A., Kaur, D., Ahmed, I.,Sogi, D. (2008). Extraction optimization of watermelon seed protein using response surface methodology. *LWT-Food Science and Technology, 41*(8), 1514-1520.

WHO, G. (2006). Guidelines for drinking water quality. *WHO: World Health Organization*.

Wink, M., Koschmieder, C., Sauerwein, M., & Sporer, F. (1997). 4.1 Phorbol Esters of J. curcas-Biological Activities and Potential Applications.

WOK, G., Slabbert, J. L., & Morgan, W. S. G. (1985). Toxicity and mutagenicity evaluation of watet coagulated with Moringa oleifera seed preparations using fish, protozoan, bacterial, coliphage, enzyme and Ames Salmonella assays. *Water Sa, 11*(1).

Wong, D. W., Camirand, W. M., Pavlath, A. E., Parris, N.,Friedman, M. (1996). Structures and functionalities of milk proteins∗. *Critical Reviews in Food Science & Nutrition, 36*(8), 807-844.

Wybraniec, S.,Mizrahi, Y. (2002). Fruit flesh betacyanin pigments in hylocereus cacti. *Journal of Agricultural and Food Chemistry, 50*(21), 6086-6089.

Wybraniec, S., Nowak-Wydra, B., Mitka, K., Kowalski, P.,Mizrahi, Y. (2007). Minor betalains in fruits of hylocereus species. *Phytochemistry, 68*(2), 251-259.

Yongabi, K. (2010). Biocoagulants for water and waste water purification: A review. *International Review of Chemical Engineering, 2*(3), 444-458.

Yongabi, K. A. (2005). *Studies on the potential use of medicinal plants and macrofungi (lower plants) in water and waste water purification.* Paper presented at the Proceedings of International E-Conference for Biotechnology and Bioengineering.

Zhang, J., Zhang, F., Luo, Y.,Yang, H. (2006). A preliminary study on cactus as coagulant in water treatment. *Process Biochemistry, 41*(3), 730-733.

Zhao, Y. X., Gao, B. Y., Shon, H. K., Cao, B. C., & Kim, J. H. (2011). Coagulation characteristics of titanium (Ti) salt coagulant compared with aluminum (Al) and iron (Fe) salts. *Journal of hazardous materials*, *185*(2), 1536-1542.

Zhu, K.-X., Sun, X.-H.,Zhou, H.-M. (2009). Optimization of ultrasound-assisted extraction of defatted wheat germ proteins by reverse micelles. *Journal of Cereal Science, 50*(2), 266-271.