

An Undergraduate Technical Research Submitted to the
College of Engineering, Architecture, and Technology
Palawan State University
Puerto Princesa City

As Partial Fulfillment of the Requirements
For Degree of
Bachelor of Science in Petroleum Engineering

October 2018

**A COMPARATIVE STUDY ON THE OIL SORPTION
CAPACITY OF CHITOSAN EXTRACTED FROM ASIAN
GREEN MUSSEL SHELLS TO SHRIMP SHELLS AS AN OIL
SPILL SORBENT**

by

JULES ALEXANDER O. ARREZA
ALPHONSE REIGH C. BORGUETA
JAN DANIEL G. MELCHOR
JOHN RAENEL G. QUILLO

College of Engineering, Architecture, and Technology
Palawan State University
Puerto Princesa City

ABSTRACT

In search for other alternatives to remove spilled oil in different bodies of water, this study was undertaken to evaluate the sorption capacity of the chitosan extracted from *Perna Veridis*, in comparison with the chitosan extracted from shrimp shells. This study assessed as to what degree of deacetylation could be obtained from the chitosan extracted from *Perna Veridis* and as to which size samples of sorbents prepared (flakes and powder) was more efficient. The water uptake capacity (g/g sorbent) and the oil retention (g oil sorbed) of the chitosan from 2 different size of samples were measured for additional data. The computed degree of deacetylation from Titration I has a value of 76.83% which is a desirable percentage for extraction and determination of chitosan's presence. The results in the experiment showed that the chitosan flakes (0.45g/g) and powder (0.39g/g) had better adsorption capacities compared to shrimp's flakes (0.38g/g) and shrimp's powder (0.28g/g). The chitosan flakes also topped its powdered form in terms of sorption capacity. Water uptake capacities of flakes and powder both obtained an amount of 1g based on the difference of wet-static and dry-static setups. The oil retention obtained by flakes was 1.67g and the powder was found to be 1.33g

Keywords: chitosan, dry-static, wet-static, degree of deacetylation

ACKNOWLEDGEMENT

The researchers would like to extend their deepest appreciation to those who have helped and supported them throughout the conduct of the study:

Their Adviser, Engr. Ryan Kristoffer C. Aguirre, for giving them advice and sharing his knowledge and ideas to improve their study;

Their Panel of Examiners, Engr. Philip Ryan B. Mague, Engr. Manuel L. Cabiguen Jr., Engr. Allan Jay L. Javier, for their suggestions, and recommendations and comments for the best outcome of the thesis.

Their parents, who helped them throughout the whole time and helped them collect the shells they needed, for their motivations, financial and moral help until the completion of the study.

Friends, especially Bea Paula Arzaga and Vince Davidson Pacañot for providing support and concerns in the best way they can, and

Almighty God who gave them strength and knowledge to be able to accomplish this requirement.

TABLE OF CONTENTS

TITLE PAGE	i
APPROVAL PAGE	ii
ACKNOWLEDGEMENT	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF EQUATIONS	vii
ABSTRACT	viii
Chapter 1: INTRODUCTION	1
Chapter 2: REVIEW OF RELATED LITERATURE	5
2.1 Related Literature	5
2.1.1 Oil Spill	5
2.1.1.1 Bunker Fuel	6
2.1.1.2 Oil Spill Occurrences in the Philippines	7
2.1.1.3 Methods of Oil Soil Cleanup	7
2.1.1.4 Sorbents	10
2.1.1.5 Criteria for Selecting Sorbents	11
2.1.2 <i>Perna viridis</i>	13
2.1.2.1 Habitat, Distribution and Reproduction	13
2.1.2.2 Uses	14
2.1.2.3 Taxonomy	15
2.2.1.2 Related Studies	15
2.2.2 Chitin and Chitosan	15
2.2.1.1 Extraction of Chitin and Chitosan	19
2.2.1.2 Characterization of Extracted Chitosan	20
2.2.2 Testing Method	23
2.2.3 Related Study I	24
2.2.4 Related Study II	26
2.2.5 Related Study III	27

2.2.6 Related Study IV	29
2.2.7 Related Study V	30
Chapter 3: CONCEPTUAL FRAMEWORK	32
Chapter 4: MATERIALS AND METHODS	35
Materials and Instruments	35
Material Preparation and Setup	36
Sample Preparation	36
Demineralization	36
Deproteinization	36
Deacetylation	37
Characterization of Chitosan	37
Experimental Setup	38
Methodological Calculations	40
Chapter 5: RESULTS AND FINDINGS	42
Chapter 6: CONCLUSION AND RECOMMENDATION	47
GLOSSARY OF TECHNICAL TERMS	50
REFERENCES	52
APPENDICES	54

LIST OF TABLES

Table 2.1.1.5	Properties of some specific oil	11
Table 2.1.2.3	Taxonomy	15
Table 2.1.1.2a	Composition of mussel shells	21
Table 2.1.1.2b	Carbon-Nitrogen content of chitin and chitosan	21
Table 2.2.5a	Tabular data of adsorption Capacity of Biopolymer flakes, biopolymer	28
Table 4.2	Materials and equipment	35
Table 5.1.2a	Adsorption Capacities	43
Table 5.1.2b	Water uptake and oil retention	43

LIST OF FIGURES

Figure 2.2a	Chemical structure of chitin	16
Figure 2.2b	Chemical structure of chitosan	16
Figure 2.2.3a	Vegetable oil removal rate of chitosan-CaCO ₃ film	25
Figure 2.2.3b	Diesel oil removal efficiency of composite films	26
Figure 2.2.5b	Adsorption capacity of biopolymer flakes, biopolymer powder and chitosan solution	29
Figure 5.1	Results of adsorption capacities	44

LIST OF EQUATIONS

Equation 2.1	Degree of deacetylation	22
Equation 2.2	Mass of oil adsorbed	28
Equation 2.3	Adsorption capacity	28

APPROVAL SHEET

This is to certify that we have supervised the preparation of and read the research paper prepared by **ARREZA, JULES ALEXANDER O., BORGUETA, ALPHONSE REIGH C., MELCHOR, JAN DANIEL G., AND QUILLO, JOHN RAENEL G.** entitled **A COMPARATIVE STUDY ON THE OIL SORPTION CAPACITY OF CHITOSAN EXTRACTED FROM ASIAN GREEN MUSSEL SHELLS TO SHRIMP SHELLS AS AN OIL SPILL ADSORBENT** and that the said paper has been submitted for final examination by the Oral Examination Committee.

Engr. Ryan Kristoffer C. Aguirre
Research Adviser

As member of the Oral Examination Committee, we certify that we have examined this paper and hereby recommend that it be accepted as partial fulfillment of the undergraduate requirement for the Degree **Bachelor of Science in Petroleum Engineering**.

**Engr. Manuel L.
Cabiguen, Jr.**
Panel Member 1

Engr. Allan Jay L. Javier
Panel Member 2

**Engr. Philip Ryan B.
Maigne**
Panel Member 3

This research paper is hereby approved and accepted by the College of Engineering, Architecture, & Technology as partial fulfillment of the undergraduate requirement for the Degree **Bachelor of Science in Petroleum Engineering**.

Engr. Elucila M. Sespeña, Ph.D.
Dean, College of Engineering, Architecture, & Technology

BIBLIOGRAPHY

A SiSrinivasan, A.; Viraraghavan, T. Bioresour. Technol. (2010), 101, 6594mple and Effective Method for Extraction of High Purity Chitosan from Shrimp Shell Waste.

Annunciado, T., Sydenstricker, T., & Amico, S. (2005). Experimental Investigation of Various Vegetable Fibers as Sorbent Materials for Oil Spills. Marine Pollution Bulletin, 1340-1346.

Aranaz, I., Mengibar, M., Harris, R., Paños, I., Miralles, B., Acosta, N., Galed, G. and Heras, Á. (2009) 'Functional

Biskup R., Jarosinska D., Rokita, B., Ulanski, P., (2012). Determination of Degree of Deacetylation of Chitosan-Comparison of Methods.

Characterization of Chitin and Chitosan', Current Chemical Biology, pp. 203-230

Dictionary-Reference. (2014). Oil Spill. Retrieved October 29, 2014, from Dictionary Reference Website: <http://dictionary.reference.com/browse/oil+spill>

Dijkers, A. (1985). Geology in Petroleum Production. Amsterdam: Elsevier Science Publishing Company Inc.

Dutta, P.K., Dutta, J., And Tripathi, V.S. (2004), Chitin and Chitosan: Chemistry, properties and applications. Journal of Scientific and Industrial, Journal of Scientific and Industrial Research, Volume 23, pages 20-31.

EPA, U. (2014). Mechanical Containment and Recovery of Oil Following A Spill. Retrieved October 22, 2014, from http://www.epa.gov/oem/docs/oil/edu/oilspill_book/chap2.pdf

EPA, U. (2014, October 29). Oil Spill Response Techniques. Retrieved October 30, 2014, from United States Environmental Protection Agency Website: <http://www.epa.gov/oem/content/learning/oiltech.htm>

InTechOpen Open Access Publisher - Open Science Open Minds | InTechOpen. (2012). Retrieved from <http://www.intechopen.com/books/biopolymers/Chitin-based-biocomposites-for-removal-ofcontaminants-from-water-a-case-study-of-fluoride-adsorptio>

Isa, Muhammed. (2013). Extraction and Characterization of Chitin and Chitosan from Mussel Shell. Civil and Environmental Research. 3. 108-114.

ITOPF. (2013). Use of Sorbent Material in Oil Spill Response. Retrieved January 11, 2015, from The ITOPF Website: <http://www.itopf.com/information-services/publications/>

Koilparambil, Divya & Rebello, Sharrel & Shanavas, Jisha. (2014). A Simple and Effective Method for Extraction of High Purity Chitosan from Shrimp Shell Waste.

Martino, A.D., Sittinger, M. and Risbud, M.V. (2005) 'Chitosan: A versatile biopolymer for orthopaedic tissue-engineering', *Biomaterials*, pp. 5983-5990.

Oil Sorbents: Testing Protocol and Certification Listing Program | Cooper | Keller (1993). Retrieved from <http://ioscproceedings.org/doi/abs/10.7901/2169-3358-1993-1-549?code=ampi-site>

Ongo, Emelda & S. Valdecañas, Candy & Jude M. Gutierrez, Bernard. (2016). UTILIZATION OF AGRICULTURAL WASTES FOR OIL SPILLS REMEDIATION.51-60. 10.17501/icoaf.2016.2106.

Padmanabhan A, Nair L. S. Chitosan hydrogels for regenerative engineering. In: Dutta P. K, editor. *Chitin and Chitosan for Regenerative Medicine*. New Delhi: Springer; 2016. pp. 3–40.

Philippines, G. (2010). Guimaras Oil Spill Incident. Retrieved October 29, 2014, from Greenpeace Philippines Website: <http://www.greenpeace.org/seasia/ph/multimedia/slideshows/the-guimarasoil-spill-incident/>

Ramirez M. A, Rodriguez A. T, Alfonso L, Peniche C. Chitin and its derivatives as biopolymers with potential agricultural applications. *Biotechnol. Appl.* 2010;27:270–276
ReliefWeb. (2013, November 30). Oil Spill in Estancia Iloilo Province, Western Visayas, Philippines Resulting from Typhoon Haiyan (Yolanda). Retrieved October 29, 2014, from ReliefWeb Website: <http://reliefweb.int/report/philippines/oil-spill-estancia-iloilo-provincewestern-visayas-philippinesresulting-typhoon>

Removal of Petroleum Spill in Water by Chitin and Chitosan | Barros | *Orbital: The Electronic Journal of Chemistry*. (2014). Retrieved from <http://www.orbital.ufms.br/index.php/Chemistry/article/view/509>

Rinaudo, M. (2006) 'Chitin and chitosan: Properties and applications', *Progress in Polymer Science*, pp. 603-632.

Speight, J. G. (2007). *The Chemistry and Technology of Petroleum* (4th ed.). Boca Raton, Florida, U.S.A.: Taylor and Francis Group LLC.

Srinivasan, A.; Viraraghavan, T. *Bioresour. Technol.* 2010, 101, 6594.

Y. S. Darmanto (2002). THE EFFECT OF CHITIN AND CHITOSAN OF CRAB SHELL ON WATER SORPTION OF ISOTHERM AND DENATURATION OF MYOFIBRILS DURING DEHYDRATION PROCESS

Chapter 1

INTRODUCTION

Crude oils and refined petroleum products consist largely of hydrocarbons, which are chemicals composed solely of hydrogen and carbon in various molecular arrangements. Crude oils contain hundreds of different hydrocarbons and other organic and inorganic substances including atoms of sulfur, nitrogen, and oxygen, as well as metals such as iron, vanadium, nickel, and chromium (U.S. EPA, 2014). It is recovered from underneath the Earth through drilling onshore and offshore. Recovered oil is transported via pipelines or tankers to refineries for processing. Petroleum derivatives or products are then transported to consumers via tankers, or large land vehicles (Speight, 2007).

Oil spilled by damaged tankers, pipelines or offshore oil rigs coats everything it touches and becomes an unwelcome but long-term part of every ecosystem it enters. It results in both immediate and long-term environmental damage. Some of the environmental damage caused by an oil spill can last for decades after the spill occurs (West, 2017). Oil spill should be eliminated as quickly as possible to prevent further damage on the local and regional ecology.

The common techniques to eliminate oil spill are the use of equipment like booms, skimmers, pumps, the use of dispersants, and the use of sorbents (U.S. EPA, 2014). Mechanical equipment and dispersants for oil spill cleanup are relatively expensive. The utilization of natural and native sorbents can, therefore, be a supplement, especially in the Philippines which is rich in these kind of resources.

There are already many studies confirming the potential of natural sorbents such as hair, poultry feather, raw cotton, saw dust, fibrous plants, wood chips, shavings and barks, to name a few. Although there are already many proven oil sorbents, it is still very important to

discover other possibilities. Oil spill can happen at a very large scale, that it might require huge quantities of sorbents. Cleanup could also last for months to years, especially if there was lack of equipment and materials for the cleanup process. New discovery for oil spill elimination is very helpful; it can be a significant addition especially if the resource is abundant; and it can be an option considering geographical factors.

Chitin, a biopolymer that comprises a linear sequence of monomeric sugars of Nacetylglucosamine. After cellulose, chitin is the most abundant biological fiber on earth. Chitin has a similar structure of cellulose, which the structural difference is the hydroxyl groups of carbon 2 is replaced by acetyl amino groups in the chitin molecule. The commercial sources of chitin are crustacean shells especially of crab, shrimp, and lobster. Chitin is also found in insects, mussels, mollusks, and in fungal cell wall (Muzarelli, 1997).

Chitin and chitosan have been evaluated by several studies as the agent for microorganism immobilization used for removal of organic pollutants such as oil and its derivatives (Bashan, 2010). Chitosan has been reported as a low cost alternative in the treatment of contaminated waters containing phenolic compounds (Milhome, 2009).

Barros conducted a research in 2014 that compared the oil adsorption capacity of chitin and chitosan in bunker oil spilled in distilled water by using chitin flakes, chitin powder, chitosan flakes, chitosan powder, and chitosan solution extracted from shrimp shells. The results from Barros' research served as the basis for our comparative study.

To find further alternatives, it is within this context that the researchers aimed to determine the oil sorption capacity of waste Asian green mussel shells as an oil spill sorbent by using shrimp shells as basis for comparison. *Perna viridis*, commonly known as the Asian green mussels, is a bivalve mussel widely distributed in the Asia-Pacific region. Mussel shell

waste could be utilized for chitosan production because it contained chitin which can be derived into chitosan through deacetylation process (AIP conference proceedings, 2016).

In this paper, the researchers studied the sorption capacity of *Perna Viridis* as an oil spill sorbent and sought answers to the following problems:

1. Will the chitosan extracted from mussel shells be an effective oil sorbent in terms of its oil sorption capacity (g,oil/g,sorbent) when compared to shrimp shells?
2. Which form of chitosan (flakes or powder) will yield more oil in terms of its oil sorption capacity?
3. What degree of deacetylation can be produced from extracting chitosan from mussel shells?

The researchers' general objective in this study was to determine the oil sorption capacity of chitosan from waste Asian Green Mussel shells as an oil spill sorbent in comparison with the extracted chitosan from shrimp shells. Specifically, the researchers also determined the degree of deacetylation and evaluated as to which size samples of sorbents prepared (flakes and powder) was more efficient. The water uptake capacity (g oil/g sorbent) and the oil retention (g oil sorbed) of the chitosan from 2 different size of samples were measured for additional data.

The shells of the Asian Green Mussels are considered to be waste products in the Philippines. A purpose of this research is to determine the potential of the Asian Green Mussel shells as an oil spill sorbent by taking into account its sorption capacity. Chitosan extracted from the shells of the Asian Green Mussels could prove to be as equally effective or even better compared to the chitosan yield from shrimp, making it a significant addition on the already

discovered green sorbents. It is more abundant than the shrimp and it is not costly to gather, process, and apply, which is a huge advantage compared to the commercial oil absorbents.

The study focused only on evaluating the effectiveness of extracted chitosan from mussels' shells to sorb oil. Waste Asian green mussels were collected from San Jose New Market and only the shells were used in the experiment. Economic impact was not included. Bunker oil was used as oil type testing. Water uptake determination was through weight difference between wet-static (water and oil) and dry-static (pure oil) system. Specific gravity and buoyancy of the sorbent material were not measured.

The study had its assumptions. The study assumed that chitosan was already proven as a potential oil sorbent regardless of its source. The chitosan extracted from *Perna Viridis* shells have varying yield and properties compared with chitosan from shrimp shells which has already been studied with regards to its potential as oil sorbent — validating the selection of sorbent material. The study also assumed that the collected waste mussel shells retained its original composition after cooking, boiling, and heating. The researchers used only 1 specific kind of oil (bunker fuel oil) for experimentation because of its availability and consistency as basis for comparison, having used the same kind of oil. Actual conditions (weather, wind direction, wave action, salt water, and oil dispersion) in an oil spill phenomenon were not simulated. Only the chitosan's oil sorption capacity and size (flaked and powdered) served as basis for comparison in determining the effectiveness of chitosan extracted from mussel shells as oil sorbent. The water uptake data served only as additional information for future studies.

Chapter 2

REVIEW OF RELATED LITERATURE AND STUDIES

This chapter exhibits a number of literature and past studies that would be beneficial in this study. Furthermore, this chapter would serve as a basis and support to every information that is required to answer the researchers' problem and objective.

2.1 Related Literature

2.1.1 Oil spill

Oil spill is the accidental release or leakage of petroleum into a body of water, as from a tanker, underwater pipeline, or an offshore oil rig, that often presents a hazard to marine life and the environment. Oceanic oil spills became a major environmental problem in the 1960s, chiefly as a result of intensified petroleum exploration on the continental shelf and the use of supertankers capable of transporting more than 450,000 metric tons (500,000 tons) of oil. Thousands of minor and several major oil spills related to well discharges and tanker operations are reported each year, with the total quantity of oil released annually into the world's oceans exceeding 1,000,000 tons (907,000 metric tons). The costs of such accidental oil spills are considerable in both economic and ecological terms. Oil on ocean surfaces is harmful to many forms of aquatic life because it prevents sufficient amounts of sunlight from penetrating and also reduces the level of dissolved oxygen. Moreover, crude oil renders feathers and gills ineffective, so that birds and fish may die from direct contact with the oil itself. Accidents to supertankers and to underwater wells and pipelines may be the cause of major oil spills, but the unintentional or negligent release of used gasoline solvents and

crankcase lubricants by industries and individuals greatly aggravates the overall environmental problem. Combined with natural seepage from the ocean floor, these sources add oil to the world's waterways at the rate of from 3,900,000 to 6,600,000 tons (3,500,000 to 6,000,000 metric tons) a year (Dictionary-Reference Website, 2014).

2.1.1.1 Bunker Fuel Oil

Bunker fuel or bunker crude is technically any type of fuel oil used aboard vessels. It gets its name from the tanks on ships and in ports that it is stored in; in the early days of steam they were coal bunkers but now they are bunker fuel tanks. Bunker fuel is graded A, B or C, with C being the thickest and most viscous, often requiring heating or blending in order to make it flow. Mixed with up to 10 per cent of a lighter fuel, such as diesel, it becomes a cheap fuel for use in shipping. No. 6 is the most common oil, that's why "bunker fuel" is often used as a synonym for the No. 6 residual fuel oil which requires heating before the oil can be pumped. Characterized as persistent oil, it is likely to spread large distances because it does not easily evaporate. It is a brown to black liquid, with a characteristic odour. Vapours are harmful if inhaled, exposure can cause eye or skin irritation. Suspected of damaging fertility, may cause organ damage through prolonged exposure. Harmful to fish, toxic to crustaceans and shellfish, very toxic to aquatic plants with potentially long-term adverse effects. Contains constituent parts with the potential to bioaccumulate. Left stagnant in storage tanks, deadly hydrogen sulphide gas can form (Dyck, 2015).

2.1.1.2 Oil Spill Occurrences in the Philippines

The Petron-chartered single hull vessel carrying 2.1 million liters of fuel oil sank in Guimaras Strait. It caused huge slick, which the Greenpeace characterized as an ecological time bomb that can cause long-term and possible irreversible damage to the environment and livelihoods of the community. Guimaras oil spill incident was the worst in Philippine history (Greenpeace Philippines, 2010).

A significant spill of heavy oil (bunker C type) occurred when Power Barge No.103 ran aground at the shores of Estancia in Iloilo during the height of typhoon Haiyan. Estimates by the management of the power barge amounted to around 800,000 liters of oil having leaked. As the ruptured tanks continued to leak and up to 600,000 liters of oil remain in the tanks, the amount of spill was increasing steadily. Urgent action was required to pump out the remaining oil or seal the holes in the tanks. Most of the spilled oil has washed ashore, contaminating the coast and mangroves up to 10 kilometers downstream. The containment booms deployed are not sufficient to effectively contain all of the free phase oil in the water. The free phase oil has been blown ashore by southeastern winds. A change of wind direction or a tropical depression could further complicate the containment of the free phase oil. A faster mechanical clean-up process with oil skimmers was urgently required (ReliefWeb, 2013).

2.1.1.3 Methods of Oil Spill Cleanup

A number of advanced response mechanisms are available for controlling oil spills and minimizing their impacts on human health and the environment. The key to effectively combating spills is careful selection and proper use of the equipment and materials best suited

to the type of oil and the conditions at the spill site. Most spill response equipment and materials are greatly affected by such factors as conditions at sea, water currents, and wind. Damage to spill-contaminated shorelines and dangers to other threatened areas can be reduced by timely and proper use of containment and recovery equipment (U.S. EPA, 2014).

Mechanical containment or recovery is the primary line of defense against oil spills in the United States. Containment and recovery equipment includes a variety of booms, barriers, and skimmers, as well as natural and synthetic sorbent materials. Mechanical containment is used to capture and store the spilled oil until it can be disposed of properly (U.S. EPA, 2014).

Containment booms are used to control the spread of oil to reduce the possibility of polluting shorelines and other resources. Booms also concentrate oil in thicker surface layers, making recovery easier. In addition, booms may be used to divert and channel oil slicks along desired paths, making them easier to remove from the surface of the water. A skimmer is a device for recovering spilled oil from the water's surface. Skimmers may be self-propelled, used from shore, or operated from vessels. The efficiency of skimmers is highly dependent upon conditions at sea. In moderately rough or choppy water, skimmers tend to recover more water than oil. Different types of skimmers offer advantages and drawbacks depending on the type of oil being recovered, the sea conditions during cleanup efforts, and the presence of ice or debris in the water. Sorbents are insoluble materials or mixtures of materials used to recover liquids through the mechanism of absorption, or adsorption, or both. Absorbents are materials that pick up and retain liquid causing the material to swell (50 percent or more). Adsorbents are insoluble materials that are coated by a liquid on its surface. To be useful in combating oil spills, sorbents need to be both oleophilic (oil-attracting) and hydrophobic (water-repellent). Although they may be used as the sole cleanup method in small spills, sorbents are most often

used to remove final traces of oil, or in areas that cannot be reached by skimmers. Sorbent materials and any oil that is removed from sorbent materials must be disposed of in accordance with approved local, state, and federal regulations (U.S. EPA, 2014).

Chemical and biological methods can be used in conjunction with mechanical means for containing and cleaning up oil spills. Dispersing agents and gelling agents are most useful in helping to keep oil from reaching shorelines and other sensitive habitats. Biological agents have the potential to assist recovery in sensitive areas such as shorelines, marshes, and wetlands (U.S. EPA, 2014).

Dispersing agents, also called dispersants, are chemicals that contain surfactants and/or solvent compounds that act to break petroleum oil into small droplets. In an oil spill, these droplets disperse into the water where they are subjected to natural processes, such as waves and currents, which help to break them down further. This helps to clear oil from the water's surface, making it less likely that the oil slick will reach the shoreline. Heavy crude oils do not disperse as well as light to medium weight oils. Dispersants should not be used on gasoline or diesel spills for example. Dispersants are most effective when applied immediately following a spill, before the lightest materials in the oil have evaporated. Biological agents are chemicals or organisms that increase the rate at which natural biodegradation occurs. Biodegradation is a process by which microorganisms such as bacteria, fungi, and yeast break down complex compounds into simpler products (U.S. EPA, 2014).

Biodegradation of oil is a natural process that slowly removes oil from the aquatic environment. However, rapid removal of spilled oil from shorelines and wetlands is necessary in order to minimize potential environmental damage to these sensitive habitats (U.S. EPA, 2014).

Physical methods are used to clean up shorelines. Natural processes such as evaporation, oxidation, and biodegradation can start the cleanup process, but are generally too slow to provide adequate environmental recovery. Physical methods, such as wiping with sorbent materials, pressure washing, and raking and bulldozing can be used to assist these natural processes (U.S. EPA, 2014).

2.1.1.4 Sorbents

Sorbents are marketed in various forms according to their composition and their intended use, but can be generalized generally as one of four types: bulk loose material (particulate); enclosed in a mesh as pillows or booms; continuous in the form of mats, sheets, booms, or rolls; and as loose fibers combined to form snares or sweeps (ITOPF, 2013).

Bulk materials (organics such as bark, peat, sawdust, cork, feather, wool, human hair; inorganics such as vermiculite, pumice, clay; synthetics such as polypropylene) are marketed as loose sorbent and serve a useful purpose to recover small spills on land (ITOPF, 2013).

Enclosed sorbent products vary in shape and volume but booms are the most common. Enclosed sorbent is typically produced using readily available organic or inorganic natural material but may also comprise individual elements of synthetic materials (ITOPF, 2013).

Continuous sorbents are primarily manufactured from synthetic materials with woven, melt-blown, polypropylene being one of the materials most commonly used during spill response (ITOPF, 2013).

Bundles or hanks of loose sorbent fibers are available that allow these oils to be recovered through a combination of adhesion to a large surface area and cohesion within the oil itself (ITOPF, 2013).

2.1.1.5 Criteria for Selecting Sorbents

In addition to the form in which the sorbent is presented and the ability of a particular material to selectively take up oil, other factors also affect a sorbent's effectiveness (ITOPF, 2013).

a) Buoyancy

For sorbents to be used effectively on floating oil, they must have and retain high buoyancy, remaining afloat even when saturated with oil and water.

b) Saturation

Sorbents can quickly become saturated by oil. Even a relatively small slick may quickly overwhelm a sorbent boom and oil may be released from the sorbent to contaminate the resource that it was intended to protect.

c) Oil Retention

One of the key aspects of the overall performance of a sorbent is its availability to retain oil.

Table 2.1.1.5 Properties of some specific oil.

Type of oil	Density	Viscosity
Kerosene	0.8 - 0.81g /mL	Less viscous
Crude oil	898 kg/m ³	Less viscous
Bunker oil (type c)	900 - 1010 kg/m ³	Viscous
Machine oil	899.0 kg/m ³ (0 degrees Celsius)	Less viscous
	888.1 kg/m ³ (20 degrees Celsius)	
	840.0 kg/m ³ (100 degrees Celsius)	

d) Strength and durability

The durability of a sorbent is important in those situations where it may be left for an extended period of time before recovery. The strength of some sorbent booms, particularly those composed of enclosed loose material, is dependent on the durability of the retaining netting material, which may break open in adverse environmental conditions.

e) Fermentation

Some organic sorbents can ferment when left in contact with water for an extended period of time.

f) Cost

The cost of sorbent products varies greatly and is primarily dependent upon the material used.

g) Availability, storage and transportation

The performance of synthetic sorbents makes their use attractive but they may not always be immediately available at the site of the spill. On the other hand, organic and inorganic sorbents may be less efficient but are more widely available.

Sorbents are bulky by nature and, in large amounts, the space required for storage can be significant.

As with storage, transportation of large volumes of sorbents can invoke logistical problems, both from the warehouse to a distribution center in the general vicinity of the spill and from there into the field where the sorbents are to be used.

2.1.2 *Perna viridis* (Asian Green Mussels)

The Asian green mussel, *Perna viridis*, is a large (> 80 mm) bivalve, with a smooth, elongate shell typical of several mytilids. It has visible concentric growth rings and a ventral margin that is distinctly concave on one side. The characteristic green coloration comes from the periostracum, the proteinaceous outer layer of the shell. It is uniformly bright green in juveniles, but dulls to brown with green margins in mature individuals. The inner surfaces of the valves are smooth and iridescent blue to bluish-green in color. A prominent, kidney-shaped retractor muscle scar is present, but the species lacks anterior adductor muscles. Close examination of the beak (i.e., where the two valves hinge together) reveals a pair of hinge teeth on the left valve that interlock with a single hinge tooth on the right valve (DeVictor and Knott undated, NIMPIS 2002, Rajagopal et al. 2005).

Tijani Isa et al investigated the chitin and chitosan composition and extraction of *Perna viridis* or common called Asian green mussels. The mussel was discovered to contain a mineral content of 51.62 % and a chitin composition was found to be 21.32 % (Tijani Isa, 2015).

2.1.2.1 *Habitat, Distribution, and Reproduction*

The Asian green mussel is found in the coastal waters of the Indo-Pacific region. However the mussels are introduced to other areas as invasive species via boat hulls and water ballasts (Rajagopal et al. 2005).

The mussel inhabits estuarine habitats and is found in densities as high as 35,000 individuals per square meter in any submerged marine object. Although vivid green in appearance, the mussels are shrouded with overgrowth and are often hard to find. The mussels

live in waters that are 11-32 °C with a wide-ranging salinity of about 18-33 ppt. *P. viridis* grows fastest at 2 meters below the surface, in high salinity and high concentration of phytoplankton although it can tolerate a range of salinity and turbid water (Rajagopal et al. 2005).

The Asian green mussel has separate sexes and fertilizes externally. There are a very few functional hermaphrodites (<0.1%). The mussel's sexual development was shown to be affected by temperature. Spawning ordinarily occurs twice a year between early spring and late autumn; however, the mussels found in the Philippines and Thailand are known to spawn all year round. The zygote transforms to a larva 7–8 hours after fertilization. The larvae stay in the water column for 10–12 days before undergoing metamorphosis into a juvenile and settling onto a surface. The juveniles become sexually mature when they are 15–30 mm in length, a size reached within 2–3 months. Growth is influenced by the availability of food, temperature, water movement, mussel's age, and caging. Cage culturing can prevent entry of predators and barnacles increases marketability but slows down the mussel's growth rate. The adult can live to up 2–3 years. Due to its fast growth, it can outcompete other fouling organisms and cause changes in marine ecological relationships. Because of its dispersed spawning nature, lack of local predators, fast growth, and high tolerance of environmental conditions, the mussel population is expected to expand in Atlantic habitats until it reaches its thermal limits (Rajagopal et al. 2005).

2.1.2.2 Uses

DeVictor and Knott (UNDATED) stated that, "*Perna viridis* is harvested commercially in the Indo-Pacific as a human food resource due to its dense and fast growth. It is not

recommended for consumption when found in areas like Tampa Bay because of its potential to harbor toxins, parasites and other health risks."

The Gulf States Marine Fisheries Commission reports in 2003 that, *P. viridis* has also been used as an indicator of biopollution of heavy metals, organochlorines, and petroleum hydrocarbons and that *P. viridis* is one of the best mussel species to test for biopollution.

NIMPIS reports in 2002 that, "*P. viridis* is an efficient filter/suspension feeder, feeding on small zooplankton, phytoplankton and other suspended fine organic material.

2.1.2.3 Taxonomy

Table 2.1.2.3 Taxonomy of *Perna viridis* (Benson, 2009).

Kingdom	Division	Class	Order	Family	Genus
Animalia	Mollusca	Bivalvia	Lamellibranchia	Mytilidae	<i>Perna</i>

2.2 Related Studies

2.2.1 Chitin and chitosan

Chitosan has drawn special interest as an effective biosorbent due to its low cost, biocompatibility, biodegradability, non-toxicity, and other unique properties such as film forming ability, chelation, adsorption and anti-microbial properties (Dutta et al, 2004). It is produced from the deacetylation of Chitin, a natural carbohydrate polymer found in the skeleton of crustaceans such as crab, shrimp and lobster. It is a cationic polysaccharide with a linear chain structure consisting of β -(1,4)-linked 2-acetamino-2deoxy- β -D-glucopyranose and 2-amino-2-deoxy- β -D-glucopyranose. The degree of deacetylation of Chitin exceeding 50%

converts the biopolymer into Chitosan. The increase of primary amine groups in Chitosan is mainly responsible for the adsorption of cations (by chelation), anions (by electrostatic attraction) or polar molecules (by interactions as ion-dipole, dipole-dipole, Van der Waals forces, etc.) (Mendez, 2010).

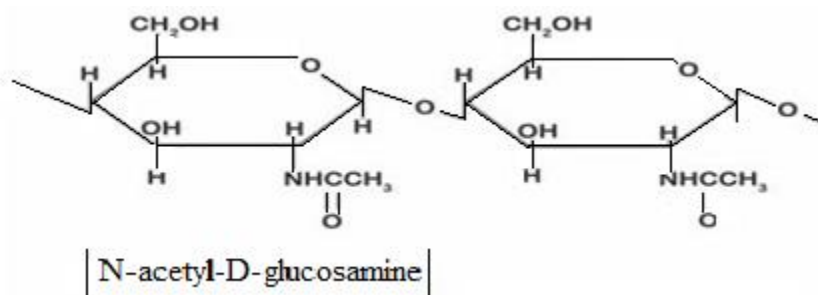


Figure 2.2.1a Chemical structure of chitin (Lertsutthiwong, 2002)

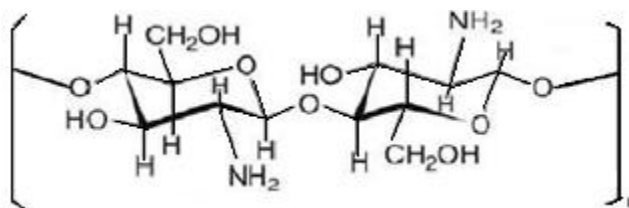


Figure 2.2.1b Chemical structure of chitosan (Lertsutthiwong, 2002)

Depending on its source, chitin occurs as two allomorphs, namely α and β forms which can be differentiated by infrared and solid-state NMR spectroscopy together with X-ray diffraction. α - Chitin is by far the most abundant; it occurs in fungal and yeast cell walls, in krills, crab tendons, and in shrimp shells. The rarer β - chitin is found in association with proteins in squid pens, vestimentiferan worms, and mussel shells (Rinaudo, 2006).

Potential and usual applications of chitin, chitosan and their derivatives are estimated to be more than 200 (Aranaz et al., 2009). The duo is the second-most abundant high molecular-weight biopolymers and is recognized as versatile, environmentally friendly raw materials (Hsu et al., 2004). Chitin is the major component in the shell of the shrimps, and

crabs, cartilage of the squid, and outer cover of insects, it also occurs as ordered crystalline microfibrils forming structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. It is also extracted from a number of other living organisms in the lower plant and animal kingdoms, serving in many functions where reinforcement and strength are required (Rinaudo, 2006). Generally, the shell of selected crustacean was reported to consist of 30-40% protein, 30-50% calcium carbonate and calcium phosphate, and 20-30% chitin (Fernandez-Kim, 2004). Depending on the processing method used to derive the chitin (Khora and Limb, 2003), and also depending on the source, its degree of deacetylation may range from 30% to 95% (Martino et al., 2005).

If the content of N-acetyl-D-glucosamine in polymer is higher than the biopolymer is chitin and if the content of glucosamine is higher than the biopolymer is chitosan (Ramirez, 2010). Chitosan is insoluble in water, basic pH solutions and organic solvents which is a major limitation of chitosan. Chitosan is soluble in dilute organic acid like acetic acid, formic acid, etc., and forms a high viscous solution. Chitosan solubility depends on the distribution of N-acetyl and free amino groups. Protonation of amino groups occurs at $\text{pH} < 6.0$ due to which chitosan becomes soluble (Padmanabhan, 2016).

Chitosan is a natural polysaccharide comprising of copolymers of glucosamine and N-acetylglucosamine, and can be obtained by the partial deacetylation of chitin. In its crystalline form, chitosan is normally insoluble in aqueous solutions above $\text{pH} 7$; however, in dilute acids ($\text{pH} 6.0$), the protonated free amino groups on glucosamine facilitate solubility of the molecule (Martino et al., 2005).

Chitosan degree of deacetylation (DDA) has often been cited as an important parameter that determines many physiochemical and biological properties of chitosans such as

crystallinity, hydrophilicity, degradation and cell response. For example, Freier et al. found in 2007 that prolonged degradation times and enhanced cell adhesion can be achieved using chitosan with a DDA close to 0% or 100% while chitosans with intermediate DDAs exhibit rapid degradation rates, but at the cost of limited cell adhesion. Hidaka et al. reported in 2010 that chitosan membranes between 65 and 80% DDA elicited marked inflammatory reactions that subsided in time with degradation of the films, granulation tissue formation, and osteogenesis while membranes made of 94% DDA chitosan showed minimal degradation, mild inflammation and minimal osteogenesis.

DDA of chitosan is generally controlled by processing of the native polymer with alkali, and with increasing time and temperature to obtain the highest DDA (>90) materials (Nuthanid et al., 2009). The molecular weight (MW) of chitosan is dependent on the initial source material (shrimp, crab, fungi, *etc.*) and can decrease with processing to increase DDA (Nuthanid et al., 2009). MW has been shown to be an important factor in chitosan properties such as crystallinity, degradation, tensile strengths and moisture content. For example, Nuthanid et al. reported in 2009 that tensile strength and moisture adsorption of chitosans with high MW_v's (600–1000 kDa) were significantly greater than those with similar DDA's but lower MW_v's (50–60 kDa). Further, Xu and Du reported in 2008 that increasing chitosan MW (10–120 kDa) increased protein encapsulation efficiencies and reduced release kinetics. However, there has been little data on the role MW on biological performance. Hamilton et al. evaluated the growth of osteoblasts on seven different chitosans (76–96 DDA and MW_v = 2400–8200 kDa) and noted there was no relationship between DDA, MW_v or growth of cells. Similarly, there were no correlations between DDA, MW or growth of keratinocytes on five different chitosans (53–97 DDA, MW_v = 735–1180 kDa). The most likely reason for the lack

of correlation in these studies is due to differences in the initial source materials and process conditions for the different production lots.

2.2.1.1 Extraction of Chitin and Chitosan

Ongo et al. investigated in 2016 the utilization of wastes for oil spill remediation through extraction of chitosan from shrimp shells by deprotenization and deacetylation using alkali treatment.

Rebello et al. conducted a research in 2014 about simple and effective method for extraction of high purity chitosan from shrimp shell waste. Shells were washed, air dried and refrigerated overnight. This was then oven dried for 4 consecutive days at 65 °C. Chitosan was prepared using a combination of three procedures. Five gram of shrimp shell waste was treated with 4% NaOH at room temperature for 24 hours. The alkali was drained from the shells and washed with distilled water repeatedly till pH dropped to neutral. This process caused deproteinization of shells. The deproteinized shells were treated with 4% HCl at room temperature for 12hours for demineralization to yield chitin. The acid was drained off from chitin, washed with distilled water and finally dried at room temperature. The process was repeated with 2% NaOH and 1% HCl. The chitin obtained still had a slight pink hue. Further decolourisation was achieved by soaking chitin in 1% potassium permanganate for 30 mins followed by 1% oxalic acid for 30 mins to 2hours. The decolourised chitin was deacetylated to form chitosan by treating with 65% NaOH for 3 days at room temperature. Alkali was drained off and washed repeatedly with distilled water till pH was lowered. Chitosan was further dried at room temperature and stored.

Tijani Isa et al. conducted a study in 2015 on the extraction of and characterization of Chitin and Chitosan from Mussel Shell. Chitosan extraction was done in four steps; pretreatment, demineralization, deproteinization, and the deacetylation steps. The shells obtained were washed thoroughly with distilled water and dried in an oven to constant weight at a temperature of 35°C. Then 100g of the sample was taken for the extraction process. The dried shells were size-reduced and soaked in 0.68 M HCl (1:10 w/v) at ambient temperature (approximately 30°C) for 6 hours after which it was washed in the acid until no bubbles were seen and no colour change was observed. The sample was then washed with distilled water until a relatively neutral was obtained and then the demineralized shell was dried to constant weight. The demineralized mussel shells were dried weighed and soaked in 0.62 M NaOH solution (1:10w/v) at ambient temperature (approximately 30°C) for 16 hours. After which the shells were then washed thoroughly with water followed with distilled water until a neutral pH was obtained. The chitin was then dried to constant weight and ground and screened with 150µm sieve. The chitin obtained was then deacetylated in 25 M NaOH (1:10 w/v) for 20 hours at 75°C. After deacetylation, the chitosan is then washed thoroughly with water followed by distilled water. The resulting chitosan was then dried to constant weight and prepared for characterization.

2.2.1.2 Characterization of Extracted Chitosan

Chitosan was also prepared from shrimp processing waste (shell) using conventional chemical process for the other crustacean species. The physicochemical properties, molecular weight (165394g/mole), degree of deacetylation (75%), ash content as well as yield (15%) of prepared chitosan indicated that shrimp processing waste (shell) are a good source of chitosan.

The water binding capacity (50.2%) and fat binding capacity (37.0%) of prepared chitosan are good agreement with the commercial chitosan. FT-IR spectra gave characteristics bands of –NH₂ at 3443cm⁻¹ and carbonyl at 1733cm⁻¹ (Islam et al., 2011).

Isa et al. studied in 2012 the Extraction and Characterization of Chitin from Nigerian Sources. The studies revealed that the shrimp had the highest yield of chitin of 8.15%, crab, crayfish and periwinkle had yields of 7.8%, 2.88% and 0.44% respectively.

Table 2.2.1.2a Composition of mussel shells

Component	Composition (%)
Protein	9.99
Mineral	51.62
Chitin	23.25
Chitosan	15.14

Table 2.2.1.2b Carbon-Nitrogen content of chitin and chitosan

S/N	Carbon (%)	Nitrogen (%)	DDA (%)	Carbon-Nitrogen ratio
1 Chitosan	13.32	2.29	60.69	5.82
2 Chitin	26.23	4.02	19.37	6.52

Source: Tijani Isa, 2015

Biskup et al. investigated in 2012 the comparison of the different methods in determining the degree of deacetylation of Chitosan. Degree of deacetylation (DD) is one of the main parameters characterizing chitosan. The most precise measurements of DD require sophisticated equipment (NMR spectrometer), not available at many laboratories worldwide working on chitosan. There is a need for low-cost, simple, yet sturdy and reliable methods and procedures for DD determination. The aim of this work was to test and compare – on the same set of chitosan samples - a few of the existing analytical techniques and provide recommendation for selecting DD determination methods. Tests were performed on four chitosans of nominal DD in the range 70 - 95%. Three different methods of titration, two

different methods of spectroscopy UV / Vis and infrared spectroscopy using various calculation approaches were used.

Most basic method of characterizing is by titration method. Dried chitosan (0.2 g) was dissolved in 20 cm³ 0.1 M hydrochloric acid and 25 cm³ deionized water. After 30 minutes continuous stirring, next portion of deionized water (25 cm³) was added and stirring continued for 30 minutes. When chitosan was completely dissolved, solution was titrated with a 0.1 mol·dm⁻³ sodium hydroxide solution using automatic burette (0.01 cm³ accuracy). Degree of deacetylation (DA) of chitosan was calculated using formula: (2) where: m – weight of sample, V₁, V₂ – volumes of 0.1 mol·dm⁻³ sodium hydroxide solution corresponding to the deflection points, 2.03 – coefficient resulting from the molecular weight of chitin monomer unit, 0.0042 – coefficient resulting from the difference between molecular weights of chitin and chitosan monomer units (Biskup, 2012).

$$DA [\%] = 2.03 \times \frac{v_2 - v_1}{m + 0.0042(v_2 - v_1)} \quad (2.1)$$

More advance and accurate method for characterizing is by the use of infrared spectroscopy. Measurements have been performed in the transmission mode, with chitosan contained in potassium bromide (KBr) tablets. Potassium bromide was mixed with chitosan in mass ratio 100:1 (200 mg KBr and 2 mg chitosan). KBr was placed in an oven at 300 °C for 24 h before mixing. Substances were mixed in agate mortar and pressed to tablet form. Tablets were dried for 24 hours at 50 °C in order to remove moisture. For every kind of chitosan 3 tablets were produced. The spectra of chitosan samples were obtained within a

frequency range of $\lambda = 400 - 4000 \text{ cm}^{-1}$, each spectrum is an average of 64 scans with a resolution of 2 cm^{-1} (Biskup, 2012).

2.2.2 Testing method

A study of Cooper et al. in 1993 comprises Testing Protocol and Certification Listing Program of Oil Spill Sorbents. The test methods are based upon those defined by the American Society for Testing and Materials and previous test methods developed by

Environment Canada for our series of reports entitled Selection Criteria and Laboratory Evaluation of Oil Spill Sorbents. This series, which was started in 1975, encompasses a number of commercially available oil spill sorbents tested with different petroleum products and hydrocarbon solvents.

The testing program will categorize the sorbents according to their operating characteristics. The main categories are oil spills on water, oil spills on land, and industrial use. The characteristics we will be evaluating with the new test protocols include initial and maximum sorption capacities, water pickup, buoyancy, reuse potential, retention profile, disintegration (material integrity), and ease of application and retrieval.

Test methods has been established to evaluate the performance of sorbents on oil and water. These procedures followed are condensed versions of the test methods outlined by the Canadian General Standards Board.

Degradation Test Static: This is a test for buoyancy, sorbent capacity, and oil retention. A sorbent sample from the static pre-test is placed on a 5 mm layer of crude oil floating on an 80 mm layer of water. The sample is left undisturbed for a period of at least 48 hours.

The sorbent is then removed and hung from a balance (Type I: hung vertically using a clip, Type II: hung horizontally using a basket). Observations pertaining to the physical condition of the sorbent are recorded. The weight of the sorbent is measured as oil drips after 15 seconds, 30 seconds, 1 minute, 2 minutes, 5 minutes, and 30 minutes. A retention profile is obtained from this data, as is the sorbent's capacity. Mechanical compression is used to extract remaining liquid from the sorbent in order to determine water pick-up.

Degradation Test: This test follows the methodology of the Degradation Pre-Test (Dynamic) with the exception that 150 ml of crude oil is introduced into the 2 L jar. Observations of the sorbent's behavior during agitation are recorded. After 15 minutes the sorbent is removed and weighed to determine oil pick-up. Mechanical compression is used to extract liquid from the sorbent to determine water pick-up for comparison with the static test.

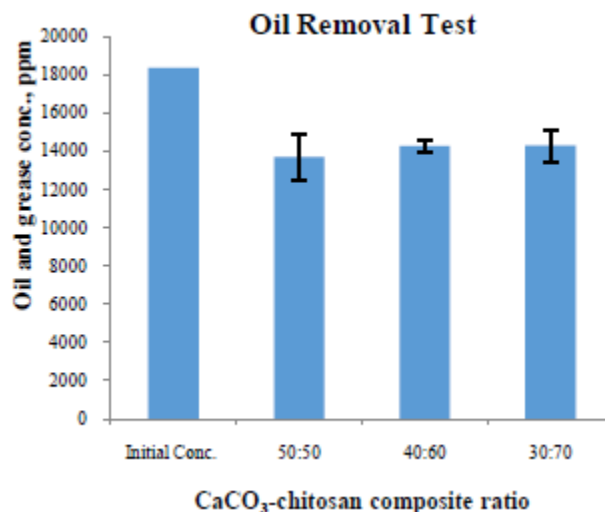
L-W Category test: This is a test for sorbent capacity and reuse. A fresh sorbent sample is placed on a 5 mm layer of crude oil floating on an 80 mm layer of water. The sample is left for a period of 15 minutes. The sorbent is then removed and left to drip for 30 seconds before being weighed (Type I: hung by clip, Type II: hung by basket). Observations pertaining to the physical conditions of the sorbent are recorded. Mechanical compression is used to extract remaining liquid from the sorbent for reuse testing and water content determination. The data is also compared with Degradation Test (Static) results. (Cooper, 1993)

2.2.3 Utilization of agricultural wastes for oil spills remediation

The study of Ongo et al. in 2016 aimed at investigating of utilization of agricultural wastes as high-value products is gaining importance not only because it reduces the volume of waste but also solves serious environmental problems. Crustacean shells and chicken eggshells

are the notable wastes generated by agro-based industries. Bioconversion of these wastes for oil spill remediation is considered a very promising and challenging approach. Chitosan from shrimp shells and Calcium Carbonate (CaCO_3) from chicken eggshells are among the excellent candidates for the adsorption of oil spills due to their unique properties such as biocompatibility, biodegradability and intrinsic porous structure. In this study, different proportions of Chitosan and CaCO_3 (50:50; 40:60 and 30:70) were varied to determine the optimum ratio that will give the highest oil removal rate. Scanning electron microscopy (SEM) showed the detailed morphology and uniform dispersion of the needle-shaped CaCO_3 on the Chitosan matrix. XRD analysis demonstrated sharp peaks at $2\theta = 9.40^\circ$, 29.7° , 37.3° which are the characteristics of crystalline Chitosan and calcite polymorph of CaCO_3 . The results showed that the 50:50 Chitosan- CaCO_3 composite exhibited a significantly high oil removal rate both for vegetable and diesel oil with an efficiency of 99%. The oil adsorption capacity of 50:50 Chitosan- CaCO_3 composite is 4.03g/g which is 4 times of its weight.

Figure 2.2.3a Vegetable oil removal/reduction rate of different Chitosan- CaCO_3 composite films.



Source: Ong, 201

Figure 2.2.3b Diesel oil removal efficiency of composite films (n=3; rsd < 10%)

Composite Film Mixture	Initial Conc. (ppm)	Final Conc. (ppm)	% Removal Rate
50:50	5000	5	99.9%
40:60	5000	15	99.7%
30:70	5000	15	99.7%

Source: Ong, 2016

2.2.4 The use of waste mussel shells for the adsorption of dyes and heavy metals

Papadimitriou et.al (2017) experimented the use of waste mussel shells for adsorption of dyes and heavy metals. Powdered mussel shells were used in standard adsorption experiments for the removal of methyl blue and methyl red as for the removal of Cr (VI), Cd and Cu. Also they were added into laboratory scale activated sludge reactors treating synthetic wastewater with hexavalent chromium, in order to investigate the potential attribution to the treatment efficiency. Adsorption experiments indicated almost 100% color removal. High removal efficiencies were observed for the metals, especially in the case of chromium and cadmium reached concentrations even below 1 mg L^{-1} . The addition of powdered mussel shells in the activated sludge processes enhanced the removal of chromium and phosphorus, while enabled the formation of heavier activated sludge flocs and thus enhanced the settling properties of the activated sludge.

2.2.5 Removal of petroleum spill in water by chitin and chitosan

Barros studied in 2014 the capacity of chitin and chitosan in adsorption of bunker fuel oil spilled in seawater by using chitin flakes, chitin powder, chitosan flakes, chitosan powder, and chitosan solution from shrimp shells. The size of these materials was determined by the USP-23 method. Chitin and chitosan flakes presented particles with size high than 0.354 mm. Chitin powder presented particles with average size of 0.354 mm, while chitosan powder had particles with average size between 0.104 and 0.075 mm. The degree of deacetylation of chitosan samples was determined by acid-base titration. In this method, a chitosan solution was prepared by adding 0.5 g chitosan (flakes or powder) in 40 mL of 0.1 mol L⁻¹ HCl solution. It was added 3 drops of methyl red to the chitosan solution, which was then titrated with standardized 0.1 mol L⁻¹ solution of NaOH and then, repeated the same titration with blank solution (0.1 mol L⁻¹ solution of HCl). The procedure was performed in triplicate. Chitosan samples showed deacetylation degrees of 82.2%. The results showed that, chitosan flakes had a better adsorption capacity by oil (0.379 ± 0.030 grams oil per gram of adsorbent),. Chitosan solution did not present such inconvenience, despite its lower adsorption capacity (0.013 ± 0.001 grams oil per gram of adsorbent). It was able to form a polymeric film on the oil slick, which allowed to restrain and to remove the oil from the samples of sea water. The study also suggests that chitosan solution 0.5% has greater efficiency against oil spills in alkaline medium than acidic medium. The process in which chitosan adsorbs oils and fats is not yet fully understood, but experimental observations indicate two types of mechanisms:

- a) Attraction of opposite charges between oil and chitosan;
- b) Interlace effect, in which chitosan acts as a network, getting involved around the drops of oil and fats and trapping them.

The polymers adsorption capacities in flakes or powder were determined using the following calculations:

$$SP = ST - SO \quad (2.2)$$

$$CP = SP / SO \quad (2.3)$$

Where:

SP= mass of oil adsorbed,

SO= mass of dry adsorbent,

ST= mass of the contents on the Petri plate

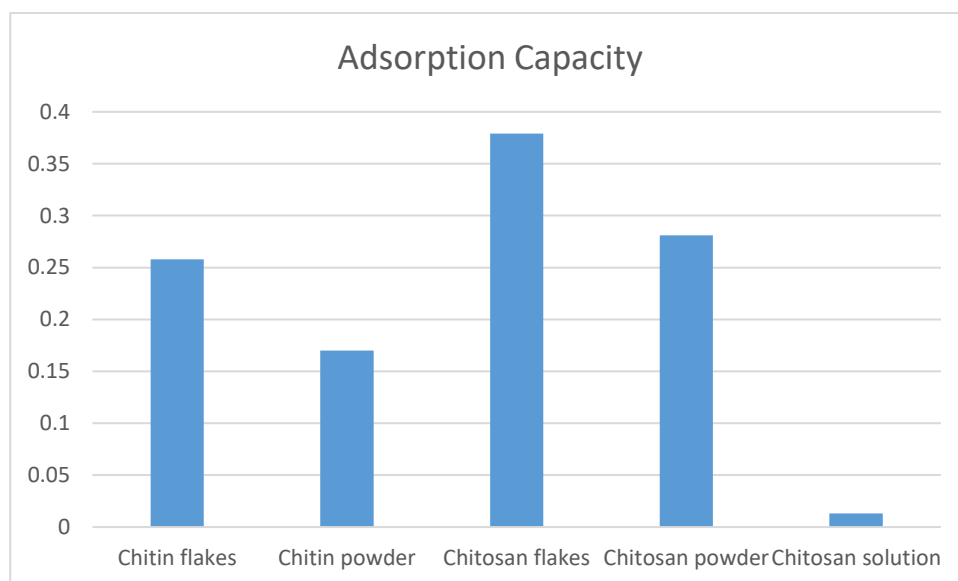
CP= adsorption capacity.

Table 2.2.5a Tabular data of adsorption capacity of biopolymer flakes, biopolymer powders and chitosan solution.

Adsorbent	Oil mass	Adsorbent amount	Adsorbed oil	Adsorption capacity
Chitin flakes	3.06 g	9.19 g	52.25 ± 4.23%	0.258 ± 0.022 g/g
Chitin powder	3.18 g	9.25 g	49.35 ± 2.01%	0.170 ± 0.004 g/g
Chitosan flakes	3.16 g	6.09 g	72.75 ± 3.97%	0.379 ± 0.030 g/g
Chitosan powder	3.08 g	8.91 g	81.27 ± 13.39%	0.281 ± 0.050 g/g
Chitosan solution	3.08 g	150.0 mL	60.52 ± 2.01%	0.013 ± 0.001 g/mL

Source: Barros, 2014

Figure 2.2.5b Adsorption capacity of biopolymer flakes, biopolymer powders and chitosan solution.



Source: Barros, 2014

2.2.6 Development of chitosan based adsorbent for the removal of residual oil from palm oil mill effluent (POME)

Hernawati conducted a study in 2011 about the modified crosslinked chitosan beads as an adsorbent to adsorb the excessive residue oil from an oily wastewater. Palm oil mill effluent (POME) from the local palm oil mill was used as a sample of an oily wastewater. Raw chitosan present some limitations such as unsatisfactory mechanical properties, poor heat resistance and soluble in dilute acid aqueous solution. Crosslinking has been proposed in this study to overcome such weakness. Three types of crosslink agent, namely glutaraldehyde (GLA), ethylene glycol diglycidyl ether (EGDE) and epichlorohydrin (ECH) were used for this purpose. Series of batch adsorption experiments were carried out using different conditions and parameters. The controlled variables were adsorbent dosage, agitation rate, contact time and pH. A detailed batch study on chitosan flakes and the crosslinked chitosan beads with

respect to its adsorption equilibrium, isotherm and kinetic study were also carried out. The best removal of residue oil was accomplished using chitosan flakes as adsorbent with adsorbent dosage of 15 g adsorbent per liter of POME, agitation rate of 100 rpm, contact time of 1 hr and pH of 6.0. Fourier transform infrared (FTIR) spectrophotometer and scanning electron microscope (SEM) images of chitosan flakes and crosslinked chitosan beads before and after the adsorption experiments were presented to prove that the residue oil had been adsorbed by these adsorbents. The Langmuir isotherm model provided the best fit for the equilibrium data in the concentration range investigated, with the maximum adsorption capacity being 131.58 mg of residue oil per gram of chitosan flakes, as obtained from the linear equation of the isotherm. The adsorption kinetics was tested using three models: pseudo-first-order, pseudo-second-order and intraparticle diffusion model. The experimental kinetics data were properly correlated with the pseudo-second-order kinetic model ($R^2=0.9996$), which provided a rate constant, k_2 , of 0.0021 g/mg.min. The research study had attested chitosan and crosslinked chitosan beads as a potential adsorbent to adsorb residual oil.

2.2.7 Chitosan microspheres (shrimp shells) applied for removal of oil from produced water in the oil industry

The discharge of oily wastewaters in the environment is steadily increasing, causing serious damages. Among various treatment methods, adsorption is generally considered the most appropriate, since it can remove both organic and inorganic pollutants. Adsorption using low-cost alternative biopolymers for removal of contaminants from wastewater has been widely investigated. In this context, chitosan has been drawing particular attention because, among its many applications, it can be used in the treatment of aqueous effluents. In this study,

microspheres were prepared by reticulation of chitosan with sodium triphosphate (STP) and studied for the treatment of water containing crude oil. The microspheres were regular and had surface pores. These microspheres were packed in treatment columns and their ability to remove oil was measured with a fluorometer, by the difference in the oil concentration before and after passing through the column. The microspheres that presented porosity about 80 % were highly efficient in oil removal, with rates above 90% (Grem, 2013)

There has been much research and studies conducted on this subject matter but we only get the information that would be more supplemental in our study. Moreover, since this chapter would serve as a supporting ground for our objectives, it would cater all the needed information for this study.

Chapter 3

CONCEPTUAL FRAMEWORK

3.1 Conceptualization

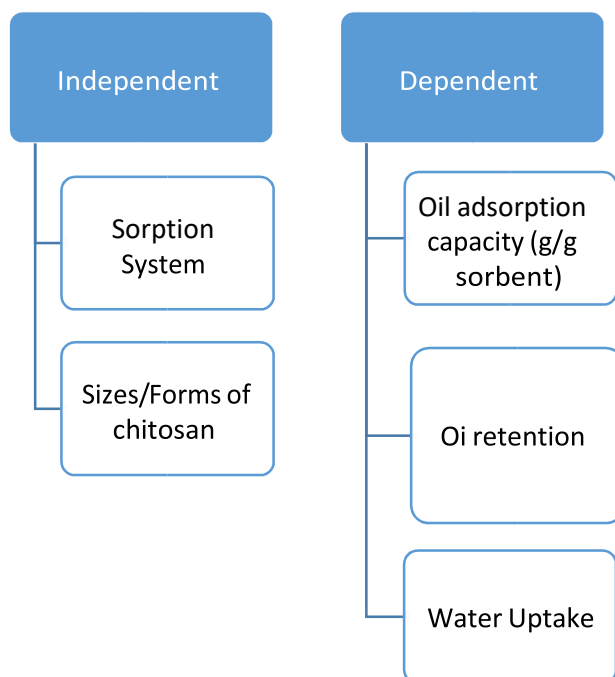
To ensure the availability and sufficiency of natural sorbents in case of an oil spill phenomenon, it is very important to discover new and abundant sources from waste materials that have good oil sorption capacity. The proponents perceived the potential of Asian green mussel shells as oil sorbent. Similar material, shrimp shell, had already been studied for its oil absorption capacity (Ong, 2016).

3.2 Research Function

Two different systems were used for the experiment: (i) dry-static system (pure oil, without agitation), and (ii) wet-static system (oil in water setup, without agitation). Dry-static system was used to determine oil adsorption capacity of sorbent, while wet-static system was used to determine water uptake and the ratio of oil to water adhered. Three trials were done in each experiment. Experiment 1 is chitosan in powdered form soaked in a solution containing bunker oil and distilled water. Experiment 2 is chitosan in flaked form soaked in a solution containing bunker and distilled water. Experiment 3 is chitosan in flaked form soaked in a solution containing pure bunker oil. Experiment 4 is chitosan in powdered form soaked in a solution containing pure bunker oil.

3.3 Conceptual Framework

Based on the paradigm below, chitosan content and the sorption systems influence the ability of the sorbent material to sorb and retain oil, as well as to take up water.



3.4 Adsorption



Figure 3.4 Process of adsorption

In Figure 3.4, the bunker oil with distilled water was sorbed by the chitosan and the separation was accomplished. In the upper product stream, the oil and distilled water adhered

to the chitosan and in the bottom stream were the remaining bunker oil and distilled water that did not adhere to chitosan.

3.5 Schematic Diagram

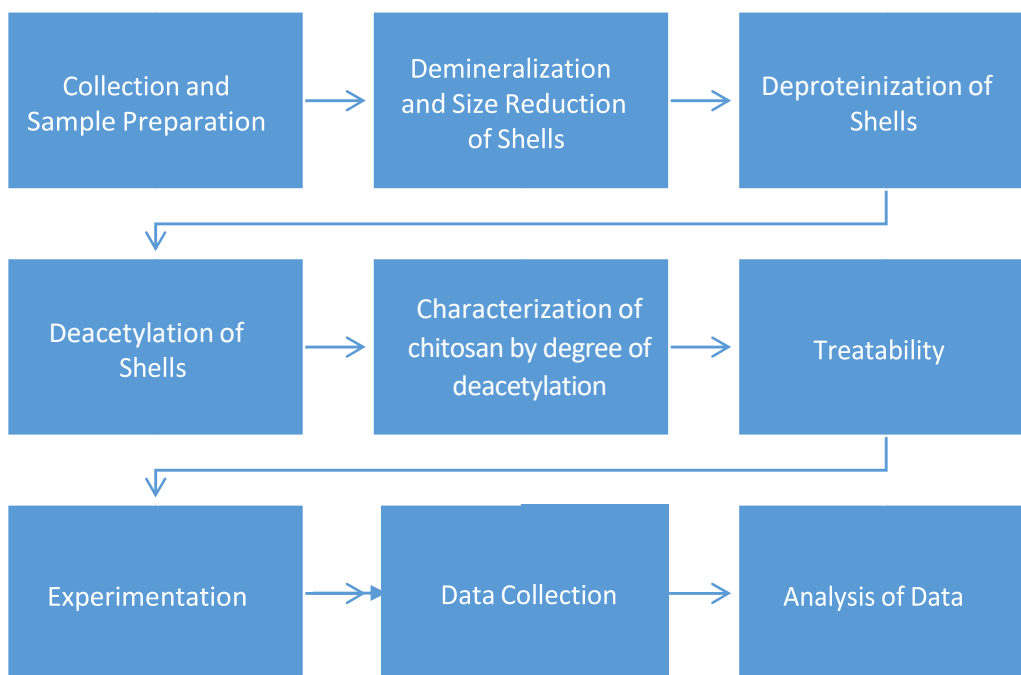


Figure 3.5b Experimentation process

Chapter 4

MATERIALS AND METHODS

4.1 Research Design

The research method that was applied is experimental. This method determined the following: oil sorption capacity (g oil/g sorbent) of Asian mussel shells, water uptake of the sorbent material, oil retention, sorption capacities of different forms of sorbents (flaked or powdered), and also the degree of deacetylation.

4.2 Materials and Instruments

Table 4.2 Materials & equipment

Consumable Materials	Laboratory Equipment
100g per form (flaked and powdered) of mussel shells	250mL and 500mL of beaker
2 setups of 57.8mL HCl/ L solution (demineralization) – 0.68 M	Glass rod
2 setups of 24.8g NaOH/ L solution (deproteinization) – 0.62 M	Funnel
2 setups of 500 g NaOH/ L solution (deacetylation) – 25 M	Safety goggles
500mL bunker fuel oil (type c) specifications in RRL	Safety gloves
Distilled water	Safety masks
6 g of extracted chitosan each for powdered and flakes	Lab gowns
Filter paper	100 μ m sieve and 350 μ m sieve
Indicator – Methyl red	1L Volumetric flask
	250mL Erlenmeyer flask
	Burette
	Burette clamp
	Induction Stove
	Cooking pots for heating
	Mesh baskets
	Pipette
	Washed bottle

4.3 Material Preparation and Set-up

4.3.1 Sample preparation:

1. Collection of 1 kg Asian mussel shells from San Jose New Market.
2. The shells were weighed to 200g then washed thoroughly with distilled water.
3. The shells were sundried to a constant weight at an ambient temperature until water was evaporated and attained its original weight.

4.3.2 Demineralization of shells and size reduction:

1. The dried shells underwent size-reduction.
2. The 200g shells were soaked in 2 setups of 0.68 M HCL (57.8 mL HCL/ L solution) to attain 1:10 w/v at ambient temperature (approximately 30° C for 6 hours after which, the setup was washed in the acid until no bubbles are seen and no color change is observed. This process caused demineralization of shells to yield chitin.

4.3.3 Deproteinization of shells:

1. The acid was drained from chitin and washed with distilled water until a relatively neutral is obtained.
2. Demineralized shells were dried to constant weight. The shells were dried weighed and soaked in 2 setups of 0.62 M NaOH solution (24.8g NaOH/ L solution) to attain 1:10 w/v at ambient temperature for 16 hours. After which the shells were washed thoroughly with

water followed with distilled water until a neutral pH is obtained. This process removed the protein content of the shell.

3. 100g of chitin were powderized with the use of mortar and pestle and screened through a 100 μ m sieve. Another 100g of chitin flakes were size-reduced and screen through 350 μ m sieve and presented with a size equal or higher than 0.354 mm while chitin powder had an average size between 0.104 mm and 0.075 mm.

4.3.4 Deacetylation of shells:

1. The 100g chitin powder and 100g chitin flakes were dried to constant weight.
2. The chitin powder obtained was deacetylated in 25 M NaOH (500g NaOH/ L solution) to attain 1:10 w/v at 75° C for 20 hours and another 25 M NaOH setup for flakes, simultaneously. After deacetylation, the chitosan is then washed thoroughly with water followed by distilled water.
3. The resulting chitosan because of this process was dried to constant weight and then prepared for characterization.

4.3.5 Characterization of chitosan:

The characterization was done using:

1. Degree of Deacetylation: Calculation of the percentage degree of deacetylation using Titration method (Biskup, 2012).

$$DA [\%] = 2.03 \times \frac{v_2 - v_1}{m + 0.0042(v_2 - v_1)} \quad (4.1)$$

Where:

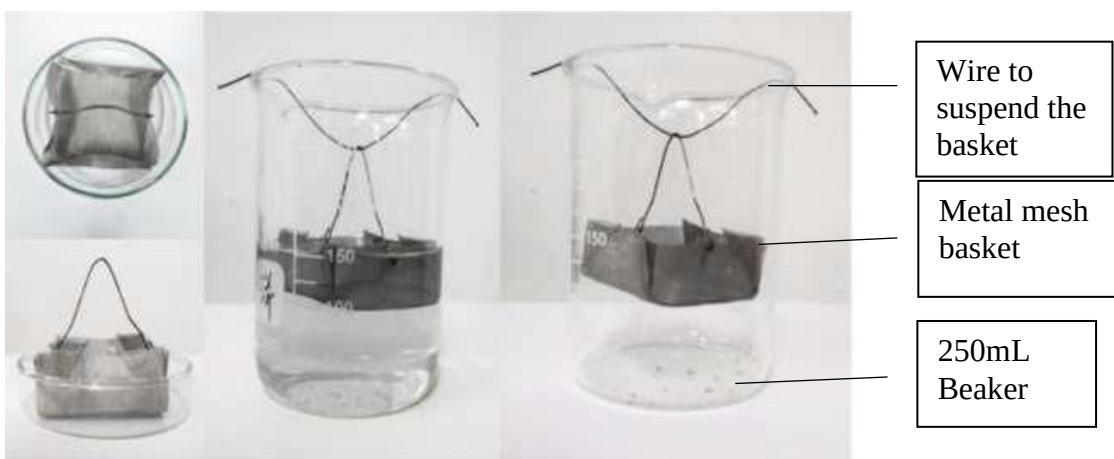
m = weight of sample

V_1, V_2 – volumes of 0.1 mol $\cdot$ mL NaOH solution corresponding to the deflection points

4.4 Experimental Procedure

The experimental procedure was based on the experimentation of a related study by Arevalo et.al where the researchers prepared 2 sorption systems, dry-static (pure oil) and wet-static (oil and water) to evaluate the oil and water uptake capacities. Water uptake was calculated by weight difference between wet-static and dry-static system. (Arevalo, 2015). The procedure was also based on the study of Cooper et.al that evaluated Testing Protocols and Certification Listing Programs for Oil Spill Sorbents.

4.4.1 Experimental Set up (Cooper, 1997)



Experiment 1: Flaked Chitosan in a Dry Static System

1. Placed the sorbent into the basket mesh
2. Soaked 6-gram sorbent on dry-static system containing 25ml bunker oil.
3. The sample was left for a period of at least 15 minutes.
4. The sorbent was then removed and hung from a balance using a metal mesh basket and let it suspend for 30 minutes to let the oil drip.
5. Weighed the sorbent after the 30-minute suspension.
6. Recorded the data.
7. Repeat steps 1 to 6 thrice to have three trials per form per setup.

Experiment 2: Powdered Chitosan in a Dry Static System

1. Placed the sorbent into the basket mesh
2. Soaked 6-gram sorbent on dry-static system containing 25ml bunker oil.
3. The sample was left for a period of at least 15 minutes.
4. The sorbent was then removed and hung from a balance using a metal mesh basket and let it suspend for 30 minutes to let the oil drip.
5. Weighed the sorbent after the 30-minute suspension Record the data.
6. Repeat steps 1 to 6 thrice to have three trials per form per setup.

Experiment 3: Flaked Chitosan in a Wet Static System

1. Placed the sorbent into the basket mesh
2. Soaked 6-gram sorbent on wet-static system containing 5mm (25ml) of bunker and 80mm (400ml) of water.
3. The sample was left for a period of at least 15 minutes.

4. The sorbent was then removed and hung from a balance using a metal mesh basket and let it suspend for 30 minutes to let the oil drip.
5. Weighed the sorbent after the 30-minute suspension.
6. Recorded the data.
7. Repeat steps 1 to 6 thrice to have three trials per form per setup.

Experiment 4: Powdered Chitosan in a Wet Static System

1. Placed the sorbent into the basket mesh
2. Soaked 6-gram sorbent on wet-static system containing 5mm (25ml) of bunker and 80mm (400ml) of water.
3. The sample was left for a period of at least 15 minutes.
4. The sorbent was then removed and hung from a balance using a metal mesh basket and let it suspend for 30 minutes to let the oil drip.
5. Weighed the sorbent after the 30-minute suspension.
6. Recorded the data.
7. Repeat steps 1 to 6 thrice to have three trials per form per setup.

4.5 Methodological Calculations

The sorption was calculated as the ratio of sorbed material to dry sorbent mass (S_o). Thus, sorption = $(S_t - S_o)/S_o$, where S_t is the total mass of sorbed samples (Annunciado et.al, 2005). The units are g oil/g sorbent for dry-static system, and g oil/g sorbent for wet-static system. The water uptake was calculated by getting the weight difference between the sorbed material from static system and the sorbed material from dry system (Annunciado et.al, 2005) at similar time, that is, the weight gain when the sorbent is soaked in a wet-static system. Since

three trials per form per system were made, sorption was calculated by getting the average of the recorded weights per form per system.

Chapter 5

RESULT AND DISCUSSION

This chapter presents the results, discussion and interpretation of data. The presentation was arranged according to the order of the research procedure and methodological calculations.

5.1 Results

These are the results gained from the research procedure of the experiment performed at Libis Road, Bgy.San Pedro, Puerto Princesa City and in the laboratories of College of Sciences and Engineering.

5.1.1 Degree of deacetylation

0.2g of dried chitosan flakes were dissolved in 20mL HCl/ L solution and 25mL of distilled water. After 30 minutes of continuous stirring, another 25mL of distilled water was added. When chitosan was completely dissolved, solution was titrated with 0.1 mol/ L solution. Degree of deacetylation of chitosan was calculated using:

$$DA [\%] = 2.03 \times \frac{v_2 - v_1}{m + 0.0042(v_2 - v_1)} \quad (5.1)$$

$$DA [\%] = 2.03 \times \frac{9-0}{0.2+0.0042(9-0)} = 76.83 \% \text{ DDA}$$

Based from the computation above, the computed % DDA has reached the range of 30% to 95% which is the basis in determination and extraction of chitosan.

5.1.2 Adsorption capacities

Table 5.1.2a Average weight and average adsorption capacity		
	Average Weight	Average Sorption Capacity (g adsorbed/g dry sorbent)
6g of flakes (Dry Static)	7.67g	0.28
6g of powder (Dry Static)	7.33g	0.22
6g of flakes (Wet Static)	8.67g	0.45
6g of powder (Wet Static)	8.33g	0.39

Table 5.1.2b Water uptake and oil retention		
	Water Uptake - g	Oil Retention - g
Flakes	1	1.67
Powder	1	1.33

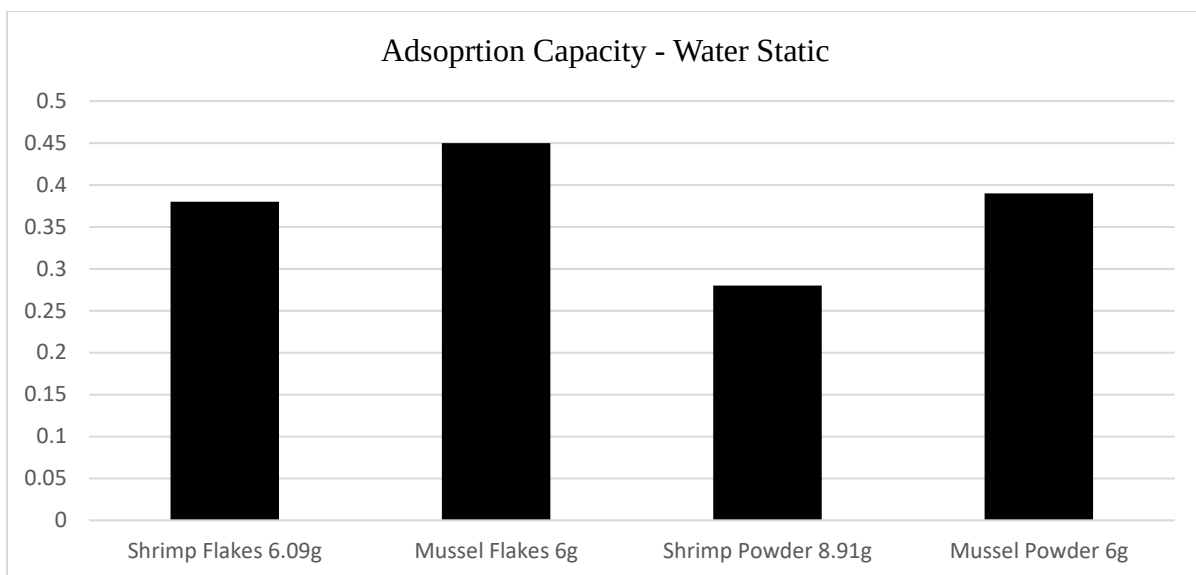


Figure 5.1 Adsorption Capacity

5.1.3 Calculations

For **Table 5.1.2a** – Average weights for flaked and powdered chitosan

Dry Static – Powdered Chitosan

$$\bar{x} = \Sigma x/n = 7g + 8g + 7g = 7.33g$$

Wet Static – Powdered Chitosan

$$\bar{x} = \Sigma x/n = 8g + 9g + 8g = 8.33g$$

Dry Static – Chitosan Flakes

$$\bar{x} = \Sigma x/n = 8g + 8g + 7g = 7.67g$$

Wet Static – Chitosan Flakes

$$\bar{x} = \Sigma x/n = 9g + 9g + 8g = 8.67g$$

For **table 5.1.2a** – Dry static (pure oil) g oil sorbed/g dry sorbent

$$SP = ST - SO$$

$$CP = SP / SO$$

WU = average weight in wet static - average weight in dry static

OR = average weight in dry static - mass of dry sorbent

Where:

SP = mass of oil sorbed

SO = mass of dry sorbent

ST = mass of sorbent after

CP = sorption capacity

WU = water uptake

OR = oil retention

Chitosan Flakes:

$$SP = 7.67\text{g} - 6\text{g} = 1.67\text{g}$$

$$CP = 1.67\text{g}/6\text{g} = 0.28\text{g/g}$$

Chitosan Powder:

$$SP = 7.33\text{g} - 6\text{g} = 1.33\text{g}$$

$$CP = 1.33\text{g}/6\text{g} = 0.22\text{g/g}$$

For **table 5.1.2a** – Wet static g sorbed/g dry sorbent

Chitosan Flakes:

$$SP = 8.67\text{g} - 6\text{g} = 2.67\text{g}$$

$$CP = 2.67\text{g}/6\text{g} = 0.45\text{g/g}$$

Chitosan Powder:

$$SP = 8.33g - 6g = 2.33g$$

$$CP = 2.33g/6g = 0.39g/g$$

Based from the computations above, the sorption capacities of flaked and powdered chitosan which resulted to 0.45g/g and 0.39g/g, respectively, yield a better sorption capacity against chitosan from shrimp shell. The water uptake of both forms have an average of 1g of sorbed water per 6g of chitosan sample. The oil retention of chitosan flakes has a value of 1.67g while the powdered form amounts to 1.33g.

Chapter 6

CONCLUSION AND RECOMMENDATION

This chapter presents the conclusions drawn from the results of the study, and researchers' recommendations for further studies in relation to their chosen research topic.

6.1 Conclusion

The following conclusions were drawn based on the results obtained from experimentation:

1. Based from the computed sorption capacities, chitosan powder and chitosan flakes extracted from *Perna Veridis*, bettered the adsorption capacities of the chitosan extracted from shrimp.
2. Sorption capacity of chitosan flakes extracted from *Perna Veridis*, sorbed 15.38% more oil than its powdered form, hence chitosan flakes is more effective than the chitosan powder.
3. The degree of deacetylation of chitosan obtained from *Perna Veridis*, has reached an accepted and standardized value for the characterization of chitosan, hence chitosan is proven present.

6.2 Recommendations

Based on the results and conclusions of this study's experiment, the following recommendations and changes are suggested for further studies:

1. A follow-up study should be done using fresh water and salt water as medium for the extraction of chitosan and how it affects the quality of chitosan extracted from *Perna Veridis*.
2. Employment of other methods for determining degree of deacetylation should be utilized, from simple, such as Titration I method, to complex ones and most precise equipment, such as ¹H NMR spectroscopy and infrared spectroscopy, to further assess the quality of the chitosan extracted from *Perna Veridis*.
3. Prolonging the soaking period of the shells for up to 3 days during the deacetylation process to yield higher %DDA and how it compares with %DDA obtained from shorter soaking time.
4. Determine the effectiveness of the chitosan extracted from *Perna Veridis* as an oil spill sorber using the criteria in selecting good sorbent materials such as the effect of buoyancy.
5. Experimentation in a more real-environment simulation wherein the presence of waves and winds are considered as extraneous variables.
6. Omit the process of demineralization to retain the Calcium carbonate inherently present in the mussel shells as it is proven in one of our related studies that it contributes in adsorbing oil spill and see how it compares with the results of this study.
7. Test out the chitosan's capability as a sorber of oil by utilizing different kinds of oil with varying densities and boiling points, from lowest to highest and how it correlates when subjected with these variables.

8. Develop a method of collecting the soaked chitosan in oil and water to yield better experimental results and lessen the possibility of human error.
9. Use a more accurate and advance heating method for better efficiency.

APPENDICES

Appendix A

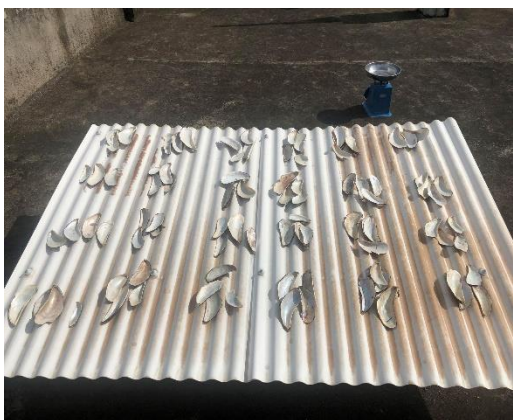
Extraction of Chitosan



Gathered shells



Washed shells using distilled water



Sun drying of shells

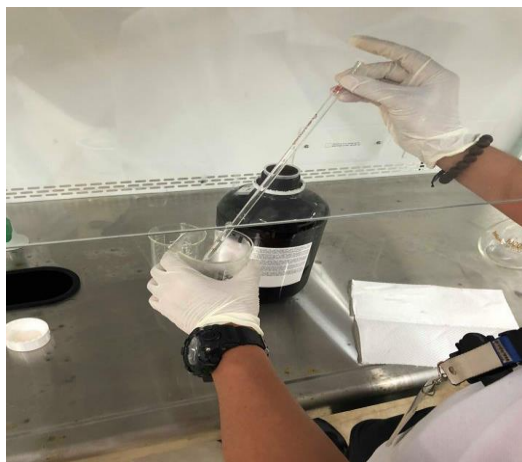


Size-reduced shells



Weighing of shell

Demineralization



Preparation of HCl solution



Soaking of shells in HCl solution

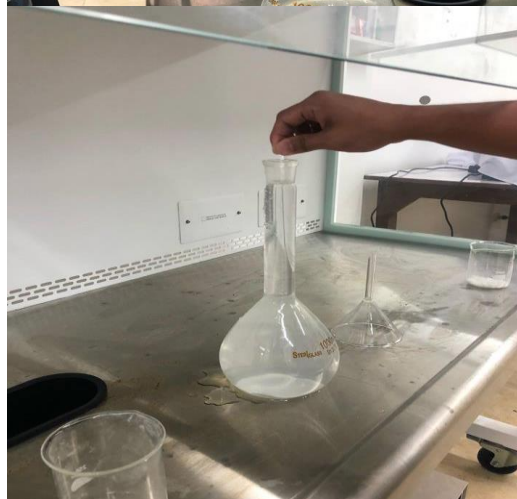
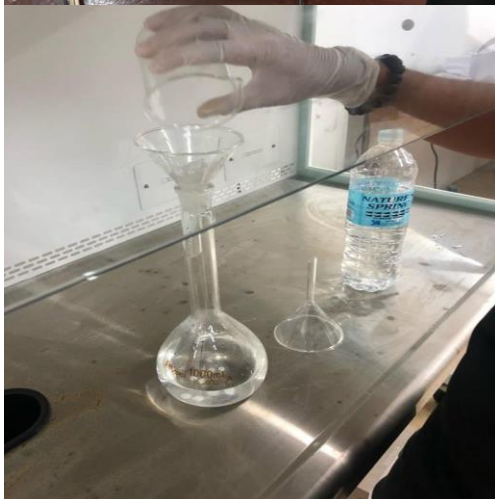


Washing-off the HCl from the shells



Drying of shell

Deproteinization



Preparation of NaOH solution



Soaking of shells in NaOH solution



Washed thoroughly and dried

Deacetylation



Size-reduced into flakes and powder



Preparation of NaOH solution



Heating of powdered shells in NaOH solution



Heating of shell flakes in NaOH solution



Draining-off the NaOH solution



Dripping-off excess NaOH solution



Rinsing of powdered shells



Rinsing of flakes



Drying of flakes and powder

Titration



Experimental Method

Experiment 1 set up for three trials: Flaked Chitosan in a Dry Static System



Weighing of sorbent



Soaking of sorbent with bunker oil for 15 minutes



Suspension of Sorbent for 30 minutes



Weighing of sorbent after suspension

Experiment 2 set up for three trials: Powdered Chitosan in a Dry Static System



Weighing of sorbent



Soaking of sorbent with bunker oil for 15 minutes

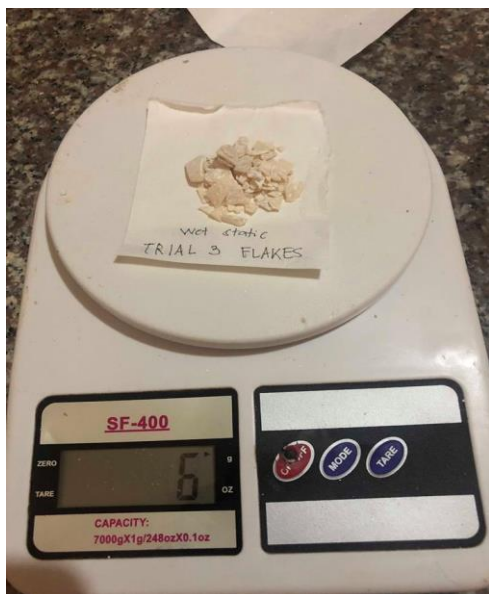


Suspension of Sorbent for 30 minutes



Weighing of sorbent after suspension

Experiment 3 set up for three trials: Flaked Chitosan in a Wet Static System



Weighing of Sorbent



Soaking of sorbent with bunker oil and water for 15 minutes



Suspension of Sorbent for 30 minutes



Weighing of sorbent after suspension

Experiment 4 set up for three trials: Powdered Chitosan in a Wet Static system



Weighing of sorbent



Soaking of sorbent with bunker oil and water for 15 minutes



Suspension of Sorbent for 30 minutes



Weighing of sorbent after suspension

Appendix B

Republic of the Philippines
PALAWAN STATE UNIVERSITY
College of Engineering, Architecture & Technology

Laboratory Apparatus Request Letter

Date: September 21, 2018

Dr. Elucila M. Sespeñe
Dean
CEAT

Dear Mam:

We humbly request to borrow equipment for titration from the College of Sciences Laboratory, for data collection of our thesis titled:

“A COMPARATIVE STUDY ON THE OIL SORPTION CAPACITY OF CHITOSAN EXTRACTED FROM ASIAN GREEN MUSSEL SHELLS TO SHRIMP SHELLS AS AN OIL SPILL ADSORBENT”

We would only use it for a day and would return it in good condition

Respectfully yours,

Arreza, Alexander Jules O.
Borgueta, Reigh Alphonse C.
Melchor, Jan Daniel G.
Quillo, John Raenel G.

Engr. Ryan Kristoffer C. Aguirre
Research Adviser

Dr. Elucila M. Sespeñe
College Dean

Appendix C

Republic of the Philippines
PALAWAN STATE UNIVERSITY
College of Engineering, Architecture & Technology

Research Defense Request Form

Date: September 25, 2018

Dr. Elucila M. Sespeñe
Dean
CEAT

Dear Mam:

We would like to request your approval on the following schedule, venue, and panel of examiners for the defense of our Research Proposal/Final Manuscript entitled:

“A COMPARATIVE STUDY ON THE OIL SORPTION CAPACITY OF CHITOSAN EXTRACTED FROM ASIAN GREEN MUSSEL SHELLS TO SHRIMP SHELLS AS AN OIL SPILL SORBENT”

Schedule (Date & Time): September 25, 2018; 12:30 PM – 2:00 PM

Venue _____

Research Committee:

1. Engr. Ryan Kristoffer C. Aguirre, Research Adviser
2. Engr. Allan Jay L. Javier, Research Reader
3. Engr. Manuel L. Cabiguen Jr., Research Reader
4. Engr. Philip Ryan B. Maigne, Research Reader

Thank you very much.

Respectfully yours,
Arreza, Alexander Jules O.
Borgueta, Reigh Alphonse C.
Melchor, Jan Daniel G.
Quillo, John Raenel G.
5th Years BSPE

Recommended by: Engr. Ryan Kristoffer C. Aguirre
Research Adviser

Noted by: Engr. Chinny July S. Baldonado
CEAT Research Coordinator

Approved: Dr. Elucila M. Sespeñe
College Dean

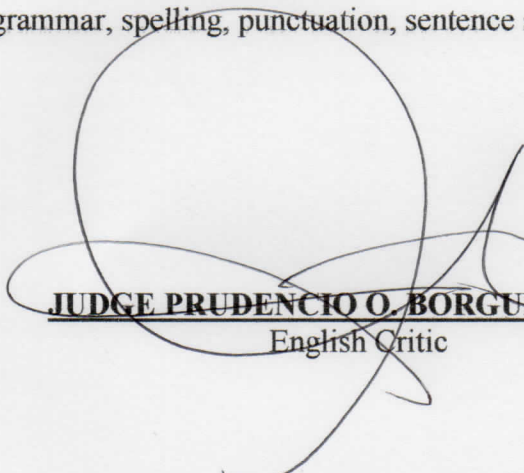
October 13, 2018

CERTIFICATION

TO WHOM IT MAY CONCERN:

This is to certify that the thesis project prepared by **JULES ALEXANDER O. ARREZA, ALPHONSE REIGH C. BORGUETA, JAN DANIEL G. MELCHOR, and JOHN RAENEL G. QUILLO** entitled, "A Comparative Study on the Oil Sorption Capacity of Chitosan Extracted from Asian Green Mussel Shells to Shrimp Shells as an Oil Spill Adsorbent" was checked and edited by the undersigned as an English Critic.

Some minor corrections were made in grammar, spelling, punctuation, sentence structure, terminology and phraseology.



JUDGE PRUDENCIO O. BORGUETA JR.
English Critic

GLOSSARY OF TECHNICAL TERMS

- Adhesion- is the tendency of dissimilar particles or surfaces to cling to one another
- Adsorb- To gather a gas, liquid or dissolved substance as a thin film on the outside surface or on internal surface within the material.
- Adsorbent- is a solid substance used to collect solute molecules from a liquid or gas.
- Adsorption capacity- the value of the amount of adsorbed substance reached in a saturated solution.
- Biodegradation- a process by which microorganisms such as bacteria, fungi, and yeast break down complex compounds into simpler products.
- Chitin- a natural carbohydrate polymer found in the skeleton of crustaceans such as crab, shrimp and lobster.
- Chitosan- is a natural biodegradable and biocompatible polysaccharide derived by deacetylation of chitin
- Deacetylation- it is simply the reverse reaction where an acetyl group is removed from a molecule.
- Demineralization- a process to reduce the content of mineral substances in tissue or organism
- Deproteinization- a process of removing protein
- Dispersant- are chemical agents (similar to soaps and detergents) that help break up an oil slick into very small droplets, which dilute throughout the water.
- Dry Static System- a system where external forces are neglected and only a pure substance is used in a solution

- Hydrophobic- are molecules that do not have a charge, meaning they are non-polar. By lacking a charge, these molecules do not have any charge-to-charge interactions that will allow them to interact with water.
- Oleophilic- a substance that has an affinity for oils and not for water
- Polypropylene- is one of those rather versatile polymers out there. It serves double duty, both as a plastic and as a fiber.
- Sorbent- a material used to absorb or adsorb liquids or gases.
- Wet Static System – a system where external forces are neglected and water is present alongside another substance