NEWFOUNDLAND SHRIMP WASTE UTILIZATION AND DISPERSANT GENERATION

by

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ABSTRACT

This research focused on the utilization of Newfoundland shrimp waste as a premium and low-cost nitrogen source for microbial growth through an enzymatic hydrolysis process. In addition, the enzymatically hydrolyzed shrimp waste was used to generate a green dispersant for crude oil dispersion in seawater. During the enzymatic hydrolysis process, an integration of response surface methodology and artificial neural network was proposed for the first time for modeling and optimization of shrimp waste hydrolysis. The utilization of shrimp waste for microbial growth was also achieved. The hydrolysis process was further optimized using the dispersant effectiveness (DE) as the response to generate a green shrimp waste based dispersant. The functional properties of the hydrolyzed product were examined. The DE and acute toxicity of the generated dispersant were evaluated. A comparison of the generated dispersant with Corexit 9500 on dispersing three types of crude oil was conducted. This research provided a promising methodology of shrimp waste management and a potential option for oil spill response.

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LIST OF SYMBOLS AND ABBREVIATIONS

ABBREVIATIONS

2FO	number 2 fuel oil
ANN	artificial neural network
ANOVA	analysis of variance
ANS	Alaska North Slope crude oil
BFT	baffled flask test
BOD	biological oxygen demand
CCD	central composite design
CCRD	central composite rotatable design
CMC	critical micelle concentration
COD	chemical oxygen demand
DCM	dichloromethane
DE	dispersant effectiveness
DFBETAS	difference in beta coefficients
DH	degree of hydrolysis
DMSO	dimethyl sulfoxide
DTAB	dodecyl trimethyl ammonium bromide
E/S	enzyme/substrate
EDTA	ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization

FOG	fat-oil-grease
Hyp	hydroxyproline
Hyp	hydroxyproline
Lys	lysine
MSE	mean squared error
OAS	osmotic adjusting solution
OD_{600}	optical density
OFAT	one-factor-at-a-time
PBC	Prudhoe Bay crude oil
PCBs	polychlorinated biphenyls
PEG 400	polyethylene glycol 400
PM	production medium
PPG	propylene glycol
RSD	relative standard deviation
RSM	response surface methodology
SDS	sodium dodecyl sulfate
Ser	serine
SLC	South Louisiana crude oil
TCA	trichloroacetic acid
TSS	total suspended solids
Tyr	tyrosine
UVS	ultraviolet spectrophotometer

SYMBOLS

Abs_{370}	absorbance at 370 nm
Abs_{400}	absorbance at 400 nm
a_i	normalized data
A_i	experimental data
A_{max}	maximum values of the datasets
A_{min}	minimum values of the datasets
b_0	constant
b_i	regression coefficient for linear effect
b_{ii}	quadratic coefficient
b_{ij}	interaction coefficient
EC_{50}	half maximal effective concentration
EI_{24}	emulsification index
H_{EL}	the height of the emulsion layer
H_S	the height of the total solution
I_j	relative importance of the *j*th input factor on the output response
N	number of data
N_h	the numbers of hidden neurons
N_i	the numbers of input neurons
W_s	connection weights between layers
X_0	value of X_i at the center point

X_i	independent variable
Y	response variable or the degree of hydrolysis
$y_{i,exp}$	experimental response
$y_{i,pred}$	network predicted results
δX	step change

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CHAPTER 1

INTRODUCTION

1.1 Background

Marine fishery has played a key role in human being's lives. The status of capture fisheries has been recorded by Food and Agriculture Organization (FAO) since 1960. In 2009, the fish production all over the world is about 80 million tonnes (FAO, 2010). Nevertheless, not all the fish production obtained is efficiently used and a portion of them is discarded as seafood waste. Large bulk of seafood processing consequently generates massive by-products and wastes. In Newfoundland, the waste generated from seafood processing plants has become a major concern (Cull, 2000). There are about 240 thousand tons of seafood landed in 2014, among which shrimp accounts for the biggest part (30%). Over 40% (w/w) of the shrimp is discarded as solid shrimp waste (Gildberg & Stenberg, 2001). Although some of such waste can be transformed into value added products, there is still a large amount being discarded as processing effluents (Jamieson et al., 2013).

The accumulated large proportion of seafood waste is usually dumped into the ocean leading to contamination in coastal areas (Adams et al., 2005; Gimeno et al., 2007; Xuemei & Hawkins, 2002). There have been a lot of studies showing that seafood processing effluents have high levels of chemical oxygen demand (COD), biological oxygen demand (BOD), fat-oil-grease (FOG), total suspended solids (TSS), pathogenic and other microflora, as well as organic matters (Park et al., 2001; Sohsalam et al., 2008). The waste may also lead to pollution of polychlorinated biphenyls (PCBs), dioxines, heavy metals and parasites if the waste dumping is uncontrolled (Blanco et al., 2007). Erondu and Anyanwu (2005) reviewed the potential

hazards and risks associated with the aquaculture industry and mentioned that the seafood waste could include environmental hazards, leading to water contamination. Islam et al. (2004) analyzed the physico-chemical and microbiological characteristics of fish and shrimp processing effluents and found that fish and shrimp processing effluents probably had adverse effects on coastal and near-shore environments. Thus, for the sake of marine environment and sustainability, resourceful and appropriate utilization methods of the seafood waste should be developed.

Offshore oil and gas development in Newfoundland and beyond has been expanding. Accordingly, it leads to the high potential of spilling oil into the sea. Spilled oil at ocean forms oil slick on the sea surface and can seriously threaten the marine environment. Oil spills have direct impact on wildlife and their habitats and long-term impact on seabirds and subsurface organisms with oil's toxicity (Nomack, 2010). Therefore, effective approaches for offshore oil spill response are highly desired. Dispersion would aid in naturally removing most of the oil from the water surface (Kingston, 2002). Consequently, dispersants have been widely used as an effective option for oil spill response (Chapman et al., 2007; Judson et al., 2010; Kujawinski et al., 2011; Lessard & DeMarco, 2000). However, there have been concerns about the toxicity and metabolism of existing chemical dispersants to the environment (Goodbody-Gringley et al., 2013; Pietroski et al., 2015). Till now, there is still a lack of sufficient environmentally friendly dispersants with low toxicity, high biodegradability and biocompatibility (Infante et al., 2004).

To fill the technical and knowledge gap of previous studies, this research explored the possibility of using shrimp waste for generating a green oil spill dispersant, which would not only enhance the utilization of shrimp waste but also provide an environmentally friendly dispersant for oil spill response.

1.2 Objectives

This research has following objectives:

(1) Integration of response surface methodology (RSM) and artificial neural network (ANN) to model and optimize Newfoundland shrimp waste hydrolysis process for microbial growth;

(2) Examination of the properties of shrimp waste hydrolysis products;

(3) Optimization of shrimp waste hydrolysis for dispersant generation;

(4) Selection of solvents for dispersant formation and determination of its acute toxicity;

(5) Evaluation of the generated shrimp waste based dispersant under multiple environment conditions; and

(6) Comparison of the shrimp waste based dispersant with synthetic dispersants (Corexit 9500).

1.3 Thesis Structure

This thesis consists of five chapters. Chapter 1 outlines the research scope, research objectives and thesis structure. Chapter 2 is the literature review of the thesis relevant topics including (1) existing shrimp waste utilization and management, (2) dispersants for oil spill response, and (3) the optimization and modeling methodology: RSM and ANN. Chapter 3 presents the studies on modeling and optimization of Newfoundland shrimp waste hydrolysis for microbial growth using RSM and ANN. Chapter 4 investigates the generation of shrimp waste based dispersant for oil spill response. Chapter 5 provides conclusions of this research and some recommendations for future studies.

This thesis contains two individual research tasks. One is to study shrimp waste hydrolysis and to apply the hydrolysates as nutrient for bacterial growth. Another one is to generate the shrimp waste based dispersant for oil spill response. The research tasks and objectives are of great significance to the fishery industry and marine environment preservation in Newfoundland, since the large amount of shrimp waste generated every year presents a big problem to deal with. Reuse of the shrimp waste into value-added products provides the potential in technological development for future industry application and environmental control.

CHAPTER 2

LITERATURE REVIEW

2.1 Shrimp Waste Utilization

2.1.1 Shrimp Waste Components

Shrimp waste generally contains 8–10% chitin, 30–40% protein, 0-14% lipids and 10–20% calcium in dry weights (Gallert & Winter, 2002; Kurita, 2006). It also consists of pigments, fatty acids, etc. These components have wide-ranging applications in medical, therapies, cosmetics, paper, pulp and textile, biotechnology and food industries. In this thesis, existing status of shrimp waste utilization in terms of chitin, protein and other components such as carotenoids and phospholipids have been reviewed.

2.1.1.1 Chitin

Chitin is a typical marine polysaccharide abundantly found in the shell of crustaceans (Kurita, 2006). It and its deacetylated derivative chitosan have been widely applied in different areas such as medical uses, food technology, photography, cosmetics, paper industry, water treatment, drug delivery, and biotechnologies (Kumar, 2000). Figure 1 gives the molecular structure of chitin and its deacetylated derivative chitosan. It can be seen that the acetyl group of each monomer is removed through a deacetylation process.

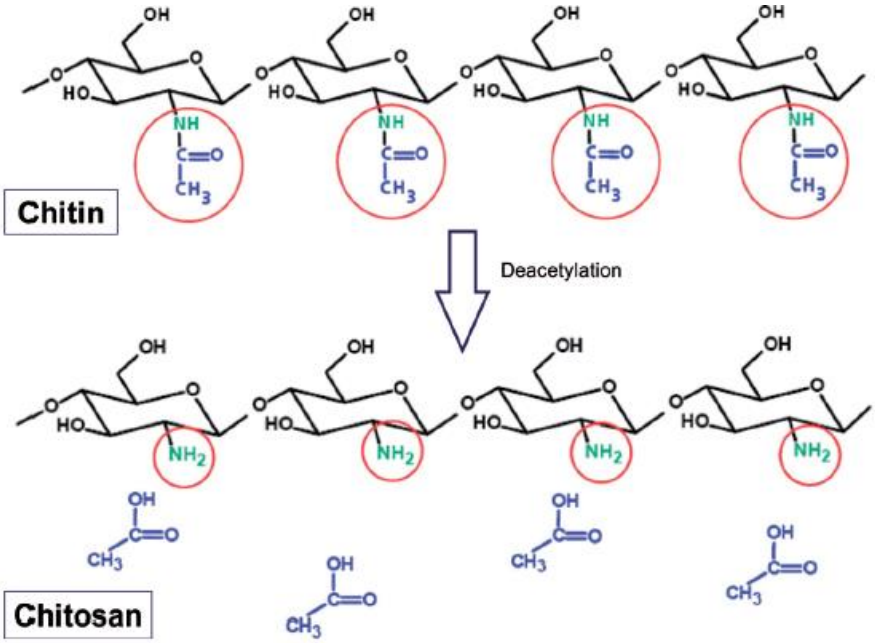


Figure 2.1 The molecular structure of chitin and chitosan (Kaur & Dhillon, 2013).

(1) Traditional chemical process of chitin extraction

The traditional procedures to obtain chitin from shrimp waste were deproteinization, demineralization, bleaching and then deacetylation (Percot et al., 2003a). Hydrochloride acid was commonly used in acid treatment to remove metal salts, particularly calcium carbonate (Kurita, 2006). NaOH was generally used in the deproteinization process to remove protein (Percot et al., 2003b). Pigments, such as carotenoid and astaxanthin were removed by strong oxidizing agents such as potassium permanganate, hydrogen peroxide and other organic solvent mixtures (Rohyami et al., 2015). The deacetylation of chitin usually took place in an alkali environment, while thermal treatment was preferred for industrial purposes (Benhabiles et al., 2012). Figure 2.2 illustrates the traditional chemical process of chitin and chitosan production.

However, usage of the above stated harsh chemicals could have negative impact on molecular weight and purified chitin's properties (Healy et al., 2003; Prameela et al., 2010). In addition, the traditional chemical process could lead to harmful effluents containing chemicals, which are non-environmental friendly (Arbia et al., 2013).

(2) Biological chitin extraction process

With the development of the green extraction methods, the biological extraction processes employing microorganisms have gained increasing attention. Moreover, fermentation has been regarded as a simpler, more productive and more environmentally friendly technique when

compared to traditional chemical techniques (Kaur & Dhillon, 2013). Healy et al. (2003) adopted anaerobic fermentation of shrimp waste to produce a purified chitin with a high calcium removal rate. The fermentation was conducted for 7 days in the presence of a mixture of selected bacteria. Khorrami et al. (2011) studied the effect of various carbon sources on lactic acid bacteria fermentation of shrimp shell and found media contained date syrup could facilitate demineralization. Similar methodologies can also be found in a couple of studies (Prameela et al., 2010； Rao & Stevens, 2005, 2006). There were also comparisons between the biological extraction method and chemical extraction method. Khanafari et al. (2008) conducted a comparative study on chitin extraction from shrimp waste using both the chemical and microbial method. The authors found that the biological extraction method was highly reproducible and required in shorter time. It was easy to manipulate, and had muchless solvent and energy demands. The outcome suggests that the microbial extraction method has the advantage over the chemical extraction process. As a result, the biological extraction method has been regarded as a promising alternative to the traditional chemical process in the chitin production (Kaur & Dhillon, 2013). Figure 2.3 shows the general biological method for extraction of chitin. This process uses organic acids and protease produced by bacteria to accomplish demineralization and deproteinization instead of the traditional chemical process, which reduces the use of harsh chemicals to a large extent (Ghorbel-Bellaaj et al., 2011).

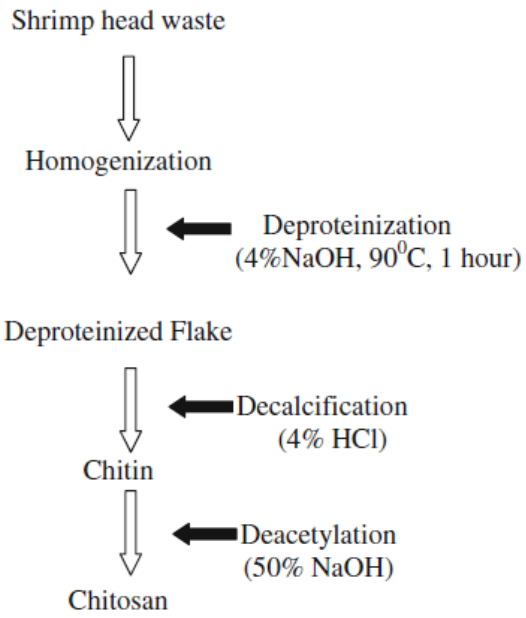


Figure 2.2 Traditional chemical process of chitin and chitosan production (Kandra et al., 2012).

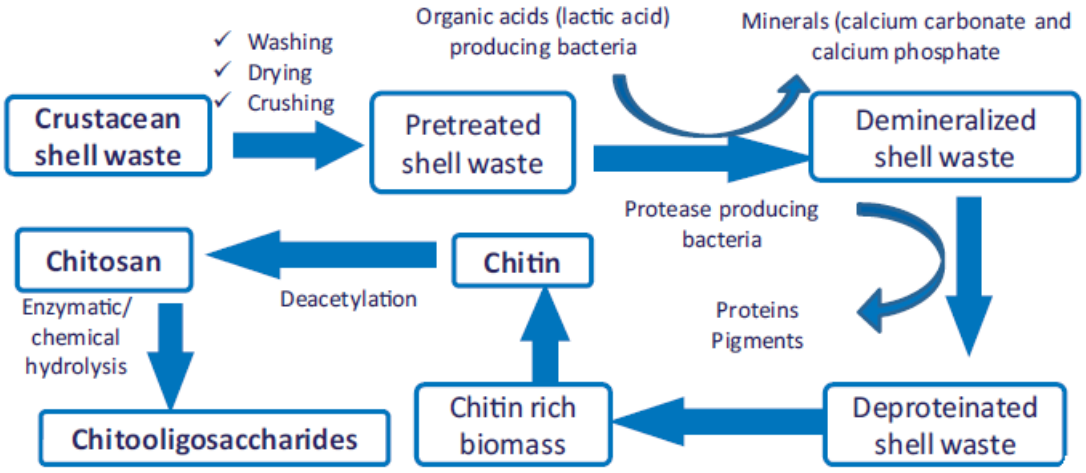


Figure 2.3 General biological method for extraction of chitin (Kaur & Dhillon, 2013).

2.1.1.2 Protein and Amino Acids

Besides chitin, the high protein content in shrimp waste has gained increasing attention to be further utilized to produce value-added products (Bueno-Solano et al., 2009; Cao et al., 2014; Fanimo et al., 2000; Okoye et al., 2005). The simplest way of utilizing shrimp waste protein is sun drying for animal feed. Okoye et al. (2005) used sundried shrimp waste meal as an animal protein source for broiler chickens in both starter and finisher diets. However, regular sun drying on the beaches may lead to environmental problems and low hygienic control during this process makes the products only useful for animal consumption (Mathew & KG, 2006). Efficient and environmentally friendly techniques have been explored with the regard of shrimp waste utilization (Kandra et al., 2012).

Hydrolysis has been widely applied to recover the protein fraction of shrimp waste (Dey & Dora, 2014; Ferrer et al., 1996; Gildberg & Stenberg, 2001; Guerard et al., 2007; Manni et al., 2010). It involves a process in which the protein is broken down into peptides and amino acids (Vidotti et al., 2003). Researchers have developed different hydrolysis processes such as autolytic hydrolysis, acidic hydrolysis, alkaline hydrolysis and enzymatic hydrolysis (Kristinsson & Rasco, 2000).

(1) Autolytic hydrolysis

Autolytic hydrolysis is promoted by the endogenous digestive enzymes. It has been confirmed to be a complex and inefficient process since different endogenous enzymes have different

demand on reaction conditions to be effective, and many endogenous enzymes were found to remain inactive when reactions began (Samaranayaka & Li-Chan, 2008). Nevertheless, the autolysis could be a cost-effective method for shrimp protein recovery as it did not need expensive exogenous enzymes. Several previous studies reported the application of autolysis in shrimp waste treatment. Eakpetch et al. (2008) reported autolytic activity of Pacific white shrimp mince at different salinity and different pH values. There was another study confirming the viscera in shrimp head could help degrade tissue proteins by endogenous enzymes (Cao et al., 2008). Cao et al. (2009) presented an effective method to recover shrimp head waste protein by means of gradual temperature increase. The autolysis hydrolysate could also be employed as food supplements in human diet. Sowmya et al. (2011) optimized the autolysis of shrimp heads for recovery of carotenoprotein using buffer solution with pH value of 8. Senphan and Benjakul (2012) found autolysis could increase the lipids extraction from hepatopancreas of Pacific whit shrimp. Cao et al. (2014) indicated that UV irradiation in combination with gradient temperatures could improve recovery of shrimp head waste protein.

(2) Acid hydrolysis

Acid hydrolysis is a process promoted by hydrochloric acid or sulfuric acid, usually at a relative high temperature (Kristinsson & Rasco, 2000). A couple of findings have documented the acid hydrolysis in dealing with protein content of shrimp waste. Jaswal (1989) investigated the acid hydrolysis of shrimp waste for producing amino acid hydrolysate. Hydrochloric acid was a confirmed to be a suitable agent for production of ninhydrin positive substances. Ferrer

et al. (1996) used hydrochloric acid to hydrolyze shrimp shell waste for obtaining glucosamine and the hydrolysate was utilized for production of single cell protein. In the study of Coward-Kelly et al. (2006), acid hydrolysis was employed to convert shrimp head waste polypeptides into amino acids, thereby enhancing the digestibility of products. In addition, acetic acid was reported to aid hydrolysis of shrimp head waste in the formulation of tilapia (*Oreochromis niloticus* Linnaeus) feed (Cavalheiro et al., 2007). Acid hydrolysis was applied to treat fish waste as well (Gao et al., 2006).

(3) Enzymatic hydrolysis

Enzymatic hydrolysis has been widely applied in recovery of the protein fraction from shrimp waste (Armenta & Guerrero-Legarreta, 2009; Babu et al., 2008; De Holanda & Netto, 2006; Gildberg & Stenberg, 2001; Manni et al., 2010; Mizani et al., 2005; Synowiecki & Al-Khateeb, 2000). An efficiency-improved enzymatic hydrolysis of shrimp head waste was conducted (Mizani et al., 2005). Triton x-100 was used along with enzyme to produce protein powder containing sufficient amounts of essential amino acids. Babu et al. (2008) conducted a comparative study of three kinds of enzymes in enzymatic isolation of protein from shrimp waste, in which trypsin reached a higher recovery rate than pepsin and papain. Klomklao et al. (2009) extracted carotenoprotein from black tiger shrimp shells using bluefish trypsin. Armenta and Guerrero-Legarreta (2009) utilized Savinase protease to enhance enzymatic hydrolysis of fermented shrimp carotenoproteins.

Alcalase, a highly efficient commercial bacterial protease, has been widely employed in the

enzymolysis of shrimp waste (Dey & Dora, 2014; Gildberg & Stenberg, 2001; De Holanda & Netto, 2006; Synowiecki & Al-Khateeb, 2000). Synowiecki and Al-Khateeb (2000) used alcalase to digest shrimp shell waste, achieving the recovery of both protein hydrolysate and chitin. De Holanda and Netto (2006) compared Alcalase and pancreatin in enzymatic hydrolysis of shrimp waste and found that alcalase was more efficient than pancratin. Guerard et al. (2007) optimized the free radical scavenging activity in alcalase-aided enzymatic hydrolysis of shrimp waste. Arancibia et al. (2015) utilized alcalase-hydrolyzed protein concentrate from shrimp waste to enhance a chitosan-based film.

As the hydrolysis process cleaved the protein into smaller peptides or free amino acids, the produced essential amino acids increases the nutritive values of the products. Table 2.1 showed the essential amino acid composition of shrimp waste hydrolysate from different studies.

Table 2.1 Essential amino acid composition of shrimp waste hydrolysates from different studies

Amino acid	*Crangon crangon* g/100g amino acids[a]	*Pandalus borealis* g/100g amino acids[b]	*Penaeus* mg/g dry mass[c]	*Penaens vannamei* mg/g shrimp head[d]	*Penaeus spp. (dry powder)* mg/g dry weight[e]
Histidine	5.01 ± 0.08	3.12 ± 0.00	11.8 ± 1.67	16.96	9.0 ± 3.2
Isoleucine	13.2 ± 0.04	5.77 ± 0.03	18.8 ± 1.87	29.91	18.3 ± 6.3
Leucine		8.86 ± 0.09	29.5 ± 1.85	48.66	11.8 ± 7.8
Lysine	6.60 ± 0.02	8.31 ± 0.32	23.8 ± 3.92	46.21	n.d.
Methionine	2.99 ± 0.10	3.30 ± 0.04	9.3 ± 0.57	16.74	16.4 ± 10.4
Phenylalanine	4.93 ± 0.065	5.55 ± 0.09	21.6 ± 2.13	31.03	10.0 ± 1.4
Threonine	5.19 ± 0.03	6.04 ± 0.02	31.6 ± 7.17	27.46	12.1 ± 1.1
Tryptophan	1.20 ± 0.03	n.d.	n.d.	8.48	n.d.
Valine	5.89 ± 0.10	6.72 ± 0.04	24.8 ± 1.84	36.16	16.9 ± 4.0

Notes: Results were presented using average ± standard deviations:

[a] Enzymatic hydrolysis using Alcalase (Synowiecki & Al-Khateeb , 2000)

[b] Enzymatic hydrolysis using Alcalase (Gildberg & Stenberg, 2001)

[c] Acid lactic fermentation (López-Cervantes et al., 2006)

[d] Autolysis for 7 hours (Cao et al., 2008)

[e] Acid hydrolysis using hydrochloric acid (Bueno-Solano et al., 2009)

2.1.1.3 Other Components of Shrimp Waste

There are other valuable fractions in shrimp waste, such as carotenoids and phospholipids. Carotenoids are bioactive substances that widely exist in a variety of products such as foods, feeds, cosmetics and pharmaceuticals (Pacheco et al., 2009). Sachindra et al. (2006) used organic solvents to recover carotenoids from shrimp waste. Cahú et al. (2012) presented a method to recover protein, chitin, carotenoids and sulfated glycosaminoglycans from shrimp waste with the aid of proteolytic endogenous enzymes (Figure 2.4). Astaxanthin, a kind of carotenoids abundant in shrimp, has a much higher antioxidant activity than other carotenoids (Radzali et al., 2014). Astaxanthin extraction from shrimp waste was achieved by lactic fermentation (Armenta‐López et al., 2002; Gimeno et al., 2007).

Phospholipids are main components of all cell membranes. They have been regarded as nutrients that have important influence on lipid digestion, transport and inflammatory reaction (Cullis & De Kruijff, 1979). Moreover, their health benefits on blood vessels, neurons and cells cannot be neglected (Küllenberg et al., 2012; Zimman et al., 2010). Research has been conducted to extract phospholipids from shrimp waste. Shen and Cheung (2014) extracted phospholipids by using solid-phase extraction with titania-coated silica core-shell composites as the sorbent.

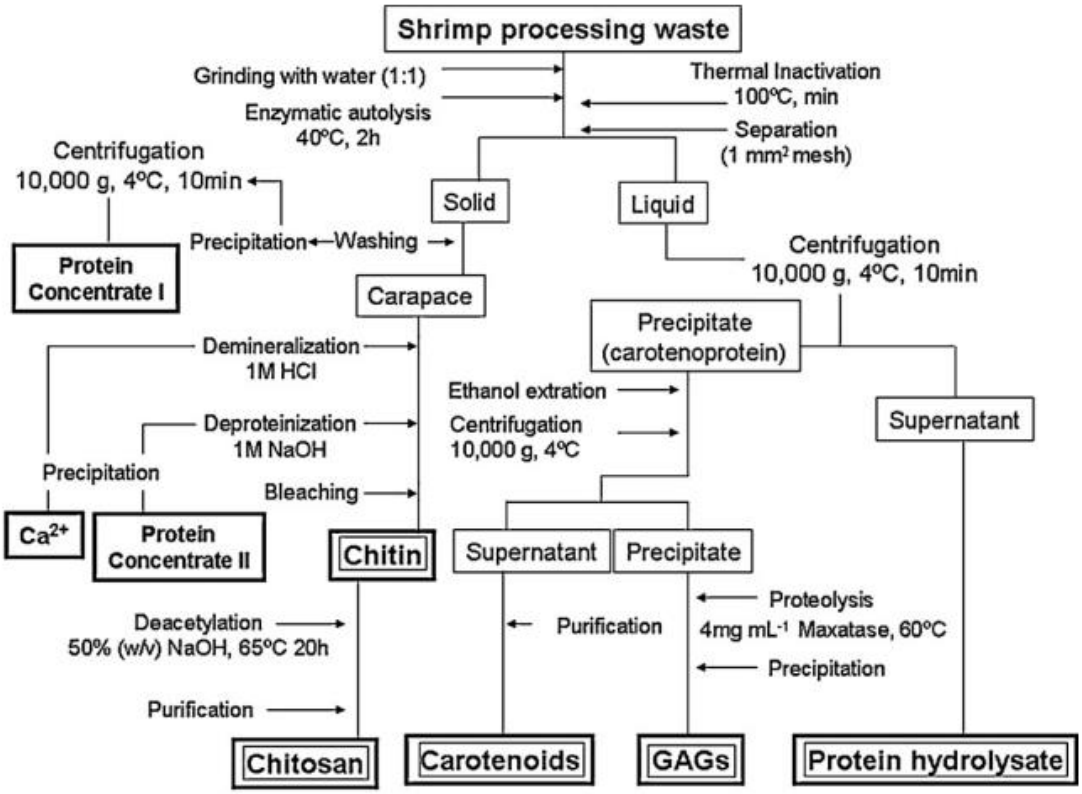


Figure 2.4 An integrated method to recover protein, chitin, carotenoids and GAGs from shrimp waste (Cahú et al., 2012).

2.1.2 Utilization of Shrimp Waste Hydrolysate

The most common utilization of the protein content from shrimp waste was for animal consumption. Shrimp waste protein hydrolysate can be served as feed ingredients in animal diets as it provides large amount of essential amino acid constituents for facilitating animal growth and maintaining physiological functions (Narayan et al., 2010). Nwanna (2003) transformed shrimp head waste into a kind of silage meal as a replacement of fish meal. The shrimp head waste was fermented together with sugar molasses by Lactobacillus plantarum and the product was co-dried with 15% feather meal as silage meal. The results of economic analysis showed incorporation of shrimp head waste meal in the aqua-feeds could be a cost-effective option in Africa (Nwanna & Daramola, 2000). Due to the high protein content, shrimp waste was also regarded as a cost-effective nitrogen source in growth media for microorganisms. Cancre et al. (1999) validated the applicability of shrimp (i.e., Pandalus borealis) waste hydrolysate to cultivate mouse fibroblast cells. The authors also confirmed the applicability of cod head hydrolysate and sardine viscera hydrolysate. Haddar et al. (2011) utilized shrimp waste powder as a sole carbon and nitrogen source for cultivating *Bacillus licheniformis* RP1 to produce proteases. Wei et al. (2015) used shrimp waste as the sole nitrogen source to synthesize N-Doped carbon quantum dots.

There were other applications of shrimp waste protein hydrolysate. Shrimp head protein hydrolysate was employed as a natural food preservative for suppressing the dehydration induced denaturation of lizardfish myofibrils (Ruttanapornvareesakul et al., 2005, 2006).

Arancibia et al. (2015) found protein concentrate from shrimp waste could improve properties of a chitosan-based film. The film became more tensile resistant and less deformable on perforation with the addition of shrimp waste concentrate protein. Till now, there has been no information on utilizing shrimp waste protein hydrolysate as oil dispersants.

2.2 Dispersants

2.2.1 How Dispersants Work

Dispersants usually consist of surfactants and solvents. They can facilitate a heterogeneous mixture of two immiscible substances like oil and water. As shown in the Figure 5, spilled oil does not mix with water and forms an oil slick on the surface of the water. Dispersants can break down the oil slick and allow oil to be dispersed into water when sprayed onto oil slicks (Lessard and DeMarco, 2000). The formation of small oil droplets makes the surface area more accessible for microbial growth. This can further enhance the consumption of oil by microbial activities and other natural processes, thereby reducing the negative impacts on marine environment (Prince and Butler, 2014).

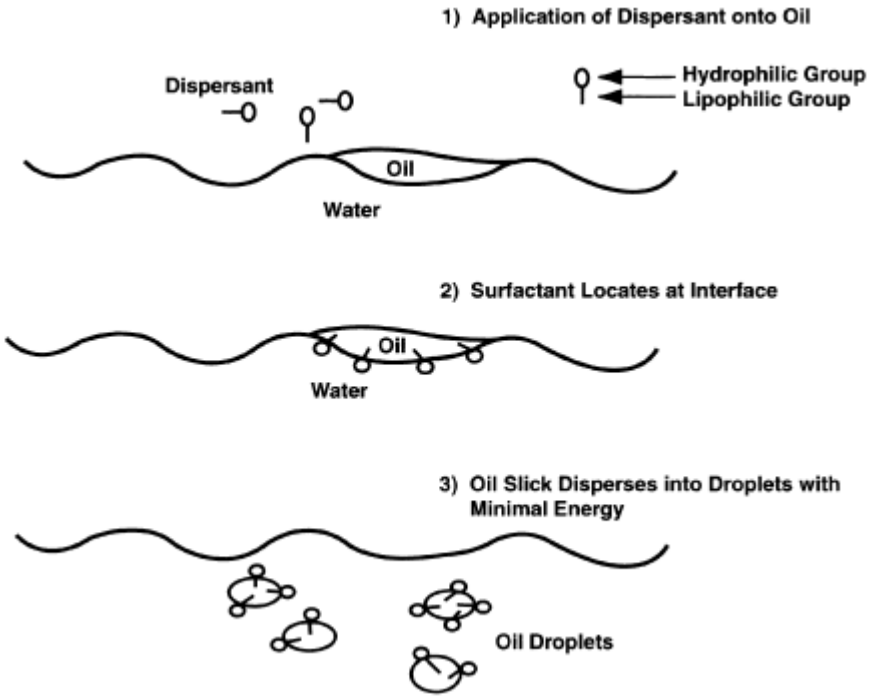


Figure 2.5 How dispersants work (Lessard & DeMarco, 2000).

2.2.2 Surfactants for Dispersant Generation

Surfactants are amphiphilic molecules, which can lower the surface tension between two liquids or between a liquid and a solid by accumulating at the interface (Schramm, 2000). These surface-active molecules can promote foam, emulsion and dispersion due to their amphiphilic nature (Cai et al., 2014). Surfactants have been applied in many fields ranging from industry, agriculture, pharmaceutics and environment remediation (Schramm, 2000; Lentz, 2003; Wong et al., 2013; Susilaningsih et al., 2013).

2.2.2.1 Petroleum-based Surfactants

(1) Classification of petroleum-based surfactants

They are usually classified as four types: anionic surfactants, cationic surfactants, nonionic surfactants and amphoteric surfactants (Myers, 2005). Anionic surfactants can release an amphiphilic anion and a cation, which is generally an alkaline metal. Anionic surfactants include sulphonates, sulphates, phosphate esters and carboxylates (Farn, 2008). Table 2.2 illustrated some examples of anionic surfactants.

Nonionic surfactants do not release ion in aqueous solution. The types of hydrophilic group are usually alcohol, phenol, ether, ester or amide. Nonionic surfactants mainly include ethoxylated linear alcohols, ethoxylated alkyl phenols, fatty acid esters, amine derivatives and amide derivatives, etc (Farn, 2008). Table 2.3 showed some examples of nonionic surfactants.

Cationic surfactants can release an amphiphilic cation and an anion in the water. Their production cost is generally higher than that of anionic surfactants due to the need of high pressure during the synthesis process. As a result, their applications are limited to bactericide and corrosion inhibition (Farn, 2008). Table 2.4 showed some examples of cationic surfactants.

Amphoteric surfactants display both anionic and cationic dissociations. This is because they have two functional groups, both anionic and cationic. The amphoteric surfactants are mild and suited for applications in personal care products (Lomax, 1996). They usually contain following types: betaines, sulfobetaines, amino acids and phospholipids (Farn, 2008). Table 2.5 showed some examples of amphoteric surfactants.

(2) Environmental concerns on petroleum-based surfactants

The presence of petroleum-based surfactants has caused certain concerns in environmental fields. Aronstein et al. (1991) found that nonionic surfactants such as $C_{12}E_4$, $C_8PE_{9.5}$, $C_9PE_{10.5}$, Tween 20 and Tween 80, could inhibit the degradation of phenanthrene in solid-water system. This was also confirmed by Laha and Luthy (1992) who found the inhibitory effect of nonionic surfactants on microbial degradation. Furthermore, petroleum-based surfactants have been found that they could reduce the adhesion of bacteria to hydrophobic surfaces in spite of their capability of enhancing hydrocarbon solubility (Efroymson & Alexander, 1991). Ortega-Calvo and Alexander (1994) found Triton X-100 inhibited cell attachment and consequently reduced the microbial activity in the biodegradation of naphthalene. Similarly, Stelmack et al. (1999) presented a study showing that petroleum-based surfactants like Triton X-100 and Dowfax

8390 could inhibit bacterial adhesion to the surfaces of compounds from soil contaminants and inhibit bacterial growth on a certain carbon source.

Some petroleum-based surfactants were comparatively studied. Shreve et al. (1995) compared the purified rhamnolipid biological based surfactant with structurally similar synthetic anionic surfactant ABS in restoring mutant *pseudomanas* organism growth. The rhamnolipid biological based surfactant demonstrated about 9 times more effective than ABS in solubilizing hydrocarbon into the aqueous phase. Similar results were also found by Kanga et al. (1997) who assessed biologically based surfactant produced by *rhodococcus* species and a representative synthetic surfactant Tween-80. The glycolipids biological based surfactant enhanced higher solubility of two-ring aromatics and displayed a lower toxicity to the organisms. Deschenes et al. (1996) evaluated the petroleum based surfactant sodium dodecyl sulfate (SDS) and a biologically based surfactant produced by *P. aeruginosa* UG2 during biodegradation of 13 of the 16 U.S. Environmental Protection Agency priority pollutant polycyclic aromatic hydrocarbons (PAHs) in a highly weathered soil previously contaminated by the wood preservatives creosote and pentacholorophenol for at least 20 years. Both surfactants are readily biodegradable. For the biodegradation of three-ring PAHs, both surfactants worked well but high concentration of petroleum based surfactant led to a lower rate. For four-ring PAHs, the petroleum based surfactant SDS was more pronounced in inhibition of biodegradation. In the study of Kanga et al. (1997), glycolipids produced by *rhodococcus* sp. H13A and a synthetic surfactant Tween 80 were employed to enhance the solubility of naphthalene and methyl-substituted naphthalenes. The results showed that both

surfactants effectively lowered the surface tension, but biologically based surfactants showed not only high capability of enhancing solubility than petroleum based surfactant but also lower aqueous toxicity. Schippers et al. (2000) evaluated sophorolipids, a type of biosurfactants, on microbial biodegradation of phenanthrene in liquid and soil suspension substrate. They show lower critical micelle concentrations (CMC) and their weight solubilization ratios were found better. Edward et al. (2003) made a toxicity comparison among three synthetic surfactants and three biological based surfactants used in oil spill remediation to two estuarine epibenthic invertebrate species. The authors concluded that the biologically based surfactants were less toxic than the synthetic surfactants.

Traditional petroleum-based dispersants usually consist of one or more petroleum-based surfactants dissolved in a solvent (NRC, 1989). Since the 1980s, environmental hazards that petroleum-based dispersants may cause have been reported (Wells, 1984; Fingas, 2002). Foght and Westlake (1982) investigated the effect of the petroleum-based dispersant Corexit 9527 on the biodegradation of Prudhoe Bay oil. The authors concluded that the addition of chemical dispersants stressed the marine environment by bringing about very high carbon to nitrogen ratio, which retarded the oil biodegradation process. Recently, Corexit EC9500A, a kind of chemical dispersant, is largely used in Gulf of Mexico oil spill on April 20, 2010 (Prince, 2015). Pietroski et al. (2015) examined the toxic effects of Corexit EC9500A and found it had negatively effect on the wetland soil microbial biomass and microbial activity.

Table 2.2 Examples of anionic surfactants

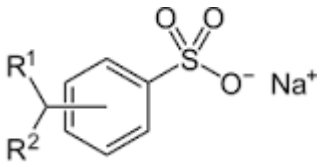
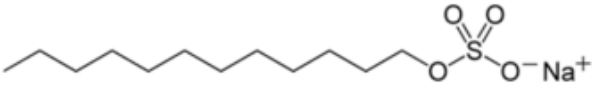
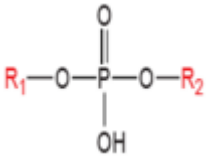
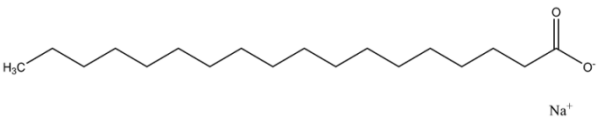
Types of anionic surfactants	Example compounds	Molecular structural formula
Sulphonates	Sodium dodecylbenzenesulfonate	 (https://en.wikipedia.org/wiki/Sodium_dodecylbenzenesulfonate)
Sulphates	Sodium dodecyl sulfate	 (http://chemistry.about.com/od/factsstructures/ig/Chemical-Structures---S/Sodium-Dodecyl-Sulfate.htm)
Phosphate esters	Phosphate diesters	 (http://www.elementis-specialties.com/esweb/esweb.nsf/pages/surfactants-anionicsurfactants)
Carboxylates	Sodium stearate	 (http://pubchem.ncbi.nlm.nih.gov/compound/sodium_palmitate#section=Top)

Table 2.3 Examples of nonionic surfactants

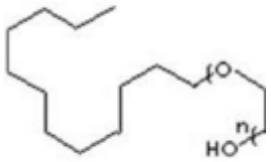
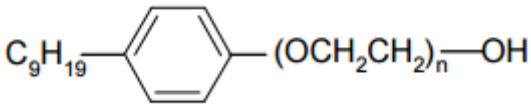
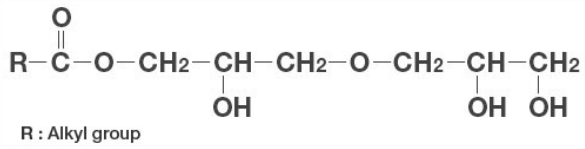
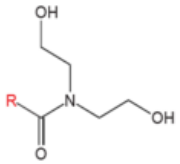
Types of anionic surfactants	Example compounds	Molecular structural formula
Ethoxylated linear alcohols	Lauryl alcohol ethoxylates	 (http://www.chemicalland21.com/specialtychem/perchem/lauryl%20alcohol%20ethoxylate.htm)
Ethoxylated alkyl phenols	Nonylphenol ethoxylate	 (http://www.arb.ca.gov/consprod/regact/2010ra/ape9016459.pdf)
Fatty acid esters	Diglycerin Fatty Acid Esters	 (http://www.sy-kogyo.co.jp/english/product/diglycerin-fatty.html)
Amide derivatives	Diethanol amides	 (http://www.elementis-specialties.com/esweb/esweb.nsf/pages/surfactants-nonionicsurfactants)

Table 2.4 Examples of cationic surfactants

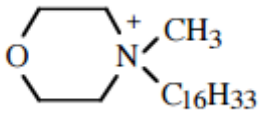
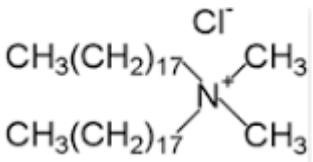
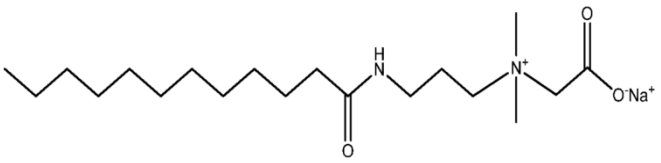
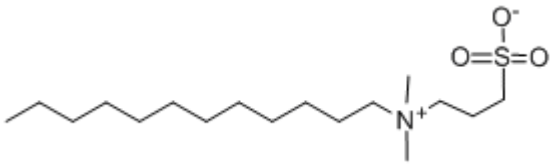
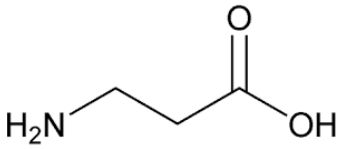
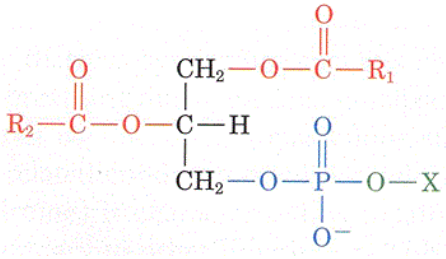
Types of anionic surfactants	Example compounds	Molecular structural formula
Morpholine compounds	N,N-Cetylmethyl morpholinium cation	 (Farn, 2008)
Quaternary ammonium salts	Distearyldimethylammonium chloride	 (https://en.wikipedia.org/wiki/Distearyldimethylammonium_chloride)

Table 2.5 Examples of amphoteric surfactants

Types of anionic surfactants	Example compounds	Molecular structural formula
Betaines	Fractionated Cocamidopropyl Betaine	 (http://www.google.com/patents/WO2014052179A2?cl=en)
Sulfobetaines	N-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate	 (http://www.chemicalbook.com/ChemicalProductProperty_EN_CB0258625.htm)
Amino acids	Aminopropionic Acid	 (http://www.uspbpep.com/usp31/v31261/usp31nf26s1_alpha-19-31.asp)
Phospholipids	Glycerophospholipid	 (https://www.studyblue.com/notes/note/n/lipids/deck/6996140)

2.2.2.2 Biologically based Surfactants

Biologically based surfactants are amphiphilic metabolites produced by microorganisms including bacteria, yeast and fungi (Satpute et al., 2010). They contain hydrophilic and hydrophobic moieties that can reduce surface tension at the air-water interface and interfacial tension among immiscible liquids or at the liquid-solid interface (Gharaei-Fathabad, 2011). The hydrophilic top could be positively or negatively charged. It usually contains hydrophilic components such as amino acids, peptides, carboxylic acids, and saccharides. The hydrophobic end usually consists of saturated, unsaturated, linear, branched, or hydroxylated fatty acids (Georgiou et al., 1992; Shekhar et al., 2014). Such features endow them with emulsifying, foaming, detergency and dispersing properties. Bioemulsifiers are usually categorized as biologically based surfactants because they exhibit emulsifying capacity, though bioemulsifiers might not reduce surface tension (Karanth et al., 1999). With low toxicity, high biodegradability and high durability under extreme conditions, biologically based surfactants are gaining increasing attention by researchers (Banat et al., 2010; Makkar et al., 2011). Due to surface-active properties, these microbial molecules could serve as agents for emulsion polymerization, wetting, foaming, phase dispersion, emulsification and de-emulsification (Desai & Banat, 1997). During the past few years, biologically based surfactants have been widely studied since they have great potential applications in many fields, specifically in the field of petrochemical industries such as enhanced oil recovery (Fiechter 1992), environmental bioremediation such as dispersion of oil spills (Saeki et al, 2009); pharmaceuticals (Gharaei-Fathabad, 2011), food processing and health care industries (Nitschke & Costa 2007;

Banat et al., 2000).

While petroleum-based surfactants are usually categorized based on the nature of their polar grouping, biologically based surfactants are classified mainly by their chemical compositions and their microbial origins (Desai & Banat, 1997; Gautam and Tyagi, 2006). The categories of biologically based surfactants mainly contain glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids, polymeric surfactants, and particulate surfactants (Desai & Banat, 1997; Rahman & Gakpe, 2008).

2.2.2.3 Protein and Amino Acid-Based Surfactants

Proteins are large surface-active biomolecules containing different types of amino acid, omnipresent in nature. They are also building blocks of life (Muheem et al., 2014). Proteins with a large quantity of apolar amino acids usually display surface activity (Kato & Nakai, 1980). In addition, protein-aided emulsions are quite useful as delivery systems in the food, pharmaceutical and cosmetics industries. Dalgleish (1997) mentioned proteins could aid emulsion of oil in water and there might be one or more small surfactants absorbed at the interface. Hu et al. (2003) reported corn oil-in-water emulsions produced by casein, whey protein isolate, soy protein isolate at pH 3.0. Chu et al. (2007) prepared β-carotene in hexane nanodispersions using soy protein. Lee and McClements (2010) achieved oil-in-water nanoemulsions containing small corn oil droplets formed by whey protein isolate. Nikiforidis et al. (2011) accomplished maize germ oil-in-water emulsions by utilizing maize germ protein. Donsì et al. (2012) designed sunflower oil-in-water nanoemulsions for food delivery system

using pea proteins.

It has been found that hydrolysis can modify the protein structure (Zhao et al., 2013). During the protein hydrolysis process, a number of peptide bonds are cleaved into lower molecular weight proteoses, peptones, peptides, and free amino acids (Chang et al., 2007), thus enhancing the protein's functional properties. De Castro et al. (2015) found enzymatic hydrolysis could improve the solubility and emulsion properties of milk proteins. Zhao and Xiong (2015) reported the surface activity of soy protein hydrolysate and the emulsion oxidative stability that interface-adsorbed peptides contributed to. Chalamaiah et al. (2010) studied the functional properties of alcalase protein hydrolysate from underutilised meriga (*Cirrhinus mrigala*) fish egg and the results suggested that the hydrolysate had good foaming capacity and emulsifying capacity. Intarasirisawat et al. (2012) presented the antioxidative properties, foam stability and emulsion capability of alcalase hydrolyzed protein hydrolysate from skipjack roe. The authors found that the low degree of hydrolysis (DH) contributed to high emulsion ability and foam stability. Gauthier and Pouliot (2003) pointed out the peptides released by enzymatic hydrolysis of whey proteins possessed antihypertensive properties for hypertensive rats and human subjects. Peptides from enzymatic hydrolysis of milk proteins were found to display immunomodulatory activities (Korhonen & Pihlanto, 2006).

Protein hydrolysis products have recently been recognized as potential green-surfactants (Arboleda et al., 2014; Singh & Dalgleish, 1998; Zhao & Xiong, 2015). Many studies have focused on the emulsifying efficacy of protein hydrolysis products, which are widely applied in

food system and drug delivery. These hydrolysis products come from diverse sources of protein, such as soy protein (Arboleda et al., 2014), peanut protein (Tang et al., 2012), quinoa seed protein (Aluko & Monu, 2003), potato protein (Cheng et al., 2014), whey protein (Foegeding et al., 2002), Atlantic cod backbone protein (Šližytė et al., 2009), yellow stripe trevally meat protein (Klompong et al., 2012), alfalfa soluble leaf protein (Lamsal et al., 2007), and skipjack roe (Intarasirisawat et al., 2012). Emulsion is a fine dispersion of one phase into another immiscible phase (e.g. water and oil). The emulsifying capabilities of protein hydrolysis products make them good surfactants in forming dispersants. Cheng et al. (2014) reported potato protein hydrolysate could facilitate soybean oil in water dispersion and the interfacial peptides were mostly short-chain peptides with two to seven amino-acids. Arboleda et al. (2014) utilized the surface hydrophobicity of soy protein hydrolysate for surface modification of materials, thereby enhancing the biocompatibility of the modified materials. Similar findings on soy protein hydrolysate were also reported (Zhao & Xiong, 2015).

In addition, synthetic surfactants that mimic natural lipoamino acids have also been regarded as green surfactants. As natural structure like amino acids and lipids show low toxicity and quick biodegradability, synthetic surfactants derived from them are preferred (Infante et al., 1997). Infante et al. (2004) proposed the combination of polar amino acids (hydrophilic moiety) and non-polar long-chain compounds (hydrophobic moiety) for building up the amphiphilic surfactants. The authors synthesized three kinds of amino acid-based surfactants with high degradability and low toxicity, which included linear chain, gemini and glycerolipid-like surfactants. Brito et al. (2009) synthesized four kinds of amino acid-based surfactants from

tyrosine (Tyr), serine (Ser), hydroxyproline (Hyp) and lysine (Lys) and found they had lower ecotoxicity than the reference surfactant dodecyl trimethyl ammonium bromide (DTAB). Brito et al. (2011) demonstrated the enhanced interfacial properties of three kinds of amino acid-based surfactants when compared with the conventional counterparts.

2.2.3 Solvents for Dispersant Generation

The solvents in dispersants mainly have two functions. The first is to facilitate the blend of surfactants to liquid solutions and the second is to enhance mixing surfactant with oil film, thereby promoting the efficiency of dispersion (Fiocco et al., 1995). The solvents used in dispersants can be classified as three types: hydrocarbon, water, and water miscible solvents (Clayton et al., 1993).

(1) Hydrocarbon-based solvents

The hydrocarbon-based solvents are usually aromatic and non-aromatic solvent systems. The earlier generation of dispersants applied in Torrey Canyon spill included aromatic compounds like benzene and xylene (Clayton et al., 1993). However, the aromatic solvents are no longer employed nowadays, due to their toxic effect on marine environment. The non-aromatic solvents are better to be used. The non-aromatic solvents generally include ethylene glycol monobutyl ether, de-aromatized kerosene and isoparaffinic-based hydrocarbon (Lewis & Daling, 2001; Versluis et al., 2004; Weyershausen & Lehmann, 2005).

(2) Water-based solvents

The water-based solvents usually possess relatively lower toxicity and they have higher efficiency in dispersing fresh oil spills and low viscosity oils due to their low affinity for oil (Clayton et al., 1993). Because the contents of surfactants are low, high application rates and supplemental mechanical enhancement are required (Fiocco & Lewis, 1999).

(3) Water miscible hydroxyl-based solvents

The water miscible solvents also have shown relative lower toxicity than that of hydrocarbon-based solvents (Fiocco & Lewis, 1999). Glycols and glycol ethers are usual contents (Clayton et al., 1993). There are much higher surfactant contents in these types of solvents, making them more effective in lower amount of application (Mackay, 1995). For example, Corexit 9527 and Corexit 9500 used in the Deepwater Horizon oil spill contained ethylene glycol monobutyl ether and propylene glycol (Hayworth & Clement, 2012; Singer et al., 1994;).

2.2.4 Dispersant-Aided Oil Spill Response

2.2.4.1 Roles of Oil Spill Dispersants

In general, physical methods have been widely adopted in oil spill response, such as booms, skimmers and in situ burning (Etkin & Tebeau, 2003). However, booms and skimmers can only be applied in calm weather conditions and they are hindered by rough weather, winds and oil slick (Lee et al, 2015). In situ burning might cause further contamination to the atmosphere (Lee et al, 2015). For instance, only 3% of the spilled oil was collected by booms and skimmers and 5% was burned in *Deepwater Horizon* oil spill response (Lubchenco et al., 2012). If spilled oil is not collected or burned, the final fate of the oil is biodegradation (Fingas, 2012). However, due to the low density of certain oil and lack of essential nutrients, microbial growth utilizing oil has been limited (Atlas & Bartha, 1972). Floating oil poses hazards to animals, subsurface organisms and some plants (Nomack, 2010). With the aim of reducing these hazards, oil spill dispersants were initially developed in the 1970s (NRC, 2005). The surfactants with fixed ratios have the capabilities of emulsifying oil so that highly viscous oil slicks can be broken up (Kujawinski et al., 2011). The concentration of dispersed oil could be about 1000 ppm at the right beginning of dispersion, which could become lower than 1 ppm within a few hours (Bejarano et al., 2013; BenKinney et al., 2011). Generally, nonionic surfactants are preferred in dispersants due to their lower water solubility and toxicity than anionic and cationic surfactants (Myers, 2005; Porter, 2013).

Dispersants have been widely used as an effective option for oil spill response (Chapman et al.,

2007; Judson et al., 2010; Kujawinski et al., 2011; Lessard and DeMarco, 2000). Specifically, in several oil spill events, dispersants were used on a large scale. During the 1992 Braer wreck in the Shetland Islands, dispersants were sprayed aerially for three days on the spilled oil (Harris, 1995). In the 1996 Sea Empress spill in South Wales, 72,000 tons of Forties blend crude oil and 480 tons of heavy fuel oil were released in to the ocean. 446 tons of petroleum based surfactants were used onto the spilled oil by aircrafts (Law & Kelly, 2004). The oil concentration was first found between 1 to 10 mg/L. But it was quickly reduced to be lower than 1 mg/L (Lunel et al, 1995). It was reported that the application of dispersants in this event avoided 57,000 to 110,000 tons of emulsified oil reaching shoreline in South Wales (SEEEC, 1998). In the 2010 Deepwater Horizon blowout in the Gulf of Mexico, two dispersants were used extensively with a total amount of 1.1 million gallons: Corexit 9527 and Corexit 9500A (Hayworth & Clement, 2012).

2.2.4.2 Protocols for Oil Spill Dispersant Testing

In the U.S. Environmental Protection Agency protocol for oil spill DE testing for treating oil spills on the open water, the swirling flask test (SFT) and baffled flask tests (BFT) are reported to give widely varying results in the hands of different testing laboratories. Sorial et al. (2004) determined the effectiveness of 18 dispersants using both EPA SFT and BFT methods done by three operators. The results showed that the coefficient of variation by the BFT was only 7.8% while that of EPA SFT was 21.9%. The average percent effectiveness of the EPA SFT was only 19.7% while 64.6% for the BFT. Besides, Holder et al. (2015) conducted a comparative

laboratory-scale DE testing of 23 Crude Oils using four testing protocols. The authors found BFT had most predictive value in dispersant usage decision of an oil spill event, sincethe testing results of BFT were inversely correlated with oil viscosity.

2.2.4.3 Environmental Factors Affecting Dispersant Effectiveness (DE)

(1) Salinity

Salinity can influence the water solubility of many compounds (Blondina et al., 1999). It has been pointed out that high salinity may result in a solubility reduction of dispersant in water, which can promote the interaction between oil and water interface (Chandrasekar et al., 2006). Studies have been focusing on salinity as an environmental factor that affects the performance of dispersants. Fingas et al. (1994) conducted laboratory experiments on the effect of salinity of three types of dispersants on three types of crude oils. The authors found DE increased as salinity increased from 0 to 45 psu. Similar results were found in other study (Chandrasekar et al., 2006). Blondina et al. (1999) tested the salinity's influence on Corexits 9527 and 9500 on dispersing several different kinds of crude oils. The results showed that Corexit 9500 was more effective than Corexit 9527 on most of the crude oils at most salinities. In addition, both dispersants had better performance when the salinity was larger than 25 ppt. Moles et al. (2002) examined the influence of two levels of salinities on two petroleum-based surfactants employed in dispersing Alaska North Slope crude oil. The results showed that both dispersants' performance was better at 22 psu than that at 32 psu at 10 ^{o}C.

(2) Mixing energy

As oil spill usually happens in flowing water like ocean, the wave and wind could impose a mixing energy on oil and water. Mixing energy provided by wave and wind can help break up oil slick into small droplets to facilitate oil dispersion. Research has been conducted about the effect of mixing energy on the DE on different kinds of crude oil. Clayton et al. (1993) reported that mixing energy could cut down the size of oil droplets. Fingas et al. (1993) found that as the mixing energy increased, DE kept increasing until a maximal was reached. Chandrasekar et al. (2006) also found this trend for dispersants on different kinds of oil including South Louisiana crude oil (SLC), Prudhoe Bay crude oil (PBC) and Number 2 Fuel Oil (2FO). Srinivasan et al. (2007) conducted BFT using Corexit 9500 and Superdispersant 25 on IFO crude oil. The authors found that DE increased steadily with mixing speeds increasing from 150 to 250 rpm at both 5 ^{o}C and 16 ^{o}C.

(3) Temperature

Temperature is another environmental factor that plays a key role in DE (Fingas et al., 1994). Water temperature can affect the physical properties of oil such as viscosity. In the northern region, low water temperature can increase the viscosity of oil and therefore decrease DE (Clayton et al. 1993). The effect of temperature on the DE of different kinds of crude oil has been studied. Moles et al. (2002) tested Corexit 9500 and 9527 on fresh Alaska North Slope crude oil (ANS) crude oil at subarctic temperatures. They found greatly decreased DE (below detection limit) under low temperature conditions. Srinivasan et al. (2007) found that the

performance of three dispersants for heavy fuel oil at 16 ºC was better than that at 5 ºC. Those dispersants' effectiveness at 5 ºC only had values ranging from 30% to 60%. However, Byford (1982) pointed out that high viscosity of crude oil might hinder the recoalescence of dispersed oil droplets. Besides, he also suggested that the high density of oil droplets decrease their buoyancy. Both circumstances might lead to better crude oil dispersion at low temperatures. A large scale Ohmsett tank test of petroleum-based surfactants under a low temperature showed the dispersants still performed effectively on dispersing Alaskan crude oils (Belore et al., 2009).

(4) Weathering

When oil was spilled, weathering was a complicated process that affects the oil dispersion (Canevari et al., 2001). During the weathering process, the lighter components of crude oil usually were evaporated, thereby increasing the viscosity of the oil. As a consequence, the effectiveness of dispersants decreased (Daling, 1988). In laboratory-scale studies, Srinivasan et al. (2007) reported that the DE decreased as the degree of oil weathering increased for Corexit 9500 on SLC, PBC and 2FO. In field-scale studies, Lewis et al. (1998) tested the DE of two petroleum-based surfactants on three kinds of weathered oil on the sea. The results showed that after several days of weathering, the oil could still be successfully dispersed by the selected dispersants. But emulsions with lower water contents reduced the performance of dispersants.

2.3 Optimization and Modeling of Experimental Systems

2.3.1 Response Surface Methodology (RSM)

One-factor-at-a-time (OFAT) experimental method studies a process by changing one independent factor at a time while keeping all the others unchanged. It can be used for selecting key parameters with their ranges of interest and operability (Bari et al., 2009). This method usually tends to be time-consuming and requires a large number of experiments for multifactor scenarios. It is unable to describe the interactions among different factors as well as to detect the true optimum to some extent (Montgomery, 2008).

To study interactive effects among different factors and find the true optimum, RSM has been widely used (Montgomery, 2008). RSM is an effective experiment-based tool to optimize a process when multiple factors and their interactions may affect the response (Rodrigues et al., 2006; Wangtueai and Noomhorm, 2009; Zheng et al., 2008). This statistics-based technique is suitable for process optimization with fitting for a quadratic surface (Myers et al., 2009). Two very useful and popular experimental designs that allow a second order model to be fit are central composite design (CCD) and Box-Behnken design (BBD). For four or more factors, CCD usually has smaller designs than BBD. Besides, BBD has limited capability for orthogonal blocking compared to CCD. In general, CCD is more preferred than BBD (Trinca & Gilmour, 2000).

Mutalik et al. (2008) adopted CCD of response surface methodology to optimize the

concentrations of four media components in enhancing biological based surfactant production from Rhodococcus spp. MTCC 2574. Bari et al. (2009) employed the OFAT method and CCD of RSM to optimize media for the improvement of production of citric acid from oil palm empty fruit bunches. See et al. (2011) used three factors, five levels of CCD design of RSM to optimize Salmon skin protein hydrolysis to obtain the maximum degree of hydrolysis using Alcalase. OFAT and RSM are usually used for evaluating the effect of independent variables, alone or interactively, and selecting optimum conditions of variables in the process.

2.3.2 Artificial Neural Network

ANN was first proposed in the 1940s (McCulloch & Pitts, 1943). It is a data processing system that imitates the human brain's way of working (Buciński et al., 2008). It combines artificial neurons that receive inputs into layers. When the input is received, the output would be calculated from the weighted input signal (Kuvendziev et al., 2014). Without knowing the detailed relationship of processing variables in advance, ANN can recognize and replicate cause-effect relationships with its capability of adaptive training and data self-organization. This makes ANN an efficient tool to study complex systems (Khajeh and Barkhordar, 2013). Due to the robustness, fault tolerance, high computational speed and self-learning capability, ANN has become as an attractive tool for non-linear multivariate modeling and predictions based on experimental datasets (Desai et al., 2005). It has been widely used in many fields of science and engineering to simulate complex physical and chemical processes (Fukuda and Shibata, 1992; Gardner and Dorling, 1998; Kasiri et al., 2008; Yi et al., 2007).

Recently, ANN has been successfully applied to model protein hydrolysis processes which involve complex reaction kinetics. Abakarov et al. (2011) employed ANN to model enzymatic hydrolysis of squid protein. In this study, reaction time and substrate concentration were inputs and reaction rate was output. The work resulted in an effective kinetic model describing the process. Li et al. (2006) modeled enzymatic hydrolysis process of Bighead Carp Muscles to optimize the hydrolysis variables on the production of antioxidant peptides. Besides, ANN was used to model the tryptic hydrolysis of pea protein isolate to make predictions of the degree of hydrolysis at three levels of temperatures. The results showed strong correlations between the experimental results and the data predicted by the model (Buciński et al., 2008).

2.3.3 Integration of RSM with ANN

It is hard to say that RSM can be applied to all optimization and modeling studies (Bas and Boyaci, 2007). As mainly used for linear and quadratic approximations, they are not suitable in highly nonlinear cases (Desai et al., 2008). It is also difficult to conduct sensitivity analysis of input parameters due to the presence of cross interactions (Lou and Nakai, 2001). On the other hand, when compared with RSM, ANN has difficulty in explaining the interaction relationship of the variables due to the employment of indefinite weights (Shao et al., 2007). This method has been criticized as a "black box" since the linearity or quadratic dependence of the transfer equations cannot be readily understood. The tendency towards overfitting the training datasets was also mentioned by the researchers (Elmolla et al., 2010; Colbourn et al.,

2011).

Generally, DOE and RSM can provide a statistically-distributed experiment design to understand the mechanisms of the system and facilitate building of a reasonable model without overfitting the datasets. since ANN can help modeling highly nonlinear cases with little restrictions while RSM cannot, the integration of ANN and RSM has been developed as an effective way to have better predictability of the process behavior through optimization. The common integration is using DOE for statistical experiment protocol followed by ANOVA analysis of RSM. ANN is then employed to model the process and the model which is in the best accordance with RSM results is selected. Shao et al. (2007) built a predictive model for the recovery of tocopherol from rapeseed oil deodorizer distillate combining ANN with RSM. Kasiri et al. (2008) optimized heterogeneous photo-Fenton process for degradation of C.I. Acid Red 14 azo dye integrating ANN and RSM.

2.4 Summary and Research Gap

In this chapter, section 2.1 reviews current status of shrimp waste utilization and application of shrimp protein hydrolysate. Section 2.1.1 discusses on shrimp waste utilization from several components like chitin, protein and amino acids and other components of shrimp waste. There are two main processes to recover chitin, namely traditional chemical process and biological extraction process. The biological extraction process shows advantages over the chemical process, such as high productivity and environmental friendliness. For the protein and amino acids recovery, three kinds of hydrolysis methods are reviewed, including autolysis hydrolysis,

acid hydrolysis and enzymatic hydrolysis. Among these methods, enzymatic hydrolysis is the most efficient one, while autolysis hydrolysis is the most cost-effective. For other components of shrimp waste, the recovery of carotenoids and phospholipids has been reviewed. Section 2.1.2 specifically reviews the application of shrimp protein hydrolysate, such as animal feed, nitrogen source for microbial growth and food preservative. As there is a large quantity of shrimp waste generated in Newfoundland every year, the enzymatically hydrolysis process of shrimp waste could be optimized and modeled to improve the disposal efficiency.

Section 2.2 reviews on the topic of oil dispersion, in which two important factors, surfactant and solvent, are examined. The dispersant-aided oil spill response was summarized. Section 2.2.1 describes multiple types of surfactants being used. The component classification and the environmental concern of petroleum-based surfactants are introduced respectively. Biologically based surfactants, such as protein-based surfactants and amino acid-based surfactants, are also reviewed. Section 2.2.2 summarizes three types of solvents commonly employed in oil dispersion technology, including hydrocarbon-based solvents, water-based solvents and water miscible hydroxyl-based solvents. Among them, the water miscible hydroxyl-based solvents are mostly used in the current application. Section 2.2.3 reviews the application of dispersants in oil spill response. The role of oil spill dispersant is introduced, which aids dispersion of floating oil that cannot be recovered by physical response methods. Section 2.2.3.2 reviews four environmental factors that affect oil spill DE including salinity, mixing energy, temperature and weathering process. The concerns about the toxicity and metabolism of existing chemical dispersants to the environment have been growing. Therefore,

more efforts are needed for generation of environmentally friendly alternatives with lower toxicity, and the protein-based dispersants could provide a good solution. To date, no information has been available regarding the use of shrimp waste hydrolysate as dispersants.

Section 2.3 gives a review on an optimization methodology and a modeling methodology of experimental systems. Section 2.3.1 reviews the traditional OFAT experimental methodology and RSM optimization methodology. Section 2.3.2 introduces the ANN modeling methodology. Section 2.3.3 introduces the advantages and disadvantages of RSM and ANN and the possibility of integrating these two methodologies. To date, no integration of RSM and ANN has been applied in optimization of shrimp waste utilization.

CHAPTER 3

NEWFOUNDLAND SHRIMP WASTE HYDROLYSIS FOR MICROBIAL GROWTH: OPTIMIZATION AND MODELING[1]

[1] The research findings in Chapter 3 have been accepted for publication by Marine Pollution Bulletin (Impact factor: 3.099) doi:10.1016/j.marpolbul.2016.05.075

3.1 Introduction

Waste generated from seafood processing plants has been a major concern in coastal Newfoundland (Adams et al., 2005) and shrimp waste represents 37% of the total seafood processing waste in the province. To date, hydrolysis has been regarded as a promising option of utilizing seafood processing waste. Enzymatic hydrolysate derived from seafood waste is regarded as a good source of protein ingredients. This kind of hydrolyzed protein could be a premium and low-cost nitrogen source for microbial growth. However, during the protein hydrolysis process, many peptide bonds are cleaved in parallel and series. This produces a complicated matrix of substrates which trigger new hydrolysis progresses (Marquez and Vázquez, 1999). In addition, thermal inactivation of enzymes at the end of hydrolysis is another sophisticated process not well understood yet. To help study factor (e.g., temperature, pH, and time) effects, understand the mechanisms of hydrolysis, predict the performance, and promote its applicability, experimental and modeling methods have been recognized as effective solutions (Morgenroth et al., 2002).

RSM is an effective experiment-based tool to optimize a process when multiple factors and their interactions may affect the response (Rodrigues et al., 2006; Wangtueai and Noomhorm, 2009; Zheng et al., 2008). Due to the incapability of handling highly nonlinear cases and difficulty in sensitivity analysis, RSM needs to be integrated with other modeling tools for system prediction to obtain a more-predictive model with less requirements on data. ANN has become an attractive tool for non-linear multivariate modeling and predictions based on experiment datasets (Desai et al., 2005). Due to the “black box” nature, ANN should be

integrated with another methodology to be able to probe the mechanism of the system. To have better predictability of the process behavior through optimization, the integration of ANN and RSM has been an effective way. To date, no integration of RSM and ANN has been applied in optimization of shrimp waste utilization.

In this chapter, to fill the above knowledge gap, the hydrolysis of northern pink shrimp (*Pandalus borealis*) waste generated in Newfoundland was used as an illustrative example. Factors including enzyme/substrate ratio (E/S), hydrolysis time, initial pH value and hydrolysis temperature were studied as system inputs. The degree of hydrolysis (DH) of shrimp waste was used as the output. RSM and ANN were integrated for system optimization through investigating the effects of inputs and their relationships with the output. A four-factor, five-level CCD design of response surface methodology was employed to evaluate both individual and synergetic effects of key factors and to optimize the DH. The experimentally determined DH for different levels of factors were then used for training the ANN to simulate the process. The significances of each input factor on the output results were determined and their effects were discussed. The optimum conditions for shrimp waste hydrolysis were also determined. Products with three different DHs were finally used as the nitrogen source to cultivate Bacillus *subtilis* N3-1P, a hydrocarbon-degrading bacterium to validate the growth efficiency related to DH.

3.2 Materials and Methods

3.2.1 Shrimp Waste Hydrolysis

3.2.1.1 Materials and Reagents

Shrimp waste including heads, shells and tails of northern pink shrimp was purchased from a local fish market in Newfoundland, Canada. The shrimp waste was grounded in a food processor (Black & Decker Model FP2700SC) and packed in plastic bags. The grounded materials were kept frozen at -18 ^{o}C. The enzyme used for the hydrolysis of shrimp waste is Alcalase 2.4L (Sigma Aldrich, U.S. $\geq$ 2.4 U/g). It is a commercial proteinase from Bacillus licheniformis, subtilisin A, inexpensive and nonspecific with endopeptidase activity. Chemicals used for medium contents were of analytical reagent grade purchased from Sigma Aldrich, Canada.

3.2.1.2 Shrimp Waste Hydrolysis

The hydrolysis of shrimp waste was done through the procedure reported by Dey and Dora (2014) with modifications. The grounded shrimp waste was thawed at room temperature for one hours and then suspended (1:1, w/v) in distilled water in a baffled colonial flask. The mixture was then heated in a water bath at 90 ^{o}C for 20 min to inactivate the indigenous hydrolyzing enzyme. Different E/S ratio of enzyme was added into each corresponding flask when the mixture was cooled to room temperature (20 ^{o}C). Next 1N HCl and 1N NaOH solution were employed to adjust the initial pH value, which was measured by a bench top pH meter. The flasks were then put into a temperature controlled water bath with a shaking rate at

110 rpm. The enzyme was deactivated by heating at 90 ^{o}C for 10 min. The samples were cooled to room temperature again and subsequently centrifuged at 10,000 rpm for 15 min. The supernatant was collected and then freeze dried to give dry powder. The dry powder was kept frozen in plastic bottles in a -80 ^{o}C freezer.

3.2.1.3 Determination of DH

DH was determined according to the method of Hoyle and Merritt (1994). The 20% trichloroacetic acid (TCA) was added to the supernatant (1:1, v/v) to create 10% TCA-soluble and TCA-insoluble fractions. The mixture was then centrifuged at 6,000 rpm at room temperature for 20 min to collect the 10% TCA-soluble supernatant. The DH was computed as the ratio of the percent of 10% TCA-soluble nitrogen versus total nitrogen of the sample. All the nitrogen content was determined using the Kjeldahl method (AOAC, 2005). Each test was performed in triplicate and the result was expressed as the mean of triplicate trials ± standard deviation (see eq. 3.1).

$$\mathrm{DH} = \frac{10\%\ \ \mathrm{TCA-soluble\ \ N\ \ in\ \ sample}}{\mathrm{Total\ \ N\ \ in\ \ sample}} \times 100\% \qquad (3.1)$$

3.2.2 RSM and ANN Design

3.2.2.1 RSM Design

RSM was used to optimize enzymatic hydrolysis of shrimp waste. According to the literature (See et al., 2011; Dey and Dora, 2014), four main factors including E/S ratio, hydrolysis time, initial pH value and hydrolysis temperature were selected for CCD. OFAT experiments were

conducted first to identify the most critical factors and their reasonable ranges (fixed level of four factors were E/S ratio = 0.5%, time = 1 h, pH = 8 and temperature = 40 ^{o}C). The main effects of critical factors on DH are shown in Figure 1.

As shown in Figure 3.1, the central points of E/S ratio, hydrolysis time, initial pH and hydrolysis temperature were set as 1.25%, 2.5 h, 8 and 50 ^{o}C, respectively. Therefore, a four-factor, five-level CCD was developed. The four independent variables and their experimental ranges are shown in Table 3.1. The variables X_i were coded as x_i according to the following relationship:

$$x_i = \frac{\mathrm{X_i} - \mathrm{X_0}}{\delta \mathrm{X}} \qquad (3.2)$$

Where, X_0 is the value of X_i at the center point and δX stands for the step change.

The CCD was comprised of 29 treatments including 2^4 factorial points, eight axial points (α = 1.41) and five replicates at the center points. DH is used as the response for the combination of the independent variables as shown in Table 3.2. Randomized experimental runs were adopted to minimize the effects of unexpected variability in the observed response. The behavioral model was estimated by the following second-order polynomial equation (Montgomery, 2008):

$$\mathrm{Y} = \ b_0 + \sum_{i=1}^{4} b_i X_i + \sum_{i=1}^{4} b_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} b_{ij} X_i X_j \qquad (3.3)$$

where, Y is the response variable or the degree of hydrolysis; $b_0$is constant; $b_i$represents the regression coefficient for linear effect; b_{ii}represents the quadratic coefficient and $b_{ij}$is the interaction coefficient.

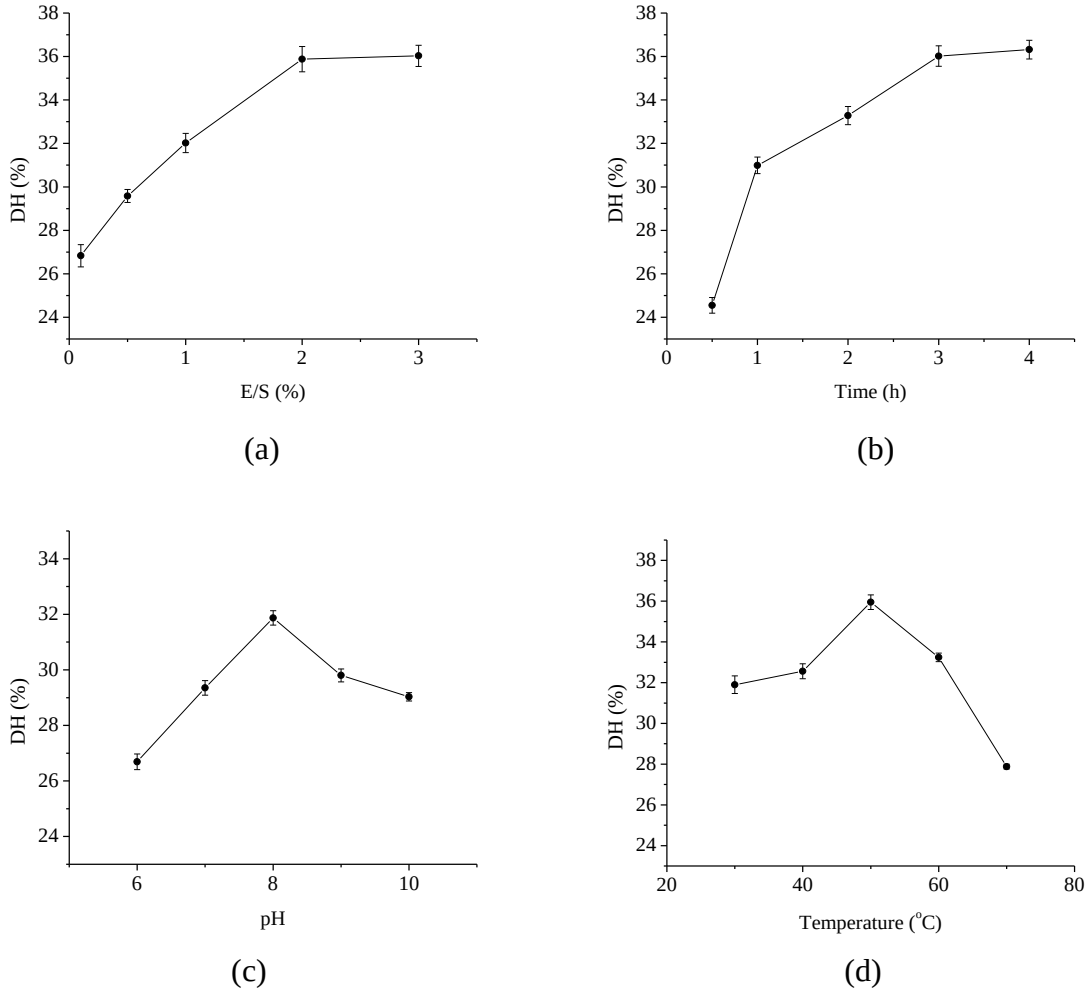


Figure 3.1 Main effect plots of OFAT experiments on (a) DH vs E/S ratio, (b) DH vs hydrolysis time, (c) DH vs initial pH, and (d) DH vs hydrolysis temperature

Table 3.1 Enzymatic hydrolysis variables and respective levels for composite central design

		Range and levels				
Independent variable	X_i	-1.41	-1	0	1	1.41
E/S ratio (%)	X_1 (A)	0.19	0.5	1.25	2	2.31
Hydrolysis time (h)	X_2 (B)	0.38	1	2.5	4	4.62
Initial pH	X_3 (C)	6.59	7	8	9	9.41
Temperature (^{o}C)	X_4 (D)	35.86	40	50	60	64.14

3.2.2.2 ANN Design

In general, ANN is a computational framework consisting of neurons and connections. Neurons are simple processing units that are grouped into layers and connected by weighted relations. Each network has an input layer and an output layer. There are one or more layers between them named hidden layers (Sarkar et al., 2009). In this study, feedforward neural network with one hidden layer trained by the backpropagation algorithm was selected. Both the experimental results of OFAT design and CCD design were used as the modeling inputs. The performance was calculated by the MATLAB (R2012b) neural network toolbox.

The numbers of neurons in the input and output layers were 4 and 1, respectively, according to the selected factors and process response. The input and hidden layers were assigned a sigmoidal transfer function which was mostly used for non-linear relationships:

$$f(x) = \frac{1}{1 + e^{-x}} \qquad (3.4)$$

The output layer activation function was linear. All the experimental data were normalized to the 0-1 range. The normalization function was shown as follows:

$$a_i = \frac{A_i - A_{min}}{A_{max} - A_{min}} \qquad (3.5)$$

where, $a_i$are normalized data; A_i are experimental data; A_{max} and A_{min} are the maximum and minimum values of the datasets.

For the determination of the number of hidden neurons, mean square error (MSE) was adopted

as the performance function of the neural network:

$$\mathrm{MSE} = \frac{\sum_{i=1}^{i=N}\left(y_{i,pred} - \ y_{i,exp}\right)^2}{N} \qquad (3.6)$$

where, N is the number of data; $y_{i,pred}$ stands for the network predicted result; $y_{i,exp}$ is the experimental response and i is the index of data.

To optimize the number of hidden neurons, each topology was employed with the trial-and-error method to improve the generalization capability. The number of hidden neurons started from 2, which was equivalent to half of the total number of input and output neurons (Abakarov et al., 2011). The experimental datasets were then divided into training, validation and testing subsets and fed to the ANN structure. A model was fitted to represent the experimental data. At last, the importance of each input variable to the response was calculated by the following equation (Garson, 1991):

$$I_j = \frac{\sum_{m=1}^{m=N_h}((|W_{jm}^{ih}| \ / \sum_{k=1}^{N_i}|W_{km}^{ih}|) \ \times |W_{mn}^{ho}|)}{\sum_{k=1}^{k=N_i}\{\sum_{m=1}^{m=N_h}(|W_{km}^{ih}| / \sum_{k=1}^{N_i}|W_{km}^{ih}|) \ \times |W_{mn}^{ho}|\}} \qquad (3.7)$$

where, I_j is the relative importance of the jth input factor on the output response; N_i and N_h are the numbers of input and hidden neurons, respectively; W_s are the connection weights between layers; i, h and o represent input, hidden and output layers, respectively; k, m and n represent input, hidden and output neurons, respectively.

3.2.3 Use of Shrimp Waste Hydrolytes for Microbial Growth

3.2.3.1 Microorganisms and Medium

Bacillus *subtilis* N3-1P screened from a petroleum hydrocarbon contaminated sample in coastal Newfoundland was selected for microbial growth. A production medium (PM) composed of $MgSO_4$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.05 g; KH_2PO_4, 3.4 g; $K_2HPO_4 \cdot 3H_2O$, 4.4 g; $(NH_4)_2NO_3$, 1 g; $FeCl_3$, 0.05 g; Glucose, 1g; NaCl, 26 g L^{-1} of distilled water, with 3% (v/v) n-hexadecane were employed for inoculation (Cai et al., 2014). A modified PM for bacteria cultivation contained 4 g L^{-1} shrimp waste hydrolysate in substitution of $(NH_4)_2NO_3$.

3.2.3.2 Inoculation and Cultivation

The bacteria were first grown in PM at 27 ^oC for 48 hours in 250 mL Erlenmeyer flasks. Then, 200 μL of the culture media were inoculated to 20 mL modified medium in 50 mL Erlenmeyer flasks, respectively. Cultivation was conducted at 27 ^oC at 200 rpm in a shaking incubator.

3.2.3.3 Measurement of Bacterial Growth

The optical density (OD_{600}) of samples was employed as the index of bacterial growth (Martone et al., 2005; Safari et al., 2012; Taskin and Kurbanoglu, 2011). Absorbance was measured at λ=600 nm using a UV-Visible spectrophotometer. Before being determined OD_{600}, all culture media were shaken for 5 s to make the media homogeneous. The

absorbance of inocula with sterile peptone water was adjusted to 0 as blank value. The measurement of OD_{600} was triplicated by sampling three times. The result was the average of three replicates.

3.3 Results and Discussion

3.3.1 CCD Experiments

Residual analysis was first conducted. It can be seen from Figure 3.2 that the normal plot gives a good linear correlation. Figure 3.3 shows a good residual vs predicted value plot with points randomly scattered. Figure 3.4 displays a good residual vs run value plot with points randomly scattered. There is no obvious trend in the plot. In Figure 3.5, the points are randomly scattered along the 45 degree line and there are no groups of points falling only above or under the line, which indicate good prediction. Figure 3.6 exhibits the externally studentized residuals. There is no outlier indicating the runs are well fitted to the model. Figure 3.7 demonstrates the difference in beta coefficients of the model. It can be seen that there are no excessive values for all the runs, representing that all the runs were good to fit.

Table 3.2 CCD design and the response

Standard order	Actual run	Code level of variables				Response
		X_1:E/S ratio (%)	X_2:hydrolysis time (h)	X_3:initial pH	X_4:temperature (^oC)	DH(%)
1	19	-1	-1	-1	-1	30.33%
2	5	1	-1	-1	-1	30.89%
3	24	-1	1	-1	-1	38.53%
4	11	1	1	-1	-1	39.45%
5	22	-1	-1	1	-1	30.85%
6	8	1	-1	1	-1	33.14%
7	15	-1	1	1	-1	41.88%
8	23	1	1	1	-1	46.16%
9	13	-1	-1	-1	1	38.89%
10	4	1	-1	-1	1	33.31%
11	10	-1	1	-1	1	44.45%
12	3	1	1	-1	1	33.83%
13	25	-1	-1	1	1	36.92%
14	12	1	-1	1	1	38.79%
15	17	-1	1	1	1	42.10%
16	14	1	1	1	1	49.27%
17	26	-1.41	0	0	0	35.94%
18	29	1.41	0	0	0	42.48%
19	6	0	-1.41	0	0	31.28%
20	1	0	1.41	0	0	42.61%
21	16	0	0	-1.41	0	41.86%
22	9	0	0	1.41	0	50.53%
23	2	0	0	0	-1.41	36.97%
24	18	0	0	0	1.41	45.44%
25	7	0	0	0	0	41.43%
26	27	0	0	0	0	40.64%
27	20	0	0	0	0	44.12%
28	21	0	0	0	0	43.25%
29	28	0	0	0	0	44.66%

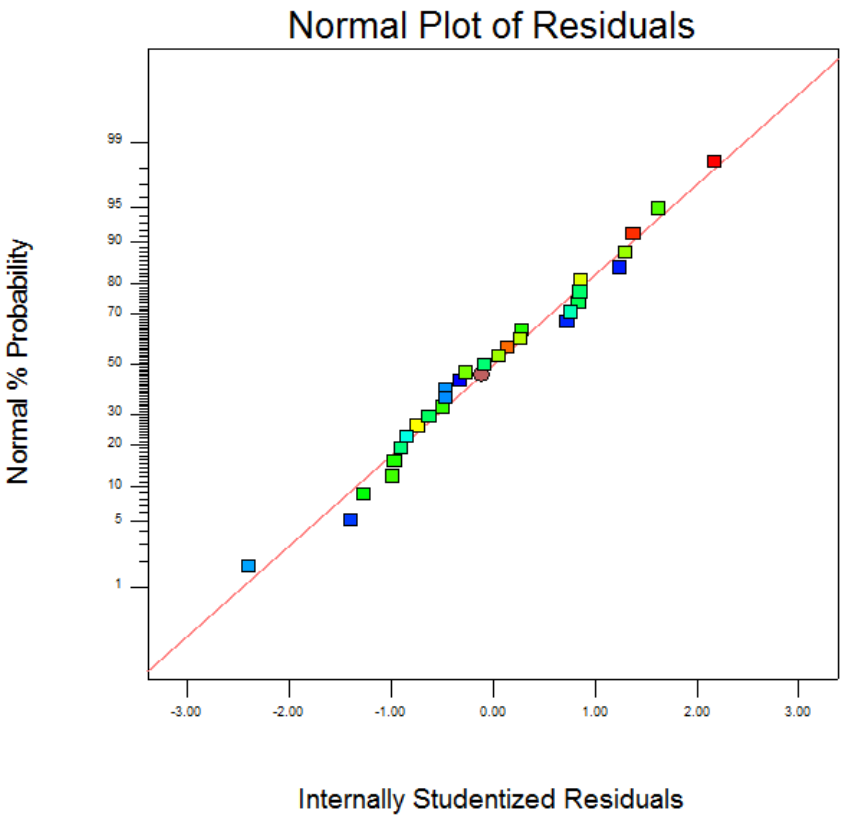


Figure 3.2 Normal plot of residuals

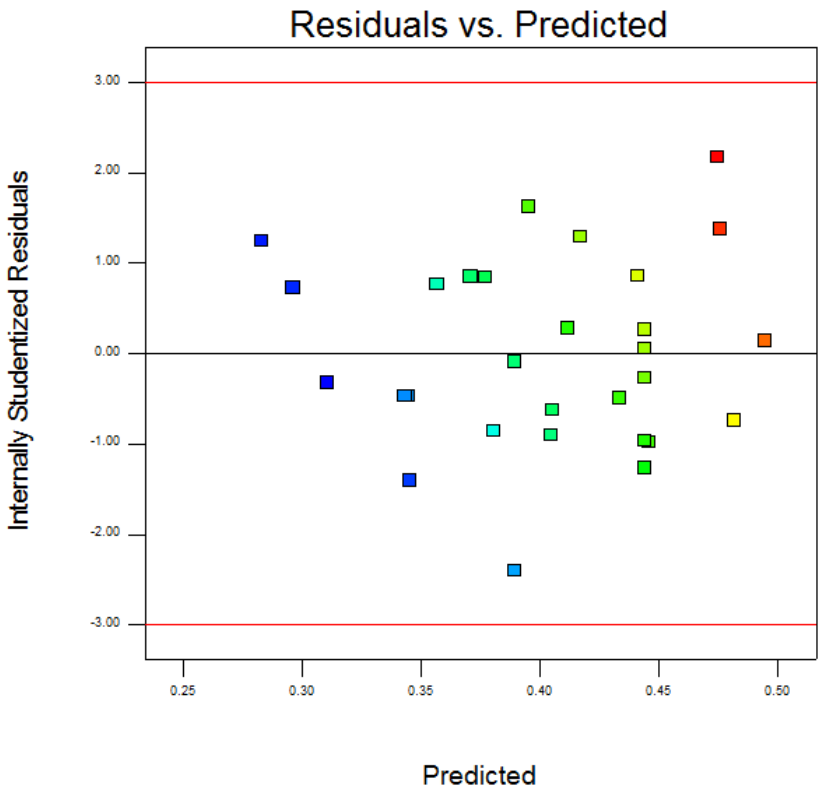


Figure 3.3 Residuals vs predicted value

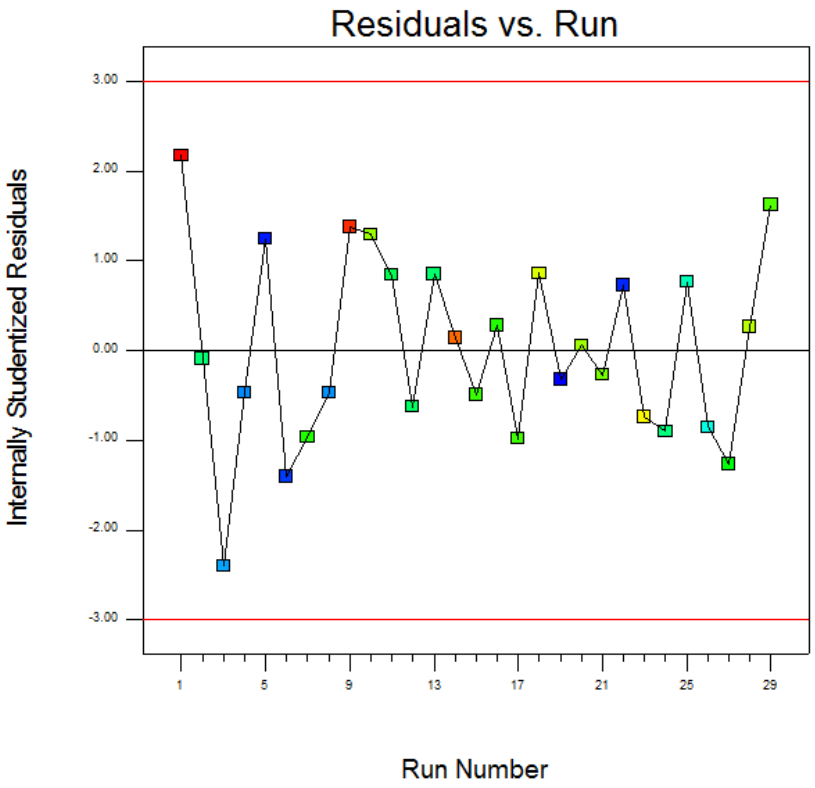


Figure 3.4 Residuals vs. run

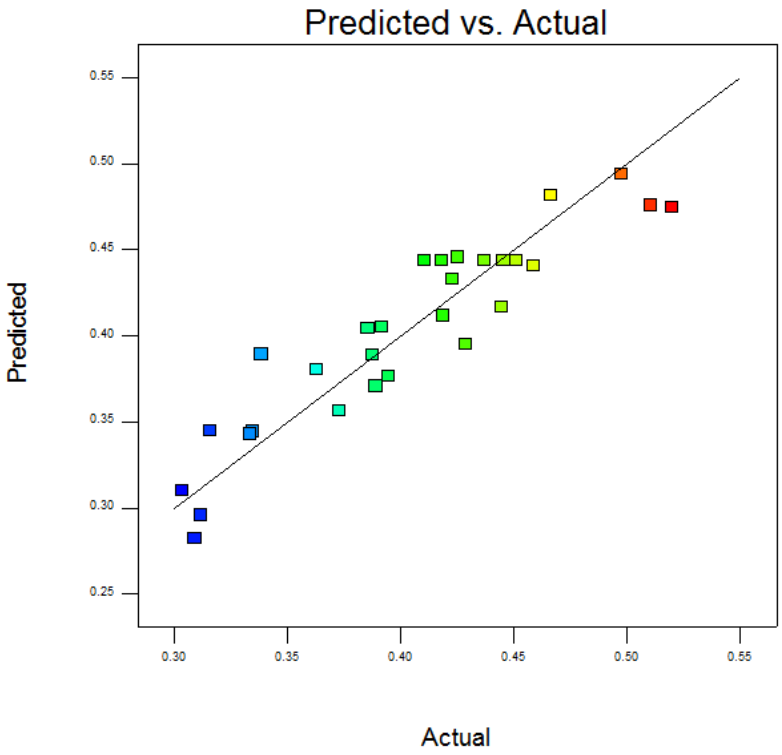


Figure 3.5 Predicted vs. actual

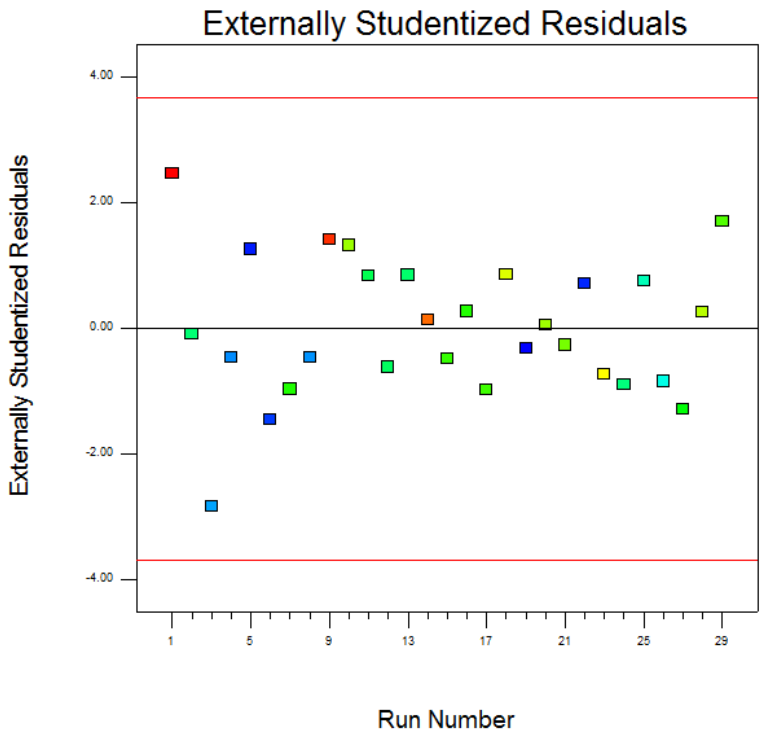


Figure 3.6 Externally studentized residuals

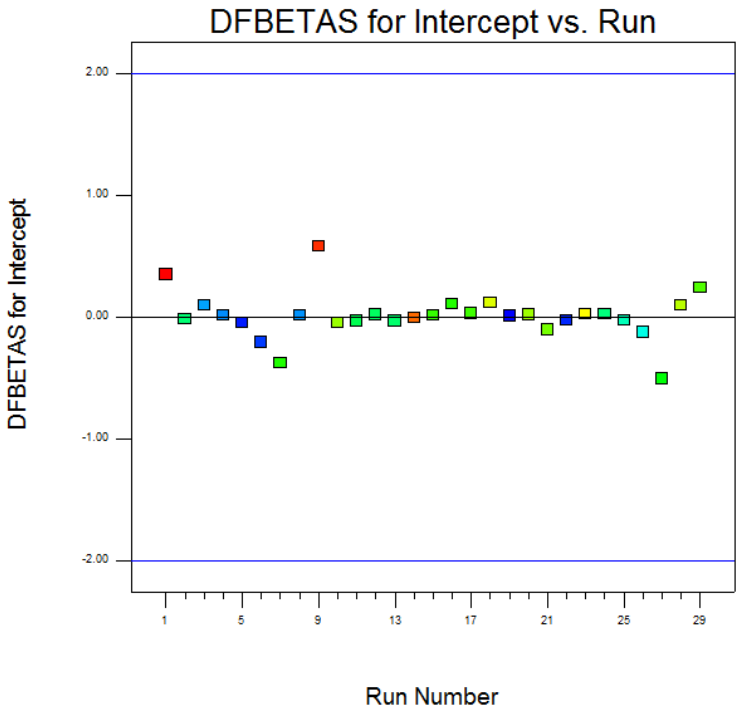


Figure 3.7 Difference in beta coefficients (DFBETAS)

3.3.1.1 RSM Optimization

The results of the CCD experiments are shown in the Table 3.2. The experimental data analysis was carried out via Design-Expert version 8.0 (Stat-ease, U.S.A.). Analysis of variance (ANOVA) is presented in Table 3.3. The 3D response surface and the 2D contour plots of the response are shown in Figure 3.8. The plots depicted the influences of two independent variables on the DH while the other two were fixed at their central levels. As shown in Table 3.3, the value of "Prob > F" of the model was way less than 0.05, indicating that the model was significantly predictive at the 0.05 significance level. The lack of fit analysis was used to test the fitness of the model. The *F*-value of the lack of fit was 3.75, which demonstrated that the lack of fit was not significant as compared to the pure error ($P>0.05$). There was a 10.45% chance that a "Lack of Fit *F*-value" this large could occur due to noise. The model was able to fit the experimental data. The adequate precision test measured the signal to noise ratio. In this case, a ratio of 12.871 was obtained. This ratio was greater than 4, which indicated an adequate signal such that the model could be used to navigate the design space. With the application of RSM, the experimental data was fitted to a quadratic polynomial model to describe the behavior of the system. The final response surface regression model was obtained as:

$$Y = 0.44 + 0.005181\ X_1 + 0.046\ X_2 + 0.023\ X_3 + 0.018\ X_4$$

$$+ 0.019\ X_1X_3 - 0.031\ X_1^2 - 0.020\ X_2^2 \qquad (3.8)$$

where, Y, X_1, X_2, X_3, X_4 were the degree of hydrolysis, E/S ratio, hydrolysis time, initial pH and

temperature, respectively.

The term “Predicted *R*-Squared” agreed well with the “Adjusted *R*-squared” (data not shown). These results suggested that the experimental results and the predicted values were in good agreement. Then, E/S ratio, hydrolysis time, initial pH and temperature were set in determined ranges (i.e. E/S ratio: 0.5% - 2%; hydrolysis time: 1 h to 4 h; initial pH: 7 - 9; hydrolysis temperature: 40 ^{o}C to 60 ^{o}C), while maximum of the response (DH) was adjusted as the goal. The optimal solution yielded by the model was: E/S ratio at 1.64%, hydrolysis time at 3.59 h, initial pH at 9 and temperature at 52.57 ^{o}C. The maximal DH was predicted to be 49.25%. Similar results of enzymatic hydrolysis of other kinds of proteins were reported in literature. Bhaskar et al.(2008) optimized enzymatic hydrolysis of visceral waste proteins of Calta by RSM with a maximum DH close to 50%. Dey and Dora (2014) optimized enzymatic hydrolysis of shrimp waste proteins with a maximum DH of 33.13%.

3.3.1.2 Effects of Parameters

Factorial effects with values of "Prob > F" less than 0.05 were considered to be significant. As shown in table 3.3, hydrolysis time (X_2), initial pH (X_3) and hydrolysis temperature (X_4) had relatively higher significant linear effects (P<0.05) on DH when compared with the E/S ratio (X_4). In addition, the quadratic effects X_1^2 and X_2^2 and the interaction effect between E/S ratio and initial pH (X_1X_3) were significant (P<0.05). All the other terms were not significant (P>0.05). Therefore, the linear effects of hydrolysis time, initial pH, hydrolysis temperature (X_2, X_3, X_4), the interaction effect between E/S ratio and initial pH (X_1X_3), and the quadratic

effect of E/S ratio and hydrolysis time (X_1^2, X_2^2) were most influential. If the experiments were conducted in the traditional method (i.e., OFAT), the significance of the interactions and quadratic effects would have been missed. Although E/S ratio did not have a strong linear effect on DH, it had a significant negative quadratic effect on the degree of hydrolysis. In response surface methodology, higher degree of polynomial means more approximation. When the region of interest is smaller, the better approximation can be obtained. It usually suffices to the quadratic level. In this study, the region of interest was curtailed to a small region based on the outputs of the OFAT experiment such that the effect only sufficed to quadratic level. This finding meant that a closely approximated value would be predicted by the model.

Silpradit et al. (2010) mentioned the significant quadratic effect of E/S ratio when they tried to optimize rice bran protein hydrolysate production using alcalase. It was also reported in the research of Dey and Dora (2014). As shown in Figure 3.8, at the high level of initial pH, the effect of E/S ratio had a positive effect on the response; while at the low level of initial pH, the effect of E/S ratio had a negative effect. This indicated a relatively significant interaction between E/S ratio and initial pH, which was in accordance with the ANOVA results. Similar results were also found in the study of Bhaskar et al. (2008), in which Catla waste was hydrolysed with alcalase. It was observed that hydrolysis time, pH and temperature had relatively higher significant effect on DH when compared with the E/S ratio.

Table 3.3 Analysis of variance (ANOVA) for response surface reduced quadratic model

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	p-value Prob > F
Model	0.0815	7	0.0116	11.8573	< 0.0001
A-E/S ratio	0.0005	1	0.0005	0.5468	0.4678
B-time	0.0421	1	0.0421	42.8990	< 0.0001
C-initial pH	0.0103	1	0.0103	10.4925	0.0039
D-temperature	0.0067	1	0.0067	6.8054	0.0164
AC	0.0058	1	0.0058	5.9145	0.0240
A^2	0.0091	1	0.0091	9.2363	0.0062
B^2	0.0037	1	0.0037	3.7858	0.0652
Residual	0.0206	21	0.0010		
Lack of Fit	0.0194	17	0.0011	3.7538	0.1045
Pure Error	0.0012	4	0.0003		
Cor Total	0.1021	28			

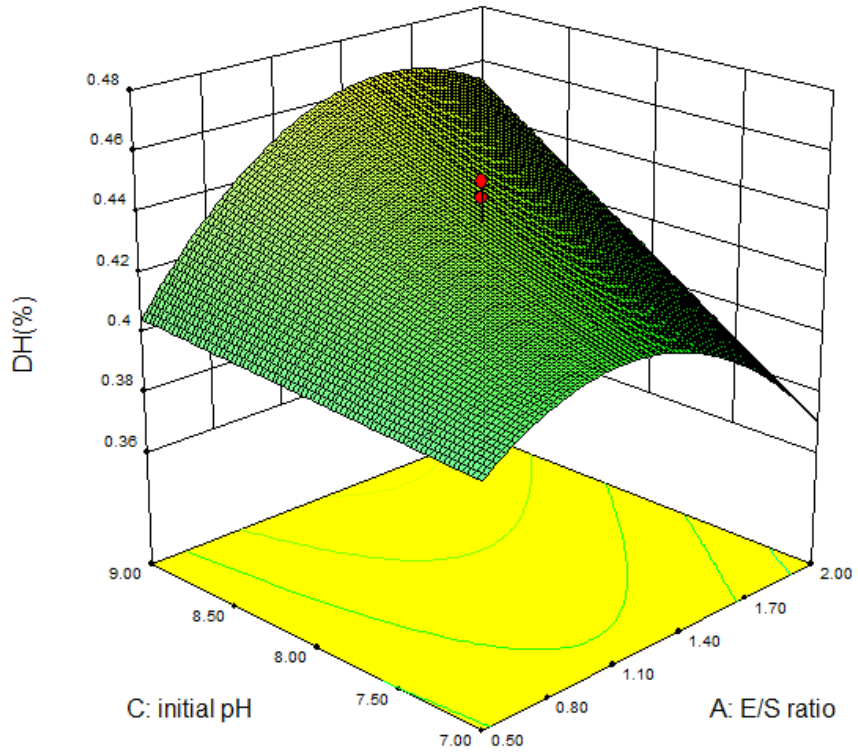


Figure 3.8 Response surface plots depicting the interaction effects of independent variables E/S ratio and initial pH on DH in enzymatic hydrolysis of shrimp waste

3.3.2 ANN Model Development

Fifty experimental datasets were used as input matrix with corresponding response as target matrix to build the neural network. Feedforward type with one hidden layer trained by the backpropagation algorithm was carried out.

3.3.2.1 Optimization of the Hidden Nodes Number

Through the trial-and-error method, the number of nodes in the hidden layer was confirmed during training. With the aim to minimize MSE, the number of neurons in hidden layer with lower MSE was desired. If the number is too small, the network may not have enough accurate capacity of predicting. If the number is too large, the model may be over-fitted and result in a slow calculation speed (Abakarov et al., 2011; Jing et al., 2014, 2015). To avoid random initialization of the weights by the software, each topology was repeated with 10 times and MSE was expressed as the mean. Figure 3.9 shows the results of network MSE versus the number of nodes in the hidden layer. It was indicated that the network error performance reached its minimum and became stable when 11 nodes were contained in the hidden layer. Therefore, 11 neurons in the hidden layer were adopted and used in further network development. The optimized neural network structure was derived as shown in figure 3.10.

3.3.2.2 Training, Validation and Testing of the Model

The experimental datasets displayed in Section 3.1 were used as inputs to the ANN model. Fifty datasets were divided into training (70%), validation (15%) and test (15%) subsets. An

inverse range scaling was carried out on the outputs to compare the model predicted results with experimental responses. Figure 3.11 illustrates the scatter regression plots of the ANN model predicted values in comparison with experimental values. The overall MSE for all datasets was 0.0000761. Figures 3.11 (a), (b) and (c) showed that the training, validation and test subsets all exhibited a good linear fit (with correlation coefficient R = 0.98, 0.99 and 0.96, respectively). In figure 3.11 (d), the line generated by ANN model closely agreed with the perfect fit line (45 degree diagonal). The correlation coefficient was 0.98, indicating that the ANN model results well fitted the experimental results. Therefore, the developed ANN model could provide high accuracy in predicting the DH of the shrimp waste hydrolysis process.

3.3.2.3 Contribution of Each Input Variable on DH

After model development, connection weights between layers were obtained for determining the relative importance of each input variable on DH. Through calculation by Equation (7), the relative importance of four input variables on the output was obtained (Table 3.4). Among the four variables, it was clear that hydrolysis time was the most influential factor in the enzymatic hydrolysis process, with relative importance of 32.79%. It was followed by initial pH and temperature, with similar relative importance of 28.98% and 26.79%, respectively. E/S ratio had the least influence. According to the ANOVA results (Table 3.3), the F values of four factors represented the relative importance of each input variable. It can be seen that hydrolysis time had the highest F value among four input variables. Initial pH and hydrolysis temperature were the next and had similar F value. E/S ratio had the smallest F value. The results obtained

from the ANN model were in good agreement with those obtained from ANOVA.

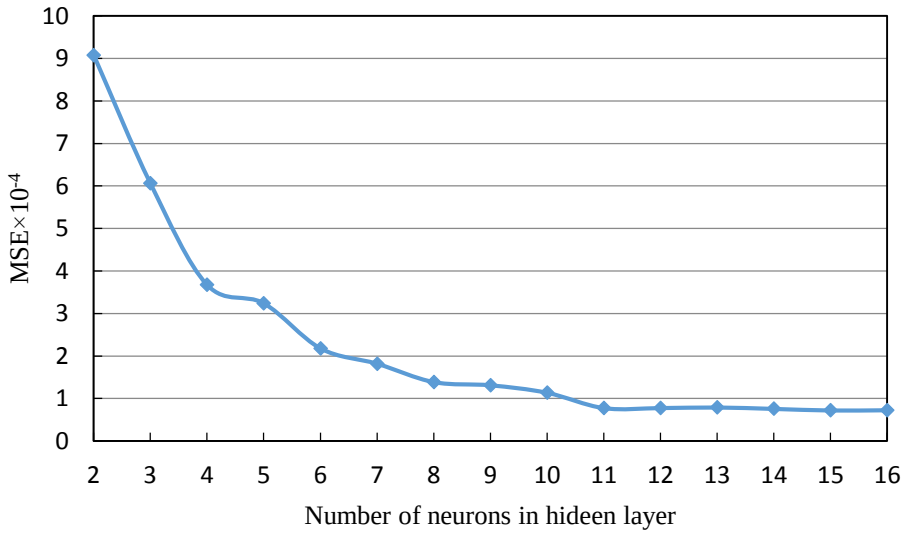


Figure 3.9 The network MSE v.s. the number of neurons in the hidden layer

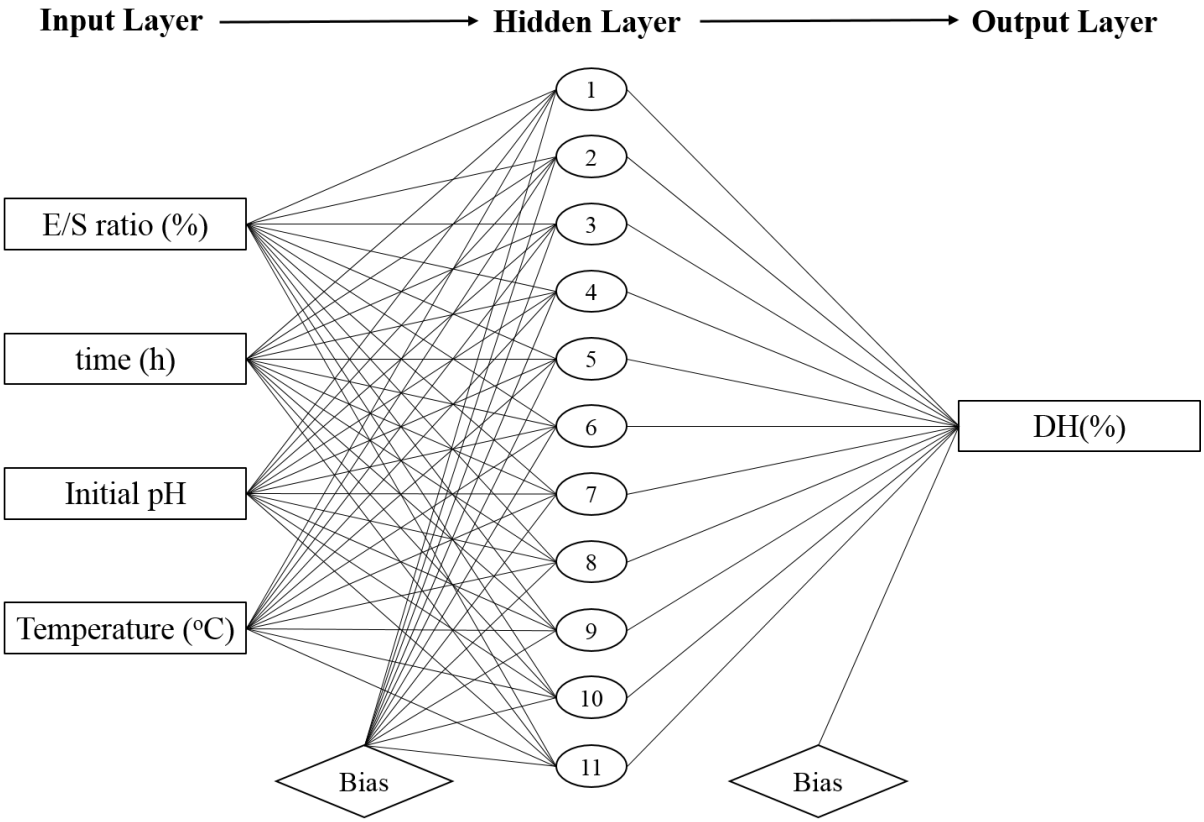


Figure 3.10 Optimized structure of the proposed ANN model

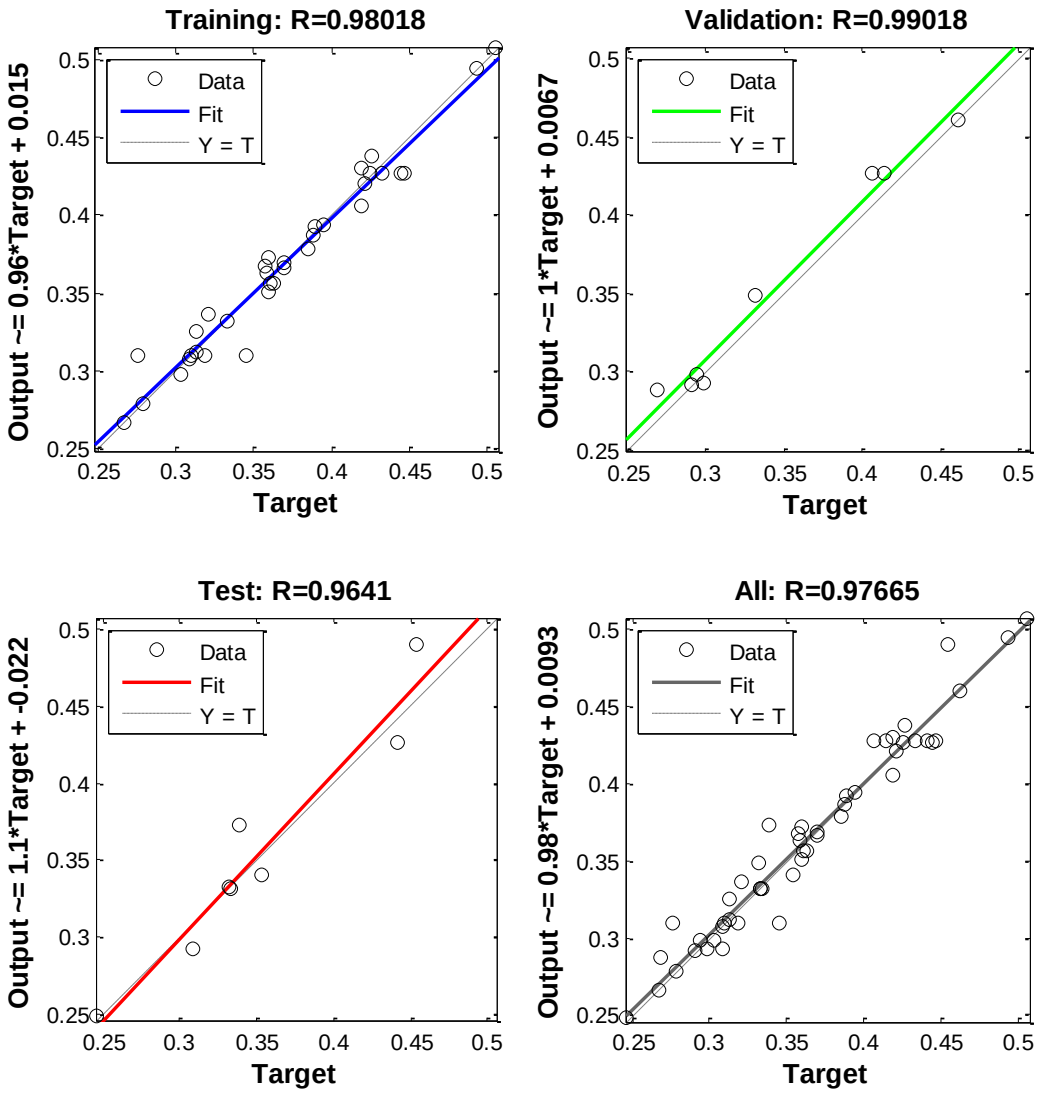


Figure 3.11 The scatter plots of ANN model predicted value in comparison with experimental values for (a) training; (b) validation; (c) testing and (d) all data sets

Table 3.4 Relative importance of input variables on the DH of shrimp waste

Input variable	Relative importance
(A) E/S ratio (%)	0.1143
(B) Hydrolysis time (h)	0.3279
(C) Initial pH	0.2679
(D) Temperature (^{o}C)	0.2898

3.3.3 RSM and ANN Model Verification

The validation experiments were conducted under the same optimal conditions (Table 3.5). The results were found to be 48.83 ± 0.30%, which were in a good agreement with the RSM-predicted results. In addition, the optimum values of the parameters obtained from RSM were fed as inputs to ANN and 48.66% of the DH was obtained as the output. These results show that both RSM and ANN methods can predict the experiment results with relatively high accuracy.

3.3.4 N3-1P Growth Curves under Three Different Conditions

The growth curves for Bacillus *subtilis* N3-1P are presented in Figure 3.12. The bacteria did grow well on a medium with three different DH of shrimp waste protein hydrolysate. For the first 36 hours, the growth curves of all three different groups displayed as the log phase. The bacterial growth of three groups all then became slow after 36 hours. The bacteria cultivated with 20% DH shrimp waste protein hydrolysate first reached the stationary phase around 72 hours. The bacteria cultivated with 30% and 40% DH shrimp waste protein hydrolysate then reached the stationary phase around 96 hours. At the beginning of each run, the amount of shrimp waste protein hydrolysate used in three different groups was the same, The group with a medium containing 40% DH of shrimp waste protein hydrolysate led to a higher cell density than the one with 30% DH. Moreover, the group with 30% DH resulted in a higher cell density than that with 20% DH. This suggested that shrimp waste protein hydrolysate with a higher DH value as nitrogen source can better facilitate microbial growth. The results were in line with the

findings of Klompong et al. (2012). They found for *S. aureus* and *E. coli*, groups containing higher DHs of yellow stripe trevally usually led to a better microbial growth.

3.4 Summary

Shrimp waste has been a major concern in coastal Newfoundland. Using enzymatic hydrolysis, the shrimp waste can be recycled and applied as a useful nutrient source for microbial growth. This study integrated RSM with ANN in the modeling and optimization of the hydrolysis process of shrimp waste. Four key factors, including E/S ratio, hydrolysis time, initial pH value and hydrolysis temperature were studied as input variables that may influence the DH. The results from RSM showed that the hydrolysis process was mainly influenced by four individual factors and the interaction effect between E/S ratio and initial pH value. DH was maximized at 48.83 ± 0.30% when four key factors were set as 1.64%, 3.59 hours, 9 and 52.57 ^{o}C, respectively. A feedforward 3-layer ANN model was developed using the experiment data to simulate this complex system. The optimal values of independent variables obtained from RSM were fed into the developed ANN model, and the results were in accordance with those from RSM. Results obtained from the validation experiments agreed well with modeling outputs from both models.

Table 3.5 Optimal values of the hydrolysis process parameters for maximum DH

Parameter	Optimum value		
	Experimental	RSM-predicted	ANN-predicted
E/S ratio (%)	1.64	1.64	1.64
Hydrolysis time (h)	3.6	3.59	3.59
Initial pH	9	9	9
Temperature (^{o}C)	52.6	52.57	52.57
DH	48.83 ± 0.30%	49.25%	48.66%

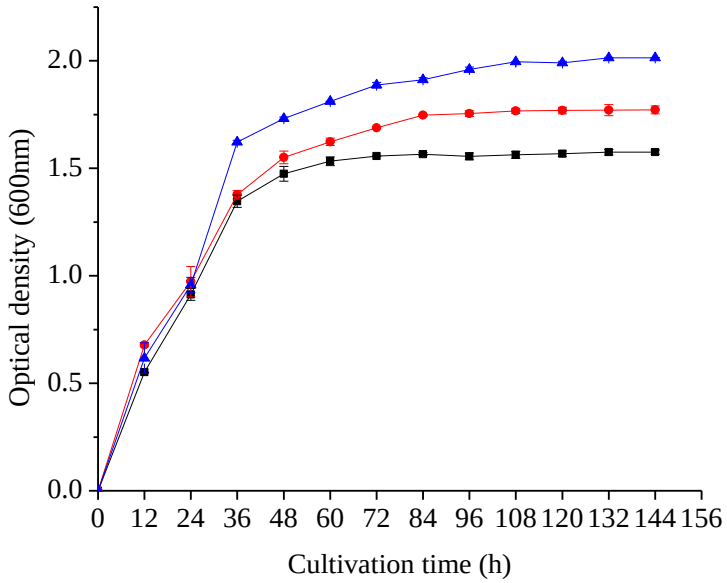


Figure 3.12 Growth curve of Bacillus *subtilis* N3-1P during cultivation in modified PM containing shrimp waste hydrolysate with different DHs: 20% (■), 30% (●), 40% (▲). Bars present standard deviations were from triplicate measurements.

CHAPTER 4

GENERATION OF SHRIMP WASTE BASED DISPERSANT FOR OIL SPILL RESPONSE[2]

[2] The research findings in Chapter 4 has been submitted to Waste Management (Impact factor: 3.829) for publication.

4.1 Introduction

Dispersants have been widely used as an effective option for oil spill response (Chapman et al., 2007; Judson et al., 2010; Kujawinski et al., 2011; Lessard and DeMarco, 2000). They can help break down the spilled oil into small droplets, thereby enhance the consumption of oil by microbial activities and other natural processes. However, there have been concerns about the toxicity and metabolism of existing chemical surface-active compounds to the environment (Goodbody-Gringley et al., 2013; Pietroski et al., 2015). Therefore, environmentally friendly alternatives with lower toxicity are much desired (Infante et al., 2004).

Protein hydrolysis products have recently been recognized to have excellent emulsifying capabilities (Arboleda et al., 2014; Singh and Dalgleish, 1998; Zhao and Xiong, 2015). Due to their low-toxicity, these natural products containing surface-active components could be potentially applied as green dispersants (Wurm and Weiss, 2014). Relatively high protein-containing resources can therefore be utilized in this regard. As there are large quantities of shrimp waste in Newfoundland, the shrimp waste with high protein content and abundance can be utilized. No information is available regarding the use of shrimp waste hydrolysate as dispersant.

The aim of this study was thus to explore the possibility of generating shrimp waste based dispersants and to examine their effectiveness on dispersing crude oil. The dispersant was obtained through a shrimp waste Alcalase-hydrolysis process. The hydrolysis process was optimized. Stability test, measurement of CMC and emulsification activity test were then

conducted to analyze the generated product. After sample analysis, four types of solvents were mixed with the hydrolysate product and examined for dispersant generation. One of the solvents was selected based on the effectiveness and toxicity of the associated dispersants to generate the final dispersant product. The effects of salinity, mixing energy and temperature on the dispersion of ANS crude oil using the generated dispersant were discussed. The DE on three kinds of crude oil including ANS crude oil, PBC and Arabian Light crude oil (ALC) were evaluated.

4.2 Materials and Methods

4.2.1 Chemicals and Materials

Alcalase 2.4L (≥2.4 U/g), dichloromethane and anhydrous sodium sulfate were obtained from Sigma Aldrich (U.S.). Instant ocean sea salt was purchased from Instant Ocean Company (U.S.A.) for making artificial seawater. Three types of crude oil (ANS, ALC and PBC) were provided by the research partner.

Shrimp waste containing heads, shells and tails of northern pink shrimp (*Pandalus borealis*) was purchased from a local fish market in Newfoundland, Canada as the starting materials. The shrimp waste was grounded in a food processor (Black & Decker Model FP2700SC) and kept frozen at -18 ^{o}C.

4.2.2 Experimental Methods

4.2.2.1 Optimization of Enzymatic Hydrolysis of Shrimp Waste

The hydrolysis procedure for treating shrimp waste was adopted from the study of Dey and Dora (2014) with modifications. The grounded shrimp waste was thawed for one hour at room temperature and suspended (1:1, w/v) in distilled water in each baffled colonial flask. The mixture was heated in a water bath at 90 ^{o}C for 15 min to inactivate the indigenous hydrolyzing enzyme. Aliquots of Alcalase enzyme was added into each flask when the mixture was cooled to room temperature. The flasks were put into a temperature controlled water bath with a shaking rate at 110 rpm. The enzyme was inactivated by raising temperature to 90 ^{o}C and maintained it for 15 min. The mixtures were then cooled to room temperature again and subsequently centrifuged at 10,000 rpm for 15 min. The supernatant was collected and freeze dried to obtain dry powder.

A three factor face-centered CCD and RSM were employed to optimize the hydrolysis process. Three factors including hydrolysis temperature, enzyme/substrate ratio and hydrolysis time were investigated. The effectiveness of dispersing Alaska North Slope crude oil was used as a response for system optimization.

The CCD was comprised of 20 treatments including 2^3 factorial points, 6 axial points and 6 replicates at the center points. The DE was used as the response for the combination of the independent variables as shown in Table 4.1. Randomized experimental runs were adopted to minimize the effects of unexpected variability in the observed response.

Table 4.1 CCD design and the response

Standard order	Actual run	Levels of variables			Response
		Temperature (ºC)	E/S ratio (%)	Time (min)	Dispersant efficiency (%)
20	1	50	1.505	90	78.88
13	2	50	1.505	30	78.99
16	3	50	1.505	90	81.08
9	4	30	1.505	90	77.61
11	5	50	0.01	90	54.55
10	6	70	1.505	90	78.38
2	7	70	0.01	30	30.7
18	8	50	1.505	90	67.52
19	9	50	1.505	90	66.35
8	10	70	3	150	71.67
7	11	30	3	150	74.22
14	12	50	1.505	150	79.7
1	13	30	0.01	30	38.3
4	14	70	3	30	74.72
5	15	30	0.01	150	37.99
6	16	70	0.01	150	64.28
12	17	50	3	90	80
15	18	50	1.505	90	70
3	19	30	3	30	50.7
17	20	50	1.505	90	72

4.2.2.2 Functional Properties of Hydrolysate Sample

Under the optimized enzymatic hydrolysis conditions, a hydrolysate sample was obtained and its properties were examined. The functional parameters were listed in below.

(1) Stability

The freeze dried hydrolysate sample was regarded as a crude surfactant and dissolved in distilled water. The stability of the dissolved sample was then investigated considering three environmental factors including salinity, pH and temperature. The salinity had 6 levels ranging from 0 psu to 50 psu. The pH value had 5 levels ranging from 3 to 11. The temperature had 5 levels ranging from 0 ^{o}C to 80 ^{o}C. The reduced surface tension was used to evaluate the stability under each set of conditions. Surface tension was measured with a surface tensiometer (DuDouy Interfacial Tensiometer, CSC Scientific). The average standard accuracy of surface tension for three measurements during calibration was around 0.5 dynes/cm.

(2) CMC

Critical micelle concentration (CMC) is the concentration of a surfactant at which micelles start to form (Carrillo et al., 1996). Beyond the CMC, the surface tension becomes constant although the concentration of a surfactant increases (Zajic and Gerson, 1979). To determine CMC, surface tension was plotted as a function of surfactant concentration. The curve's slope exhibited a sudden change at the CMC. The CMC test usually helps to determine the saturated concentration of surfactant concentration so that the solution surface property won't change

beyond this point (Cai et al., 2011).

(3) Emulsification Activity

The emulsification activity on seven types of oil was evaluated. Each sample was prepared by mixing 5 mL hydrolysate product and 5 mL oil with a vortex shaker for 2 min and leaving to stand for 24 hours. The calculation of emulsification index (EI_{24}) was done using the following equation:

$$EI_{24} = H_{EL}/ H_S * 100\% \quad (4.1)$$

where, H_{EL} was the height of the emulsion layer and H_S was the height of the total solution.

4.2.2.3 Solvent Selection of Dispersant Generation Using Hydrolysate Sample

Four solvents including water, polyethylene glycol 400 (PEG 400), propylene plycol (PPG), and dimethyl sulfoxide (DMSO) were selected to mix with the hydrolysate sample (i.e., crude surfactant) respectively for generating dispersions. The ratio of the crude surfactant to each solvent was 1:1 (w/v). DE and acute toxicity were used as the parameters for solvent selection.

(1) Crude oil standard curve and BFT for DE measurement

Standard crude oil solutions were prepared for calibrating the ultraviolet spectrophotometer (UVS). 5μL, 10μL, 15μL, 20μL and 25μL of ANS crude oil were added using a syringe to 30

mL of dichloromethane (DCM), respectively. These ANS-DCM solutions were used as standard references representing DE of 20%, 40%, 60%, 80% and 100%. The procedure was also applied to ALC and PBC crude oil to generate ALC-DCM and PBC-DCM standard references. UVS was employed to measure the absorbance. The absorbance of the extracts was measured at three wavelengths: 340, 370 and 400 nm, respectively. The area under the absorbance vs. wavelength curve between 340 and 400 nm was regarded as the relative concentration of dissolved oil (Chandrasekar et al., 2006). It was determined by the following equation using the trapezoidal rule:

$$\text{Area} = \frac{(\text{Abs}_{340}+\text{Abs}_{370})\times 30}{2} + \frac{(\text{Abs}_{370}+\text{Abs}_{400})\times 30}{2} \quad (4.2)$$

DE could be calculated as the ratio of the area of dispersed oil to the area of total oil added to the system (equals to the corresponding volume of oil dissolved in DCM). The standard curve was plotted using the value of area to DE (not shown in this paper). The coefficient of determination was all larger than 0.99.

BFT for DE was modified from the method developed by Sorial et al. (2004). Firstly, in a baffled flask, 120 mL of artificial seawater was prepared by dissolving “Instant Ocean” sea salt at room temperature. 100 μL of crude oil was carefully added to the surface of seawater using a 100 μL pipette and dispersant was then added onto the center of oil slick. Next, the flask was shaken at a rotation speed of 200 rpm on an orbital shaker (Southwest Science Company). After shaking for 10 min, the flask was left to stand still for another 10 min. Then 2 mL of the mixture was discarded from a stopcock at the bottom of the flask before collecting 30 mL of the

sample into a 50 mL measuring cylinder. The 30 mL sample was then poured into a separatory funnel and extracted with 5 mL of DCM for three times. Anhydrous sodium sulfate was added into the extract to remove water that may be contained in solvent. At last, the extract was adjusted to a volume of 20 mL for further analysis. The seawater/oil mixture BFT was conducted as a quality control group, and the seawater/dispersants mixture BFT was conducted as a blank control group.

(2) Microtox acute toxicity test

Microtox® Model 500 analyzer was employed to determine the acute toxicity of dispersant to luminescent bacterium *Vibrio fischeri* according to the standard protocol for the Microtox® basic test. The diluent, osmotic adjusting solution (OAS) and reconstitution solution were purchased from Modern Water Company. The data were collected and analyzed by MicrotoxOmni® 4.1 software. Turbidity and color correction were conducted according to the method reported by Campisi et al. (2005). The half maximal effective concentration (EC_{50})-5 min and EC_{50}-15 min were obtained. EC_{50}-5 min means the concentration of a sample that causes a 50% decrease in the light emitted by the bacteria after 5 min of exposure (la Farré et al., 2001).

4.2.2.4 Performance of Produced Dispersant under Different Environmental Conditions

Once a solvent was selected, it was then mixed with the crude surfactant at a ratio of 1:1 (v:w) to obtain the dispersant product. DE of the produced dispersant using ANS crude oil was then

evaluated under multiple environmental conditions including different salinities, mixing energies and temperatures. The salinities had six levels ranging from 0 psu to 50 psu. Shaking speed was used to represent mixing energies, which had five levels ranging from 150 rpm to 250 rpm. The temperatures had five levels ranging from 4 ^{o}C to 80 ^{o}C. In addition, DE of the produced dispersant on dispersing three different crude oils at 4 ^{o}C and 22 ^{o}C were compared with those of Corexit 9500.

4.2.2.5 Statistical Analysis

Each test was repeated for three times. Each presented result was the average of three replicates. The precision of DE was determined by calculating the relative standard deviation (RSD). In this study, all the RSD values were less than 15%, implying that the obtained data are acceptable (Venosa et al., 2002).

4.3 Results and Discussion

4.3.1 Optimization of Enzymatic Hydrolysis

The results of the CCD experiments were shown in the Table 4.1. The experimental data analysis was carried out via Design-Expert version 8.0 (Stat-ease, U.S.A.). The 3D response surfaces of the response were shown in Figure 4.1. After ANOVA analysis (results not shown), enzyme/substrate ratio, hydrolysis time and hydrolysis temperature were significant factors on DE. The interaction effects among the factors were not significant. DE of Corexit 9500 on ANS was then determined as an optimization reference, which was 78 ± 1.2%. With a target range of

DE larger than 78%, 45 optimal combinations of three factors were obtained. Regarding of the enzyme's cost, the solution with the smallest use of enzyme was selected from the sets. The selected production conditions were E/S ratio at 1%, hydrolysis time at 2.23 h and temperature at 62.5 ^{o}C. The corresponding DE was 78.1 ± 1.0%.

4.3.2 Hydrolysate Sample Analysis

4.3.2.1 Stability

The stability of the selected hydrolysate product on surface tension reducibility was examined. The surface tension of water is around 72 dynes/cm at room temperature. The addition of the product could reduce the surface tension to a great extent. In this test, the concentration of the tested product was 100 g/L. Figure 4.2a displayed the effect of salinity on the surface tension reducibility of the product. The reduced surface tension remained stable through all tested levels of salinity. Figure 4.2b demonstrated the effect of pH value on the surface tension reducibility of the product. It can be seen that the product's surface tension reducibility decreased from 41 dynes/cm to 31 dynes/cm as pH value increased from 3 to 11. These results indicated that acidic environment could enhance the product's surface tension reducibility while alkaline environment could impair the capability of reducing surface tension. Figure 4.2c illustrated the effect of temperature on the surface tension reducibility of the product. Although the stability was relative low at 0 ^{o}C and 80 ^{o}C, the surface tension reducibility could still reach a value larger than 27.5 dynes/cm. These results suggested the stable surface tension reducibility of the product. It was beneficial for applications under different conditions of

temperature, salinities and pH, such as harsh ocean environments.

4.3.2.2 CMC

CMC is an important characteristic of a surfactant. It is defined as the concentration of a surfactant above which micelles form and all additional surfactant added to the system go to micelles.

The hydrolysis of protein can generate peptides that have shorter chains with excellent surface activity (Adjonu et al., 2014; Intarasirisawat et al., 2012). The surface tension reduction potential of such peptides has been documented in the literature. Innocente et al. (1998) reported proteose peptone 3 produced from the milk fat globule membrane protein could reduce air/water surface tension to as low as 32.11 dynes/cm. Kezwoń et al. (2015) found that moderately hydrolyzed collagen could lower air/water surface tension to 53.9 dynes/cm. In this study, the air/water surface tension reduction capability of shrimp waste based product was studied. The plot of surface tension against different shrimp waste based product concentration is presented in Figure 4.3. It shows that the surface tension decreased as the product's concentration increased, until reaching the lowest, 33.23 dynes/cm. While at this point, CMC was 19.9 g/L. According to the study of Gong et al. (2014), the solution of chemical dispersant Corexit EC9500A had a surface tension of 41.67 dynes/cm at CMC of 22.5 mg/L. This showed that shrimp waste based surfactant had an excellent capacity in lowering surface tension.

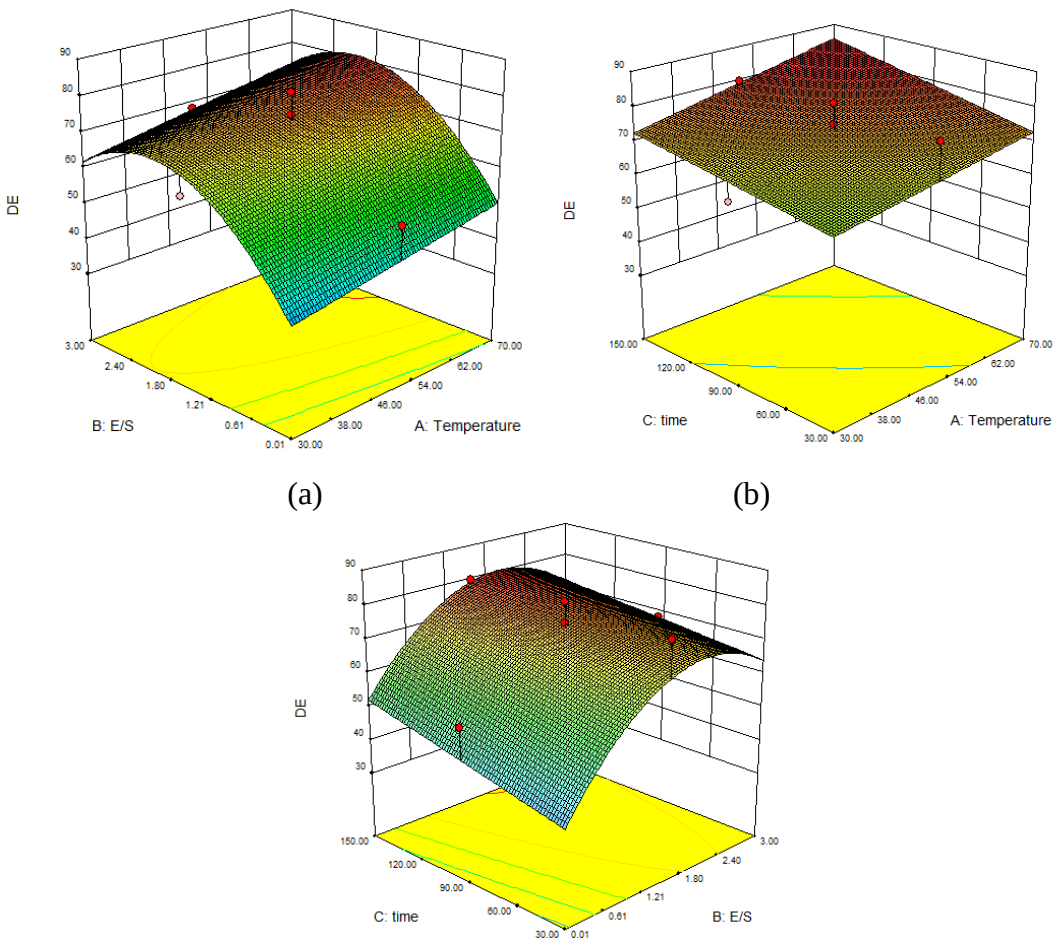


Figure 4.1 Response surface on different combination of two factors: (a) E/S ratio and temperature; (b) time and temperature; (c) time and E/S ratio

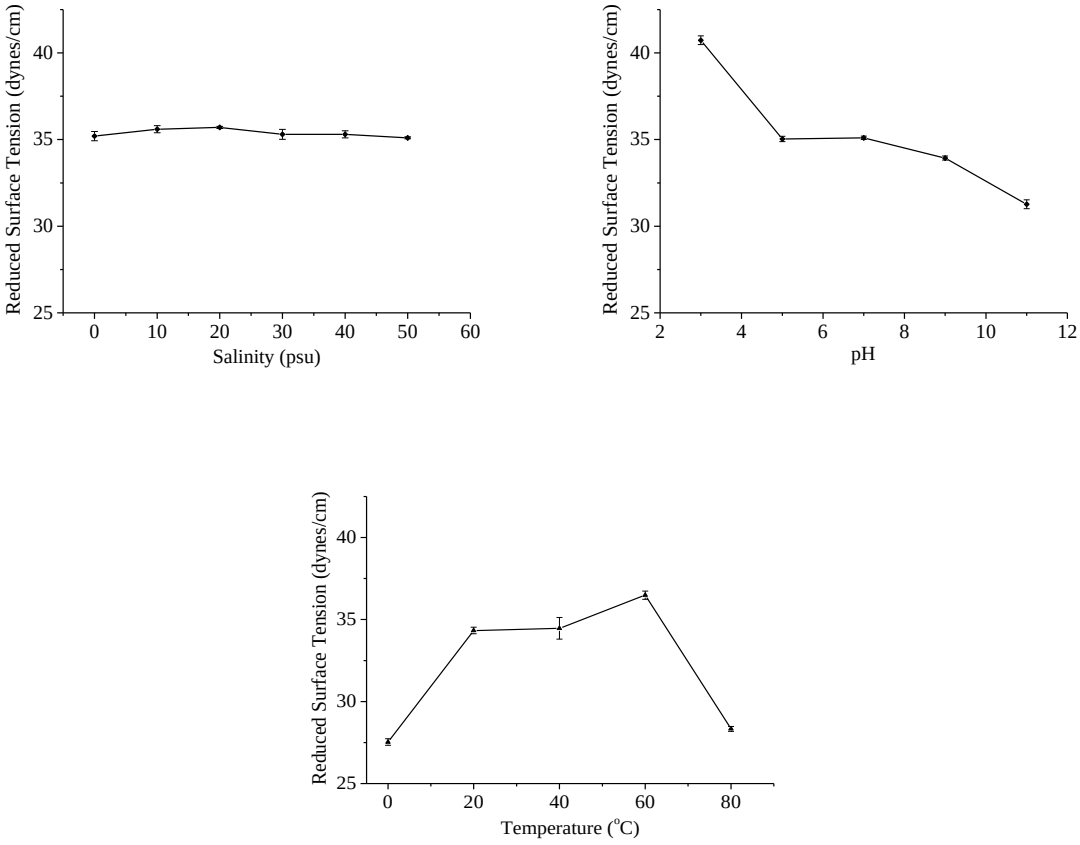


Figure 4.2 Stability test: (a) reduced surface tension vs salinity; (b) reduced surface tension vs pH; (c) reduced surface tension vs temperature.

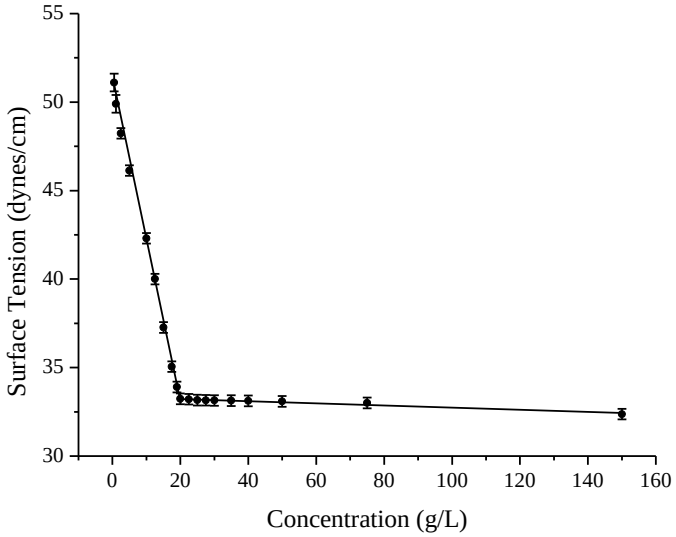


Figure 4.3 Surface tension vs shrimp waste based product concentration

4.3.2.3 Emulsification Activity

The emulsification activity of the hydrolysate surfactant product was tested on seven types of oil including hexadecane, mineral oil, diesel, toluene, dodecane, kerosene, and motor oil. The results are shown in Figure 4.4. The product was able to form stable emulsification on hexadecane, dodecane and kerosene while it had low emulsification activity on mineral oil, diesel and motor oil. The results indicate the potential use of the surface-active product in emulsifying petroleum hydrocarbons.

4.3.3 Selection of Solvent for Dispersant Generation

The hydrolysate surfactant product was mixed with four types of solvents including water, PEG 400, PPG and DMSO. DE of all dispersants were examined and compared in Figure 4.5. The concentration of the powder surfactant product applied for testing DE was 1/5 of CMC, which was about 4 g/L. The results showed that with same amount of powder, water-based dispersant had higher DE than the others.

The results of acute toxicity were shown in Table 4.2. It clearly indicated that the toxicity of each chemical solvent based dispersant was about 6-10 times higher than that of the water-based dispersant. All the four generated dispersants had values of the acute toxicity thousand times less than that of Corexit 9527 with a value of 4.9-12.8 mg/L according to literature (George-Ares & Clark, 2000). The shrimp waste based dispersant had thus shown a great potential as an environmental-friendly option for oil spill response.

The aforementioned results indicated that water was a good solvent to mix with the hydrolysate surfactant for dispersant generation and thus employed for the following experiments. The advantages of water as the solvent when compared with the other chemical solvents were higher DE, lower acute toxicity and lower cost.

4.3.4 DE under Different Simulated Environmental Conditions

4.3.4.1 Salinity

Salinity can influence the water solubility of many compounds. It has been pointed out that high salinity may result in a solubility reduction of dispersant in water, which can promote the interaction between oil and water interface (Mackay et al., 1984). Salinity has been an important environmental factor that affecting the performance of dispersants (Chandrasekar et al., 2006; Moles et al., 2002). In this study, salinities ranging from 0 psu to 50 psu were investigated, which can represent estuaries water at different salinity levels. Figure 4.6 demonstrated DE of the shrimp waste based dispersant on ANS crude oil under different salinities at room temperature. In Figure 4.6, "SWBD" represented shrimp waste based dispersant and "9500" represented Corexit 9500.

Results indicated that DE increased as salinity increased from 0 psu to 20 psu, after which DE at 20 psu was more pronounced than that at 30 psu to 50 psu. Moles et al. (2002) also found that Corexit 9527 exhibited higher DE on ANS crude oil at 22 psu as compared to other levels of salinity at room temperature. Similar results were also presented by Blondina et al. (1999).

They found Corexit 9500 had the maximum dispersion efficacy at salinity around 20-25 psu for Forcados crude oil, ALC, PBC and Arabian Medium crude oil.

4.3.4.2 Mixing Energy

As oil spill usually happens in flowing water like ocean, the waving sea water and wind could impose a mixing energy on oil and water. Mixing energy provided by wave and wind can help break up oil slick into small droplets, which facilitates oil dispersion. Figure 4.7 showed DE of the shrimp waste based dispersant on ANS crude oil under different shaking speeds at room temperature. When the shaking speed was less than 200 rpm, there was a great increase in DE, which indicated that mixing energy below 200 rpm had a significant effect on DE. When the shaking speed was higher than 200 rpm, the increase in DE became less distinctive as shaking speed increased. This may indicate that DE was not affected too much by mixing energy after mixing energy achieved a relative high level. Similar results were also presented in chemical dispersants' performance on crude oil. Fingas et al. (1993) found that as the mixing energy increased, DE kept increasing until a maximal was reached. Srinivasan et al. (2007) conducted BFT using Corexit 9500 and Superdispersant 25 on IFO crude oil. They found DE increased steadily with mixing speeds increasing from 150 to 250 rpm at both 5 ^{o}C and 16 ^{o}C. Chandrasekar et al. (2006) also found this trend for dispersants on different kinds of oil including SLC, PBC and 2FO. This implied that the performance of both the shrimp waste based dispersant and some chemical dispersants was similarly affected by mixing energy.

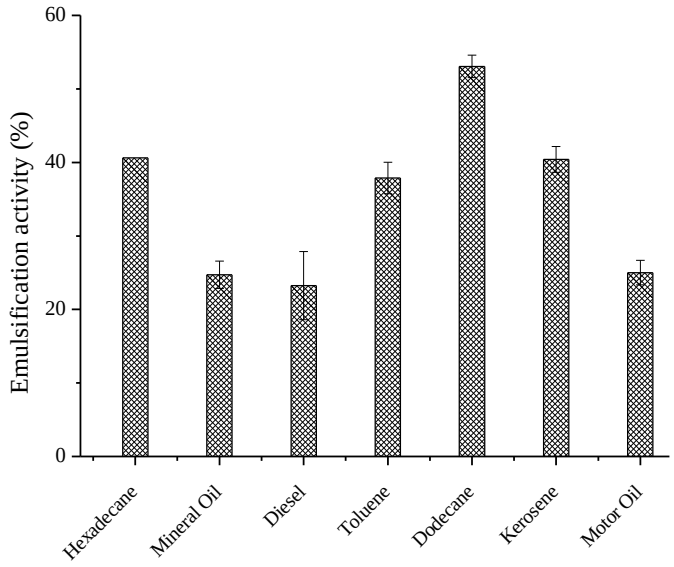


Figure 4.4 Emulsification activity of the product on 7 types of oil

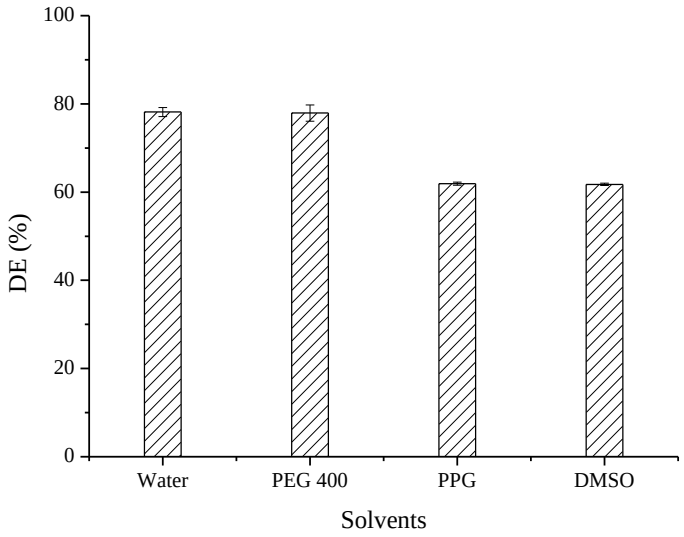


Figure 4.5 DE of dispersants with four types of solvents

Table 4.2 Acute toxicity of solvents

Solvents	Acute Toxicity (EC_{50})	
	5 min	15 min
Water	17.96 g/L	20.30 g/L
PEG 400	2.75 g/L	2.62 g/L
Propylene Glycol	1.99 g/L	2.08 g/L
Dimethyl Sulfoxide	3.11 g/L	3.34 g/L

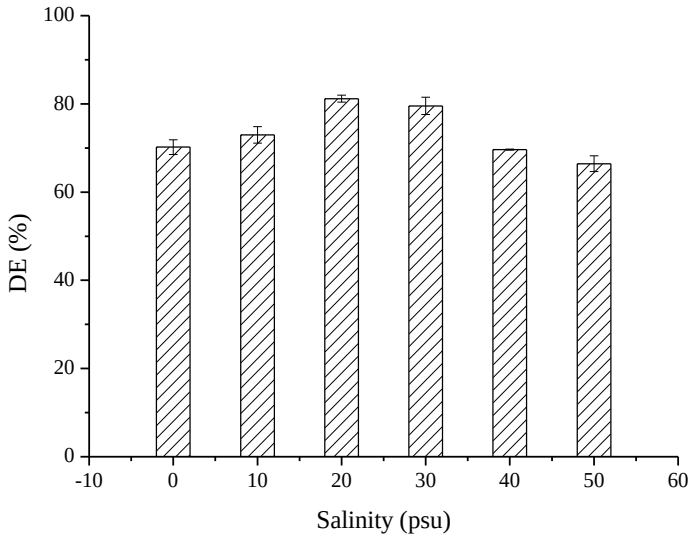


Figure 4.6 DE vs salinity (200 rpm, 22^{o}C)

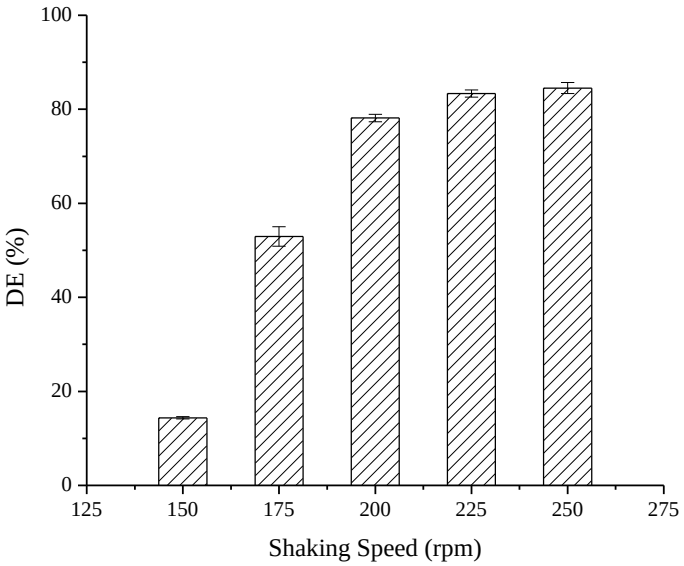


Figure 4.7 DE vs shaking Speed (35 psu, 22^{o}C)

4.3.4.3 Temperature

Temperature is another environmental factor that plays a key role in impacting DE. Water temperature can affect the physical properties of oil such as viscosity. Low water temperature can increase oil viscosity thus decrease DE. Moles et al. (2002) tested Corexit 9500 and 9527 on fresh ANS crude oil at subarctic temperatures. They found a greatly decreased DE (below detection limit) under low temperature. Srinivasan et al. (2007) found the performance of three dispersants for heavy fuel oil at 16 ^{o}C was better than that at 5 ^{o}C. DE at 5 ^{o}C only had values ranging from 30% to 60%. In this study, low temperature (i.e., 4 ^{o}C, representing the North Atlantic harsh environment's temperature) and another four temperature levels (22 ^{o}C, 40 ^{o}C, 60 ^{o}C, 80 ^{o}C) were selected. Figure 4.8 showed DE of the shrimp waste based dispersant on ANS crude oil under different temperatures. It can be seen that the DE value on ANS crude oil at 4 ^{o}C was lower than those at other temperature levels. As temperature increased, DE increased and reached as high as 81% at 40 ^{o}C. Afterwards, DE remained around 80% at higher temperature as 60 ^{o}C and 80 ^{o}C. In general, the shrimp waste based dispersant was effective on dispersing ANS crude oil even under low temperature. The product had a great potential to be applied in cold regions.

4.3.5 Comparison with Synthetic Dispersant Corexit 9500

The effectiveness of shrimp waste based dispersant and Corexit 9500 on dispersing three different types of crude oil at 22 and 4 ^{o}C were showed in Figure 4.9 and Figure 4.10, respectively. The amount of Corexit 9500 used was 1 CMC, namely 22.5 mg/L. Results

indicated that for all three kinds of crude oil, both shrimp waste based dispersant and Corexit 9500 displayed a better dispersion capability at 22° C than that at 4 °C. At 22 °C, the shrimp waste based dispersant had a similar dispersion capability on PBC or ANS to Corexit 9500, which was around 80%. It showed a better dispersion capability on ALC than Corexit 9500 with 5% higher DE. At 4 °C, the shrimp waste based dispersant had a 10% higher DE on PBC than Corexit 9500 and a similar dispersion capability on ALC to Corexit 9500, which was around 77%. For ANS, Corexit 9500 was 5% more effective than the shrimp waste based dispersant. These results suggested that the generated shrimp waste based dispersant, an environmentally friendly alternative, had a comparable oil dispersing capacity to that of Corexit 9500.

4.4 Summary

Through enzymatic hydrolysis, shrimp waste was utilized and optimized for generating surfactant with CMC of 19.9 g/L and surface tension at 33.23 dynes/cm. The product showed stable surface properties under different temperature, salinity and pH levels. Water was proved to be a good solvent for mixing with the hydrolysate surfactant product to generate the green dispersant. DE of the shrimp waste based dispersant on ANS crude oil under three factors including salinity, mixing energy and temperature with different levels was examined. Results indicated that the dispersant could achieve effective dispersion even at low salinity and under low temperature. Its performance was further compared with a popular dispersant Corexit 9500 on dispersing efficacy of PBC, ALC and ANS crude oil. The shrimp waste based dispersant

showed a comparable dispersion capacity.

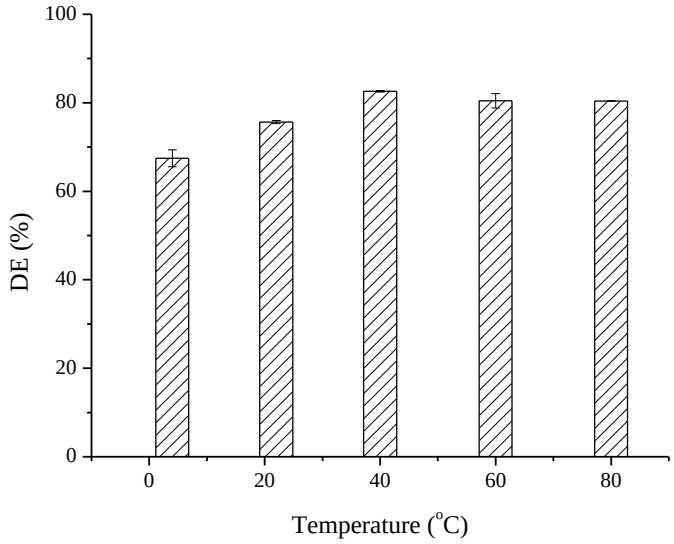


Figure 4.8 DE vs temperature (35 psu, 200 rpm)

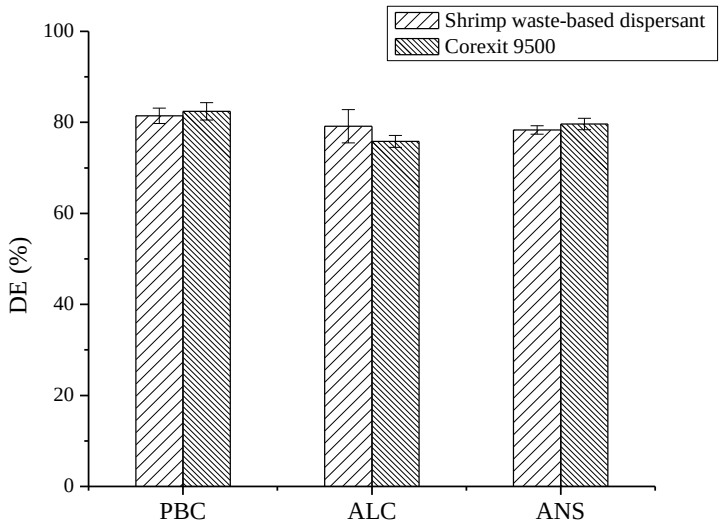


Figure 4.9 DE of shrimp waste based dspersant and Corexit 9500 with three different crude oil (35 psu, 200 rpm, 22ºC)

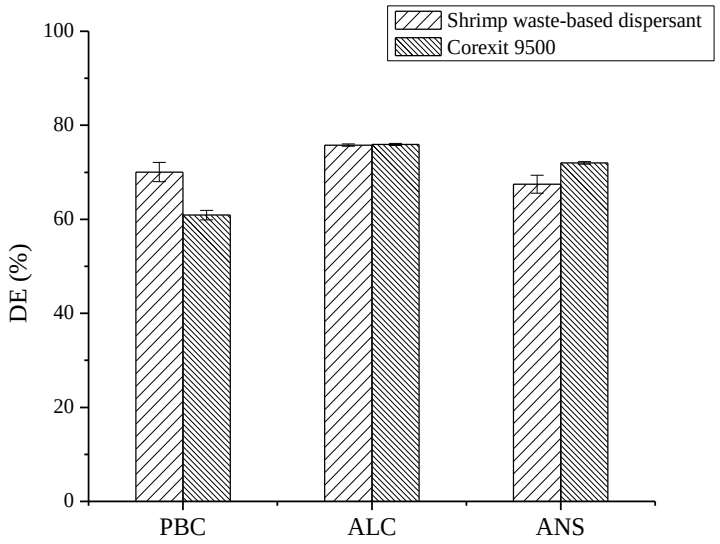


Figure 4.10 DE of shrimp waste based dispersant and Corexit 9500 with three different crude oil (35 psu, 200 rpm, 4^{o}C)

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

In this thesis work, RSM with ANN was integrated in the optimization and modeling of Newfoundland shrimp waste hydrolysis. The results from RSM showed that the hydrolysis process was mainly influenced by four individual factors (i.e. E/S ratio, hydrolysis time, initial pH value, and hydrolysis temperature) and the interactive effect between E/S ratio and initial pH value. Besides, a feedforward 3-layer ANN model was developed using the experiment data to simulate this complex system. Results obtained from the validation experiments agreed well with the modeling outputs of both models. The research results improved the understanding of shrimp waste hydrolysis process and contributed to shrimp waste utilization with high efficiency;

In addition, this study investigated the potential of using shrimp waste hydrolysate to generate a green dispersant for oil spill treatment. The hydrolysis process was first optimized based on the DE of the product. The surfactant product displayed stable surface properties under multiple environment conditions. Water was identified as the best solvent for dispersant generation in comparison with three chemical solvents. Compared with a popular dispersant Corexit 9500 on dispersing efficacy of PBC, ALC and ANS crude oil, the shrimp waste based dispersant exhibited a comparable dispersion capacity. This outcome suggested a promising application of shrimp waste in producing green dispersants for oil spill response even in cold seawater. The significantly less toxicity and effective dispersion should make this dispersant is an environmental-friendly alternative competitive to the currently used dispersant systems.

5.2 Research Contributions

(1) In this study, the integration of RSM with ANN was first applied in optimization and modeling of Newfoundland shrimp waste hydrolysis. The research is contributive to the technological development on effective shrimp waste utilization, in which will be of benefit for marine environmental improvement and sustainable stewardship of low-cost seafood wastes. It also validated the effectiveness of using the shrimp waste hydrolysate as nutrient source for microbial growth.

(2) This study investigated on utilizing shrimp waste for generating crude oil dispersant for the first time. It offers a marine waste management option which is applicable for Newfoundland and beyond, and can be adopted by oil industries as a viable technique for treating offshore oil spill problems.

5.3 Recommendations

(1) During the shrimp waste enzymatic hydrolysis process, enzyme usage is the main source of cost. In the future study, optimization study of the ratio of enzymatic dosage versus protein content should be conducted to maximize the cost-effectiveness of this method. New kinds of cost-effective enzymes for enzyme hydrolysis should be explored and developed as well.

(2) Shrimp waste hydrolysate can be a nitrogen source for growth of hydrocarbon-degrading bacteria. Some of the bacteria are also biosurfactant producers. Future work can focus on the potential of producing biosurfactants through bacteria cultivation utilizing shrimp waste

hydrolysate.

(3) As the shrimp waste based dispersant could enhance dispersion of ANS, PBC and ALC crude oil, experiments on other kinds of crude oil can be further conducted and compared with existing dispersants. The output can help to aid the generation of guidances for oil spill response.

(4) Based on the OFAT result of factors that have effect on dispersing process, DOE and RSM can be applied to further investigate the interactive effect among factors. These will help modeling and predict the process of dispersant application. For example, the plans for oil spill response can be adjusted according to different environment conditions.

(5) Biodegradation of the dispersed crude oil can be further studied. During the process, the fate and transport of both crude oil and dispersants can be studied. The environment assessment during the biodegradation phase can be conducted as well.

(6) Mechanism of dispersion formation by shrimp waste based dispersant can be further investigated with the focus on the extraction of selective contents from shrimp waste based dispersant to make the dispersant more effective.

References

Abakarov, A., Teixeira, A., Simpson, R., Pinto, M., & Almonacid, S. (2011). Modeling of squid protein hydrolysis: artificial neural network approach. Journal of Food Process Engineering, 34(6), 2026-2046. DOI: 10.1111/j.1745-4530.2009.00567.x

Adams, S., Bose, N., Hawboldt, K., & Husain, T. (2005, June). Environmental monitoring of fish plant effluent in coastal Newfoundland. In Oceans 2005-Europe (Vol. 2, pp. 1062-1067). IEEE. DOI: 10.1109/OCEANSE.2005.1513205

Adjonu, R., Doran, G., Torley, P., & Agboola, S. (2014). Formation of whey protein isolate hydrolysate stabilised nanoemulsion. *Food Hydrocolloids*, *41*, 169-177.

Aluko, R. E., & Monu, E. (2003). Functional and bioactive properties of quinoa seed protein hydrolysates. *Journal of Food Science*, *68*(4), 1254-1258.

AOAC (2005). Official methods of analysis (16th ed.). Washington, DC: Association of Official Analytical Chemists.

Arancibia, M. Y., Alemán, A., López-Caballero, M. E., Gómez-Guillén, M. C., & Montero, P. (2015). Development of active films of chitosan isolated by mild extraction with added protein concentrate from shrimp waste. *Food Hydrocolloids*, *43*, 91-99.

Arbia, W., Arbia, L., Adour, L., & Amrane, A. (2013). Chitin extraction from crustacean shells using biological methods-A review. *Food Technology and Biotechnology*, *51*(1), 12.

Arboleda, J. C., Rojas, O. J., & Lucia, L. A. (2014). Acid-generated soy protein hydrolysates and their interfacial behavior on model surfaces. *Biomacromolecules*, *15*(11), 4336-4342. http://dx.doi.org/10.1021/bm501344j

Armenta, R. E., & Guerrero-Legarreta, I. (2009). Amino acid profile and enhancement of the enzymatic hydrolysis of fermented shrimp carotenoproteins. *Food chemistry*, *112*(2), 310-315.

Armenta‐López, R., Guerrero, I. L., & Huerta, S. (2002). Astaxanthin extraction from shrimp waste by lactic fermentation and enzymatic hydrolysis of the carotenoprotein complex. *Journal of food Science*, *67*(3), 1002-1006.

Aronstein, B. N., Calvillo, Y. M., & Alexander, M. (1991). Effect of surfactants at low concentrations on the desorption and biodegradation of sorbed aromatic compounds in soil. Environmental science & technology, 25(10), 1728-1731.

Atlas, R. M., & Bartha, R. (1972). Degradation and mineralization of petroleum in sea water: limitation by nitrogen and phosphorous.*Biotechnology and Bioengineering*, *14*(3), 309-318.

Babu, C. M., Chakrabarti, R., & Sambasivarao, K. R. S. (2008). Enzymatic isolation of carotenoid-protein complex from shrimp head waste and its use as a source of carotenoids. *LWT-Food Science and Technology*, *41*(2), 227-235.

Banat, I. M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M. G., Fracchia, L., ... & Marchant, R. (2010). Microbial biosurfactants production, applications and future potential. *Applied Microbiology and Biotechnology*, *87*(2), 427-444.

Bari, M. N., Alam, M. Z., Muyibi, S. A., & Jamal, P. (2009). Improvement of production of citric acid from oil palm empty fruit bunches: Optimization of media by statistical experimental designs. Bioresource technology, 100(12), 3113-3120. DOI:

10.1016/j.biortech.2009.01.005.

Bas, D., & Boyaci, I. H. (2007). Modeling and optimization II: comparison of estimation capabilities of response surface methodology with artificial neural networks in a biochemical reaction. Journal of Food Engineering, 78(3), 846-854. DOI: 10.1016/j.jfoodeng.2005.11.025

Bejarano, A. C., Levine, E., & Mearns, A. J. (2013). Effectiveness and potential ecological effects of offshore surface dispersant use during the Deepwater Horizon oil spill: a retrospective analysis of monitoring data.*Environmental monitoring and assessment*, *185*(12), 10281-10295.

Belore, R. C., Trudel, K., Mullin, J. V., & Guarino, A. (2009). Large-scale cold water dispersant effectiveness experiments with Alaskan crude oils and Corexit 9500 and 9527 dispersants. *Marine pollution bulletin*, *58*(1), 118-128.

Benhabiles, M. S., Salah, R., Lounici, H., Drouiche, N., Goosen, M. F. A., & Mameri, N. (2012). Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste. *Food hydrocolloids*, *29*(1), 48-56.

BenKinney, M., Brown, J., Mudge, S., Russell, M., Nevin, A., & Huber, C. (2011, March). Monitoring effects of aerial dispersant application during the MC252 Deepwater Horizon Incident. In *International Oil Spill Conference Proceedings (IOSC)* (Vol. 2011, No. 1, p. abs368). American Petroleum Institute.

Bhaskar, N., Benila, T., Radha, C., & Lalitha, R. G. (2008). Optimization of enzymatic hydrolysis of visceral waste proteins of Catla (Catla catla) for preparing protein

hydrolysate using a commercial protease. Bioresource technology, 99(2), 335-343. DOI: 10.1016/ j.biortech.2006.12.

Blanco, M., Sotelo, C. G., Chapela, M. J., & Pérez-Martín, R. I. (2007). Towards sustainable and efficient use of fishery resources: present and future trends. *Trends in Food Science & Technology*, *18*(1), 29-36.

Blondina, G. J., Singer, M. M., Lee, I., Ouano, M. T., Hodgins, M., Tjeerdema, R. S., & Sowby, M. L. (1999). Influence of salinity on petroleum accommodation by dispersants. *Spill Science & Technology Bulletin*, *5*(2), 127-134. http://dx.doi.org/10.1016/s1353-2561(98)00048-6

Brito, R. O., Marques, E. F., Silva, S. G., do Vale, M. L., Gomes, P., Araújo, M. J., ... & Vinardell, M. P. (2009). Physicochemical and toxicological properties of novel amino acid-based amphiphiles and their spontaneously formed catanionic vesicles. Colloids and Surfaces B: Biointerfaces, 72(1), 80-87.

Brito, R. O., Silva, S. G., Fernandes, R. M., Marques, E. F., Enrique-Borges, J., & do Vale, M. L. C. (2011). Enhanced interfacial properties of novel amino acid-derived surfactants: Effects of headgroup chemistry and of alkyl chain length and unsaturation. *Colloids and Surfaces B: Biointerfaces*,*86*(1), 65-70.

Buciński, A., Karamać, M., Amarowicz, R., & Pegg, R. B. (2008). Modeling the tryptic hydrolysis of pea proteins using an artificial neural network. LWT-Food Science and Technology, 41(5), 942-945. DOI: 10.1016/j.lwt.2007.06.021

Bueno-Solano, C., López-Cervantes, J., Campas-Baypoli, O. N., Lauterio-García, R.,

Adan-Bante, N. P., & Sánchez-Machado, D. I. (2009). Chemical and biological characteristics of protein hydrolysates from fermented shrimp by-products. *Food chemistry*, *112*(3), 671-675.

Byford, D. C. (1982). The development of dispersants for application at low temperatures. In *Proceedings of the Fifth Arctic Marine Oilspill Program Technical Seminar* (pp. 339-254).

Cahú, T. B., Santos, S. D., Mendes, A., Córdula, C. R., Chavante, S. F., Carvalho, L. B., ... & Bezerra, R. S. (2012). Recovery of protein, chitin, carotenoids and glycosaminoglycans from Pacific white shrimp (Litopenaeus vannamei) processing waste. *Process Biochemistry*, *47*(4), 570-577.

Cai, L., Gochin, M., & Liu, K. (2011). A facile surfactant critical micelle concentration determination. *Chemical Communications*, *47*(19), 5527-5529.

Cai, Q., Zhang, B., Chen, B., Zhu, Z., Lin, W., & Cao, T. (2014). Screening of biosurfactant producers from petroleum hydrocarbon contaminated sources in cold marine environments. Marine pollution bulletin, 86(1), 402-410. DOI:10.1016/j.marpolbul.2014.06.039

Campisi, T., Abbondanzi, F., Casado-Martinez, C., DelValls, T. A., Guerra, R., & Iacondini, A. (2005). Effect of sediment turbidity and color on light output measurement for Microtox® Basic Solid-Phase Test. *Chemosphere*, *60*(1), 9-15. http://dx.doi.org/10.1016/j.chemosphere.2004.12.052

Cancre, I., Ravallec, R., Van Wormhoudt, A., Stenberg, E., Gildberg, A., & Le Gal, Y. (1999).

Secretagogues and growth factors in fish and crustacean protein hydrolysates. *Marine Biotechnology*, *1*(5), 489-494.

Canevari, G. P., Calcavecchio, P., Becker, K. W., Lessard, R. R., & Fiocco, R. J. (2001, March). Key parameters affecting the dispersion of viscous oil. In *international oil spill conference* (Vol. 2001, No. 1, pp. 479-483). American Petroleum Institute.

Cao, W., Tan, C., Zhan, X., Li, H., & Zhang, C. (2014). Ultraviolet irradiation and gradient temperature assisted autolysis for protein recovery from shrimp head waste. *Food chemistry*, *164*, 136-141.

Cao, W., Zhang, C., Hong, P., & Ji, H. (2008). Response surface methodology for autolysis parameters optimization of shrimp head and amino acids released during autolysis. *Food chemistry*, *109*(1), 176-183.

Cao, W., Zhang, C., Hong, P., Ji, H., Hao, J., & Zhang, J. (2009). Autolysis of shrimp head by gradual temperature and nutritional quality of the resulting hydrolysate. *LWT-Food Science and Technology*, *42*(1), 244-249.

Carrillo, P. G., Mardaraz, C., Pitta-Alvarez, S. I., & Giulietti, A. M. (1996). Isolation and selection of biosurfactant-producing bacteria. *World Journal of Microbiology and Biotechnology*, *12*(1), 82-84. http://dx.doi.org/10.1007/bf00327807

Cavalheiro, J. M. O., de Souza, E. O., & Bora, P. S. (2007). Utilization of shrimp industry waste in the formulation of tilapia (Oreochromis niloticus Linnaeus) feed. *Bioresource Technology*, *98*(3), 602-606.

Chalamaiah, M., Rao, G. N., Rao, D. G., & Jyothirmayi, T. (2010). Protein hydrolysates from

meriga (Cirrhinus mrigala) egg and evaluation of their functional properties. *Food Chemistry*, *120*(3), 652-657. http://dx.doi.org/10.1016/j.foodchem.2009.10.057

Chandrasekar, S., Sorial, G. A., & Weaver, J. W. (2006). Dispersant effectiveness on oil spills–impact of salinity. *ICES Journal of Marine Science: Journal du Conseil*, *63*(8), 1418-1430. http://dx.doi.org/10.1016/j.icesjms.2006.04.019

Chang, C. Y., Wu, K. C., & Chiang, S. H. (2007). Antioxidant properties and protein compositions of porcine haemoglobin hydrolysates. *Food Chemistry*, *100*(4), 1537-1543. http://dx.doi.org/10.1016/j.foodchem.2005.12.019

Chapman, H., Purnell, K., Law, R. J., & Kirby, M. F. (2007). The use of chemical dispersants to combat oil spills at sea: A review of practice and research needs in Europe. *Marine Pollution Bulletin*, *54*(7), 827-838. http://dx.doi.org/10.1016/j.marpolbul.2007.03.012

Cheng, Y., Chen, J., & Xiong, Y. L. (2014). Interfacial Adsorption of Peptides in Oil-in-Water Emulsions Costabilized by Tween 20 and Antioxidative Potato Peptides. *Journal of agricultural and food chemistry*, *62*(47), 11575-11581. http://dx.doi.org/10.1021/jf5038135

Chu, B. S., Ichikawa, S., Kanafusa, S., & Nakajima, M. (2007). Preparation of protein-stabilized β-carotene nanodispersions by emulsification–evaporation method. *Journal of the American Oil Chemists' Society*, *84*(11), 1053-1062.

Clayton, J. R., Payne, J. R., Farlow, J. S., & Sarwar, C. (1993). *Oil spill dispersants: mechanisms of action and laboratory tests* (No. PB-93-236578/XAB). Science Applications International Corp., San Diego, CA (United States).

Colbourn, E.A., Roskilly, S.J., Rowe, R.C., and York, P. (2011). Modelling formulations using gene expression programming – A comparative analysis with artificial neural networks. European Journal of Pharmaceutical Sciences, 44(3), 366–374.

Coward-Kelly, G., Agbogbo, F. K., & Holtzapple, M. T. (2006). Lime treatment of shrimp head waste for the generation of highly digestible animal feed. *Bioresource technology*, *97*(13), 1515-1520.

Cull, D. Quantitative comparison of levels of organic wastes from four major sources at four scales along the Newfoundland coastline. MSc. Thesis. Memorial University of Newfoundland. St. John's, NL. 2000.

Cullis, P. T., & De Kruijff, B. (1979). Lipid polymorphism and the functional roles of lipids in biological membranes. *Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes*, *559*(4), 399-420.

Dalgleish, D. G. (1997). Adsorption of protein and the stability of emulsions.*Trends in Food Science & Technology*, *8*(1), 1-6.

Daling, P. S. (1988). A study of the chemical dispersibility of fresh and weathered crude oils. In *Proc. 11th AMOP Seminar. Ottawa: Environment Canada* (pp. 481-499).

de Castro, R. J. S., Bagagli, M. P., & Sato, H. H. (2015). Improving the functional properties of milk proteins: focus on the specificities of proteolytic enzymes. *Current Opinion in Food Science*, *1*, 64-69. http://dx.doi.org/10.1016/j.cofs.2014.12.004

De Holanda, H. D., & Netto, F. M. (2006). Recovery of components from shrimp (Xiphopenaeus kroyeri) processing waste by enzymatic hydrolysis.*Journal of Food*

Science, *71*(5), C298-C303.

Desai, J. D., & Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. *Microbiology and Molecular biology reviews*, 61(1), 47-64.

Desai, K. M., Survase, S. A., Saudagar, P. S., Lele, S. S., & Singhal, R. S. (2008). Comparison of artificial neural network (ANN) and response surface methodology (RSM) in fermentation media optimization: case study of fermentative production of scleroglucan. Biochemical Engineering Journal,41(3), 266-273. DOI: 10.1016/j.bej.2008.05.009

Desai, K. M., Vaidya, B. K., Singhal, R. S., & Bhagwat, S. S. (2005). Use of an artificial neural network in modeling yeast biomass and yield of β-glucan. Process Biochemistry, 40(5), 1617-1626. DOI: 10.1016/j.procbio.2004.06.015

Deschenes, L., Lafrance, P., Villeneuve, J. P., & Samson, R. (1996). Adding sodium dodecyl sulfate and Pseudomonas aeruginosa UG2 biosurfactants inhibits polycyclic aromatic hydrocarbon biodegradation in a weathered creosote-contaminated soil. Applied microbiology and biotechnology, 46(5-6), 638-646.

Dey, S. S., & Dora, K. C. (2014). Optimization of the production of shrimp waste protein hydrolysate using microbial proteases adopting response surface methodology. Journal of food science and technology, 51(1), 16-24. DOI: 10.1007/s13197-011-0455-4

Doerffer, J.W. (1992). *Oil spill response in the marine environment*. United Kingdom: Pergamon Press.

Donsì, F., Annunziata, M., Vincensi, M., & Ferrari, G. (2012). Design of nanoemulsion-based delivery systems of natural antimicrobials: effect of the emulsifier. *Journal of*

biotechnology, *159*(4), 342-350.

Dufossé, L., De La Broise, D., & Guerard, F. (2001). Evaluation of nitrogenous substrates such as peptones from fish: a new method based on Gompertz modeling of microbial growth. Current microbiology, 42(1), 32-38. DOI: 10.1007/s002840010174

Eakpetch, P., Benjakul, S., Visessanguan, W., & Kijroongrojana, K. (2008). Autolysis of Pacific white shrimp (Litopenaeus vannamei) meat: characterization and the effects of protein additives. *Journal of food science*,*73*(2), S95-S103.

Efroymson, R. A., & Alexander, M. (1991). Biodegradation by an Arthrobacter species of hydrocarbons partitioned into an organic solvent. Applied and Environmental Microbiology, 57(5), 1441-1447.

Elmolla, E.S., Chaudhuri, M., and Eltoukhy, M.M. (2010). The use of artificial neural network (ANN) for modeling of COD removal from antibiotic aqueous solution by the Fenton process. Journal of Hazardous Materials, 179, 127–134.

Erondu, E. S., & Anyanwu, P. E. (2005). Potential hazards and risks associated with the aquaculture industry. *African Journal of Biotechnology*, *4*(13).

Etkin, D. S., & Tebeau, P. (2003, April). Assessing progress and benefits of oil spill response technology development since Exxon Valdez. InInternational Oil Spill Conference (Vol. 2003, No. 1, pp. 843-850). American Petroleum Institute., P. (2003, April). Assessing progress and benefits of oil spill response technology development since Exxon Valdez. *In International Oil Spill Conference* (Vol. 2003, No. 1, pp. 843-850). American Petroleum Institute.

Fanimo, A. O., Oduguwa, O. O., Onifade, A. O., & Olutunde, T. O. (2000). Protein quality of shrimp-waste meal. *Bioresource Technology*, *72*(2), 185-188.

FAO. (2010). Review of the state of world marine fishery resources. FAO Fisheries Technical Page 3.

Farn, R. J. (Ed.). (2008). *Chemistry and technology of surfactants*. John Wiley & Sons.

Ferrer, J., Paez, G., Marmol, Z., Ramones, E., Garcia, H., & Forster, C. F. (1996). Acid hydrolysis of shrimp-shell wastes and the production of single cell protein from the hydrolysate. Bioresource Technology, 57(1), 55-60.

Fiechter, A. (1992). Biosurfactants: moving towards industrial application. *Trends in biotechnology*, *10*, 208-217.

Fingas, M. F. (2002). White Paper on Oil Spill Dispersant Effectiveness Testing in Large Tanks. Prince William Sound Regional Citizens' Advisory Council.

Fingas, M. (2012). *The basics of oil spill cleanup*. CRC Press.

Fingas, M. F., Kyle, D. A., Holmes, J. B., & Tennyson, E. J. (1993, March). The effectiveness of dispersants: Variation with energy. In *International Oil Spill Conference* (Vol. 1993, No. 1, pp. 567-574). American Petroleum Institute. http://dx.doi.org/10.7901/2169-3358-1993-1-567

Fingas, M. F., Kyle, D. A., Wang, Z., Ackerman, F., & Mullin, J. (1994, June). Testing of oil spill dispersant effectiveness in the laboratory. In *ARCTIC AND MARINE OILSPILL PROGRAM TECHNICAL SEMINAR* (pp. 905-905). MINISTRY OF SUPPLY AND SERVICES, CANADA.

Fiocco, R. J., & Lewis, A. (1999). Oil spill dispersants. *Pure and Applied Chemistry*, *71*(1), 27-42.

Fiocco, R. J., Lessard, R. R., Canevari, G. P., Becker, K. W., & Daling, P. S. (1995). *The impact of oil dispersant solvent on performance* (No. CONF-9410157--).

Foegeding, E. A., Davis, J. P., Doucet, D., & McGuffey, M. K. (2002). Advances in modifying and understanding whey protein functionality. *Trends in Food Science & Technology*, *13*(5), 151-159.

Foght, J. M., & Westlake, D. W. S. (1982). Effect of the dispersant Corexit 9527 on the microbial degradation of Prudhoe Bay oil. Canadian Journal of Microbiology, 28(1), 117-122.

Fukuda, T., & Shibata, T. (1992). Theory and applications of neural networks for industrial control systems. IEEE Transactions on Industrial Electronics, 39(6), 472-489. DOI: 10.1109/41.170966

Gallert, C., & Winter, J. (2002). Solid and liquid residues as raw materials for biotechnology. *Naturwissenschaften*, 89(11), 483-496. http://dx.doi.org/10.1007/s00114-002-0372-5

Gao, M. T., Hirata, M., Toorisaka, E., & Hano, T. (2006). Acid-hydrolysis of fish wastes for lactic acid fermentation. *Bioresource technology*, *97*(18), 2414-2420.

Gardner, M. W., & Dorling, S. R. (1998). Artificial neural networks (the multilayer perceptron)—a review of applications in the atmospheric sciences. Atmospheric environment, 32(14), 2627-2636. DOI: 10.1016/S1352-2310(97)00447-0

Garson, D. G. (1991). Interpreting neural network connection weights. Artificial Intelligence

Expert, April (1991), pp. 47–51.

Gautam, K. K., & Tyagi, V. K. (2006). Microbial surfactants: a review. *Journal of Oleo Science*, *55*(4), 155-166.

Gauthier, S. F., & Pouliot, Y. (2003). Functional and biological properties of peptides obtained by enzymatic hydrolysis of whey proteins. *Journal of Dairy Science*, *86*, E78-E87.

George-Ares, A., & Clark, J. R. (2000). Aquatic toxicity of two Corexit® dispersants. *Chemosphere*, *40*(8), 897-906.

Georgiou, G., Lin, S. C., & Sharma, M. M. (1992). Surface–Active Compounds from Microorganisms. *Nature Biotechnology*, *10*(1), 60-65.

Gharaei-Fathabad, E. (2011). Biosurfactants in pharmaceutical industry: A mini-review. American Journal of Drug Discovery and Development, 1(1), 58-69.

Ghorbel-Bellaaj, O., Jellouli, K., Younes, I., Manni, L., Salem, M. O., & Nasri, M. (2011). A solvent-stable metalloprotease produced by Pseudomonas aeruginosa A2 grown on shrimp shell waste and its application in chitin extraction. *Applied biochemistry and biotechnology*,*164*(4), 410-425.

Gildberg, A., & Stenberg, E. (2001). A new process for advanced utilisation of shrimp waste. *Process Biochemistry*, 36(8), 809-812. http://dx.doi.org/10.1016/s0032-9592(00)00278-8

Gimeno, M., Ramírez-Hernández, J. Y., Mártinez-Ibarra, C., Pacheco, N., García-Arrazola, R., Bárzana, E., & Shirai, K. (2007). One-solvent extraction of astaxanthin from lactic acid fermented shrimp wastes. *Journal of agricultural and food chemistry*, *55*(25), 10345-10350.

Gong, Y., Zhao, X., O'Reilly, S. E., Qian, T., & Zhao, D. (2014). Effects of oil dispersant and oil on sorption and desorption of phenanthrene with Gulf Coast marine sediments. *Environmental Pollution*, *185*, 240-249.

Goodbody-Gringley, G., Wetzel, D. L., Gillon, D., Pulster, E., Miller, A., & Ritchie, K. B. (2013). Toxicity of Deepwater Horizon source oil and the chemical dispersant, Corexit® 9500, to coral larvae. *PLoS One*, *8*(1), e45574.

Guerard, F., Sumaya-Martinez, M. T., Laroque, D., Chabeaud, A., & Dufossé, L. (2007). Optimization of free radical scavenging activity by response surface methodology in the hydrolysis of shrimp processing discards. *Process Biochemistry*, *42*(11), 1486-1491.

Haddar, A., Hmidet, N., Ghorbel-Bellaaj, O., Fakhfakh-Zouari, N., Sellami-Kamoun, A., & Nasri, M. (2011). Alkaline proteases produced by Bacillus licheniformis RP1 grown on shrimp wastes: Application in chitin extraction, chicken feather-degradation and as a dehairing agent. *Biotechnology and Bioprocess Engineering*, *16*(4), 669-678.

Hao, L., Benxian, S., & Xinzhong, C. (2005). RESEARCH AND EVALUATION ON HIGH EFFICIENCY SERIES OIL SPILL DISPERSANT [J]. *Speciality Petrochemicals*, 2005.

Harris, C. (1995, February). The Braer Incident: Shetland Islands, January 1993. In *International Oil Spill Conference* (Vol. 1995, No. 1, pp. 813-819). American Petroleum Institute.

Hayworth, J. S., & Clement, T. P. (2012). Provenance of Corexit-related chemical constituents found in nearshore and inland Gulf Coast waters. *Marine pollution bulletin*, *64*(10), 2005-2014.

Healy, M., Green, A., & Healy, A. (2003). Bioprocessing of marine crustacean shell waste. *Acta biotechnologica*, *23*(2‐3), 151-160.

Holder, E. L., Conmy, R. N., & Venosa, A. D. (2015). Comparative Laboratory-Scale Testing of Dispersant Effectiveness of 23 Crude Oils Using Four Different Testing Protocols*. *Journal of Environmental Protection*, *6*(6), 628.

Hoyle, N. T., & Merritt, H. John (1994). Quality of fish protein hydrolysate from herring (Clupea harengus). Journal of Food Science, 59(1), 76-79. DOI: 10.1111/j.1365-2621.1994.tb06901.x

Hu, M., McClements, D. J., & Decker, E. A. (2003). Lipid oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate, and soy protein isolate. *Journal of Agricultural and Food Chemistry*, *51*(6), 1696-1700.

Infante, M. R., Pérez, L., Pinazo, A., Clapés, P., Morán, M. C., Angelet, M., ... & Vinardell, M. P. (2004). Amino acid-based surfactants. *Comptes Rendus Chimie*, *7*(6), 583-592.

Infante, M., Pinazo, A., & Seguer, J. (1997). Non-conventional surfactants from amino acids and glycolipids: structure, preparation and properties. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 123, 49-70.

Innocente, N., Corradini, C., Blecker, C., & Paquot, M. (1998). Emulsifying properties of the total fraction and the hydrophobic fraction of bovine milk proteose-peptones. International Dairy Journal, 8(12), 981-985.

Intarasirisawat, R., Benjakul, S., Visessanguan, W., & Wu, J. (2012). Antioxidative and functional properties of protein hydrolysate from defatted skipjack (Katsuwonous pelamis)

roe. *Food chemistry*, *135*(4), 3039-3048. http://dx.doi.org/10.1016/j.foodchem.2012.06.076

Islam, M. S., Khan, S., & Tanaka, M. (2004). Waste loading in shrimp and fish processing effluents: potential source of hazards to the coastal and nearshore environments. *Marine Pollution Bulletin*, *49*(1), 103-110.

Jamieson, B. L., Gonçalves, A. A., & Gagnon, G. A. (2013). Evaluation of treatment options for Atlantic Canadian seafood processing plant effluent. Journal of Environmental Engineering and Science, 8(4), 448-460. DOI: 10.1680/jees.2013.0045

Jaswal, A. S. (1989). Methodology investigations for the production of amino acid hydrolysate from shrimp waste. *Canadian Institute of Food Science and Technology Journal*, *22*(5), 460-463.

Jing, L., Chen, B., & Zhang, B.Y. (2014). Modeling of UV-induced photodegradation of naphthalene in marine oily wastewater by artificial neural networks. Water, Air, & Soil Pollution, 225(4), 1-14. DOI: 10.1007/s11270-014-1906-0.

Jing, L., Chen, B., Zhang, B.Y., & Li, P. (2015). Process Simulation and Dynamic Control for Marine Oily Wastewater Treatment using UV Irradiation. Water Research, 81, 101-112, DOI: 10.1016/j.watres.2015.03.023.

Judson, R. S., Martin, M. T., Reif, D. M., Houck, K. A., Knudsen, T. B., Rotroff, D. M., ... & Dix, D. J. (2010). Analysis of eight oil spill dispersants using rapid, in vitro tests for endocrine and other biological activity. *Environmental science & technology*, *44*(15), 5979-5985.

Kandra, P., Challa, M. M., & Jyothi, H. K. P. (2012). Efficient use of shrimp waste: present and future trends. *Applied microbiology and biotechnology*,*93*(1), 17-29.

Kanga, S. A., Bonner, J. S., Page, C. A., Mills, M. A., & Autenrieth, R. L. (1997). Solubilization of naphthalene and methyl-substituted naphthalenes from crude oil using biosurfactants. Environmental science & technology, 31(2), 556-561.

Karanth, N. G. K., Deo, P. G., & Veenanadig, N. K. (1999). Microbial production of biosurfactants and their importance. *Current Science*, *77*(1), 116-126.

Kasiri, M. B., Aleboyeh, H., & Aleboyeh, A. (2008). Modeling and optimization of heterogeneous photo-fenton process with response surface methodology and artificial neural networks. Environmental science & technology, 42(21), 7970-7975. DOI: 10.1021/es801372q

Kato, A., & Nakai, S. (1980). Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins.*Biochimica et Biophysica Acta (BBA)-Protein Structure*, *624*(1), 13-20.

Kaur, S., & Dhillon, G. S. (2013). Recent trends in biological extraction of chitin from marine shell wastes: a review. *Critical reviews in biotechnology*,*35*(1), 44-61.

Kezwoń, A., Chromińska, I., Frączyk, T., & Wojciechowski, K. (2015). Effect of enzymatic hydrolysis on surface activity and surface rheology of type I collagen. *Colloids and Surfaces B: Biointerfaces*. http://dx.doi.org/10.1016/j.colsurfb.2015.05.017

Khajeh, M., & Barkhordar, A. (2013). Modelling of solid-phase tea waste extraction for the removal of manganese from food samples by using artificial neural network approach.

Food chemistry, 141(2), 712-717. DOI: 10.1016/j.foodchem.2013.04.075

Khanafari, A., Marandi, R. E. Z. A., & Sanatei, S. (2008). Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods. *Iranian Journal of Environmental Health Science & Engineering*, *5*(1), 1-24.

Khorrami, M., Najafpour, G. D., Younesi, H., & Amini, G. H. (2011). Growth kinetics and demineralization of shrimp shell using Lactobacillus plantarum PTCC 1058 on various carbon sources. *Iran J Ener Environ*, *2*, 320-5.

Kingston, P. F. (2002). Long-term environmental impact of oil spills. *Spill Science & Technology Bulletin*, *7*(1), 53-61.

Klomklao, S., Benjakul, S., Visessanguan, W., Kishimura, H., & Simpson, B. K. (2009). Extraction of carotenoprotein from black tiger shrimp shells with the aid of bluefish trypsin. *Journal of food biochemistry*, *33*(2), 201-217.

Klompong, V., Benjakul, S., Kantachote, D., & Shahidi, F. (2012). Use of protein hydrolysate from yellow stripe trevally (Selaroides leptolepis) as microbial media. Food and Bioprocess Technology, 5(4), 1317-1327. DOI: 10.1007/s11947-010-0402-9

Silpradit, K., Tadakittasarn, S., Rimkeeree, H., Winitchai, S., & Haruthaithanasan, V. (2010). Optimization of rice bran protein hydrolysate production using alcalase. In Asian Journal of Food and Agro-Industry (Vol. 3, No. 2, pp. 221-231). King Mongkut's University of Technology Thonburi (KMUTT). Korhonen, H., & Pihlanto, A. (2006). Bioactive peptides: production and functionality. *International dairy journal*, *16*(9), 945-960.

Kristinsson, H. G., & Rasco, B. A. (2000). Fish protein hydrolysates: production, biochemical,

and functional properties. Critical reviews in food science and nutrition, 40(1), 43-81. DOI: 10.1080/10408690091189266

Kujawinski, E. B., Kido Soule, M. C., Valentine, D. L., Boysen, A. K., Longnecker, K., & Redmond, M. C. (2011). Fate of dispersants associated with the Deepwater Horizon oil spill. *Environmental science & technology*, *45*(4), 1298-1306.

Küllenberg, D., Taylor, L. A., Schneider, M., & Massing, U. (2012). Health effects of dietary phospholipids. *Lipids Health Dis*, *11*(3), 1-16.

Kumar, M. N. R. (2000). A review of chitin and chitosan applications.*Reactive and functional polymers*, *46*(1), 1-27.

Kurita, K. (2006). Chitin and chitosan: functional biopolymers from marine crustaceans. *Marine Biotechnology*, *8*(3), 203-226.

Kuvendziev, S., Lisichkov, K., Zeković, Z., & Marinkovski, M. (2014). Artificial neural network modelling of supercritical fluid CO 2 extraction of polyunsaturated fatty acids from common carp (Cyprinus carpio L.) viscera. The Journal of Supercritical Fluids, 92, 242-248. DOI: 10.1016/j.supflu.2014.06.007

la Farré, M., García, M. J., Tirapu, L., Ginebreda, A., & Barceló, D. (2001). Wastewater toxicity screening of non-ionic surfactants by Toxalert® and Microtox® bioluminescence inhibition assays. *Analytica chimica acta*, *427*(2), 181-189. http://dx.doi.org/10.1016/s0003-2670(00)01022-9

Laha, S., & Luthy, R. G. (1992). Effects of nonionic surfactants on the solubilization and mineralization of phenanthrene in soil–water systems. Biotechnology and Bioengineering,

40(11), 1367-1380.

Lamsal, B. P., Koegel, R. G., & Gunasekaran, S. (2007). Some physicochemical and functional properties of alfalfa soluble leaf proteins. *LWT-Food Science and Technology*, *40*(9), 1520-1526.

Law, R. J., & Kelly, C. (2004). The impact of the "Sea Empress" oil spill.*Aquatic Living Resources*, *17*(03), 389-394.

Lee, Kenneth (chair), Michel Boufadel, Bing Chen, Julia Foght, Peter Hodson, Stella Swanson, Albert Venosa. (2015). Expert Panel Report on the Behaviour and Environmental Impacts of Crude Oil Released into Aqueous Environments. Royal Society of Canada, Ottawa, ON. ISBN: 978-1- 928140-02-3

Lee, S. J., & McClements, D. J. (2010). Fabrication of protein-stabilized nanoemulsions using a combined homogenization and amphiphilic solvent dissolution/evaporation approach. *Food Hydrocolloids*, *24*(6), 560-569.

Lentz, R. D. (2003). Inhibiting water infiltration with polyacrylamide and surfactants: Applications for irrigated agriculture. *Journal of soil and water conservation*, *58*(5), 290-300.

Lessard, R. R., & DeMarco, G. (2000). The significance of oil spill dispersants. *Spill Science & Technology Bulletin*, *6*(1), 59-68.

Lewis, A., & Daling, P. (2001). Oil spill dispersants. *Consultant ALOS. SINTEF, Staines*.

Lewis, A., Crosbie, A., Davies, L., & Lunel, T. (1998). Large scale field experiments into oil weathering at sea and aerial application of dispersants.

Li, L., Wang, J., Zhao, M., Cui, C., & Jiang, Y. (2006). Artificial neural network for production of antioxidant peptides derived from bighead carp muscles with alcalase. Food technology and biotechnology, 44(3), 441.

Liu, Q., Kong, B., Xiong, Y. L., & Xia, X. (2010). Antioxidant activity and functional properties of porcine plasma protein hydrolysate as influenced by the degree of hydrolysis. *Food Chemistry*, *118*(2), 403-410. http://dx.doi.org/10.1016/j.foodchem.2009.05.013

Lomax, E. G. (Ed.). (1996). *Amphoteric surfactants* (Vol. 59). CRC Press.

López-Cervantes, J., Sánchez-Machado, D. I., & Rosas-Rodríguez, J. A. (2006). Analysis of free amino acids in fermented shrimp waste by high-performance liquid chromatography. *Journal of Chromatography A*, *1105*(1), 106-110.

Lou, W., & Nakai, S. (2001). Application of artificial neural networks for predicting the thermal inactivation of bacteria: a combined effect of temperature, pH and water activity. Food Research International, 34(7), 573-579. DOI: 10.1016/S0963-9969(01)00074-6

Lubchenco, J., McNutt, M. K., Dreyfus, G., Murawski, S. A., Kennedy, D. M., Anastas, P. T., ... & Hunter, T. (2012). Science in support of the Deepwater Horizon response. *Proceedings of the National Academy of Sciences*, *109*(50), 20212-20221.

Lunel, T., Swannell, R., Rusin, J., Wood, P., Bailey, N., Halliwell, C., ... & McDonagh, M. (1995). Monitoring the effectiveness of response operations during the Sea Empress incident: a key component of the successful counter-pollution response. *Spill Science & Technology Bulletin*, *2*(2), 99-112.

Mackay, D. (1995). Effectiveness of chemical dispersants under breaking wave conditions. *ASTM Special Technical Publication*, (1252), 310-340.

Mackay, D., Chau, A., Hossain, K., & Bobra, M. (1984). Measurement and prediction of the effectiveness of oil spill chemical dispersants. *Oil Spill Chemical Dispersants, Research, Experience and Recommendations, ASTM STP*, *840*, 38-54.

Makkar, R. S., Cameotra, S. S., & Banat, I. M. (2011). Advances in utilization of renewable substrates for biosurfactant production. *AMB express*, *1*(5), 1-19.

Manni, L., Ghorbel-Bellaaj, O., Jellouli, K., Younes, I., & Nasri, M. (2010). Extraction and characterization of chitin, chitosan, and protein hydrolysates prepared from shrimp waste by treatment with crude protease from Bacillus cereus SV1. *Applied Biochemistry and Biotechnology*, *162*(2), 345-357.

Marquez, M. C., & Vázquez, M. A. (1999). Modeling of enzymatic protein hydrolysis. Process biochemistry, 35(1), 111-117. DOI: 10.1016/S0032-9592(99)00041-2

Martone, C. B., Borla, O. P., & Sánchez, J. J. (2005). Fishery by-product as a nutrient source for bacteria and archaea growth media. Bioresource technology, 96(3), 383-387. DOI: 10.1016/j.biortech.2004.04.008

Mathew, P., & KG, R. N. (2006). Ensilation of shrimp waste by Lactobacillus fermentum. *Fishery Technology*, *43*(1).

McCulloch,W.W., & Pitts,W. (1943). A logical calculus of ideas imminent in nervous activity. *Bulletin of Mathematical Biophysics*, 5, 115–133.

Mizani, M. A. R. Y. A. M., Aminlari, M., & Khodabandeh, M. (2005). An effective method for

producing a nutritive protein extract powder from shrimp-head waste. *Food science and technology international*, *11*(1), 49-54. http://dx.doi.org/10.1177/1082013205051271

Moles, A., Holland, L., & Short, J. (2002). Effectiveness in the laboratory of Corexit 9527 and 9500 in dispersing fresh, weathered, and emulsion of Alaska North Slope crude oil under subarctic conditions. *Spill Science & Technology Bulletin*, *7*(5), 241-247.

Montgomery, D. C. (2008). Design and analysis of experiments. John Wiley & Sons.

Morgenroth, E., Kommedal, R., & Harremos, P. (2002). Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment-a review. Water Science & Technology, 45(6), 25-40.

Muheem, A., Shakeel, F., Jahangir, M. A., Anwar, M., Mallick, N., Jain, G. K., ... & Ahmad, F. J. (2014). A review on the strategies for oral delivery of proteins and peptides and their clinical perspectives. *Saudi Pharmaceutical Journal*.

Mutalik, S. R., Vaidya, B. K., Joshi, R. M., Desai, K. M., & Nene, S. N. (2008). Use of response surface optimization for the production of biosurfactant from Rhodococcus spp. MTCC 2574. *Bioresource technology*, 99(16), 7875-7880.

Myers, D. (2005). *Surfactant science and technology*. John Wiley & Sons.

Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2009). Response surface methodology: process and product optimization using designed experiments (Vol. 705). John Wiley & Sons.

Narayan, B., Velappan, S. P., Zituji, S. P., Manjabhatta, S. N., & Gowda, L. R. (2010). Yield and chemical composition of fractions from fermented shrimp biowaste. *Waste*

Management & Research, *28*(1), 64-70.

Nikiforidis, C. V., Karkani, O. A., & Kiosseoglou, V. (2011). Exploitation of maize germ for the preparation of a stable oil-body nanoemulsion using a combined aqueous extraction–ultrafiltration method. *Food Hydrocolloids*,*25*(5), 1122-1127.

Nitschke, M., & Costa, S. G. V. A. O. (2007). Biosurfactants in food industry. *Trends in Food Science & Technology*, *18*(5), 252-259.

Nomack, M. (2010). Environmental impacts of oil spills. Retrieved from http://www.eoearth.org/view/article/158443

NRC (National Research Council). 1989. Using Oil Spill Dispersants on the Sea. National Academy Press, Washington, DC.

NRC (National Research Council). 2005. *Oil Spill Dispersants: Efficacy and Effects;* National Academy Press: Washingon, DC.

Nwanna, L. C. (2003). Nutritional value and digestibility of fermented shrimp head waste meal by African catfish Clarias gariepinus. *Pakistan Journal of nutrition*, *2*(6), 339-345.

Nwanna, L. C., & Daramola, J. A. (2000, September). Harnessing of shrimp head waste in Nigeria for low cost production of tilapia, Oreochromis niloticus (L.). In *Tilapia aquaculture in the 21st Century. Proc. from the 5th Intl. Symp. on Tilapia in Aquaculture. Rio de Janeiro, Brasil* (pp. 174-178).

Okoye, F. C., Ojelowa, G. S., & Njoku-Onu, K. (2005). Evaluation of shrimp waste meal as a probable animal protein source for broiler chicken. *Int. J. Poult. Sci*, *4*, 458-461.

Ortega-Calvo, J. J., & Alexander, M. (1994). Roles of bacterial attachment and spontaneous

partitioning in the biodegradation of naphthalene initially present in nonaqueous-phase liquids. Applied and environmental microbiology, 60(7), 2643-2646.

Pacheco, N., Garnica-González, M., Ramírez-Hernández, J. Y., Flores-Albino, B., Gimeno, M., Bárzana, E., & Shirai, K. (2009). Effect of temperature on chitin and astaxanthin recoveries from shrimp waste using lactic acid bacteria. *Bioresource technology*, *100*(11), 2849-2854.

Park, E., Enander, R., Barnett, S. M., & Lee, C. (2001). Pollution prevention and biochemical oxygen demand reduction in a squid processing facility. *Journal of Cleaner Production*, *9*(4), 341-349.

Percot, A., Viton, C., & Domard, A. (2003a). Characterization of shrimp shell deproteinization. *Biomacromolecules*, *4*(5), 1380-1385.

Percot, A., Viton, C., & Domard, A. (2003b). Optimization of chitin extraction from shrimp shells. *Biomacromolecules*, *4*(1), 12-18.

Pietroski, J. P., White, J. R., & DeLaune, R. D. (2015). Effects of dispersant used for oil spill remediation on nitrogen cycling in Louisiana coastal salt marsh soil. *Chemosphere*, 119, 562-567. http://dx.doi.org/10.1016/j.chemosphere.2014.07.050

Porter, M. R. (2013). *Handbook of surfactants*. Springer.

Prameela, K., Murali Mohan, C., Smitha, P. V., & Hemalatha, K. P. J. (2010). Bioremediation of shrimp biowaste by using natural probiotic for chitin and carotenoid production an alternative method to hazardous chemical method. *International Journal of Applied Biology and Pharmaceutical Technology*, *1*(3), 903-910.

Prince, R. C. (2015). Oil spill dispersants: boon or bane?. *Environmental science & technology*, *49*(11), 6376-6384.

Prince, R. C., & Butler, J. D. (2014). A protocol for assessing the effectiveness of oil spill dispersants in stimulating the biodegradation of oil. *Environmental Science and Pollution Research*, *21*(16), 9506-9510.

Radzali, S. A., Baharin, B. S., Othman, R., Markom, M., & Rahman, R. A. (2014). Co-solvent selection for supercritical fluid extraction of astaxanthin and other carotenoids from Penaeus monodon waste. *Journal of oleo science*, *63*(8), 769-777.

Rahman, P. K., & Gakpe, E. (2008). Production, characterisation and applications of biosurfactants-Review. *Biotechnology*.

Rao, M. S., & Stevens, W. F. (2005). Quality parameters of chitosan derived from fermentation of shrimp biomaterial using a drum reaction. *J. chem. technol. Biotechnol*, *80*, 1080-1087.

Rao, M. S., & Stevens, W. F. (2006). Fermentation of shrimp biowaste under different salt concentrations with amylolytic and non-amylolytic Lactobacillus strains for chitin production. *Food Technology and Biotechnology*, *44*(1), 83-87.

Rodrigues, L., Teixeira, J., Oliveira, R., & Van Der Mei, H. C. (2006). Response surface optimization of the medium components for the production of biosurfactants by probiotic bacteria. Process Biochemistry, 41(1), 1-10. DOI: 10.1016/j.procbio.2005.01.030

Rohyami, Y., Zulkipli, R. A., Safitri, A. D., Pratiwi, N. W., & Sholihah, R. A. (2015). Adsorption of Hexavalent Chromium from Aqueous Solutions by Chitosan/Chitosan Beads. *Adsorption*.

Ruttanapornvareesakul, Y., Ikeda, M., Hara, K., Osako, K., Orawan, K. and Nozaki, Y. (2005). Effect of shrimp head protein hydrolysates on the state of water and denaturation of fish myofibrils during dehydration. *Fisheries Science*, 71: 220–228.

Ruttanapornvareesakul, Y., Ikeda, M., Hara, K., Osatomi, K., Osako, K., Kongpun, O., & Nozaki, Y. (2006). Concentration-dependent suppressive effect of shrimp head protein hydrolysate on dehydration-induced denaturation of lizardfish myofibrils. Bioresource technology, 97(5), 762-769. DOI: 10.1016/j.biortech.2005.03.038

Sachindra, N. M., Bhaskar, N., & Mahendrakar, N. S. (2006). Recovery of carotenoids from shrimp waste in organic solvents. *Waste Management*,*26*(10), 1092-1098.

Saeki, H., Sasaki, M., Komatsu, K., Miura, A., & Matsuda, H. (2009). Oil spill remediation by using the remediation agent JE1058BS that contains a biosurfactant produced by Gordonia sp. strain JE-1058. *Bioresource Technology*, *100*(2), 572-577.

Safari, R., Motamedzadegan, A., Ovissipour, M., Regenstein, J. M., Gildberg, A., & Rasco, B. (2012). Use of hydrolysates from yellowfin tuna (Thunnus albacares) heads as a complex nitrogen source for lactic acid bacteria. *Food and Bioprocess Technology*, *5*(1), 73-79. http://dx.doi.org/10.1007/s11947-009-0225-8

Samaranayaka, A. G., & Li-Chan, E. C. (2008). Autolysis-assisted production of fish protein hydrolysates with antioxidant properties from Pacific hake (Merluccius productus). Food Chemistry, 107(2), 768-776. DOI: 10.1016/j.foodchem.2007.08.076

Sarkar, B., Sengupta, A., De, S., & DasGupta, S. (2009). Prediction of permeate flux during electric field enhanced cross-flow ultrafiltration—a neural network approach. Separation

and purification technology, 65(3), 260-268. DOI: 10.1016/j.seppur.2010.04.018

Satpute, S. K., Banpurkar, A. G., Dhakephalkar, P. K., Banat, I. M., & Chopade, B. A. (2010). Methods for investigating biosurfactants and bioemulsifiers: a review. *Critical reviews in biotechnology*, *30*(2), 127-144.

Schippers, C., Gessner, K., Müller, T., & Scheper, T. (2000). Microbial degradation of phenanthrene by addition of a sophorolipid mixture. Journal of Biotechnology, 83(3), 189-198.

Schramm, L. L. (2000). *Surfactants: fundamentals and applications in the petroleum industry*. Cambridge University Press.

Sea Empress Environmental Evaluation Committee. (1998). *The environmental impact of the Sea Empress oil spill: final report*. Stationery Office.

See, S. F., Hoo, L. L., & Babji, A. S. (2011). Optimization of enzymatic hydrolysis of Salmon (Salmo salar) skin by Alcalase. International Journal of Food Research, 18(4), 1359-1365.

Senphan, T., & Benjakul, S. (2012). Compositions and yield of lipids extracted from hepatopancreas of Pacific white shrimp (Litopenaeus vannamei) as affected by prior autolysis. *Food chemistry*, *134*(2), 829-835.

Shao, P., Jiang, S. T., & Ying, Y. J. (2007). Optimization of molecular distillation for recovery of tocopherol from rapeseed oil deodorizer distillate using response surface and artificial neural network models. *Food and Bioproducts Processing*, *85*(2), 85-92.

Shekhar, S., Sundaramanickam, A., & Balasubramanian, T. (2014). Biosurfactant producing microbes and its potential applications: A Review. *Critical Reviews in Environmental*

Science and Technology, 45:14, 1522-1554.

Shen, Q., & Cheung, H. Y. (2014). TiO2/SiO2 Core–Shell Composite-Based Sample Preparation Method for Selective Extraction of Phospholipids from Shrimp Waste Followed by Hydrophilic Interaction Chromatography Coupled with Quadrupole Time-of-Flight/Mass Spectrometry Analysis. *Journal of agricultural and food chemistry*, *62*(36), 8944-8951.

Shreve, G. S., Inguva, S., & Gunnam, S. (1995). Rhamnolipid biosurfactant enhancement of hexadecane biodegradation by Pseudomonas aeruginosa. Molecular marine biology and biotechnology, 4(4), 331-337.

Singer, M. M., George, S., Jacobson, S., Lee, I., Tjeerdema, R. S., & Sowby, M. L. (1994). Comparative toxicity of Corexit® 7664 to the early life stages of four marine species. *Archives of Environmental Contamination and Toxicology*, *27*(1), 130-136.

Singh, A. M., & Dalgleish, D. G. (1998). The emulsifying properties of hydrolyzates of whey proteins. *Journal of Dairy Science*, *81*(4), 918-924.

Šližytė, R., Mozuraitytė, R., Martinez-Alvarez, O., Falch, E., Fouchereau-Peron, M., & Rustad, T. (2009). Functional, bioactive and antioxidative properties of hydrolysates obtained from cod (Gadus morhua) backbones.*Process Biochemistry*, *44*(6), 668-677.

Sohsalam, P., Englande, A. J., & Sirianuntapiboon, S. (2008). Seafood wastewater treatment in constructed wetland: Tropical case. *Bioresource technology*, *99*(5), 1218-1224.

Sorial, G. A., Venosa, A. D., Koran, K. M., Holder, E., & King, D. W. (2004). Oil spill dispersant effectiveness protocol. II: Performance of revised protocol. *Journal of*

environmental engineering, *130*(10), 1085-1093.

Sowmya, R., Rathinaraj, K., & Sachindra, N. M. (2011). An autolytic process for recovery of antioxidant activity rich carotenoprotein from shrimp heads.*Marine biotechnology*, *13*(5), 918-927.

Srinivasan, R., Lu, Q., Sorial, G. A., Venosa, A. D., & Mullin, J. (2007). Dispersant effectiveness of heavy fuel oils using baffled flask test. *Environmental Engineering Science*, *24*(9), 1307-1320.

Stelmack, P. L., Gray, M. R., & Pickard, M. A. (1999). Bacterial adhesion to soil contaminants in the presence of surfactants. Applied and environmental microbiology, 65(1), 163-168.

Susilaningsih, D., Okazaki, F., Yopi, Y., Widyastuti, Y., & Harayama, S. (2013, December). Isolation and Screening of Surfactant-producing Bacteria from Indonesian Marine Environments and Its Application on Bioremediation. In *ANNALES BOGORIENSES* (Vol. 17, No. 2, pp. 43-53).

Synowiecki, J., & Al-Khateeb, N. A. A. Q. (2000). The recovery of protein hydrolysate during enzymatic isolation of chitin from shrimp Crangon crangon processing discards. *Food chemistry*, *68*(2), 147-152. http://dx.doi.org/10.1016/s0308-8146(99)00165-x

Tang, L., Sun, J., Zhang, H. C., Zhang, C. S., Yu, L. N., Bi, J., Zhu, F., Liu, S. F., & Yang, Q. L. (2012). Evaluation of physicochemical and antioxidant properties of peanut protein hydrolysate. PLoS ONE 7(5): e37863. http://dx.doi.org/10.1371/journal.pone.0037863

Taskin, M., & Kurbanoglu, E. B. (2011). Evaluation of waste chicken feathers as peptone source for bacterial growth. Journal of applied microbiology,111(4), 826-834. DOI:

10.1111/j.1365-2672.2011.05103.x

Trinca, L. A., & Gilmour, S. G. (2000). Difference variance dispersion graphs for comparing response surface designs with applications in food technology.*QUALITY CONTROL AND APPLIED STATISTICS*, *45*(4), 447-452.

Venosa, A. D., King, D. W., & Sorial, G. A. (2002). The baffled flask test for DE: a round robin evaluation of reproducibility and repeatability. *Spill Science & Technology Bulletin*, *7*(5), 299-308. http://dx.doi.org/10.1016/s1353-2561(02)00072-5

Versluis, J. F. A., van Buijtenen, J. P., Melkert, J. A., & Bogers, P. (2004, January). Thermal Stability Fuel Additives for Civil Aviation Applications. In*ASME Turbo Expo 2004: Power for Land, Sea, and Air* (pp. 65-71). American Society of Mechanical Engineers.

Vidotti, R. M., Viegas, E. M. M., & Carneiro, D. J. (2003). Amino acid composition of processed fish silage using different raw materials. *Animal Feed Science and Technology*, *105*(1), 199-204.

Wangtueai, S., & Noomhorm, A. (2009). Processing optimization and characterization of gelatin from lizardfish (Saurida spp.) scales. LWT-Food Science and Technology, 42(4), 825-834. DOI: 10.1016/j.lwt.2008.11.014

Wei, J., Wang, H., Zhang, Q., & Li, Y. (2015). One-pot hydrothermal synthesis of N-doped carbon quantum dots using the waste of shrimp for hydrogen evolution from formic acid. *Chemistry Letters*, *44*(3), 241-243.

Wells, P.G., 1984. The toxicity of oil spill dispersants to marine organisms: a current perspective. In: Allen, T.E. (Ed.), Oil Spill Chemical Dispersants: Research, Experience,

and Recommendations, STP 840. American Society for Testing and Materials, Philadelphia, PA.

Weyershausen, B., & Lehmann, K. (2005). Industrial application of ionic liquids as performance additives. *Green Chemistry*, *7*(1), 15-19.

Wong, P. T., Wang, S. H., Ciotti, S., Makidon, P. E., Smith, D. M., Fan, Y., ... & Baker Jr, J. R. (2013). Formulation and characterization of nanoemulsion intranasal adjuvants: effects of surfactant composition on mucoadhesion and immunogenicity. *Molecular pharmaceutics*, *11*(2), 531-544.

Wurm, F. R., & Weiss, C. K. (2014). Nanoparticles from renewable polymers. Frontiers in chemistry, 2.

Xuemei, Z., & Hawkins, S. J. (2002). Interactions of aquaculture and waste disposal in the coastal zone. *Journal of Ocean University of Qingdao*, *1*(1), 8-12.

Yi, Q. X., Huang, J. F., Wang, F. M., Wang, X. Z., & Liu, Z. Y. (2007). Monitoring rice nitrogen status using hyperspectral reflectance and artificial neural network. Environmental science & technology, 41(19), 6770-6775. DOI: 10.1021/es070144e

Zhao, J., & Xiong, Y. L. (2015). Interfacial peptide partitioning and undiminished antioxidative and emulsifying activity of oxidatively stressed soy protein hydrolysate in an O/W emulsion. *LWT-Food Science and Technology*, *61*(2), 322-329.

Zhao, J., Xiong, Y. L., & McNear, D. H. (2013). Changes in structural characteristics of antioxidative soy protein hydrolysates resulting from scavenging of hydroxyl radicals. *Journal of food science*, *78*(2), C152-C159.

http://dx.doi.org/10.1111/1750-3841.12030

Zheng, H., Shen, X., Bu, G., & Luo, Y. (2008). Effects of pH, temperature and enzyme-to-substrate ratio on the antigenicity of whey protein hydrolysate prepared by Alcalase. International dairy journal, 18(10), 1028-1033. DOI: 10.1016/j.idairyj.2008.05.002

Zimman, A., Chen, S. S., Komisopoulou, E., Titz, B., Martínez-Pinna, R., Kafi, A., ... & Graeber, T. G. (2010). Activation of aortic endothelial cells by oxidized phospholipids: a phosphoproteomic analysis. *Journal of proteome research*, *9*(6), 2812-2824.

APPENDICES

APPENDIX A: Standard calibration curves for BFT

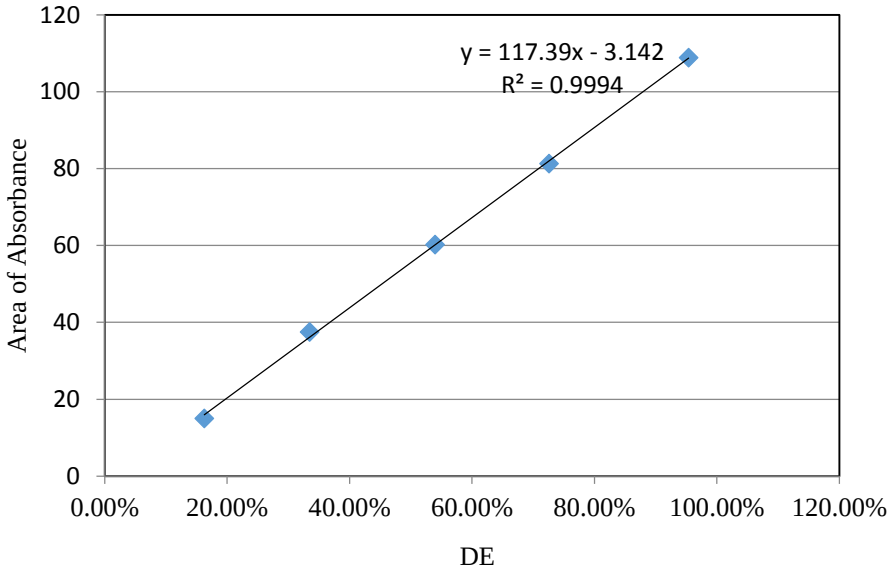


Figure A-1 ANS crude oil standard curve

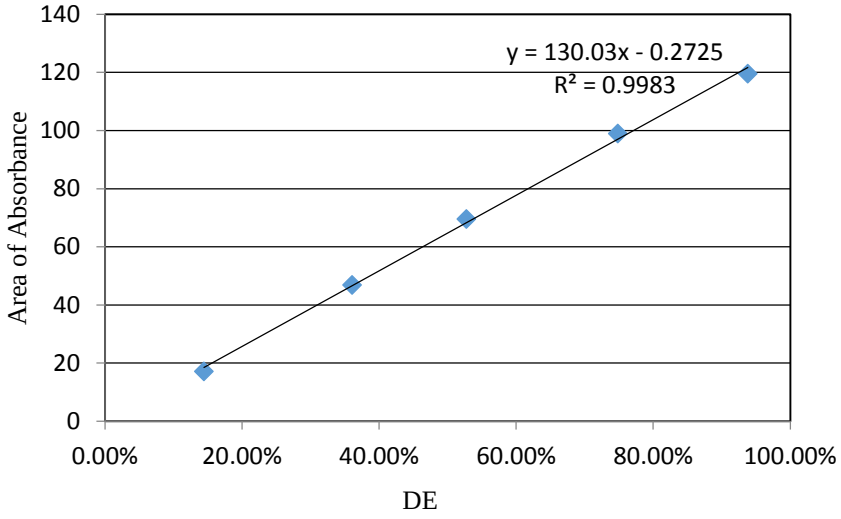


Figure A-2 PBC crude oil standard curve

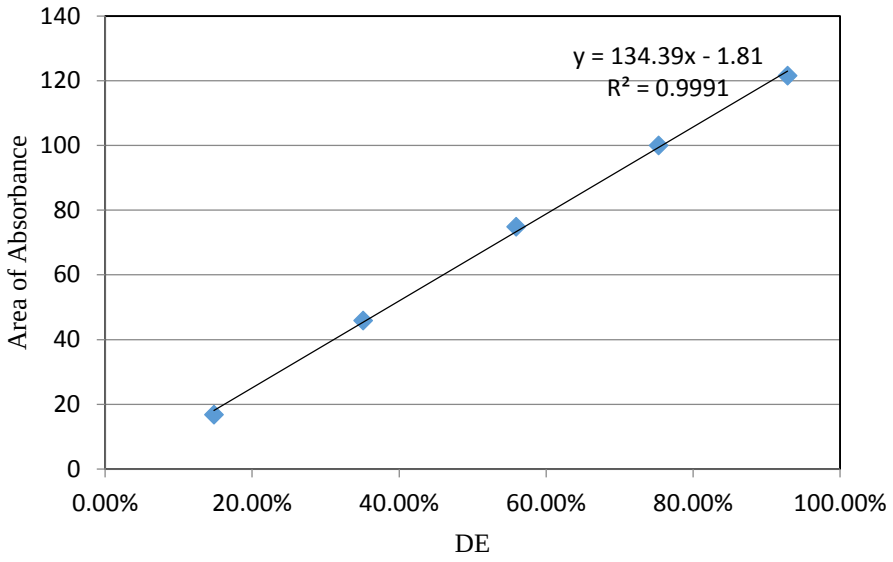


Figure A-3 ALC crude oil standard curve

APPENDIX B: Experiment system and facilities

Figure B-1 Temperature-controlled water bath shaker

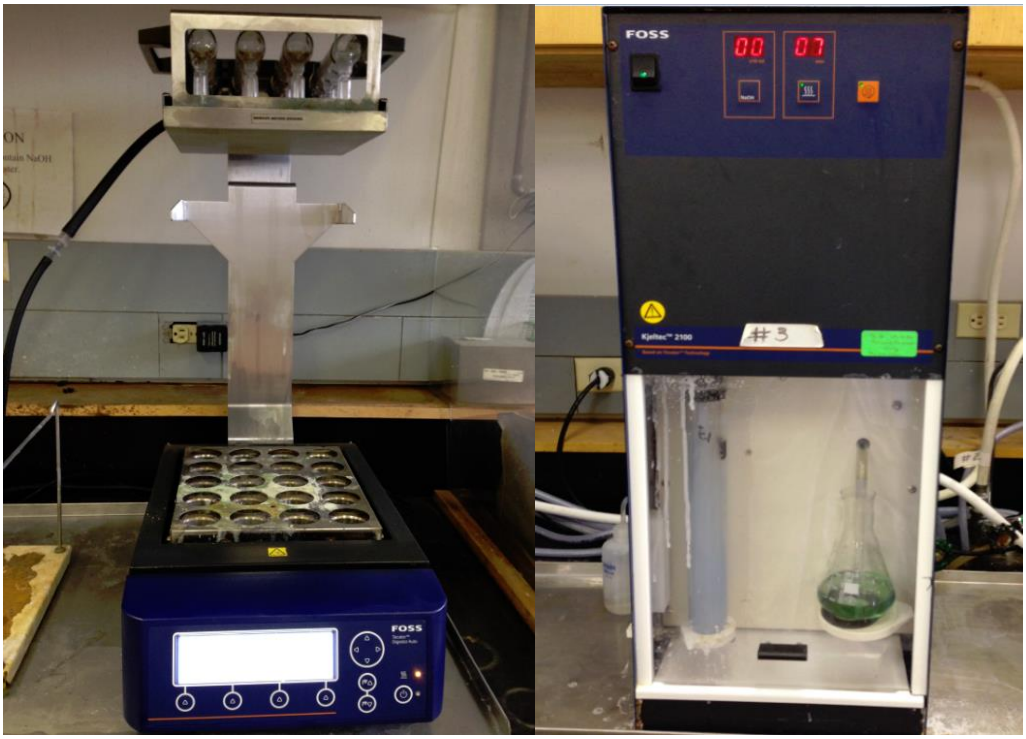


Figure B-2 Digestion system (left) and distillation system (right) for Kjeldahl Nitrogen determination

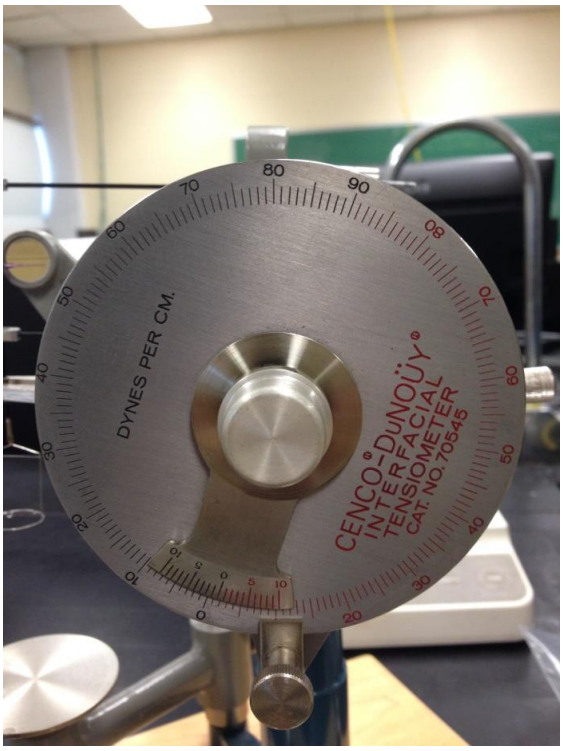


Figure B-3 Tensiometer for surface tension measurement

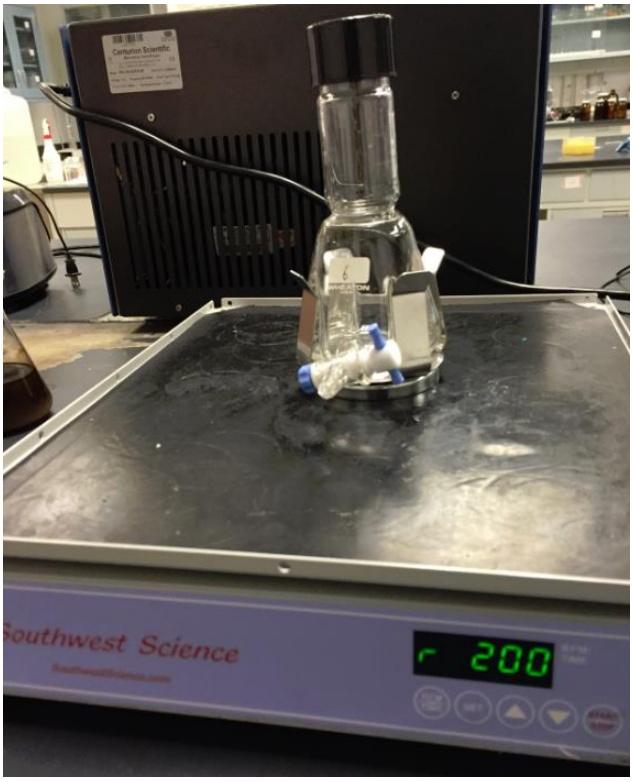


Figure B-3 BFT flask and shaker

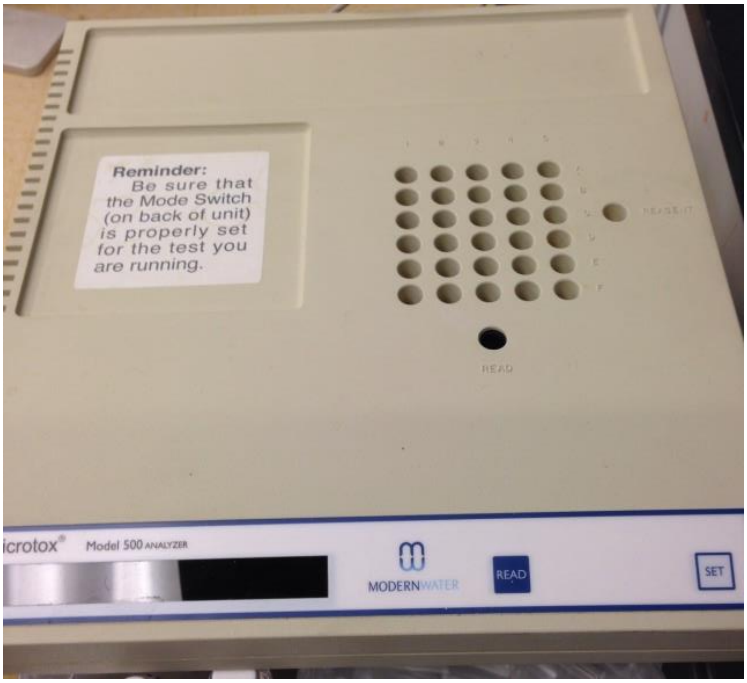


Figure B-4 Microtox model 500 analyzer

APPENDIX C: Images of ANS crude oil dispersed by shrimp waste based dispersant

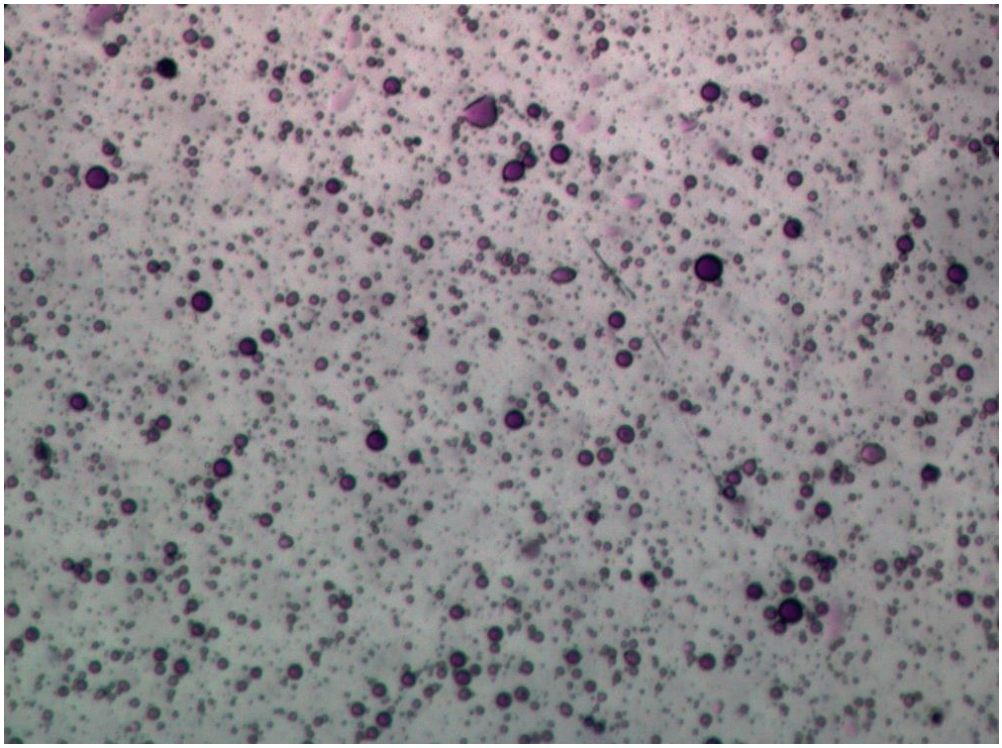


Figure C-1 ANS Crude Oil Dispersed by shrimp waste based dispersant under microscope