DEVELOPMENT OF HERDING AGENTS FOR MARINE OIL SPILL RESPONSE

by

©Miao Yu

A Thesis Submitted to the

School of Graduate Studies

In partial fulfillment of the requirements for the degree of

Master of Engineering

Civil Engineering

Faculty of Engineering and Applied Science

Memorial University of Newfoundland

October 2021

St. John's Newfoundland and Labrador

ABSTRACT

This thesis tackles the development of herders as marine oil spill treating agents. In the first section of the thesis, a biosurfactant-based bioherder was generated and examined for marine oil spill response. The biosurfactant was produced by *Rhodococcus erythropolis*, a strain isolated from the North Atlantic Ocean. A commercial chemical herder, USN cold water blend, was used for performance comparison. Factors affecting herding effectiveness, including temperature, salinity, and oil/herder ratio, were investigated using the Design of Experiment methodology. Statistical analysis was employed to study the interactions among the factors on herding ability. Experimental results confirmed that the developed bio-herder can serve as an effective marine oil spill treating agent, and temperature and herder/oil ratio are significant factors affecting oil herding effectiveness.

In the second section of the thesis, molecular design was conducted to obtain environmentally friendly herder products for marine oil spill response. The quantitative structure-activity relationship between molecular structures of herders and their activities (herding ability and toxicity) was firstly established using 3D-QSAR modeling. Span surfactants were selected as the model key components of herders. Two QSAR (CoMFA and CoMSIA) models were constructed, and ten molecularly modified span compounds were obtained. Molecular docking was carried out to further examine the biodegradability of one newly designed molecular.

Through both studies, new herders (bio-herder and molecular modified herder) were generated, which are useful in herd production. In addition, the associated herding performance and environmental impacts were evaluated. Overall, this thesis work contributes new knowledge to the field and provides more sustainable options for marine oil spill response operations.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere thanks to my supervisors, Drs. Baiyu Zhang and Bing Chen. They have not only patiently provided me training in critical thinking and independent research, but also motivated me to establish vision and innovation through my Master study.

I would also like to acknowledge the Northern Region Persistent Organic Pollution Control (NRPOP) Laboratory, the Faculty of Engineering and Applied Science, the School of Graduate Studies at the Memorial University of Newfoundland for providing financial and technical support for my research. My special thanks go to Dr. Qinhong Cai, Dr. Zhiwen Zhu, Dr. Xing Song, Wenwen Gu, Yiqi Cao, Qiao Kang, and Lidan Tao for their inspiration and guidance. I want to express my deepest gratitude to my lab colleagues, Jingjing Ling, Yunwen Tao, Fei Wu, Guihua Dong, Min Yang, Xixi Li, Xudong Ye, Jiheng Hu, Dr. Hongjing Wu, Dr. Bo Liu, and Dr. Xiaying Xin, for their precious friendships and support during my study.

Last but not least, I would like to thank my parents and my boyfriend for their love and support as always. My gratitude also goes to all my family members and friends. I would not accomplish this study without their love and support.

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LIST OF SYMBOLS AND ABBREVIATIONS

QSAR	Quantitative Structure-Activity Relationship
CMC	Critical Micelle Concentration
DOE	Design Of Experiment
KOA	Octanol-Air Partition Coefficients
KOW	Octanol/Water Partition Coefficient
MUN	Memorial University Of Newfoundland
NRPOP	Northern Region Persistent Organic Pollution Control
PAHS	Polycyclic Aromatic Hydrocarbons
FTIR	Fourier Transform Infrared
CCNY	City Collage Of New York
ANS	Arctic North Slope
HLB	Hydrophilic-Lipophilic Balance
IC_{50}	Inhibitory Concentration
EC_{50}	Effective Concentration

ISB	In Situ Burning
PAHs	Polycyclic Aromatic Hydrocarbons
USN	United States Navy
PM	Production Medium
MGDG	Monogalactosyldiacylglycerol
SDS	Sodium Dodecyl Sulfate
THL	Trehalose Tetraester
HOR	Herder Oil Ratio
LOO	Leave One Out
PCNs	Polychlorinated naphthalene
POPs	Persistent Organic Pollutions

CHAPTER 1 INTRODUCTION

1.1 Background

Oil spills have drawn concern globally due to their impacts on the marine environment. In 1989, over 36,000 seabirds died immediately after the Exxon Valdez oil spill, and the majority of rescued 1,800 living oiled seabirds died after they were brought to rehabilitation centers (Piatt & Ford, 1996). In 2010, the Deepwater Horizon oil spill was claimed as the largest record in the history of the petroleum industry (Bly, 2011). Over 700 thousand tons of crude oil were released (Commission, 2011). This oil spill has caused over $5.5 billion loss to fishing and tourism industries, millions of seabirds and billions of fish eggs were killed, and population shift of microorganisms, phytoplankton, and other flora was observed (Corn, 2010; Hagerty, 2010; Widger et al., 2011). In 2004, between 10,000 and 16,000 birds died in the Terra Nova oil spill (Wilhelm et al., 2007). With the estimated undiscovered potential of 6 billion barrels of oil, Newfoundland and Labrador take up about 40% of Canada’s conventional light crude oil output with promising prospects (Turner et al., 2010). The province thus needs to be well prepared for any potential accidental oil spills.

A variety of response technologies, including physical/mechanical and chemical countermeasures, have been developed to help to take prompt actions once any accidental oil spill happens to reduce the associated impact on marine systems, fisheries and local communities. The boom is a mechanical device used to prevent spilled oil from spreading to surrounding areas. Once being constrained within a specific area by the boom, the oil can be recovered, processed, or concentrated so that it can be recovered, burned, or otherwise treated (Fingas, 2010). The oil skimmer is a mechanical device consisting of a disc, a belt, a drum, and a brush. It is designed to be combined with a boom to remove oil from the water surface without changing its performance so that it can be reprocessed and reused (Schwartz, 1979; Schulze, 1998; Hammoud, 2006). In-situ burning is a thermal method for oil spill repair, which can achieve a higher oil removal efficiency with minimal special equipment (Dave & Ghaly, 2011). About 250,000 barrels of floating oil were mitigated during 411 separate burn events (DWH-NRDA, 2015). However, these traditional mechanical/physical countermeasures have the shortcomings such as narrow operating time windows, fire protection requirements for booms, oil slick thickness requirements for ignition, interference caused by currency and wind, heat loss during burning, and insufficient separation (US EPA, 2012).

Mechanical countermeasures usually recover just a small fraction of the spilled oil and other techniques, such as chemical treating agents, are available to enhance oil spill response capabilities (Walker et al., 1995). Awareness of employing oil spill treating agents with surfactants as critical components has been raised, leading to the production and application of dispersants and herding agents. As a promising alternative response technique, the application of dispersants has been well recognized, especially after the Deepwater Horizon oil spill event (Walker et al., 2003). During the Deepwater Horizon oil spill, about 1.84 million gallons of Corexit 9500 and 9527 dispersants were applied from the sea surface and the subsea to disperse the oil (Graham et al., 2011). Herding agents are another group of oil spill treating agents, sometimes referred to as oil collecting agents. It can be sprayed onto the water surrounding an oil spill to thicken the spilled oil layer so as to further facilitate the in-situ burning treatment (Buist et al., 2008a).

1.2 Statement of Problems

Currently available chemical herders are mixtures of synthesized surfactants (including hydrocarbon-based, silicone-based, and fluoro-surfactants) and solvents (Buist et al., 2010a; Fingas, 2014). The ingredients of chemical herders are considered to be of significant toxicity and/or persistency in the environment (Imai et al., 1994; Mullin et al., 2016; Niedobová et al., 2016; Barron et al., 2020). The negative impacts of surfactant-based chemical oil treating agents on marine life and the environment raised increasing concerns, especially after the Deepwater Horizon oil spill (Beyer et al., 2016; Li et al., 2016). Chemical herding agents used in enhancing in-situ burning mainly remain in the water column after usage. Corexit 9580, as one of the common commercialized herders, increased the toxicity of the oil in the water column (Bhattacharyya et al., 2003). Due to its tendency to remain in the water, the risk of marine species intake is rising. The 72h-EC50 (median effective concentration) value of Corexit 9580 on marine species *Skeletonema costatum* was 135.3mg/L (Hansen et al., 2014). Sorbitan monolaurate, part of the sorbitan ester family, is the main ingredient of one chemical named Thickslick 6535. It was found that sorbitan ester could increase the mortality rates in rats and potentially induce cutaneous irritation in humans (Fitzhugh et al., 1960; Elder, 1985). Hence, more environmentally friendly and biodegradable surfactants need to be generated as marine oil spill treating agents. Biosurfactants has been used in various industries as an alternate to chemical surfactants.

Biosurfactant-based herding agents (Bio-herders) have significant potential as an alternative herder with better overall performance. Biosurfactants are surface-active materials produced by microorganisms (George-Ares et al., 1999). They can be applied in various industries as detergents, solubilizing agents, wetting and spreading agents, oil/water emulsifying agents, or antifoaming agents based on their hydrophilic-lipophilic balance (HLB) and chemical structures (Cai et al., 2014). Some biosurfactants have high spreading pressures since they are mixtures of congeners with hydrophobic moieties (Cai et al., 2014). Due to this high spreading force, they can spread rapidly on the water surface into a molecular layer and contact spilled oil slicks. In recent years, biosurfactants have been attracting a broad interest since they are biodegradable, less toxic, and stable in harsh environments (Al-Bahry et al., 2013; Alvarez et al., 2015; Patel et al., 2019). The commercialized biosurfactant Rhamnolipids has lower toxicity than chemical surfactants (Lang & Wagner, 1987). According to Lang and Wagner (1987), the Rhamnolipids surfactants have a lower IC_{50} value than chemical surfactants on marine flagellates microalgae. Glycolipids biosurfactants produced by *Rhodococcus* species were reported to have less aqueous toxicity than Tween-80 (Kanga et al., 1997). As studied by Marqués et al. (2009), trehalose tetraester (THL) produced by *Rhodococcus* species was less irritating than sodium dodecyl sulfate (SDS). It is thus desired to develop biosurfactant-based bio-herding agents with better performance and more friendliness to the environment. However, bio-herders have rarely been studied with extremely limited publications on bio-herder production and the associated herding effectiveness evaluation.

On the other hand, greener chemical herders can also be obtained through chemical structure modification using tools like 3D-QSAR modeling and molecular docking. 3D-QSAR studied the relationship between chemical properties and their molecular structures

(Akamatsu, 2002). It is a widely used tool in predicting environmental performance parameters of chemicals and design of environmentally friendly molecular. Molecular docking is an essential tool in computer-assisted drug design and structural molecular biology (Morris & Lim-Wilby, 2008). It can be used to predict the predominant binding of a ligand with a protein from a known three-dimensional structure. 3D-QSAR and molecular docking have the potential to be applicable for generating environmentally friendly chemicals. Four environmentally friendly ditridecyl phthalate derivatives were generated from the constructed 3D-QSAR model by evaluating their toxicity and estrogen combined activities (Du et al., 2020; Li et al., 2020a). Two environmentally friendly polychlorinated naphthalenes (PCNs) with lower toxicity were selected from established QSAR models (Gu et al., 2020b). And then, the biodegradation ability of these two PCNs derivatives further analyzed by binding the molecular with selected PAH degradation enzymes. Hydrocarbon-based chemical herder Span series are commonly used for in-situ burning enhancement (Buist et al., 2006). Herding agents are mainly detected on water surface post burning; this could cause concern for using chemical herder in the marine environment (Gray et al., 2014; Place et al., 2016). Greener chemical herder with molecular structure improved through computer-assisted modeling and molecular docking has not been reported. Hence, this study is amied at filling the knowledge gap by generating more environmentally friendly, biodegradable chemical herders as marine oil spill treating agents.

1.3 Objectives

To fill the research gaps identified in section 1.2, this thesis aims to develop environmentally friendly herders as marine oil spill treating agents. It entails two tasks: (1)

generation of a bio-herder using a biosurfactant producing *Rhodococcus* strain, and (2) development of green chemical herding agents with improved molecular structures using 3D-QSAR modeling and molecular docking.

Rhodococcus erythropolis M25, a strain obtained from mutation of an isolated species from the North Atlantic Ocean (Cai et al., 2016; Lv et al., 2016), was used to produce the biosurfactant-based bioherder. A commercial chemical herder, USN cold water blend, was used for performance comparison. Factors affecting herding effectiveness, including temperature, salinity, and oil/herder ratio, were investigated using the Design of Experiment methodology. Statistical analysis was employed to study the interactions among the factors on herding ability.

The molecular design was conducted to obtain green chemical herder products for marine oil spill response. Quantitative structure-activity relationships between molecular structures of herders and their activities (herding ability and toxicity) were established using 3D-QSAR modeling. Span surfactants were selected as the key model components of herders. Two QSAR (CoMFA and CoMSIA) models were constructed to obtain molecularly modified span compounds. Molecular docking was then adopted to further examine the biodegradability of any newly designed molecular. This is the first study using QSAR on herder structure modification and performance improvement.

Through both tasks, herder production was expected to be advanced with new herders (bio-herder and molecular modified herder) generated. In addition, the associated herding performance and environmental impacts were evaluated. The thesis outputs would help contribute to knowledge in the field and provide more options for sustainable marine oil spill response.

1.4 Thesis Structure

This thesis consists of five chapters. Chapter 2 describes the background of herding agents, as well as their structures and application as biosurfactants in oil spill response, including physicochemical properties and applications in oil pollution mitigation. In this chapter, the QSAR modeling classification, model design, and environmental application are reviewed. Chapter 3 is a study of the development of biosurfactant-based bio-herders by *Rhodococcus erythropolis* strain M25. This chapter also investigates the significant environmental factors affecting the performance of herders. Chapter 4 describes the 3D-QSAR studies to aid the production of green chemical herders with better herding performance through molecular modification. Finally, Chapter 5 presents the conclusions, research accomplishments, and contributions to knowledge, as well as recommendations for future work.

CHAPTER 2 LITERATURE REVIEW

2.1 Herding Agents for Marine Oil Spill Response

2.1.1 Herding theory

Herding agents usually consist of surfactants and solvents. In dealing with oil spill, herding agents facilitate in-situ burning technique by increasing the oil slick thickness. The chemical surfactant in the herding agent prevents oil from spreading with a character of larger spreading pressure than crude oil (Buist et al., 2010a). Spilled oil forms an oil slick on the water surface and has the tendency to spread into the sheen slick. Sprayed herding agents can prevent the oil slick from spreading and keep the thickness greater than 2-3mm. This critical oil slick thickness is key to the implementation of in-situ burning response (Buist et al., 2011a).

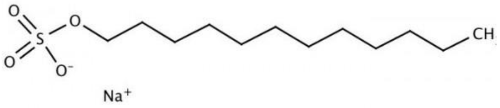
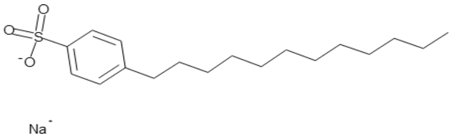
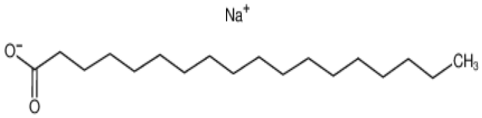
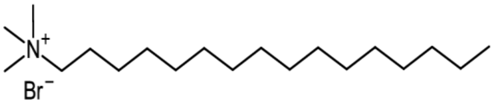
2.1.2 Chemical surfactants

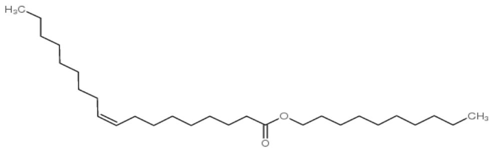
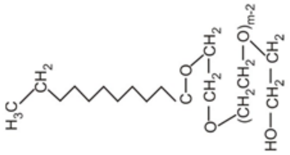
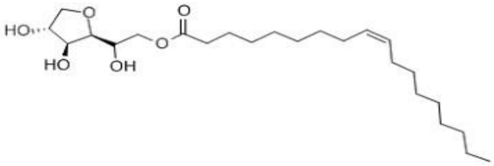
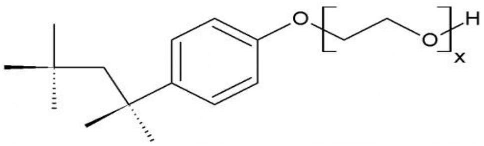
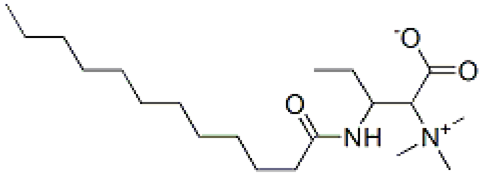
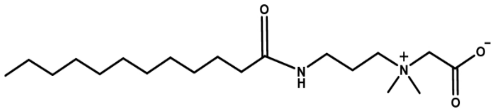
Surfactants are amphiphilic molecules that can lower the surface tension between two liquid phases or at the liquid-solid interface(Schramm, 2000). Surfactants are chemical compounds with both hydrophobic groups (or tails) and hydrophilic groups (or heads). As surface-active compounds, effective surfactants can reduce the surface tension between the air and water from 72 to 30 mN/m or lower (Schramm, 2000). The ability of surfactants to influence the properties of surfaces and interfaces could benefit the application in various fields.

Surfactants have been applied in many fields, ranging from food industry, agriculture, pharmaceuticals, personal care products, and environmental remediation (Dziezak, 1988; Schueller & Romanowski, 1998; Schramm et al., 2003). Surfactants can be added as food coating modifiers in the food production and processing industry. For example, surfactants such as polysorbate 60 and sorbitan monostearate were used to blend and stabilize the fat and cocoa butter in chocolate coating (Dziezak, 1988). Surfactants can be used in increasing the production rate of commercially grown vegetable crops in sandy soil. Surfactants used in sandy soil can enhance the water retention and nutrition capacity by reducing the sandy soil water repellency (Sarvaš, 2003; Ghebru et al., 2007). Surfactants also can be used in pharmaceutical suspensions as sodium lauryl sulfate, and docusate sodium are used to lower the surface tension between the suspended agent and suspending medium (Kulshreshtha et al., 2009). In the cosmetics industry, surfactants such as alkyl polyglyosides were used to improve the stability of micro-emulsions to reduce skin irritation and to create the formulation of oil-in-water emulsion (Rieger, 2017). Another application of surfactant is in the petroleum industry, where surfactant can be used to form oil-in-water emulsification, differential sticking prevention, shale-swelling inhibitors, and foaming/ defoaming addition (Quintero, 2002). Surfactants also have potential in oil spill remediation for both marine and sub-surface environments (Owoseni et al., 2014; Li et al., 2016).

Chemical surfactants can be classified according to their chemical structures into four main categories: anionic, cationic, nonanoic, and amphoteric or zwitterionic (Myers, 1991; Schramm et al., 2003). 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 (Stache, 1995). Cationic surfactants release amphiphilic cations and anions in water (Rubingh, 1990). Nonionic surfactants do not release ions in an aqueous solution. The types of hydrophilic groups usually include alcohols, phenols, ethers, esters, and amides. Nonionic surfactants mainly include ethoxylated linear alcohols, ethoxylated alkyl phenols, fatty acid esters, amine derivatives, and amide derivatives (Cross, 1987). Amphoteric or zwitterionic surfactants display both anionic and cationic dissociations. This is because they have two functional groups, both anionic and cationic (Lomax, 1996). Anionic surfactants are generally with negative charges while the cationic surfactants are usually carrying positive charges. They usually contain the following types: betaines, amino acids, and phospholipids. The classification has been listed in Table 2.1.

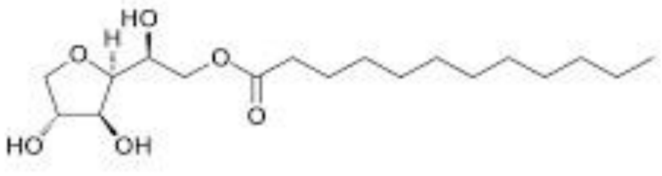
Table 2.1 Classification of chemical surfactants

Class	**Type**	**Example**	**Molecular structure formula**
Anionic [-]	Sulfate	Sodium dodecyl sulfate (SDS)	
	Sulfonate	Sodium dodecyl benzene sulfonate	
	Carboxyl	Sodium stearate	
Cationic [+]	Quaternary ammonium halides	Cetyltrimetylammonium bromide (CTAB)	
		Laurylamine hydrochloride	

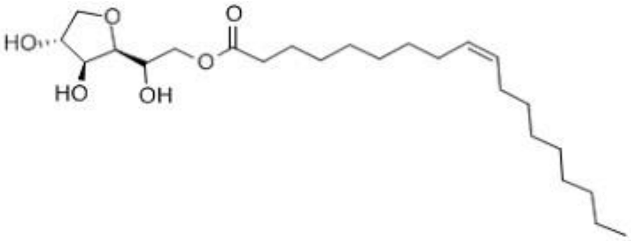
Non-ionic [0]	Fatty Acid Alkyl Esters	Decyl Oleate	
	Polyoxyethylene alcohol	Alkylpolyoxyethylenes	
	Polyol ester	Spans 80	
	Polyethylene	Triton X-100	
Amphoteric or zwitterionic [+/-]		Lauramidopropyl betaine	
		Cocamidopropyl betaine	

2.1.3 Chemical herders

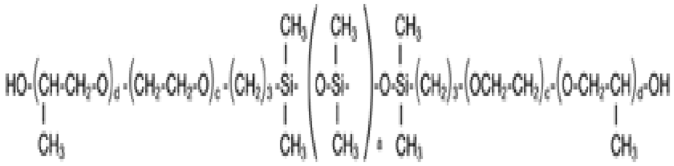
Commercial herding agents have been used for over 30 years (Buist et al., 2008b). Hydrocarbon-based (i.e., Corexit 9580, original U.S. Navy cold water blend, warm-water herder blend, OC-5), silicone-surfactant based (i.e., Silsurf A108, Silsurf A004D), and second-generation fluorosurfactant PolyFox™ PF151 are the most popular chemical herders (Buist et al., 2010a; Fingas, 2014). U.S. Navy cold water blend was successfully used in thickening oil slick excess of 3 mm and worked in the presence of ice (Buist, 2010). Silsurf A004D is another propriety silicone surfactant mixture that has been tested to show higher herding efficiency (Buist et al., 2010a). Gupta et al. (2015) attempted to develop an ecofriendly green herder, resulting from retraction oil slicks around 500% at 5°C and 700% (20°C). Bonheyo et al. (2017) reported that aggregators could be used both as sorption and herders in oil spill response. Zhou (2020) recently proposed that phytanic acid and monogalactosyldiacylglycerol (MGDG) can be used as eco-friendly herders under calm sea conditions. Figure 2.1 shows the structure of selected chemical herders.



USN cold water blend (TS6535)



USN warm water blend



$$\mathrm{HO{-}\left(\underset{\underset{CH_3}{|}}{CH}{-}CH_2{-}O\right)_d{-}\left(CH_2{-}CH_2{-}O\right)_c{-}\left(CH_2\right)_3{-}\overset{\overset{CH_3}{|}}{\underset{\underset{CH_3}{|}}{Si}}{-}\left(O{-}\overset{\overset{CH_3}{|}}{\underset{\underset{CH_3}{|}}{Si}}{-}\right)_a{-}O{-}\overset{\overset{CH_3}{|}}{\underset{\underset{CH_3}{|}}{Si}}{-}\left(CH_2\right)_3{-}\left(OCH_2{-}CH_2\right)_c{-}\left(O{-}CH_2{-}\underset{\underset{CH_3}{|}}{CH}\right)_d{-}OH}$$

Silsurf A0004up (OP-04)

Figure 2.1 Exemplar structures of predominant chemical herders

2.1.4 Applications of herders in oil spill response

In general, booms, skimmers, and in situ burning are widely adapted in oil spill response through physical mechanisms (Etkin & Tebeau, 2003). However, only 3% of the spilled oil was collected by booms and skimmers, and 5% was burned in the Deepwater Horizon oil spill response (Lubchenco et al., 2013). As a matter of fact, booms, skimmers, and in-situ burning can only be applied in calm weather conditions, and they are hindered by rough weather, winds, and oil slick (Lee et al., 2015). For instance, an oil slick can only be ignited when its thickness is greater than 2-3 mm in order to keep the heat. About 2-3% of the heat generated by the combustion is returned to the oil layer, where it causes additional vapors to escape and burn. When the oil layer is thinner than 1-2 mm, the heat is lost to the water. As such, not enough vapors are released and combustion cease (Buist et al., 1994). Hence, research for developing in-situ burning enhancement technique is warranted.

Chemical herders have been widely tested as oil collecting agents to improve in situ burning in the marine environment. Herding agents can increase the oil slick thickness by lowering the water and air interfacial tension. A series of laboratorial and in-field tests have been conducted to prove the ISB enhancement ability of chemical herders. For instance, Buist et al. (2010b) tested the herder (USN) in the field in situ burning test at the Barents Sea (Arctic Ocean). More than 90% of oil recovery efficiency was reached in the burning trial. Aggarwal et al. (2017) compared two herding agents (OP40/TS6535) during the in situ burning study in Alaska (Pacific Ocean). The enhanced burning efficiency was up to 84%. Rojas et al. (2020) has recently studied the two commonly chemical herders (OP40/TS6535) in a lab-scale setting. SL Ross lab and Ohmsett tested the herding ability and in situ burning for multiple years with multiple testing scales (Buist et al., 2006; Buist et al., 2007; Buist et al., 2008a). Some of the current applications in the past decade were summarized in Table 2.2.

Table 2.2 Examples of current herder studies

Location	**Year**	**Herders**	**Oils**	**Scare**	**Key points**
SL Ross Lab	2003	EC 9580	ANS	1m^2	2-4 mm
SL Ross Lab	2004/2005	EC 9580 USN OC-5	ANS Gas Oil	1 m^2/ 10 m^2 wave tank	3-4 mm/ Burning test
CRREL Basin	2005	UNS	Hydrocal	81 m^2	USN effective up to 70% ice cover
Ohmsett	2006	UNS	Blend Ewing Bank/ Arab Medium	1000 m^2	3-4 mm
Prudhoe Bay Pool	2006	USN	Kupurak	25 m^2	Burning test/USN effective in brash
SL Ross Lab	2007/2008	PF151N Silsurf	ANS Kuparuk	1 m^2/	Silsurf best both static/ dynamic test

		Siltech	No.2Fuel oil	10 m^2 DFP	
SL Ross Lab	2008	USN	ANS	1 m^2	Best solvent is 2-ethyl 1-butanol
Barents Sea	2008	USN	Heidrun	1700 m^2	Burning test 90+% removal efficiency
SL Ross/ Ohmsett	2008/2009	USN	ANS Kupruk Oseberg	2-4000 m^2	Improves weir skimmer recovery rates
SL Ross Lab	2010	USN		Spray test	SSC 0002 nozzle selected
SL Ross Lab	2011	USN OC-5 Silsurf	ANS Kuparuk No.2 Fuel Oil	1 m^2 10 m^2 DFP 4000 m^2	Silicone herders retained small burning slicks
DESMI-AFTI	2013	Water &Canola		Spray test	Helicopter application

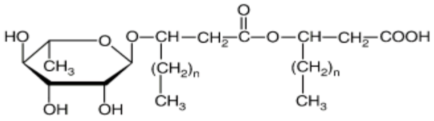
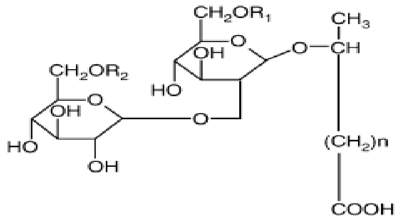
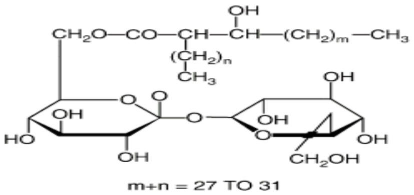
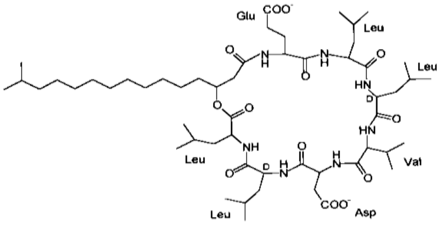
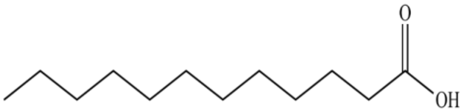
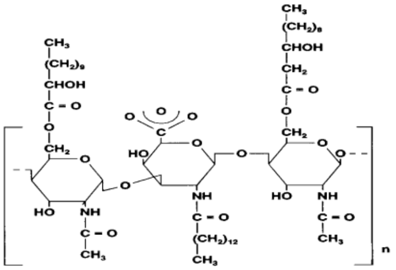
		Surrogates			
Alaska	2017	OP40 TS6535	ANS	Field-scale	73% to 84%
Denmark	2017	OP-04	DUC crude oil	1 m^2 19 m^2	Burning test 42-85%
Arctic Lab	2017	Aggregators	Crude oil	1100L tank	Herding and absorption together
BSEE	2018	TS6535 OP-04	ANS	1 m^2	1-3 mm
Denmark	2019/2020	TS6535 OP-04	ANS Grane		1–5 mm
CCNY	2020	Phytanic acid MGDG	Crude oil	0.09 m^2	Potential herding agents

2.2 Biosurfactants for Oil Spill Mitigation

2.2.1 Biosurfactants

Biosurfactants are surface-active compounds produced extracellularly by microorganisms (George-Ares et al., 1999). Such surface-active biomolecules are superior alternatives for chemical surfactants due to their unique properties (Mukherjee et al., 2006; Geys et al., 2014). Biologically based surfactants fall in categories containing glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids, polymeric surfactants, and particulate surfactants (Desai & Banat, 1997; Gudiña et al., 2016). Biosurfactants can be classified into two groups based on their molecular weights. Low-molecular mass biosurfactants, such as lipopeptides, glycolipids, and proteins, can effectively reduce the surface/interfacial tension. Those with high molecular masses can stabilize emulsions but usually do not reduce surface tension (e.g., polysaccharides, lipopolysaccharides proteins, or lipoproteins) (Rosenberg & Ron, 1999). Biosurfactants can also be characterized based on the nature of the charge on individual polar moiety. For instance, negatively charged biosurfactants are mainly due to sulphur or sulphonate groups. Cationic biosurfactants contain positive charges that come from cationic groups such as quaternary ammonium. Amphoteric biosurfactants have both positively and negatively charged moieties in the same molecules (Rahman & Gakpe, 2008). Some of these classified common biosurfactants and their corresponding producers are summarized in Table 2.3 (Mulligan & Giggs, 1993; Silva et al., 2014).

Table 2.3 Classification and examples of biosurfactants

Head Group	Biosurfactant	Molecular Structure	Microorganism
Glycolipids	Rhamnolipids	 Monorhamnolipids	*Pseudomonas aeruginosa*
	Sophorolipids	 Acidic sophorolipid	*Torulopsis bombicola,* *Corynebacterium batistae*
	Trehaloselipids	 Trehalose monomycolates	*Rhodococcus erythropolis,* *Mycobacterium sp*
Lipopeptides	Surfactin		*Bacillus subtilis,* *Bacillus pumilus A*
Fatty acids	Fatty acid		*Corynebacterium lepus*
Polymeric	Emulsan		*Acinetobacter calcoaceticus*

2.2.2 Applications of Biosurfactants in oil pollution remediation

Marine oil spills often occur during onshore or offshore activities, causing serious ocean pollution. Oil pollutions can be found in various oil operation stages, ranging from muds, produced waste discharge, accident oil spills by ship, and leaking from the pipeline, storage tanks. Those spilled oil has a massive impact on human, surrounding environment, and ecosystem. Ivshina et al. (2013) pointed out that oil spill responses are sustainable physical, chemical, and biological procedures for aquatic and non-aquatic phases. Spilled oil on the water surface could be removed physically using a boomer and skimmer. Part of spilled oil can be removed by in-situ burning. Weathering is a complex process for oil that is exposed longer in the air under sunshine, including spreading, evaporation, dispersion, emulsification, oxidation, dissolution, biodegradation, and sedimentation. Weathered oil can be found at bottom sediment, sandy beach, rocky shoreline, and coastlines (Lee et al., 2015; Fingas, 2016). Biosurfactants have attracted considerable interest in the environmental biotechnologies and industries, including potential oil recovery applications, oil spills control, and bioremediation of contaminated soil (Makkar & Rockne, 2003; Patel et al., 2019).

Offshore oil spills can be treated by using dispersants. Dispersants are chemically synthesized and can be defined as a blend of surfactants, solvents, and additives (Kujawinski et al., 2011). Biosurfactants are environmentally friendly since they can also be readily biodegraded and less damaging to the environment than the more recalcitrant chemical surfactants (Pacwa-Płociniczak et al., 2011). Rhamnolipid is a commercial purified biosurfactant that has been studied as an alternative dispersant. Holakoo (2001) tested the feasibility of Rhamnolipids for dispersing oil slicks at 25 °C and a salinity of 35 ppt. It was found that the dispersion efficiency decreased when temperatures and salinity were lowered. Saeki et al. (2009) examined the dispersion ability of the culture broth of *Gordonia* sp. strain JE-1058 through baffled flask testing. It showed a potential to be applied as an oil spill dispersant and stimulated the degradation of weathered crude oil (ANS 521). Glycolipids produced by *Rhodococcus* species H13-A have the ability to enhance the solubility of naphthalene and its methyl-substituted derivatives (Kanga et al., 1997). Trehalose lipid is a non-reducing disaccharide in which the two glucose units are linked and an a-1,1-glycosidic structure (Franzetti et al., 2010). Trehalose lipid produced by *Rhodococcus* species has potential applications in oil recovery, control of oil spills, and bioremediation of contaminated soil (Makkar & Rockne, 2003; Patel et al., 2019). The application of trehalose lipids in oil spill response was summarized in Table 2.4.

Table 2.4 Trehalose lipid production and application

Microorganism	Type of Biosurfactant	Application
Rhodococcus erythropolis 3C-9	Trehalose lipid	Oil spill cleanup operations
Rhodococcus ruber strain IEGM AC219	Trehalose lipids	Bioremediation of crude oil contaminated soil
Rhodococcus erythropolis P6-4P	Trehalose lipid	Biodispersant of crude oil spill
Rhodococcus Erythropolis SP. SB-1A	Trehalose lipid	Biodispersant of crude oil spill
R. wratislaviensis BN38	Trehalose tetraester	Bioremediation applications
Micrococcus luteus BN56	Trehalose tetraester	Bioremediation of oil-contaminated environments
Nocardiopsis lucentensis MSA04	Glycolipid	Bioremediation of marine environment
Pseudozyma hubeiensis	Glycolipid	Bioremediation of marine oil pollution
Mycobacterium flavescens strain EX-91	Glycolipids	Bioremediation of oil-contaminated wastewater
Rhodococcus strain H13-A	Glycolipids	Bioremediation of polycyclic aromatic hydrocarbons (PAHs)
Gordonia sp. strain BS29	Glycolipids	Bioremediation of enhancing crude oil contaminated soil

Oil pollutants in soil system can be controlled by biosurfactants through enhanced soil washing or bioremediation. During a soil washing process, a biosurfactant can be applied to improve the mobilization/solubilization of hydrocarbons, increase the contact angle, and reduce the capillary force between soil and oil (Deshpande et al., 1999; Luna et al., 2009). The number of surfactant micelles in water increases with the solubilization increases, because the contaminants partitioning into the hydrophobic core of surfactant micelles. Thus, surfactant concentrations well above the critical micelle concentration (CMC) are critical for this solubilization enhancement to be significant in soil washing (Deshpande et al., 1999). Biosurfactants have been reported as soil washing agents in soil remediation by Kavitha et al. (2014); Zhu et al. (2016); Mulani et al. (2017), and Arelli et al. (2018). In these reports, biosurfactants were used to treat oil pollution and showed advantages such as biodegradability and low toxicity. As soil washing agents, biosurfactants were also characterized into groups by different molecular weights and functions. The low-molecular-weight compounds can lower the surface tension. On the other hand, high-molecular-weight compounds were used as stabilizing agents (Das et al., 2017). Chemical dispersants were deemed toxic to the environment in the studies by Pietroski et al. (2015). And Pi et al. (2017) studied the contribution of chemical dispersants on crude oil biodegradation and concluded that biosurfactants were more effective at bioremediation of oil-contaminated marine environment.

Soil remediation has been widely used to remove heavy metal, petroleum hydrocarbons, and volatile organic matters from the contaminated site (Guerin, 2015; Koshlaf & Ball, 2017). For enhanced bioremediation, biosurfactants can interact with bacterial cells to alter cell hydrophobicity, and facilitate the access of the cells to water-solubilized hydrocarbons, large oil droplets, and pseudo-solubilized or emulsified oils (Franzetti et al., 2010). Rhamnolipids, lichenysins, and surfactin are the few biosurfactants found to be successful in the remediation of oil contamination (Mulani et al., 2017). Biosurfactant generated microbars have been reported isolated by scientists from various locations. Luna et al. (2009) isolated a new biosurfactant produced by *Candida glabrataform* in Indiana coastal area. A new biosurfactant producer *Exiguobacterium* sp. Strain N4-1P was first published by Cai et al. (2014). Malavenda et al. (2015) isolated the Arctic and Antarctic bacteria producing biosurfactants using hydrocarbons as carbon sources. The application of soil recovery in the Arctic area is limited, significantly affected by temperature, sea ice, sunlight regime, suspended sediment plumes (Vergeynst et al., 2018). The contaminated soil type, contaminant, remediation methods, biosurfactant, producer, and removal rate are summarized in Table 2.5.

Table 2.5 Summery of biosurfactant application in the soil environment

Site/soil types	Contaminant	Remediation Methods	Biosurfactant/ Producer	Remove efficiency	Reference
Sand from shore of Ravenna (Italy)	intermediate fuel oil 180 (IFO-180)	Soil washing	Rhamnolipids/ sophorolipids	97%	Arelli et al. (2018)
Shoreline sediment	petroleum hydrocarbon	Soil flushing	Dispersant		Guerin (2015)
Oil industrial zones soil	Pyrene	Bioremediation	*Pseudomonas aeruginosa SP4*	84.6%	(Jorfi et al., 2013)
Sandy/ find sand soil/ clay/ clay loam	Crude oil	Soil washing	*B.lichenifo* ETW 5P *rmis*	>85%	(Kavitha et al., 2014)
Contaminated soil with wastewater of oil refinery plant	Crude oil	Biodegradation	*Achromobacter kerstersii* LMG3441	53%	(Kazemzadeh et al., 2020)
Soil slurry	Crude oil	Bioremediation	rhamnolipid (JBR-425)		(Kumari et al., 2018)
Beach sand	Motor oil	Soil washing (flask scale)	*Candida glabrata* UCP 1002	84%	(Luna et al., 2009)
Soil	petroleum hydrocarbon	Bioremediation	*Aspergillus fumigatus*	90%	(Martins et al., 2009)

Sea sand	Weathered crude oil (ANS 521)	Soil washing	glycolipidtype	80%	Saeki et al. (2009)
Soil	Crude oil	Bioremediation	mussel shells (Bioaug-SC)		Sheppard et al. (2014)
saline marsh soil	Crude oil	Biodegradation	*Proteobacteria and Bacteroidetes*	80.9%	Wei et al. (2020)
Marine sediments	Crude oil	In-situ bioremediation (Field study)		50%	Zhao et al. (2018)
Loam soil	Crude oil	Bioremediation	Rhamnolipid	86.97%	Zhang et al. (2011)
Soil (silty loam)	Crude oil	Soil washing	*B. subtilis* N3-4P	58% /65.2 %	Zhu et al. (2016)

2.2.3 Potentials of biosurfactant based bioherders

The negative impacts of surfactant-based chemical oil treating agents on marine ecosystems and environments have raised increasing concerns, especially after the Deepwater Horizon oil spill (Beyer et al., 2016; Li et al., 2016). Chemical herding agents used in enhancing in-situ burning mainly remain in the water column post application. Due to the tendency of chemical herder towards remaining in the water, the risk of marine species intake is rising. The acute toxicity of one common chemical herder (Corexit 9580) was sensitive to Artic marine species and increased the toxicity of the oil in the water column (Bhattacharyya et al., 2003; Hansen et al., 2014). Sorbitan monolaurate is the main ingredient of the chemical herder, named Thickslick 6535. As the part of the sorbitan ester family, the use of sorbitan monolaurate could increase the mortality rate of rats during an oral feeding period of 23 days (Fitzhugh et al., 1960). Sorbian esters also have the potential to induce cutaneous irritation in humans, and they can cause sensitization in patients with damaged skin (Elder, 1985). As reported, sorbitan laurate is a tumor promoter in mouse skin with 10 percent or greater concentrations. As discussed above, current surfactants have significant toxic effects, and they remain persistent in the environment for an undesirably long period. Therefore, development of safer and less toxic alternative surfactants is needed.

Biosurfactants that are produced by microorganisms during their growth have been suggested as a promising and renewable source. Biosurfactants are found to be less toxic, more effective, and stable at extreme pH (e.g., pH 1.0 or 14.0 at 40℃), temperature, and salinity, and better at enhancing biodegradation (Kretschmer et al., 1982; Muthusamy et al., 2008). It was found that the toxicity of Rhamnolipids biosurfactant is lower than those of chemical surfactants, according to the tests of IC_{50} on marine flagellates microalgae (Elucidation et al., 1987). According to Kanga et al. (1997), the Tween-80 system was approximately 50% more toxic than the biosurfactant system. It was reported that Glycolipids produced by Rhodococcus species H13-A with a higher EC_{50} exhibited lower aqueous toxicity than Tween-80. As studied, trehalose tetraester (THL) produced by *Rhodococcus* sp. 51T7 is less irritating than the commercial chemical surfactant. The cellular toxicity testing results showed that IC_{50} of THL is higher than those obtained with sodium dodecyl sulfate (SDS) for both keratinocytes and fibroblast (Marqués et al., 2009). It is thus desired to develop biosurfactant-based bio-herding agents with better performance and more friendliness to the environment.

Biosurfactants have gained considerable interest in environmental biotechnologies and industries. The potential applications of biosurfactants are in oil recovery, biodegradation and detoxification of industrial effluents, control of oil spill, and bioremediation of contaminated soil, and other areas like cosmetics, pharmaceutical, and food applications (Makkar & Rockne, 2003; Patel et al., 2019). It was reported in 2020 that the global biosurfactants market was estimated to be US$4.5 billion and is expected to reach US$6.5 billion by 2027. The annual growth rate is 3.5%, significantly higher than the overall surfactant market (2%) (Scenario, 2011). They had shown a potential for unique applications, including the biological control of pests in medicine, pharmaceutics, cancer treatment, and wound healing (Stipcevic et al., 2006; Piljac et al., 2007). Previous studies have proved the capability of producing biosurfactants with great potential for future applications (Cai et al., 2014; Cai et al., 2015). Additionally, biosurfactants can be produced from industrial wastes, by-products, and cheap raw materials such as fish and shrimp wastes (Luna et al., 2017; Zhu et al., 2020).

As reviewed in Section 2.2, biosurfactants have been used in generating dispersants for marine oil spill response. However, bio-herders have rarely been studied, particularly in terms of bio-herder production and the associated herding effectiveness evaluation. It is timely and important to develope bio-herders as better alternatives. Biosurfactant-based herding agents with the prospective features of high effectiveness, low toxicity, and persistency can be a promising option.

2.3 3-D QSAR Aided Molecular Design and Its Environmental Applications

2.3.1 Introduction

Quantitative structure–activity relationship (QSAR) modeling is a well-established computational approach to conduct chemical data analysis. QSAR models can be developed by establishing empirical, linear, or non-linear relationships between chemical descriptors values and properties or bioactivities of molecules(Muratov et al., 2020). Chemical descriptor values can be computed based on molecular structures, while properties and bioactivities can be obtained through the experimental measurements of those molecules. Developed models can be applied to predict or design novel chemicals with desired properties The QSAR approach provides a rapid analytical tool to fill data gaps for limited or absent experimental information (Dearden, 2017). A brief history of early QSAR methodologies was summaries by Verma et al. (2010).

According to Verma et al. (2010), QSAR models can be classified by dimensionality or types of chemometric methods. The QSAR methods are often categorized into 6 classes (from 1D to 6D-QSAR) based on the structural representation or by the way descriptor values are derived. QSAR models can also be categorized into linear methods (e.g., linear regression, multiple linear regression, partial least-squares, principal component analysis/regression) and non-linear methods (e.g., artificial neural networks, k-nearest neighbors, and bayesian neural nets). It depends on the type of employed correlation technique, which establishes a relationship between structural properties and biological activity.

The 3D-QSAR methods can be classified by various criteria (Table 2.6). Comparative Molecular Field Analysis (CoMFA) is a powerful method modified from the DYnamic Lattice-Oriented Molecular Modeling System (DYLOMMS) by combining the two existing techniques, GRID and partial least squares (PLS) (Cramer et al., 1988). To overcome certain limitations of CoMFA, Comparative Molecular Similarity Indices Analysis (CoMSIA) was then developed. CoMSIA can simultaneously consider steric, electrostatic, hydrophobic, and hydrogen bonding properties. CoMSIA can overcome the prerequisite of involving complete ligand training sets by a tailor-made scoring function. CoMSIA uses atom-type specific interaction fields to ease the interpretation of PLS results. In addition to the enthalpic contribution, the methodology is also expected to include the entropic effects resulting from (de-)solvation, since structural knowledge from experimentally determined complexes is converted into statistical pair potentials. CoMFA and CoMSIA are two commonly used methods for 3D-QSAR modeling.

Table 2.6 3D-QSAR classification

Based on	Classification	examples
Intermolecular modeling	Ligand-based 3D-QSAR	CoMFA, CoMSIA, COMPASS, GERM, CoMMA, SoMFA
	Receptor-based 3D-QSAR	COMBINE, AFMoC, HIFA, CoRIA
Alignment criterion	Alignment-dependent 3D-QSAR	CoMFA, CoMSIA, GERM, COMBINE, AFMoC, HIFA, CoRIA
	Alignment-independent 3D-QSAR	COMPASS, CoMMA, HQSAR, WHIM, EVA/CoSA, GRIND
Chemometric technique	Linear 3D-QSAR	CoMFA, CoMSIA, AFMoC, GERM, CoMMA, SoMFA
	Non-linear 3D-QSAR	COMPASS, QPLS

2.3.2 QSAR aided molecular design

The process of QSAR modeling can be divided into three stages, which are development, model validation, and application (Muhammad et al., 2018).

Development: 3D-QSAR model design starts with gathering model compounds from literature sources for both training and testing sets. The training set is used to construct the model, while the testing set is used to validate the model. The 2D molecular structures of chemicals obtained from the database such as PubMed. These 2D structures are turned into 3D models using software such as Sybyl or Gaussian. The generated 3D structures from the software of the complex were then subjected to energy minimization and geometry optimization. Molecular descriptors can be physicochemical properties calculated by the computational software, such as constitutional, electronic, geometrical, hydrophobic, lipophilicity, solubility, steric, quantum chemical, and topological descriptors. Multivariate analysis (e.g., multi linear regression, partial least square) can be carried out to correlatie molecular descriptors with observed activity.

Model validation: The developed models are internally validated by the leave-one-out (LOO) cross-validation technique implemented in the software. The LOO technique eliminates one compound from the data set randomly in each cycle and builds the model using the rest of the compounds. Thus, the model formed is used for predicting the activity of the eliminated compound. This process is repeated untill all the compounds are eliminated. Meanwhile, developed models can be validated externally by judging the prediction of test set activity values.

Application: QSAR analysis is a ligand-based drug design method developed more than 50 years ago by Hansch and Fujita (1964). Constructed 3D-QSAR model is used to find a statistically significant correlation between the chemical structure and continuous or categorical/binary biological/toxicological property using regression and classification techniques. This computational method has been applied successfully in different areas such as drug development, pharmacy, and toxicity studies (Abramenko et al., 2020). QSAR methods are typically applied in predicting potential toxicity outcomes for in vitro cell cultures or in vivo animal test systems. It is used to well understand the toxicity end point (e.g., mutagenicity, developmental toxicity, cancer), biokinetic, and encompass multiple mechanisms and pathways (Mostrag-Szlichtyng & Worth, 2010; Cherkasov et al., 2014). Many function annotation proteins are relevant for pharmaceutical design because they may be enzymes of different classes that could become drug targets. QSAR modeling thus can be used to predict the enzyme classification on drug target (Concu et al., 2010). One QSAR model was developed with 794 compounds to predict the plasma protein binding for drug development (Ghafourian & Amin, 2013). The plasma protein binding causes significant changes in the volume of distribution, clearance, and drug half-life for pharmacokinetics characterization of drugs. Besides, the relationships between molecular structures and physical/chemical properties are also widely studied in other fields, such as predicting acute and chromic toxicity, genotoxicity and carcinogenicity of pollutants, and organic compounds adsorption (Lapenna et al., 2010; Serafimova et al., 2010; Gui et al., 2021).

2.3.3 QSAR in environmental applications

3D-Quantitative Structure-Activity Relationship (3D-QSAR) studies the relationship between chemical properties and their molecular structures. 3D-QSAR is a widely used tool in predicting environmental performance parameters of chemicals and design environmentally friendly chemicals.

As a powerful technique, the QSAR method has been widely applied in toxicology by many researchers. For example, Devillers (2004) predicted the acute toxicity of organophosphorus pesticides on rats using the developed QSAR model. The QSAR model was developed with 51 chemicals as the training set, and the partial least squares and artificial neural network analysis methods were used for rate LD_{50} value prediction. Toropov et al. (2017) established a QSAR model for predicting the acute toxicity of 116 pesticides on rainbow trout. Furthermore, researchers also developed linear regression QSAR models to evaluate the acute toxicity (EC_{50}) of a set of biocides collected from different sources on the freshwater crustacean Daphnia magna (Marzo et al., 2020). Zhao et al. (2004) precited the polycyclic aromatic hydrocarbons solubility using 3D-QSAR models. Lu et al. (2001) constructed QSAR models using hydrophobicity parameter $\log K_{ow}$ to determine the substituted benzene toxicity on algae. Li et al. (2020b) studied the toxicity of quinolone antibiotics by K_{ow} 3D-QSAR modeling. Toxicity and biological enrichment for polychlorinated naphthalene (PCNs) were studied by 3D-QSAR modeling (Gu et al., 2017; Gu et al., 2020a).

QSAR models can also be used to study the migration and allocation behaviors of organic pollutions. Khan et al. (2019) established the QSAR ecotoxicity model for the fish mortality endpoint using 1121 organic compounds. Gui et al. (2021) designed the QSAR model to predict the adsorption affinity of organic compounds on plastics in the aquatic environment. The adsorption affinity of 13 organic compounds on polyethylene and chlorinated polyethylene was compared. Two QSAR models were developed to predict k_{O3} (O_3 reaction rate constants) of various organic chemicals with multiple linear regression and support vector machine methods (Huang et al., 2020). Chen et al. (2016) used the established model to predict the mitigation ability for PCBs. The octanol-air partition coefficient (K_{oa})can also be used along with K_{ow} (octanol-water partition)to study the bioaccumulation and bioconcentration for POPs in aquatic and identifying biomagnifying chemicals in terrestrial food-chains (Arnot & Gobas, 2003; Gobas et al., 2003). On the other hand, Roberts (1991) studied the acute lethal toxicity data for a range of anionic and nonionic surfactants by QSAR modeling to predict by calculated log K_{ow} values. As studied by Liu et al. (2020b), the toxicity of 20 amine surfactants on Daphnia magna was tested to complete the toxicity data of amine surfactants. Limited studies on 3D-QSAR have been focused on bacteria's surfactant attachment to the membrane (Campbell et al., 1999). No study on using 3D-QSAR was conducted in predicting K_{ow} and K_{oa} for herders.

Furthermore, QSAR can be used to develop a globally harmonized system (GHS) by predicting the hazardous properties for selected hazard classes within the GHS regulation (Quintero et al., 2012) and assessing risks (Pradeep et al., 2016). The QSAR model developed by Pandey et al. (2020) studied the acute toxicity and key structure related to the toxicity of 85 environmental transformation product pesticides. More importantly, this model can also provide an early warning of their potentially detrimental effects on fishes for regulatory purposes.

The developed QSAR model can be used for designing new chemicals. Hydrophobicity, electronic and steric effects have been identified as highly important parameters for the modeled toxicity, which can provide structure modification substrate position as modification guidelines (Lapenna et al., 2010). For example, phthalic acid ester (PAE), which is often used as a plasticizer to provide stability for plastic products,is ubiquitous environmental pollutant (Singh et al., 2017). Four environmentally friendly ditridecyl phthalate derivatives were screened out by evaluating their toxicity and estrogen combined activities predicted by the 3D-QSAR model (Du et al., 2020; Li et al., 2020a). As a typical persistent organic pollutant, polychlorinated naphthalenes (PCNs) are toxic to the environment. Gu et al. (2020a) established a multiactivity 3D-QSAR based on the comprehensive evaluation index of PCNs and selected 2 derivatives with significantly comprehensive evaluation index reduced as environmently friendly PCNs. Fluoroquinolone is the most widely used synthetic antibacterial agent in clinical practice, which has led to a series of toxic side effects on organisms (Liu, 2010). Hou et al. (2020) used the CoMISA method to construct a 3D-QSAR model with the plasma protein binding rate as the dependent variable for prediction. It was found that a variety of environmentally friendly trovafloxacin derivatives with lower plasma protein binding rates are designed. It was reported that QSAR modeling can also modify enzyme molecules, such as deacetylate enzyme, histone deacetylase 6, and human's 4-hydroxyphenylpyruvate dioxygenase (Chu et al., 2020; Liu et al., 2020a).

2.4 Summary

This chapter started with a literature review on herding agents for marine oil spill response. Background information of herding agents, including definition, herding theory, and commonly used herding agents were introduced. Properties of chemical surfactants were also summarized. Chemical surfactants can be classified based on their structure properties, molecular mass into different groups. The wide applications of chemical surfactants to food industry, oil recovery and environmental remediation were discussed. Subsequently, the review extended to the chemical herders and their working principles. The current studies of chemical herding agents in oil spill mitigation and their herding performance evaluation were introduced.

Biosurfactants, including their physiochemical properties, classification, and their special advantages compared with chemical surfactants were then summarized. The feasibility of biosurfactants for oil spill response was supported by a review of current experimental studies using biosurfactants such as Trehalose lipids and Rhamnolipids. Applications of biosurfactants for oil pollution remediation and the need for generating novel bioherders were discussed. Potential and knowledge gaps in the bioherder development were identified.

Finally, this chapter included a comprehensive review of QSAR modeling. QSAR models are designed to predict complicated physicochemical /biological properties of chemicals from their experimental or calculated properties. The mathematical model of quantitative structure-activity relationship was reviewed, including classifications and exemplar model methods. Then, the QSAR modeling development, validation, and application were discussed as modeling design stages. 3D-QSAR models were widely applied in drug development, prediction of toxicity, prediction of metabolism, and biokinetics studies. Recent applications of QSAR modeling in environmental studies were summarized. It was demonstrated that 3D-QSAR modelling could be used for molecular design to obtain greener chemicals. The challenges of using computational tools for chemical herder structural design and improvement were identified. Based on the literature review, this thesis work was focus on filling the knowledge gaps by generating more environmentally friendly, biosurfactant-based herders and biodegradable chemical herders as marine oil spill treating agents.

CHAPTER 3 BIOHERDER GENERATED FROM RHODOCOCCUS AS MARINE OIL SPILL TREATING AGENTS

3.1 Introduction

Marine oil spills can cause ocean pollutions during oil operation onshore and offshore activities (Li et al., 2016). Oil pollution happens at various operational stages, ranging from crude oil release tanks, offshore platforms, drilling rigs, and wells. It can also happen as crude oil spills of refined petroleum products and their by-products, bunker fuel used by large ships, and oily white refuse or waste oil spills (Zhang et al., 2019). Oil spills might also occur due to many reasons through the exploration, extraction, and transportation processing (Lee et al., 2015). Most oil spills were caused by leaking tankers, and the majority of total oil release occurrences were at small scales (Fingas, 2011). In the past decade, the overall amount of spilled oil worldwide has surpassed 1 billion gallons, with 6 million tons of oil released into the oceans per year (Abdul-Hamid et al., 2013). The Deepwater Horizon oil spill happened in 2010 claimed the largest record in the history of the petroleum industry; there were over 700 thousand gallons of crude oil released (Bly, 2011; Commission, 2011).

Marine oil spills have negative impacts on both shoreline and offshore environments. The selection of proper response options can help to reduce the impacts caused by incidents where a large volume of oil is released into the ocean. A previous study indicated that only 5% of the released oil was burned during the Deepwater Horizon oil spill response (Lubchenco et al., 2013). When the oil layer is thinner than 1-2 mm, the heat is lost to the water. There is no enough vapors released to facilitate combustion (Buist et al., 1994). Hence, a technique assisting in-situ burning by increasing the oil slick thickness is needed.

Herding agents can thicken oil slicks by reducing the surrounding water-air surface tension. The most popular commercialized herders are hydrocarbon-based herders (e.g., Corexit 9580, original U.S. Navy cold water blend, warm-water herder blend, OC-5), silicone-surfactant based herds (e.g., Silsurf A108, Silsurf A004D), and second-generation fluorosurfactant PolyFox™ PF151 (Buist et al., 2010a; Fingas, 2014). The effectiveness of herding agents can be defined as the percentage change in the oil slick area under a sufficient period (Buist et al., 2008a). The operational conditions of herding agents have been investigated among various environmental factors, including oil type, herder type, temperature, water salinity, and the wave and ice present (Buist et al., 2006; Buist, 2010; Buist et al., 2010a).

An environmental concern has been raised based on the properties and fate of herding agents in the water column (Gray et al., 2014). To face the challenge, this study is focused on the generation of a biosurfactant-based bioherder produced by *Rhodococcus* species for marine oil spill response. The bioherder was generated using the microorganisms *Rhodococcus* M25 strain screened from North Atlantic Ocean and genetically modified in the NRPOP lab (Cai et al., 2014; Lv et al., 2016). After generation using this hyper-production *Rhodococcus* M25 strain, the bioherder was characterized. Herding effectiveness was compared with the chemical herder to demonstrate its performance. Further, we tested its feasibility for oil herding under different operational conditions. This study demonstrated that biosurfactant-based herder can be used as an alternative marine oils pill treating agent.

3.1 Methodologies

3.1.1 Biosurfactant production and characterization

The biosurfactant producing *Rhodococcus* M25 strain is genetically mutated by Cai et al. (2016). The stock strain was frozen and preserved at -80 ℃ in Marine Broth. The strain was cultured on Marine Broth Agar for 3day. Then bacteria were cultivated in the Production Medium (PM) composed of $MgSO_4$, 0.2 g; $CaCl_2·2H_2O$, 0.05 g; KH_2PO_4, 3.4 g; $K_2HPO_4·3H_2O$, 4.4 g; $(NH_4)_2NO_3$, 1 g; $FeCl_3$, 0.05 g; Glucose, 1g; NaCl, 26 g in 1L of distilled water, with 3% (v/v) diesel incubation was maintained at 30 °C while shaking at 200 rpm (Cai et al., 2014).

The crude biosurfactant product was generated following the pre-developed protocol (Kuyukina et al., 2001; Cai et al., 2016). After seven days of cultivation, a thick emulsion layer was formed on the surface. All broth, including the water phase and oil phase, was collected for freeze-and-thaw treatment to break the emulsion. The upper organic phase and the lower water phase were stored at -20 °C for further extraction. Separated biosurfactants including bacteria cells was collected. The solution that contains biosurfactant was first washed by petroleum ether to remove oil, and then sodium sulfate was added to absorb water. This step was repeated at least three times until no oil and water were left. Then biosurfactant was extracted with chloroform and methanal (2:1, v/v). Sonication was performed to detach cells from biosurfactants. This procedure was repeated several times until a clear solution appeared in the lower phase. All lower phases were collected, combined, and subjected to concentration by rotary evaporation. The crude biosurfactant products were collected and stored at -20 °C for further analysis and testing.

The biosurfactant extraction schematic was shown in Fig. 3.1. Fourier transform infrared (FT-IR) spectroscopic analysis of the bioherder was conducted on a Bruker Alpha instrument.

3.1.2 Herding experiments

A customized plastic tray was filled with water up to 1.5 – 2 cm, and crude oil was poured into the center of the water surface. The oil was allowed to spread on water surface to equilibrium in 20 min, forming a thin film of oil on water. The overhead digital camera was mounted in a customized rig to take photos for further analysis automatically. The experiment setup is described in Fig. A-1 in the Appendix. Crude biosurfactant was added into the system gently from the corner. Photographs were taken every minute for at least 20 min. Herding performance was evaluated at three salinity and three temperature, so as to investigate the impact of these conditions on the herded oil slick thickness over time. The captured images were processed using ImageJ software to determine an oil slick area, which was used to calculate the change rate in thickness with time (Buist et al., 2008a; Gupta et al., 2015).

Change rate = (Initial Area-Final area)/Initial area*100% (1)

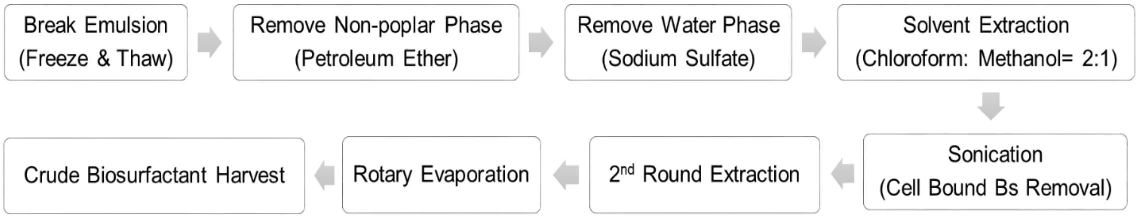


Figure 3.1 Biosurfactant extraction process

As mentioned previously, this experiment relies closely on a stable external environment. An experiment chamber may be considered for further experiment. According to the results of a preliminary test, the camera will lose focus after a long period of overhead hanging. By setting the camera in the manual focus mode or using a fixed lens, this problem can be solved. Since the focus of the camera may change due to various reasons (e.g., change in rig location, change of the battery, removal of the SD card, and setting the camera to manual focus mode, etc.), the size of the object in the photo may be different. This may impact the scale length in Image J. To ensure the objective is the same size, the camera must be in the same setting and each set of photos use the same scale. The herding effectiveness of bio-herder was examined and further compared with hydrocarbon-based chemical herder (Span) and silicon-based chemical herder (Silsurf).

3.1.3 DOE analysis

Design of experiments (DOE) is a statistical approach to plan, conduct, analyze, and interpret controlled tests, and to evaluate the factors that control the value of a parameter or a group of parameters. Factorial factor design is used to evaluate the impacts and interactions of the variables on the response.

The response of this design is the herding effectiveness, calculated as the change rate of the oil slick thickness over experimental time. Input variables are those factors that impact the herding performance, including water temperature (A), water salinity (B), oil (C), herder dose (D), and Approach (E). The range of temperature (A) was selected between 4 °C and 24 °C. Low temperature was applied exam if the bioherder can work effectively in cold seawater. The higher level was set as room temperature. Because of the equipment limitation in the lab, temperatures lower than 0 °C cannot be achieved. So, 4 °C was used to representing the cold environment and 24 °C as the room temperature. For factor B (salinity), a lower value of 0% represents freshwater, while a higher value of 3.5% is artificial seawater. Distilled water was used to prepare artificial seawater. ANS (Arctic North Slope) crude oil was selected as testing oil. The amount of oil (C) was scaled down from a small-scale bench test (Buist et al., 2010). Different initial oil slick thickness was obtained by adding different amounts of ANS (Arctic North Slope) crude oil. However, the minimal amount of oil poured onto the experiment's water surface was 200 µl. Since the image process software cannot calculate oil slick less than this thickness, numeric factors were set at the middle level when experimenting with center points. The herder dose (D) was set according to the previous study. The adding approach is designed to see if the herder adding technique has impact on the experimental results. Adding point "1" was located at the right lower corner of a pert dish, while "2" included current corner "1" and its diagonal corner. Adding herder at 2 locations required the coordination of hands and eyes. Selected factors are summarized in Table 3.1.

Since the performance of herding agents relies on the calmness of weather conditions, all experiments were carried out in the room with no wind or draft. The surface of the benchtop was leveled to eliminate the effect of gravity. Since the amount of oil used here was relatively small, experiments were conducted under an open-air environment without the need for a fumehood. All experiments were conducted when no other people were around. The door of the lab was open to reduce the air vapor concentration. A steady room temperature was kept, and heatrelease from surrounding operating machines (e.g., oven) was avoided.

Table 3.1 Factors summary

Factor	Name	Unites	Low Actual	High Actual	Low Coded	High Coded
A	Temperature	°C	4	24	-1	1
B	Salinity	%	0	3.5	-1	1
C	Oil amount	µl	360	720	-1	1
D	Herder Dose	µl	5	15	-1	1
E	Approach		1	2	-1	1

3.2 Results and Discussion

3.2.1 Bioherder production and characterization

Bioherder harvested from PM media cultivated after seven days and extracted by a mixture solvent of chloroform and methanol. The bioherder produced by *Rhodococcus erythropolis* M25 was identified as trehalose lipids (Cai et al., 2016). According to the composition of the product, the hydrophobic tails of trehalose lipids are primarily consisted of fatty acid chains of C16:0, C16:2ω4 and C18:1ω9 (Cai, 2019). FTIR analysis（Fig. 3.2）of this crude bioherder was conducted to examine the function groups present in the molecular structure. The FTIR spectrum shows the characteristic vibrational modes of different functional groups, including carboxylic groups (3500-2500 cm^{-1} (br), carboxyl O-H stretch, 1649.84 cm^{-1}, carboxyl –C=O stretch; 1063.76~990.48, carboxyl –C–O stretch) and alkyl groups (2922.37~2852.11 cm^{-1}, alkyl C–H stretch; 1432.83~1302.61 cm^{-1}, alkyl –C–H bending).

3.2.2 Bioherder performance evaluation

Bioherder performance was evaluated based on the herding effectiveness and efficiency. The herding effectiveness is the area change rate between time 0 and time 20 min. The herding efficiency is the area change rate within 1 min after introducing the bioherder into the system. The herding effectiveness was calculated using Equation 1. Area values were measured from photos captured by the overhead camera. Each photo was converted to black and white images, and the pixels were measured using the software Image J. Every measurement is based on the same scale setting (10 mm) marked on the side of the testing pan. The initial area is the measurement of the picture shot right before addition of bioherder. Original and black and white images in Fig 3.3 show an example of the image process by Image J. The measurement of this oil slick for run # 8 is 4470.23 mm^2. The area of the oil slick at time t is measured and calculated followed the same procedures.

The herding effectiveness of bioherder under different temperatures, salinity, herder-oil ratio (HOR) was examined. The change in herding effectiveness for different HOR in artificial seawater and freshwater are illustrated in Fig. 3.4 and Fig. 3.5, respectively. For the different water salinity under the same temperature, both figures indicate the same trend that herding effectively goes up with the increase of HOR. The herding effectiveness for both fresh and saltwater increased sharply at the first 3 min, and then gradually increased.

As can be seen from Fig. 3.4, the difference in area change rate with large HOR is smaller than that between the lower HOR. At 20min, the oil slick area change rate was declined by 3.5% (from 86.82% to 83.82%) when HOR was decreased by half (from 1:24 to 1:48). The oils lick change rate was lowered by 25% when HOR was decreased to 1/3 of its original level (from 1:24 to 1:72). Simultaneously, the change rate was dropped by 56% when HOR was decreased to 1/4 of its original level (from 1:24 to 1:144). On the other hand, the impact of HOR on herding effectiveness on freshwater was slightly different (Fig. 3.5). At 20min, the oil slick area change rate was declined by 7% (from 89.33% to 82.32%) when HOR was decreased from 1:24 to 1:72. It seems that there was no significant change in herding effectiveness when the HOR was dropped by 1/3 or ¼ . This indicates that the performance of the herder itself has a higher impact on the herding effectiveness. When using a higher performance herder, only a small amount is needed.

Fig. 3.6 shows the impact of salinity on herding effectiveness for a different level of HOR. The most effective herding rate for 20 min is 89.33% at room temperature for freshwater. The most rapid bioherder reaction at the first minute is 65% on saltwater at room temperature. It seems that the herding is increased with decreasing HOR. There is no significant difference in change rate when changing HOR from 1:24 to 1:72 in both fresh and saltwater. The impact of the herder itself is higher than the impact of salinity. There is a significant change of salinity on the change rate when the HOR is low. It was observed that salinity has a significant impact on the performance when a smaller herder dose is applied to the system. This indicates that when using herding agents in saltwater, the amount of herd per unit oil should be increased.

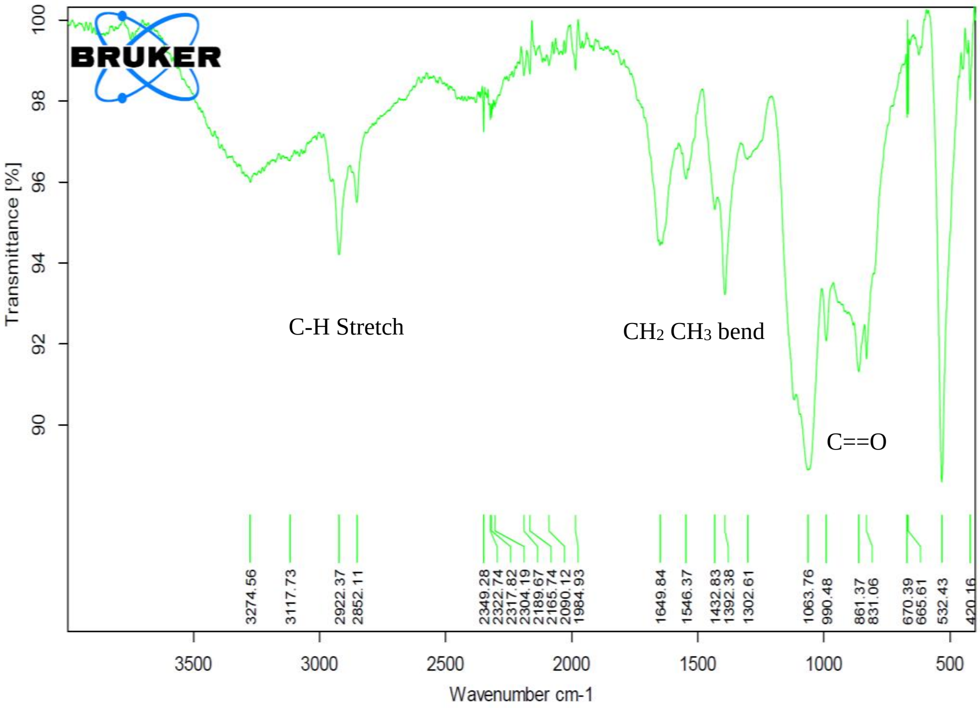
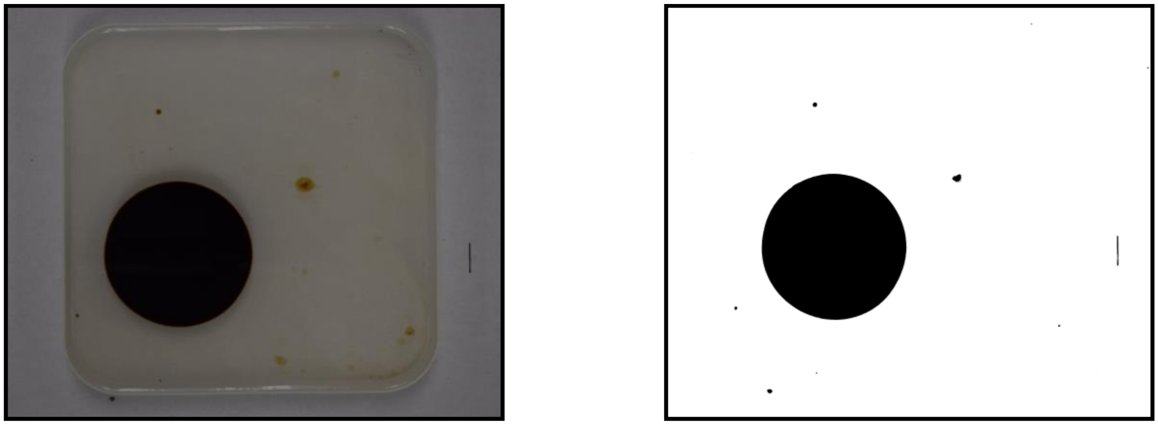


Figure 3.2 FTIR spectrum of bioherder.



(a) (b)

Figure 3.3 Example of calculating oil slick area using Image J (t = 20min).

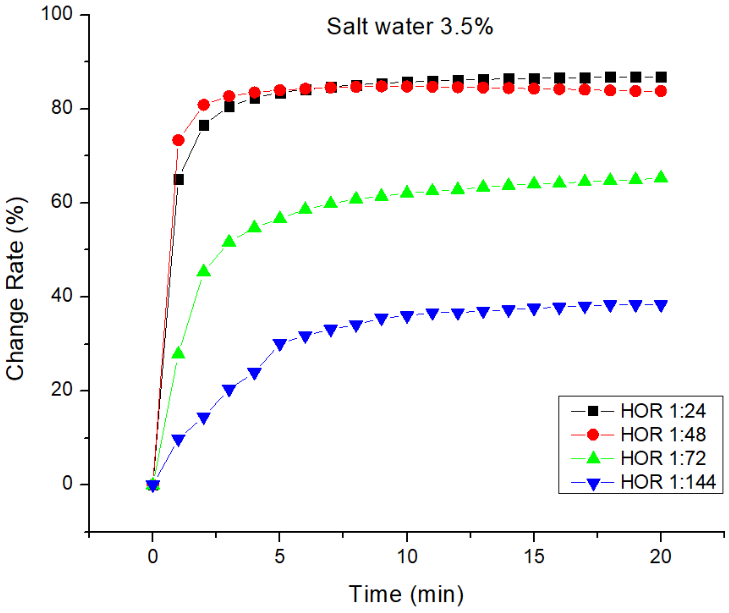


Figure 3.4 Herding efficacy of fresh water and salter eater at room temperature.

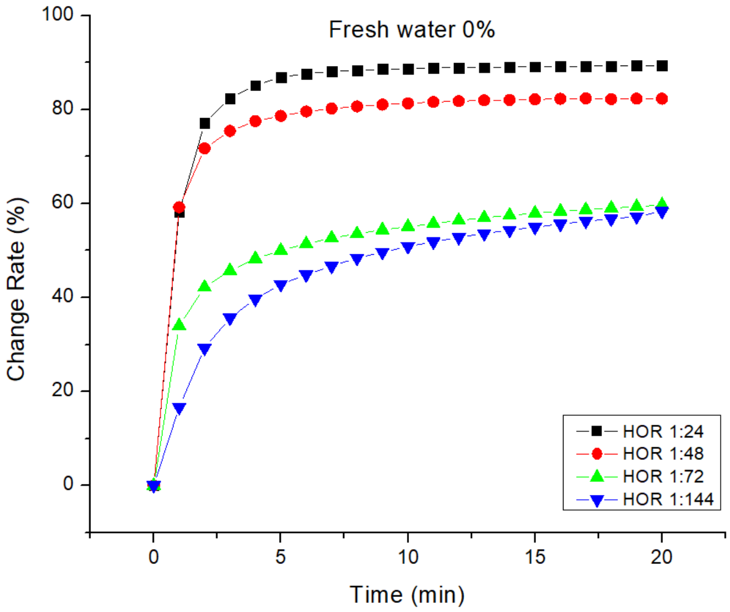


Figure 3.5 Herding efficacy of fresh water and salter eater at room temperature.

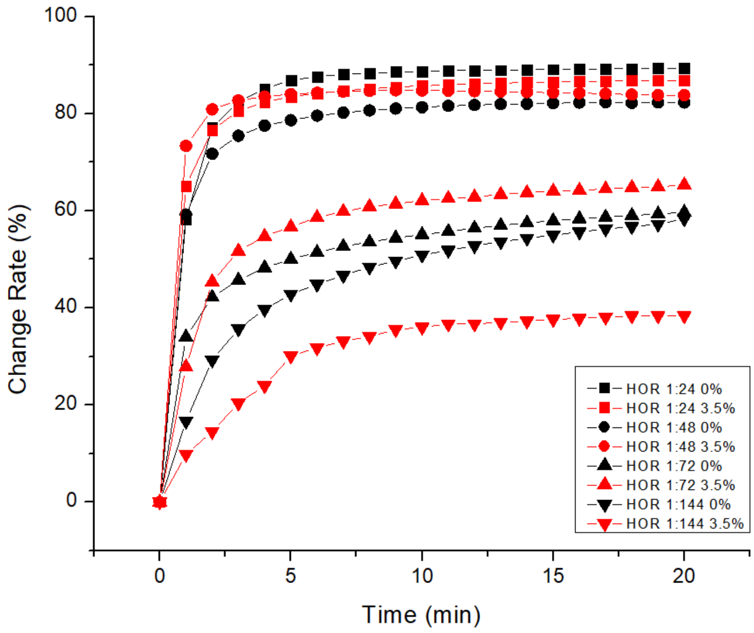


Figure 3.6 Comparison of salinity on herding effectiveness at room temperature

Performance of both the bioherder and the chemical herder were compared using the same lab setting and procedures. Hydrocarbon-based chemical herder span 20, silicon-based chemical herder silsurf, and crude bioherder produced by *Rhodococcus* were used at the same dose following the standard protocols. According to Buist et al. (2010a), the herder dose applied to the water surface is 150 ug/m2. This dose converted this customized testing system to 2.16 ul, making the HOR is 1:333 (Table 3.2). This HOR is not set for bioherder performance in the last session, because it required a large-scale pan to allow oil spreading. This series of experiments was carried out under the steady room conditions, with no wind or heat interpretation. The performance evaluation period was shortened, since the chemical herder reacted rapidly. The chemical herder increased the oil slick thickness quickly and tended to push the oil toward the wall of testing pans. It can be assumed that chemical herder reacts quickly and interacts with oil slick before it forms a monolayer on the water surface. This needs to be proven by testing on a large-scale system. As can be seen from Table 3.2, the change rate of silsurf herder is higher than span and the bioherder. Detailed experiment results are listed in the Appendix.

Table 3.2 Performance comparison of chemical and bioherder under the same condition

	Span	Silsurf	Bioherder
Temperature °C	24	24	24
Herder dose µl	2.16	2.16	2.16
HOR	1:333	1:333	1:333
Water salinity %	3.5	3.5	3.5
Change rate %	89	93	74

3.2.3 Bioherder applications for marine oi spill response

The impacts of environmental factors on herding performance and their interactions are evaluated using DOE results. Five factors of experiment design and results are summarized in Table 3.3. Two levels of factors design analysis were carried out for both 1 min (efficiency) and 20 min (effectiveness). This ANOVA analysis was performed under a 0.05 confidence level. According to Table 2.4, significant effects are A (Temperature), D (Herder dose), and their interaction AD. Main factors C (oil amount), E (adding location), the other two factor interactions, and other quadric terms are not significant. The lack of fit is not significant, indicating the model is a good fit. Besides, the difference between adjusted R^2 (0.8790) and predicted R^2 (0.8059) is less than 0.2. Adeq precision is 16.0870, which indicates an adequate signal. Since a signal-to-noise ratio of greater than 4 is desirable. This model can be used to navigate the design space.

According to Table 3.5, significant effects are A (Temperature), D (Herder dose). Main factors B (salinity), factors C (oil amount), E (adding location), the other two factor interactions, and other quadric terms are not significant. The lack of fit is not significant, indicating the model is a good fit. In addition, the difference between adjusted R^2 (0.8633) and predicted R^2 (0.7344) is less than 0.2. Adeq precision is 14.0966, which indicates an adequate signal. Since a signal-to-noise ratio of greater than 4 is desirable. This model can be used to navigate the design space.

In order to verify that the ANOVA is reliable, several requirements must be met. 1) The sample is normally distributed; 2) variances are constant; 3) experimental runs are randomized and independent. The Design Expert provides all the necessary diagnosis plots, which are shown in Fig. 3.7 and Fig. 3.8.

Table 3.3 DOE experiment variables and results

Standard Order	Actual run	Levels of Variables					Response	
		Temperature (℃)	Salinity （%）	Oil (µl)	Herder (µl)	Approach	Change Rate (%)	
							1 min	20 mins
19	1	14	1.75	540	10	1	24.31	81.75
20	2	14	1.75	540	10	2	34.02	87.06
3	3	4	3.5	360	5	1	4.16	17.25
12	4	24	3.5	360	15	1	65.16	86.82
5	5	4	0	720	5	1	5.37	22.34
18	6	14	1.75	540	10	2	20.24	69.77
4	7	24	3.5	360	5	2	27.86	65.36
6	8	24	0	720	5	2	16.65	58.35
11	9	4	3.5	360	15	2	16.55	56.00
2	10	24	0	360	5	1	34.02	59.74
7	11	4	3.5	720	5	2	5.99	40.92
17	12	14	1.75	540	10	1	35.68	75.24

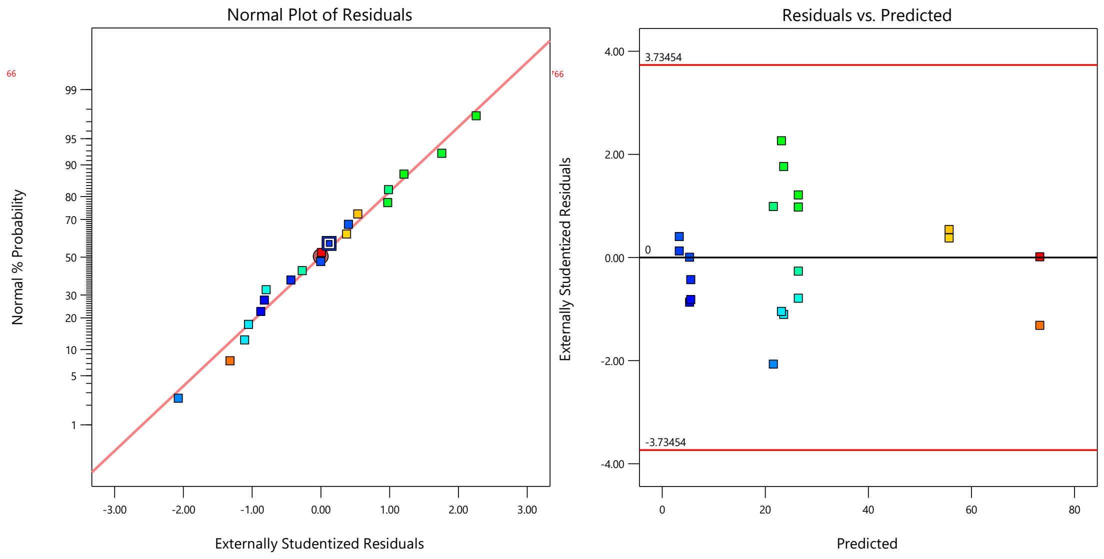
9	13	4	0	360	15	1	2.75	47.84
1	14	4	0	360	5	2	-0.21	44.97
8	15	24	3.5	720	5	1	9.79	38.38
14	16	24	0	720	15	1	59.22	82.32
10	17	24	0	360	15	2	58.17	89.33
13	18	4	0	720	15	2	0.32	51.45
16	19	24	3.5	720	15	2	73.38	83.82
15	20	4	3.5	720	15	1	35.78	66.23

Table 3.4 ANOVA table for factorial design sign at 1min

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	9015.30	5	1803.06	28.61	< 0.0001	significant
A-Temperature	4675.69	1	4675.69	74.18	< 0.0001	
B-salinity	243.21	1	243.21	3.86	0.0697	
D-herder dose	2695.71	1	2695.71	42.77	< 0.0001	
AD	1016.21	1	1016.21	16.12	0.0013	
BD	384.48	1	384.48	6.10	0.0270	
Residual	882.43	14	63.03			
Lack of Fit	723.02	12	60.25	0.7559	0.6974	not significant
Pure Error	159.41	2	79.71			
Cor Total	9897.73	19				
Adjusted R^2	0.8790					
Predicted R^2	0.8059					
Adeq precision	16.0870					

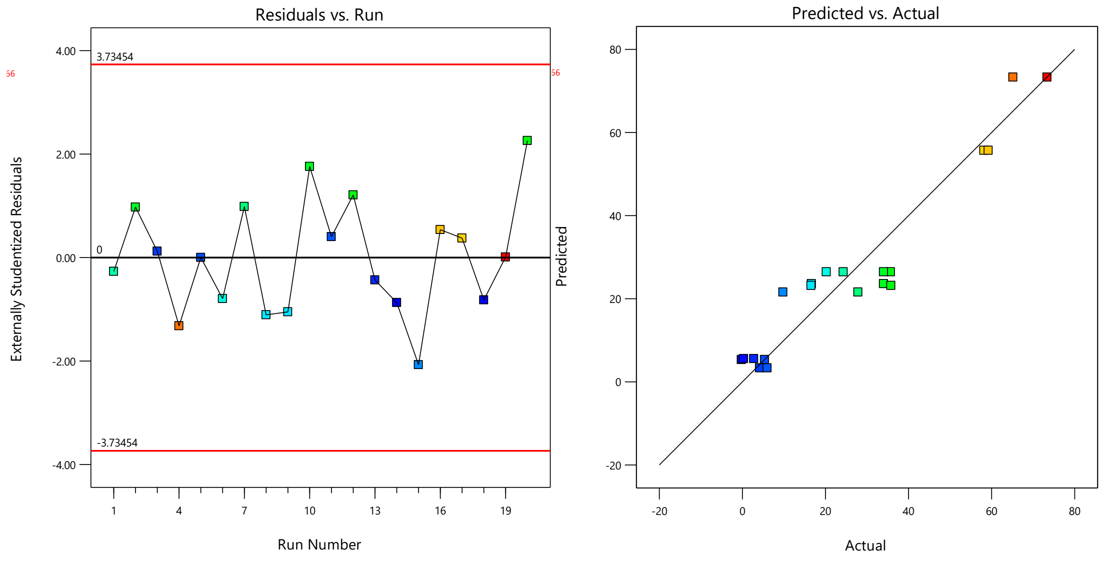
Table 3.5 ANOVA table for factor design at 20 min

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	6461.82	4	1615.45	27.85	< 0.0001	significant
A-Temperature	2945.92	1	2945.92	50.79	< 0.0001	
D-herder dose	2930.08	1	2930.08	50.51	< 0.0001	
E-Locatiuon	300.00	1	300.00	5.17	0.0406	
DE	346.96	1	346.96	5.98	0.0295	
Curvature	1541.61	2	770.81	13.29	0.0007	
Residual	754.08	13	58.01			
Lack of Fit	583.55	11	53.05	0.6222	0.7559	not significant
Pure Error	170.53	2	85.27			
Cor Total	8757.51	19				
Adjusted R^2	0.8633					
Predicted R^2	0.7344					
Adeq precision	14.0966					



a) Normal Plot

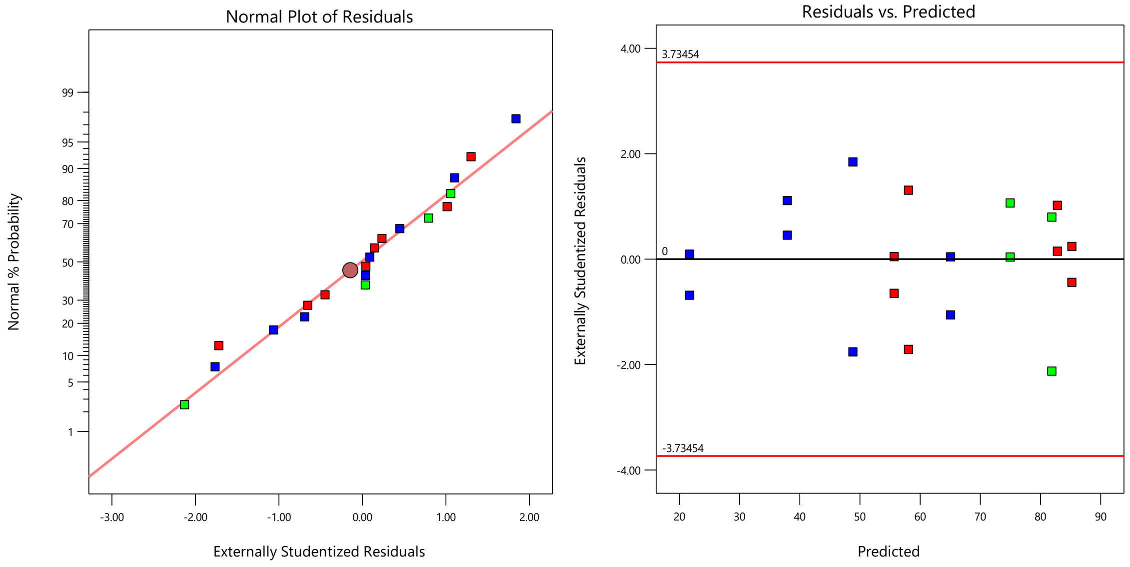
b) Residuals vs. predicted



c) Run random plot

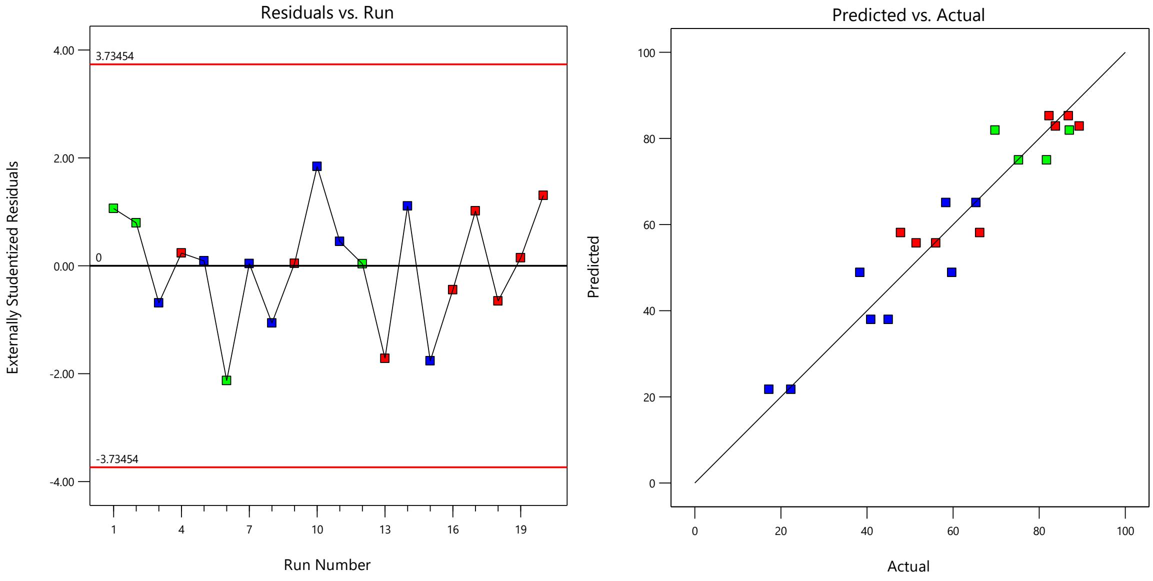
d) Predicted vs. actual

Figure 3.7 Assumption check for herder efficiency model.



a) Normal Plot

b) Residuals vs. predicted



c) Run random plot

d) Predicted vs. actual

Figure 3.8 Assumption check for herder effectiveness model.

3.3 Summary

This study has demonstrated that the biosurfactant can be used as herding agents for marine oil spill response. Temperature and herder/oil ratio were found to exert high impacts on the herding performance. The biosurfactant produced by *Rhodococcus erythropolis* M25 as a herding agent was examined, and the influences of environmental and operational factors (including, temperature, herder dose, spilled oil amount, water salinity, and operation location) on its performance were investigated. A five-factor fractional design was applied to investigate the importance of these factors and their impact on herding effectiveness and efficiency. The results of this study showed that a higher temperature and a larger amount of herder could result in a higher oil slick thickness changing rate. Different water salinity under the same temperature led to the same trend; that is, the herding effectively goes up with the increase of HOR. Large-scale testing need to be further conducted for evaluating the applicability of the developed bioherder in the field.

CHAPTER 4 3D-QSAR AIDED DEVELOPMENT OF HERDING AGENTS FOR MARINE OIL SPILL RESPONSE

4.1 Introduction

In situ burning (ISB) is an efficient physical oil spill response technique, which has been used to remove 4-10% of spilled oil (Lee et al., 2015). Physical conditions such as wind speed (wind speeds ≤ 2 m/s), wave height, the thickness of the oil (2-3 mm), oil type, the degree of weathered oil, and the degree of emulsified oil can limit the applicability of ISB. A strategy to enhance ISB is to increase the oil slick thickness by herder or boomers. Boomers can contain burning by blocking breaking waves and loose ice. Chemical herder has been widely tested as oil collecting agents to improve ISB in the marine environment. A series of lab and in-field tests has shown that chemical herders enhanced ISB at various locations, such as Barents Sea (Buist et al., 2010b), Alaska (Aggarwal et al., 2017), SL Ross lab, and Ohmsett (Buist et al., 2006; Buist et al., 2007; Buist et al., 2008a).

The most popular chemical herders are hydrocarbon-based herders (i.e., Corexit 9580, original U.S. Navy cold water blend, warm-water herder blend, OC-5), silicone-surfactant based (i.e., Silsurf A108, Silsurf A004D), second-generation fluorosurfactant PolyFox™ PF151 (Buist et al., 2010a; Fingas, 2014). The chemical surfactants in the herding agent prevent oil from spreading due to their ability to provide larger spreading pressure than crude oil (Buist et al., 2011b). Span series is the most common key component found in hydrocarbon-based chemical herders, such as the original U.S. Navy cold water blend (Span20), ThickSlick 6536 (Span 20), and warm water blend (Span80) (Fingas, 2014). The surfactant can reduce the surface tension of surrounding water from 70 mN/m to 20-30 mN/m (SLRossEnvironmental, 2015) and the best herding agents have spreading pressures in the mid-40 mN/m range, which is higher than the spreading pressure (10-20 mN/m) in most crude oil (Buist et al., 2011b). Critical micellar concentration (CMC) is another important parameter for surfactant performance; with the lower CMC of surfactant in the water, less surfactant will be required to reduce the system's free energy to form micelles. Standard free energy of micellization (ΔG°_{mic}) can be calculated as the CMC's natural logarithm (Peltonen et al., 2001; Bhardwaj et al., 2016). Hence, standard free energy is used to represent the herding ability.

The distribution of chemical herder after burning can cause environmental concerns. According to various ISB tests conducted by Buist et al. (2008b), more than 90% of the oil was combusted in herder-enhanced burning testing. The herder was mainly detected on the water surface, and there was no significant increase in smoke plume analysis (Buist et al., 2018). The herder after burning still retains the ability to re-herder oil, but cannot be ignited again. Studies have shown the burning residual from ISB is not as toxic as the weathered oil itself (Daykin et al., 1994; Blenkinsopp et al., 1996). However, herders in water after burning have the potential to remain in water for a longer period; the concertation will increase with dissolution in water. A more environmentally friendly herding agent with lower solubility, lower migration ability, higher herding performance, and higher biodegradation potential is needed.

3D-QSAR studies the relationship between chemical properties and their molecular structures. 3D-QSAR is a widely used tool in predicting environmental performance parameters of chemicals and design environmentally friendly molecules. The octanol-water partition (K_{ow}) can predict the solubility of organic compounds and their toxicity in water and soil. Zhao et al. (2004) carried out a QSAR modeling study to predict polycyclic aromatic hydrocarbons solubility. Lu et al. (2001) constructed QSAR models using hydrophobicity parameter $\log K_{ow}$ to determine the toxicity of substituted benzenes on algae.

Li et al. (2020b) studied the toxicity of quinolone antibiotics by establishing K_{ow} 3D-QSAR models. Toxicity and biological enrichment for polychlorinated naphthalene (PCNs) were studied by 3D-QSAR (Gu et al., 2017; Gu et al., 2020a). The octanol-air partition coefficient (K_{oa}) can be used as an independent variable in 3D-QSAR modeling for mobility and bioactivity. Chen et al. (2016) predicted the mitigation ability for PCBs. The K_{oa} value can also be used along with K_{ow} to study the bioaccumulation and bioconcentration for POPs in aquatic systems and identification and biomagnification of chemicals in terrestrial food-chains (Arnot & Gobas, 2003; Gobas et al., 2003). Limited papers have been reported to use 3D-QSAR to study bacteria's surfactant attachment to the membrane (Campbell et al., 1999). There has been no study reported on using 3D-QSAR to predict K_{ow}, K_{oa}, for herders.

The work in this chapter was aimed to study the quantitative structure-activity relationship between surfactant toxicity and its molecular structure. In this study, QSAR models were constructed with 3D descriptors according to the values of $\log K_{ow}/\log K_{oa}$ for 22 span surfactants. Two types of QSAR methods—CoMFA and CoMSIA—were used to predict the $\log K_{ow}/\log K_{oa}$ values of the remaining 9 span surfactant and investigate the relationship between the span surfactant structures and their ability. Furthermore, eight modified compounds were designed based on Span-31. Molecular docking was used to study the biodegradability of the newly designed molecules.

4.2 Methodologies

4.2.1 Construction of 3D-QSAR models for predicting K_{ow} and K_{oa}

Prediction of K_{ow} values for span-based chemical herders: The Sybyl-x 2.0 software was used to establish the 3D-CoMFA and 3D-CoMSIA models. Those target physical characteristics (K_{ow} & K_{oa}) of the span-based chemical herders related to environmental pollution were obtained from the research EPIWEB4.1. We chose to use the logarithm of K_{ow} ($\log K_{ow}$) as experimental input data. To establish the 3D-QSAR models, structural parameters and $\log K_{ow}$ values were used as independent and dependent variables. The whole dataset contains 31 compounds, including 22 compounds as the training set for 3D-QSAR model generation and 9 compounds as the testing set for model validation. The selection of the training set and testing set is based on the ratio of 3:1. Compounds have the capacity to represent the structural diversity and cover a wide range of $\log K_{ow}$ values to be chosen as a training set.

Molecular structure modeling and alignment of Spans: The 3D-QSAR and molecular alignment were performed using the ASybyl-x 2.0 molecular modeling software package. The comparative molecular field analysis (CoMFA) and the comparative molecular similarity index analysis (CoMSIA) models were selected for conducting the 3D-QSAR analysis. The 3D structure of each compound in the dataset was generated using the Sketch Molecule module in Sybyl (Gu et al., 2017). The generated structures were not the most stable conformations, so minimized structures were used as initial conformations for molecular alignment. The geometries of these compounds were subsequently optimized using the Tripos force field (Clark et al., 1989) with Gasteige–Huckel charges (Gasteiger & Marsili, 1980). Repeated minimizations were performed using the Powell method with a maximum of 10,000 iterations to achieve an energy convergence gradient value of 0.005 $kJ.mol^{-1}$. To derive the best possible 3D-QSAR statistical model, ligand-based alignment was employed in this study. In this process, the Span-31molecule, which has the most complex structure, was used as the template to align the other compounds using the Align Database command in Sybyl. All of the molecules were well aligned.

Evaluation and validation of the CoMFA and CoMSIA models: The model stability and predictive ability can be evaluated both internally and externally. Sybyl software offers LOO cross-validation, no-cross-validation, and scrambling stability text procedures, where q^2, R^2, Q^2, and cSDEP values can represent the internal predictive ability and robustness of the models. Linear regression analysis using Origin was used as an external predictive ability evaluation, which provided the R^2 and slope as evaluation criteria. According to the models, the 3D-QSAR contour map of descriptors can provide the guideline for molecular modification (structure modification substrate position). Modified molecules providing lower predicted $\log K_{ow}$, and $\log K_{oa}$ values were chosen to confirm substitute functional groups. The relationship between chemical hydrophobicity and toxicity has been shown for nearly 100 years in both mammals and fish, using the log of the octanol: water partition coefficient (K_{ow}). The models that predict and evaluate the K_{oa} were constructed followed the same procedures discussed above.

Table 4.1 Molecular field contribution of the CoMFA and CoMSIA models

		S	**E**	**H**	**D**	**A**
K_{ow}	CoMFA	0.779	0.221	-	-	-
	CoMSIA	0.262	0.157	0.360	0.068	0.152
K_{oa}	CoMFA		1			
	CoMSIA	0.144	0.254	0.342	0.058	0.2

4.2.2 Molecular modification of Span derivatives

Contour maps for two models can be obtained from Sybyl software. Two descriptor fields (steric and electrostatic field) from the CoMFA contour map were selected to determine substitution sites and groups that affect the $\log K_{ow}$ and $\log K_{oa}$ value for Spans. At the same time, five descriptor fields were selected for CoMSIA model contour maps, including steric, electrostatic, hydrophobic, hydrogen bonds, and donor-acceptor fields. According to this analysis, the Span derivatives were designed using Span-31 as template molecules.

4.2.3 Evaluation of the modified Span-31 derivatives

<u>Gibbs free energy:</u> Two environmental parameters, solubility (predicted by $\log K_{ow}$) and mobility and bioactivity (predicted by $\log K_{oa}$) of the Span-31 derivatives, were evaluated using the multi-activity 3D-QSAR model. The total energy of Span-31 and its derivatives were calculated by Gaussian 09 software as a stability parameter. The calculated Gibbs free energy was used as the functional characteristics to evaluate the herding ability of Span-31 and its derivatives. With the lower CMC of surfactant in the water, less surfactant is required to reduce the system's free energy to form micelles. Standard free energy of micellization ΔG°_{mic} was calculated according to the Clausius–Clapeyron equation.

$$\Delta G^{\circ}_{mic} = RT \ln cmc \tag{2}$$

<u>Biodegradable potential:</u> The total score of Molecular docking will be used to evaluate the biodegradable potential of modified molecules. The molecular docking method was carried out using Discovery Studio 4.0, and the Span-31 derivatives were loaded into the same software. PAH biodegrading enzymes dioxygenase were used for the molecular docking performance, where the LibDock store (total score) functions were obtained. The template

proteins (1O7P and 4J5I) were defined as the receptor molecules using the LibDock module. Protein defining, Potential binding sits editing, and Dock Ligands (LibDock) functions were then selected as described in the literature (Gu et al., 2020b). The LibDock scores rank the various binding capacities of the Span31 molecule to the proteins; the higher total score value corresponds to a stronger the binding force.

4.3 Results and Discussion

4.3.1 Performance evaluation of CoMFA and CoMSIA models for K_{ow} prediction

Tables 4.1 and 4.2 represent the molecular field's contribution rate and the statistical results of the obtained models, respectively. The internal predictive ability and robustness of the developed models were evaluated by LOO cross-validation, no-cross-validation, and scrambling stability text procedures. The q^2, R^2, Q^2, and cSDEP values represent the models' internal predictive ability and robustness. The CoMFA model is considered reliable and acceptable if q^2 is greater than 0.50, and R^2 is greater than 0.90 (Golbraikh & Tropsha, 2002). Generally, the R^2 of the model will be larger than the interaction cross-validated value (q^2). The model is considered to overfit the data when R^2 is larger than q^2 by more than 25% (Leach, 2001). The overall predictive ability of the CoMFA and CoMSIA models was externally validated by predicting the activity of an independent test set of compounds (the compounds not included in the original training set). The predictive ability of the models was presented by the predicted R^2 (R^2pred > 0.6), the standard error of prediction (SEP) of the test set (Golbraikh & Tropsha, 2002). Our CoMFA model for $\log K_{ow}$ had an optimum number (n) of 9, a cross-validated q^2 of 0.816 (>0.5), a non-cross-validated R^2 of 1.000 (>0.9), an SEE of 0.510, an F value of 44842.404, a Q^2 of 0.638, a cSDEP of 5.538, and a dQ^2/dr^2yy of 1.608, illustrating that this model has suitable fitting predictive abilities. CoMFA analysis indicated that the corresponding percentages of the variance explained by steric and electrostatic fields were 79.90% and 21.1%, verifying electrostatic interactions were the major contribution to the $\log K_{ow}$ of Spans.

The CoMSIA defines explicit hydrophobic and hydrogen bond donor and acceptor descriptors in addition to the steric and electrostatic fields in CoMSIA. Our CoMSIA model had an optimum n of 10, a cross-validated q^2 of 0.839(>0.5), a non-cross-validated R^2 of 1 (>0.9), an SEE of 0.027, an F value of 142752.408, a Q^2 of 0.555, a cSDEP of 6.645, and a dQ^2/dr^2yy of 2.083. These statistical indexes were reasonably high, indicating that the CoMSIA model has a strong predictive ability. It was also found that the electrostatic field makes a higher contribution (26.2%) to the Spans $\log K_{ow}$ values than that of the steric (15.7%) but smaller than the hydrophobic field (36.0%). Alternatively, the steric field's impact on Span $\log K_{ow}$ values was reduced when the hydrophobic field was introduced.

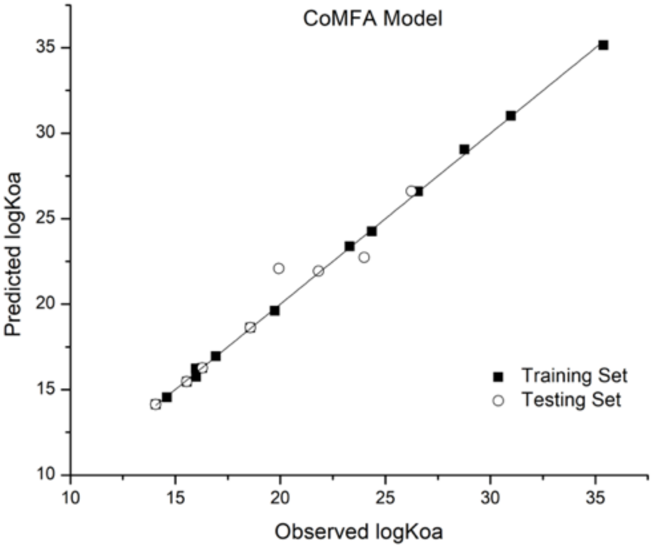
Table 4.2 Statistical parameters of the CoMFA and CoMSIA models

K_{ow}	**n**	**q^2**	**SEE**	**R^2**	**F**	**R^2_{predic}**	**SEP**	**Q^2**	**cSDEP**	**dQ^2/dr^2yy**
CoMFA	9	0.816	0.51	1	44842.404	0.927	1.39	0.638	5.538	1.608
CoMSIA	10	0.839	0.027	1	142752.408	0.887	1.731	0.553	6.645	2.083
K_{oa}										
CoMFA	6	0.843	0.152	1	5848.310	0.935	1.129	0.672	4.703	1.014
CoMSIA	8	0.862	0.066	1	22811.417	0.934	1.136	0.530	5.538	1.311

4.3.2 Performance evaluation of CoMFA and CoMSIA models for K_{oa} prediction

Tables 4.1 and 4.2 represent the contribution rate of the molecular field and the statistical results of the obtained models, respectively. The CoMFA model is considered reliable and acceptable if q^2 is greater than 0.50, and R^2 is greater than 0.90 (Golbraikh & Tropsha, 2002). Generally, the R^2 of the model will be larger than the interaction cross-validated value (q^2). The model is considered to overfit the data when R^2 is larger than q^2 by more than 25% (Leach 2001). Our CoMFA model for $\log K_{oa}$ has an optimum n of 6, a cross-validated q^2 of 0.843 (>0.5), a non-cross-validated R^2 of 1.000 (>0.9), an SEE of 0.152, an F value of 5848.31, a Q^2 of 0.672, a cSDEP of 4.703, and a dQ^2/dr^2yy of 1.014, illustrating that this model has both suitable fitting predictive abilities. CoMFA analysis indicates that the corresponding percentages of the variance explained by steric and electrostatic fields were 100%, verifying electrostatic interaction was the major contribution to the $\log K_{oa}$ of Spans.

The CoMSIA defines explicit hydrophobic and hydrogen bond donor and acceptor descriptors in addition to the steric and electrostatic fields in CoMSIA. Our CoMSIA model has an optimum n of 10, a cross-validated q^2 of 0.862(>0.5), a non-cross-validated R^2 of 1 (>0.9), an SEE of 0.066, an F value of 22811.417, a Q^2 of 0.53, a cSDEP of 5.538, and a dQ^2/dr^2yy of 1.311. These statistical indexes were reasonably high, indicating that the CoMSIA model had a strong predictive ability. It was also found that the hydrophilic made a higher contribution (34.2%) to the Spans $\log K_{oa}$ values than that of the steric (14.4%) and electrostatic (25.4%).



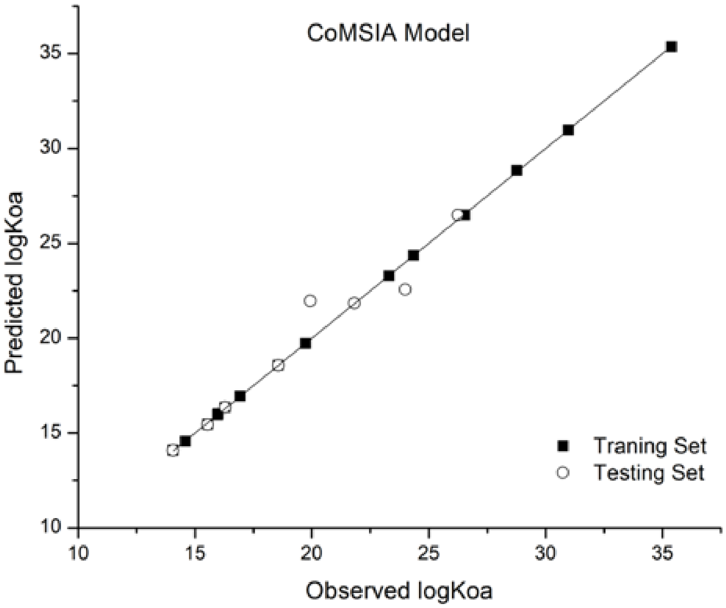


Figure 4.1 The plot of observed vs. predicted logK_{oa} values using CoMFA /CoMSIAmodel.

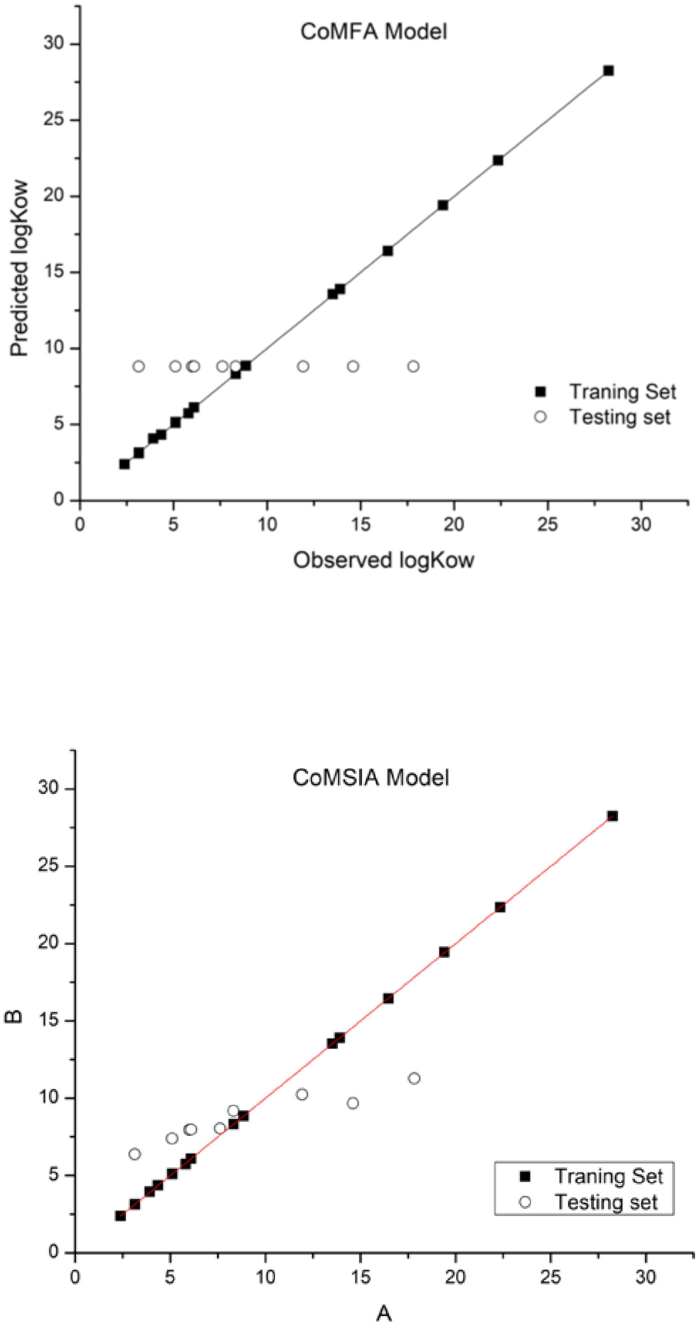


Figure 4.2 The plot of observed vs. predicted logK$_{ow}$ values using CoMFA/CoMSIA model.

4.3.3 Validation of CoMFA and CoMSIA models

According to Golbraikh and Tropsha (2002), q^2 alone is not a suitable parameter to estimate the prediction capability of QSAR models, and external validation should be applied. External validation was also conducted to assess further the reliabilities and the predictive ability of the built model (Li et al., 2013). The test set containing 9 compounds was used for this validation. According to Table 4.2, R^2pred values of 0.927 (>0.6) and 0.887 (>0.6), SEP values of 1.39, and 1.731 were achieved, verifying the good external predictive ability of the two models for K_{ow}. Meanwhile, R^2pred values of 0.935 (>0.6) and 0.934 (>0.6), SEP values of 1.129and 1.136, verifying the good external predictive ability of the two models for K_{oa}.

Further analysis of the $\log K_{ow}$ predicted by the CoMFA and CoMSIA models revealed a fine linear dependence (R^2 of 0.99995 and 0.99999, respectively) among calculated values and predicted values. Figures 4.2 shows that the slopes of the linear equations for calculated and predicted values were 0.9997 and 0.99998, respectively. External validation for the K_{oa} model can also be found in Figures 4.1, R^2 of 0.99956 and 0.99992, and the slopes of the linear equations for calculated and predicted values were 0.99932 and 0.99997, respectively.

Both CoMFA and CoMSIA models were used to predict results, and the calculated and predicted $\log K_{ow}$ / $\log K_{oa}$ values Table 4.3.

From the statistical parameters and fitting degree of verification, the CoMFA and CoMSIA models were considered reliable and acceptable (Table 4.2). Further analysis of the $\log K_{ow}$ predicted by the CoMFA and CoMSIA models revealed a fine linear dependence among experimental values and predicted values. The statistical indexes of the CoMFA and

CoMSIA models were reasonably high, indicating that the two models reached the internal inspection and external validation standard of models. The validations suggested that the models exhibit optimum stability and good predictive power. Thus, the two models can be used to predict the logK_{ow} of the same types of compounds. According to the contribution rates of the descriptor fields, the two models mutually verified and proved that electronic effects primarily influence the logK_{ow}. According to the 3D isogram of the CoMFA and CoMSIA models, the information for the modification of Spans was consistently obtained. Even though the models exhibited a satisfactory fitting ability and acceptable predictive ability, only the CoMFA model contained two descriptor fields (steric and electrostatic fields), presenting certain limitations in analyzing the effect of the descriptor field and the modification of the information on the compounds. The CoMSIA model contained five descriptor fields, which provided a more comprehensive understanding of the effect on the physical and chemical properties of Spans. It is noted that in this model, the five descriptor fields play an important role in renovating Spans and designing new types of compound molecules. For example, an analysis of the hydrophobic field revealed that hydrophobic groups on the head and tail would decrease the logK_{ow}.

Table 4.3 Predicted logK_{ow} / logK_{oa} values of Span from the CoMFA and CoMSIA models.

	logK_{ow}			**logK_{oa}**		
No.	Obs	Pred		Obs	Pred	
		CoMFA	CoMSIA		CoMFA	CoMSIA
Span-2[a]	3.15	3.16	3.15	14.07	14.14	14.09
Span-3[a]	3.15	3.10	3.13	14.07	14.14	14.08
Span-4[a]	3.15	3.10	3.13	14.07	14.14	14.08
Span-5[a]	3.15	3.16	3.15	14.07	14.14	14.09
Span-6[b]	3.15	8.81	6.37	14.07	14.14	14.09
Span-7[a]	3.92	4.08	3.95	14.59	14.55	14.57
Span-8[a]	5.12	5.16	5.13	15.55	15.47	15.46
Span-9[a]	5.12	5.11	5.11	15.55	15.47	15.45
Span-10[a]	5.12	5.11	5.11	15.55	15.47	15.45
Span-11[b]	5.10	8.81	7.40	15.55	15.47	15.45
Span-12[a]	2.39	2.39	2.39	16.93	16.95	16.95
Span-13[a]	6.10	6.12	6.11	16.28	16.27	16.35
Span-14[a]	6.10	6.12	6.11	16.28	16.27	16.35
Span-15[a]	6.10	6.12	6.11	16.28	16.27	16.35

Span-16[b]	6.00	8.81	7.97	16.28	16.27	16.37
Span-17[b]	6.10	8.81	7.97	16.28	16.27	16.35
Span-18[a]	5.81	5.73	5.75	15.99	15.75	16.02
Span-19[a]	4.35	4.34	4.37	15.97	16.23	15.97
Span-20[b]	7.62	8.81	8.04	19.93	22.08	21.97
Span-21[a]	8.86	8.86	8.86	19.74	19.62	19.72
Span-22[b]	11.93	8.81	10.24	21.82	21.94	21.85
Span-23[a]	13.52	13.57	13.52	24.35	24.26	24.37
Span-24[b]	14.60	8.81	9.67	24.00	22.73	22.56
Span-25[a]	6.00	8.81	7.97	23.30	23.38	23.29
Span-26[a]	16.46	16.41	16.44	26.55	26.60	26.49
Span-27[b]	17.83	8.81	11.29	26.25	26.60	26.49
Span-28[a]	19.41	19.41	19.44	28.76	29.06	28.84
Span-29[a]	28.25	28.25	28.24	35.39	35.16	35.36
Span-30[a]	22.35	22.35	22.35	30.97	31.02	30.97
Span-31[*]	8.33	8.81	9.18	18.57	18.62	18.57

Note: a = training set, b = testing set, * = template molecule

4.3.4 Molecular modification and enzyme docking for evaluating biodegradability of the developed herders

Analysis of the substitution characteristics based on the contour maps obtained for the multi-effect 3D-QSAR models.

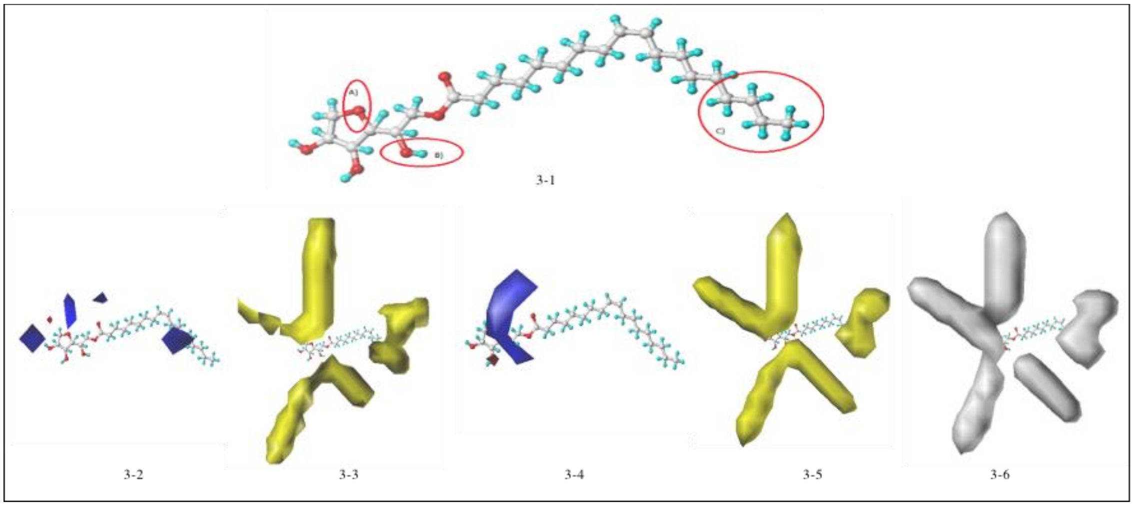


Figure 4.3 K_{ow} Contour maps of the CoMFA/CoMSIA model, electrostatic fields (3-2/3-4), static fields (3-3/3-5), and hydrophobic (3-6).

Figures 4.3 displays the contour maps of the CoMFA and CoMSIA models based on the template molecule Span-31 for K_{ow} prediction. The diagrams were helpful in identifying the important regions where variations in the steric, electrostatic, hydrophobic around the compound explain differences in the $\log K_{ow}$ values of Spans. In the steric field (3-3/3-5), the sterically favored region is shown in green, and the sterically unfavored region is in yellow. In the electrostatic field (3-2/3-4), blue-colored contours represent regions where the positive charge increases the $\log K_{ow}$ values. In contrast, red-colored regions display areas where the negative charge enhances $\log K_{ow}$ values. Moreover, the yellow and white contours depict hydrophobic and hydrophilic favored regions (3-6), respectively.

The CoMFA steric contour map is displayed in Fig. 4.3. The region A (1-O- position) and end of the tail (Region C) show larger yellow contours, suggesting that if large volume groups were introduced at these positions, the $\log K_{ow}$ values would decrease. A blue block above the 1-O (Region A) and 5-C- position (Region B) was observed in the contour maps (Fig. 4.3(3-3)), which means that the negative charge located there will decrease the $\log K_{ow}$ values. Besides, the contour map (Fig. 4.3(3-2)) of the electrostatic field of the CoMFA model showed that the red region was mainly distributed at positions 1-O- (Region A) and 5-C- (Region B). This indicates that introducing positive substituents at these positions was conducive to reducing the $\log K_{ow}$ values for Spans. In the CoMSIA steric contour map (Fig. 4.3 (3-5)), the yellow area was near 1O- (Region A), 5C- (Region B), and tail (Region C). Therefore, the introduction of large groups at the 1-O- and 5-C- positions will reduce the $\log K_{ow}$. Fig.4.3 (3-6) shows the 1-O-, 5-C, and the tail was covered by hydrophilic favored white contour, so hydrophilic groups will lower the $\log K_{ow}$ value.

By comparing contour maps of the CoMFA and CoMSIA models, we found that the modified information generated by the two models was consistent. A synthetic analysis of the effect of steric, electrostatic, and hydrophobic fields in the CoMSIA model showed that the common 1-O- and 5-C-were covered by yellow contours of the steric contour map, blue contours of the electrostatic contour map, and white hydrophobic contour map. Based on these findings, the 1O and 5C atoms were affected by steric, electrostatic, and hydrophobic fields; that is, it was conducive to reducing the K_{ow} value of larger volume, negative electrophilic, or hydrophobic groups introduced at this position. Lower water solubility function groups (-F, -Cl, -Br), Hydrocarbyl (-CH, $-CH_2$, $-CH_3$), Nitro ($-NO_2$), and hydrophobic hydroxyl (-OH) group were selected as modified groups to be embedded in the target congener (Span-31). The replacement groups and prediction results are shown in Table 4.4.

Similarly, the counter maps of $\log K_{oa}$ CoMFA/CoMSIA models (Fig. 4.4) show that the same region is covered by static field (4-2/4-4), electrostatic field (4-3), and hydrophilic field (4-5). Large volume hydrophilic function groups with negative chares will be introduced to minimize the $\log K_{oa}$ value.

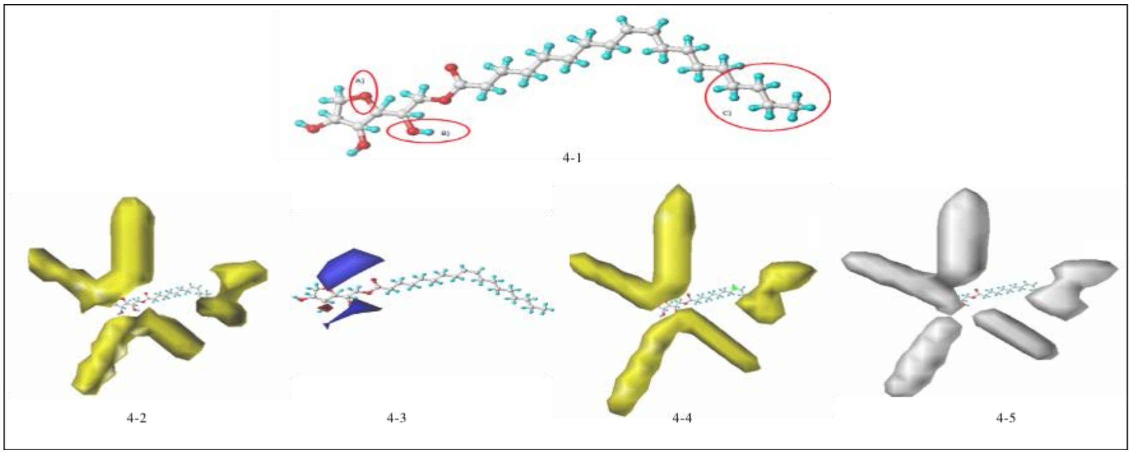


Figure 4.4 K_{oa} Contour maps of CoMFA model, steric fields (4-2); CoMSIA model, steric fields (4-4); electrostatic fields (4-3); hydrophobic(4-5).

4.3.5 Evaluation of practicability and biodegradability of selected Span31 derivatives

Ten span31 derivatives were designed using this technique to calculate water solubility (predicted by $\log K_{ow}$), mobility and bioactivity (predicted by $\log K_{oa}$), stability parameter (predicted by Total energy), herding ability (predicted by Gibbs free energy), and biodegradability (predicted by LibDock score). Based on these results, 4 span31 derivatives (1-Cl-31, 5-SH-31, 1-CH_2-31, 1-S-31) had $\log K_{ow}$ values lower than those of the target molecule. Bioconcentration, transport and fate values decreased, while their biodegradability increased (Table 4.4). Predicted K_{oa} value decreased by CoMFA/CoMSIA were the molecules 31-Cl, 31-F, 31-Br, 31-CH_2, 31-NH, and 31-SH (5C). Predicted K_{ow} value decreased for molecules 31-Cl, 31-F, 31-CH_2, 31-NH, 31-SH (5C), and 31-S. Total energy decreased for molecular 31-Cl, 31-F, and 31-SH (5C). Increased for molecules 31-CH2. Gibbs free energy decreased for 31-Cl, but increased for 31-SH (5C) and 31-CH_2. The derivatives of 1-Cl-31, 5-SH-31, 1-CH2-3, 1-S-31 were more easily degraded than span31 and were therefore selected using the scoring functions.

As can be seen in Table 4.4, the CoMSIA model predicted that when the 1-O- position was replaced by -Cl, -S, and -CH_2, the 5-C position was replaced with -SH, the $\log K_{ow}$ values of modified Span-SH compounds decreased significantly compared with span-31, indicating that the introduction of functional groups is the reason for the descending K_{ow} values of the modified compounds. When the 1O position was replaced with the halogen atom SH, the K_{ow} value increased significantly. This is consistent with the 1O atom being affected by steric, electrostatic, and hydrophobic fields and is also consistent with the electrostatic interaction being the major contributor to the $\log K_{ow}$ of Spans, meaning that the steric field has little influence that can be ignored.

The total energy (stability parameter) (Xu et al., 2016) and Gibbs free energy (functional parameter) (Peltonen et al., 2001) of Span31 derivatives were calculated using Gaussian 09. Table 4.4 shows that when the 1O position of Span31 was replaced by the Cl group (31-Cl), the total energy of 31 decreased significantly. However, the Gibbs free energy decreased only by -1.311% compared with 31. When the 1O position of span31 was replaced with -CH_2, -NH, and -Si, the total energy decrease of the modified compounds was 16.407~48.918%. The change rate of the Gibbs Free energy was 4.596% ~-1.070%, thus explaining that the effect on the degree of total energy and the energy gap of modified compounds was smaller.

Molecular docking was performed between the PAH degrading enzyme and the span31 derivatives, and scoring functions were obtained. The span31 derivatives that were more easily degraded than span31 were selected using the scoring functions. The molecular docking method was used to calculate the total-score values for the interaction between 4 of the span31 derivatives and 1O7P (Pseudomonas putida) /4J5I (Mycobacterium) to determine the degradability of these compounds. Compared with the target molecule, the solubility, mitigation and allocation behavior values associated with the new span31 derivatives were observed to decrease. However, the biodegradability increased by 1.35% - 8.88%.

4.4 Summary

This study successfully created the environmental parameter prediction model for herding agents span31. For K_{ow}, the obtained CoMFA and CoMSIA models exhibited q^2 of 0.816 and 0.839, r^2 of 1 and 1, SEP of 1.39 and 1.731, and r^2 pred of 0.927 and 0.887, respectively, indicating satisfactory fitting ability and acceptable predictive ability for the 31spans. Similarly, the obtained CoMFA and CoMSIA models can successfully predict K_{oa} with q^2 of 0.843 and 0.862, r^2 of 1 and 1, SEP of 1.129 and 1.136, and r^2 pred of 0.935 and 0.934. The two models externally verified and proved that the electrostatic field plays a dominating role in Spans $\log K_{ow}$ values. In an electrostatic field, the introduction of electropositive groups in the 1-O and 5-C positions of Span31 can significantly reduce the $\log K_{ow}$ and $\log K_{oa}$ values and increase the hydrophobic strength. Modified compounds based on contour maps exhibited smaller $\log K_{ow}$ values compared with targeted span31. At the same time, there was no significant difference between the calculated total energy and the energy gap of span31. Biodegradability of new molecular is increased, increasing the ability to burn residue recovery in oil spill response. The increased Gibbs free energy of modified molecule indicates the newly designed molecule has lower CMC. Using a lower concentration of these new herding agents can provide the same performance level in the oil spill response.

Table 4.4 Structural modification of new Spna31 molecules, derivatives predicted values of the logK$_{ow}$, logK$_{oa}$, total energy, Gibbs free energy, total-score by CoMFA/CoMSIA modules and molecular docking

Compounds	Predicted values of logK$_{oa}$		Predicted values of logK$_{ow}$		Total energy (a.u.)	Change rate of energy (%)	Gibbs	Change rate of Gibbs (%)	Docking total score	Change rate of total score (%)	Docking total score	Change rate of total score (%)
	CoMFA	CoMSIA	CoMFA	CoMSIA					1O7P		4J5I	
31	18.624	19.654	8.810	9.180	-1392.371	0	0.586	-	114.495	0	135.643	0
1-Cl-31	17.081	17.800	8.760	7.378	-1777.285	-27.64	0.578	-1.311	-	-	-	-
1-F-31	17.039	16.325	7.309	4.509					-	-	-	-
1-Br-31	15.689	17.028	8.832	5.905	-3888.809	-179.29	0.577	-1.511	-	-	-	-
5-CH$_3$-31	19.579	20.093	8.810	9.604								
1-CH$_2$-31	16.756	16.833	6.253	5.622	-1356.456	2.58	0.613	4.596	124.657	8.88	133.264	-1.75
1-NH-31	18.105	16.414	7.548	5.357								
5-SH-31	16.609	18.097	5.421	6.937	-1317.161	5.40	0.587	0.183	116.036	1.35	128.511	-5.26
1-Si-31	16.586	21.376	2.809	10.577	-1606.610	-15.39	0.580	-1.070	117.042	2.22	136.005	0.27
1-S-31	19.168	16.457	6.329	5.359	-1715.345	-23.20	0.583	-0.518	118.151	3.19	149.141	9.95
5-NH$_2$-31	19.109	18.037	9.004	7.393	-1372.497	1.42	0.602	2.667	119.431	0.043	140.677	0.037

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

This thesis focused on the generation of environmentally friendly surfactant-based herding agents for marine oil spill response. It includes the production of an alternative biosurfactant-based bio-herder and a green span-based chemical herder. The effectiveness of these newly developed herding agents for marine oil spill response has also been demonstrated under various conditions.

The first study began with the usage of *Rhodococcus erythropolis* M25 to form a biosurfactant based bio-herder. Five operational/environmental factors based on the literature review and previous trial tests were identified and tested using a 25-1 half fractional design. Results indicated that the oil slick thickness change rate increases with the increasing temperature under both higher and lower herder dose levels. It is obvious that the change rate increases more rapidly with the increasing temperature when the herder dose is at a higher level. This indicated that a higher temperature and a greater amount of herder could provide a higher oil slick thickness changing rate. The results proved the hypothesis that the biosurfactant-based product can be used as an effective herding agent, and the temperature and herder/oil ratio have a high impact on the bio-herding effectiveness.

The second study successfully created the environmental parameter prediction model for molecular modification of the herding agent Span 31. Two QSAR (CoMFA and CoMSIA) models were constructed to predict the solubility and mobility and bioactivity of span series herders. The 3D structures of thirty-one model compounds were gathered from literature sources for training (22) and testing (9) sets. The octanol-water partition (K_{ow}) can be used as the independent variable to predicate organic compound solubility and toxicity in water.

And the octanol-air partition coefficient (Koa) is used as independent variables for mobility and bioactivity. Both the CoMFA and CoMSIA models were validated as reliable and acceptable with good predictive power. By comparing contour maps of the CoMFA and CoMSIA models, it was found that the modified information generated by the two models was consistent, and electronic effects primarily influence the $\log K_{ow}$ and $\log K_{oa}$. Through molecular design, ten molecularly modified span compounds were obtained with lower $\log K_{ow}$ and $\log K_{oa}$. Gibbs free energy can be used to evaluate the herding ability of span derivatives. The increased Gibbs free energy of modified molecular indicates that the newly designed molecule has a lower CMC. Using a lower concentration of these new herding agents can provide the same performance level in the oil spill response. Molecular docking was then undertaken to examine the biodegradability of a newly designed span derivative. Biodegradability of new molecule is increased with the increasing the ability to burn residue recovery in oil spill response. This is the first study using QSAR for herder structure modification and performance improvement.

Through both tasks, herder production was advanced with new herders (bio-herder and molecular modified herder) generated. Besides, the associated herding performance and environmental impacts were evaluated. The thesis outputs contribute knowledge to the field and provide more sustainable marine oil spill response operations options. The results of this thesis point to opportunities to find effective and green herding agents with less negative environmental impacts on marine oil spill response. The research would benefit governments, oil industries, and oil spill responders by providing promising oil spill herding options. The outputs would also benefit coastal communities by controlling environmental risks to preserve a healthy living environment.

5.2 Research Contributions

(1) This study, for the first time, used genetically modified biosurfactant producers to generate a bio-herders product. The product was proved to be capable of herding crude oil slick effectively.

(2) This study constructed an eco-friendly structure of Span-based herder with improved herding performance and potential biodegradability. This is the first study using 3D-QSAR integrated molecular docking for herder structural modifications.

5.3 Recommendations for Future Work

(1) This study has explored the potential of using biosurfactants produced by *Rhodococcus erythropolis* to form bio-herding agents under calm conditions. However, the herding effectiveness in harsh marine environments under complicated conditions (e.g., with rough waves, under low temperatures, with ice) should be further examined.

(2) It is worth studying the relationships between structures of bio-herders and the associated herding effectiveness through further identification of bio-herder functional groups using high-performance liquid chromatography-mass spectrometry (HPLC-MS), gas chromatography - mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) analysis.

(3) Pilot-scale (e.g., wave tank) examination of the bio-herding effectiveness should be conducted.

(4) The stability and effectiveness of the bio-herder during enhancing in-situ burning operations should be conducted. The integration of herding and in-situ burning would help to demonstrate the applicability of the bio-herder as the treating agent to deal with real-world marine oil spills.

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APPENDIX

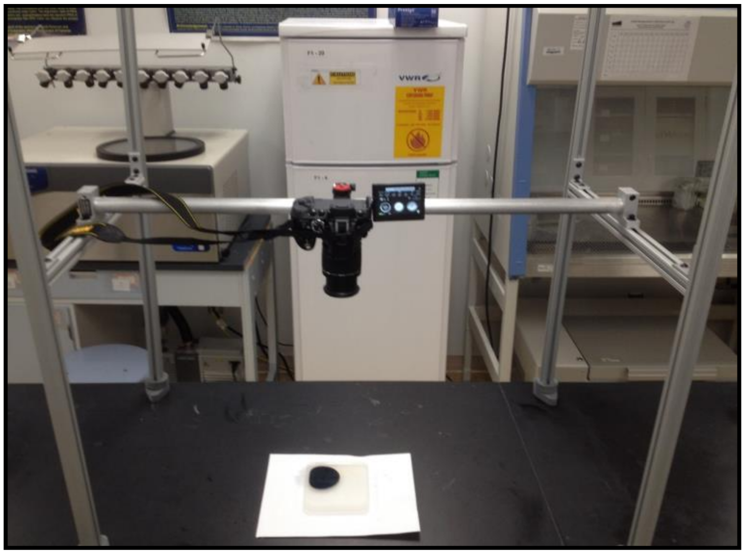

Figure A.1 Experiment Setup

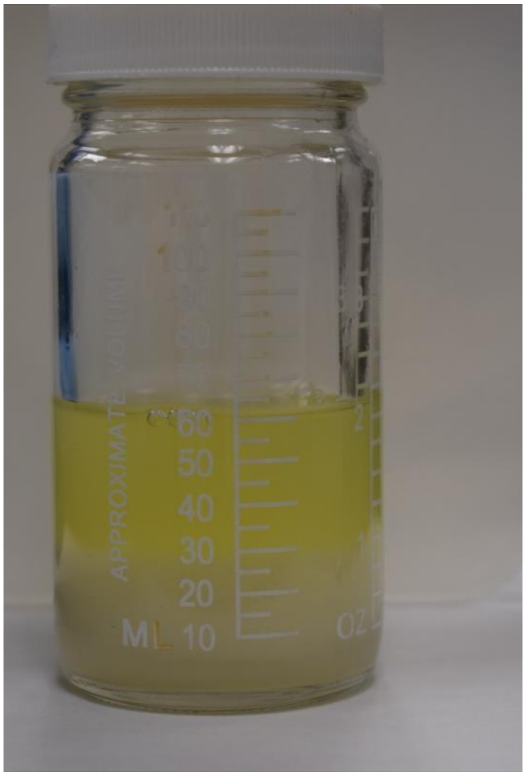

Figure A.2 Biosurfactant before extraction

Figure A.3 Biosurfactant cultivation in the flask

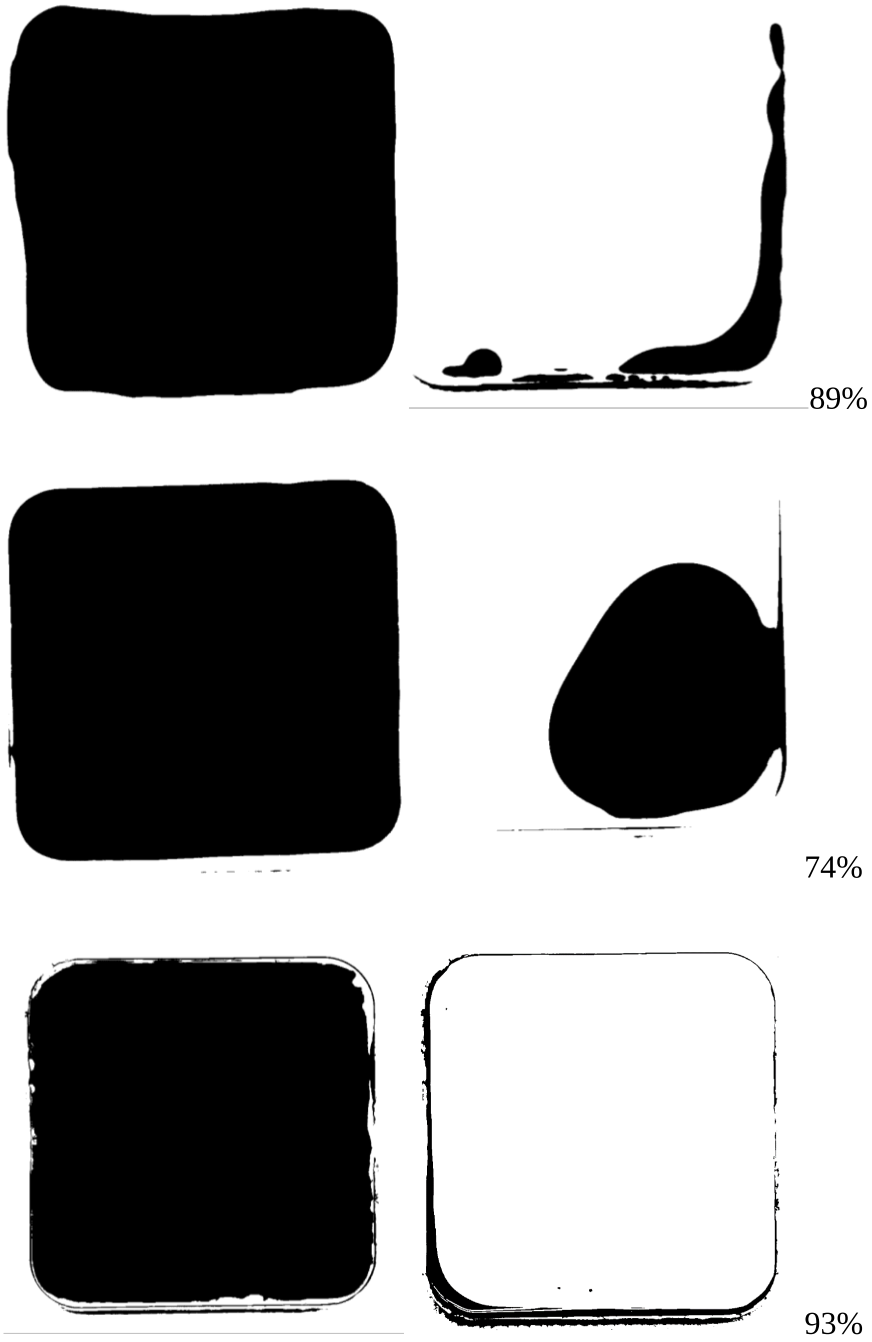

Figure A.4 Comparison of chemical and biological herder performance