Microcapsules-based multifunctional coatings for anticorrosion and antibacterial applications

Chong, Yong Bing

2019

Chong, Y. B. (2019). Microcapsules-based multifunctional coatings for anticorrosion and antibacterial applications. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/89652>

<https://doi.org/10.32657/10220/47801>

**NANYANG
TECHNOLOGICAL
UNIVERSITY**

SINGAPORE

**MICROCAPSULES-BASED MULTIFUNCTIONAL COATINGS FOR
ANTICORROSION AND ANTIBACTERIAL APPLICATIONS**

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SCHOOL OF MECHANICAL AND AEROSPACE ENGINEERING

2019

**MICROCAPSULES-BASED MULTIFUNCTIONAL COATINGS FOR
ANTICORROSION AND ANTIBACTERIAL APPLICATIONS**

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A thesis submitted to the Nanyang Technological University
in partial fulfilment of the requirement for the degree of
Doctor of Philosophy

2019

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

28 January 2019

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Date

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Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

28 January 2019

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Date

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Professor Yue Chee Yoon

Authorship Attribution Statement

This thesis contains material from 1 paper published in the following peer-reviewed journal where I was the first author.

Chapter 4 is published as Y.B. Chong, H. Zhang, C. Y. Yue, and J. Yang, "Fabrication and release behavior of microcapsules with double-layered shell containing clove oil for antibacterial applications," *ACS Applied Materials & Interfaces*, vol. 10, pp. 15532-15541, 2018.

The contributions of the co-authors are as follows:

- Prof. Yang and Prof. Yue provided the initial project direction and edited the manuscript drafts.
- I prepared the manuscript drafts and the final manuscript was revised by Prof. Yang and Prof. Yue.
- I co-designed the study with Dr. He Zhang and performed all laboratory work at the School of Mechanical and Aerospace Engineering.
- I conducted the laboratory works and different materials characterizations.
- Dr. He Zhang assisted in the interpretation of the SEM images, FTIR and UV-Vis release test results.

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Acknowledgement

First and foremost, I would like to express my most sincere gratitude and appreciation to my supervisor, Prof. Yue Chee Yoon and my ex-supervisor, Prof. Yang Jinglei for their guidance, encouragement and patience in leading me through my studies in NTU. Their unselfish sharing of knowledge and guidance has played a significant role in achieving my current stage of research work and guiding me to the completion of this PhD thesis.

Furthermore, I would like to acknowledge Nanyang Technological University for the financial support and the research scholarship of this project.

Collaboration is always a big part in research. Hence, I wish to thank my colleagues and friends- Dr. Zhang He, Dr. Sun Dawei, Dr. Wei Yuefan, Dr. Zhang Xin, Dr. Tan Chuan Hao and Dr. An Jinliang who have guided me well through my research with their outstanding knowledge and experiences in research. I would like to express my appreciation to Prof. Christopher Shearwood for his unselfish sharing of knowledge on the antimicrobial test and conducts.

Moreover, I wish to especially acknowledge Ms. Koh Joo Luang and Ms. Wang Hui for their continuous support and all the other technical staffs at the School of MAE for their aid in my research work.

Last but not least, I would like to express my heartfelt gratefulness to my family for their continuous support and understanding, and to my girlfriend for her encouragement and support throughout my research work.

Abstract

Corrosion and biofouling are among the most challenging issues in the marine industry which has caused huge economic losses annually. The complex marine environment has caused severe corrosion problem due to the damage of paint or coating and biofouling problem due to the accumulation of micro- and macro-organisms on the coatings. One of the most common approaches to treat biofouling is the use of biocides, which was found to pose negative impact to the environment and human health. Additionally, the encapsulation of corrosion inhibitors for anticorrosion properties of the coatings involves the use of toxic organic solvent, which raises concern over environmental and health issues. Hence, a more environmental friendly solution to solve both corrosion and biofouling problems is needed.

Clove oil as a natural antimicrobial agent was successfully encapsulated through interfacial and in-situ polymerizations. The release of clove oil from the double layered PU/PUF microcapsules fitted well against the Baker-Lonsdale model, indicating diffusion as the release mechanism. The calculation of Baker-Lonsdale constant (k_B) values validates the feasibility to fabricate microcapsules with a wide range of diffusivity up to 8 times difference, indicating the versatility of this microencapsulation technique for a vast range of applications. In particular, the release rate decreases with increasing PU shell thickness and increasing surface roughness. Additionally, the microcapsules possess excellent antibacterial activities against different marine bacterial strains – *Escherichia coli*, *Vibrio coralliilyticus* and *Exiguobacterium aestuarii*.

Multifunctional microcapsules containing clove oil and 8-hydroxyquinoline (8-HQ) with good mechanical strength, anticorrosion and antibacterial features were fabricated. Accelerated corrosion test indicates the formation of inhibition layer based on the reaction between the released 8-HQ compound and the steel substrate, which retards corrosion attack. Additionally,

these microcapsules possess excellent antibacterial effect against *E. coli*, *V. coralliilyticus* and *E. aestuarii*. These results successfully validate the ideas to encapsulate two different functional agents – 8-HQ as the corrosion inhibitor and clove oil as the natural antimicrobial agent in a single robust microcapsule, eliminating the use of toxic organic solvent to dissolve solid 8-HQ compound and serving both anticorrosion and antibacterial functions simultaneously.

To further improve the functionality of the microcapsules, the shell interface was decorated with zinc oxide (ZnO) nanosheets, which render the microcapsules close to superhydrophobic properties, with a contact angle of around $144.2 \pm 4.0^\circ$. Additionally, epoxy coatings with embedded ZnO-decorated microcapsules indicates improved anticorrosion performance due to the better affinity between the rough surface morphology of ZnO nanosheets decorated microcapsules and the matrix. The microcapsules possess improved antibacterial properties due to the release of zinc ions and the direct contact between the ZnO nanosheet and the bacterial cell membrane. The results successfully validate the hypothesis that microcapsules shell interface can be designed to incorporate additional functionality as well as improving the existing functionalities originated from the encapsulated core materials.

Antifouling field test was conducted at the Sebarok Island, Singapore for duration up to 45 days. In general, microcapsules-based epoxy coatings indicate better antifouling performances as compared to the blank epoxy coating and epoxy coatings with clove oil without encapsulation. However, the antifouling effect of the microcapsules-based coatings generally deteriorates as the immersion period in the seawater increases to 45 days. Barnacles, tubeworms, algae and bryozoans were successfully identified as the fouled macro-organisms on the coatings. Overall, microcapsules-based coatings were examined and proven to be a potential environmental friendly solution for corrosion and biofouling problems in the marine industry.

Table of contents

Contents

Statement of Originality	I
Supervisor Declaration Statement	II
Authorship Attribution Statement	III
Acknowledgement	IV
Abstract	V
Table of contents	VII
List of Figures	XI
List of Tables	XVI
List of Abbreviations	XVII
Chapter 1 Introduction	1
1.1 Background of research	1
1.1.1 Background of biofouling	1
1.1.2 Background of corrosion	4
1.2 Objective of research	5
1.3 Scope	7
1.4 Report Outline	8
Chapter 2 Literature review	10
2.1 Existing antifouling coatings	10
2.1.1 Biocides-based coatings	10
2.1.2 Foul release coating	15
2.2 Natural alternatives	16
2.2.1 Enzymes	17
2.2.2 Surface chemistry	19
2.2.3 Bioinspired coating	21
2.2.4 Essential oils	23
2.3 Existing anticorrosion coatings	27
2.3.1 Surface modification	27
2.3.2 Corrosion inhibitors	30
2.3.3 Inorganic nanoparticles	32
2.4 Microencapsulation techniques	33

2.4.1 Antifouling related researches	34
2.4.2 Anticorrosion related research	39
2.4.3 Release models	46
2.5 Summary and motivation	49
Chapter 3 Materials and Experiments	52
3.1 Materials	52
3.2 Synthesis of Microcapsules	52
3.2.1 Fabrication of double layer PU/PUF shell microcapsules containing clove oil	52
3.2.2 Fabrication of PMF shell microcapsules containing 8-HQ and clove oil	54
3.2.3 Fabrication of ZnO decorated microcapsules through hydrothermal route	56
3.3 Characterization of microcapsules	58
3.3.1 Surface and shell morphology	58
3.3.2 Microcapsules content analysis	59
3.3.3 Core fraction analysis	59
3.3.4 Release profile analysis	60
3.3.5 Antibacterial test	62
3.3.6 Wettability analysis	64
3.3.7 Mechanical property analysis	64
3.3.8 X-ray photoelectron spectroscopy	65
3.4 Preparation and testing of coatings	65
3.4.1 Preparation of PDMS coating with embedded microcapsules	65
3.4.2 Preparation of epoxy coating with embedded microcapsules	66
3.4.3 Anticorrosion test	67
3.4.4 Antibacterial test	67
3.4.5 Marine fouling field test	68
Chapter 4 Synthesis, Characterization and Release Study of double layer PU/PUF shell microcapsules containing clove oil	71
4.1 Motivation	71
4.2 Shell formation mechanism	73
4.3 Characterization of double layered PU/PUF shell microcapsules	75
4.3.1 Morphology and shell structure	75
4.3.2 Microcapsules contents	76
4.3.3 Core Fraction	77
4.4 Parameter studies	78
4.4.1 Number of shell layers	78

4.4.2 Amount of PU reactants	80
4.4.3 Length of PUF deposition time	81
4.5 Tuning of Release Profiles	84
4.5.1 Fitting of the release profiles	84
4.5.2 Influence of the number of shell layers	87
4.5.3 PU shell thickness	88
4.5.4 Morphology of PUF shell	89
4.5.5 Comparison of Baker-Lonsdale constant (k_B)	91
4.6 Antibacterial activity of the microcapsules	95
4.7 Summary	103
Chapter 5 Development of Multifunctional Microcapsules Containing 8-HQ and Clove Oil	105
5.1 Motivation	105
5.2 Characterization of multifunctional microcapsules	106
5.2.1 Formation mechanism	106
5.2.2 Morphology and shell structure	107
5.2.3 Microcapsules content	109
5.2.4 Mechanical strength	110
5.3 Antibacterial test of the multifunctional microcapsules	111
5.3.1 <i>E. coli</i>	111
5.3.2 <i>V. coralliilyticus</i>	114
5.4 Antibacterial test of multifunctional coating	115
5.5 Anticorrosion test of multifunctional coating	118
5.5.1 Visual inspection	118
5.5.2 SEM analysis of the scratched region	120
5.5.3 FTIR and XPS analysis of the inhibition layer formed	122
5.5.4 Long term storage of microcapsules	125
5.5.5 Long term anticorrosion performance of the coatings	127
5.6 Summary	129
Chapter 6 Development of ZnO nanosheets decorated microcapsules	131
6.1 Motivation	131
6.2 Characterization of ZnO nanosheets decorated microcapsules	133
6.2.1 Shell morphology	133
6.2.2 EDX analysis	134
6.2.3 XRD analysis	135

6.2.3 Contact angle measurement	136
6.3 Antibacterial test of the microcapsules	138
6.3.1 Time-kill test	138
6.3.2 Improvement of antibacterial effect	141
6.4 Anticorrosion test	144
6.4.1 Tafel plot	144
6.4.2 Long term anticorrosion performance	148
6.5 Summary	149
Chapter 7 Field test of marine fouling at Sebarok Island, Singapore	151
7.1 Motivation	151
7.2 Antibacterial test against marine biofilm-forming bacteria	152
7.3 Antibacterial test of coatings against seawater bacteria	155
7.4 Characterization of the antifouling performance	158
7.4.1 Visual inspection	159
7.4.2 SEM characterization	174
7.5 Summary	182
Chapter 8 Conclusion and recommendation for future works	184
8.1 Conclusion	184
8.2 Recommendation for future works	189
8.2.1 Synergistic effect with other essential oils and corrosion inhibitors	189
8.2.2 Long term antifouling performance of microcapsules-based coatings	190
8.2.3 Incorporation of fabricated microcapsules in commercial coatings	191
References	192
List of Publications	209

List of Figures

Chapter 1

Figure 1.1 Development processes of marine fouling.	2
--	---

Chapter 2

Figure 2.1 Schematic illustration of biocide-based coatings	11
Figure 2.2 Scanning electron micrographs of natural textured surfaces.....	22
Figure 2.3 Locations and mechanisms related to antibacterial activity of EOs.....	24
Figure 2.4 Chemical structure of eugenol, which is the main component of clove oil	26
Figure 2.5 The autonomic healing concept of microcapsule-based system.....	40

Chapter 3

Figure 3.1 Fabrication process of double PU/PUF layer microcapsules containing clove oil	53
Figure 3.2 Procedure summary of double layer PU/PUF microcapsules containing clove oil.....	54
Figure 3.3 Fabrication process of PMF shell microcapsules containing clove oil and 8-HQ	55
Figure 3.4 Procedure summary of PMF shell microcapsules	56
Figure 3.5 Fabrication process of ZnO nanosheets decorated PMF shell microcapsules.....	57
Figure 3.6 Procedure summary of ZnO nanosheets decorated PMF shell microcapsules.....	58
Figure 3.7 Calibration curve for absorbance at different clove oil concentration	61
Figure 3.8 Setup of the release profile analysis test.....	61
Figure 3.9 Marine field test setup at Sebarok Island, Singapore.	69

Chapter 4

Figure 4.1 Formation of polyurethane shell through interfacial polymerization.....	74
Figure 4.2 Characterizations of double PU/PUF shell microcapsules under FESEM.....	75
Figure 4.3 FTIR spectra of clove oil, double layer PU/PUF shell and core material	77
Figure 4.4 Core content of microcapsules fabricated under different parameters	78
Figure 4.5 Comparison of single layer PU shell and double layer PU/PUF shell microcapsules.	79
Figure 4.6 Effect of the amount of PU reactants on PU shell thickness	81
Figure 4.7 Morphology of microcapsules fabricated under different parameters.	82
Figure 4.8 Fitting of release profile using different models.	86
Figure 4.9 Comparison of release profiles of single PU shell and double PU/PUF shell microcapsules and FESEM images before and after complete release of clove oil.	87
Figure 4.10 Influence of different parameters on the release profiles	90
Figure 4.11 Comparison of Baker-Lonsdale constant (k_B) values.....	94
Figure 4.12 Time-kill test against <i>V. Coralliilyticus</i> for synthesized microcapsules with different surface roughness at different time interval.....	96
Figure 4.13 Time-kill test against <i>E. aestuarii</i> for synthesized microcapsules with different surface roughness at different time interval.....	97
Figure 4.14 Time-kill test against <i>E. coli</i> for synthesized microcapsules with different surface roughness at different time interval.	98
Figure 4.15 Time-kill test against marine biofilm-forming bacteria for synthesized microcapsules with different surface roughness at different time interval.	99
Figure 4.16 Log reduction curve of antibacterial time-kill test results.....	100
Figure 4.17 Identification of chemical compounds in clove oil using GC-MS analysis.	102

Chapter 5

Figure 5.1 Overall reaction scheme of melamine-formaldehyde.....	107
Figure 5.2 Characterizations of PMF shell microcapsule under FESEM.....	108
Figure 5.3 FTIR spectra of clove oil, 8-HQ, mixture of clove oil and 8-HQ, and core material.	109
Figure 5.4 Single-capsule compression test results of PMF shell microcapsules.....	111
Figure 5.5 Time-kill test results against <i>E. coli</i> at different time intervals.....	113
Figure 5.6 Comparison of antibacterial effect of microcapsules containing different core material: clove oil, 8-HQ dissolved in toluene and 8-HQ dissolved in clove oil.....	113
Figure 5.7 Time-kill test results against <i>V. coralliilyticus</i> at different time intervals	114
Figure 5.8 Fabrication of epoxy with partially embedded microcapsules	115
Figure 5.9. Multifunctional coatings with antibacterial and anticorrosion features	116
Figure 5.10 Zone inhibition test against seawater bacteria for different coatings	117
Figure 5.11 Optical images of coatings after one day immersion in 3.5 wt% NaCl solution.....	119
Figure 5.12 Optical images of the underlying steel substrates after 10 days immersion in 3.5 wt% NaCl solution.	121
Figure 5.13 SEM images of underlying steel surface after removing the coating with acetone.	121
Figure 5.14 Comparison of FTIR spectra of insoluble complex and 8-HQ compound.....	122
Figure 5.15 XPS analysis of the insoluble complexes.....	124
Figure 5.16 Predicted chemical structures for the insoluble complexes.....	124
Figure 5.17 Effect of microcapsules storage period on the anticorrosion performance.	126
Figure 5.18 Long-term stability of the fabricated microcapsules in anticorrosion coating.	128

Chapter 6

Figure 6.1 Characterizations of ZnO nanosheets decorated microcapsules under FESEM.	133
Figure 6.2 EDX analysis to confirm the presence of ZnO nanosheets on the shell material.....	134
Figure 6.3 XRD analysis of PMF shell and ZnO nanosheets decorated microcapsules.....	135
Figure 6.4 Comparison of the surface morphologies between PMF shell microcapsules and ZnO nanosheets decorated PMF shell microcapsules.	137
Figure 6.5 Hierarchical structures of the microcapsules.....	138
Figure 6.6 Time-kill test results against <i>E. coli</i> at different time intervals.....	140
Figure 6.7 Time-kill test results against <i>E. aestuarii</i> at different time intervals.	140
Figure 6.8 Time-kill test results against <i>V. coralliilyticus</i> at different time intervals.	141
Figure 6.9 Time-kill test against <i>E. coli</i> for synthesized microcapsules with different shell materials with and without ZnO nanosheets	142
Figure 6.10 Time-kill test against <i>E. coli</i> at different time interval with and without direct contact of ZnO nanosheets decorated microcapsules.....	143
Figure 6.11 Tafel plots after immersion in 3.5 wt% NaCl solution for a period of 10 days.	146
Figure 6.12 SEM images of the underlying substrate after 10-day immersion in 3.5 wt% NaCl solution.....	146
Figure 6.13 Underlying substrate after 5 months of immersion in 3.5 wt% NaCl solution.	148

Chapter 7

Figure 7.1 Time-kill test against marine biofilm-forming bacteria for double layered PU/PUF shell microcapsules with different surface roughness.	153
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Figure 7.2 Time-kill test against marine biofilm-forming bacteria for PMF shell multifunctional microcapsules and ZnO nanosheets decorated PMF shell microcapsules.	154
Figure 7.3 Zone inhibition test results of coatings against the seawater bacteria.....	157
Figure 7.4 Optical images showing the marine field test results for different coatings	161
Figure 7.5 Comparison of marine field test results after 30 days of immersion in seawater.....	167
Figure 7.6 Time-kill test results against seawater bacteria for different PDMS coatings.	172
Figure 7.7 Microimages of the barnacles collected on the immersed coatings.	176
Figure 7.8 Microimages of barnacles with different sizes found on different coatings.	176
Figure 7.9 Micromages of the algae found on the blank epoxy coating.....	177
Figure 7.10 Microimages of the tubeworms attached onto the immersed coating	178
Figure 7.11 Microimages of the diatoms found onto the immersed coating	179
Figure 7.12 Microimages of bryozoans attached on the immersed coating	180

List of Tables

Table 3.1 Composition of marine agar 2216 and marine broth 2216 (without agar).	61
Table 4.1 Calculation of k_B values for microcapsules fabricated under different parameters	92
Table 6.1 Electrochemical corrosion measurements from Tafel plots of different coatings	147
Table 7.1 Inhibition zone produced by different types of antibacterial coating	157
Table 7.2 Fouled percentage coverage of immersed coatings at Sebarok Island, Singapore	168
Table 7.3 Presence of different fouling organisms on different immersed coatings	181

List of Abbreviations

8-HQ	8-hydroxyquinoline
BDO	1,4-butanediol
CDP	Controlled depletion polymer
CFU	Colony forming unit
CR	Corrosion rate
DCOIT	4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one
DI	Deionized water
EDX	Energy dispersive X-ray spectroscopy
<i>E. coli</i>	<i>Escherichia coli</i>
EIS	Electrochemical impedance spectroscopy
<i>E. aestuarii</i>	<i>Exiguobacterium aestuarii</i>
FESEM	Field emission scanning electron microscope
FRC	Foul release coating
FTIR	Fourier-transform infrared spectroscopy
GC-MS	Gas chromatography-mass spectrometry
GRAS	Generally recognized as safe
HCl	Hydrochloric acid
HDI	Hexamethylene diisocyanate
MPY	Mils per year
NaOH	Sodium hydroxide
NaCl	Sodium chloride
PMF	Poly(melamine-formaldehyde)

PU	Polyurethane
PUF	Poly(urea-formaldehyde)
PVA	Polyvinyl alcohol
SDBS	Sodium dodecylbenzenesulfonate
SEM	Scanning electron microscope
SPC	Self-polishing copolymer
<i>V. coralliilyticus</i>	<i>Vibrio coralliilyticus</i>
XPS	X-ray photoelectron spectroscopy
XRD	X-ray powder diffraction
ZnO	Zinc Oxide

Chapter 1 Introduction

1.1 Background of research

1.1.1 Background of biofouling

Biofouling is an unsolved problem that has caused huge economic losses, which occurs worldwide from the offshore oil and gas industry to the hulls of ships. In the context of this research work, biofouling can also be termed as marine fouling as most of the fouling problems investigated are related to the marine industry. Marine fouling can be defined as the undesirable accumulation of micro-organisms and macro-organisms such as the plants and animals on the substrate immersed in the sea water [1]. These biological settlements have caused severe problem to the marine industry in term of its operating cost. The adverse effects caused by marine fouling include higher frictional resistance and higher fuel consumption of the ships and deterioration of coating or paint that may introduce other problems such as corrosion and discoloration [2]. Among these adverse effects, higher fuel consumption of the ship was found to be most significant from the economical perspective. High level of fouling can increase drag, reducing the overall hydrodynamic pressure of the ship and increasing its fuel consumption by up to 30%. This contributes to a much higher operating cost and economic losses worldwide. Additionally, increasing fuel consumption also exerts negative impact to the environment with increasing amount of greenhouse gases emission.

Hence, comprehensive understanding on the process of biofouling formation is essential to tackle the marine fouling problems. The development process of biofouling can be divided into few steps of growth as shown in Figure 1.1. As a whole, there are two types of adhesion, which are reversible and irreversible adhesions [3]. Reversible adhesion usually happens within seconds with the formation of conditioning film on the immersed surface, consisting of organic polymers

such as proteins and polysaccharides. Subsequently, this film allows the settlement of both diatoms and bacteria, creating a biofilm matrix. The adhesion at this primary stage is reversible because only weak attractions, such as Van der Waals and electrostatic interactions are formed. After a longer period of time, sufficient nutrients from the liquid currents fully develop the biofilm formation. The biofilm provides sufficient food for the secondary colonizers, which usually consists of spores of macroalgae and protozoans. Subsequently, the tertiary colonizers or the macrofoulants such as macroalgae, sponges and barnacles also attached onto the surface.

One of the earliest approaches to tackle biofouling problem was simply to scrape or wash biological settlement off the hulls of the ships. However, this method is only effective towards a small range of species. Additionally, it is time consuming and ineffective to remove many other species that firmly attached onto the ship hulls over time. Hence, antifouling coatings have emerged to prevent biological settlement instead of cleaning or scraping them every few months [1, 3-8]. Different approaches were introduced to combat with the attack of marine fouling and that include the use of biocides [9], self-polishing coating [4] and foul release coating [3].

Figure 1.1 Development processes of marine fouling, which can be divided into two phases, reversible (within seconds) and irreversible adhesion (up to months) [3]

Among all of these, the use of tributyltin self-polishing copolymer (TBT-SPC) paints has been the most successful coating in deterring marine fouling. TBT compound was found to be very effective biocides against a broad spectrum of microorganisms in the marine environment. However, it was discovered that TBT-SPC systems causes severe negative impacts to the environment. The deleterious effect of TBT compounds released to the marine environment was first discovered in Arcachon bay (France) at the end of 1970s [10, 11]. It was shown to have caused malformations in different species including *Crassostrea gigas* and *Nucella sp.* International Maritime Organization (IMO) also reported on the accumulation of TBT compound in mammals and debilitation of the immunological defenses in fish. These facts had led to the ban of use of tributyltin (TBT)-releasing paints and coatings by the International Maritime Organization (IMO) and the Marine Environmental Protection Committee (MEPC) starting from 2008 [12, 13].

The ban of TBT-SPC paints has raised the importance of examining and omitting the environment toxicity of different antifouling coatings before using it in real industrial applications. Different types of antifouling coatings were developed, such as foul release coating [3] , zwitterionic polymers [14-16], enzyme [7, 17, 18] and bioinspired superhydrophobic surfaces [19-22] that possess low surface energy and hierarchical structure. Additionally, natural compounds such as the essential oils were found to have significant antimicrobial function [23]. Recent research focus was hence diverted to the discovery of natural antifouling compounds to replace existing toxic antifouling agents such as copper oxide, Irgarol 1051, Diuron and DCOIT [24]. The use of essential oils as a natural and environmental friendly antifouling alternative was examined in this research work.

1.1.2 Background of corrosion

Corrosion protection has been the focus of scientific study for more than 100 years as corrosion has caused huge economic losses annually. The economic losses resulted from corrosion problem adds up to US \$2.2 trillion, which is over 3% of the world's GDP [25]. Moreover, this figure only reflects the direct cost of corrosion, which does not include the waste of resources, loss of production and workplace injury resulting from corrosion. Corrosion problem has spread across many different industries, including the automotive, aerospace, and marine industries. To mitigate these corrosion problems, different corrosion protection approaches were introduced. Traditional approaches for corrosion protection include cathodic protection, use of corrosion inhibitor, material selection based on their potential difference and coating as a barrier protection [26-28]. For the past decades, self-healing coating has also achieved significant research progress in the field of corrosion protection with the use of microcapsules in different coating or paint [29-35]. The concept of self-healing coating is a novel method for corrosion protection. When the coating is damaged, micro-cracks will be formed and serve as a site of attack from external corrosion electrolyte to cause severe corrosion problem over time. However, with the introduction of microcapsules in a self-healing coating, this problem is solved as the micro-cracks are repaired autonomously to block the passage and retard the corrosion process.

Among all the traditional approaches, coating and paint are the most common method to act as a barrier between underlying material and harsh surrounding environment especially in the marine environment. Corrosion inhibitors are usually incorporated into these coatings by direct loading, in which corrosion inhibitors are directly mixed into the coating mixture and applied onto the underlying material [27]. The disadvantage of direct loading is the interaction between

corrosion inhibitors and the coating matrix, which may degrade the coating and cause it to lose its barrier property eventually. To solve this problem, reservoirs are needed to prevent this interaction as well as controlling the release of corrosion inhibitors over time to prolong the lifetime of its anticorrosion function. Hence, encapsulation of the corrosion inhibitors has become an important technique to solve the problem of undesirable interaction with the polymer matrix. In particular, the microencapsulation of corrosion inhibitors such as 2-mercaptobenzothiazole and quinolone derivatives, was shown to have efficient corrosion protection towards aluminum alloy AA2024-T3 [36] and mild steel [37, 38]. This technique provides a shelter for the inhibitors to prevent the interaction between inhibitor compound and the paint chemistry. However, the use of toxic organic solvents such as toluene and xylene to dissolve these compounds for encapsulation may cause negative environmental impact [39, 40]. Therefore, an improved encapsulation process with the elimination of toxic solvents is essential to promote a more environmental friendly route for the effective use of corrosion inhibitors.

1.2 Objective of research

The objective of this research is to create a microcapsules-based multifunctional coating with both antifouling and anticorrosion features. In this study, clove oil was selected as the natural antifouling agent while 8-hydroxyquinoline (8-HQ) was used as the corrosion inhibitor.

Clove oil is natural which could potentially eliminate the use of toxic biocides nowadays. However, clove oil as a volatile [41, 42] chemical and its chemical instability [43] causes the needs of microencapsulation. Polymer microencapsulation techniques – interfacial and in situ polymerization [29, 31, 44-46], were shown to produce robust microcapsules without agglomeration problems. Hence, these techniques are implemented in this study to fabricate microcapsules with different shell structure and properties.

8-HQ was shown to be an effective corrosion inhibitor for different substrates, which include mild steel [47], cold rolled steel [48] magnesium alloy [49] and aluminum alloy [50]. However, previous research emphasized on the use of 8-HQ in the corrosive solution, which is not practical for the use of many applications. In order to incorporate the 8-compound into the coating, several recent studies proposed to encapsulate 8-HQ to allow corrosion inhibition properties at the site of corrosion attack [47, 51-53]. However, 8-HQ as a solid compound was encapsulated using organic solvent, such as xylene and toluene, which are toxic [39, 40]. Based on this research gap, this study proposes to encapsulate 8-HQ by dissolving in clove oil, which is more natural at the same time achieving a multifunctional functionality of anticorrosion and antibacterial properties.

Microencapsulation is essential to protect both clove oil and 8-HQ from undesirable interaction with the polymer matrix and premature leakage. Additionally, the release of the core materials can be controlled through tuning different reaction parameters during the fabrication process of the microcapsules. Hence, this study will also contribute to a better understanding of the controlled release behavior in a microcapsule system, which can be tuned through manipulating several reaction parameters.

My research can be divided into four different parts that eventually leads to my final goal, which is to fabricate a multifunctional coating containing microcapsules with both anticorrosion and antibacterial features. The first step of this research is to fabricate microcapsules containing clove oil as a multifunctional agent, particularly for its antimicrobial effects. Additionally, the release profile of the fabricated microcapsules was investigated based on the manipulation of different reaction parameters. Following this study, the second step involves the fabrication of multifunctional microcapsules containing both clove oil and 8-HQ. Antibacterial and anticorrosion tests were carried out systematically to determine both antibacterial and

anticorrosion properties of these microcapsules. The third step of this research is to fabricate microcapsules with the deposition of zinc oxide nanosheets on the shell material, rendering the microcapsules close to superhydrophobic properties as well as improved antibacterial and anticorrosion properties based on the rough and hierarchical structure resulted from the deposition of zinc oxide nanosheets. Lastly, different types of coatings based on different types of microcapsules were fabricated and tested against the marine environment to determine the antifouling behavior of the coatings.

1.3 Scope

The fabrication of double layer PU/PUF shell microcapsules containing clove oil was mainly accomplished by optimizing the parameters of different microencapsulation process. To confirm successful encapsulation, the microcapsules were characterized via Scanning Electron Microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR) and Ultraviolet-visible (UV-Vis) Spectroscopy. The process parameters including the amount of shell forming materials and the reaction duration were investigated systematically and evaluated in term of the core content and release behavior of the core material through different polymer shells. The release behavior of the core materials from the microcapsules was characterized using UV-Vis Spectroscopy. First of all, clove oil of different concentration was examined to get a calibration curve according to Beer-Lambert Law. The release of clove oil was then characterized by measuring the absorbance of the peak at wavelength of 280 nm. The antibacterial properties of the fabricated microcapsules were examined through ASTM E2315 time-kill test against different types of marine bacteria.

Additionally, the fabrication of PMF shell microcapsules containing clove oil and 8-HQ was successfully achieved through in situ polymerization. The successful encapsulation of both clove

oil and corrosion inhibitor was characterized via Scanning Electron Microscope (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). The mechanical strength of the microcapsules was examined through the single capsules compression test and compared against different types of microcapsules examined through the similar method in previous research. Subsequently, the effect of 8-HQ as the corrosion inhibitor was evaluated based on the anticorrosion performance of the scratched epoxy coating with embedded microcapsules through accelerated anticorrosion test in 3.5 wt% NaCl solution.

ZnO nanosheets were fabricated onto the microcapsules shell to study on the additional functionalities incorporated on the shell interface. The wetting behaviors of the microcapsules were studied and compared against the microcapsules without the deposition of ZnO nanosheets. The improvement of antibacterial effect through the modification of the shell materials can be examined through the comparison of these modified microcapsules with the microcapsules without any modification to the shell materials. Additionally, the anticorrosion properties are compared similarly to examine the effect of ZnO nanosheets on the final anticorrosion performance of the microcapsules-based coatings.

In this research work, the antibacterial tests were conducted according to the ASTM E2315 time-kill test. The antibacterial effect of different types of microcapsules was generally evaluated based on the quantification colony forming unit (CFU). To examine the on-site antifouling performance of the microcapsules-based coatings, antifouling field tests were performed at an island under collaboration with a Singapore local company.

1.4 Report Outline

Chapter 1 introduces the background of corrosion and biofouling problem in marine industry and highlights the objective of this project in solving these problems.

Chapter 2 reviews the existing antifouling and anticorrosion coatings, current natural alternatives for antifouling applications and different types of microencapsulation techniques related to antifouling and anticorrosion applications. Based on the review, a summary was presented to emphasize the motivation of this project.

Chapter 3 describes the materials used, the preparation of different types of microcapsules, characterization methods for the fabricated microcapsules and different testing procedures of the microcapsules-based coatings.

Chapter 4 describes the synthesis, characterization and release study of the double layer PU/PUF shell microcapsules containing clove oil. The release profile of the microcapsules with different reaction parameters was investigated and the antibacterial performance of the microcapsules was evaluated.

Chapter 5 presents the synthesis and characterization of multifunctional microcapsules containing 8-HQ and clove oil. The results of anticorrosion and antibacterial test of the microcapsules and coatings were presented based on different materials characterization techniques.

Chapter 6 describes the synthesis and characterization of ZnO nanosheets decorated microcapsules. Similarly, the antibacterial and anticorrosion tests based on these microcapsules were presented.

Chapter 7 concluded this research work by conducting marine fouling field test on the coatings based on different types of microcapsules fabricated in this study. The field test was conducted in Sebarok Island, Singapore under the collaboration with a local company.

Chapter 8 concludes the main findings of this research work and describes possible future work for this study.

Chapter 2 Literature review

2.1 Existing antifouling coatings

2.1.1 Biocides-based coatings

Biofouling has become one of the most important issues in the maritime industry. Many different types of antifouling coatings have been developed to alleviate the damage and the traditional methods include the use of biocides to disrupt the cell activities or cause cell death directly. Antifouling biocides can be defined as the active ingredients in antifouling coatings or paints that prevent the settlement and adhesion of either micro- or macro-organisms to the substrate surface. Tributyltin (TBT) based compounds were found to be a broad spectrum of biocides that possess excellent antifouling effect towards a wide variety of fouling organisms. However, several studies revealed the adverse effect of TBT-based compounds towards the aquatic life due to its high toxicity [10, 11]. The use of tributyltin (TBT)-releasing paints and coatings were banned by the International Maritime Organization (IMO) and the Marine Environmental Protection Committee (MEPC) starting from 2008 [12, 13].

Biocide release rate is the key element to the environmental effects and it can be determined using standard protocols provided by American Society for Testing and Materials (ASTM) and International Standards Organization (ISO). One of the methods to control the release rate of antifouling biocides is through adsorption or microencapsulation of these biocides to prevent premature leaching and to control the release rate of biocides over time [54].

Figure 2.1 Schematic illustration of biocide-based coatings: (a) Insoluble matrix or contact leaching coatings; (b) Soluble matrix or Controlled Depletion Polymer coatings [3].

Due to the ban of organotin compounds, tin-free biocide-based coatings can be subdivided into three main categories: contact leaching coating, soluble/controlled depletion polymer (CDP) coatings and self-polishing copolymer (SPC) coatings. The differences between these coatings are illustrated in Figure 2.1 [3].

Contact leaching coating can also be known as an insoluble matrix as it uses high molecular weight binders that are insoluble in seawater, which include acrylics, vinyls, epoxy and chlorinated rubber polymers [1]. Due to their good mechanical strength characteristics, high amount of biocides can be incorporated into it. The binder is insoluble in seawater and seawater will spread through the pores that are left empty and dissolve the next biocide particles deeper inside the coating. However, the efficiency of this coating will deplete as the release rate of biocides gradually decreases with time. The efficiency of this type of coatings usually last between 12 and 24 months [55].

Soluble matrix coating utilizes soluble matrix paints containing biocides. In seawater, both biocides and soluble binder will be dissolved and released. These coatings only last for about 12 to 15 months as the erosion and release rates are very rapid at the early stage of exposure to

seawater [55]. To prolong the efficiency of soluble matrix coating, controlled depletion polymer (CDP) coating was introduced, in which the soluble binder is reinforced with synthetic organic resins which aid to control the hydration and dissolution of the binders. CDP coating prolong the efficiency of soluble matrix coating to up to 36 months.

Self-polishing copolymer (SPC) coatings are based on acrylic or methacrylic copolymers which are easily hydrolysable in seawater [3]. These copolymers are blended with some biocides and control the leaching rate of biocides through controlling the binder erosion rate [56]. SPC coating undergoes few mechanisms to release the fouling organisms. First of all, water diffuses into the coating and dissolves biocidal particles. However, hydrophobic copolymer matrix does not allow the penetration of seawater into the empty pores, as previously mentioned in soluble matrix coating. As the methacrylic ester group is hydrolyzed under alkaline conditions, seawater dissolves more biocidal particles, causing more leached layer and causing the copolymer matrix to be easily erodible by seawater for subsequent release of biocidal particles (self-polishing effect) [3]. Even though it is similar to the soluble matrix coating, the advantage of SPC is that the release rate of biocides can be controlled by adjusting the degree of polymerization of the copolymer matrix.

Therefore, non-toxic antifouling coatings have attracted significant interest, particularly the use of low surface energy fouling release coatings (FRCs). On top of using low surface energy materials, the other approaches include controlling the wettability, surface topography, roughness and elasticity to develop FRCs [57].

The use of TBT based antifouling coatings was initially replaced by other biocides including Irgarol, Diuron, Sea-Nine 211, copper pyrithiones, zinc pyrithiones and 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT). A wide range of chemical is used as antifouling biocides and

possesses different properties and environment effects from one another. Specifically, a good antifouling biocide is one that is effective in preventing biological settlement without persisting at a concentration higher than that can cause negative impact to the environment [58]. This can be achieved either by controlling the release rate of biocides to the environment or through rapid transformation following release from the surface. Over the past century, the important biocides that were widely used include copper oxide, Irgarol 1051, Diuron, DCOIT and etc. For these biocides, the key processes that are important to understand the environmental impacts include degradation of these biocides, the specific mechanisms of degradation, partition into sediments and uptake into organisms [58].

In term of environmental impact, extensive research on the presence and accumulation of different antifouling compounds were accomplished. For example, copper oxide leaches into the water in the form of free copper ion (Cu^+) and immediately oxidized to Cu^{2+} to form complexes with inorganic and organic ligands. However, the proportion of free ions, inorganic or organic complexes of copper varies greatly with water properties, such as pH and salinity of sea water [59]. Moreover, copper will also adsorb onto suspended particulate matter (SPM) and eventually accumulate in the sediments. Hence, copper concentrations are usually higher in the sediments as compared to that in the water column [60]. Therefore, in areas of high sediment such as marinas and harbors, resuspension of sediment can release bioavailable copper into the water, thereby increasing the risk to aquatic life and causing negative impacts to the aquatic environment.

Irgarol 1051 does not degrade easily in water and has a half-life of 100 to 350 days [61]. Moreover, it also has high persistence in anaerobic sediments by either adsorbing to sediment particles or associated with paint particles [62]. Similar to copper oxide, resuspension of sediments contaminated with Irgarol 1051 release the bioavailable Irgarol 1051 into the water

column. On a contrary, diuron persists in water but much less persistent in sediment with a half-life of 14 days [61]. It is present at high concentration in sea water but only weakly adsorbed to sediments and detected at low concentrations in sediments [61, 63]. DCOIT was examined to be a labile compound as compared to copper oxide, Irgarol 1501 and Diuron and degrades more rapidly in sea water and sediment [61, 62]. DCOIT undergoes biotic degradation under both aerobic and anaerobic conditions through the cleavage of the isothiazolone ring and subsequent oxidation of the alkyl metabolites. Moreover, DCOIT has a short half-life of less than 24 hours in sea water and half-life of less than 12 hours in sediments [64].

However, previous studies have shown the toxicity of these compounds [61, 65-68] against different types of marine organisms, resulting in a need to completely eliminate the use of toxic biocides. Irgarol as one of the most commonly used biocides after the ban of TBT compound, was found to be highly toxic against the freshwater macrophytes due to its bioaccumulative potential in the macrophytes [65]. Diuron was discovered to be slightly toxic against mammals and moderately toxic against the aquatic invertebrates. 3,4-dichloroaniline, which is the principal product of biodegradation of diuron, exhibits higher toxicity as compared to diuron [67]. Additionally, Sea-Nine 211 was determined to be highly toxic against the embryos and larvae of three different marine invertebrates- the bivalve *Mytilus edulis*, the sea-urchin *Paracentrotus lividus* and the ascidian *Ciona intestinalis* [24]. DCOIT is the biocidal ingredient of the commercial antifouling agent Sea-Nine 211 and its toxicity against different types of marine organisms was studied. For instance, 28-day exposure to 1 µg/L of DCOIT induces apoptosis in the testicular germ cells of the mummichog *Fundulus heteroclitus*, indicating the impairment of the reproductive performance [69]. Furthermore, the metal phrithiones- copper and zinc pyrithiones were shown to be the most toxic antifouling compound against the suspension-

cultured fish cells derived from the chinook salmon *Oncorhynchus tshawytscha* embryos, as compared to the other antifouling compounds tested, including Sea-Nine 211, Diuron and Irgarol [66].

2.1.2 Foul release coating

Foul release coating (FRCs) is an antifouling coating with low surface energy to weaken the ability of organisms to form a strong interfacial bond with the surface. The two major types of FRCs are fluoropolymer and silicone based polymer coating. There are two important criteria for FRCs: low surface energy and low elastic modulus, which allows the interface between organism adhesives and the coating surface to fail. The smoothness of the surface at molecular level also allows the organisms to be detached due to low surface energy when the moving ships reach a critical velocity [70].

Additionally, previous research has shown the influence of thickness on the elastic modulus of the coating. Typically, the application thickness of silicone coating is 150 μm while that of fluoropolymer coating is 75 μm . A thicker silicone coating is usually more successful as it requires less energy to break the bond between the foulants and the coating. For thicker silicone coating, detachment of organisms occurs through a peeling fracture mechanism while it occurs through shearing associated mechanism for thinner fluoropolymer coatings [71]. Several studies were conducted to discuss the influence of different mechanical factors such as surface energy, elastic modulus, composition variables of coating and coating thickness on the effectiveness of silicone and fluoropolymer based foul release coatings [72-74].

Foul release coating has an advantage over biocide-based antifouling coatings due to its unique approach of pure physical deterrent effects. It possesses antimicrobial activity against a broad spectrum of micro- and macro-organisms without incurring the issues of biodegradation,

legislative standards and fees necessary to register an active antifouling compound [5]. However, the disadvantage of FRCs is the requirement of moving boat with a critical speed velocity to dislodge the organisms. When the vessels are not used regularly, the biological communities can establish and form heavy fouling onto the surface.

Recently, another type of low surface energy synthetic surface named slippery liquid-infused porous surface (SLIPS) was created based on the inspiration from *Nepenthes* pitcher plants [20]. This technique is based on the use of nano/microstructured substrates to lock in place the infused lubricating fluid to form an inert “slippery” interface. Different surfaces with the concept of SLIPS were fabricated such as the preparation of microporous butyl methacrylate-ethylene dimethacrylate (BMA-EDMA) surfaces via UV-initiated free-radical polymerization [75]. Following by the infusion of fluorocarbon lubricants into the porous microtexture, a strong liquid-repellent slippery surface with good fouling-resistant properties was generated.

2.2 Natural alternatives

Due to the toxicity of organotin compounds such as tributyltin (TBT) and triphenyltin, many countries had progressed to ban the application of antifouling coatings based on these organotin compounds since early 2008. This had increased the demand for environmental friendly and non-toxic antifouling coating and compounds. Besides organotin compounds, several other biocides including copper-based, zinc-based and silver atoms have negative impact on the environment and need to be replaced by new environmental friendly antifouling coatings or technologies [61, 63, 65, 67, 68].

2.2.1 Enzymes

Enzymes are considered as environmental friendly as they are rapidly biodegraded and hence eliminated the problem of bioaccumulation. As an environmentally friendly solution, several strategies of the use of enzymes to prevent biofouling were proposed, which include biocidal effect of the enzymes, the attack on the adhesives used for settlement and the disruption of the biofilm matrix, generation of biocides and interference with intercellular communications [76].

First, some of the enzymes were found to exhibit biocidal effect similar to the conventional biocides. In particular, chitinase was proven to be effective against the barnacles, through the decomposition of chitin, an essential element for the barnacle exoskeleton [77]. However, the chitinase was not effective against several other biofouling organisms. Additionally, hydrogen peroxides were produced via the enzyme-mediated conversion of starch. The antifouling coatings releasing hydrogen peroxides through this process were found to exhibit excellent antifouling effect based on the field experiment results [78, 79]. Hydrogen peroxide induces oxidative damage in living cells, which eventually causes cell death. Moreover, hydrogen peroxide disproportionate rapidly into oxygen and water at the seawater pH range [80]. Hence, the bioaccumulation is not an issue, promoting the use of hydrogen peroxides as an environmentally friendly solution to replace the use of conventional toxic biocides.

Second, the enzymes are capable of disrupting the adhesives required for the settlement of microorganisms. According to previous research, during the settling stage of organisms such as cell and spore, an adhesive is required to be released to wet the surface entirely [81]. The researchers concluded that the degradation of adhesives by the use of enzymes can be achieved through two ways- repelling of organisms both during and after the settlement stage. As most of the adhesives degrading enzymes are hydrolases and need water for activity, it was concluded

that the primary mechanism lies onto either preventing settlement or weakening the attachment strength of the organisms to allow them to be more easily removed through hydrodynamic forces.

The use of proteases to degrade the adhesives used for settlement was demonstrated [18, 82, 83]. It is evident from previous studies that the strength of adhesion could be decreased through the actions of different types of proteases. For example, the adhesion strength and settlement of *Ulva* zoospores was inhibited by the serine-protease while the settlement of *Balanus amphitrite* cyprid larvae and diatom *Navicula perminuta*, was also decreased through the application of a wide range of proteases [18]. Additionally, the use of proteases from deep-sea bacteria was proven to be effective in inhibiting the larval attachment of the bryozoan *Bugula neritina* L. The application of the proteases in the field experiments have also shown promising results as excellent antifouling effect of proteases and trypsin incorporated into a water-soluble paint matrix were observed [82].

Third, some enzymes are capable of disrupting the biofilm formation, which is significant and essential for microfouling. Extracellular polymeric substance (EPS) is the essential element in biofilm matrix. Due to the complexity of this polymer network structure, the combination of different enzymes such as the lyases and hydrolases, is required to successfully disintegrate the polymeric networks constituting the biofilm matrix [84]. Additionally, protein is another important element in a biofilm matrix. Hence, the proteases used to prevent adhesion was found to be effective in disrupting the biofilm matrix, which was evident for a strain of *Pseudoalteromonas* [17]. However, due to the complicated network in a biofilm matrix, degrading a key component of the biofilm matrix does not necessarily disrupt the entire biofilm matrix. The explanation for this phenomenon was that other components in the biofilm matrix may assist to promote the proliferation of different organisms [85]. Based on these results, it can

be concluded that the use of enzymes alone may not serve as a comprehensive environmentally friendly solution to tackle the unsolved biofouling problem.

2.2.2 Surface chemistry

The principles of superhydrophobicity were employed in the field of antifouling coatings. According to previous research, superhydrophobic coatings minimize the wetting area of the surface through the presence of an air film constructed through the hierarchical micro- and nanostructures, and the treatment of low surface energy materials [86]. Additionally, these coatings show no detrimental side effects, eliminating the use of common toxic antifouling agents such as Diuron, DCOIT and Irgarol [61, 63, 65, 67, 68]. To transfer the principle of superhydrophobicity into the use of biofouling prevention, two different steps were proposed [87]. First, a superhydrophilic surface can be fabricated to maximize the contact with water and thus, minimizing the contact with biological matter. Second, underwater superhydrophobicity can be designed to support an air film between the surface and the water. The presence of this superhydrophobic rough surface minimizes the probability that biological organisms attach onto the surface due to the reduction of wetted areas. However, the long term effect of the superhydrophobic coatings remains an issue for the on-site applications due to the harsh and complicated marine environment. It was shown that the superhydrophobic surface delays fouling occurrence better as compared to the hydrophobic surfaces. The effect was observed only during the beginning short period exposure to the fouling environment and both films lost their antifouling properties after a long period of exposure to the seawater [88].

Zwitterion compounds are defined as chemical compounds containing both positive and negative charges with a total net charge of zero, such as the poly (sulphobetaine) and poly (carboxybetaine). Due to this characteristic, an electrostatically induced hydration layer is

formed on top of the zwitterionic materials, rendering them high resistant properties against nonspecific protein adsorption, bacterial adhesion and biofilm formation [14]. In general, the strong electrostatically induced hydration layer creates a hydrophilic surface which prevents the formation of strong interfacial bond between the secreted proteoglycan bioadhesives secreted by the marine organisms and the fouling substrate [89]. The use of zwitterionic polymer as an environmentally benign fouling resistant marine coating was demonstrated through its settlement-inhibiting effect against a marine species. Coating based on poly (sulfobetaine methacrylate) (polySBMA) were grafted onto glass surfaces through surface-initiated atom transfer radical polymerization (ATRP). This zwitterionic polymer-based coating inhibits the settlement of spores of the green marine algae, *Ulva* as well as reducing the adherent strength of both spores and diatoms [90]. Additionally, polySBMA was also grafted on hydrophobic polymer membranes to hydrophilize the membrane for better resistance to protein fouling [15].

Carbohydrates as an interesting family of biomolecules were successfully immobilized onto solid surfaces for the development of new biomimetic materials that can reduce unspecific adsorption of protein on the carbon surfaces [91]. These coatings are environmentally benign and highly stable as compared to other chemical species. Additionally, these carbohydrate coatings were fabricated on poly (ether sulfone) (PES), which demonstrates lower fouling in a complex aqueous environments that are rich in biomass such as the wastewater effluent, as compared to the bare PES membrane surfaces [92]. Subsequently, the performance of carbohydrates immobilized fouling resistant coatings in the marine environment were studied on three different substrates: stainless steel, nylon and PES. According to the field test results in a coastal environment for 20 days, the carbohydrate coatings show good promise as an environmentally friendly solution for reducing adhesion and retention of marine foulants [22].

Even though different types of surface chemistry were introduced on the substrate to prevent fouling, more marine fouling field test trials should be conducted to evaluate the antifouling performance of these coatings under the complex marine environment. This is an essential step to evaluate the stability of these coatings in achieving long term antifouling performance.

2.2.3 Bioinspired coating

Bioinspired coatings can be defined as the coatings which mimic the structure from the natural elements. In the marine environment, several marine organisms such as the shark and mussels possess natural antifouling defense. As a result, research was conducted to investigate the natural surface microtopographies of these marine organisms and to determine the factors that influence the antifouling behaviors [93, 94]. Figure 2.2 shows different natural textured surface of the marine environment, which include spinner shark skin, Galapagos shark skin, mussel shell and crab shell [95]. On the basis of Figure 2.2, it is evident that the microtopography of the marine organisms certainly plays an important role in their respective antifouling performance. Additionally, the superhydrophobicity of the plant leaves such as the lotus leaf, was investigated [96]. Previous research has identified two main types of surface structures in the natural plant leaves with superhydrophobicity such as the lotus, rice, ramee and taro leaves [97]. According to the SEM studies, the first type of surface structure is the micro- and nanostructure (binary structure) and the second type is micro-line structure (unitary structure).

Inspired by the superhydrophobicity properties from the natural, previous research focused on fabricating these structures on different substrates through the use of different methods. Among all the natural surfaces, the surface structures of the lotus leaves were studied most extensively

Figure 2.2 Scanning electron micrographs of natural textured surfaces of: (a) Spinner shark skin; (b) Galapagos shark skin; (c) Mussel shells (*M. edulis*) and (d) crab shell (*C. pagurus*) [95].

and several methods were proposed on different substrates. A superhydrophobic polystyrene (PS) film was prepared using the versatile electrohydrodynamics method through forming a novel composite structure consisting of porous microspheres and nanofibers [98]. Additionally, artificial lotus leaf structure was prepared on poly(dimethylsiloxane) (PDMS) using nanocasting process by directly replicating the lotus leaf structure for the first time, eliminating the use of conventional route of chemical synthesis [99]. A soft-lithographic imprinting approach to fabricate superhydrophobic lotus-leaf-like surface was introduced by replica molding [100]. Lotus-like papillary structures were successfully replicated on the imprinted surface according to SEM characterization.

Other than investigating the surface topography of the natural antifouling surfaces and simply fabricating the structures on different substrates, field test trials were conducted to examine

further the antifouling performance of different marine organisms. For example, *Mytilus galloprovincialis* mussel shells were proven to significantly reduce the settlement of barnacle larvae in a fourteen-week field exposure trials [101]. A novel marine coating with hierarchically wrinkled surface topographies (HWST) of different wrinkles length scale was fabricated and field tested in seawater [6]. According to the field test results, coatings with flat topographies were fouled after a short time of immersion period (4-15 weeks). However, the novel HWST coatings were free of biofouling after 18 months of immersion in the seawater, without any attachment of barnacles. These results suggest the importance of surface topographies against barnacle fouling and the potential use of this novel HWST coating as an environmentally friendly defense against marine fouling. However, there are still a limited number of field test studies for the bioinspired coatings fabricated based on the natural surface structure. Hence, future research should focus more onto the testing of the bioinspired coatings in the seawater, to illustrate the antifouling performance of these coatings for the marine industry.

2.2.4 Essential oils

Essential oils (EOs) have shown antibacterial activity against *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli*, *Shigella dysenteria*, *Bacillus cereus* and *Staphylococcus aureus* at levels between 0.2 and 10 $\mu\text{l ml}^{-1}$. The ban of organotin compounds (tributyltin and triphenyltin) has directed more focus of research on the antimicrobial activity of various plant oil extracts and their components. A number of EO components including carvacrol, thymol, eugenol and etc., were identified to contribute to the antibacterial activity of different essential oils [102]. The essential oils (EOs) that possess strong antibacterial activity usually contain a high percentage of phenolic compounds such as eugenol and thymol. Additionally, essential oils

derived from aromatic medical plants was found to exhibit significant antimicrobial effects against bacteria, yeast, fungi and viruses [23].

The antibacterial mechanism of the EOs is based on the hydrophobicity of EOs that enables them to partition in the lipids of cell membrane through hydrophobic interactions between EOs and the membrane. The locations or mechanisms that are related to the antibacterial activity of EOs are described in Figure 2.3.

According to Figure 2.3, there are several different explanations about the antibacterial activity of EOs, which includes degradation of cell wall [103], damage to cytoplasmic membrane [104, 105], damage to membrane protein [104, 106], leakage of cell contents [106, 107], coagulation of cytoplasm [108] and depletion of the proton motive force [104, 109]. When the structure and membrane of bacteria cell are disrupted, leakage of ions and cell contents will then occur. At the early stage, a certain amount of leakage can be tolerated but extensive loss of cell contents and ions will lead to cell death eventually.

Figure 2.3 Locations and mechanisms related to antibacterial activity of EOs [102].

The high percentage of phenolic compounds such as eugenol and carvacrol was found to exhibit significant antimicrobial activity. Moreover, the importance of the presence of the hydroxyl groups in these compounds was proven as well [110]. However, the specific position of the hydroxyl group on the phenolic ring does not affect the antimicrobial activity strongly.

The major drawbacks of natural product based antifouling coating include the more tedious process of producing large quantities of natural product and thereby preventing large scale industrial applications. Moreover, many researches about natural antifouling compound focused on either microfoulers (bacteria, fungi or microalgae) or macrofoulers (barnacles, larval settlement) and these natural compounds may possess a narrow spectrum of antifouling activities. However, these drawbacks do not halt the progress of this research work as the focus now would be to fabricate an environmental friendly antifouling coating due to the raise of concern about human health since the ban of organotin compounds, particularly the ban of tributyltin (TBT) based coatings.

Clove oil is an essential oil extracted from *Syzygium aromaticum*. It is one of the essential oils that were found to exhibit significant antimicrobial effects against a wide range of bacteria strains [111]. The chemical composition of clove oil was characterized using gas chromatography-mass spectrometry (GC-MS) analysis and was found to have 36 different components. Among the components, eugenol (88.58%) was the main component in clove oil, followed by eugenyl acetate (5.62%) and many others [112]. Clove oil is widely used in clinical dentistry in root canal therapy and temporary fillings, owing to its antimicrobial activity against oral bacteria. Additionally, the price of clove oil is also at the lower end among the essential oils. According to Sigma Aldrich, the price of some commonly used essential oils such as lavender

(S\$ 323/kg), spearmint (S\$ 224/kg), peppermint (S\$ 267/kg), tea tree (S\$ 234/kg) and cinnamon oil (S\$ 1170/kg) is higher as compared to that of the clove oil at only S\$ 163/kg.

The antimicrobial activity of clove oil was contributed by its main component, eugenol. The molecular formula of eugenol is $C_{10}H_{12}O_2$ and its chemical structure is shown in Figure 2.4.

Figure 2.4 Chemical structure of eugenol, which is the main component of clove oil

A study on the antibacterial activity of eugenol and its mechanism of bactericidal action against *Salmonella typhi* was conducted [105]. Based on the study, the antibacterial activity of eugenol was the result of damaged cytoplasmic membrane and subsequent leakage of intracellular constituents. Moreover, the mechanism of antimicrobial activity of clove oil was accessed through observing the changes in membrane composition by assaying for the leakage of protein and lipid using Bradford [113] and van Handel's [114] method respectively. It was revealed that both cell wall and membrane of the bacteria was significantly damaged [115].

Other than its antimicrobial function, clove oil was found to possess mosquito repellent activity. In particular, clove oil was found to repel malaria, filarial and yellow fever vectors for a period of 60-180 minutes [116, 117]. The safe concentration of clove oil as a natural repellent was found to be 0.5% as it can cause skin irritation at high concentration [118]. Moreover, in

another study on the comparative repellency of 38 essential oils against mosquito bites, clove oil possessed the longest duration of 100% repellency (between 2-4 hours) against all three species of mosquitos tested [119]. Overall, clove oil with antimicrobial activities against a wide range of bacteria, anti-mosquito properties and cheaper price as compared to many other essential oils, was selected in this study.

However, clove oil was found to be volatile and chemically unstable in the presence of light, air, moisture and high temperature. Due to these problems, most essential oils including clove oil are not suitable for long-term use. Hence, encapsulation of clove oil to prevent its interaction with the surrounding environment is essential to prolong its lifetime and maintain its chemical stability.

2.3 Existing anticorrosion coatings

2.3.1 Surface modification

Superhydrophobic coatings are one of the most studied anticorrosion coatings. The idea of superhydrophobic surface originated from the nature, such as the lotus [120], red rose petal [121], cicada wings [122] and butterfly wings [123]. Superhydrophobicity is usually defined by the surfaces with water contact angle of $\theta > 150^\circ$, which repels the liquid due to low surface energy and rough surface morphology [86]. Low surface free energy can be achieved through chemical compositions. However, low surface energy alone was proven to be ineffective in fabricating superhydrophobic surfaces [124]. Hence, the surface morphology was investigated and proven to be essential in achieving superhydrophobic surfaces. In particular, rough surface morphology can trap air between the hierarchical micro- and nanostructure of the surfaces, which will eventually improve the water contact angle values [120].

According to previous research, many techniques and materials have been used to fabricate superhydrophobic coatings. As mentioned previously, a superhydrophobic coating is a coating with low surface energy and rough surface morphology. As a result, the preparation of superhydrophobic coatings can be divided into two categories, which are fabricating a rough surface on a low surface energy material and treating a rough surface with a material of low surface energy [125]. Additionally, several techniques and processes have been employed to fabricate superhydrophobic coatings, which include sol-gel process [126, 127], layer-by-layer (LbL) assembly [128, 129] and etching [130, 131].

Through sol-gel process, a superhydrophobic and super-oleophilic silica film was fabricated on stainless steel mesh with the use of tetraethoxysilane (TEOS) and methyltriethoxysilane (MTES) as precursors [126]. Through the combination of the hierarchical structure of silica film and the low surface energy functional group, a superhydrophobic surface was successfully constructed through the sol-gel process. Similarly, layer-by-layer (LbL) assembly was utilized to fabricate superhydrophobic coatings through the combination of these two factors. Branchlike structure of Ag aggregates was fabricated on the matrix of a LbL polyelectrolyte multilayer to form a rough surface structure. With further chemisorption of a self-assembled monolayer of n-dodecanethiol, a superhydrophobic coating with a contact angle of 154° and a tilt angle lower than 3° was achieved [128]. Additionally, etching is a simple and inexpensive process to generate superhydrophobic coatings with rough structure. A superhydrophobic surface was fabricated on AA2024 aluminum alloys through chemical etching using hydrochloric acid [130]. According to the study, it was observed that the etching time can be controlled to achieve different degree of roughness.

Corrosion is often defined as the destruction of metal due to chemical reactions, such as the attack of chloride ions from the seawater. Hence, superhydrophobic coatings prevent the water from being absorbed onto the coating, which eventually reduces the possibility of attack by the corrosive ions. Previous research has shown the use of superhydrophobic coatings on different substrates, which include steel, aluminum, copper and magnesium alloy. For example, the use of electrodeposited superhydrophobic conducting polythiophene coating was effective in protecting the underlying steel substrate [132]. Moreover, a rough and highly porous organosilica aerogel-like film was prepared on the aluminum surface. The superhydrophobicity nature of the film prevents the infiltration of water into the film and hence limits the exposure of corrosive elements to the metal surface [133]. Superhydrophobic coatings were also fabricated onto metal substrate. For example, a novel super-hydrophobic film was fabricated onto the copper wafer by chemically absorbing myristic acid (n-tetradecanoic). The corrosion resistance behavior of the copper was improved remarkably with the superhydrophobic film, owing to the formation of a composite interface with flower-like surface nanostructures [134]. Additionally, a superhydrophobic film was successfully deposited on the magnesium alloy AZ31 through chemical vapor deposition (CVD) process. According to the electrochemical impedance spectroscopy (EIS) test results, the deposition of the super-hydrophobic film was proven to improve the anticorrosion resistance performance of magnesium alloy AZ31 [135].

However, the durability of the superhydrophobic coatings is an important issue to be addressed before the implementation stage for practical applications. The stability of the superhydrophobic coatings under different conditions was studied extensively. In particular, to target the use of these coatings in a marine environment, several studies were conducted to examine the stability of the superhydrophobic surfaces on different substrates such as copper, aluminum and carbon

steel in the seawater environment [134, 136-139]. These studies have shown to fabricate superhydrophobic coatings that can conserve their superhydrophobicity even after a long duration of immersion period in the seawater.

2.3.2 Corrosion inhibitors

Corrosion is a process between metals and corrosive medium, which involves the movement of metal ions of metal into solution at the active areas (anode), passage of electrons from metal to an acceptors (such as oxygen and hydrogen ions) at less active area (cathode), an ionic current in the solution and an electronic current in the metal [27]. The use of corrosion inhibitors is very popular to prevent the reaction between metals and the media by adsorption of ions and molecules onto metal surface.

Conventional corrosion inhibitors such as chromate and arsenic based compounds were found to be toxic and highly carcinogenic. As a result, alternative corrosion inhibitors are required to replace these compounds. Over the past two decades, extensive research and development have led to the discovery of new corrosion inhibitors that are based on different organic compounds, especially those with π bonds, heteroatoms (P, N, S and O) showed significant inhibition efficiency [140]. These organic corrosion inhibitors can form coordination bonds with the metal surface because of the availability of lone electron pairs and π bonds, leading to the formation of inhibition layer [141].

The organic compound corrosion inhibitors for steels in an acidic environment include acetylenic alcohols, aromatic aldehydes, benzimidazoles, nitrogen-containing heterocycles, quinoline derivatives, quaternary salts and etc [142]. A total of eight benzimidazole derivatives were identified as the corrosion inhibitors for mild steel in 1 M hydrochloric acid [143]. Similar, several benzimidazole derivatives such as 2-mercaptobenzimidazole and 2-methylbenzimidazole,

and the original benzimidazole compound were compared on their respective corrosion inhibition efficiency. According to the results, 2-mercaptobenzimidazole was found to be the most effective corrosion inhibitors for mild steel in 1 M hydrochloric acid solution [141]. On the other hand, the imidazoline-based compound is one of the most studied corrosion inhibitors under the group of nitrogen-containing heterocycles organic compound. 2-undecyl-1-ethylamino imidazoline (2UEI) was found to be an effective corrosion inhibitor for N80 mild steel in CO₂-saturated 3% NaCl solutions as the inhibition efficiency increases with the increase of 2UEI concentration [144]. Similarly, an imidazoline derivative was found to be an effective mixed-type inhibitor that suppressed both anodic and cathodic corrosion processes by its adsorption on API X65 steel in CO₂-saturated 3% NaCl solutions [145].

Additionally, the use of quinoline derivatives as corrosion inhibitors in acidic media was studied extensively to replace the highly carcinogenic chromate and arsenic compounds. Commercially available pharmaceutically active compound 5-Nitro-8-Hydroxy Quinoline was determined to be an effective corrosion inhibitor on mild steel in 1M hydrochloric acid solution [37]. Another quinoline derivative, 3-formyl-8-hydroxyquinoline was proven to inhibit corrosion of mild steel in hydrochloric acid medium [146]. To further understand the corrosion inhibition mechanism of quinoline derivatives, Monte Carlo simulation and theoretical calculations were also employed to confirm and determine the adsorption process of the inhibitors [147]. Recently, 8-hydroxyquinoline (8-HQ) was proven to be an alternative green and sustainable corrosion inhibitor on X60 steel for the acidizing oilfield uses. As an outstanding chelating and complexing agent, 8-HQ was reported to act as a mixed-type inhibitor and protect the steel surface by forming an inhibition layer through the complexation of the functional groups in 8-HQ with the iron atoms present on the steel surface [148].

2.3.3 Inorganic nanoparticles

Inorganic oxide nanoparticles such as SiO_2 , ZrO_2 , Al_2O_3 , TiO_2 , ZnO and CeO_2 possess good chemical stability and provide corrosion protection to metal substrate. Using sol-gel method, steel coated with SiO_2 and TiO_2 were prepared and an improved anticorrosion properties was observed through the addition of these layers [149]. Additionally, ZrO_2 possess good chemical stability and high hardness to render the metal substrate with improved anticorrosion properties. ZrO_2 coatings were successfully deposited on 304 stainless steel by sol gel method with the use of zirconium propoxide as the precursor and densified in air and in oxygen-free (argon or nitrogen) atmosphere [150]. According to the potentiodynamic polarization curves, the deposition of ZrO_2 coatings extends the lifetime of the material by a factor of eight under very aggressive environment. In addition, to further improve the properties of ZrO_2 coatings, the ZrO_2 layers were heat-treated at 400 °C and 800 °C [151]. The final ZrO_2 layers were homogenous, crack-free and the corrosion resistance of the mild steel was increased by a factor of 6.3 and 2.3 respectively. Al_2O_3 coatings were deposited on the 316 type stainless steel using sol-gel method to improve the corrosion resistance of 316 type stainless steel [152]. Similar deposition of Al_2O_3 coatings on the mild steel was applied on the mild steel using sol-gel method and the corrosion current density was reduced by 5 orders of magnitude as compared to the pure substrate without Al_2O_3 coatings [153]. Thin Al_2O_3 coatings were grown by atomic layer deposition at 160 °C on steel surface. Electrochemical analysis of the ultra-thin aluminum oxide coatings have shown decreasing corrosion rate with increasing thickness of the Al_2O_3 film [154].

ZnO is considered as non-toxic and hence environmental-benign coatings can be fabricated using these nanoparticles. Moreover, zinc oxide is also on the U.S. Food and Drug Administration's list of generally regarded as safe (GRAS) substances. Previous research has

shown the use of ZnO nanoparticles for improved coating barrier properties. Nano ZnO-containing epoxy-based coatings were prepared by co-deposition of ZnO nanoparticles and epoxy-based cathodic electrodeposition paint [155]. Unlike the non-stabilized coating, it was found that the ZnO-stabilized coatings maintain their protective properties after a prolonged exposure to UV light. Additionally, the anticorrosion performance of the epoxy-polyamide nanocomposites containing different loadings of ZnO nanoparticles was improved. According to the results, better anticorrosion performance through the addition of ZnO nanoparticles was attributed to two reasons [156]. First, ZnO nanoparticles increase the barrier properties by preventing electrolyte diffusion into the coating matrix. Second, the coating resistance against hydrolytic degradation is improved. Similar results were achieved by incorporating ZnO nanoparticles into polyurethane coating [157]. Polyurethane coating containing ZnO nanoparticles was exposed to salt spray laboratory test for 500 h. As compared to the blank polyurethane coating, coatings modified with 2.0 wt% ZnO nanoparticles possess enhanced anticorrosion performance by restricting the diffusion of electrolyte. Additionally, large surface area of the well dispersed nano sized ZnO particles absorb more resin on its surface, which eventually enhances the density of the coatings and improves the anticorrosion performance.

2.4 Microencapsulation techniques

Microencapsulation serves as a powerful technique in different applications primarily to protect the encapsulated core materials, render controlled release behavior and prolongs the lifetime of the applications. In this section, the use of microencapsulation techniques for antifouling and anticorrosion related applications is discussed in details.

2.4.1 Antifouling related researches

Encapsulation techniques were employed in different antifouling or antimicrobial related researches, which include the encapsulation of enzyme, biocides, essential oils as well as the modification of microcapsules shell materials.

2.4.1.1 Encapsulation of enzyme

Enzymes are considered as environmental friendly solution to prevent biofouling as discussed earlier due to the ability to be rapidly biodegraded. However, the short lifetimes of enzymes limit their use in practical applications. As a result, several approaches were proposed to improve enzyme stability, which include enzyme immobilization, enzyme modification and enzyme encapsulation [158]. These approaches stabilize the enzyme activity by preventing the unfolding and denaturation of the enzyme molecules. Among these approaches, enzyme encapsulation was among the most studied enzyme immobilization methods. In particular, sol-gel method was the most popular approach in encapsulating enzymes. The first approach of enzyme encapsulation was through encapsulating enzymes in silica matrices using the typical synthetic protocol with tetramethylorthosilicate (TMOS) or tetraethylorthosilicate (TEOS) as the precursor [159]. As compared to other approach in immobilizing, tighter confinement of the enzymes through this encapsulation technique can be achieved as the enzymes are added from the early stage of silicate formation.

Additionally, a novel method to encapsulate enzyme with the addition of D-glucose as the pore-forming agent was proposed [160]. Nanoporous silica host was fabricated and the pore size and volume generally increased with increasing amount of D-glucose. The enzymatic activity of acid phosphatase (ACP) encapsulated in the nanoporous host was significantly improved over that without the addition of D-glucose. However, the harsh conditions required for chemical

synthesis limit the practicality of these applications. As a result, biomimetic silica support catalyzed by silaffin polypeptides from diatoms was formed at neutral pH and ambient temperature and pressure [161]. The quick gelation process resulted in negligible loss of enzyme activity. Additionally, results have shown high percentage of enzyme immobilization and the immobilized enzyme was proven to be more stable as compared to the free enzymes.

To further improve the biomimetic approach, protamine-templated biomimetic hybrid capsules encapsulating enzymes were produced [50]. Biomimetic silicification process was performed on the pre-encapsulated β -glucuronidase (GUS) in liquid-core alginate (Alg) capsules, resulting in the final alginate/protamine/silica hybrid capsules with no loss of enzymatic activity after 10 reaction cycles. The relative activity of GUS encapsulated in hybrid capsules was proven to be higher than that encapsulated in Alg capsules and that of the free enzyme. To realize the use of encapsulated enzymes in practical application, enzyme-based antifouling paints were proposed as a bio-based and non-accumulating alternative. A hydrogen peroxide-producing system containing hexose oxidase (HOX), glucoamylase and starch was tested in artificial seawater [7]. In particular, the biomimetic encapsulation for HOX through polyethylenimine-templated silica co-precipitation resulted in 46% higher activity as well as better stability in artificial seawater. Hence, the encapsulation of the enzymes promises a sustained release of HOX and prolongs the antifouling action in a self-polishing coating.

2.4.1.2 Encapsulation of biocide

Premature leakage and degradation were among the problems in the use of biocides and the release of the biocides needs to be controlled to achieve the required antifouling activity across an extended period of usage. These concurrent losses of biocides cause the need of the addition of higher initial levels of biocides, causing environmental issues as the biocides are

fundamentally proven to be toxic. Hence, encapsulation of biocides was introduced and investigated in different studies to solve these problems.

Biocides such as the isothiazolinone based compounds were successfully encapsulated within modified silica frameworks [162]. The porous frameworks inhibit the aqueous extraction of the biocides and the controlled delivery of the biocides facilitates a dynamic equilibrium to maintain the minimum inhibitory concentration to deliver longer effective activity over an extended period of time. Additionally, the encapsulation of toxins within hundred micron-sized particles was observed to reduce the amount of biocides required to achieve 90% mortality of the freshwater zebra mussel *Dreissena polymorpha* in a 12 hours treatment by a factor of approximately three [163]. Among different types of biocides, water based biocides are prone to excessive leaching and hence high concentrations of these biocides are required to ensure successful protection against biodeterioration. Therefore, sodium benzoate as model water soluble biocides was encapsulated and proven to be effective against two brown rot species *Coniophora puteana* and *Serpula lacrymans* [164]. The encapsulation successfully prolongs the lifetime of biocides and extends its effect based on the slow diffusion from the capsules. Several biocides were also encapsulated using silica nanocapsules. In particular, biologically-active compounds 2-mercaptobenzothiazole (MBT) and 4,5-dichloro-2-octyl-4-isothiazolin-3-one (DCOIT) as some of the most commonly used biocides in the market, were encapsulated using silica nanocapsules [9]. The antibacterial efficacy against *E. coli* was achieved through the successful encapsulation of MBT and DCOIT in the silica nanocapsules. Recently, some of the commercial antifouling compounds such as Irgarol 1051, Ecomea and zinc pyrithione were successfully encapsulated in biodegradable poly(lactic acid) (PLA) nanoparticles [165]. However, despite the successful encapsulation of these commercial biocides, the antifouling properties of the coating containing

these nanoparticles were not presented. Hence, future work should be focused on more application based testing to evaluate the antifouling performance.

Other than for antifouling purposes, biocides were encapsulated for several other applications which require prolonged resistance to microbiological growth. Halloysite biocide nanosystem was developed based on a natural halloysite nanotube-based carrier with biocide activity for construction materials protection [166]. According to the outdoor testing, the system was effective in preventing biological growth for duration of two years' outdoor exposure. Similarly, biocides were encapsulated in porous silica microparticles to control the release rate and increase the lifetime of the encapsulated compound in a wood paint. Under accelerated weathering tests, the microparticles were shown to prolong the biocidal effect of the fungicide.

2.4.1.3 Encapsulation of clove oil

The volatile nature [41, 42] and chemical instability of clove oil [43] under the presence of light, air and high temperature causes the need of encapsulation for protection from the harsh surrounding environment and to prevent premature release and degradation of clove oil.

Recently, liposome-encapsulated clove oil was examined and proven to have antimicrobial activity for *S. aureus* by utilizing pore-forming toxins (PFTs) to activate clove oil release to reach the bacteria [167]. Moreover, eugenol rich clove extract was encapsulated using solid lipid carriers. Lipid formulations containing clove oil were spray dried to encapsulate the volatile and poor water soluble compounds, eugenol and eugenyl acetate to form solid redispersible powders [168]. Gelatin-based microcapsules containing clove oil were prepared according to the coacervation phase method, using protein gelatin as the external polymer. These microcapsules were then attached onto the fibers of fabric and found to be effective at reducing the live House Dust Mites (HDMs) [169]. Besides that, biodegradable microcapsules based on chitosan were

synthesized by the single coagulation process. These biodegradable microcapsules containing clove oil were successfully synthesized and prolonged the release of clove oil [170].

However, on the basis of the characterization of the synthesized microcapsules from the previous studies, high agglomeration tendency and irregular shape of the microcapsules remain a challenge for the aforementioned techniques. These problems affect undesirably the delivery and release of the encapsulated core, as well as the incorporation of dispersed microcapsules into different functional coatings. Hence, a better encapsulation process to produce robust microcapsules with no agglomeration issue is required to improve the practical uses of the fabricated microcapsules containing clove oil.

2.4.1.4 Modification of microcapsule shell

The shell of the microcapsules serves as another interface which can be modified for multifunctional properties of the microcapsules. According to previous research, several studies were conducted to improve the properties of the microcapsules by depositing additional functionality on the shell material. Silver nanoparticles were distributed on the surface to increase wall toughness and strength [171]. Additionally, the thermal stability of the phase change material microcapsules was improved. Another study fabricated multifunctional microencapsulated phase change materials with silver/silica double-layered shell with thermal energy storage, electrical conduction and antimicrobial effectiveness [172]. With higher latent-heat storage and good thermal regulating efficiency, the microcapsules were also found to exhibit high antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*. Silica shell microcapsules with silver nanoparticles deposited on the interior wall of the silica shell were fabricated using a two-step process [173]. The slow release of Ag^+ ions through diffusion

through the mesopore channels in the shell renders the AgNPs@silica microcapsules with excellent antibacterial activity against *E. coli* for long periods over two months.

Other than depositing silver nanoparticles, multifunctional microcapsules containing *n*-eicosane were fabricated using zinc oxide nanoparticles [174]. Hence, other than the energy storage properties from the encapsulated core material, the shell material with zinc oxide nanoparticles also serves additional functionalities of photocatalysis and antibiosis.

2.4.2 Anticorrosion related research

Microencapsulation techniques were widely used in different anticorrosion system, in which the use of microcapsules in self-healing coatings was a well-known research. Additionally, corrosion inhibitors were also encapsulated to protect the corrosion inhibitors from reacting with the surrounding matrix and prevent corrosion upon in situ damage of the microcapsules.

2.4.2.1 Self-healing coatings

Self-healing materials have received great attention over the past decade due to their ability to sense the damage and heal the crack immediately. The working mechanisms of self-healing materials are classified into two: intrinsic and extrinsic self-healing. Intrinsic self-healing describes the ability to heal cracks by the polymers themselves while extrinsic self-healing involves the use of healing agents to close the cracks. The healing agents are stored in reservoirs, which can be divided into microcapsule-based, hollow tube-based and microvascular-based reservoirs. Among these three reservoirs, microcapsules are the most commonly used because it is more versatile and can be used in both bulk polymer composites and thin polymer coatings.

Figure 2.5 The autonomic healing concept of microcapsule-based system [32]

The autonomous healing concept of a microcapsule-based system is demonstrated in Figure 2.4. When the host polymer matrix suffers from damage and crack starts to form, the microcapsules containing healing agents are ruptured. The healing agent will be released into the crack plane through capillary action. The healing agent contacts the catalyst embedded in the host polymer matrix and polymerizes to seal the crack [32].

The first generation of microcapsule-based system focused on a two part system consisting of Grubbs' catalyst and encapsulated healing agent, DCPD [31]. When crack forms in the host matrix, encapsulated DCPD will be released to react with the Grubbs' catalyst and undergo ring opening metathesis polymerization (ROMP) to heal the crack.

The mechanism of microcapsule-based system usually involves the encapsulation of healing agent and dispersion of catalyst in the host polymer matrix. However, Cho *et al.* demonstrated a different mechanism by encapsulating the catalyst instead of the healing agents [34]. The healing agent, hydroxy end-functionalized polydimethylsiloxane (HOPDMS) and polydiethoxysiloxane (PDES) mixture was phase separated in the matrix while the catalyst, di-n-butyltin dilaurate

(DBTL) was encapsulated into polyurethane microcapsules. This new material system introduced a new and environmentally more stable healing chemistry.

Among these anticorrosion systems based on embedded capsules, isocyanate-based system has emerged as one of the topics that attract a lot of research attentions over the last decade. Isocyanates were found to be a potential catalyst-free healing agent for use in humid environment. Microencapsulation of isophorone diisocyanate (IPDI) was done using interfacial polymerization of polyurethane (PU) as the shell material [29]. The formation of polyurethane shell is through the reaction between toluene diisocyanate (TDI) prepolymer and 1,4-butanediol. The large difference in reactivity between IPDI and TDI allows the reaction of TDI to form polyurethane shell and successful encapsulation of IPDI as core content.

Besides that, several other isocyanates were encapsulated for the purpose of self-healing anticorrosion coating. That includes the facile microencapsulation of hexamethylene diisocyanate (HDI) as core materials through interfacial polymerization, which is similar to the procedure mentioned above [45]. Results show significant corrosion retardancy in the self-healing coating containing these microcapsules under an accelerated corrosion process. Moreover, thiol isocyanate chemistry with a dual capsule strategy was also studied recently for the development of extrinsic self-healing epoxy materials [175]. This strategy involves encapsulation of both thiol and isocyanate and upon rupture of these microcapsules, amine groups in the epoxy serves as a catalyst for the addition reaction between thiol and isocyanate to heal the cracks.

Microcapsules containing isocyanates were proven to be effective in self-healing of cracks and retardancy of corrosion. Overall, isocyanates were proven to be potential healing agents for various healing chemistries as they are highly reactive towards many functional groups including

amine, alcohol and thiol groups. Due to the high reactivity of isocyanates with water, several efforts were conducted on these microcapsules to improve its properties, especially its stability and shelf life. Double layer isocyanate-based microcapsules with excellent thermal and non-polar solvent resistance were fabricated to withstand weak polar solvents (hexanes and xylene) for up to 24 days [176]. Moreover, the weight loss of these microcapsules after 60 minutes of isothermal treatment at 100 °C was reduced by almost 90% as compared to pure isocyanates without encapsulation. The effects of functionalizing compound on the microcapsules containing isocyanates on the microcapsule properties such as stability were accessed. In particular, the use of hexamethylenedisilazane (HDMS) as functionalizing compound results in microcapsules with high isocyanates content up to 85 wt% and also maintain the core content after one day immersion in water [177]. This improvement was significant as compared to the microcapsules with a non-functionalized shell, where the initial isocyanate content drops rapidly from 49 to 15wt%.

Methods were developed to prepare microcapsules containing reactive amines for potential applications in self-healing polymers. Early in year 2010, amine core microcapsules were prepared using interfacial polymerization of an isocyanate and an amine stabilized by an inverse Pickering emulsion [178]. The self-healing chemistry is similar to the epoxy bulk material, where the preparation of amine-containing microcapsules enables the creation of self-healing epoxies through the epoxide-functionalized healing agents.

Besides that, self-healing thermoset using encapsulated epoxy-amine healing chemistry was achieved through dual-capsule strategy, where the first capsule contains a modified aliphatic polyamine and the second capsule contain a diluted epoxy monomer [179]. Healing efficiency of 91% was achieved with 7 wt% amine capsules and 10.5 wt% epoxy capsules. Moreover, the long

term stability of the healing system shows 68% healing efficiency after aging for 6 months. This improved stability has shown promising future for industrial applications.

Other than polymer microcapsules, etched hollow glass bubbles (HGBs) with through-holes at micron level were used to store epoxy and amine solution to achieve self-healing in epoxy matrix [180]. This two-part epoxy-amine chemistry is similar to what was discussed above. However, the use of HGBs gives much stronger containers than the polymeric microcapsules. Systematic studies on the properties of healing agent carriers and self-healing performance were carried out to show this promising technique for self-healing epoxy coating [46, 181]. Moreover, this epoxy-amine chemistry was also used in recovering the interfacial bonding strength of glass fiber through functionalizing the surface of the fiber with microcapsules containing various concentrations of reactive epoxy resin and ethyl phenylacetate (EPA) solvent [182]. For the mechanism, debonding of the fiber/matrix interface ruptures the capsules and the solvent swells the matrix to increase the chance that residual amine from the matrix will polymerize the epoxy healing agents to seal the crack.

2.4.2.2 Encapsulation of corrosion inhibitors

Microencapsulation of corrosion inhibitors is needed to prevent undesirable interactions between corrosion inhibitors and the coating matrix, which result in reduction of inhibition efficiency and weakening of coating barrier properties. For example, the uncontrollable leaching of corrosion inhibitors over time due to surrounding electrolytic environment may reduce the lifetime of the coating with corrosion inhibition effect.

Quinoline compounds were introduced to have corrosion inhibition effect. In the past few years, research has been done to encapsulate quinoline compounds in different types of reservoirs. 8-Hydroxyquinoline (8-HQ) was researched extensively due to its multifunctional

properties, which include antiseptic, disinfectant, pesticide, antibacterial and corrosion inhibition properties. Encapsulation of 8-HQ using ceria nanocontainers were introduced and it showed significant anticorrosion effect against AA2024 [51]. In particular, higher corrosion current triggers the release of 8-HQ from the nanocontainers to suppress the corrosion activities. Moreover, it was found that ceria chloride (CeCl_3) and 8-HQ can inhibit the corrosion of pure aluminum [183]. Encapsulation of 8-HQ was also done using titania nanocontainers [52]. The incorporation of these nanocontainers with 8-HQ loaded into epoxy coating was found to exhibit anticorrosion properties against aluminum alloy (AA) 2024-T3. Recently, polymer microcapsules were used to encapsulate 8-HQ to control its release into the coating matrix. Interfacial polymerization method was introduced to encapsulate 8-HQ through the reaction of toluene diisocyanate (TDI) and polyamidoamine (PAMAM) dendrimer to form polyurea shell [184]. By using the PAMAM dendrimer, it was found to improve thermal stability of the polyurea microcapsules.

Besides the encapsulation of 8-HQ, extensive research was carried out on the encapsulation of another efficient corrosion inhibitor, 2-Mercaptobenzothiazole (MBT). Encapsulation of 2-MBT was done by the synthesis of polyurea microcapsules for corrosion protection aluminum alloy (AA) 2024 [185]. These polyurea microcapsules were fabricated through the reaction between TDI and diethylenetriamine (DETA). Moreover, the low solubility of 2-MBT in aqueous solutions plays an important role in the extent of its release. It was found that the release was low at neutral environment, while its release is much higher at both acidic and alkaline conditions. This trend matches well with the localized nature of corrosion processes in AA 2024, which marks the potential of these microcapsules in natural corrosion environment. However, the loading content of 2-MBT was low, which is only about 5%, calculated based on

thermogravimetry (TG) measurement. Nanocapsules containing 2-MBT as corrosion inhibitor was also fabricated through direct encapsulation of 2-MBT in emulsion drops assembled with polyelectrolyte shells [186].

Smart nanocapsules containing inhibitors were investigated for the corrosion protection of copper [187]. Three different types of nanocapsules: polyelectrolyte modified halloysite nanotubes (PHN), polyelectrolyte modified SiO₂ nanoparticles (PSN) and polyelectrolyte nanocapsules (PNC) were fabricated with loaded benzotriazole as corrosion inhibitor. It was discovered that the rate of benzotriazole release depends on the polyelectrolyte shell components and the quantity of corrosion inhibitor. Other than that, clay nanotube was also investigated to encapsulate benzotriazole as the corrosion inhibitor [188]. The benzotriazole loaded halloysite was exposed to the solution of Cu(II) ions to form the tube end stoppers for purpose of control release of benzotriazole. By forming stopper complexes of different strengths, the release of benzotriazole becomes tunable in the range of ten to hundreds of hours. Moreover, layer-by-layer assemble was implemented as a technique to entrap corrosion inhibitor on the outside of microcapsules. Benzotriazole was successfully loaded on the outside of urea-formaldehyde microcapsules by layer-by-layer assembling using polyelectrolytes [189]. This method offers an innovative idea of using “one” capsule to achieve both simultaneous crack healing and corrosion inhibition on exposed substrate. Similarly, silica nanoparticles covered layer-by-layer with polyelectrolyte layers and layers of benzotriazole as the corrosion inhibitors were introduced in a hybrid sol-gel film to act as a corrosion protection systems for metallic substrates [190]. These researches highlight the role of core-shell interface in achieving additional functionalities from the microcapsules or nanoparticle templates.

2.4.3 Release models

Significant advances have been achieved in the field of drug release and several mathematical models were derived and proposed to explain the release behavior of different dosage forms. The rapid advancement in materials design and engineering has also highlighted the needs of understanding for different polymer used in the controlled delivery field. Hence, this section serves to provide insights into several release models that are relevant to this study. Mathematical evaluation of the release kinetics aids in better understanding the release mechanisms through experimental results.

First of all, zero-order release kinetics is a release model in which the drug release rate is constant over a period of time without depending on the active agent concentration [191]. The equation of zero-order model is shown below:

$$\frac{M_t}{M_\infty} = kt \quad (2.1)$$

where k is the zero order constant, M_t is the released amount at time t and M_∞ is the total amount released.

The requirement to achieve zero-order release kinetics is to ensure that the reservoir material remains saturated with the drug. Hence, the constant release rate of the system (zero-order kinetics) only applies during the saturation of core materials and the drug release rate decreases rapidly below the saturation point. For instance, the release of tryptophan and theophylline from the biphasic polymer hydrogels demonstrates zero-order kinetics with constant release rate only until approximately 67-88% of the excess drug was released and the subsequent release becomes time-dependent [192]. Additionally, continuous zero-order release over an extended period of time was observed too. In particular, long-term constant release of HIV microbicide TMC120

from silicone elastomer vaginal rings was observed over 71 days period, with a constant release of 136 µg/day [193].

Additionally, Higuchi model is among the earliest proposed model to describe the drug dissolution in a matrix system [194, 195], in which Fickian diffusion was identified as the release mechanism. The most general equation of Higuchi model describes the linear relationship between the fraction of active agents released and the square root of time, which is shown below:

$$\frac{M_t}{M_\infty} = k_H t^{0.5} \quad (2.2)$$

where k_H is the Higuchi dissolution constant, M_t is the released amount at time t and M_∞ is the total amount released.

In particular, there are several assumptions related to the use of Higuchi model as a release model [196]. Most importantly, the initial drug concentration in the system has to be much higher than the solubility of the drug. The diffusion is assumed to be unidirectional and the thickness of the matrix is much larger than the size of the drug molecules. Additionally, the diffusivity is considered to be constant and hence, the polymer matrix studied cannot significantly swell upon contact with water.

Several drug loaded devices had proposed Higuchi model to define their respective release kinetics. For example, bioactive biomaterials with silicone elastomer as the polymer matrix and metronidazole as the model antibacterial drug were produced as a novel self-lubricating silicone biomaterial [197]. The release kinetics of this silicone systems were accessed and found to be fitted well against the Higuchi model. Additionally, the release kinetics of quinupramine from the ethylene-vinyl acetate (EVA) copolymer membrane follow the Higuchi model, in which the amount of quinupramine released is proportional to the square root of time [198].

Baker-Lonsdale model is a mathematical model used for drug release, which was derived based on Higuchi model in particular for spherical matrices [199]. This model is slightly different from the Higuchi model discussed earlier where the release occurs through a homogeneous planar matrix, such as the slab and cylinder matrices. The fully derived equation of Baker-Lonsdale model is shown below:

$$\frac{3}{2} \left[1 - \left(1 - \frac{M_t}{M_\infty} \right)^{\frac{2}{3}} \right] - \frac{M_t}{M_\infty} = \frac{3D_m C_{ms}}{r_0^2 C_0} t = k_B t \quad (2.3)$$

where D_m is the diffusion coefficient, C_{ms} is the drug solubility in the matrix, r_0 is the radius of the microcapsules, M_t is the released amount at time t , M_∞ is the total amount released and C_0 is the initial drug concentration in the matrix. k_B is defined as the Baker-Lonsdale constant, which is used to fit the release profile.

Additionally, the time at which the device becomes exhausted of the active agents, t_∞ can be derived as the following:

$$t_\infty = \frac{r_0^2 C_0}{6DC_{ms}} \quad (2.4)$$

The Baker-Lonsdale model was derived from a monolithic dispersion system, which consists of a dispersion of active agents in a rate-limiting polymer matrix. According to Baker-Lonsdale model, diffusion is identified as the main release mechanism, in which the compounds are released by dissolution into the polymer medium and subsequent diffusion to the surface of the microcapsules or microspheres. Previous research has shown the use of Baker-Lonsdale model in fitting different release mechanisms for both microcapsules and microspheres. For instance, the release behaviors for pH-responsive and photoresponsive – poly(acrylic acid)/azobenzene microcapsules containing rhodamine B were in excellent agreement with the Baker-Lonsdale model, indicating diffusion as the controlled release mechanism [200]. Additionally, a sustained

release of agrochemical named Clothianidin was achieved by a coating layer of PU through polymerization reaction between polyol and isocyanate [201]. The release studies indicate that the release behaviors of these microspheres agree well with the Baker-Lonsdale model and the spherical shape of the microspheres remain stable without any change in its framework.

Lastly, Ritger-Peppas model is another release model that is used to describe drug release from the polymeric system through the power law [202]. The equation for the Ritger-Peppas model is a simple exponential relation as shown below:

$$\frac{M_t}{M_\infty} = at^n \quad (2.5)$$

where a is a constant and n is the release exponent, M_t is the released amount at time t and M_∞ is the total amount released.

This model is usually used to study the release of a polymeric system when the release mechanism is unclear or when more than one release mechanism is present for the particular polymeric system [191]. For instance, when $n = 0.5$, it can be defined as the Higuchi model in which diffusion is the main release mechanism. On the contrary, when $n = 1$, the model is non-Fickian and the drug release can be related to the zero order release kinetics, in which the release mechanisms are governed by the swelling or relaxation of polymer chains.

2.5 Summary and motivation

Corrosion and biofouling were found to be two of the most severe problems in the maritime industry due to the high cost of replacement and maintenance. Hence, applying a multifunctional coating with both anticorrosion and antifouling properties is an efficient and innovative method to protect the material especially for the applications in the marine industry. Corrosion inhibitors and antifouling compounds were usually directly added into the coating. However, the

undesirable interaction of these compounds with the coating matrix may cause premature loss of the functional effects. Additionally, the emergence of different microencapsulation techniques allows the encapsulation of both corrosion inhibitors and antifouling compound. Recently, mesoporous silica nanoparticles (MSNs) was applied as delivery tools for self-healing coatings which consist of BTA (corrosion inhibitor) and benzalkonium chloride (BC) [203]. This pH/sulfide ion responsive release system loaded with both corrosion inhibitors and biocides has highlighted the importance of multifunctional coatings. Many other encapsulation techniques including in-situ polymerization and interfacial polymerization can also be used to encapsulate natural antifouling compound and control its release to extend the lifetime of the coating.

Since the ban of organotin compounds especially the TBT-SPC system, there has been extensive research on the discovery of more environmental friendly approaches for antifouling coatings. Hence, natural antimicrobial compounds such as essential oils had become the research focus nowadays. However, these volatile essential oils can be oxidized easily and premature degradation will decrease its efficiency as antimicrobial agents. One way to tackle this challenge is through encapsulation of essential oils to prolong its lifetime and prevent premature degradation. This leads to the research focus of this work, which is to fabricate microcapsules containing clove oil and quantify its release profile under the influence of different reaction parameters. Moreover, the antibacterial activity of the fabricated microcapsules against different marine bacterial strains was conducted to determine its potential use in treating marine biofouling problems.

Recently, one of the most promising research areas includes the incorporation of other functionalities such as antifouling and self-cleaning properties into anticorrosion coatings to achieve a multifunctional smart coating. Microencapsulation technique is widely used in self-

healing materials but its versatility is not fully explored in antifouling materials. Moreover, the shell material of the microcapsules offers another interface to incorporate other functionalities into these microcapsules. For example, other antifouling compounds such as metal oxide nanoparticles can be deposited onto the shell material to achieve more intensive antifouling efficiency of the microcapsules. Hence, microencapsulation is a promising technique for this research work to fabricate multifunctional coatings with both antifouling and anticorrosion features. The focus of my work will be mainly on the use of different microencapsulation techniques in encapsulating different functional compounds and fabricating multifunctional coating with both antifouling and anticorrosion features.

Chapter 3 Materials and Experiments

3.1 Materials

4,4'-diphenylmethane diisocyanate (MDI) prepolymer Suprasec 2644, was obtained from Huntsman. Urea was obtained from Promega. Melamine, 37 wt% formaldehyde solution, resorcinol, ammonium chloride, 2.5wt% EMA surfactant, 1,4-butanediol, clove oil, 8-hydroxyquinoline (8-HQ), sodium dodecyl benzenesulfonate (SDBS), polyvinyl alcohol (PVA), sodium hydroxide (NaOH) and hydrochloric acid solution (HCl, 0.1M), zinc acetate dehydrates ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) and ammonium hydroxide were purchased from Sigma Aldrich. Marine broth 2216 and marine agar 2216 were purchased from BD, Difco. Epolam 5015 and hardener 5015 used as epoxy matrix were supplied by Axson. All the chemicals used in this report were received without further purification.

3.2 Synthesis of Microcapsules

3.2.1 Fabrication of double layer PU/PUF shell microcapsules containing clove oil

Double layered shell microcapsules containing clove oil were fabricated through a combination of interfacial and in situ polymerization. The whole process is illustrated in Figure 3.1. In the first step, interfacial polymerization was employed to fabricate the inner polyurethane (PU) shell of the microcapsules. Subsequently, a second step of in situ polymerization was applied to fabricate the outer polyurea-formaldehyde (PUF) shell of the microcapsules.

For the first step of interfacial polymerization, a solution of 50 ml of deionized (DI) water with 3 wt% of gum Arabic as surfactant was prepared and heated up to 40 °C in a 250ml beaker that was immersed in a temperature controlled water bath. Under an agitation rate of 800 rpm, the oil phase mixture of 2g Suprasec 2644 and 10g clove oil was added dropwise into the above surfactant solution and the ensuing emulsification was allowed to take place for 20 minutes.

Next, 2g of 1,4 butanediol (BDO) was then added slowly into the system to initiate interfacial polymerization to form the PU inner shell. The interfacial polymerization process was allowed to react for 3 hours to form the single layer PU shell microcapsules. The resulted suspension of microcapsules slurry was rinsed with DI water for several times to remove the excessive reactants from the suspension.

Subsequently, in situ polymerization was applied to form the outer PUF shell of the microcapsules. 2g of urea, 0.5g of ammonium chloride and 0.5g of resorcinol were dissolved in 60 ml of DI water and heated up to 55 °C in a 250ml beaker immersed in water bath. This is followed by adjusting the pH value of the system to 3.5 through the addition of NaOH and HCl solutions. Next, the polyurethane microcapsules from the first step were dispersed into the system followed by adding 5g of 37 wt% formaldehyde solution to initiate the in situ polymerization reaction. The system was left to react for 30 minutes before rinsing with DI water for several times and air drying for 12h before future characterization. The schematic representation to fabricate the double layer PU/PUF shell microcapsules containing clove oil is shown in Figure 3.1. The preparation process is summarized in Figure 3.2.

Figure 3.1 Fabrication process of double PU/PUF layer microcapsules containing clove oil

Figure 3.2 Summary of the fabrication procedure of double layer PU/PUF microcapsules containing clove oil through interfacial and in situ polymerization.

3.2.2 Fabrication of PMF shell microcapsules containing 8-HQ and clove oil

8-hydroxylquinoline (8-HQ) dissolved in clove oil was successfully encapsulated using in-situ polymerization of melamine–formaldehyde as the shell material [204]. Mixture of 3.75g melamine and 9.5g of 37 wt% formaldehyde solution was prepared and the pH value of the final mixture was adjusted to 8.5-9.0 using NaOH solution. The pre-polymer solution of melamine-formaldehyde was then put into a water bath of 70 °C under magnetic stirring for 1 hour on a programmable hotplate. For the preparation of the surfactant solution, 0.6g of sodium dodecylbenzenesulfonate (SDBS) and 0.09g of polyvinyl alcohol (PVA) were mixed thoroughly in 30ml of DI water and placed into a water bath of 55 °C on a programmable hotplate. Subsequently, the prepared pre-polymer solution of melamine-formaldehyde was added into the

above surfactant solution and allowed to settle for a few minutes. The core material of 20% 8-HQ dissolved in clove oil solution was prepared by mixing 2.125g of 8-HQ compound with 8.5g of clove oil. Strong mechanical vortex mixing was employed to ensure thorough dissolution of 8-HQ compound in the liquid clove oil. Under an agitation rate of 200 rpm, the core material was added drop wise into the system and the ensuing emulsification was allowed to take place for 1 hour. After stabilizing the emulsification, the pH value of the system was then slowly tuned to about 4.3 by adding 10 mL of 1M acetic acid at a very slow rate of 0.1 ml/min using the syringe pump system. Subsequently, the system was left to react for another 3 hours at 55 °C. To remove the excessive reactants, the obtained suspension of microcapsules slurry was rinsed with DI water for 5 times. Finally, the collected microcapsules were air dried for 12 hours and sieved to remove debris before further analysis. The schematic of the fabrication processes of PMF shell microcapsules containing 8-HQ and clove oil is shown in Figure 3.3 and summarized in Figure 3.4.

Figure 3.3 Fabrication processes of PMF shell microcapsules containing clove oil and 8-HQ

Figure 3.4 Summary of the fabrication procedure of PMF shell microcapsules containing 20 wt% 8-HQ dissolved in clove oil through in situ polymerization.

3.2.3 Fabrication of ZnO decorated microcapsules through hydrothermal route

ZnO nanosheets decorated microcapsules were synthesized via a solution method at 60 °C. the zinc acetate dehydrates ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) aqueous solution (0.05 M) was prepared and neutralized with ammonia hydroxide until a clear solution was achieved. The as-prepared PMF

shell multifunctional microcapsules from Section 3.2.2 were transferred into 50ml of the prepared aqueous solution containing 0.55g of $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$. Subsequently, the solution with 1 g of PMF shell microcapsules was stirred homogeneously at 200 rpm in a water bath of 60 °C for 1h to allow the growth of ZnO nanosheets onto the PMF shell. After reacting for 1h, the obtained suspension of microcapsules slurry was rinsed with DI water for 5 times and subsequently air dried for 12 hours at RT. The schematic of fabrication procedure of depositing ZnO nanosheets on the PMF shell interface is shown in Figure 3.5 and summarized in Figure 3.6.

Figure 3.5 Fabrication processes of ZnO nanosheets decorated PMF shell microcapsules.

Figure 3.6 Summary of the fabrication procedure of ZnO nanosheets decorated PMF shell microcapsules through solution method.

3.3 Characterization of microcapsules

3.3.1 Surface and shell morphology

The morphology, size, core shell structure and shell thicknesses of the fabricated microcapsules were studied using Field Emission Scanning Electron Microscope (FESEM) (Joel, Model: JSM-7600F, Japan). Firstly, the collected microcapsules were scattered on a conductive

tape. Some of them were then lightly crushed with a razor blade so that the cross-section and inner structure of the shell can be observed. Meanwhile, the size distributions of the microcapsules were calculated using ImageJ based on at least 150 specimens in the obtained FESEM images.

3.3.2 Microcapsules content analysis

Fourier Transform infrared (FTIR) spectra were obtained using FTIR spectrometer (Varian 3100) to examine the chemical structure of different samples and confirm the successful encapsulation of different core materials. Different samples, including pure clove oil, microcapsules containing clove oil and the shell materials were prepared. The shell materials can be obtained by washing crushed microcapsules with acetone for several times. The use of acetone flushes away the core materials, leaving behind the shell materials upon air drying. Subsequently, the testing samples were prepared by grinding the microcapsules with potassium bromide (KBr), followed by transmitting infrared light through the samples to scan and observe the chemical properties of the microcapsules.

3.3.3 Core fraction analysis

The core content of the prepared microcapsules was determined by the extraction method using ethanol as the extracting solvent. Initially, 0.1g of microcapsules (W_1) was weighed and poured into a folded filter paper. The microcapsules were subsequently squeezed firmly to completely release the encapsulated clove oil. The clove oil was then extracted from the broken microcapsules using ethanol. This extraction process was repeated for 5 times to ensure complete extraction and the remaining weight (W_2) of shell material was measured carefully. The whole core fraction analysis test was conducted for 3-5 times to determine the average value. Lastly, the calculation of core content is based on the equation below:

$$\text{Core content (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (3.1)$$

3.3.4 Release profile analysis

The release of clove oil from the microcapsules was characterized using Ultraviolet-Visible (UV-Vis) Spectroscopy with the use of Beer-Lambert law. According to Beer-Lambert law, the absorbance values obtained from the UV-Vis measurement is directly proportional to the concentration of the chemicals used. Figure 3.7 shows the absorption curve of clove oil with different concentrations at the wavelength of 280 nm. As shown in Figure 3.7, the calibration curve generated determines the equation to calculate the amount of clove oil released based on the absorbance value measurement through UV-Vis spectroscopy.

To set up the release study, microcapsules containing clove oil were placed in a dialysis bag that was subsequently submerged in a centrifuge tube with DI water as the dissolution medium. The setup of the release study is shown in Figure 3.8. At different time intervals, 2 ml aliquot was taken out and an equal amount of fresh DI water was added into the centrifuge tube. Subsequently, the solution samples at different time intervals were analyzed for their clove oil content using UV-Vis Spectrophotometer (at $\lambda = 280$ nm) and the amount of clove oil released was calculated based on Beer-Lambert law as described above. To ensure consistency and accuracy of results, all measurements were carried out in triplicate.

Figure 3.7 Calibration curve for absorbance at different clove oil concentration according to Beer-Lambert law

Figure 3.8 Setup of the release profile analysis test

3.3.5 Antibacterial test

The antibacterial activities of the fabricated microcapsules containing clove oil were evaluated based on ASTM E2315 against *V. coralliilyticus*, *E. aestuarii*, *E. coli* and the collected marine biofilm-forming bacteria using a time-kill test method [205]. Cultures of *V. coralliilyticus*, *E. aestuarii* and *E. coli* were prepared overnight at 30°C in marine broth 2216. The bacterial suspensions were all diluted in Phosphate Buffered Saline (PBS) solution to a concentration of approximately 1×10^6 cfu/ml as the starting bacteria concentration for the time-kill test. Meanwhile, 0.2g of microcapsules containing clove oil (0.2g) was placed in a dialysis bag with 2 ml of deionized (DI) water. Subsequently, this dialysis bag containing microcapsules was brought into contact with 20 ml of prepared PBS solution containing 1×10^6 cfu/ml of *V. Coralliilyticus*, *E. aestuarii* and *E. Coli* respectively at room temperature.

To further investigate the potential antifouling effect of the fabricated microcapsules, marine biofilm-forming bacteria were prepared by swapping off from the surface of on-site contaminated samples. Steel plate with epoxy coatings (5cm×5cm×1cm) was immersed in natural seawater near Sebarok Island for 2 weeks. Subsequently, the steel plate was collected and washed aseptically using filtered seawater for several times to remove the unattached bacteria from the surface. A sterile surface swab was then rubbed across the sampling area of 5cm×5cm with the purpose to scrap the marine biofilm-forming bacteria off from the surface. Similar to the culture method for individual marine bacteria, these bacteria on the surface swab were suspended in the marine broth 2216 and grown overnight at 30 °C. For the marine biofilm-forming bacteria, 0.4g of microcapsules containing clove oil was used in order to treat a wider range of marine bacteria in the suspension, which may require different Minimum Inhibition Concentration (MIC) values.

To evaluate the antibacterial performance of the fabricated microcapsules containing clove oil, an aliquot was removed at different time intervals and appropriate dilutions were made using PBS solution. The standard plate count method was adopted whereby the diluted bacterial

Table 3.1 Composition of marine agar 2216 and marine broth 2216 (without agar).

Composition	Formulation (g/L)
Sodium Chloride	19.45
Magnesium Chloride	8.8
Peptone	5.0
Sodium Sulfate	3.24
Calcium Chloride	1.8
Yeast Extract	1.0
Potassium Chloride	0.55
Sodium Bicarbonate	0.16
Ferric Citrate	0.1
Potassium Bromide	0.08
Strontium Chloride	0.034
Boric Acid	0.022
Disodium Phosphate	0.008
Sodium Silicate	0.004
Sodium Fluoride	0.0024
Ammonium Nitrate	0.0016
Agar	15

suspension was spread uniformly on marine agar 2216 plates. The plates were incubated overnight at 30°C before quantification of the number of colony forming unit (CFU) to determine the surviving organisms. Based on the results, the antibacterial activities of microcapsules containing clove oil can be determined and compared between different formulations.

3.3.6 Wettability analysis

The wettability of the microcapsules was examined by measuring the contact angle of an epoxy coating with microcapsules spread onto the epoxy surface. Partially embedded microcapsules on epoxy coating were successfully prepared by controlling the curing time of the epoxy. Subsequently, the contact angle measurement was conducted on a contact angle goniometer (Attension, KSV Instrument). The volume of DI water droplet used was 5 μL . After dropping the water droplets, the contact angle was measured and the water droplet was subsequently removed using a clean tissue paper. To obtain an average value, the contact angles were measured on a total of five different spots on the coating covered by microcapsules. Additionally, a few samples were prepared to ensure the repeatability of the measurements. The final contact angle values of the PMF shell microcapsules and ZnO nanosheets decorated PMF shell microcapsules were measured and compared to determine the effect of ZnO nanosheets on the final wettability of the microcapsules.

3.3.7 Mechanical property analysis

The mechanical properties of the microcapsules were conducted using the single-capsule compression test by Keller and Sottos [206]. Based on the good dispersibility of the fabricated microcapsules, single microcapsule was easily put at the end of the rod. Images of the single microcapsule were taken prior to the compression test for the diameter measurement of the microcapsules tested. The loading rate for the stepper actuator (Physik Insurmente M-230S) was

2 $\mu\text{m/s}$ and controlled via a computer interface. The load was acquired by a 0.5N load cell (FUTEK). The normalized maximum strength, σ_{max} of the microcapsules can be calculated using the formula below [29]:

$$\sigma_{\text{max}} = \frac{4P_{\text{max}}}{\pi(D_o^2 - D_i^2)} \quad (3.2)$$

where P_{max} is the maximum load, D_o and D_i are the outer and inner diameter of the PMF shell microcapsules respectively.

3.3.8 X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) was conducted to examine the presence and chemical state of the elements on the corrosion inhibition layer. The formation of inhibition layer was scrap off from the scratched region of the fabricated anticorrosion coating with microcapsules containing 8-HQ and clove oil. XPS analysis of the sample was then performed by using a Kratos Axis Supra Spectrometer with an Al $K\alpha$ excitation radiation (1486.6 eV). The scanned size of the sample is 300*700 μm with an emission current of 15 mA. The wide and spectrum scans were conducted at 1 eV and 0.1 eV step size respectively.

3.4 Preparation and testing of coatings

3.4.1 Preparation of PDMS coating with embedded microcapsules

Polydimethylsiloxane (PDMS) coatings were prepared using Sylgard 184 silicone elastomer kit based on the recommended mixing ratio of 10:1 for silicone elastomer base and curing agent. Fabricated microcapsules were added into the mixture at the required weight percentage (10 or 20 wt%) at ambient temperature and degassed in the vacuum for 15 minutes. Subsequently, the degassed PDMS coating with embedded microcapsules was coated onto the cover slips (21 $\times$ 21 mm^2) to a thickness of about 300-500 μm . PDMS coatings were cured at room temperature for

48h before further characterization on the antibacterial properties. The softer and less cross-linked nature of PDMS matrix as compared to the epoxy matrix facilitates earlier release of the encapsulated antibacterial agents – clove oil and 8-HQ. Hence, it would be more suitable to demonstrate the antibacterial properties of coatings with embedded microcapsules in the lab.

3.4.2 Preparation of epoxy coating with embedded microcapsules

Firstly, the epoxy coating is prepared based on the recommended mixing ratio of 10:3 for epoxy resin (EPolam 5015, AXSON) and hardener 5015. Subsequently, fabricated microcapsules were added into the mixture at different weight percentage (10 and 20 wt%) at ambient temperature and degassed in the vacuum for 15 minutes. Meanwhile, a few pieces of steel panel ($50 \times 50 \times 2 \text{ mm}^3$) were polished with sand paper, degreased with acetone, and finally washed with distilled water. After drying, the steel panel was coated with the degassed epoxy coating with embedded microcapsules to a thickness of about 300-500 μm . The coating was cured at room temperature for 24 hours before conducting antibacterial and anticorrosion test on it. EPolam 5015 epoxy coating were commonly used for marine application. Hence, it is suitable to demonstrate the antifouling properties in the seawater.

To prepare multifunctional coating with half-embedded microcapsules, the preparation of the epoxy coating is similar except for the curing process. The epoxy coating is prepared based on the recommended mixing ratio as described above and with different weight percentage of fabricated microcapsules as required. After degassing in the vacuum for 15 minutes, these coatings were cured at 40 $^{\circ}\text{C}$ for 2.5 hours. To coat an additional layer of half-embedded microcapsules, microcapsules are spread on the top of the coating. Due to the viscous nature of the coating after curing for 2.5h at 40 $^{\circ}\text{C}$, approximately half of the microcapsules are embedded

in the coating while the other half are exposed above the coating surface. The coating was cured at room temperature for another 24 hours before further testing.

3.4.3 Anticorrosion test

To evaluate the anticorrosion performance, the coatings with embedded microcapsules were manually scratched using razor blade while the uncoated area of the steel plates were tightly covered using waterproof adhesive tape to ensure corrosion attack only at the scratched regions. Subsequently, the coating is immersed in 1M NaCl solution for 24 hours. The scratched areas were investigated and characterized using SEM to examine the anticorrosion performance. Other than scratching the coatings, anticorrosion tests were also conducted on coatings without scratching them. In particular, the anticorrosion performance of the coatings can be examined by investigating the underlying substrate by removing the coating through sonication in acetone solution. Subsequently, the degree of corrosion for the underlying substrate can be studied through SEM.

Besides SEM observation, Tafel test was carried out to examine the anticorrosion performance. The current density from Tafel plots can be compared to determine the coatings with the best anticorrosion performance. Additionally, to evaluate the formation of inhibition complexes at the scratched regions, XPS was done to examine the composition of the organic compounds formed.

3.4.4 Antibacterial test

The antibacterial tests of the coatings were conducted based on two different tests – zone inhibition test and time-kill test. Zone inhibition test was used to compare the ability of different coatings in inhibiting microbial growth. Seawater bacteria were grown overnight at 30 °C to form a very cloudy bacteria suspension, which was then spread over the marine agar plate using a sterile swab. Subsequently, the antibacterial coatings containing different types of

microcapsules were placed at the center of the agar plate, with the effective antibacterial surface in contact with the marine agar. The marine agar plates were then incubated at 30 °C for 24 hours with the presence of the antibacterial coatings. To evaluate the antibacterial performances of the coatings, the inhibition zone was then compared among the coatings with different types of microcapsules and formulations.

Time-kill test was conducted similar to the procedure in ASTM E2315 time-kill test. Cultures of seawater bacteria collected from Sebarok Island, Singapore were prepared overnight at 30 °C in marine broth 2216. The bacterial suspensions were then diluted in Phosphate Buffered Saline (PBS) solution to a concentration of approximately 1×10^4 cfu/ml as the starting bacteria concentration for the time-kill test. Subsequently, the antibacterial coatings were brought into contact with 10 ml of prepared PBS solution containing 1×10^4 cfu/ml of seawater bacteria at room temperature. To evaluate the antibacterial performance of the antibacterial coatings, an aliquot of 50 μ L was removed at different time intervals and aseptically spread on the marine agar 2216 plates. The plates were incubated overnight at 30°C before quantification of the number of colony forming unit (CFU) to determine the surviving organisms.

3.4.5 Marine fouling field test

Immersion studies were carried out by attaching the steel plates onto a PVC pipe with drilled holes using a steel hook. The setup of the immersion test is shown in Figure 3.9. According to Figure 3.7, the coatings were immersed at a depth of approximately 1m below the water surface, considered optimal for rapid biofouling process [207]. The zoomed-in image of the steel plates hanging on the PVC pipe with the use of steel hook was taken on-site before immersing the samples into the seawater. Approximately a total of 11 holes were drilled on the PVC pipe with 10cm between the holes. This is essential to prevent the collision between the steel plates during

the entire marine fouling field test. Subsequently, a nylon rope was passed across the PVC pipe and both ends of the rope were tied on the structure at the port area. Additionally, two floats were tied on the rope at approximately 1m above the immersed samples, to ensure a submergence level of 1m regardless of the sea level during the entire immersion period.

The samples were immersed in the seawater for duration of 15, 30 and 45 days respectively. At each time interval, the samples were collected and transported back to the laboratory for further characterization. Prior to the characterization, the samples were gently rinsed using the seawater collected from the immersion site to remove the loosely bind biomass. Several methods were used to examine the degree of fouling, which include visual inspection and SEM analysis.

Figure 3.9 Marine field test setup for antifouling coatings with different types of embedded microcapsules and formulations at Sebarok Island, Singapore.

The fouled coverage percentage was calculated with the use of software ImageJ while the presence of different macro-organisms was investigated through SEM characterizations. The antifouling performance of the coatings was examined based on the fouling coverage and the presence of macro-organisms on the surface, in accordance to ASTM D6990 – Standard Practice for Evaluating Biofouling Resistance and Physical Performance of Marine Coating Systems. According to the ASTM standards, the types of fouling organisms observed on the coatings were recorded as a guideline on the difference between the fouling organisms attached onto the control coatings and the microcapsules-based coatings. Additionally, the estimated percentage of the coating surface covered by fouling organisms is based only on the attachment of primary biofouling settlement, which refers to the biofouling attached directly to the coating surface but not biofouling attached to other fouling organisms (secondary fouling). The estimated fouled coverage percentage and the presence of different types of fouling organisms observed on the coating surface were determined and tabulated.

Chapter 4 Synthesis, Characterization and Release Study of double layer PU/PUF shell microcapsules containing clove oil

4.1 Motivation

Biofouling is defined as the settlement of marine organisms onto any surface immersed in seawater. Existing antifouling coatings in the market involve the use of toxic antifouling agents such as Irgarol, Diuron, copper pyrithione, zinc pyrithione and 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT), as the successors for tributyltin (TBT) compound which was banned due to its toxicity [208, 209]. However, the toxicity of these antifouling agents was proven as they can cause severe negative environmental impacts [61, 65, 67, 210]. To eliminate the use of these toxic biocides, more environmental friendly alternatives are desired.

Essential oils (EOs) are natural compound produced from the plant extracts and have gained wide attention recently due to their multifunctional properties such as antimicrobial, antioxidant, antifungal, anti-inflammatory and mosquito repellent [211]. Among the wide range of EOs, clove oil is an essential oil extracted from *Syzygium aromaticum* that exhibits significant antimicrobial effects against a wide range of bacterial strains [111]. Additionally, clove oil also possesses other properties such as antibiofilm [212, 213], antioxidant [214], antifungal [215], anti-inflammatory [216], antiviral [217] and insect repellent activity [117].

Most of the studies on clove oil focused on medicinal, food industry and food packaging applications. However, little or no research was conducted on examining the potential use of clove oil in antifouling applications or for other applications such as coating for outdoor facilities. Additionally, clove oil has several disadvantages, including its volatility [41, 42] and chemical instability [43] under the presence of light, air and high temperature, which have severely halted

its practical applications. Hence, encapsulation is essential to protect clove oil from the harsh surrounding environment and prevent premature release and degradation of clove oil.

Previous research accomplishes the encapsulation of clove oil through different methods, such as by using liposomes [167], single coagulation [170], spray dried [168], coacervation phase [169] and envelope binding methods [218]. However, some problems were surfaced, such as high agglomeration tendency and irregular shape of the microcapsules, which affect the release of clove oil for practical uses. In contrast, polymer microencapsulation techniques were proven to produce robust microcapsules with regular shapes, which are used extensively in a wide range of applications [29-32, 34, 46, 173, 219]. Hence, in this study, interfacial and in situ polymerization techniques are utilized to fabricate microcapsules containing clove oil. The release profile of clove oil from the microcapsules is also relatively important as insufficient release may cause ineffectiveness while excessive release may result in skin irritation or negative impacts to human health. Hence, the release profile of the fabricated microcapsules is explored in this study.

Herein, double layer PU/PUF shell microcapsules containing clove oil were fabricated through a combination of interfacial and in situ polymerization methods. The release profile of the fabricated microcapsules was studied and some insights into tuning the release profile through manipulating several parameters were discussed both qualitatively and quantitatively. To our knowledge, there is no existing study focusing on the release behavior of a double layer PU/PUF shell microcapsules containing clove oil and this study serves to provide a new approach on tuning the release profile through manipulating the parameters on both shell layers. Additionally, the antibacterial test of the microcapsules and coatings were investigated systematically and the effect of the release rate on the antibacterial properties of the microcapsules was examined.

4.2 Shell formation mechanism

During the first step of interfacial polymerization, the mixture of clove oil and Suprasec 2644 as the oil phase was emulsified into finer liquid droplets in a 3 wt% Gum Arabic surfactant aqueous solution. Gum Arabic contains glycoprotein and polysaccharides. The content of glycoprotein contains amino functional group and tends to react with isocyanates [220]. Hence, a thin shell was formed at the oil/water interface after 20 minutes of emulsification. However, the structure of this thin shell is very loose and it collapses in less than half an hour under exposure to the environment. Some BDO was then added into the continuous phase to react with PU prepolymer at the oil-water interface to form a polyurethane (PU) shell through the reaction between NCO groups in the PU prepolymer and OH groups in the diol [45]. The reaction was left for 3 hours to allow diffusion of diol across the PU shell for further shell thickening. Figure 4.1 shows the formation mechanism of PU shell through the reactions between PU prepolymer and BDO at the oil-water interface.

However, the single layer PU shell is porous and the release of clove oil from this porous PU shell is rapid. Additionally, the microcapsules tend to aggregate upon drying due to the leakage of clove oil from the porous PU shell. According to Figure 4.7a1, the inner PU layer is formed through agglomeration of PU nanoparticles, which are the result of the reaction between isocyanates and diol. Hence, the voids between these nanoparticles contribute to a porous PU layer which eventually allows rapid release of clove oil across the shell membrane. Moreover, the single layer PU shell has low mechanical strength due to its loose and porous structure.

Figure 4.1 Formation of polyurethane shell through interfacial polymerization [29]

Some cracks were observed on the microcapsules after storage in the vial for several days, which will be discussed later in this chapter. Hence, a second layer of cross-linked polymer was fabricated to decrease and control the release rate, and prolongs the service lifetime of these microcapsules. Under an acidic condition, urea-formaldehyde tends to polymerize at 55 °C. This behavior was used in a polymerization technique to fabricate microcapsules with a dense poly-(urea-formaldehyde) (PUF) shell [221]. At pH 3.5 and a reaction temperature of 55 °C, the polymerization process was initiated as soon as formaldehyde solution was added into the solution containing urea. As the molecular weight of PUF increases, it starts to deposit on the PU shell. With increasing reaction time for UF deposition, microcapsules with higher surface roughness can be formed.

4.3 Characterization of double layered PU/PUF shell microcapsules

4.3.1 Morphology and shell structure

The morphology, core shell structure and shell thickness of the microcapsules were examined using FESEM. Figure 4.2 shows the characterization results of the fabricated double layered PU/PUF shell microcapsules. According to Figure 4.2a, the resultant microcapsules are spherical

Figure 4.2 Characterizations of double PU/PUF shell microcapsules under FESEM showing (a) overview of double PU/PUF shell microcapsules containing clove oil; (b) shell morphology of individual microcapsule; (c) cross-sectional view of the shell showing inner porous PU and outer dense PUF shells; (d) histogram of the microcapsules' diameter distribution

in shape with an average diameter of $102 \pm 17 \text{ }\mu\text{m}$ (measured using software ImageJ). Additionally, the size distribution of the microcapsules based on 150 individual microcapsules is shown in Figure 4.2(d).

Figure 4.2(c) shows the cross-sectional view of the broken shell, consisting of an inner porous PU layer and an outer dense PUF layer. This confirms the existence of a core-shell structure of the microcapsules with distinctive layers of PU and PUF in the shell. It is apparent that the outer surface of the microcapsules is very rough due to the dense deposition of urea-formaldehyde (UF) nanoparticles while the inner PU layer of the microcapsules appears to be more porous and loose. The overall shell thickness of the double layer PU/PUF microcapsules is around $1.2 \text{ }\mu\text{m}$, which consists of a $0.86 \pm 0.16 \text{ }\mu\text{m}$ inner PU layer and a $0.41 \pm 0.07 \text{ }\mu\text{m}$ outer PUF layer.

4.3.2 Microcapsules contents

FTIR spectra of pure clove oil, core material extracted from the microcapsules, and shell material were examined. According to Figure 4.3, it can be seen that the spectrum of clove oil is similar to that of the core material extracted from the fabricated microcapsules. Hence, this indicates that clove oil was successfully encapsulated.

The absorption band in the region of $3000 \text{ to } 3500 \text{ cm}^{-1}$ represents the OH-stretching in clove oil. For the spectrum of PU/PUF shell, the absorption band at 2260 cm^{-1} represents the NCO stretch characteristics. This indicates that the NCO functional groups in the shell were not exhausted completely during the reaction process and the highly crosslinked PUF outer layer prevents its diffusion across the shell to react with water molecules outside [176].

Figure 4.3 FTIR spectra of clove oil, double layered PU/PUF shell and core material (clove oil). Identical spectra of clove oil and core material confirm successful encapsulation of clove oil.

4.3.3 Core Fraction

The core fraction of the microcapsules fabricated under different parameters was examined based on the solvent extraction method. The core fractions of the microcapsules with different amount of PU reactants added and different length of PUF deposition time are shown in Figure 4.4. According to Figure 4.4a, increasing amount of PU reactants decreases the core fraction of the final microcapsules. This is related to the formation mechanism of PU shell, in which higher amount of PU reactants contributes to thicker PU shell. As a result, the overall weight of the microcapsules increases and the fraction of clove oil encapsulated become comparatively lower. According to Figure 4.4b, similar trend is also observed for the microcapsules fabricated with

Figure 4.4 Core content of microcapsules fabricated under different parameters: (a) amount of PU reactants and (b) length of PUF deposition time.

different length of PUF deposition time in which longer PUF deposition time also decreases the core fraction of the microcapsules. As the length of PUF deposition time is prolonged, the surface roughness increases with higher amount of PUF nanoparticles deposited. Hence, the overall weight of the microcapsules increases and the fraction of encapsulated clove oil become lower. The effect of these parameters on the final microcapsules was investigated and discussed in the following sections.

4.4 Parameter studies

4.4.1 Number of shell layers

The characterization of fabricated microcapsules with single layer PU shell and double layer PU/PUF shell was conducted to determine the differences on their shell properties. Figure 4.7(a1-3) shows the FESEM images of single layer PU shell microcapsules. According to Figure 4.7a1, the inner PU layer is formed through the agglomeration of PU nanoparticles, which are the result of the reaction between isocyanates and diol. Hence, the voids between these nanoparticles contribute to a porous PU layer which eventually allows rapid release of clove oil across the shell

Figure 4.5 Comparison of single layer PU shell and double layer PU/PUF shell microcapsules: (a) Single layer PU shell microcapsule and (b) the cross-sectional view of the PU shell; (c) Double layer PU/PUF shell microcapsule and (d) the cross-sectional view of the double layer PU/PUF shell showing distinct inner PU and outer PUF shells.

membrane. As a result, the single layer PU shell appears to be more porous and the release of clove oil happens more rapidly, causing agglomeration of single layer PU shell microcapsules before practical uses. Additionally, these microcapsules possess low mechanical strength due to the loose and porous nature of the PU shell. Based on Figure 4.7a2, some cracks were observed on the microcapsules after storage in the vial for several days. In order to improve the

mechanical strength of the microcapsules and prevent premature release of clove oil, a second layer of cross-linked PUF shell was fabricated on top of the inner PU shell.

According Figure 4.5, the surface morphology and cross-sectional view of single PU shell and double layer PU/PUF shell microcapsules are totally different. Figure 4.5(d) confirms the successful deposition of an outer PUF shell onto the initial PU shell shown in Figure 4.5(b). The outer PUF shell appears to be more cross-linked and denser as compared to the inner PU shell, which is more porous. Additionally, the surface morphology of these two types of microcapsules totally different.

Through the comparison between Figure 4.5c and 4.5d, the surface morphology of the double layer PU/PUF shell microcapsules was found to be much rougher as compared to the microcapsules with single PU shell. The difference can be attributed to the formation mechanism of the outer PUF shell, in which the random deposition of PUF nanoparticles renders the final microcapsules with a rough surface morphology.

4.4.2 Amount of PU reactants

The effect of the amount of PU reactants on the inner PU shell thickness of the microcapsules was investigated systematically. According to Figure 4.6, the PU shell thickness increases along with the increase in the amount of PU reactants added during the interfacial polymerization process. To be specific, the amount of PU reactants (Suprasec 2644 and BDO) added was 1g, 2g and 3g each respectively, resulting in PU shell thickness of 252 ± 82 nm, 444 ± 122 nm and 859 ± 161 nm as shown in Figure 4.6. Figure 4.7(c1-3) clearly shows this trend through the FESEM images of PU shell thickness of different formulations. This phenomenon can be explained by the formation mechanism of PU shell through interfacial polymerization process. During the

Figure 4.6: Effect of the amount of PU reactants (Suprasec 2644 and BDO) added on the PU shell thickness of the fabricated microcapsules.

interfacial polymerization process, clove oil as the core material was mixed with MDI based urethane prepolymer Suprasec 2644 to form the oil phase. Meanwhile, the addition of BDO in the aqueous phase initiated the polymerization reaction at the oil-water interface, through the reaction between the hydroxyl group in the aqueous phase and the isocyanate group in the oil phase. As the formation of PU shell is based on the reaction between these two PU reactants – Suprasec 2644 and BDO, the PU shell thickness increases when higher amount of diol in the aqueous phase diffuses and reacts with higher amount of isocyanates.

4.4.3 Length of PUF deposition time

The length of PUF deposition time is another effect that was studied on its effect against the release profile of clove oil from the fabricated microcapsules. Specifically, the influence of the

Figure 4.7 FESEM images of microcapsules fabricated under different parameters: single layer PU shell with low mechanical strength as proven by: (a1) inner PU layer consists of PU nanoparticles across the shell; (a2) crack observed on single layer PU shell; (a3) magnification of the crack indicating low mechanical strength. FESEM images of microcapsules fabricated under different parameters: (b1-3) increasing roughness of surface morphology at increasing length of PUF deposition time; (c1-3) increasing PU shell thickness at increasing amount of PU reactants

length of PUF deposition time on the surface morphology of the microcapsules was investigated. According to the characterization using FESEM as shown in Figure 4.7 (b1-3), the surface

morphology of the microcapsules becomes rougher with the increase in PUF deposition time. This trend is related to the increasing amount of PUF nanoparticles deposited on the inner PU shell as the PUF reaction time is prolonged from 15 minutes to 1 hour. Based on the formation mechanism of PUF shell, the reaction between urea and formaldehyde was initiated under an acid catalyzed environment. As the reaction time is prolonged, PUF nanoparticles with increasing weight tend to deposit on the shell to form a dense layer of PUF shell. As the deposition happens randomly, a rough surface morphology is achieved and the degree of roughness increases with longer PUF deposition time. Hence, it is proven that the surface morphology can be tuned through controlling the length of PUF deposition time.

4.5 Tuning of Release Profiles

According to the studies in previous section, it is apparent that the number of shell layers, PU shell thickness and the surface morphology of the final microcapsules can be controlled through manipulating several reaction parameters. In this section, the release profiles of the microcapsules were examined and compared against different reaction parameters as described above. Recently, the environmental impact of different chemicals used has become one of the most important concerns across many industries. Slow release of the functional agent encapsulated may compromise the performance of the targeted applications while rapid release of the core material may shorten the lifetime of the microcapsules. Hence, knowledge about the release rate of clove oil from the fabricated microcapsules is essential before the implementation stage of these microcapsules into different applications.

4.5.1 Fitting of the release profiles

Over the past few decades, significant advances have been achieved in the field of drug release and several different models were derived to explain different release behavior of different dosage forms. In this study, several release models were compared, which include zero order [192] , Ritger-Peppas [202, 222], Higuchi [194, 195] and Baker-Lonsdale [199] models. Below are the equations related to different release models:

Zero-order Model:

$$\frac{M_t}{M_\infty} = kt \quad (4.1)$$

where k is the zero order constant.

Ritger-Peppas Model:

$$\frac{M_t}{M_\infty} = at^n \quad (4.2)$$

where a is a constant and n is the release exponent.

Higuchi Model:

$$\frac{M_t}{M_\infty} = k_H t^{0.5} \quad (4.3)$$

where k_H is the Higuchi dissolution constant.

Baker-Lonsdale Model:

$$\frac{3}{2} \left[1 - \left(1 - \frac{M_t}{M_\infty} \right)^{\frac{2}{3}} \right] - \frac{M_t}{M_\infty} = \frac{3D_m C_{ms}}{r_0^2 C_0} t = k_B t \quad (4.4)$$

where D_m is the diffusion coefficient, C_{ms} is the drug solubility in the matrix, r_0 is the radius of the microcapsules, M_t is the released amount at time t , M_∞ is the total amount released and C_0 is the initial drug concentration in the matrix. In this study, k_B is the Baker-Lonsdale constant, which is used to fit the release profile.

According to Figure 4.8, the release profiles are fitted against four different models described above – Zero order, Ritger-Peppas, Higuchi and Baker-Lonsdale models. R-squared values are calculated to evaluate the goodness of fit of different models. According to the results of R-squared values, the release profile of double layer PU/PUF shell microcapsules was fitted best against the Baker-Lonsdale model. This model was derived from the Higuchi model to describe the drug release behavior specifically for microcapsules and microspheres [199]. Based on this model, the release mechanism from these double layer PU/PUF shell microcapsules is determined to be primarily diffusion across the polymeric shell. Additionally, the FESEM images in Figure 4.9 further confirm diffusion as the main release mechanism of clove oil as the shell remains intact after complete release of clove oil. The only difference of the microcapsules after complete release is their shrinkage as a result of the complete release of encapsulated clove oil.

Figure 4.8 Fitting of release profile using different models: (a) Zero-order model, (b) Ritger-Peppas model, (c) Higuchi model, (d) Baker-Lonsdale model and the correlation coefficients of corresponding kinetic models.

Figure 4.9 Comparison of release profiles of microcapsules with single layer PU shell and double layer PU/PUF shell and the FESEM images of the microcapsules before and after complete release of clove oil.

4.5.2 Influence of the number of shell layers

As discussed previously, the difference of the shell structure of single layer PU shell and double layer PU/PUF shell was shown in Figure 4.5. Additionally, the premature release of single layer PU shell microcapsules urged the need to fabricate an additional outer layer of dense PUF shell to prevent the rapid release of clove oil and eliminate the agglomeration problem. The comparison of release profiles of microcapsules with double layer PU/PUF shell and single layer PU shell are shown in Figure 4.10a. Based on the comparison, the release rate of clove oil is comparatively lower from double layered PU/PUF shell microcapsules. Owing to the dense structure of PUF layer, the release of clove oil from the microcapsules is prolonged and complete

release takes around one week, much longer as compared to single PU layer microcapsules which take only 1 day to completely release the encapsulated clove oil.

The difference of release rate can be explained based on the structure and mechanical strength of the microcapsules. As discussed previously, the porous structure and poor mechanical strength of the single layer PU shell leads to some cracks, which decrease the shelf life of these microcapsules. This was clearly shown in the FESEM images of Figure 4.7 (a1-3). These factors cause severe agglomeration of the microcapsules and eventually hinder further manufacturing processes for potential industrial applications. The fabrication of an additional PUF shell through in situ polymerization successfully prolongs the release of clove oil and extends the lifetime of these microcapsules. This is apparent in Figure 4.2c, in which an additional dense PUF shell is observed to prevent premature release of clove oil.

4.5.3 PU shell thickness

The PU shell thickness was successfully manipulated by controlling the amount of PU reactants added. According to Figure 4.7 (c1-3), increasing amount of PU reactants added increases the PU shell thickness of the microcapsules. In this section, the effect of PU shell thickness on the release profiles of the fabricated microcapsules was investigated. It is apparent from the release profile in Figure 4.10c that the release rate of clove oil is slower at increasing amount of PU reactants added.

Higher amount of PU reactants results in a thicker PU shell. This phenomenon affects the release profile of the microcapsules as a thicker shell increases the distance clove oil requires to diffuse before being released from the microcapsules, rendering them with slower release rate. This explanation is further strengthened by the excellent fitting of release profiles against the Baker-Lonsdale model, which determines diffusion as the main release mechanism of clove oil.

Overall, the release profiles and FESEM images of the microcapsules fabricated through adding different amount of PU reactants have successfully proven the feasibility of controlling PU shell thickness and fabricating microcapsules with different release rate.

4.5.4 Morphology of PUF shell

According to previous section, the morphology of PUF shell can be controlled by varying the length of PUF deposition time, resulting in microcapsules with different degree of roughness. In this section, the effect of the surface morphology of PUF shell on the release profiles of the microcapsules was examined. The release profiles in Figure 4.10b clearly demonstrate the effect of the degree of roughness of the outer PUF shell on the release profiles of the fabricated microcapsules. According to the release profiles, the highest release rate of clove oil was observed from the microcapsules with low surface roughness which was fabricated with 15 minutes of PUF deposition time. However, when the PUF deposition time was extended to 30 minutes and 1 hour to fabricate microcapsules with medium and high surface roughness, the release rates became significantly lower and complete release occurs only after a week of immersion in water.

This phenomenon can be explained by the formation mechanism of PUF shell and the diffusion of clove oil across microcapsules with different surface roughness. As the PUF deposition time increases, the in situ polymerization of PUF nanoparticles extends. As a result, increasing molecular weight of more PUF nanoparticles causes more PUF nanoparticles to deposit on the shell.

Figure 4.10 Influence of (a) the number of shell layers; (b) the morphology of PUF shell; (c) the PU shell thickness on release profiles.

Due to the stacking of PUF nanoparticles, microcapsules with higher surface roughness were fabricated and this increases the diffusion distance of clove oil across the shell membrane. As the diffusion of clove oil occurs from every dimension of the spherical microcapsules, rougher surface morphology sustains the release of clove oil and decreases the release rate of clove oil at longer PUF reaction time.

4.5.5 Comparison of Baker-Lonsdale constant (k_B)

According to the fitting of release profiles against different release models, it was concluded that the release profiles of the double layered PU/PUF shell microcapsules containing clove oil fitted best to the Baker-Lonsdale model. On the basis of Baker-Lonsdale model equation 4.4, the constant k_B comprises of several different parameters which include the diffusion coefficient (D_m), the drug solubility (C_{ms}), radius of the microcapsules (r_o) and the initial drug concentration in the matrix (C_o).

Referring to Figure 4.7, it is apparent that the size of the microcapsules is not affected by the change in the reaction parameters such as the length of PUF deposition time and the amount of PU reactants. Additionally, the amount of clove oil encapsulated is the same for all studies. Hence, it can be assumed that the values of r_o , C_{ms} and C_o are the same for all the fabricated microcapsules and the diffusion coefficient (D_m) is the only primary factor affecting the value of k_B through the change in surface morphology and PU shell thickness.

Table 4.1 summarizes the values of k_B for microcapsules fabricated under different parameters – number of shell layers, degree of surface roughness and PU shell thickness respectively. It is worth to note that each of the release profiles fits well with the Baker-Lonsdale model with an average R-squared value of above 0.98. Hence, it can be assumed that all the microcapsules fabricated under different reaction parameters possess release profiles that fit well against the Baker-Lonsdale model. Additionally, this result further strengthens the previous argument that diffusion is the main mechanism for the release of clove oil based on the good fitting against Baker-Lonsdale model. According to Table 4.1, the value of k_B is the highest with a single PU layer shell and the lowest when the PU shell thickness was increased to 859 ± 161 nm. The highest k_B value is almost 8 times higher than the lowest k_B values, indicating the versatility of

Table 4.1 Calculation of k_B values for microcapsules fabricated under different parameters – number of shell layers, PU shell thickness and degree of surface roughness.

Factors influencing release profile		Baker-Lonsdale constant $k_B (10^{-3})$	R^2
Number of shell layers	Single PU	19.5	0.96
	PU/PUF	4.07	0.98
PU shell thickness	252 $\pm$ 82 nm	7.43	0.99
	444 $\pm$ 122 nm	4.07	0.98
	859 $\pm$ 161 nm	2.54	0.99
Degree of surface roughness	Low	16.6	0.98
	Medium	4.07	0.98
	High	3.02	0.99

this method to fabricate microcapsules with a wide range of release rate through manipulating several reaction parameters.

Subsequently, to further investigate on the trend of the change in diffusivity based on different parameter change, several graphs were plotted as shown in Figure 4.11. Figure 4.11 indicates the trend of k_B values with the change in the number of shell layers, degree of surface roughness and PU shell thickness. In particular, k_B values decreases with increasing number of shell layers,

degree of surface roughness and PU shell thickness. As the value of k_B is proportional to diffusion coefficient, it can be concluded that the diffusivity of clove oil from the microcapsules decreases with the additional shell layers, rougher surface morphology and thicker PU shell thickness.

According to the Baker-Lonsdale model, the release mechanism involves dissolution of the active agents in the polymer medium and followed by diffusion to the surface of the microcapsules or microspheres [199]. Hence, based on the high correlation coefficient of the release profiles to the Baker-Lonsdale model, the release mechanism of the double layered PU/PUF microcapsules can be predicted. It can be assumed that the encapsulated clove oil is released from the microcapsules by dissolving into the polymer shell materials and subsequently diffuses to the surface of the outer PUF shell. Hence, on the basis of this model, the influence of microcapsules surface morphology and PU shell thickness can be explained qualitatively.

First, as the surface morphology becomes rougher, the stacking of the PUF nanoparticles on the shell surface increases the roughness of the surface morphology as well as the average distance clove oil is required to diffuse to the surface of the PUF shell. Similarly, when PU shell thickness increases, the diffusion of clove oil to the outer PUF shell surface becomes slower due to the additional distance it requires to diffuse across the shell materials. Hence, the diffusivity decreases with both increasing surface roughness and PU shell thickness, as supported by diffusion mechanism suggested by the Baker-Lonsdale model and the calculation of Baker-Lonsdale constant, k_B . Additionally, it is evident from Figure 4.9 that the diameter of the microcapsules remains the same before and after the release test without significant swelling, which agrees well with the assumptions made for the Baker-Lonsdale model. As the Baker-Lonsdale model highlights on the diffusion of active agents across the polymer matrix, it is

reasonable to assume similar mechanism for the release of clove oil across the double layered PU/PUF shell based on the excellent agreement with high correlation coefficients for all release profiles under different parameters. To summarize, both qualitative and quantitative analysis of the Baker-Lonsdale model successfully validates the feasibility to tune the release profiles by manipulating the reaction parameters.

In conclusion, this research work successfully investigates the release profiles of a double layered PU/PUF shell microcapsules containing clove oil and contributes to the understanding on manipulating the release behavior through tuning the number of shell layers, degree of surface roughness and PU shell thickness. The qualitative analysis based on the FESEM images and quantitative analysis based on the equation of Baker-Lonsdale model successfully validates the idea that the release profiles of the double layered PU/PUF shell can be tuned by manipulating the number of shell layers, degree of surface roughness and PU shell thickness, resulting in a wide range of k_B values with 8 times difference between the highest and lowest values.

Figure 4.11 Comparison of Baker-Lonsdale constant (k_B) values for microcapsules (a) with different number of shell layers and (b) with different degree of surface roughness and PU shell thicknesses.

4.6 Antibacterial activity of the microcapsules

The antibacterial activity of the microcapsules against different types of marine bacteria was accessed using time-kill test method based on ASTM E2315. Several marine bacteria were chosen as the representative marine bacteria in this study.

First of all, bacteria species belonging to the genera *Vibrio* were usually used as the representative of marine bacteria to test the effectiveness of antifouling agents or paint coatings [223]. *V. coralliilyticus* is a globally distributed bacterium with temperature dependent pathogenicity in coral and was found in marine biofilm [224]. Hence, antibacterial activity of fabricated microcapsules against *V. coralliilyticus* was examined to serve as one of the preliminary indications on the potential of the fabricated microcapsules containing clove oil to treat biofouling. Additionally, *Exiguobacterium* is another genus isolated from the marine biofilm [225, 226] and hence *E. aestuarii* isolated from the marine environment is used in this study as another representative of marine bacteria. Furthermore, *E. coli* was found in the marine biofilm on the ship hulls according to the previous study [227]. Therefore, *E. coli* is included as another bacterium examined to determine the potential of the fabricated microcapsules to treat marine biofouling problem. Moreover, *E. coli* is among the widely used bacteria in many different antibacterial studies to examine the efficacy of different antibacterial agents or coatings. As a result, these three bacteria – *V. coralliilyticus*, *E. aestuarii* and *E. coli*, were used as the individual representative marine bacteria associated with the formation of marine biofilm. Additionally, the marine biofilm-forming bacteria collected from the on-site contaminated sample serves as a comprehensive indication on the effectiveness of the fabricated microcapsules in treating biofouling problem.

The release profiles were proven to be affected by different reaction parameters - the amount of PU reactants added and the length of PUF deposition time, which contribute to microcapsules with different PU shell thickness and surface roughness respectively. The antibacterial effect was examined based on the standard plate count method using marine agar 2216 plates. As shown in Figures 4.12-4.15, the antibacterial effect of the fabricated microcapsules was apparent with the

Figure 4.12 Time-kill test against *V. Coralliilyticus* for synthesized microcapsules with different surface roughness at different time interval: (a1) 0h; (a2) 4h; (a3) 8h; (a4) 12h; (a5) 18h; (a6) 24h for control sample without any microcapsules; (b1) 0h; (b2) 2h; (b3) 3h; (b4) 4h (b5) 6h; (b6) 24h for microcapsules with low surface roughness; (c1) 0h; (c2) 2h; (c3) 3h; (c4) 4h; (c5) 6h; (c6) 24h for microcapsules with high surface roughness. Scale bar is 2cm.

decrease in the number of colonies formed with increasing contact time between the fabricated microcapsules and the marine bacteria test. Moreover, the effect of microcapsules surface roughness on the antibacterial effect was investigated.

Figure 4.13 Time-kill test against *E. aestuarii* for synthesized microcapsules with different surface roughness at different time interval: (a1) 0h; (a2) 3h; (a3) 7h; (a4) 9h; (a5) 24h; (a6) 32h for control sample without any microcapsules; (b1) 0h; (b2) 3h; (b3) 7h; (b4) 9h (b5) 24h; (b6) 32h for microcapsules with low surface roughness; (c1) 0h; (c2) 3h; (c3) 7h; (c4) 9h; (c5) 24h; (c6) 32h for microcapsules with high surface roughness. Scale bar is 2cm.

Figure 4.14 Time-kill test against *E. coli* for synthesized microcapsules with different surface roughness at different time interval: (a1) 0h; (a2) 3h; (a3) 6h; (a4) 9h; (a5) 24h; (a6) 32h for control sample without any microcapsules; (b1) 0h; (b2) 3h; (b3) 6h; (b4) 9h (b5) 24h; (b6) 32h for microcapsules with low surface roughness; (c1) 0h; (c2) 3h; (c3) 6h; (c4) 9h; (c5) 24h; (c6) 32h for microcapsules with high surface roughness. Scale bar is 2cm.

Figure 4.15 Time-kill test against marine biofilm-forming bacteria isolated from on-site contaminated samples for synthesized microcapsules with different surface roughness at different time interval: (a1) 0h; (a2) 3h; (a3) 6h; (a4) 9h; (a5) 24h; (a6) 32h for control sample without any microcapsules; (b1) 0h; (b2) 3h; (b3) 6h; (b4) 9h (b5) 24h; (b6) 32h for microcapsules with low surface roughness; (c1) 0h; (c2) 3h; (c3) 6h; (c4) 9h; (c5) 24h; (c6) 32h for microcapsules with high surface roughness. Scale bar is 2cm.

Figure 4.16 Log reduction curve of antibacterial time-kill test results for fabricated microcapsules containing clove oil with different surface roughness against (a) *V. coralliilyticus*; (b) *E. aestuarii*; (c) *E. coli* and (d) marine biofilm-forming bacteria isolated from on-site contaminated samples.

Based on the standard plate count method, Figure 4.16 summarizes the antibacterial time-kill test results of the fabricated microcapsules with different surface roughness against *V. coralliilyticus*, *E. aestuarii*, *E. coli* and marine biofilm-forming bacteria isolated from on-site contaminated sample. Specifically, the complete killing of the bacteria for microcapsules with low roughness occurs at a much earlier contact time as compared to that for microcapsules with high roughness. According to Figure 4.16, the same trend can be retrieved from the antibacterial

activity against all the marine bacteria tested. The bacteria were also proven to remain viable for the control sample without the addition of the fabricated microcapsules, indicating the entire antibacterial mechanism as a result of the released clove oil from the fabricated microcapsules.

According to the time-kill test results summarized in Figure 4.16, it was clearly shown that the release rate of clove oil based on different surface roughness, greatly affects the antibacterial activity of the fabricated microcapsules. To further investigate the origin of the excellent antibacterial effect of clove oil, gas chromatography-mass spectroscopy (GC-MS) analysis was conducted. The results in Figure 4.17 show that eugenol is the main component while other minority chemical compounds include eugenol acetate, caryophyllene and etc. The GC analysis is shown in Figure 3.3, where most of the additional peaks for clove oil were observed at acquisition time between 12 to 15 minutes. Among these peaks, the peak at 13.4 minutes is the most obvious peak and it corresponds to the presence of eugenol based on NIST library data. Hence, this GC-MS analysis confirms the presence of eugenol, which is an essential compound for this study. Other chemical compounds found in eugenol include eugenol acetate and caryophyllene, which was found at acquisition time of 14.8 mins and 14.1 mins respectively.

Figure 4.17 Comparison between gas chromatograph of 10 ppm clove oil and blank column to identify the chemical compounds in clove oil using GC-MS analysis.

On the basis of GC-MS analysis results, it can be assumed that the antibacterial effect of the released clove oil from the microcapsules originates from its main constituent – eugenol. Additionally, according to previous research, the antibacterial mechanism of eugenol is related to the hydrophobicity of the phenolic structure, which allows these hydrophobic molecules to partition into the cell membrane through passive diffusion across the cell membrane. As a result, the cell membrane is disrupted and permeabilized [115, 228, 229]. Subsequently, the increasing transport of potassium and ATP out of the cells causes cell death [230]. Thus, microcapsules with higher release rate of clove oil cause higher frequency of interaction between eugenol and the cell membrane, resulting in earlier complete kill of the marine bacteria as compared to

microcapsules with lower release rate of clove oil. Nevertheless, this study has successfully demonstrated the effect of microcapsules surface roughness on the antibacterial effect of the fabricated microcapsules, proving its versatility for different applications requiring different release and bacteria killing rates.

4.7 Summary

Double layer PU/PUF shell microcapsules containing clove oil was successfully fabricated through the combination of interfacial and in situ polymerization. Significant results achieved through this study are summarized as the following:

- The core-shell structure of the fabricated microcapsules was confirmed through FESEM characterization, showing distinct PU and PUF shell with $0.86 \pm 0.16 \text{ }\mu\text{m}$ inner PU layer and a $0.41 \pm 0.07 \text{ }\mu\text{m}$ outer PUF layer. The resultant microcapsules are spherical in shape with an average diameter of $102 \pm 17 \text{ }\mu\text{m}$ (measured using software ImageJ).
- The release profiles of the microcapsules fitted well against the Baker-Lonsdale model, which determines diffusion as the primary release mechanism. Moreover, the release rate can be tuned through the change in the PU shell thickness and the microcapsules surface morphology by manipulating the amount of PU reactants and the length of PUF deposition time.
- Quantitative analysis based on the calculation of Baker-Lonsdale constant (k_B) values validates the feasibility to fabricate microcapsules with a wide range of diffusivity up to 8 times difference, indicating the versatility of this microencapsulation technique to be employed for a vast range of applications. Hence, the method of tuning the release profile through manipulating the number of shell layers, degree of surface roughness and shell thickness was successfully validated and can be used for other microcapsules or matrices.

- Excellent antibacterial activity of the microcapsules against *E. coli*, *V. coralliilyticus*, *E. aestuarii* was determined through ASTM E2315 time-kill test, which is the first study on the use of volatile clove oil in killing marine bacteria. Additionally, the excellent antibacterial properties of the microcapsules containing clove oil indicate their potential as an environmental friendly solution for antifouling applications.
- GC-MS analysis successfully validated the presence of eugenol compound as the main component in clove oil, which allows these hydrophobic eugenol molecule to partition into the cell membrane and eventually cause cell death.

Chapter 5 Development of Multifunctional Microcapsules Containing 8-HQ and Clove Oil

5.1 Motivation

Corrosion remains as an unsolved challenging problem, which has caused huge economic losses annually, especially for the marine industry due to the corrosive environment. Corrosion inhibitors have been used extensively for corrosion protection through adsorption of ions and molecules onto metal surface, to minimize the reaction between metal and corrosive medium [142, 231, 232]. Previous research has shown that corrosion inhibitors are usually organic compounds with π bonds, heteroatoms (P, N, S and O) [140]. In recent years, extensive research has been done on the microencapsulation of corrosion inhibitors. Encapsulation of 8-hydroxyquinoline (8-HQ) was successful using ceria nanocontainers [51], titania nanocontainers [52] and polyurea shell [184, 185]. However, organic solvent such as toluene and xylene was used to dissolve the 8-HQ compound for encapsulation. This is undesirable as the release of these organic solvents can potentially cause potential environmental hazards and health problems [39, 40]. Hence, a natural alternative should be used to eliminate the use of these toxic organic solvents. Additionally, the mechanical properties of the fabricate microcapsules should be investigated and improved to withstand harsh industrial manufacturing process.

In the marine industry, biofouling is another major problem remains unsolved. Since the ban of tributyltin (TBT) compound which was banned due to its toxicity [208, 209], the most commonly use antifouling agent today includes Irgarol, Diuron, copper pyrithione, zinc pyrithione and 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT). However, recent research has shown the toxicity of these antifouling agent: Irgarol [61, 65], Diuron [67], DCOIT [24, 68], copper and zinc pyrithiones [66, 210]. Therefore, current research focuses on the use of natural compound to

treat biofouling problem. As discussed in the previous chapter, clove oil has been identified as an environmentally friendly solution that can potentially treat biofouling problem.

Furthermore, encapsulation of different types of actives in a single microcapsule serving multiple purposes has become a step forward in the field of multifunctional coatings. Recently, a multifunctional coating combining self-healing and antifouling features was fabricating through the application of mesoporous silica nanoparticles (MSNs) as the delivery tools for both corrosion inhibitor and biocide [203]. This work has proven the potential of multifunctional coatings, especially with the combination of multifunctional coating combining both anticorrosion and antifouling features due to the existence of both corrosion and biofouling problems in marine industry. Hence, the motivation of this study is to fabricate multifunctional microcapsules containing 8-HQ as the corrosion inhibitor dissolved in clove oil as a natural antifouling compound, to simultaneously tackle both corrosion and biofouling problem in the marine industry. This study serves to impose a new idea of encapsulating two different functional agents – corrosion inhibitors and antimicrobial agents in a single microcapsule, which contributes to multifunctional properties and eliminating the use of toxic solvent to dissolve the solid corrosion inhibitors compound for encapsulation.

5.2 Characterization of multifunctional microcapsules

5.2.1 Formation mechanism

In-situ polymerization of poly (melamine-formaldehyde) (PMF) shell is based on the pre-polymer solution of this polymer heated at 70 °C for 1 hour before added into the emulsion system and eventually deposit on top of the oil droplets. The overall reaction scheme of melamine-formaldehyde (MF) pre-polymer is illustrated in Figure 5.1.

individual microcapsules. The microcapsules size distribution is shown in Figure 5.2(d). Additionally, the microcapsules possess a rough surface morphology as shown in Figure 5.2(b), formed through the deposition of melamine-formaldehyde nanoparticles on the stable core material droplet as the PMF molecular weight increases. The cross-sectional view of the microcapsules shown in Figure 5.2(c) confirms the core-shell structure of the fabricated

Figure 5.2 Characterizations of PMF shell microcapsules under FESEM showing (a) spherical microcapsules without any agglomeration problem; (b) Rough surface morphology; (c) Dense PMF shell with shell thickness of $3.67 \pm 0.66 \mu\text{m}$; (d) Microcapsules size distribution, giving an average size of $98 \pm 15 \mu\text{m}$.

microcapsules. Based on the cross-sectional view, the fabricated PMF shell appears to be very dense with a thickness of $3.67 \pm 0.66 \mu\text{m}$.

5.2.3 Microcapsules content

The successful encapsulation of both 8-HQ and clove oil was determined through FTIR characterization. According to Figure 5.3, the FTIR spectra of clove oil, 8-HQ, mixture of clove oil and 8-HQ and the core material extracted from the fabricated microcapsules were compared. The results have shown similar spectrum of the core material extracted to the mixture of clove oil and 8-HQ, proving the successful encapsulation of this mixture. The addition of 8-HQ compound into clove oil is confirmed through the peak near 1580 cm^{-1} , which corresponds to $\text{C}=\text{N}$

Figure 5.3 FTIR spectra of clove oil, 8-HQ, mixture of clove oil and 8-HQ and the core material extracted from the fabricated multifunctional microcapsules.

stretching. Additionally, the peak at 1400 cm^{-1} and 1090 cm^{-1} represents C-N stretching. These peaks confirm the presence of nitrogen in the mixture, based on the addition of 8-HQ compound and is essential for corrosion inhibition properties.

5.2.4 Mechanical strength

The mechanical strength of the fabricated microcapsules was investigated using the single-capsule compression test [206]. Single microcapsule was placed under the compression rod and the diameter of the tested microcapsules was measured based on the known diameter of the compression rod, which is 2 mm. Figure 5.4 shows the load-displacement curve of the PMF shell microcapsules under compression and the maximum load of the tested microcapsules was $18.81 \pm 0.92\text{ mN}$. Based on the load-displacement curve, it is apparent that the PMF shell microcapsules display brittle failure mode. Additionally, the normalized maximum strength, $\sigma_{\max}$ of the microcapsules is calculated using the formula below [29]:

$$\sigma_{max} = \frac{4P_{max}}{\pi(D_o^2 - D_i^2)} \quad (5.1)$$

where $P_{\max}$ is the maximum load, D_o and D_i are the outer and inner diameter of the PMF shell microcapsules respectively.

The normalized maximum strength of the fabricated PMF shell microcapsules is $30.21 \pm 3.3\text{ MPa}$ based on the formula above. This calculated strength value was then compared the strength of microcapsules measured using the same single-capsule compression test setup, which are urea-formaldehyde shell [206], polyurethane shell microcapsules [29] and etched hollowed glass bubbles (HGBs) [180].

Figure 5.4 Single-capsule compression test results with (a) Load-displacement curve of the fabricated PMF shell microcapsules; (b) Comparison of normalized maximum strength between microcapsules with different shells and the fabricated PMF shell microcapsules.

According to Figure 5.4(b), the strength of the fabricated PMF shell microcapsules is an order of magnitude and 5 times higher than the strength of PUF and polyurethane shell microcapsules respectively. Additionally, the strength is also more than twice the strength of hollow glass bubbles (HGBs), which was previously shown to possess high strength as a non-polymer reservoir. These comparisons demonstrate the robustness of the fabricated PMF shell microcapsules, which may eventually broaden the practical use of these microcapsules in a wider range of applications.

5.3 Antibacterial test of the multifunctional microcapsules

5.3.1 *E. coli*

The antibacterial activity of the fabricated multifunctional microcapsules was examined systematically based on the ASTM E2315 time-kill test. As the antibacterial activity originates from the released core material, the effect takes place gradually as the contact time prolongs.

According to Figure 5.5, the antibacterial effect was clearly observed as contact time prolongs while the bacteria without the addition of microcapsules can grow healthily without any problem.

Clove oil is an essential oil with eugenol as its main constituent. Excellent antibacterial behavior of the microcapsules originates from the interaction between the released eugenol compound and the bacteria cell membrane, which disrupts and permeabilizes the membrane to cause leakage of nutrients required for healthy cell growth [115, 229, 230]. Additionally, 8-HQ was proven to exhibit antimicrobial activity against a wide range of bacteria strains based on its ability to chelate metal ions that are essential for the metabolic processes [234]. In summary, both clove oil and 8-HQ exhibit antibacterial effect against *E. coli*.

To further understand the antibacterial mechanism and the potential synergistic effect from the mixture of clove oil and 8-HQ, the antibacterial effect of the fabricated microcapsules containing 20 wt% 8-HQ dissolved in clove oil was compared against that of microcapsules containing only clove oil and 8-HQ respectively. Figure 5.6 shows the bacterial viability of *E. coli* at different time interval for different types of microcapsules. It is apparent that all three types of microcapsules with different core materials – clove oil, 8-HQ and mixture of clove oil and 8-HQ, exhibit excellent antibacterial behavior with similar trend. In particular, the antibacterial effect of PMF shell microcapsules containing only clove oil was observed earliest as compared to the other two. Hence, it can be assumed that clove oil plays a relatively important role for the antibacterial effect of the fabricated microcapsules. Even though there is no vivid synergistic antibacterial effect based on the mixture of these two compounds, the presence of 8-HQ does not compromise the antibacterial effect of the released clove oil from the microcapsules. In conclusion, clove oil was proven to be an effective antibacterial agent in the fabricated microcapsules, other than serving as an environmental friendly solvent for encapsulating 8-HQ.

Figure 5.5 Time-kill test results against *E. coli* at different time intervals: (a1) 0h; (a2) 4h; (a3) 8h; (a4) 11h; (a5) 24h; (a6) 48h for control sample without any microcapsules; (b1) 0h; (b2) 4h; (b3) 8h; (b4) 11h; (b5) 24h; (b6) 48h for synthesized microcapsules. Scare bar represents 2cm.

Figure 5.6 Comparison of antibacterial effect of microcapsules containing different core material: clove oil, 8-HQ dissolved in toluene and 8-HQ dissolved in clove oil.

5.3.2 *V. coralliilyticus*

Similar to *E. coli*, the antibacterial effect of the fabricated multifunctional microcapsules was observed against *V. coralliilyticus*, a marine bacteria species that belongs to the genera *Vibrio*, which were usually used as the representative of marine bacteria to test the effectiveness of antifouling agents or paint coatings [223]. *V. coralliilyticus* is a globally distributed bacterium with temperature dependent pathogenicity in coral and was found in marine biofilm [224]. Hence, antibacterial activity of fabricated microcapsules against *V. coralliilyticus* was examined to serve as one of the preliminary indications on the potential of the fabricated microcapsules containing clove oil to treat biofouling.

Figure 5.7 Time-kill test results against *V. coralliilyticus* at different time intervals: (a1) 0h; (a2) 1h; (a3) 3h; (a4) 5h; (a5) 7h; (a6) 24h for control sample without any microcapsules; (b1) 0h; (b2) 1h; (b3) 3h; (b4) 5h; (b5) 7h; (b6) 24h for synthesized microcapsules. Scale bar represents 2cm.

According to Figure 5.7, the antibacterial effect against *V. coralliilyticus* was also observed as the contact time prolongs. The antibacterial mechanism is similar to that for *E. coli*, which is related to the gradual release of clove oil and 8-HQ from the microcapsules.

5.4 Antibacterial test of multifunctional coating

Multifunctional coatings were fabricated based on the addition of the fabricated microcapsules partially embedded in epoxy coating matrix. According to Figure 5.8, the microcapsules are partially embedded in the epoxy matrix by spreading the microcapsules on the epoxy surface after curing for 3 hours at 40 °C. As the epoxy matrix is partially cured, it becomes more viscous and prevents the microcapsules from completely sinking to the bottom. As a result, an epoxy coating with partially embedded microcapsules can be synthesized as shown in Figure 5.8(a).

The mechanism of the multifunctional coating for both antibacterial and anticorrosion features was illustrated in Figure 5.9. The time-kill antibacterial test from the previous section has demonstrated the excellent antibacterial properties of the fabricated microcapsules. Similarly, the antibacterial mechanism of the multifunctional coating is shown in Figure 5.9(a), depicting the

Figure 5.8 Fabrication of epoxy with partially embedded microcapsules through controlling the curing time of the epoxy matrix.

Figure 5.9 Mechanism of multifunctional coatings with antibacterial and anticorrosion features: (a) antibacterial effect of the coating based on the released clove oil from the microcapsules; (b) upon mechanical damage to the coating, the 8-HQ dissolved in clove oil is released from the broken microcapsules; (c) the reaction of 8-HQ with the exposed steel substrate forms an insoluble complex to act as a passivation layer to retard corrosion attack.

released clove oil from the microcapsules, which eventually disrupts the cell membrane and causes cell death [115, 229, 230]. Additionally, the multifunctional coating based on the fabricated multifunctional microcapsules possesses anticorrosion feature simultaneously, protecting the underlying substrate from corrosion attack during physical damage which exposes the substrate to the corrosive medium. The anticorrosion mechanism of the multifunctional coating is illustrated in Figure 5.9 (b) and (c). Figure 5.9(b) demonstrates the release of 8-HQ dissolved in clove oil from the broken microcapsules upon mechanical damage to the coating. Immediately after the breakage of microcapsules, the released 8-HQ compounds react with the iron atom from the steel substrate to form insoluble complexes that act as a barrier between steel surface and corrosive medium. As shown in Figure 5.9(c), the protective barrier retards corrosion attack through preventing or minimizing the dissolution of corrosion species such as Cl^- ions and

O₂ gas. The adsorption of these molecules is through chemisorption, which involves the charge sharing or transfer from the nitrogen molecules to the iron atom from the metal surface to form a coordinate type bond [141].

In the previous section, time-kill test results have successfully indicated the excellent antibacterial performance of the fabricated microcapsules against both *E. coli* and *V. coralliilyticus*. Subsequently, zone inhibition test was conducted on the epoxy coating with partially embedded multifunctional microcapsules containing clove oil and 8-HQ against *E. coli*. The schematic representation of the coating is shown in Figure 5.9(a). On the basis of SEM images in Figure 5.8, approximately half of the multifunctional microcapsules were exposed to the outside of the surface while another half is embedded in the coating. The exposure of the microcapsules outside the surface of the epoxy matrix allows the diffusion of clove oil and 8-HQ from the microcapsules to eventually cause cell death of the marine bacteria tested.

Figure 5.10 Zone inhibition test against *E. coli* for (a) epoxy coating containing fabricated multifunctional microcapsules showing a clear inhibition zone without the growth of *E. coli* and (b) blank epoxy coating showing no inhibition zone.

According to Figure 5.10, a clear inhibition area was observed around the multifunctional coating while the bacteria remain viable around the blank epoxy coating without any inhibition area. According to the zone inhibition test results, it can be concluded that epoxy coating containing the fabricated multifunctional microcapsules possess excellent antibacterial performance against *E. coli*, one of the commonly found bacteria on the ship hull [227]. The antibacterial performance of the coatings originates from the released core materials from the microcapsules submerged on the surface of the coating, which manage to diffuse across the marine agar and inhibit the growth of *E. coli* around the coating, forming a clear inhibition area as shown in Figure 5.10(a). In conclusion, the zone inhibition test results confirm on the antibacterial mechanisms proposed in the schematic diagram as shown in Figure 5.9(a), which originates from the presence of partially embedded microcapsules in the system.

5.5 Anticorrosion test of multifunctional coating

5.5.1 Visual inspection

To evaluate the anticorrosion performance of the multifunctional coating, different types of coating – epoxy coating with 8-HQ compound directly added through dissolving in xylene, blank epoxy coating and multifunctional coating with embedded multifunctional microcapsules, were prepared. Subsequently, these coatings were manually scratched using a razor blade and immersed in a 3.5 wt% NaCl aqueous solution at room temperature. After immersion for 24h, visual inspection was carried out on these coatings on their respective degree of corrosion. According to Figure 5.11, severe rust was formed and observed in the scratched regions of both blank epoxy coating and epoxy coating with 8-HQ compound directly added while the scratched

areas of the multifunctional coating with embedded microcapsules were completely free of rust. It is apparent that the scratched region of the multifunctional coating contains some black color solids, which may be responsible for the superior anticorrosion behavior over the other two coatings. The superior anticorrosion performance of the multifunctional coating originates from the presence of encapsulated 8-HQ compound, possessing excellent anticorrosion properties based on the formation of inhibition layer, which corresponds to the black color solids observed in Figure 5.11(c).

Both pure epoxy coating and epoxy coating with 8-HQ compound directly added into the epoxy matrix demonstrates poor anticorrosion properties. The scratches made on these coatings expose the underlying substrate to the corrosive medium immediately after the scratches were made. Unlike the multifunctional coating, the black color inhibition layer was not present to retard the corrosion attack by blocking the active sites and hence severe rust was observed.

Figure 5.11 Optical images after one day immersion in 3.5 wt% NaCl solution for (a) epoxy coating with 8-HQ directly added during the epoxy curing process, (b) epoxy coating and (c) epoxy coating with 20 wt% of PMF shell multifunctional microcapsules.

In particular, it is relatively important to note the absence of anticorrosion properties of the epoxy coating with 8-HQ compound directly added. Despite the addition of 8-HQ compound, the anticorrosion performance was not observed. This is due to the undesirable interaction between 8-HQ and the epoxy matrix, resulting in the loss of corrosion inhibition effect of 8-HQ. This is apparent from the color of the epoxy coating with 8-HQ compound directly added, which is red in color showing the premature reaction between 8-HQ compound and the epoxy matrix during the curing process. Hence, this highlights the importance of encapsulating 8-HQ compound to render the coating with excellent in-situ anticorrosion properties at the damage sites of the coatings.

5.5.2 SEM analysis of the scratched region

To further evaluate the anticorrosion performance, the coatings were immersed in 3.5 wt% NaCl solution for up to 10 days. Subsequently, the coatings were removed through a sonication process in acetone solution to allow detailed studies on the degree of corrosion on the underlying steel surfaces. According to Figure 5.12, the improved anticorrosion performance based on the addition of multifunctional microcapsules containing clove oil and 8-HQ was observed. Figure 5.12(a) shows the corroded areas around the scratched region. This is due to the penetration of the corrosive medium across the scratch, causing more loss of materials around the scratches. The corroded areas around the scratched regions were further confirmed through the zoomed-in SEM images as shown in Figure 5.13(a). It is apparent from the SEM images that more materials were corroded around the scratched regions of the blank epoxy coating resulting in a rougher surface with material losses. This indicates the poorer sealing effect of the blank epoxy coating which allows the penetration of corrosive medium.

Figure 5.12 Optical images of the underlying substrate after 10 days immersion in 3.5 wt% NaCl solution for (a) epoxy coating and (b) epoxy coating with embedded PMF shell microcapsules containing clove oil and 8-HQ; (c) SEM image showing the dark color inhibition layer formed by the reaction between 8-HQ compound and the steel surface.

Figure 5.13 SEM images of underlying steel surface after removing the coating with acetone for (a) blank epoxy coating and (b) epoxy coating with 20 wt% PMF shell multifunctional microcapsules after 10 days of immersion in 3.5 wt% NaCl solution.

On the contrary, these corroded areas were not observed around the scratched regions of the fabricated multifunctional coatings. The improved anticorrosion performance around the scratched regions is confirmed through the SEM images shown in Figure 5.12(b). Because of the

presence of inhibition layer as shown in Figure 5.11(c), the penetration of corrosive medium is inhibited by the formation of black color complexes due to the reaction between 8-HQ compound and the iron atom of the steel surface. As a result, the corrosion attack is retarded and better anticorrosion performance was achieved. According to Figure 5.13(b), the zoomed in SEM images of the region around the scratched region of the fabricated multifunctional coating show less or no material loss as compared to the corroded areas around blank epoxy coating as shown in Figure 5.13(a). Additionally, Figure 5.12(c) shows the formation of dark color inhibition layer on the steel surface outside of the scratched regions. The formation of these black dots is related to the slow diffusion of 8-HQ compound from the microcapsules, to react with the steel surface. These inhibition layers can further improve the anticorrosion performance of the coatings by enhancing the barrier properties of the multifunctional coatings.

5.5.3 FTIR and XPS analysis of the inhibition layer formed

The newly formed insoluble complexes were examined using FTIR spectroscopy. The insoluble complex formed at the scratched region were scrapped and collected for FTIR

Figure 5.14 (a) Comparison of FTIR spectra of insoluble complex and 8-HQ compound; (b) Zoomed-in spectra in the range of 1000 to 2000 cm^{-1} .

characterization. Referring to the FTIR spectra in Figure 5.14, there are several shifts in the absorption peaks for the insoluble complex formed as compared to the original 8-HQ compound. Based on the comparison, the peak occurred at 1093 cm^{-1} for 8-HQ, which corresponds to the C-O vibrations, is shifted to 1107 cm^{-1} for the insoluble complex. Additionally, the shift in the absorption peak, from 3142 to 3427 cm^{-1} indicates the change of OH bond in the newly formed insoluble compound. As a result, the shift of these peaks indicates the interaction of oxygen atom with the metal surface atoms. Moreover, the absorption peaks at 1506 and 1579 cm^{-1} are due to C=N stretching in the original 8-HQ compound. These peaks are slightly shifted to 1494 and 1575 cm^{-1} respectively, indicating the change of vibration frequencies of this bond resulted from the interaction of nitrogen atom with the metal surface atoms. Hence, the chemical structure of the insoluble complexes formed based on the interaction of 8-HQ compound and the steel substrate is predicted as shown in Figure 5.16, similar to what was reported in the previous studies [49, 235, 236].

Additionally, XPS analysis was conducted to further confirm the chemical structure of the newly formed insoluble complexes. According to Figure 5.15(b), a high energy peak was observed at the binding energy of 397 eV , which corresponds to the presence of nitrogen in metal nitrides chemical state. Hence, the presence of bonding between nitrogen and the iron atom is confirmed through the XPS analysis. Moreover, a peak was observed at around 709 eV in the XPS spectrum shown in Figure 5.15(a), which corresponds to the presence of iron atom in the chemical state of FeO. Therefore, the reaction between iron atom and oxygen from the 8-HQ compound is confirmed as well. Based on the confirmation of the presence of iron and nitrogen atoms through XPS analysis, the chemical structure of the inhibition layer is predicted to be that

shown in Figure 5.16, which agrees well with the chemical structure suggested in the previous research [49, 235, 236].

Figure 5.15 XPS analysis of the insoluble complexes showing the presence of (a) iron atom in the chemical state of FeO with binding energy of 790.6 eV and (b) nitrogen atom in the chemical state of metal nitride with binding energy of 397 eV.

Figure 5.16 Predicted chemical structures for the insoluble complexes based on literature [49, 235, 236], supported by FTIR and XPS analysis respectively.

5.5.4 Long term storage of microcapsules

Long term storage of the microcapsules is closely related to the quality of encapsulation of the fabricated microcapsules, in which microcapsules with dense polymer shells prevent the leakage of the encapsulated agents over an extended period of time. In this section, the effect of the microcapsules' long term storage stability on the anticorrosion performance was investigated. The multifunctional microcapsules were stored in a vial for different period of time before being used in the epoxy coating for anticorrosion purposes. Subsequently, these coatings with microcapsules of different storage period were scratched and immersed in 3.5 wt% NaCl solution for anticorrosion test. After one day of immersion, the anticorrosion performance is evaluated based visual inspection on the formation of dark color inhibition layer at the scratched region.

Figure 5.17 shows the anticorrosion results of anticorrosion coating with microcapsules of 50, 70 and 100 days of storage period. According to the visual inspection of these anticorrosion coatings, it is apparent that dark color inhibition layer still forms at the scratched region. However, the amount of dark color inhibition layer formed in the scratched region of the anticorrosion coating containing microcapsules after 100 days of storage period is comparatively less and some rust were observed at the scratched region. Nevertheless, anticorrosion coatings with microcapsules after 50 and 70 days of storage period both show excellent anticorrosion performance with a dense inhibition layer formed as shown in Figure 5.17(a) and 5.17(b). No rust was observed for these two samples.

The explanation for the difference in the anticorrosion performance is related to the loss of core material as the storage period increases. According to Figure 5.17(d), it vividly indicates the decrease in core fraction of the microcapsules when the storage period increases from 50 to 70 and eventually 100 days. Nevertheless, the microcapsules possess excellent storage stability as it

demonstrates excellent anticorrosion performance after 70 days of storage period with core fraction maintained at more than 55%. The excellent storage stability of the microcapsules is attributed by the dense PMF shell as shown in Figure 5.2(c), which prevents the premature release of the core material before further processing.

Figure 5.17 Effect of microcapsules storage period: (a) 50 days; (b) 70 days and (c) 100 days on the anticorrosion performance and (d) the core fraction of the microcapsules after different storage period in the vial. Scale bar is 5mm.

5.5.5 Long term anticorrosion performance of the coatings

The anticorrosion performance of the coatings was successfully demonstrated above. The formation of the passivation layer at the scratched region was proven to retard corrosion. However, the purpose of a coating is to protect the underlying substrate for an extended period of time. Hence, the long term anticorrosion performance of the fabrication coatings which depends on the stability of the microcapsules in the coating is important for potential practical applications. Long term anticorrosion performance is essential to ensure that the substrate is protected from corrosion attack for the entire period of applications. Thus, in this study, the long term anticorrosion performance of the epoxy coating containing 20 wt% of multifunctional microcapsules was examined by scratching the coating after immersion in 3.5 wt% NaCl solution for up to a month. In order to achieve excellent long term anticorrosion performance, the long term stability of the microcapsules under a corrosive environment is essential to protect and prevent the premature release of 8-HQ compound from the coatings.

Figure 5.18 illustrates the long-term effectiveness of the anticorrosion coating with embedded multifunctional microcapsules as the dark color complexes as the inhibition layers are formed upon scratching even after 1 month of immersion in 3.5% NaCl solution, retarding the corrosion attack and protecting the underlying substrate. Comparing to the epoxy coating which dense rust was formed on the scratches made, the formation of dark color insoluble complexes successfully retard corrosion attack. Hence, it is concluded that the fabricated multifunctional microcapsules possess excellent stability in the salt water without premature release of 8-HQ compound, allowing in-situ sealing of the scratched region with the formation of dark color complexes. Based on the results, the long term effectiveness of the fabricated anticorrosion coating can

prolong the lifetime of these coatings and successfully protects the substrate for an extended period of service time.

Figure 5.18 Long-term stability of the fabricated microcapsules in anticorrosion coating allows the effective formation of dark color complexes after multiple scratches made on the: (a1) 1st day, (a2) 15th day and (a3) 30th day of immersion in 3.5% NaCl solution. Rust formation was clearly observed in epoxy coating after multiple scratches made on the: (b1) 1st day, (b2) 15th day and (b3) 30th day of immersion in 3.5% NaCl solution.

5.6 Summary

8-HQ as the corrosion inhibitor and clove oil as the natural antimicrobial agent were successfully encapsulated through in situ polymerization reaction. The significant achievements from this study include the followings:

- Fabricated microcapsules are spherical in shape with a diameter of 98 ± 15 μm .
- The PMF shell is very dense with a thickness of 3.67 ± 0.66 μm , rendering the microcapsules higher mechanical strengths as compared to previously reported PU and PUF shell microcapsules, and hollow glass bubbles (HGBs), based on the single-capsule compression test.
- The microcapsules possess excellent antibacterial activity against different types of marine bacteria such as *E. coli*, *V. coralliilyticus* and *E. aestuarii*, indicating the potential use in treating marine fouling problems.
- Zone inhibition and time-kill test results have shown excellent antibacterial performance of the coatings containing the fabricated microcapsules against the seawater bacteria isolated from Sebarok Island and grown in the lab using marine broth 2216.
- Epoxy coating containing the fabricated microcapsules show excellent anticorrosion performance through the formation of inhibition layer to retard corrosion attack near the scratched regions. The inhibition layer was formed through the reaction between 8-HQ compound and the iron atom on the steel surface, which were confirmed through FTIR and XPS studies.
- The microcapsules have a good shelf life as the anticorrosion effect was observed with microcapsules stored in vial for up to 70 days. Additionally, the anticorrosion effect

remains effective at the scratched regions of epoxy coating containing the fabricated microcapsules, up to 30 days of immersion in 3.5 wt% NaCl solution.

- On the basis of excellent antibacterial and anticorrosion properties of the fabricated multifunctional microcapsules, this study successfully validates the feasibility of the ideas to encapsulate two different functional agents – 8-HQ as the corrosion inhibitor and clove oil as the natural antimicrobial agent in single robust microcapsules with good mechanical properties. Additionally, this study proposes an entirely new method of encapsulating the solid corrosion inhibitor compounds by dissolving them in a natural antimicrobial agent rather than a toxic organic solvent such as toluene and xylene, contributing to a more environmental friendly fabrication method.

Chapter 6 Development of ZnO nanosheets decorated microcapsules

6.1 Motivation

Previous research has indicated the use of microencapsulation techniques in different antifouling and anticorrosion applications, primarily aiming to sustain the release and prevent the premature leakage of the encapsulated active agents [7, 51, 184, 237]. As discussed in the previous chapter, microcapsules containing different types of core material – clove oil and 8-HQ are capable of functioning as multifunctional microcapsules for both antibacterial and anticorrosion purposes. In short, the works discussed in the previous chapters generally focused on encapsulating different types of core materials to achieve different functionalities. However, the microcapsules shell interface as another interface to improve on the multifunctional properties of the microcapsules, were not explored.

Recently, multifunctional materials have become the focus of many research fields due to their ability to deliver several functions simultaneously. According to the previous research, several studies focused on improving the properties of the microcapsules by depositing additional functionality on the shell material. For instance, silver nanoparticles were distributed on the surface to increase wall toughness and strength, at the same time improving the thermal stability of the phase change material microcapsules [171]. In another study, multifunctional microcapsules with silver/silica double-layered shell containing phase change materials were fabricated with thermal energy storage, electrical conduction and antimicrobial effectiveness [172]. With higher latent-heat storage and good thermal regulating efficiency, the microcapsules were also found to exhibit high antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*. Other than depositing silver nanoparticles, multifunctional microcapsules containing *n*-

eicosane were fabricated using zinc oxide nanoparticles [174]. Hence, other than the energy storage properties from the encapsulated core material, the shell material with zinc oxide nanoparticles also serves additional functionalities of photocatalysis and antibiosis.

ZnO is a widely used versatile material possessing different characteristics, including its piezoelectric, photochemical and high photocatalytic activity. It is widely assumed to be low cost, non-toxic and biocompatible. ZnO is listed as “generally recognized as safe” (GRAS) by the U.S. Food and Drug Administration. Hence, similar to the use of natural clove oil as discussed in Chapter 4, ZnO is a promising material to replace the toxic antibacterial and antifouling agents in use, such as Diuron, Irgarol and DCOIT. Moreover, ZnO is extensively used in cosmetic, pharmaceutical and medical applications, indicating no harmful effect against human health. Another remarkable application of ZnO is as a photocatalyst for environmental protection due to its high photocatalytic generation of hydrogen peroxide [238, 239]. Additionally, ZnO has been widely used to exhibit antibacterial activities against a wide range of both Gram-positive and Gram-negative bacteria, including *E. coli*, *Salmonella*, *Listeria monocytogenes*, *Vibrio cholerae* and *Staphylococcus aureus* [240-242]. Among these bacteria, it is interesting to note that ZnO nanoparticle possess excellent antibacterial activities against one of the most commonly found marine bacteria, *V. cholera*.

In this study, a novel type of multifunctional microcapsules with zinc oxide (ZnO) nanosheets decorated PMF shell was synthesized via a solution method. This study aims to explore the use of the microcapsules shell interface to incorporate additional functionalities on the microcapsules. It is hypothesized that the deposition of ZnO nanosheets on the shell interface can further improve the antibacterial performance of the microcapsules as well as incorporating other functionalities due to the surface structure resulted from their random deposition.

6.2 Characterization of ZnO nanosheets decorated microcapsules

6.2.1 Shell morphology

The shell morphology of the microcapsules with the deposition of ZnO nanosheets was studied using FESEM characterization. According to Figure 6.1, the microcapsules appear to be spherical in shape, similar to the microcapsules fabricated in the previous two works.

Figure 6.1 Characterizations of ZnO nanosheets decorated microcapsules under FESEM showing (a) overview of the fabricated microcapsules with spherical shape; (b) rough surface morphology based on the deposition of ZnO nanosheets; (c) enlarged image of the ZnO nanosheets showing hierarchical structure; (d) histogram for microcapsule's size distribution.

However, the major difference lies on the surface morphology of the microcapsules shell. Figure 6.1(b) clearly shows the rough surface morphology of the microcapsules as a result of the random deposition of ZnO nanosheets on the microcapsule shell. Additionally, a hierarchical structure was observed as shown in Figure 6.1(c). Based on the use of imageJ on calculating the size of microcapsules fabricated, the microcapsules possess a diameter of $128.6 \pm 22 \text{ }\mu\text{m}$.

6.2.2 EDX analysis

An EDX spectrum was collected on the yellow color spots containing the hierarchical structure as shown in the SEM images on the top right of Figure 6.2. According to the EDX spectrum, the presence of both Zn and O was confirmed, indicating the successful deposition of ZnO on the microcapsules shell surface.

Figure 6.2 EDX analysis to confirm the presence of ZnO nanosheets on the shell material

6.2.3 XRD analysis

Figure 6.3 shows the XRD patterns of the pristine PMF shell microcapsules as the control and ZnO nanosheets decorated PMF shell microcapsules. The broaden peak located at around 22.5 °, as shown in Figure 6.3, can be identified as melamine-formaldehyde ($C_4H_8N_6O$), which is the major chemical component of the microcapsules shell. After deposited with ZnO nanosheets, the intensity of it is weakened, and sharper and stronger diffraction peaks appear as shown in Figure 6.3. The peaks located at around 31.7 °, 34.5 °, 36.2 °, 47.5 °, 56.7 °, 62.8 ° and 67.9 ° can be indexed to planes (100), (002), (101), (102), (110), (103) and (112) of a hexagonal phase structured ZnO (PDF 36-1451) respectively, indicating the successful synthesis of ZnO structures on the shell material.

Figure 6.3 XRD analysis of PMF shell microcapsules and ZnO nanosheets decorated PMF shell microcapsules confirms the deposition of hexagonal phase structured ZnO on the PMF shell materials.

6.2.3 Contact angle measurement

To illustrate the effect of ZnO nanosheets deposition on the final microcapsules, the contact angle measurement was carried out to determine the wettability of the microcapsules. Different types of microcapsules: ZnO nanosheets decorated PMF shell microcapsules and PMF shell microcapsules were compared against their contact angle measurement. Both types of microcapsules were spread evenly on the epoxy coating with approximately half of the microcapsules exposed above the surface by controlling the curing process of the epoxy coating.

According to Figure 6.3, the surface morphologies of these two types of microcapsules were compared. As discussed earlier in Chapter 5, the PMF shell of the microcapsules was formed based on the deposition of PMF nanoparticles as the molecular weight of the PMF polymers increases under an acidic environment. As a result, it is apparent from Figure 6.4(b) that the PMF shell has only a low degree of roughness due to the random deposition of PMF nanoparticles. Nevertheless, Figure 6.4(c) and 6.4(d) illustrates a much rougher surface morphology due to the synthesis of ZnO nanosheets deposited on the PMF shell. According to Figure 6.5, a hierarchical structure was observed on the ZnO nanosheets, which consists of both micro- and nanoscale roughness, contributing to a high contact angle value of $144.2 \pm 4.0^\circ$, very close to the range of superhydrophobicity (contact angle above 150°). On the contrary, the contact angle of the coating with PMF shell microcapsules is only $120.8 \pm 4.3^\circ$, comparatively lower than that of the coating with ZnO nanosheets decorated microcapsules.

Figure 6.4 Comparison of the surface morphologies between PMF shell microcapsules and ZnO nanosheets decorated PMF shell microcapsules: (a) Overview of the PMF shell morphology; (b) Rough morphology constructed by the random deposition of PMF nanoparticles; (c) Overview of the ZnO nanosheets decorated PMF shell morphology; (d) Hierarchical structure owing to the deposition of the ZnO nanosheets.

Figure 6.5 Hierarchical structures of the microcapsules with (a) microscale roughness and (b) nanoscale roughness based on random deposition of ZnO nanosheets.

On the basis of SEM characterization and contact angle measurement, it was proven that the deposition of ZnO nanosheets successfully render the microcapsules with a close to superhydrophobic properties through the presence of hierarchical structure, which can trap sufficient air between the structures. Hence, the contact angle measurement results successfully validates the hypothesis that an additional functionality – superhydrophobicity was successfully incorporated onto the microcapsules which originally possess only anticorrosion and antibacterial properties as discussed in Chapter 5. The superhydrophobic properties of the ZnO nanosheets decorated microcapsules could establish a wider range of applications such as self-cleaning and antifouling applications [30].

6.3 Antibacterial test of the microcapsules

6.3.1 Time-kill test

The antibacterial activity of the microcapsules against different types of marine bacteria were examined using time-kill test method based on ASTM E2315. Similar to the previous chapters,

marine bacteria including *E. coli*, *V. coralliilyticus* and *E. aestuarii* were tested against the fabricated microcapsules to determine the potential of these microcapsules in treating marine fouling problem.

The antibacterial results are based on the colony count using the standard plate count method. The presence of white dots (colony) on the agar plates represents the growth of bacteria in which higher number of colonies count represents higher number of surviving bacteria. Figure 6.6 shows the antibacterial effect from the microcapsules against *E. coli* as the contact time prolongs. For the control sample without any addition of the fabricated microcapsules, the viability of *E. coli* is confirmed as the bacteria grow healthily without any reduction in the colony count. On the contrary, excellent antibacterial effect was observed with microcapsules added, in which the effect is mainly due to the presence of ZnO nanosheet on the microcapsules surface. Similar trend was observed for the antibacterial test against other bacteria, which include *E. aestuarii* and *V. coralliilyticus*, in which the antibacterial test results are shown in Figure 6.7 and 6.8 respectively.

According to the previous research, several antibacterial mechanisms of ZnO were proposed. First, the disruption of the cell membrane due to the cell internalization of ZnO was concluded to be one of the main antibacterial mechanisms, which causes leakage of cellular content and eventually cell death. This is due to the production of reactive oxygen species (ROS) such as hydroxyl groups (OH[•]), peroxide (O₂^{2•}) and hydrogen peroxide (H₂O₂), which are formed due to the interaction between ZnO nanosheets and water [243, 244]. Additionally, the direct contact between ZnO nanoparticles and the bacteria membrane was determined to be another important factor that may cause physical damage to the cell membrane [245, 246].

Figure 6.6 *E. coli* growth for control sample without any microcapsules at different time interval: (a1) 0h; (a2) 3h; (a3) 6h; (a4) 9h; (a5) 24h; (a6) 32h; and the time-kill test results for ZnO nanosheets decorated microcapsules against *E. coli* at different time interval: (b1) 0h; (b2) 2h; (b3) 4h; (b4) 8h; (b5) 11h; (b6) 24h. Scale bar is 2cm.

Figure 6.7 *E. aestuarii* growth for control sample without any microcapsules at different time interval: (a1) 0h; (a2) 3h; (a3) 7h; (a4) 9h; (a5) 24h; (a6) 32h; and the time-kill test results for ZnO nanosheets decorated microcapsules against *E. aestuarii* at different time interval: (b1) 0h; (b2) 3h; (b3) 5h; (b4) 7h; (b5) 9h; (b6) 24h. Scale bar is 2cm.

Figure 6.8 *V. coralliilyticus* growth for control sample without any microcapsules at different time interval: (a1) 0h; (a2) 1h; (a3) 3h; (a4) 5h; (a5) 7h; (a6) 24h; and the time-kill test results for ZnO nanosheets decorated microcapsules against *E. aestuarii* at different time interval: (b1) 0h; (b2) 1h; (b3) 2h; (b4) 5h; (b5) 7h; (b6) 24h. Scale bar is 2cm.

6.3.2 Improvement of antibacterial effect

Previous section has clearly shown the excellent antibacterial activity of ZnO nanosheet decorated shell microcapsules against different marine bacterial strains. This section serves to investigate the improvement of ZnO nanosheet and the antibacterial mechanism originated from ZnO nanosheets.

The antibacterial test of PMF shell microcapsules from Chapter 5 was compared against that of ZnO nanosheet decorated PMF shell microcapsules. According to Figure 6.9, the complete kill of *E. coli* was observed much earlier with the modification of the shell material using ZnO nanosheets. In particular, the complete kill of *E. coli* was observed at the 5th hour of contact with the ZnO nanosheets decorated microcapsules as compared to a much slower 24-48 hours of contact time required for PMF shell microcapsules. As the deposition of ZnO nanosheets is the

only difference between these two types of microcapsules, ZnO is hence determined to have successfully improved the overall antibacterial effect of the microcapsules. These results successfully validate the hypothesis that the deposition of ZnO nanosheets renders the microcapsules improved antibacterial performance, which is essential to exert greater antibacterial effect through multiple routes – release of core materials and presence of antibacterial materials on the shell surface.

To further illustrate the antibacterial mechanism of the fabricated microcapsules with ZnO nanosheets decorated shell, time-kill test against *E. coli* for microcapsules separated from the bacterial suspension using a dialysis bag is compared against those with microcapsules without the use of dialysis bag. According to Figure 6.10, the bactericidal effect of the ZnO nanosheets occurred faster when there is a direct contact (without the use of dialysis bag) between the microcapsules and *E. coli* suspension.

Figure 6.9 Time-kill test against *E. coli* for synthesized microcapsules with different shell material with and without ZnO nanosheets at different time interval: (a1) 0h; (a2) 1h; (a3) 2h; (a4) 4h; (a5) 6h; (a6) 11h for ZnO nanosheet decorated PMF shell and (b1) 0h; (b2) 2h; (b3) 6h; (b4) 11h; (b5) 24h; (b6) 48h for PMF shell.

Under direct contact between ZnO nanosheets and *E. coli*, the complete killing of the bacteria was observed at the contact time of only 8h. However, separating the microcapsules from the bacteria suspension using a dialysis bag prevents the physical contact from the ZnO nanosheets and delays the complete killing of *E. coli*. As a result, the antibacterial effect of the microcapsules relies solely on the release ROS based on the interaction between ZnO nanosheet and water and hence the bactericidal effect occurs later. According to this comparison, it can be confirmed that the physical contact between the ZnO nanostructure and the bacterial cell membrane vividly affects the antibacterial effect of ZnO nanosheet, which is in agreement with the previous research [245, 246].

Figure 6.10 Time-kill test against *E. coli* at different time interval with (a) microcapsules separated from the bacterial suspension using a dialysis bag and (b) microcapsules with direct contact to the bacterial suspension.

Additionally, this antibacterial effect based on the physical contact between microcapsules surface and the bacterial cell membrane was not observed for the aforementioned microcapsules – double layer PU/PUF shell microcapsules and PMF shell microcapsules. Hence, it can be concluded that the rough ZnO nanosheets on the surface improves the overall antibacterial effect through its unique structure in disrupting the cell membrane.

6.4 Anticorrosion test

Anticorrosion test was conducted on epoxy coating with embedded ZnO nanosheets decorated PMF shell microcapsules by comparing against pure epoxy coating and epoxy coating with PMF shell microcapsules. This study serves to examine the effect of ZnO nanosheets on the overall anticorrosion performance of the coatings.

6.4.1 Tafel plot

Tafel plot is based on electrochemical kinetics that relates overpotential rate and the electrochemical reaction rate. The Tafel equation indicates that the change of overpotential is proportional to the log of the resulting corrosion current. Subsequently, the current intensity can be determined to calculate the rate of corrosion.

According to the Tafel plots in Figure 6.11, the corrosion current (I_{corr}) and corrosion potential (E_{corr}) can be obtained by extrapolating both the cathodic and anodic curves for the respective corrosion process of different coatings. The intersection point of the cathodic and anodic curves provides the corrosion potential and corrosion current for the particular coating tested. Additionally, the corrosion rate (CR) is calculated using the equation below:

$$CR(in MPY) = 0.13 \times I_{corr} \times \frac{EW}{\rho} \quad (6.1)$$

in which CR is the corrosion rate in mils per year (MPY), I_{corr} is the corrosion current density (mA/cm^2), EW is the equivalent weight of mild steel and ρ is the density of mild steel (g/cm^3).

The mild steel equivalent weight is estimated to be 28.25 based on previous research [247]. According to ASTM (G. 102-89 of 1999) practice, the calculation of equivalent weight (EW) only involves elements above 1 mass percent. Hence, iron (Fe) is the only element considered for the calculation of EW for mild steel, which is estimated to be 28.25.

On the basis of the Tafel plots in Figure 6.11, it can be concluded that both epoxy coatings containing ZnO nanosheets decorated PMF shell microcapsules and PMF shell microcapsules possess improved anticorrosion properties as compared to the blank epoxy coating. As discussed earlier in Chapter 5, the addition of corrosion inhibitors – 8-HQ compound improves the anticorrosion properties of the coating upon scratching by forming an inhibition layer through its reaction with the iron atom on the steel surface. Similarly, without scratching the coating, the slow release of 8-HQ compound may contribute to the improved anticorrosion properties of the coatings. Figure 6.12 shows the underlying steel surfaces after removing the epoxy coating containing 20 wt% of PMF shell microcapsules using acetone. It was apparent that dark color inhibition layers were formed onto the surface of the underlying steel substrate, which prevents the penetration of the corrosive medium and minimize the corrosion attack. The slow diffusion of 8-HQ compound from the microcapsules reacts with the iron atom on the steel surface, resulting in the formation of corrosion inhibition layer as shown in Figure 6.12b.

Figure 6.11 Tafel plots of blank epoxy coating, epoxy coating with PMF shell microcapsules and epoxy coating with ZnO nanosheets decorated PMF shell microcapsules after immersion in 3.5 wt% NaCl solution for a period of 10 days.

Figure 6.12 SEM images of the underlying substrate after 10-day of immersion in 3.5 wt% NaCl solution showing (a) single dark color inhibition layer and (b) multiple dark color inhibition layer across the surface formed by the reaction between 8-HQ compound and the steel surface.

The electrochemical corrosion parameters including the corrosion potential (E_{corr}), corrosion current (I_{corr}) and corrosion rate (CR) are summarized and tabulated in Table 6.1. According to the electrochemical corrosion measurements results, the current density and corrosion rate is the lowest for epoxy coatings containing ZnO nanosheet decorated PMF shell microcapsules. The improved anticorrosion properties can be explained by the larger surface area of the rough surface resulting from the deposition of ZnO nanosheets [156]. The formation of ZnO nanosheets on the microcapsules surface increase the barrier properties as the large surface area of the ZnO nanosheets absorb more resin on its surface, which eventually enhances the density of the coatings and prevents electrolyte diffusion into the coating matrix.

Table 6.1 Electrochemical corrosion measurements from Tafel plots for different coatings - blank epoxy coating, epoxy coating with PMF shell microcapsules and epoxy coating with ZnO nanosheets decorated PMF microcapsules.

	E_{corr} (mV)	I_{corr} (mA/cm ²)	CR (MPY)
Blank epoxy coating	-286.3	1.279×10^{-7}	5.98×10^{-8}
Epoxy coating with PMF shell microcapsules	-318.8	6.694×10^{-8}	3.13×10^{-8}
Epoxy coating with ZnO-PMF shell microcapsules	-276.1	5.253×10^{-8}	2.46×10^{-8}

6.4.2 Long term anticorrosion performance

To evaluate the long term anticorrosion performance of coating through the addition of ZnO nanosheets decorated microcapsules, the tested coatings were immersed into 3.5 wt% NaCl solution for up to 5 months. According to Figure 6.13(a), it is apparent that the underlying substrate of the blank epoxy coating is heavily corroded as the diffusion of the corrosive medium through the coating causes severe corrosion attack on the steel surface. On the contrary, the steel surface is less corroded for epoxy coating containing PMF shell microcapsules containing 8-HQ and clove oil, as shown in Figure 6.13(b). Several black dots were observed on the surface due to the reaction of 8-HQ released from the microcapsules and the iron atom from the steel surface. The anticorrosion performance of the coating was further improved through the addition of ZnO nanosheets decorated PMF shell microcapsules. It is apparent from Figure 6.13(c) that the corroded areas were reduced significantly as compared to the corroded areas observed on both blank epoxy coating and epoxy coating containing PMF shell microcapsules without the

Figure 6.13 Underlying substrate after 5 months of immersion in 3.5 wt% NaCl solution for (a) blank epoxy coating; (b) epoxy coating containing 20 wt% PMF shell microcapsules and (c) epoxy coating containing 20 wt% ZnO nanosheets decorated PMF shell microcapsules.

deposition of ZnO nanosheets. The improved anticorrosion effect is mainly due to the better affinity of the microcapsules with the coating matrix, which provides improved barrier properties to prevent the diffusion of corrosive ions across and into the coating.

6.5 Summary

ZnO nanosheets were successfully deposited onto PMF shell microcapsules through hydrothermal methods. The significant achievement from the decoration of ZnO nanosheets on the shell surface is summarized as following:

- The deposition of ZnO nanosheets was confirmed through FESEM due to the apparent presence of ZnO nanosheets covering the PMF shell surface. Additionally, EDX and XRD analysis also proven the presence of zinc and oxygen element.
- Rough surface and hierarchical structure resulted from the deposition of ZnO nanosheets render the microcapsules with close to superhydrophobic properties, an additional functionality not exposed from the previous chapters.
- ZnO nanosheets decorated PMF shell microcapsules also possess excellent antibacterial effect against several marine bacteria such as *E. coli*, *V. coralliilyticus* and *E. aestuarii*. The antibacterial effect was improved through the deposition of ZnO nanosheets as earlier complete kill of the bacteria was observed.
- Direct contact of the bacteria with the ZnO nanosheets decorated microcapsules reduce the duration required to achieve complete kill, indicating physical contact between the ZnO nanosheets and bacteria cell membrane to be an important antibacterial mechanism.
- Tafel plots determine better anticorrosion effect of epoxy coating containing ZnO nanosheets decorated PMF shell microcapsules as compared to blank epoxy coating and epoxy coating containing PMF shell microcapsules. Improved affinity of the

microcapsules to the coating matrix is contributed by the rough ZnO nanosheets, which further prevents the diffusion of corrosive ions across the coating.

- In summary, the deposition of ZnO nanosheets have successfully improved the existing anticorrosion and antibacterial performance of the microcapsules, at the same time incorporating close to superhydrophobic properties to the microcapsules. The results successfully validate the hypothesis that microcapsules shell interface can be designed to incorporate additional functionality as well as improving the existing functionalities originated from the encapsulated core materials.

Chapter 7 Field test of marine fouling at Sebarok Island, Singapore

7.1 Motivation

Previous chapters focused on illustrating the fabrication and characterizations of different types of microcapsules with different shell and core materials. Additionally, the functionalities of the microcapsules were demonstrated through anticorrosion and antibacterial tests against different types of marine bacteria. However, experiment done in a lab setting cannot sufficiently confirm the practicality for their industrial applications such as for the marine industry specifically. Marine fouling remains as a complicated problem which involves many different types of micro- and macro-organisms. The antifouling agent in use today include Irgarol, Diuron, copper pyrithione, zinc pyrithione and 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT), as the successors for tributyltin (TBT) compound which was banned due to its toxicity [208, 209]. However, the toxicity of these antifouling agents currently in use has become an important concern as they can cause severe negative environmental impacts [61, 65, 67, 210]. Hence, more environmental friendly alternatives are desired to replace the use of these toxic antifouling agents.

According to the previous studies, field test is essential to determine the antifouling effect against different types of marine settlement under seawater environment. The use of proteases as a novel antifoulant against larval settlement was studied by incorporating these proteases in a water-soluble matrix and exposing it to larval settlement in a field experiment [82]. Additionally, the effect of roughness of polysiloxane surfaces on the antifouling performance was studied by immersing the samples in seawater for 1 and 2 months respectively. Subsequently, the antifouling performance was evaluated based on the coverage percentage of different macro-organisms such as the algae, barnacles, bryozoans, ascidians and tubeworms [88]. The effect of aryldiazonium saccharide coatings on biomass accumulation on the surfaces of different

materials was investigated by immersing in coastal waters over a 20 days period [22]. The performance of the coating was subsequently justified through different techniques including infrared spectroscopy, light microscopy, scanning electron and He-ion microscopy.

To examine the effectiveness of the fabricated microcapsules containing the proposed natural antifouling agent- clove oil, antifouling field test in the marine seawater is essential. In this chapter, the marine field tests were conducted at Sebarok Island (1.206 °N, 103.8 °E), Singapore under the collaboration with Singaport Cleanseas Pte Ltd. Specimens with epoxy coatings containing different types of fabricated microcapsules were immersed into the ocean for a certain period of time before characterization of the coatings for their antifouling performance. The characterization in this study mainly focuses on the visual inspection and SEM characterization. Visual inspection allows the calculation of surface coverage percentage with fouling organisms through the use of ImageJ software while SEM characterization confirms the structure of the fouling organisms such as the barnacles, tubeworms and algae which were found onto the immersed coatings in the seawater.

7.2 Antibacterial test against marine biofilm-forming bacteria

Previous chapters have shown the antibacterial test of different types of microcapsules containing clove oil as the antifouling agent against several marine bacterial strains, such as *E. coli*, *E. aestuarii* and *V. coralliilyticus*. However, there is a wide range of marine bacteria that may contribute to the formation of marine biofilm, which may eventually cause other micro and macro-organisms to attach to the surface. Thus, antibacterial test against marine biofilm-forming bacteria extracted and swapped off from an on-site contaminated sample is a more comprehensive indication on the potential use of these microcapsules in tackling antifouling problems.

The antibacterial test against the marine biofilm-forming bacteria was conducted based on the time-kill test procedure. 0.4g of different types of the fabricated microcapsules containing was brought into contact with the marine biofilm-forming bacteria suspension with a concentration of approximately 1×10^6 cfu/ml. According to Figure 7.1, it is apparent that the marine bacteria were killed as the contact time between the double layered PU/PUF shell microcapsules containing clove oil and the bacteria suspension increases. On the contrary, the bacteria remain viable in the control sample without the addition of the microcapsules. Hence, it can be proven

Figure 7.1 Time-kill test against marine biofilm-forming bacteria for synthesized microcapsules with different surface roughness at different time interval: (a1) 0h; (a2) 2h; (a3) 4h; (a4) 6h; (a5) 24h; (a6) 32h for control sample without any microcapsules; (b1) 0h; (b2) 2h; (b3) 4h; (b4) 6h; (b5) 24h; (b6) 32h for microcapsules with low surface roughness; (c1) 0h; (c2) 2h; (c3) 4h; (c4) 6h; (c5) 24h; (c6) 32h for microcapsules with high surface roughness. Scale bar is 2cm.

that the fabricated microcapsules containing clove oil are indeed effective against the isolated marine biofilm-forming bacteria from an on-site contaminated sample, but not only to the individual marine bacteria tested in the previous chapters.

Similarly, antibacterial effect of the PMF shell microcapsules containing 8-HQ and clove oil was also shown according to Figure 7.2. The release of both 8-HQ and clove oil from the microcapsules successfully damages the cell membrane of the seawater bacteria and causes cell death. Additionally, as discussed previously in Chapter 6, the deposition of ZnO nanosheets on

Figure 7.2 Time-kill test against marine biofilm-forming bacteria at different time interval with (a) PMF shell multifunctional microcapsules; (b) ZnO nanosheets decorated PMF shell microcapsules separated from the bacterial suspension using a dialysis bag and (c) ZnO nanosheets decorated PMF shell microcapsules with direct contact to the bacterial suspension. The scale bar is 2 cm.

the PMF shell microcapsules results in excellent antibacterial effect as compared to the original PMF shell. Similar trend is observed against the marine biofilm-forming bacteria isolated from the on-site contaminated samples as shown in Figure 7.2. Additionally, with the direct contact of these microcapsules to the bacteria suspension, the complete kill of the bacteria was observed earlier due to the physical contact between the nanosheets and the cell membrane of the bacteria. In particular, when 0.4g of ZnO nanosheets decorated PMF shell microcapsules were in physical contact with 20ml of bacteria suspension with a concentration of approximately 1×10^6 cfu/ml, the complete kill of the marine biofilm-forming bacteria was observed at the 3rd hour while the complete kill was observed at only the 5th hour when the microcapsules were physically separated from the bacteria suspension.

7.3 Antibacterial test of coatings against seawater bacteria

Zone inhibition test was conducted on the coating with microcapsules half embedded on the surface of epoxy coating. This can be achieved by controlling the curing time of epoxy matrix. In particular, the fabricated microcapsules were spread onto the epoxy coating after curing for 3 hours at 40 °C. As the epoxy is partially cured, the viscosity increases and serves as a force to prevent the microcapsules from sinking into the coating. As a result, epoxy coatings with microcapsules approximately half embedded on the surface were successfully fabricated as confirmed by the SEM images shown in Figure 5.8.

Epoxy coatings with half embedded microcapsules were used in the lab setting to demonstrate the antibacterial behavior originated from the microcapsules. As a high number of bacteria is introduced in the antibacterial test, the diffusion of the encapsulated antimicrobial agents across the exposed microcapsules shell will be sufficient to demonstrate the antibacterial effect against the fast-growing bacteria in the zone inhibition test. Hence, epoxy coatings with half-embedded

microcapsules were utilized to accelerate the release of encapsulated core materials to demonstrate the antibacterial effect originated from different types of microcapsules fabricated in this study.

Additionally, exposing the microcapsules on the surface of epoxy coating is related to the antifouling mechanism of epoxy coatings with fully embedded microcapsules. According to a commercialized product “Coppercoat”, a high performance antifouling system that can last for more than 10 years was designed through the use of epoxy matrix containing pure copper. The erosion rate of the antifouling system based on epoxy coating is about 0.25 mil/year [248]. Hence, the slow erosion rate of the epoxy coating renders the antifouling system a longer period of effective use and control the amount of effective antifouling agent released to the environment. During the erosion process, the microcapsules originally fully embedded in the coating will become exposed, causing a similar structure of half-embedded microcapsules on the surface of epoxy coating eventually. Therefore, a zone inhibition test of epoxy coatings with half-embedded microcapsules is demonstrated here to evaluate the potential antifouling performance through proving the antibacterial properties of the coatings against the seawater bacteria.

According to the zone inhibition test, all epoxy coatings with half-embedded microcapsules including double layered PU/PUF shell microcapsules containing clove oil, PMF shell microcapsules containing clove oil and 8-HQ, and ZnO nanosheets decorated PMF shell microcapsules containing clove oil and 8-HQ, possess antibacterial properties against the seawater bacteria. As shown in Figure 7.3, clear inhibition zones were observed around all three epoxy coatings containing different types of half-embedded microcapsules. The antibacterial effect of these coatings originates from the released core materials- clove oil and 8-HQ from the half-embedded microcapsules as well as from the presence of ZnO nanosheets for the ZnO

nanosheets decorated microcapsules. These results act as a supplementary for the time-kill test results of the microcapsules from the previous chapters and successfully demonstrate the antibacterial properties of the epoxy coatings containing the fabricated microcapsules.

Figure 7.3 Zone inhibition test results for (a) blank epoxy coatings; (b) epoxy coating with half-embedded double layered PU/PUF microcapsules containing clove oil; (c) epoxy coating with half-embedded PMF shell microcapsules containing clove oil and 8-HQ and (d) epoxy coating with half-embedded ZnO nanosheets decorated microcapsules. Scale bar is 5mm.

Table 7.1 Inhibition zone (mm^2) produced by different types of half-embedded microcapsules antibacterial coating (21mm*21mm).

Types of half-embedded microcapsules	Inhibition area (mm^2)
Double layered PU/PUF microcapsules containing clove oil	859.5 ± 82.9
PMF shell microcapsules containing clove oil and 8-HQ	1277.3 ± 219.1
ZnO nanosheets decorated PMF shell microcapsules	906.8 ± 76.6

Additionally, the antibacterial effect of the coatings was compared by measuring the inhibition area without any bacterial growth. On the basis of Table 7.1, epoxy coatings with half-embedded PMF shell microcapsules containing clove oil and 8-HQ possess the largest inhibition area among the three. This phenomenon successfully proves on the antibacterial properties of 8-HQ, which was released and diffused across the marine agar to inhibit the growth of seawater bacteria around the coating. As a result, the inhibition area is larger as compared to that of the epoxy coatings with half-embedded double layered PU/PUF microcapsules containing only clove oil. On the contrary, the inhibition area of the epoxy coatings containing half-embedded ZnO nanosheets decorated microcapsules is not the largest among the three. Possible explanation for this phenomenon includes the limited diffusion of zinc ions across the marine agar and the lower core fraction for ZnO nanosheets decorated microcapsules as compared to the other two types of microcapsules. ZnO nanosheets are not released and always remain intact on the surface of the microcapsules. Hence, the antibacterial effect based on the release of zinc ions and physical contact of the nanosheets is more focused on the bacteria seawater directly in contact with the testing surface of epoxy coatings containing half-embedded microcapsules, causing a smaller inhibition zone around the coatings. Nevertheless, inhibition zones were observed for all three epoxy coatings containing different types of half-embedded microcapsules as shown in Figure 7.3.

7.4 Characterization of the antifouling performance

The coatings with different types of microcapsules and formulation were immersed into the seawater at Sebarok Island, Singapore (1.206167, 103.796210). Epoxy coating (Epilam 5015 resin) were used for the antifouling tests, as epoxy coatings are largely used in marine coatings today for protection against corrosion, fouling and abrasion [249-252]. Additionally, previous

research has shown the use of epoxy resin as the polymer matrix for different antifouling paints containing different antifouling agents such as the metallic copper and fatty acid derivatives [253, 254]. The slow erosion rate of epoxy resin controls and reduces the amount of antifouling agents released from the antifouling coatings, eliminating any negative environmental impact that may arise upon excessive release of the antifouling agents. Hence, epoxy resin is used as the polymer matrix in this antifouling field test.

Control sample with blank epoxy coating was exposed to the same environment as the testing epoxy coatings containing different types of fabricated microcapsules. After immersion for a certain period of time (15, 30 and 45 days), the coatings were retrieved from the immersion site and accessed accordingly. Evaluation of the antifouling performance was assessed according to the ASTM D6990, Standard Practice for Evaluating Biofouling Resistance and Physical Performance of Marine Coating Systems [255]. In particular, all the coatings including the control and testing epoxy coatings were retrieved from the site after different immersion period. Subsequently, the visual percentage of coverage by the micro- and macro-organisms was determined. According to the previous research, some of the most common micro- and macro-organisms involved in the biofouling process include barnacles, oysters, mussels, tubeworms and various types of algae, which correlate well with the organisms attached onto the coatings tested in this study.

7.4.1 Visual inspection

After retrieving the samples from the immersion site, the samples were transferred back to the lab for further characterization and evaluation of the antifouling performance. The evaluation of antifouling performance was done based on the visual inspection and determination of the fouled surface coverage using ImageJ software. In particular, the image was adjusted using color

threshold and the fouled surface area is measured accordingly using the wand tool to calculate the percentage of surface that was fouled by the marine organisms. The summary of the visual inspection results is shown by comparing the optical images of the coatings after different immersion period – 15, 30 and 45 days in the seawater. Additionally, the types of macro-organisms found on different types of coatings were recorded accordingly and confirmed through SEM characterization.

Figure 7.4 Optical images showing the marine field test results for different coatings after 15, 30 and 45 days of immersion in the seawater: (a) epoxy coatings with double layered PU/PUF shell microcapsules containing clove oil; (b) epoxy coatings with PMF shell microcapsules containing clove oil and 8-HQ; (c) epoxy coatings with ZnO nanosheets decorated PMF shell microcapsules; (d) epoxy coatings with 10% clove oil directly added without encapsulation and (e) blank epoxy coatings. Scale bar is 1cm.

7.4.1.1 Antifouling performance of blank epoxy coatings

Blank epoxy coatings as the control samples were immersed in the seawater for a duration of 15, 30 and 45 days respectively. Subsequently, the fouling conditions of the coatings were summarized as shown in Figure 7.4. According to the visual inspections shown in Figure 7.4e, the fouled percentage of the blank epoxy coatings increases as the immersion period prolongs from 15 to 45 days. The fouling organisms mainly composed of the algae, which formed a dense network on the blank epoxy coatings. This phenomenon is especially vivid for the samples after 45 days of immersion as shown in Figure 7.4(e3), in which a dense network of algae completely covers the coating surface. The specific structure of the algae and other possible macro-organisms were confirmed through SEM characterization, which will be discussed later.

7.4.1.2 Antifouling performance of epoxy coatings containing double PU/PUF shell microcapsules containing clove oil

Double layered PU/PUF shell microcapsules containing clove oil were fabricated as discussed in Chapter 4. The antibacterial tests of the microcapsules against different types of marine bacteria have indicated the potential use of these microcapsules for antifouling applications. Therefore, 10 wt% double layered PU/PUF shell microcapsules containing clove oil were mixed

into epoxy coating to fabricate a potential antifouling coating for the antifouling field test at Sebarok Island, Singapore. According to Figure 7.4a, the overall antifouling performances of the fabricated coatings are generally better than the blank epoxy coating after 15, 30 and 45 days of immersion in the seawater respectively. However, it is apparent that the antifouling performance deteriorates when the immersion time prolongs, as evidenced from Figure 7.4(a1) to (a3). The fouled coverage of the coating was very low after 15 days of immersion while some algae were observed on the coatings after 30 days of immersion. Subsequently, some other macro-organisms which include the tubeworms and bryozoans are attached onto the coatings after 45 days of immersion. The deterioration of antifouling effect can be attributed to the depleting amount of clove oil released from the microcapsules. Nevertheless, the change in fouling degree for epoxy coatings with double layered PU/PUF shell microcapsules containing clove oil over 45 days of immersion in seawater remains comparatively constant as compared to the other coatings shown in Figure 7.5, indicating the controlled release behavior of this coating.

7.4.1.3 Antifouling performance of epoxy coatings containing PMF shell microcapsules containing clove oil and 8-HQ

Robust multifunctional PMF shell microcapsules containing clove oil and 8-HQ were fabricated as discussed in Chapter 5, which possess both anticorrosion and antibacterial properties. According to the time-kill test results, these microcapsules are effective against several marine bacteria, which indicate their potential use to prevent marine biofilm formation and eventually biofouling. These multifunctional microcapsules were dispersed into the epoxy coating and subsequently immersed into the seawater for antifouling field test study. Figure 7.4b shows the antifouling field test results of the epoxy coating containing 20 wt% of multifunctional microcapsules, in which Figure 7.4(b1) to (b3) corresponds to the visual inspection of the coating

after immersion in the seawater for 15, 30 and 45 days respectively. At 15 days of immersion, the coating is almost free of fouling and only some algae were observed after 30 days of immersion. However, a variety of fouling organisms such as tubeworms, bryozoans and barnacles were found on the samples after 45 days of immersion in the seawater, indicating the deteriorating effect of the antifouling coating based on the multifunctional PMF shell microcapsules containing clove oil and 8-HQ. The deterioration of the antifouling performance can be related to the depleting amount of clove oil and 8-HQ released from the coatings.

7.4.1.4 Antifouling performance of epoxy coatings containing ZnO nanosheets decorated PMF shell microcapsules

According to Chapter 6, the decoration of the PMF shell with ZnO nanosheets improved the overall antibacterial effect of the microcapsules through the release of zinc ions and the physical contact of the hierarchical structure of ZnO nanosheets with the bacterial cell membrane. Similar to the other fabricated microcapsules, the ZnO nanosheets decorated PMF shell microcapsules possess excellent antibacterial behavior against a wide range of marine bacterial strain as well as the seawater bacteria isolated from Sebarok Island, Singapore. Epoxy coatings containing these microcapsules were then tested for their antifouling performance in the seawater for duration up to 45 days. According to Figure 7.4d, the coating is almost free of marine fouling after 15 days of immersion in the seawater with a small amount of algae attached on the surface. Subsequently, more algae were observed onto the coatings after 30 days of immersion. However, the epoxy coatings containing ZnO nanosheets decorated microcapsules were severely fouled by a large amount of algae and several other macro-organisms such as the tubeworms and barnacles after 45 days of immersion in the seawater. The huge increase of the fouling degree indicates the short

term antifouling performance of the coatings, which may be related to the poor stability and rapid deposition of ZnO nanosheets in the seawater environment [256].

7.4.1.5 Antifouling performance of epoxy coatings containing 10 wt% clove oil

To illustrate the influence and importance of microencapsulation process, epoxy coatings containing 10% of clove oil directly added into the epoxy matrix were prepared. Figure 7.4c summarizes the antifouling field test results of these coatings after immersing in the seawater for 15, 30 and 45 days respectively. After 15 days of immersion in the seawater, algae and sediments were found on the coatings, covering a large percentage of the coating surface as shown in Figure 7.4(c1). Additionally, small barnacles were found to have attached onto the coating after 15 days of immersion in the seawater. Subsequently, bigger barnacles were observed after 30 days of immersion. After 45 days of immersion in the seawater, the coating surface is further covered by a larger amount of algae and other macro-organisms such as the barnacles and tubeworms. The influence of microencapsulation of clove oil on the final antifouling performance of the coating is further discussed in the next section.

7.4.1.6 Influence of microencapsulation on the antifouling performance

The antifouling performance of epoxy coatings containing 10 wt% of microencapsulated clove oil, epoxy coatings containing 10 wt% clove oil without encapsulation and blank epoxy coating after 30 days of immersion in the seawater was compared as shown in Figure 7.5 to illustrate the importance of encapsulating clove oil. On the basis of Figure 7.5(a) and (b), the fouled percent coverages of the coatings are similar. However, there are some macro-organisms including barnacles and tubeworms found on the epoxy coating with clove oil directly added, which were not found on the surface of the coating with fabricated microcapsules containing clove oil.

The importance of the encapsulation of clove oil is illustrated based on the comparison between epoxy coating with microcapsules containing clove oil and epoxy coating with clove oil added directly without encapsulation, after immersion in the seawater for 30 days. Through the comparison of the surface fouling, the antifouling performance of the epoxy coating containing 10 wt% of double layered PU/PUF shell microcapsules is the best. The antifouling performance originates from the addition of microcapsules containing clove oil because a dense layer of algae was observed on the blank epoxy coating, which indicated the poorer antifouling effect of the coating without the fabricated microcapsules. However, no hard macro-organisms such as the barnacles were observed on the blank epoxy coating. The absence of barnacles on blank epoxy coating may be attributed by the formation of dense algae on the blank epoxy coating. Due to the dense algae settlement, barnacles fail to attach on the surfaces as the chemistry of the surface becomes different and the settlement of barnacles through the cement adhesives became difficult [83]. Therefore, the barnacle cannot be easily attached onto the surface as it may not be favorable to adhere on the fouled algae organisms.

On the contrary, barnacles and tubeworms were observed on the epoxy coating with clove oil directly added without encapsulation, after immersion for 30 days in the seawater. As compared to epoxy coating with microcapsules containing clove oil, the antifouling performance of the epoxy coating with clove oil directly added appears to be poorer and hence, the importance of encapsulation is highlighted. On the basis of these results, it can be inferred that microencapsulation of clove oil is essential to protect clove oil from any undesirable interaction with the epoxy matrix, which may compromise the antifouling performance of the coatings as evidenced from the presence of barnacles only after 15 days of immersion in the seawater, on epoxy coatings containing clove oil without encapsulation.

Figure 7.5 Comparison of marine field test results after 30 days of immersion in seawater of (a) epoxy coating containing 10 wt% fabricated microcapsules with clove oil, (b) epoxy coating containing 10 wt% clove oil directly added into the coating without encapsulation, showing two barnacles (marked by red box) attached firmly on the coating and (c) blank epoxy coating with a dense algae layer on the surface. Scale bar is 1 cm.

7.4.1.7 Calculation of the fouled percentage coverage of the immersed coatings

The antifouling performances of the immersed coatings were evaluated according to the visual inspection. Based on the images taken, the fouled percentage coverage of the immersed coatings was calculated with the use of ImageJ software. In particular, the images are adjusted using the color threshold technique to generate images with a specific color range representing the fouled areas. Subsequently, the fouled area can be selected and measured accordingly using ImageJ software. By dividing the measured area with the total surface area of the coatings, the fouled percentage coverage of the immersed coating can then be calculated. The fouled percentage coverage results are summarized in Table 7.2.

Table 7.2 Fouled percentage coverage of immersed coatings at Sebarok Island, Singapore

Types of microcapsules in epoxy coatings	Fouled Percentage Coverage (%)		
	15 days	30 days	45 days
10 wt% double layered PU/PUF microcapsules containing clove oil	43.6	46.1	51.0
20 wt% PMF shell microcapsules containing clove oil and 8-HQ	8.30	51.6	52.3
20 wt% ZnO nanosheets decorated PMF shell microcapsules	13.6	51.8	81.3
10 wt% clove oil without encapsulation	65.0	48.3	67.8
Blank epoxy	48.0	65.1	67.9

According to Table 7.2, the antifouling performance of the microcapsules-based epoxy coatings is better as compared to both blank epoxy coatings and epoxy coatings containing clove oil without encapsulation after 15, 30 and 45 days of immersion respectively. The results successfully validates on the hypothesis that the antifouling performance of the coatings is improved through the incorporation of microcapsules containing antimicrobial agents that are effective against the marine bacteria, disrupting the marine biofilm formation and exerting antifouling behavior against the marine organisms. The antifouling performance of the microcapsules-based epoxy coatings is especially obvious after 15 days of immersion while the antifouling effect deteriorates at increasing immersion period in the seawater.

First of all, the fouled percentage of epoxy coatings with 10 wt% double layered PU/PUF microcapsules containing clove oil remains relatively constant over 45 days of immersion in the seawater. This could be related to the controlled release of clove oil from the coatings, which render the coatings with evenly distributed antifouling effect across the surface and a sufficient amount of clove oil at different time interval for preserving the antifouling effect. Some algae were observed on the coatings after 15 days of immersion; however, other macro-organisms such as the barnacles and tubeworms were found attached on the coatings after 45 days of immersion, signifying the deteriorating antifouling performance against the macro-organisms. Nevertheless, it is apparent from Figure 7.4 that the amount of fouled organisms attached is significantly less as compared to the blank epoxy coating, as supported by the calculation of fouled percentage in Table 7.2. Hence, it can be concluded that epoxy coatings with 10 wt% of double layered PU/PUF microcapsules containing clove oil possess a superior antifouling effect compared to blank epoxy coating without microcapsules.

Secondly, the fouled percentage of epoxy coatings with 20 wt% PMF shell microcapsules containing clove oil and 8-HQ was only 8.3% after 15 days of immersion with the presence of a small amount of algae, indicating the best antifouling performance among the coatings tested. However, as the immersion period prolongs to 30 and 45 days, the fouled percentage increases with the attachment of more algae and other macro-organisms. The excellent antifouling performance of the coatings after 15 days of immersion may be originated from the release of both clove oil and 8-HQ at the early stage, which successfully combats against the marine fouling attack. Additionally, as the antifouling performance of epoxy coatings with PMF shell microcapsules containing clove oil and 8-HQ is better as compared to that with double layered PU/PUF microcapsules containing only clove oil, it is hypothesized that the addition of 8-HQ is

the primary reason for the excellent antifouling performance at the early stage of immersion. A possible explanation of this phenomenon could be related to the lower molecular weight of 8-HQ (145.15 g/mol) as compared to the molecular weight of clove oil (205.6 g/mol).

The fouled percentage of epoxy coatings containing 20 wt% of ZnO nanosheets decorated microcapsules possesses a similar trend as the PMF shell microcapsules, with an exceptional antifouling performance as compared to the blank epoxy coating after 15 days of immersion in the seawater. The fouled percentage was initially low with only 13.6% after 15 days of immersion in the seawater. Similar to what was discussed earlier, the release of clove oil and 8-HQ plays an important role on the excellent antifouling performance. Additionally, it can be hypothesized that the deposition of ZnO nanosheets renders excellent antibacterial performance towards the marine bacteria as discussed in Chapter 7.2, in which the release of zinc ions from the coatings successfully prevents biofouling at the early stage. However, the fouled percentage rapidly increases to 81.3% after 45 days of immersion, which is a phenomenon not observed from other microcapsules-based epoxy coatings. The severe fouling of the coatings can be explained by the weak stability of ZnO nanosheets in the seawater environment [256], which causes the short lifetime of the coatings in the seawater for antifouling purposes.

Based on the antifouling performance results of different microcapsules-based coating, it was hypothesized that the presence of 8-HQ and ZnO nanosheets is essential to the antifouling performance of the coatings at the early stage of immersion in the seawater. Hence, to further examine on this hypothesis, time-kill tests were conducted using a polymer matrix which allows faster release of the encapsulated agents. To satisfy this condition, PDMS was selected as the polymer matrix used for the time-kill tests as it possesses higher free volume as compared to epoxy, which facilitates earlier release of the encapsulated core materials from the coatings to

exert antibacterial effect during the lab test. Free volume is generally defined as the free space between the entangled polymer chains, which allows the diffusion of molecules across or within the polymer [257]. One of the most used calculation for calculating the specific free volume (V_f) of a polymer is defined below:

$$V_f = V_{SP} - 1.3V_W = \frac{1}{\rho} - 1.3V_W \quad (7.1)$$

where V_{SP} is the specific volume of a polymer which is equal to $1/\rho$ where ρ is the polymer density (g/cm^3) and $1.3V_W$ is the occupied volume in which V_W is the sum of increments of individual Van der Waals volumes.

In the time-kill test, SYLGARD 184 Silicone Elastomer (PDMS) was used as the coating matrix and the polymer density after cured is 1.03 g/cm^3 , which is lower as compared to that of the epoxy coatings used for the antifouling test (Epolam 5015) with a polymer density of 1.12 g/cm^3 after cured. Hence, on the basis of equation 7.1 which indicates the inversely proportional relationship between the specific free volume and polymer density, it can be assumed that the PDMS coating used in this time-kill test has a higher free volume as compared to the epoxy coatings, to facilitate higher amount of released molecules.

Figure 7.6 shows the time-kill test results of PDMS coatings containing different types of microcapsules. It is apparent from the results that only PDMS coatings with PMF shell microcapsules and ZnO nanosheets decorated microcapsules possess antibacterial effect against the seawater bacteria while the effect is absent for the blank PDMS coating and PDMS coatings with double layered PU/PUF microcapsules containing clove oil. These results agree well with the antifouling field test results in Table 7.2, in which the antifouling performance of the coatings containing PMF shell microcapsules and ZnO nanosheets decorated microcapsules is better at the early stage of the test – 15 days of immersion in the seawater.

Figure 7.6 Time-kill test results against seawater bacteria for (a) control blank PDMS coating; (b) PDMS coating containing double layered PU/PUF shell microcapsules; (c) PDMS coating containing PMF shell microcapsules and (d) PMDS coating containing ZnO nanosheets decorated microcapsules. The sequence of 1-6 for each test represents the time interval of 0, 3, 6, 9, 24 and 48 hours respectively, in which excellent antibacterial effect against the seawater bacteria was observed for PDMS coatings containing PMF shell and ZnO nanosheets decorated microcapsules. Scale bar is 2 cm.

On the basis of the time-kill test results, it is shown that the addition of 8-HQ compound and ZnO nanosheets in the formulation of the microcapsules renders the microcapsules-based coatings excellent antibacterial effect against the seawater bacteria, which further validates the

antifouling field test results at the early stage (15 days) of immersion in the seawater. The lab-based time-kill test results using PDMS as a polymer matrix with a higher free volume successfully validates the field test results, indicating an important finding on the key roles of 8-HQ and ZnO nanosheets to combat biofouling at the early stage of immersion.

Another important finding from the antifouling field test is related to the importance of encapsulation of clove oil for improved antifouling performance. Epoxy coatings containing 10 wt% of clove oil without encapsulation shows poor antifouling performance as 51.70% of the surface was fouled after 15 days of immersion into the seawater. Subsequently, the fouling percentage increases to 65.03% after 45 days of immersion, which is almost similar to the blank epoxy coating. Additionally, macro-organisms such as the barnacles were observed much earlier (after 15 days of immersion) on the epoxy coatings containing 10 wt% of clove oil without encapsulation. The presence of barnacles was only observed after 45 days of immersion for epoxy coatings with 10 wt% double layered PU/PUF microcapsules containing clove oil. Hence, on the basis of these observations, it can be concluded that the microencapsulation of clove oil successfully improves the overall antifouling performances of the coatings. Without encapsulation, the undesirable interaction of clove oil with the epoxy matrix during the curing process can cause premature loss of antifouling properties of the clove oil. The volatile nature of the clove oil may also cause partial loss due to evaporation during the curing process. Thus, microencapsulation of clove oil was proven to be essential to prevent the undesirable interactions with the coating chemistry as well as the premature loss of volatile clove oil.

7.4.2 SEM characterization

SEM studies were carried out to identify the presence of different macro-organisms on the different specimens immersed in the seawater by examining on the specific structure of the macro-organisms based on literature.

7.4.2.1 Identification of barnacles

The attachment of barnacles was observed on many structures, which include the rock surfaces, ship hull, pier structure, deep sea applications and etc [258, 259]. Hence, barnacles were identified as one of the most commonly found fouling organisms. As one of the most commonly found fouling macro-organisms, the structure of the barnacles was studied. Barnacles are usually protected by a few calcareous plates, forming a volcano-like structure [258]. Based on the antifouling field test, Figure 7.7(a) shows the barnacle structure with a volcano-like structure, similar to what was reported. Additionally, the top of the barnacles is usually covered by another two plates, which will open during the feeding process to allow the cirri limbs to wave into the water current and retrieve food sources. The plates covering the top of the barnacles were also found in the barnacles collected as shown in Figure 7.7(b), which confirms the presence of barnacles as the fouling organisms.

According to Figure 7.5(b), barnacles were observed onto the coatings with clove oil directly added into the coating after 30 days of immersion in the seawater. Additionally, after a prolonged period of immersion in the seawater (45 days of immersion), barnacles were also found on the epoxy coating containing double layered PU/PUF shell microcapsules. However, based on the SEM images of the barnacles as shown in Figure 7.8, the barnacles found on the epoxy coating containing double layered PU/PUF shell microcapsules after 45 days of immersion were significantly smaller (331.1 μm) as compared to those found on epoxy coating

with clove oil directly added only after 30 days of immersion (1.793 mm). The significant difference in size of the barnacles show the superior antifouling properties of the microcapsules-based epoxy coatings comparing to the epoxy coating with clove oil directly added as the barnacles generally increase in size as they grow on the substrates [260].

Due to the released clove oil from the microcapsules and eventually from the coatings, the attachment of barnacles was inhibited up to 30 days. However, the antifouling effect deteriorates as the immersion period prolong, primarily due to the depleting effective amount of clove oil released, which allows the growth of barnacles after 45 days of immersion in the seawater. On the contrary, large barnacles as shown in Figure 7.7b were found on the epoxy coatings with clove oil directly added after 30 days of immersion in the seawater, primarily due to the premature undesirable interaction of clove oil with the epoxy causing the loss of antifouling effect of the clove oil.

The presence of barnacles illustrates the poorer antifouling effect of the coating as compared to the coating containing 10 wt% of double layered PU/PUF shell microcapsules containing clove oil. For the coatings with clove oil directly added into the coating matrix, two reasons may have contributed to the loss of antifouling effect, as indicated from the growth of barnacles. There are two possible reasons to explain this scenario. First, the undesirable interaction between the coating matrix and clove oil may cause the loss of antifouling effect of the clove oil prior to its release from the coating. Second, the uneven distribution of clove oil across the coating due to the aforementioned undesirable interaction with the coating matrix may result in the growth of barnacles on certain parts of the coating. Through encapsulation of clove oil in a polymer shell, the undesirable interaction can be eliminated by preventing the direct contact between them. Additionally, robust microcapsules without any agglomeration issues promote homogeneous

mixing of the microcapsules in the coating and render the coating antifouling effect across the entire surface.

Figure 7.7 Microimages of the barnacles collected on the immersed coatings showing (a) a calcareous dense shell and (b) the covering plates which function to protect the barnacles and open for feeding process.

Figure 7.8 Microimages of barnacles with different sizes found on different coatings: (a) smaller barnacle (331.1 μm) on epoxy coatings with double layered PU/PUF microcapsules after 45 days of immersion and (b) larger barnacle (1.793 mm) on epoxy coatings with clove oil directly added after 30 days of immersion.

7.4.2.2 Identification of algae

Algae is one of the most commonly found soft macrofouling organisms [89, 261]. According to Figure 7.9, green algae were observed on some coatings after 15 and 30 days of immersion in the seawater. As the immersion time prolongs, the degree of coverage by the algae increases and a dense network of algae can be observed on the fouled surface. As shown in Figure 7.9(b), the SEM images of the algae found on the blank epoxy coatings after 30 days of immersion indicates the formation of a dense network based on the interconnected algae structure.

Figure 7.9 Microimages of the algae found on the blank epoxy coating: (a) some algae with sediments around after 15 days of immersion, (b) dense network of algae after 30 days of immersion.

7.4.2.3 Identification of tubeworms

According to literatures, tubeworms were identified as another type of fouling organisms which live in calcareous tubes which protect their soft bodies (the worms) [262, 263]. Hence, these organisms are named as tubeworms based on the structure with the worms inside a tube. Tubeworms were also observed on some of the coatings tested in the seawater. Figure 7.10 shows the structure similar to the tubeworms, with a clear tube-like structure attached firmly onto the coating. Additionally, the calcareous tube of the tubeworms is brittle in nature and breaks into pieces upon damage as shown in Figure 7.10(b). In this study, tubeworms were identified as one of the most frequently found fouling organisms that attached on the surface of the immersed coatings. In particular, tubeworms were observed on all the coatings tested in this study after 30 days of immersion.

Figure 7.10 Microimages of the tubeworms attached onto the immersed coatings showing (a) a calcareous tube which is brittle and breaks into pieces upon damage as shown in (b).

7.4.2.4 Identification of diatoms

Diatoms were identified as one of the most commonly found fouling microorganisms on the coatings and substrates immersed in the aquatic environment including fresh and marine waters [264]. The diatoms have a siliceous cell wall, which together with the organic layers and components, form the diatom frustules [265]. The frustules are highly ornamented, which allows the formation of a wide range of shapes. The diatoms are generally divided into two types, which are known as the centric and pennate diatoms respectively. The centric diatoms are round in shape and radially symmetrical while the pennate diatoms are elongated and tend to appear bilaterally symmetrical, which were all similar to what was found on the immersed coatings in this study as shown in Figure 7.11(a) and (b). Researchers have estimated up to 2 million species of diatom that can be found on the Earth and more species are still being discovered in many ongoing researches. According to previous literatures, some of the most commonly found diatoms species include *Amphora*, *Achnanthes*, *Navicula* and etc [265].

Figure 7.11 Microimages of the diatoms found onto the immersed coatings in the seawater, confirming the presence of (a) centric with a circular shape and (b) pennate diatoms with an elongated shape.

7.4.2.5 Identification of bryozoans

Bryozoans are tiny colonial organisms which were identified as another common fouling macro-organism. There are different forms of bryozoan colonies, which can be encrusting, arborescent or free living. Additionally, zooids are defined as the individuals within the bryozoan colonies.

According to the SEM images in Figure 7.12, the structure attached on the coatings after immersion in the seawater is similar to what was found for bryozoans based on previous research [266]. Figure 7.12(a) shows the branching zooecium onto the sediments firmly. Zoomed-in image in Figure 7.12(b) clearly shows the presence of zooids and oral spine which resembles the horns of the deers. On the basis of the bryozoans found at Green Island, Taiwan, the possible bryozoan species found in this study could be *Amastigia tricervicornis* sp. nov. The naming of this species is based on the presence of structures similar to a deer's antler, in which *cervus* means deer and *cornus* means horn. Nevertheless, based on previous research on the structure and morphologies of bryozoans, it was confirmed that bryozoans were observed on the coatings in this field test study.

Figure 7.12 Microimages of bryozoans attached on the coatings after immersion in the seawater showing (a) branched structure and (b) U-shaped guts.

7.4.2.6 Summary of macro-organisms found on immersed coatings

Table 7.3 Presence of different fouling organisms on different immersed coatings

Epoxy coating content	Immersion period (days)	Fouling organisms				
		Algae	Diatoms	Barnacles	Tubeworms	Bryozoans
10 wt% double layered PU/PUF microcapsules containing clove oil	15	✓				
	30	✓	✓			
	45	✓	✓	✓	✓	
20 wt% PMF shell microcapsules containing clove oil and 8-HQ	15	✓				
	30	✓	✓			
	45	✓	✓	✓	✓	✓
20 wt% ZnO nanosheets decorated PMF shell microcapsules	15	✓				
	30	✓	✓			
	45	✓	✓	✓	✓	✓
10 wt% clove oil without encapsulation	15	✓	✓	✓		
	30	✓	✓	✓	✓	
	45	✓	✓	✓	✓	✓
Blank epoxy	15	✓	✓			
	30	✓	✓	✓	✓	✓
	45	✓	✓	✓	✓	✓

FESEM characterization of the micro- and macro-organisms found on the immersed coatings has confirmed the presence of barnacles, tubeworms, diatoms, algae and bryozoans. The results correlate well with previous studies, which identified these organisms to be those commonly found fouling organisms from the marine environment [8, 267]. The presence of different types of fouling organisms onto different immersed coatings is summarized in Table 7.3.

7.5 Summary

Antifouling field test is an essential step in realizing the practical application of these microcapsules to tackle the biofouling problems in the marine industry. Hence, this chapter focuses on the antifouling field test at Sebarok Island, Singapore for different epoxy coatings containing different types of microcapsules fabricated. The coatings were immersed for different period of time – 15, 30 and 45 days respectively. Subsequently, the antifouling performance of the immersed coatings was evaluated based on the fouled coverage percentage through visual inspection and the use of ImageJ software. Additionally, SEM characterizations of the fouled organisms were conducted, which investigated on the presence of algae, diatoms, barnacles, tubeworms and bryozoans on the immersed coatings. The antifouling performance of different immersed coatings is summarized as below:

- Microcapsules-based epoxy coatings possess better antifouling performance against the blank epoxy coatings, in which the difference is obvious at the earlier stage of immersion period (15 days). However, the antifouling effect of the microcapsules-based coatings generally deteriorates as the immersion period in the seawater increases to 45 days.
- At the early stage of antifouling field test, it was hypothesized that the presence of 8-HQ and ZnO nanosheets in the microcapsules formulation improves the antifouling effect of epoxy coatings containing PMF shell microcapsules and ZnO nanosheets decorated

microcapsules respectively. Time-kill test results using PDMS coating with higher free volume successfully validates this hypothesis by demonstrating excellent antibacterial results against the seawater bacteria for both PMF shell microcapsules containing clove oil and 8-HQ and ZnO nanosheets decorated microcapsules.

- Clove oil directly added into the epoxy matrix may cause premature loss of antifouling function due to undesirable interaction between clove oil and the epoxy matrix. Additionally, premature loss of clove oil through evaporation may occur during the curing process. As a result, the fouled coverage percentage of this coating is similar to the blank epoxy coating, showing no antifouling effect as well as highlighting the importance of encapsulating clove oil.
- Barnacles, tubeworms, algae and bryozoans were among the fouling organisms observed onto the immersed coatings in the seawater at Sebarok Island, Singapore. The presence of all the macro-organisms were mostly observed after 45 days of immersion in the seawater for the microcapsules-based epoxy coatings while most of the macro-organisms were observed after 15 or 30 days of immersion for the blank epoxy coatings and epoxy coatings containing clove oil without encapsulation.
- Marine environment is complex and harsh. Hence, future research should focus on combining these fabricated microcapsules containing clove oil with other existing natural antifouling technique to achieve synergistic and long term antifouling effect.

Chapter 8 Conclusion and recommendation for future works

8.1 Conclusion

The background of corrosion and biofouling and the progress of research in the field of corrosion inhibitor, antifouling coatings and microencapsulation techniques were reviewed systematically. Current problems of corrosion and biofouling especially in the marine industry where these two issues occur simultaneously remained unsolved to date. Hence, the motivation of this research is to fabricate microcapsules-based multifunctional coatings with both antifouling and anticorrosion features. This research work successfully fabricated different types of microcapsules containing different active agents, such as clove oil as the natural antimicrobial agent and 8-HQ as the corrosion inhibitors. Additionally, the microcapsules shell interfaces were decorated with ZnO nanosheets to improve the antibacterial and anticorrosion properties of the microcapsules while incorporating additional functionalities such as close to superhydrophobicity properties.

My research work can be summarized based on the significant findings from each chapter:

1. Double-layered PU/PUF shell microcapsules containing clove oil were successfully fabricated through interfacial and in situ polymerization methods.
 - The release profiles of the fabricated microcapsules were characterized using UV-Vis Spectrometry, based on the Beer-Lambert law. The release rate of clove oil can be tuned by manipulating the amount of PU reactants added and the length of PUF deposition time. By fitting the release profiles against different models, the release profile of the fabricated microcapsules fits best against the Baker-Lonsdale model, which determines diffusion as the primary mechanism for release.

- This study contributed to a comprehensive understanding on the release characteristics of double layered PU/PUF shell microcapsules containing clove oil. The quantitative analysis based on the calculation of Baker-Lonsdale constant (k_B) values validates the feasibility to fabricate microcapsules with a wide range of diffusivity up to 8 times difference, indicating the versatility of this microencapsulation technique to be employed for a vast range of applications.
 - Antibacterial test results of the double-layered PU/PUF shell microcapsules containing clove oil against different types of marine bacteria including *E. coli*, *V. coralliilyticus* and *E. aestuarii* proposed for the first time the use of volatile clove oil to kill marine bacteria, indicating the potential use as an environmental friendly solution for marine antifouling applications..
2. 8-Hydroxyquinoline (8-HQ) as the corrosion inhibitor and clove oil as the antifouling agent were successfully encapsulated using in-situ polymerization of poly (melamine-formaldehyde) (PMF) as the shell material.
- Robust microcapsules were fabricated with a dense PMF shell, in which the strength of the fabricated PMF shell microcapsules is an order of magnitude, 5 times higher and twice the strength of PUF shell microcapsules, polyurethane shell microcapsules and hollow glass bubbles respectively, leading to excellent pot life of the microcapsules up to 70 days without excessive leakage.
 - Epoxy coatings containing the fabricated microcapsules demonstrate excellent anticorrosion effect by forming an inhibition layer on the scratched region to protect the underlying substrate from corrosion attack.

- Excellent antibacterial activity of the fabricated microcapsules against the marine bacteria *E. coli*, *V. coralliilyticus* and *E. aestuarii* was achieved through the release of encapsulated core materials.
 - Excellent anticorrosion and antibacterial properties of the fabricated microcapsules successfully validate the feasibility of the proposed idea to encapsulate two different functional agents – 8-HQ as the corrosion inhibitor and clove oil as the natural antimicrobial agent in single robust microcapsules with good mechanical properties. A new method of encapsulating the solid corrosion inhibitor compounds by dissolving them in a natural antimicrobial agent rather than a toxic organic solvent such as toluene and xylene is proposed as a more environmental friendly fabrication method.
3. ZnO nanosheets were successfully fabricated on the PMF shell microcapsules containing 8-HQ and clove oil through solution method.
- The successful deposition of ZnO nanosheets on the microcapsules surface was confirmed through FESEM, EDX and XRD analysis, in which the hierarchical structure of the ZnO nanosheets render the microcapsules close to superhydrophobic properties, with a contact angle of $144.2 \pm 4.0^\circ$.
 - As compared to PMF shell microcapsules, the physical contact of ZnO nanosheets with the bacteria cell membrane and the release of zinc ions improved the antibacterial effect.
 - ZnO nanosheets successfully improved the anticorrosion effect through better affinity with the coating matrix to prevent the diffusion of corrosive medium across the coating.

- Results above successfully validate the hypothesis that microcapsules shell interface can be utilized to incorporate additional functionality as well as improving the existing functionalities originated from the encapsulated core materials.
4. Antifouling field test was conducted at Sebarok Island, Singapore to examine the on-site antifouling performance of different microcapsules-based coating.
- Time-kill test results indicate excellent antibacterial properties of all three types of microcapsules against the seawater bacteria isolated from Sebarok Island, Singapore while zone inhibition test results clearly demonstrate the antibacterial performance of the epoxy coatings with all three types of half-embedded microcapsules.
 - The antifouling performance of microcapsules-based epoxy coatings were proven to be better as compared to the blank epoxy coatings, indicating the antifouling properties of the fabricated microcapsules with clove oil, 8-HQ and ZnO nanosheets.
 - Antifouling performance of the microcapsules-based epoxy coatings was obvious at the early stage of immersion especially for microcapsules with the presence of 8-HQ and ZnO. Subsequently, time-kill test results using PDMS coating with higher free volume successfully validates this hypothesis by demonstrating excellent antibacterial results against the seawater bacteria in a lab setting for both PMF shell microcapsules containing clove oil and 8-HQ and ZnO nanosheets decorated microcapsules.
 - Encapsulation of clove oil was demonstrated to be an important factor for the improved antifouling performance of the coatings. This phenomenon can be explained by the uneven distribution of clove oil across the coating due to their undesirable interaction with the coating matrix or some premature loss during the curing process, which may result in the growth of barnacles on certain parts of the coating.

- The antifouling effect of microcapsules-based coatings generally deteriorates after 45 days of immersion in the seawater, primarily due to the depleting amount of clove oil and 8-HQ released from the coatings, indicating the needs of future work in tackling the complex marine biofouling process.

This research work has successfully validated the use of natural clove oil to kill various types of marine bacterial strains, indicating the potential to serve as an environmental friendly antifouling solution. A thorough understanding on tuning the release profiles of the microcapsules was also achieved based on both qualitative and quantitative analysis. Additionally, the use of clove oil to dissolve 8-HQ for encapsulation proposed a more environmental friendly fabrication method to produce multifunctional microcapsules with both anticorrosion and antibacterial properties. The innovative idea of designing the microcapsules shell interface with ZnO nanosheets introduces new functionalities to the microcapsules as well as improving the existing antibacterial and anticorrosion properties. To examine the ideas from the lab setting, antifouling field test was conducted and successfully validates the better antifouling performance of the microcapsules-based coatings. To conclude, this research work involving the use of microcapsules successfully proposes a new perspective into a more environmental friendly solution for the corrosion and biofouling problems in the marine industry.

8.2 Recommendation for future works

8.2.1 Synergistic effect with other essential oils and corrosion inhibitors

The entire research work focuses on the use of clove oil as a natural antimicrobial agent with excellent antibacterial properties against several marine bacteria. According to the literature review, there are many other essential oils that are widely used, which include cinnamon, lavender, lemongrass, rosemary and thyme oils. All these essential oils were proven to exhibit antibacterial properties against a wide range of bacteria. Hence, the future work can focus on the synergistic effect of clove oil with other essential oils to target a wider range of marine bacteria that may be present in the marine environment. Different mixture ratio can be investigated to determine the optimum antibacterial or antifouling performance based on the synergistic effect between different essential oils. This study will serve as one of the future directions to extend the use of essential oils in potentially treating marine biofouling problems.

8-HQ was encapsulated in this study as a corrosion inhibitor that is effective against mild steel. Additionally, previous research has shown the use of 8-HQ as the corrosion inhibitors for pure aluminum, aluminum alloys and magnesium alloys. However, the use of corrosion inhibitors is one of the most traditional anticorrosion approaches. As a result, there are many existing corrosion inhibitors such as the aromatic aldehydes, benzimidazoles, nitrogen-containing heterocycles and quaternary salts. Moreover, natural corrosion inhibitors have been one of the key topics recently which include the use of different plant extracts for corrosion inhibition studies. Therefore, the synergistic effect of 8-HQ with other existing natural corrosion inhibitors can be studied to further improve the anticorrosion performance of the microcapsules-based coatings.

8.2.2 Long term antifouling performance of microcapsules-based coatings

Based on the field test study in Chapter 7, the antifouling effect of the microcapsules-based coatings was demonstrated to be improved as compared to the blank epoxy coating and epoxy coating with clove oil directly added into the coating matrix up to 30 days of immersion in the seawater. However, macro-organisms such as the barnacles and tubeworms were observed on the microcapsules-based coatings after 45 days of immersion. This phenomenon indicates the deteriorating antifouling performance of the microcapsules-based coatings as the immersion time prolongs, most likely due to the depleting amount of clove oil and 8-HQ released.

As discussed in Chapter 4, the release profiles of double layer PU/PUF shell microcapsules were studied and analyzed. Hence, to further improve the long term antifouling performance of the microcapsules-based coatings, the release profiles of the microcapsules-based coating will be studied in the future to determine the amount of encapsulated core materials released at different immersion period and different marine environment. For instance, the temperature of seawater near the surface is higher as compared to that near the seabed. By analyzing these factors, the antifouling performance and lifetime of these coatings can be evaluated for a wider range of potential antifouling applications.

Alternatively, other types of coating matrix with higher or slower dissolution rate can be studied to replace the epoxy matrix, which is known to be waterproof after cured and hence a very slow dissolution rate. For instance, the fabricated microcapsules can be incorporated into the self-polishing coatings available in the market to increase the release rate of the effective antifouling agents from the coatings. Subsequently, the fabricated microcapsules-based coatings can be applied to real industrial applications under different marine environment to evaluate the antifouling effect under practical industrial uses especially for the marine industry.

8.2.3 Incorporation of fabricated microcapsules in commercial coatings

As discussed in Chapter 4-7, the fabricated microcapsules were incorporated into epoxy coating for the subsequent antibacterial, antifouling and anticorrosion tests. The results were generally improved based on the addition of different types of microcapsules, as proven from the respective test results. The lab test results have shown the improvement of antibacterial and anticorrosion properties while the field test trials have indicated the improvement of antifouling behaviors through the addition of the microcapsules.

However, according to the marine antifouling field test, the antifouling effect deteriorates after 45 days of immersion. This phenomenon may be attributed to two different reasons. First, the release rate of clove oil may be too slow, which may cause the insufficient release of clove oil to deter marine fouling. The remedy for this reason has been analyzed and discussed in Chapter 8.2.2 above. Second, the use of fabricated microcapsules containing clove oil alone may not be effective against the harsh and complex marine environment. Hence, the antifouling effects can be further improved by combining the fabricated microcapsules with other existing technologies. In summary, the research findings from this study can be utilized to complement other existing antifouling solutions to boost the antifouling performance and extend the lifetime of the antifouling coatings. The future work will focus on the synergistic effect of fabricated microcapsules with other existing antifouling, antibacterial and anticorrosion coatings.

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List of Publications

1. **Yong Bing Chong**, He Zhang, Chee Yoon Yue, and Jinglei Yang, "Fabrication and release behavior of microcapsules with double-layered shell containing clove oil for antibacterial applications," *ACS applied materials & interfaces*, vol. 10, pp. 15532-15541, 2018
2. Yuefan Wei, **Yong Bing Chong**, Hejun Du, Junhua Kong, and Chaobin He, "Loose yarn of Ag-ZnO-PAN/ITO hybrid nanofibres: Preparation, characterization and antibacterial evaluation," *Materials & Design*, vol. 139, pp. 153-161, 2018/02/05/ 2018.
3. Dawei Sun, **Yong Bing Chong**, Ke Chen, and Jinglei Yang, "Chemically and thermally stable isocyanate microcapsules having good self-healing and self-lubricating performances," *Chemical Engineering Journal*, vol. 346, pp. 289-297, 2018.
4. **Yong Bing Chong**, He Zhang, Xin Zhang, Chee Yoon Yue and Jinglei Yang. "Robust multifunctional microcapsules with antibacterial and anticorrosion features." (Manuscript finished).
5. **Yong Bing Chong**, Yuefan Wei, Chee Yoon Yue, Jinglei Yang. "ZnO-decorated superhydrophobic microcapsules with excellent antibacterial properties." (Manuscript in preparation).