SYNTHESIS OF METAL-TANNATE COMPLEXES AND THEIR APPLICATION AS ANTIFOULANT FOR FISH CAGE NETS

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by

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LIST OF ABBREVIATION AND SYMBOLS

LIST OF ABBREVIATION

AAS	Atomic absorption spectroscopy
Al-T	Aluminium-tannate
Al-TA	Aluminium-tannic acid
ASW	Artificial Seawater
CAE	Catechin equivalent
Cu-OM	Copper omadine
Cu-T	Copper-tannate
Cu-TA	Copper-tannic acid
EDX	Energy Dispersive X-Ray
FB 4	*Pseudoalteromonas lipolytica* strain LMEB 39
FB 9	*Pseudoalteromonas* ruthenica strain KMM300
FB 13	*Bacillus aquimaris* strain TF-12
FBN 2	*Vibrio parahaemolyticus* strain DHC22
Fe-T	Iron-tannate
Fe-TA	Iron-tannic acid
FTIR	Fourier transform infrared spectroscopy
GAE	Gallic acid equivalent
IMO	International Maritime Organization
LC_{50}	Lethal concentration 50
MT	Mangrove tannin
Pt	Platinum
SEM	Scanning electron microscopes
TBT	Tributyltin
UV-Vis	Ultraviolet and Visible Absorption Spectroscopy

LIST OF SYMBOLS

cp	Centipoises
df	Dilution factor
w/v	weight per volume
v/v	volume per volume

SINTESIS KOMPLEKS LOGAM-TANAT DAN PENGGUNAANNYA SEBAGAI ANTIKOTORAN UNTUK JARING SANGKAR IKAN

ABSTRAK

Biokotoran marin (marine biofouling) adalah organisma marin yang melekat pada permukaan yang direndam dalam air laut dengan pembentukannya berpotensi dipengaruhi oleh beberapa proses kimia, fizikal dan biologi. Biokotoran marin ini sangat menjejaskan hasil industri akuakultur kerana meengotorkan jaring ikan. Oleh itu jaring sangkar ikan perlu dilitupi dengan cat antikotoran yang mengandungi agen antikotoran untuk mengelakkan pembentukan biokotoran marin. Agen antikotoran yang berasaskan logam seperti tributilstanum adalah berbahaya kepada alam semulajadi dan telah diharamkan oleh Pertubuhan Maritim Antarabangsa (IMO). Objektif utama kajian ini adalah untuk mencari alternatif bagi agen antikotoran yang lebih mesra alam dengan mensintesis kompleks ion logam-tanat sebagai agen antikotoran yang berasaskan sumber semulajadi. Dalam kajian ini, tiga agen antikotoran baru telah disintesis dengan mengkomplekskan tanin daripada kulit kayu bakau daripada spesies *Rhizophora apiculata* dengan beberapa ion logam iaitu kuprum (Cu), ferum (Fe) dan aluminium (Al). Kondisi optimum penghasilan kompleks logam-tanat dikaji pada beberapa keadaan iaitu kesan dos logam, pH, suhu dan masa tindak balas. Dos logam yang optimum bagi kuprum-tanat (Cu-T) adalah 1: 2, yakni 2 dos logam Cu per tanin bakau (MT) manakala bagi kedua- dua ferum-tanat (Fe-T) dan aluminium-tanat (Al-T) adalah 1:3 dos logam per MT. Suhu 60 ºC, pH 5 dan masa tindak balas 30 minit adalah kondisi yang optimum bagi Cu-T dan Fe-T. Bagi kompleks Al-T pula kondisi optimum pengkompleksan adalah pada, pH 4.5, 45 ºC dan masa tindak balas 30 minit merupakan keadaan optimanya. Sifat kompleks logam-tanat dicirikan dengan menggunakan analisis spektroskopi inframerah Fourier (FTIR), analisis mikroskop elektron pengimbas dengan serakan tenaga X-ray (SEM-EDX), spektroskopi penyerapan atom (AAS), ujian ketoksikan menggunakan anak udang *Artemia salina* sebagai organisma kawalan dan ujian antimikrobial. Berdasarkan ujian ketoksikan, semua kompleks adalah toksik kerana nilai LC_{50} dibawah 1.0 mg mL^{-1}, dengan mengikut urutan iaitu daripada kompleks yang paling toksik kepada yang kurang toksik: kuprum oksida (Cu_2O) (0.018 mg mL^{-1}) > kuprum-asid tanik (Cu-TA) (0.020 mg mL^{-1}) > Cu-T (0.029 mg mL^{-1}) > kuprum omadin (Cu-OM) (0.036 mg mL^{-1}) > ferum-asid tanik (Fe-TA) (0.045 mg mL^{-1}) > aluminium-asid tanik (Al-TA) (0.053 mg mL^{-1}) > Fe-T (0.064 mg mL^{-1}) > Al-T (0.077 mg mL^{-1}) > asid tanik (TA) (0.14 mg mL^{-1}) > MT (0.21 mg mL^{-1}). Ujian antimikrobial menunjukkan kompleks Cu-T dan Cu-TA menunjukkan zon perencatan yang lebih baik melebihi 1.0 cm berbanding dengan kompleks Fe dan Al. Rendaman rumusan cat antikotoran ke dalam air laut menunjukkan prestasi yang baik sehingga tempoh dua bulan. Jaring ikan yang dilitup dengan cat antikotoran yang mengandungi 15% Cu-T sebagai agen antikotoran memberikan keputusan yang lebih baik berbanding Cu-TA, Cu_2O, dan Cu-OM. Manakala, jaring ikan yang dilitupi dengan cat antikotoran dengan komposisi Fe-T, Fe-TA, Al-T dan Al-TA tidak menunjukkan perbezaaan yang ketara antara satu sama lain. Oleh itu, Cu-T boleh menjadi pilihan terbaik untuk dijadikan sebagai alternatif agen antikotoran untuk cat antikotoran dalam mengawal biokotoran marin bagi jaring sangkar ikan di kawasan tropika.

SYNTHESIS OF METAL-TANNATE COMPLEXES AND THEIR APPLICATION AS ANTIFOULANT FOR FISH CAGE NETS

ABSTRACT

Marine biofouling is the attachment of colonized marine organisms on surfaces that are immersed into the seawater in which its formation is influenced by several potential chemical, physical and biological processes. This biofouling significantly affect the outcome of aquaculture industry due to the fouling of the fish nets. Therefore, antifoulant is used in the formulation of antifouling paint in order to avoid the settlement of marine biofouling. Previous metal-based antifoulant like tributyltin tends to be harmful to the natural environment and has been banned by the International Maritime Organization (IMO). The main objective of this research is to find an option for more ecofriendly antifoulant by synthesizing some metal ion-tannate complexes which are natural products based antifoulants that have antifouling properties. In this study, three new antifoulants were synthesized by complexing tannin from mangrove bark of *Rhizophora apiculata* species with metal ions which were copper (Cu), iron (Fe) and aluminium (Al). The optimization of metal tannate complexation was studied under various conditions including the effect of metal dosage, pH, temperature and reaction time. The optimum dosage conditions for copper-tannate (Cu-T) was 1: 2 dosage ratio of Cu ion per mangrove tannin (MT) meanwhile both iron-tannate (Fe-T) and aluminium-tannate (Al-T) were 1:3 dosage ratio of ion per MT. A temperature of 60 ºC, pH 5 and 30 minutes reaction time were obtained as the optimum conditions for both Cu-T and Fe-T. Meanwhile, for Al-T complex it was at pH 4.5 with temperature of 45 ºC and 30 minutes reaction time.

The properties of this metal tannate complexes were characterized by using Fourier Transform Infrared spectroscopy (FTIR), Scanning Electron Microscopy with energy dispersive X-ray (SEM-EDX), Atomic Absorption spectroscopy (AAS), the toxicity test using brine shrimp, *Artemia salina* as control organism and antimicrobial test. Based on the toxicity tests, all complexes were considered toxic due to the LC_{50} value which is below 1.0 mg mL^{-1}, by means followed the order from the most toxic to the least toxic: copper oxide (Cu_2O) (0.018 mg mL^{-1}) > copper-tannic acid (Cu-TA) (0.020 mg mL^{-1}) > Cu-T (0.029 mg mL^{-1}) > copper omadine (Cu-OM) (0.036 mg mL^{-1}) > iron-tannic acid (Fe-TA) (0.045 mg mL^{-1}) > aluminium-tannic acid (Al-TA) (0.053 mg mL^{-1}) > Fe-T (0.064 mg mL^{-1}) > Al-T (0.077 mg mL^{-1}) > tannic acid (TA) (0.14 mg mL^{-1}) > MT (0.21 mg mL^{-1}). Antimicrobial tests showed Cu-T and Cu-TA gave better inhibition zone with more than 1.0 cm of inhibition zone compared to Fe and Al complexes. The immersion of the paint formulations into the seawater showed good performance until two months of life span. Fish nets coating with antifouling paint containing 15% Cu-T as antifoulant gave better results followed by Cu-TA, Cu_2O, and Cu-OM. Meanwhile, fish nets that were coated with antifouling paint with the composition of Fe-T, Fe-TA, Al-T and Al-TA showed no significant differences among them. Thus, Cu-T can be the best candidate to be employed as an alternative antifoulant for antifouling paint in order to control the marine biofouling for fish cage nets in tropical areas.

CHAPTER 1

INTRODUCTION

1.1 Biofouling Phenomena

Biofouling is a process that occurs when marine organism colonized the surfaces that immersed in the marine environment. The undesirable accumulation of microorganisms and macroorganisms, plants and animals on artificial surfaces immersed in seawater can be defined as marine biofouling (Yebra *et al*., 2004). Marine biofouling is mainly classified into two main groups which is microfouling and macrofouling. Microfouling or also known as slime consists of biofilm formation from bacteria like unicellular algae, protozoa and fungi. Meanwhile, macrofouling can be divided into soft fouling like soft coral and seaweed and hard fouling like barnacles, mussels and tubeworms.

This biofouling phenomenon created a lot of problems to the marine environment. It is a natural process which affects both living organisms and man-made surfaces such as ship hulls, aquaculture cages, pipelines and filtration systems in industries and others. In the case of ship hull the accumulation of this fouling increase the frictional resistance of a ship moving through water, resulting in lower speeds, greater fuel consumption and poorer maneuverability (Turner, 2010). In fact, it is also increase the dry-docking operations frequency and deterioration of the ship coating so that corrosion of the materials is favoured (Yebra *et al*., 2004).

In the case of fish net cages the biofouling will cause the weight of cages to be increase and this will lead to the physical damage to the nets and decrease the size of net holes. Thus, the surface area will be increased and caused water flow and oxygen supply disruption. Therefore, biofouling need to be removed by high pressure water jet and this process is time consuming and laborious. In local fish farming industries, the fish net cages used must be change and clean for every 5 to 8 days. Generally, biofouling is hardly to be remove and also very costly.

The current methods used for removing the attachment of biofouling are mechanical cleaning, air or sun drying, antifouling paint and others. In mechanical cleaning, the common way used are like brushing, scrapping and high power water jet. The air or sun drying will kills the biofouling but not removing them. Antifouling paint is containing antifoulant and design to release the toxic substances to the marine environment. Antifoulant should be toxic only to target organisms at environmental concentrations. The most common antifoulant used are copper oxide, copper omadine also known as copper pyrithione and zinc oxide.

The best antifoulant is one that is effective in preventing fouling of the painted surface at low concentration and longer protection. The use of copper as antifoulant in antifouling paint can avoid the attachment of biofouling. However, higher amount of copper used can cause detrimental environmental effects. The common antifoulant like copper oxide consists of copper up to 50% (Macleod and Eriksen, 2007). Thus, this amount of copper need to be reduces for the safety of marine environment.

In this research, an alternative antifoulant with lower amount of metal will be study by complexation the metal ion with tannin. The tannin that being used is the value added of mangrove bark which is the waste product from charcoal industry. In Malaysia, *Rhizophora Apiculata* is one of the two main mangrove species found in Malaysia and mainly used in the charcoal industry. The mangrove bark is riches with tannin sources. Tannins are naturally water-soluble polyphenolic compounds that have high molecular mass (>500) and potentially to precipitate protein (Miriam *et al*., 2007). Tannins are classified as hydrolysable tannins and condensed tannins. Based on their chemical structure, they can readily complex with metal ions forming the corresponding tannates (Belloti *et al*., 2010). Many studies have reported that metal ions such as Cu, Fe and Al can successfully complexes with tannin to form metal-tannate (Miriam et al., 2006; Malesev and Kuntic 2007).

1.2 Problem Statement

Antifouling paints are formulated to avoid the settlement of marine biofouling organisms on fish cage net and most of them contain antifoulant in their formulation. Due to the banning of tributyltin (TBT) as most effective antifoulant in antifouling paint by IMO, copper and copper based antifouling paints are coming into use again. However, their uses are being restricted because recent reports have shown high levels of copper in marine waters. According to bibliographic data (Depountis *et al*., 2006), the toxic limit of copper for the fishes is 0.01 to 1.7 mg mL^{-1}**.** New alternative antifoulant which contained much lower amount of copper is needed to be developed. Recently, natural products are being employed as an alternative for conventional metallic based antifoulant. Mangrove barks of *Rhizophora apiculata* contain high amount of tannin and is abundantly available as waste product from the charcoal industry. Tannin, have been shown to have antimicrobials properties which are required as an antifoulant (Zucker, 1983; Scalbert, 1991). Furthermore, tannin are found to be narcotic to naupliar larvae of Balanus amphitrite (barnacle) and others fouling organisms (Miriam *et al.,* 2007). Thus, complexs of metal ions with tannin and its inclusion in an antifouling paint would produce a synergetic antifoulant which able to prevent the attachments of marine biofouling on the fish cage net.

1.3 Objectives

The aim of this research is to develop new antifoulant based on tannin for the protection of marine biofouling for fish net in fish farming industry. Metal-tannate complexes were synthesized and characterized based on its physical and chemical properties. Their performances as antifoulant were evaluated throughout antifouling paint formulation by immersion in seawater as well as by toxicity test against brine shrimp and antimicrobial test. In order to achieve this aim, the research will be divided into several objectives as follow:

1. To optimize the extraction of tannin from mangrove bark (*Rhizophora apiculata*).
2. To characterize the tannin extract with FTIR spectroscopy, total phenolic content, the flavonoid content and condensed tannin content.
3. To optimize the production of all metal-tannate complexes (copper-tannate, iron-tannate, and aluminium-tannate) based on metal dosage, pH, temperature and time.
4. To characterize metal-tannate and metal-tannic acid complexes by FTIR spectroscopy, SEM-EDX analysis and AAS.
5. To evaluate the toxicity of the metal-tannate and metal-tannic acid complexes against brine shrimp, *Artemia salina* as control organism and the antimicrobial test.
6. To formulate antifouling paint with the inclusion of metal-tannate and metal-tannic acid complexes as an antifoulant.
7. To evaluate the potential of metal-tannate and metal-tannic acid complexes as antifoulant in real sea water.

CHAPTER 2

LITERATURE REVIEW

2.1 Marine Biofouling

Fouling are living organisms (biofouling) or non-living matters that are usually attached on submerged solid surfaces. Biofouling is a complex sequence of events influenced by several chemical, physical and biological processes. Marine biofouling can be defined as the undesirable accumulation of microorganisms and macroorganisms on artificial surfaces immersed in seawater (Miriam *et al*., 2007). A surface that is immersed in the marine environment will be immediately covered by dissolved chemical compounds that adsorb on the surface and evolve to a macromolecular film (Wahl, 1989; Miriam *et al*., 2006). This is followed by a process of biofouling where the macromolecular film on the surface is colonized by microorganisms, algal spores and invertebrate larvae (Miriam *et al*., 2006).

The severity of biofouling process depends on the marine environment. It is most likely affected by two major factors which are geographical location and water conditions (Yebra *et al*., 2004). The influence of geographical location plays a significant role since it cannot be changed or modified in order to control the growth of biofouling. In a tropical climate area, there is only a small seasonal change, thus biofouling may occur throughout the year. Meanwhile, in seasonal areas the grow of biofouling occur based on the temperature, at low temperature certain biofouling will grow completely and others will grow in warm temperature period.

Another important factor is the water conditions. The temperature, salinity, and depth are part of the water conditions that which contribute to the intensity of biofouling growth. According to Woods Hole Oceanographic Institute (1952), biofouling is widely known to be generally heavier in regions with high temperatures. Contrary, at low salinity the growth rate and the maximum size attained and can cause malformations. In addition, the depth of water consequently affected the biofouling growth rate. Biofouling is more favorable to occur in coastal areas compared to deep water area or oceanic waters.

The attachment of marine biofouling on the surface that is immersed in the sea water occurred in few main stages. According to a study performed by Chamber *et al*. 2006, the first stage is known as conditioning. During this stage the organic and inorganic molecules are attracted to the surface after immersion in between one minute to one hour. Second stage of marine biofouling involved the attachment of microorganisms through extracellular polymeric substances (EPS). This stage will occur between 1 hour to 24 hours, forming microbial biofilm. The transition form of microbial film to a more complex biofilm occurred in the third stage. In a week, the colonization of multicellular spores, microalgae, and sediments can be observed.

Finally, the last stage involves the growth of larger marine invertebrate for instance barnacles, mussels, and macro-algae (seaweed). The approximate time for the presence is between two to three weeks. Figure 2.1 shows the summary of marine biofouling stages.

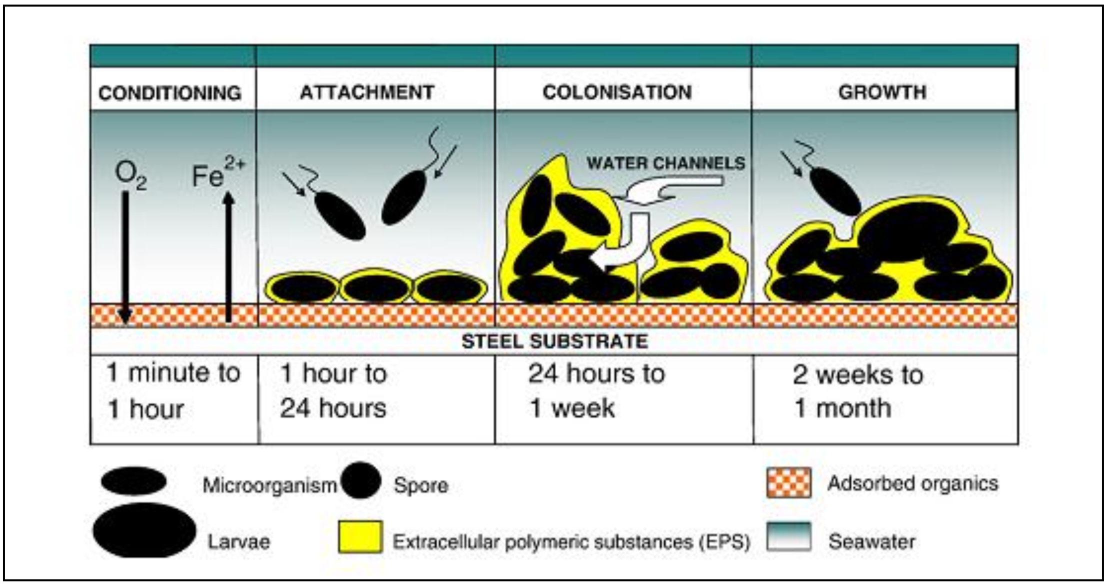

Figure 2.1: Marine biofouling stages. (Chambers *et al*., 2006)

2.1.1 Microfouling

Marine biofouling is mainly classified into two main groups which is microfouling and macrofouling. The anassemblage of attached cells sometimes referred as microfouling, biofilm or slime. When a chemically inert substrate is immersed in seawater an almost immediate accumulation of organic carbon residues are adsorbed onto the wetted surface, composition of which depends on the ions, glycoprotein, humic acid and fulvic acids available in the liquid phase (Chambers *et al*., 2006).

This process occurred by physical forces like electrostatic interaction and van der waals forces. A molecular ‘conditioning’ film occurred within minutes of immersing a clean surface in water.

According to Chambers *et al*. 2006, contact and colonization between the microorganism and the surface is promoted by the movement of water through Brownian motion, sedimentation and convective transport, although organisms can also actively seek out substrates due to propulsion using flagella. This will enhance the microorganisms to adhere to the immersed surface to form biofilm. Biofilm is formed at this early stage of colonizers. Bacteria are among the first organisms that will be attached to the surface. In addition, they also form biofilm which is complex, clusters and three dimensional in nature and serve as a focus for the attachment and growth of other organisms, such as invertebrates, sessile plants, and animals (Zardus *et al*., 2008).

Biofilm will act as a diffusion barrier, interrupted by the ions and water flows to and from the substrate surface (Chambers *et al*., 2006). A compact biofilm may be achieved until 500 µm in thickness due to the rapid rise of attached cells colonies. In biofilm, the bacteria, diatoms and other microorganisms are bound together. However, diatoms which are unicellular algae are more predominant. They adhered to the surfaces by secreting sticky EPS via an elongated slit, the raphe, in one or both valves. Review by Yebra *et al*. 2004, stated that more particles and organisms are trapped by the EPS such as polysaccharides, proteins, lipids and nucleic acid and the roughness of the irregular microbial colonies. Algal spores, barnacle cyprids, marine fungi and protozoa, are likely to be included and some of this may be attracted by sensory stimuli. The biofilm formation will be followed by macrofoulers. In particular condition, biofilm will give biochemical signal with the larvae employed in selecting a settlement site, attaching to it, and undergoing metamorphosis (Dahms *et al*., 2004). Larval settlement of marine organisms and attachment of algal spores are enhanced by biofilm.

2.1.2 Macrofouling

Macrofouling is the consequences fouling created by the microbial colonies of biofilm. It occurred at the final stage of marine biofouling process which involved the settlement and the growth of the larger marine invertebrate, together with the growth of macroalgae (Davies and Williamson, 1995; Fernando and Carlos, 2008). In the case of macrofouling, it can be divided into two subgroup which is soft fouling and hard fouling. Soft fouling consists of algae and invertebrate such as sponges, soft corals, anemones, and tunicates and hydroids. On the contrary, hard fouling comprises of invertebrates such as barnacles, mussels, and tube worms (Callow and Callow, 2002). Figure 2.2 shows the characteristics of macrofouling organisms.

Groups	Algae (plants)	Invertebrates (animals)							
Subgroups	(a) green (b) brown and (c) red	Hard shell organisms				Grass type organisms	Small bush organisms	Spineless organisms	
Designation	(a)Enteromorpha, Ulva and Cladophora, (b) Ectocarpus, and Fucus, and (c) Ceraminum	Balanus	Barnacles	Molluscs	Fouling bryozoans	Hydroids or bryozoans	Hydroids or bryozoans	Ascidians	Sponges and sea anemones
Example of typical aspect									
Designation	Green algae	Balanus	Calcareous polychaetes	Molluscs	Fouling bryozoans		Bryozoans	Ascidians	
Example of typical aspect									
Short description	Only plants that become attached to immersed surface: (a) closed to surface; (b) at mid depth (c) at depth	Attaced trunco-conical or cylindrical crustaceans	Barnacles are Balanus that are fixed to surfaces via a stem	Bivalves containing a spineless animal in their interior	Calcareous incrustations that multiply from a central individual	Organisms that cover surfaces with an open grass or fur	Like bushes of several centimetre and with branches	Constituted by a spineless bag with two tubular openings or starry plates	Spineless and spongy aspect (sponges) and sea anemones

Figure 2.2: Characteristics of macrofouling organisms. (Almeida *et al*., 2007)

According to a review performed by Chambers *et al*. 2006, adhesion and settlement are also a key stage in the life cycle of marine organisms, so the evolutionary pressure to colonise a surface is great. The driving force of adhesion can be considered as being made up of contributions from the interfacial tension between the organism and the substratum, organism and the liquid and between the substratum and the liquid. Hence, Petrucci and Rosellini (2005) stated that barnacles (Crustacea), mussels (Molluscs), sepulids (Anellids) and hydroids (Coelenterates) are adhering as the fouling layer reaches the maximum development.

It is most likely that, balanus is the most important crustaceous fouling species. This species has a planktonic larva that can produce a strong extracellular material at the surface called cement, and adheres strongly to many materials. Cyprid is formed at the critical larval developmental stage of the barnacle and a temporary adhesive is used while exploring the surface for a place to settle and permanently adhered (Khandeparker and Anil, 2007). Barnacles adhere by using a hydrophobic protein which crosslinks using cysteine residues. There are many factors which can influence the settlement of barnacles, a key attribute being the presence of other barnacles through the remains of old exoskeletons or newly settled cyprids.

Another important macrofouling species are mussels which are widely known as bivalve species. This species tend to have two shells and form big colonies on a great number of materials. In view of Callow and Callow (2002), it is stated that, mussels use byssus threads composed mainly of collagen but have, in contrast to barnacles, a hydrophilic polyphenolic adhesive protein which crosslink in an oxidation–reduction reaction that occurs in the presence of an enzymatic catalyst. When the optimal substrate is detected it transforms into the immobile, settled cell phase which can permanently anchor itself and germinate producing a new plant. The adhesion of species to a substrate is an important aspect of biofouling for if this process could be prevented, fouling could be controlled. Figure 2.3 shows the development of marine biofouling.

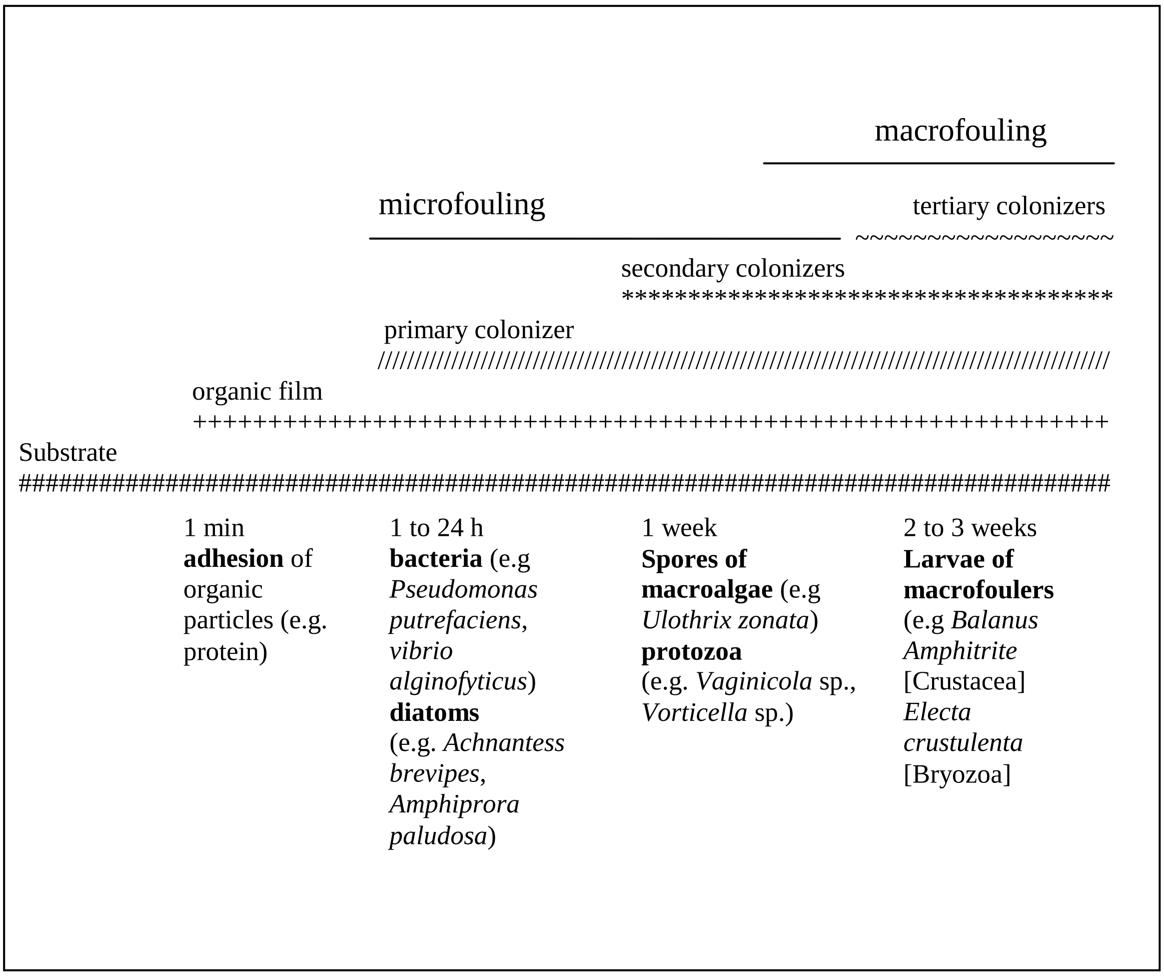

Figure 2.3: The development of marine biofouling. (Yebra *et al*., 2004)

2.1.3 The Effect of Marine Biofouling

Marine biofouling is a natural process which affects both living organisms and man-made surfaces such as ship hulls, aquaculture cages, pipelines and filtration systems in the industries and others. Fouling on many static structures may compromise safety by reducing stability and concealing structural defects (Turner, 2010). Maritime transportation cost significantly increased due to biofouling effect. The importance of maritime transportation in the global freight trade is unmistakable, particularly in terms of tonnage as it handles about 90% of the global exchange (Rodrigue, 2006). The globalization of production and trade are concomitant as one cannot function without the other. Since 70s, the scale, volume and efficiency of the international trade have continued to increase (Hellio and Yebra, 2009).

According to Marechal and Hellio (2009), the major trading routes are going via tropical and/or sub-tropical areas and consequently ships will face at some point of their voyage some very high fouling pressure. Sailing across oceans, ships are confronted with significantly different environmental conditions from tropical waters to cold or temperate waters within a few days, leading for the need of active hull protection against a wide range of organisms. In view of ship hull cases the accumulations of slime, algae and animals increase the frictional resistance of a boat moving through water, resulting in lower speeds, greater fuel consumption and poorer maneuverability (Turner, 2010). A view by Abbott *et al*. (2000) stated that, the growth of organisms on a vessel hull increases frictional drag which reduces the ship speed or requires increased power and fuel consumption to maintain speed.

Hence, slime films alone can impart the powering penalties of 21%, with heavy calcareous biofouling which increases this penalty to 86% (Schultz, 2007). Figure 2.4 shows the effect of marine biofouling onto ship hulls. In general, it can be concluded that high frictional resistances, is due to the roughness which leads to an increase of weight and subsequent potential speed reduction and loss of maneuverability. In order to compensate for this, higher fuel consumption is needed, which causes increased emissions of harmful compounds. It may also entail a need for heavier and less energetically efficient machinery. The increase in fuel consumption which can be up to 40% and in voyage overall costs as much as 77%. Besides that, an increase of the frequency of dry-docking operations also occurred and this surely caused the time lost and resources are wasted when remedial measures are applied. Moreover, a large amount of toxic wastes is also generated during this process.

Figure 2.4: The effect of marine biofouling onto ship hulls.

It also leads to the deterioration of the coating so that corrosion, discoloration, and alteration of the electrical conductivity of the material are favored. Due to this effect, an introduction of species into the environment where they were not naturally present which is invasive on non-native species may occur. Consequently, fish farm fouling is a growing, global phenomenon (Hodson *et al*., 2000), and it is widely accepted that fouling in the aquaculture industry is an expensive problem (Enright, 1993; Hodson *et al*., 1997; Nikolaou *et al*., 2014). There are several positive attributes of biofouling; for example, the manipulation of fouling for seeding mussel lines (Mallet and Carver, 1991; Richard *et al*., 2007). Due to marine biofouling, aquaculture and fish farming industry facing a lot of problems such as decreasing of fish net size holes, water flow disruption, restricted of nutrient exchange and others. Therefore, this marine biofouling must be control using environmentally friendly antifouling technologies.

2.2 Fish Farming Industry

Fish farming industry is a method of raising fish in an enclosed cage in open waters such as lakes, rivers, manmade lakes, coastal waters and offshore. In 2005, 62 countries had provided data on cage aquaculture. On the basis of reported information, the major cage culture producers in 2005 included China, Norway Chille, Japan, United Kingdom, Vietnam, Canada, Turkey, Greece, Indonesia, Philippines, Korea, Australia, Denmark, Malaysia, and Thailand (Halwart *et al*., 2007). From Figure 2.5 it can be seen that the major cage aquaculture producers was dominated by China.

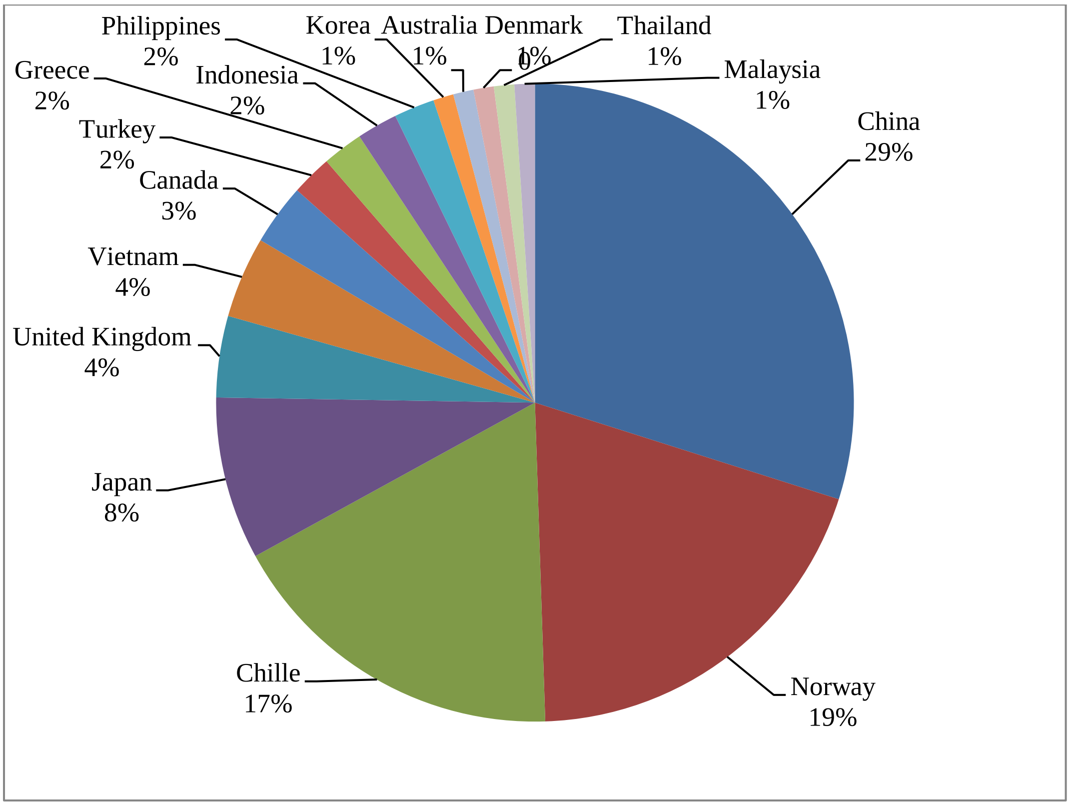

Figure 2.5: Major cages aquaculture in country globally (Halwart *et al*., 2007).

This industry is quite cheap since no water management is needed since it depends on nature. There are two types of cages used in this fish farming industry which is fixed and floating cages. Hence, the dimension and net mesh size are dependent on the cultured fish. At the early stage, hapa cage which is the smallest mesh size net is used for cultured fish. Then the nursery cage is used as the fish grow bigger and lastly, as the cultured fish reach marketable size, the grow-out cage is used.

In order to choose the netting material there are few factors to be considered. The netting material must be durable, resistant to abrasion, and has high breaking strength. The netting material also should maintain its shape and not heavy to be handled. Therefore, synthetic fibres such as polyethylene, polyester, polypropylene, and polyamide are more preferred. These types of fibres are light and easily to fold. Polyamide and polyethylene are available locally. However, polyamide is more costly than polyethylene. In general, polyethylene is more convenient to be used as it is cheaper and has better protection against damage.

According to Thomas (2009), in designing the net structures require several forces to be considered; the main being static and dynamic loads. Static loads include maintenance, operations and the weight of the structure like net and other structural parts. Meanwhile, dynamic loads include forces generated by wind above the water surface, waves at the air-water interface, and currents (particularly tidal currents) in the water. Besides that, the collection of floating debris, collision with water craft or large predators or other similar conditions, may affect the corrosion and fouling. Fouled nets create twice the resistance to the tidal current compared to the clean nets (Milne, 1970; Mark *et al*., 2006).

Biofouling effects to the fish farming industry are largely detrimental. Biofouling can reduce the cage volume by constricting the net openings due to hydrodynamic forces on a fouled net (Phillippi *et al*., 2001). Further structural stress as well as a reduction in cage buoyancy and increased net deformation also occurred as the the weight of cages can severely increase (Milne, 1970; Beveridge, 1996; Phillippi *et al*., 2001). Beveridge (1996), stated that biofouling also caused physical damage to the nets and decreases the mesh size of the net. Thus, the surface area will be increased and caused water flow disruption. As a consequence, the nutrient exchange and waste removal will be restricted (Eckman *et al*., 2001).

Another important effect is that oxygen supply will be disrupted and decreases the dissolved oxygen levels due to the respiratory activity of fouling organisms themselves (Cronin *et al*., 1999; Mark *et al*., 2006). The effect of bifouling may indirectly caused further stress to stock by affording habitat to a range of diseases and parasites for example, 'netpen liver disease' (Andersen *et al*., 1993; Mark *et al*., 2006) and amoebic gill disease (Tan *et al*., 2002). The increased in disease and parasites due to the development of fouling adds to the concerns over the use of combatant chemicals, such as cypermethrin, azamethiphos and emamectin benzoate, which are used for their treatment but have, potentially, detrimental environmental impacts (Waddy *et al*., 2002).

Once the effects occurred it will indirectly increased the cost of fish farming in terms of cleaning and repairing processes. Maintenance of cages involves net changing, cleaning and mending. The net changing depends on the mesh size of the net due to the fouling that will cause the net to clog. In the other hand, the net cleaning process is needed regularly in order to prevent excessive fouling being attached to the net and causing net damages and loss of fish. This cleaning is also related to the mesh net size since the smaller the mesh size, the heavier the fouling rate. Fouling need to be removed by high pressure water jet and this process is time consuming and laborious. Cage mending is a process where panel and rope replacement or partial replacement with rejoining is required. In this case, it is due to the damaged net panels or frequently uses the weakened ropes. Figure 2.6 shows the effect of marine biofouling onto fish net.

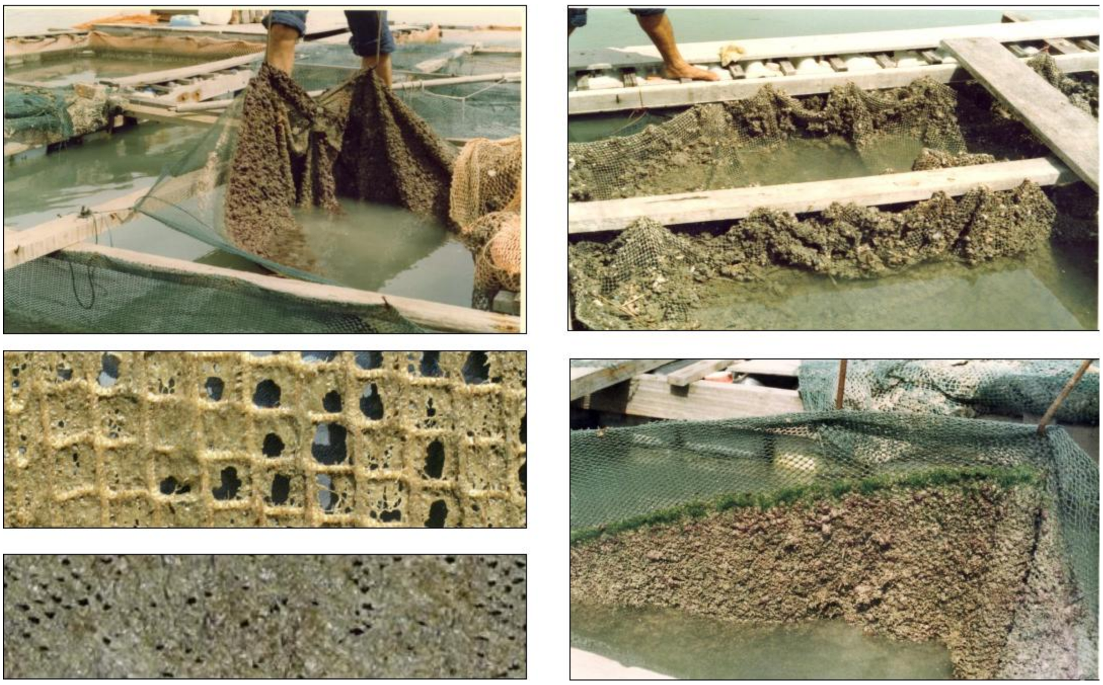

Figure 2.6: The effect of marine biofouling onto fish nets.

2.3 Antifouling Technologies

In recent decades, a lot of methods have been developed to control the marine biofouling. Figure 2.7 shows the antifouling technology to control the growth of biofouling. The antifouling technology is divided into two major groups of using non-coating and coating methods. Non-coating consists of three sub groups which is electrochemical, cleaning in water or land and others such as natural grazers, temperature, low or high pH. Previous antifouling methods that have been used are cleaning in water or land (Willemsen, 2005). In the light of fish farming industry and aquaculture, mechanical cleaning involves brushing, scrapping, cleaning by using hand or with high power water jets on site, and air or sun drying. This common technique to control biofouling will kill most of the fouling organisms but may increase organic loading to the sites. Moreover, this type of method requires labour intensive. Figure 2.8 shows the mechanical cleaning method to control biofouling.

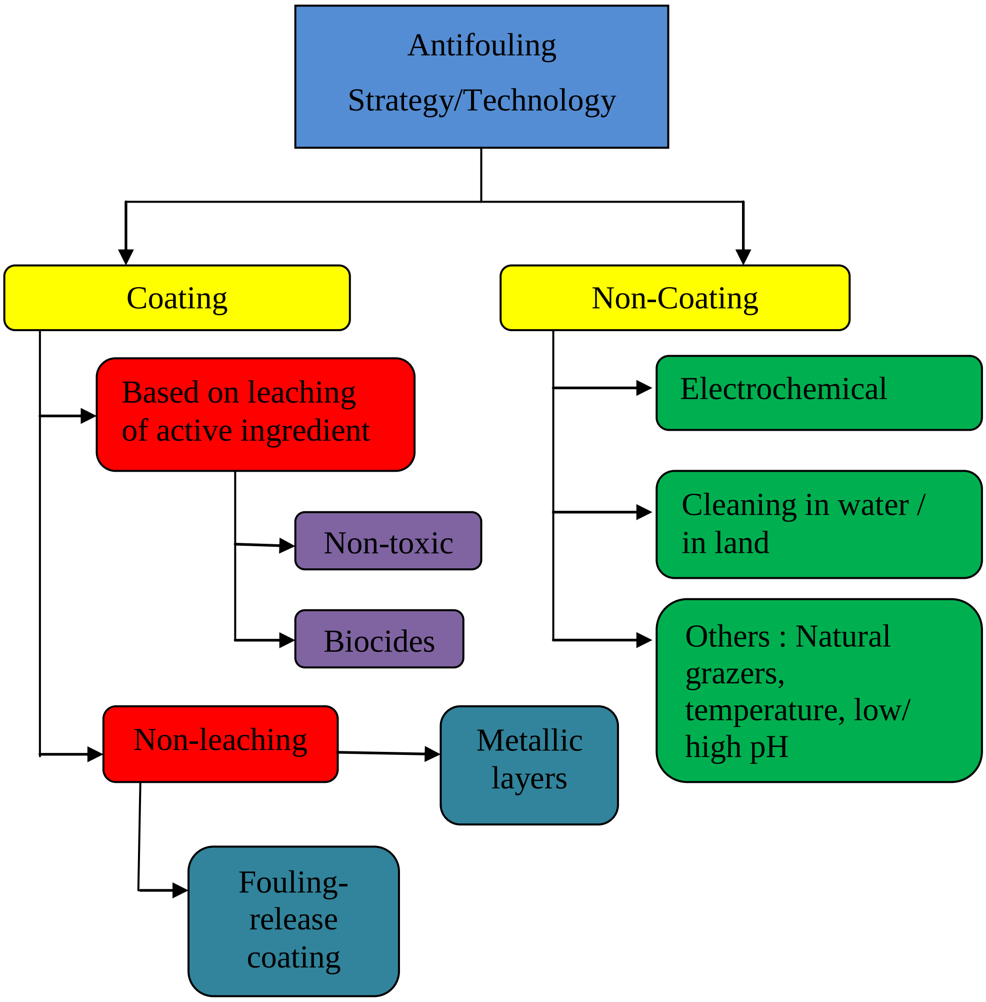

Figure 2.7: Antifouling strategies to control the growth of biofouling (Willemsen, 2005).

Figure 2.8: The mechanical cleaning method to control bioflouling.

In the case of air or sun drying method, the heat or desiccation kills the unwanted aquatic organisms but does not remove fouling completely. Magnetic or electrical forces, electrolysis, surface change and dissolute or precipitate toxic ions are the examples for the electrochemical method. Other non-coating method like natural grazers and avoidance is when cultures are removed or repositioned during heavy fouling settlement. Coating based on leaching of active ingredient included biocides for instance copper oxide or organic biocides) and non-toxic like enzymes, natural compounds, ‘living’ coatings (microorganisms). Non-leaching is classified as coating subgroup consisting of fouling release coating, metallic layers and others.

Examples of fouling release coatings are silicone based like silicone PDMS, flour-silicones and nanotechnology based materials. Metallic layers include organometallic coating and metal-cladding. Other non-leaching methods are fast polishing, ‘spiky’coatings (physical deterrence), removable foils, hydrogels, and contact activity. Besides that, new fish net cages design and materials (Menton and Allen, 1991; Willemsen, 2005) also can be applied in order to control the fouling problem.

Coating is the conventional way to handle biofouling. Antifouling coatings usually will resist the adhesion of biofouling or degrade or kill them. Antifouling coating requires few properties to be considered such as durability, adhesion, effect on corrosion, ease of application, smoothness, drying time, colour and the availability including the cost. Nowadays, several strategies for designing effective antifouling coatings have been studied. Fortunately, most of these strategies are helpful in combating the problem of fouling but several of them are also associated with shortcomings related to stability, toxicity, or the method of fabrication. It has recently been shown that the most successful modern techniques have involved coating with metal containing antifouling paint (Thomas and Brooks, 2010).

2.4 Antifouling Paint

In view of the variety of problems that occur due to marine biofouling, antifouling paint was one of the best effective methods to overcome the problem. In 1950s, Tributyltin (TBT) contained in self-polishing coatings was regarded as the most efficient antifouling protection between dry docking episodes. The efficiency of TBT, especially in "self-polishing" formulations, was remarkable, and the application of TBT-based paints rapidly expanded. Added bonuses also included the fact that it did not cause galvanic corrosion on aluminium hulls, it was colourless, and periods between dry-docking were extended (Readman, 2006).

However, despite their efficiency and versatility, it was found that they have disastrous effects on the marine environment like their accumulation in mammals and the weakening of fish immunological systems (Almeida *et al*., 2007). TBT was subsequently found to be highly toxic to molluscs at very low concentrations and has caused substantial environmental damage, including damage to shellfish aquaculture (Mark *et al*., 2006). Moreover, the trials with TBT coatings on fish cages showed significant adverse metabolic and growth effects upon farmed salmon (Short & Thrower, 1986; Davies & Mackie, 1987; Mark *et al*., 2006). The effect of organotin antifouling residues also were found in various fish species (Kannan *et al.*, 1995; Mark *et al*., 2006).

As already been stated by Mark *et al*. (2006), in 1990 encouragement legislation to control potential adverse impacts associated with use of TBT compounds in anti-fouling paints was issued. As a result, in October 2001, when the IMO took stock of the adverse effects of TBT on the marine environment, an order was issued banning the use of this type of biocides in the manufacturing of antifouling paints as of 1st January 2003, and the presence of these paints on ship surfaces as of 1st January 2008 (Almeida *et al*., 2007). The environmental problem created by TBT will take many years to recover. On the contrary, more environmentally acceptable antifoulants has been developed (Hodson *et al.*, 2000).

However, there is still no approaches has been able to match the cost or benefit relationship offered by organotin antifouling formulations (Abbott *et al.*, 2000; Cowling *et al.*, 2000), so implementation of the total ban proposed by the IMO is to be difficult. Hence, copper based antifouling paint has been developed in order to replace the TBT. In copper-based antifouling coatings, copper is added as copper oxide (Cu_2O), thiocyanates (CuSCN) or sulfides (Cu_2S), resulting in different