HEXADECYLTRIMETHYLAMMONIUM-SILVER-KAOLINITE AS A MARINE ANTI-BIOFOULING PAINT ADDITIVE

MURNI BINTI NOOR AL AMIN

UNIVERSITI TEKNOLOGI MALAYSIA

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ABSTRACT

Copper-based anti-biofouling paint coating has been used to combat biofouling on man-made artificial marine structures for several decades. Despite its widespread use, the massive accumulation of copper and its compounds causes a serious threat to marine ecosystems. Thus, the search for efficient and environmentally friendly anti-biofouling coating has become more significant. Therefore, this current study focused on developing an anti-biofouling paint additive employing the active compounds, silver ion (Ag^+) and surfactant hexadecyltrimethylammonium (HDTMA) embedded in the kaolinite clay structure which is designated as Organo-silver-kaolinite (Or-Ag-Kao). The developed paint additive is intended for applications in marine artificial structures. The Or-Ag-Kao was prepared by adding Ag-kaolinite with HDTMA via a cation exchange process. The prepared samples; Ag-Kao, HDTMA-Kao and Or-Ag-Kao were characterized using ATR-FTIR and EDX. In this study, these analyses confirmed that the Ag^+ and HDTMA were successfully intercalated into the kaolinite without affecting their original structure. The samples were then incorporated into the commercial paint matrix to test for their anti-bacterial adhesion and anti-biofilm efficiency as an anti-biofouling coating additive against isolated marine biofouling bacteria, *Bacillus niabensis* and *Alteromonas litorea*. It was observed Or-Ag-Kao paint coating efficiently reduced the bacterial adhesion of *B. niabensis* (91.51%) and *A. litorea* (87.53%), as compared to commercial paint without additive. The Or-Ag-Kao paint coating also showed excellent anti-biofilm on *B. niabensis* and *A. litorea* with EPS mean volume of $0.219 \pm 0.129 \times 10^5$ μm^3 and $0.151 \pm 0.052 \times 10^5$ μm^3, respectively. A weak interaction was observed between the model bacteria and Or-Ag-Kao with commercial paint coated surface with the mean lateral detachment force 139.4004 nN (*B. niabensis*) and 146.2251 nN (*A. litorea*). Major contact surface area reduction was also observed in Or-Ag-Kao paint coating (0.275 μm^2 in *B. niabensis* and 0.391 μm^2 in *A. litorea*) indicated that Or-Ag-Kao paint coating successfully minimized surface bacterial attachment. As a conclusion, the broad-spectrum effectiveness of Or-Ag-Kao paint coating minimized bacterial adhesion and biofilm formation may provide an environmental-friendly solution for marine biofouling. It is envisaged that the newly developed Or-Ag-Kao has high potential to be commercialized as an alternative marine anti-biofouling coating replacing the present traditional antifouling paints.

ABSTRAK

Lapisan cat anti-biopenempelan semula jadi berasaskan tembaga telah digunakan bagi menangani biopenempelan semula jadi pada struktur laut buatan selama beberapa dekad. Walaupun digunakan secara meluas, pengumpulan tembaga dan sebatiannya secara besar-besaran mampu menyebabkan ancaman serius terhadap ekosistem marin. Oleh itu, pencarian lapisan anti- biopenempelan semula jadi yang cekap dan mesra alam menjadi lebih penting. Oleh itu, kajian semasa ini difokuskan kepada pembangunan bahan tambahan cat anti-biopenempelan semula jadi menggunakan sebatian aktif, ion perak (Ag^+) dan surfaktan heksadesiltrimetil amonium (HDTMA) yang dibenam dalam struktur tanah liat kaolinit yang dinamakan sebagai Organo-perak-kaolinit (Or-Ag-Kao). Cat anti-biopenempelan semula jadi yang dibangunkan akan diaplikasikan kepada struktur buatan marin. Or-Ag-Kao disediakan dengan menambahkan Ag-kaolinit dengan HDTMA melalui proses pertukaran kation. Sampel yang disediakan; Ag-Kao, HDTMA-Kao dan Or-Ag-Kao dicirikan menggunakan ATR-FTIR dan EDX. Dalam kajian ini, analisis ini mengesahkan bahawa Ag^+ dan HDTMA telah berjaya dimuatkan ke dalam struktur kaolinit tanpa menjejaskan struktur asalnya. Sampel dimasukkan ke dalam matriks cat komersial bagi menguji kecekapan anti-bakteria dan anti-biofilem sebagai bahan tambahan bagi lapisan cat anti-biopenempelan semula jadi terhadap bakteria biopenempelan semula jadi yang telah dipencilkan, *Bacillus niabensis* dan *Alteromonas litorea*. Diperhatikan bahawa lapisan cat Or-Ag-Kao dengan cekap mengurangkan lekatan bakteria *B. niabensis* (91.51%) dan *A. litorea* (87.53%) berbanding dengan cat komersial tanpa bahan tambahan. Lapisan cat Or-Ag-Kao juga menunjukkan anti-biofilem yang sangat baik terhadap *B. niabensis* dan *A. litorea* dengan purata isipadu EPS masing-masing bernilai $0.219 \pm 0.129 \times 10^5$ μm^3 dan $0.151 \pm 0.052 \times 10^5$ μm^3. Interaksi yang lemah juga diperhatikan diantara model bakteria dan permukaan bersalut cat komersial Or-Ag-Kao dengan daya pemisahan lateral 139.4004 nN (*B. niabensis*) dan 146.2251 nN (*A. litorea*). Pengurangan luas permukaan sentuhan yang besar juga diperhatikan di lapisan cat Or-Ag-Kao (0.275 μm^2 di *B. niabensis* dan 0.391 μm^2 di *A. litorea*) menunjukkan bahawa lapisan cat Or-Ag-Kao berjaya meminimumkan lekatan bakteria permukaan. Sebagai kesimpulan, lapisan cat Or-Ag-Kao mempunyai spektrum keberkesanan yang luas dalam meminimumkan lekatan bakteria dan pembentukan biofilem mampu menyediakan penyelesaian yang mesra alam untuk biopenempelan semula jadi marin. Adalah dijangka bahawa, Or-Ag-Kao yang dibangunkan ini berpotensi tinggi untuk dikomersialkan sebagai lapisan anti-biopenempelan semula jadi marin alternatif menggantikan cat anti-biopenempelan semula jadi berasaskan tembaga yang ada sekarang.

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LIST OF ABBREVIATIONS

2-D	-	Two dimensional
3-D	-	Three Dimensional
AF	-	Anti-fouling
AFM	-	Atomic Force Microscope
Ag-Kao	-	Silver modified kaolinite
ANOVA	-	Analysis of variances
ATR-FTiR	-	Reflectance-Fourier Transform Infrared
BLAST	-	Basic Local Alignment Search Tool
Br	-	Bromide
Ca	-	Calcium
CA	-	Mean contact angle
CCD	-	Charge coupled device
CFD	-	Computational fluid dynamics
CFU	-	Colony forming units
Cl	-	Chloride
CLSM	-	Confocal Laser Scanning Microscope
CO	-	Carbon monoxide
CV	-	Crystal violet
CW	-	Calcofluor White M2R
CW	-	Calcofluor White M2R
DCA	-	Diiodomethane contact angle
DNA	-	Deoxyribonucleic acid
DVLO	-	Derjaguin, Verwey, Landau and Overbeek
E	-	East
e.g.	-	*exempli gratia* to mean "for example"
eDNA	-	Extracellular deoxyribonucleic acid
EDX	-	Energy-Dispersive X-ray
EPS	-	Extracellular polymeric substances
Eq.	-	Equation
FIB	-	Focused ion beam

GCA	-	Glycerol contact angle
H_2O	-	Chemical formula for water
HDTMA	-	Hexadecyltrimethylammonium
i.e.	-	*id est* to mean "that is" or "in other words."
IMO	-	International Maritime Organization
Kao	-	Kaolinite
KBr	-	Potassium Bromide
LAS X	-	Leica Application Suite X
MA	-	Marine Agar
Max	-	Maximum
MB	-	Marine broth
Mg	-	Magnesium
N	-	North
Na^+	-	Sodium ions
NCBI	-	National Centre for Biotechnology Information
NIS	-	Indigenous species
OD	-	Optical density
Or-Ag-Kao	-	Organo-silver-kaolinite
Or-Kao	-	Organo-kaolinite
PBS	-	Phosphate-buffered saline
PI	-	Propidium iodide
PS	-	Paint surface
QAC	-	Quaternary ammonium compounds
q-PCR	-	Quantitative polymerase chain reaction
QSI	-	Quorum sensing inhibitors
rRNA	-	16S ribosomal DNA
Sb	-	Antimony
SD	-	Standard deviation of negative control
SE	-	Surface energy
SEM	-	Scanning Electron Microscope
Si	-	Silica
sp.	-	Species

TBT-SPC	-	Tributyltin self-polishing
Ti	-	Titanium
TPT	-	Tributyltin
UV	-	Ultraviolet
VCA	-	Video contact angle
vdW	-	van de Waals
WCA	-	Water contact angle
A. litorea	-	*Alteromonas litorea*
B. niabensis	-	*Bacillus niabensis*
et al.	-	*et alii* to mean "and others"
etc.	-	*et cetera* to mean "and other similar things", or "and so forth"
I. baltica	-	*Idiomarine baltica*
M. communis	-	*Marinomonas communis*
P. aeruginosa	-	*Pseudomonas aeruginosa*
S. lutimaris	-	*Salinimonas lutimaris*
Ag^{+}	-	Silver ion
CO_2	-	Carbon dioxide
Cu^{2+}	-	Copper ion
Fe^{2+}	-	Iron divalent ion
Zn^{+}	-	Zinc ion
$(CH_3)_3N(CH2)_{15}CH_3Br$	-	Chemical formula for Hexadecyltrimethylammonium bromide
$AgNO_3$	-	Silver nitrate
$Al\ (OH)_3$	-	Aluminium hydroxide
Al_2O_3	-	Chemical formula for Aluminium oxide
$Al_2Si_2O_5(OH)_4$	-	Chemical formula for kaolinite
$MgCl_2$	-	Chemical formula for Magnesium chloride
NMs	-	Nanomaterials
NO_X	-	Nitrogen oxides
OD_{avcNeg}	-	Mean Optical density of negative control
OD_c	-	Cut-off Optical density
ODc	-	Cut-off OD

$SiNO_3$	-	Silicon Nitride
SiO_2	-	Chemical formula for Silicon dioxide
SO_X	-	Sulphur oxide

LIST OF SYMBOLS

\$	-	Dollar
%	-	Percentage
~	-	Approximatively equal to
±	-	Range of values that a measured quantity may have
^{o}	-	Degree
^{o}C	-	Degree Celsius
µL	-	Microliter
µm	-	Micrometre
µm	-	Micrometre
µm^2	-	Micrometre square
µm^3	-	Micrometre cube
CFU/mL	-	Colony forming unit per millilitre
cm	-	Centimetre
cm^{-1}	-	One per centimetre
eV	-	Electronvolt
g	-	Acceleration of gravity
G	-	Gram
h	-	Hours
k	-	Cantilever spring constant
kb	-	Kilobyte
kHz	-	Kilohertz
L	-	Cantilever length
L	-	Litre
m	-	Meter
mg	-	Milligram
min	-	Minutes
mJ/m^2	-	Millijoule per metre square
mL	-	Millilitre
mm	-	Millimetre
mM	-	Millimolar

N/m	-	Newton-metre
Nm	-	Nanometre
nm/V	-	Nano meter per voltage
nN	-	Nano newton
pH	-	Potential of hydrogen
Ra	-	Arithmetic mean roughness
rpm	-	Revolutions per minute
Rq	-	Root-Mean-Square roughness
s	-	Seconds
S	-	Sensitivity of applied cantilever
S	-	Sensitivity of applied cantilever
V	-	Voltage
$V_{deflection}$	-	Additional compression of the cantilever during bacterial detachment process
$V_{setpoint}$	-	User defined cantilever compression
V_{total}	-	Total vertical deflection
w/v	-	Weight per volume
α	-	Alpha
γ	-	Gamma
θ	-	Angle
θ and Ø	-	Angles of cantilever tip bending due to counterforce from bacterial cell to tip during detachment
λ_{emit}	-	Emission wavelength
λ_{excite}	-	Excitation wavelength

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CHAPTER 1

INTRODUCTION

1.1 Research Background

The ocean is the largest part on earth that contains an enormous amount of bacteria and always exposed to a variety of stress. In adapting these environmental challenges, marine bacteria usually form multicellular aggregates, known as biofilm. It is commonly visualized as a slimy layer that contains bacterial consortia embedded in an extracellular matrix. Biofilm development is a complex dynamic process forms gradually over time starting from the formation of a conditioning layer followed by bacterial adhesion, bacterial growth and biofilm expansion (Armbruster and Parsek, 2018). Biofilm formation have numerous advantages for bacteria development but detrimental to human and many industrial processes (Muhammad *et al.*, 2020).

Biofilm formation on marine man-made artificial structures such as ship body, vessels, cooling water systems, sensors, oil rigs and pipelines are a common ecological phenomenon. Surface colonization and subsequent biofilm formation and development provide numerous advantages to microorganism survival, but they can also cause serious deleterious problems to marine industries. Biofilm formation on marine structures contribute to biofouling, a process of deposition of living and non-living matter on man-made artificial structures. Biofilm formation mediates the attachment of other undesirable of microscopic (algae) or macroscopic (barnacles, mussels) organisms on surfaces. Biofouling formation interfere with process performance (Bucs *et al.*, 2018), cause material damage by the microbial attack (Fitridge *et al.*, 2012; Turan *et al.*, 2016), decrease product quality and quantity (Skovhus *et al.*, 2017), contamination of antifouling chemicals (Wang *et al.*, 2017) and shortening the life-time of plant components due to extended cleaning (Abioye *et al.*, 2019).

Another biofouling-related issue of concern is the effect of the introduction of species into a new environment, non-indigenous species (NIS) where they are not naturally present (invasive species) by the long-range transport of water in ballast tanks (Ojaveer *et al.*, 2018). Fouling communities developed in the submerged vessel communities have higher potential to be spread beyond their native distribution (Katsanevakis *et al.*, 2013). These invasive species may cause unwanted consequences for the environment, economics, and human health (Fletcher *et al.*, 2013; Lezzi *et al.*, 2018; Bonanno and Orlando-Bonaca, 2019).

Many conventional approaches such as mechanical cleaning, ultraviolet radiation and traditional chemical biocides have been utilized to counter biofilm effects in marine environment, but they are less effective. The use of antifouling paints with organometallic compounds or synthetic biocides might be the best way to combat biofilm and prevent biofouling (Salta *et al.*, 2013). For many years, tributyltin (TBT) was widely used as the active components in antifouling (Eklund and Watermann, 2018). TBT which is the most effective antifoulant has been used as a paint additive since the 1960s (Chambers *et al.*, 2006; Korkut and Atlar, 2012). However, due to its toxic effect on the wider marine environment, the application of TBT coatings was prohibited by Marine Environment Protection Committee (MEPC) of the International Maritime Organization (IMO) in September 2008 (Wang *et al.*, 2017).

After the ban on the use of TBT coating, copper has been increasingly used as the main biocide ingredient in antifouling paint coatings to tackle marine biofouling. Although copper is less toxic than TBT compounds, the extensive use of copper-based antifouling paints has led to the accumulation of copper and its compounds in the marine environment particularly beginning to pose a serious environmental problem (Srinivasan and Swain, 2007; Neira *et al.*, 2011; Siddiqui *et al.*, 2015). Previous studies shown that massive accumulation of copper ions concentrations toxic to aquatic organisms (Matthiessen *et al.*, 1999; Katranitsas *et al.*, 2003; Bao *et al.*, 2010). Studies have shown that dissolved copper may affect not only target species but also give negative effects on other marine species by disrupting enzymatic activity and increasing mortality (Katranitsas *et al.*, 2003; Bao *et al.*, 2010; Lindgren *et al.*, 2018).

The widespread environmental concerns of copper containing biocides had leading to the implementation of new, very strict regulations on limitation in application and residue levels of copper-containing biocides for boat, yacht and ship hulls. The new regulation on copper-based antifouling paints application was decided after considering serious consequences of biofouling and the benefits of effective copper-based antifouling paints that use safe and approved copper concentrations. Even though countries like Japan, Denmark and Sweden had prohibited the use of copper in anti-fouling systems (Dekinesh, 2018), many counties in the world including European Union, France, New Zealand, Canada, and United State still approved the use of copper-based antifouling coatings in their waters with requirement of a complete scientific risk assessments and additional safety measurements. The scientific risk assessments are including the eco-toxicology tests and environment impacts tests (Source:http://echa.europa.eu/information-on-chemicals/biocidal-active-substances) while the additional safety measurements are including safe operational procedures for the application, removal, enhanced labelling, and maximum residue levels in products entering the food chain (McNeil, 2018).

Due to the restrictions on toxic biocides active ingredients usage in antifouling paints and increasing consumer environmental concern, the study of marine biofilm and the strategies to combat biofouling is currently one of the most important fields of research. Current research has developed more approaches as potential environmentally-friendly alternatives to solve biofilm and biofouling problems on man-made artificial marine structures (Li and Ning, 2019). Novel approaches such as quorum sensing inhibitors (Song *et al.*, 2020; Muras *et al.*, 2021), antimicrobial peptides (Doiron *et al.*, 2018); Saha *et al.*, 2020) , enzymes (Aykin *et al.*, 2019; Mehrabi *et al.*, 2020), non-biocides compounds (Ventura *et al.*, 2017; Almeida *et al.*, 2018), bio-surfactants (Kim *et al.*, 2015; Vladkova, 2018), microorganism metabolites (Wang *et al.*, 2017; Li and Ning, 2019), nanomaterials (NMs) (Gutner-Hoch *et al.*, 2018; Figueiredo *et al.*, 2020) and biomimetic surfaces (Selim *et al.*, 2020; He *et al.*, 2020) had studied to solve biofilm formation on marine man-made artificial structures. However, many of these approaches still have limitations in application and efficiency (Tian *et al.*, 2021).

Therefore, this research aims to offer a promising solution to the biofouling problem in its initial stage of bacterial adhesion and biofilm formation on surfaces. A considerable focus of this research lies in the development of anti-biofouling paint additive with an emphasis on active compounds, silver ion (Ag^+) and hexadecyltrimethylammonium (HDTMA) embedded in kaolinite clay structure. The aim is to transfer the developed anti-biofouling paint coating on man-made artificial structures in marine environment. Immobilization of cationic surfactants from the quaternary ammonium compounds (QAC) such as HDTMA in silver-kaolinite, termed as organo-silver-kaolinite clay (Or-Ag-Kao) may not only improve the antibacterial activity of the unmodified clay (Malachova *et al.*, 2011; Salim and Malek, 2017; Nur Aryantie *et al.*, 2019) but it also improve the absorption properties of the clay (Bagherifam *et al.*, 2014; Malik *et al.*, 2018). Kaolinite good controlled-release delivery system that could minimize cation residues in the environment and maintain toxic concentration of the loaded cation in close approximate to target microorganisms (Holešová *et al.*, 2013; (Abukhadra and Allah, 2019). The loading of Ag^+ and HDTMA onto the carrier system such as kaolinite may prevent the excessive release hence suppress the toxic side effects toward the marine ecology system as they are released into the environment at a controllable rate. Immobilization of HDTMA in silver modified kaolinite is also an alternative to prevent the formation of insoluble precipitates, AgCl when the Ag^+ is released into the seawater comes into contact with chloride anions (Karel *et al.*, 2015). Studies have shown that QACs such as HDTMA may act as the counter ions due to the great affinity towards chloride ions (Malek *et al.*, 2013).

1.2 Problem Statement

Biofouling in maritime causes environmental disasters and billions of dollars are being consumed annually in the shipping industry. Drag and surface smoothness decreases when fouling organisms attach to the hulls of the vessel resulting an increase in the hydrodynamic weight and subsequently lead to a reduction in top speed and loss of maneuverability (Lindholdt *et al.*, 2015). The effect of higher frictional resistance also increased fuel consumption (Cao *et al.*, 2011), increased bunker costs at a constant speed of the vessel or reduced speed at a constant fuel consumption which later lead to additional expenses due to time delays or lost earnings (Dafforn *et al.*, 2011; Selim *et al.*, 2017) . Moreover, biofouling can also cause an increase of the frequency of dry-docking operations either due to the need of additional hull cleaning or due to costly additional hull repair or coating replacement (Desher, 2018). Regarding to environmental impact, biofouling initiate transfer of invasive species into a new environment and has led to a new danger for the different oceans. Transported invasive species would potentially damage the conserved biodiversity to the particular new environment (Shevalkar *et al.*, 2020). Thus, efforts of managing biofouling are beneficial to both economically and to the environment.

The most effective and easy way to overcome biofouling problem is application of antifouling coatings containing varying concentrations and mixtures of biocides (Miller *et al.*, 2020). Presently, the most commonly used biocide in antifouling paints is cooper oxide (Gu *et al.*, 2020). Though copper oxide is less toxic than tin, its effect on marine ecology is gradually increasing as it continues to accumulate (Siddiqui *et al.*, 2015). This condition has caused much concern from regulators and researchers. The massive accumulation of copper ions concentrations in marine environment would significantly link to serious environmental hazard (Corcoll *et al.*, 2019). Thus, the search for efficient and environmentally friendly anti-biofouling coating has become an even more significant issue (Selim *et al.*, 2015).

Therefore, this research focused on investigating the anti-bacterial adhesion and anti-biofilm properties of the modified kaolinite, Or-Ag-Kao against isolated marine biofilm-forming bacteria. The finding would reveal the modified kaolinite potential as a new highly effective, broad-spectrum and environmental-friendly paint additive that can be applied in marine artificial structures. The working hypothesis was that immobilization of active compounds, silver ions and HDTMA in kaolinite structures would enhance the anti-bacterial adhesion and anti-biofilm properties of the paint and concurrently reduced the environmental hazard due to silver accumulation in marine environment. To date, no research has been done on producing new paint formulations containing Or-Ag-Kao to reduce or inhibit the biofilm formation on surfaces submerged in the seawater.

1.3 Objectives of the Study

In this study, the kaolinite sample's ability in reducing or minimizing biofilm formation were evaluated qualitatively and quantitatively. Hence, the following objectives were put forward:

(a) To characterize the prepared modified kaolinite samples and compared with the commercial paint.

(b) To select a paint coating technique that can minimize bacteria attachment on paint surfaces.

(c) To isolate, characterize and identify the attached marine bacterial on painted surfaces exposed to natural seawater under hydrodynamic condition.

(d) To evaluate the anti-bacterial adhesion and anti-biofilm efficiency of the modified kaolinite sample (with/without paint) against Gram-positive and Gram-negative marine biofilm-forming bacteria.

(e) To investigate the lateral detachment force between the individual Gram-positive and Gram-negative marine biofilm-forming bacteria from modified kaolinite + commercial paint surfaces.

1.4 Scopes of the Study

The research was initiated by characterizing the prepared kaolinite samples: Kao (raw kaolinite), Ag-Kao (silver-kaolinite), HDTMA-Kao/Or-Kao (surfactant modified kaolinite), Or-Ag-Kao (surfactant-silver-kaolinite) and commercial paint using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTiR) Spectroscopy and Energy-Dispersive X-ray (EDX) Spectroscopy. Characterization of the prepared kaolinite samples and commercial paint would provide information about the chemical elements present in the samples. Determinations of the sample's chemical elements constitution would be beneficial in generating correlation of the present elements and the anti-bacterial adhesion and anti-biofilm effects.

Subsequently, four different paint coating methods: brush, roller, dipping and air spray were evaluated to determine the most unfavourable surface condition that can reduce marine bacteria formation. The paint surface conditions were evaluated based on the paint surface physical properties and its microbial growth properties. The physical properties of the paint surfaces were analysed using Atomic Force Microscope (AFM), Eclipse Ni-U Upright Microscope and VCA Optima instrument. Meanwhile, the microbial growth properties of the surfaces were evaluated from the Colony Forming Unit (CFUs) counting. This was followed by the selection of coating technique that was applied to deposit the kaolinite samples incorporated into commercial paint. The selected coating technique would provide an inconducive environment for bacterial biofilm development which will influence the selection of modified kaolinite sample to minimize the bacterial adhesion.

Biofilm formation was induced when bacterial cells attach on a surface. The type of bacterial cells that attached on a surface depend on the surface structure. Therefore, the marine bacteria were isolated from exposed surfaces in natural filtered seawater at a standard temperature and flow rate for a setting period. The isolated bacteria were subjected to series of biochemical test and biofilm assay before identified using molecular identification (16S rRNA sequencing). Next, the isolated bacteria EPS components were determined by using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTiR) Spectroscopy. Later, the EPS productivity of the isolated biofilm-forming marine bacteria were determined and compared using Jiao *et al.* (2010) method.

Anti-bacterial bioassay was conducted to determine the effectiveness of the anti-biofilm paint derived from modified kaolinite in inhibiting or reducing the biofilm development of the isolated biofilm-forming marine bacteria. From the anti-bacterial bioassay, two species of the bacteria with superior biofilm formation capability were selected to represent the Gram-negative and Gram-positive group of bacteria. Confocal Laser Scanning Microscope (CLSM) analysis was performed to enumerate the bacterial cells on the paint surface. CLSM analysis would provide clearer and specific information on bacterial attachment and biofilm formation as CLSM can observed living cells, dead cells and EPS.

The investigation on the strength of the bacterial attachment on paint surfaces were evaluated via interaction forces of cell-substrate surface interaction using AFM. For this purpose, lateral detachment force method in contact mode imaging was used to quantify the strength. Both qualitative and quantitative information collected from single bacterial cell interaction forces will determine which modified kaolinite sample was most effective in preventing biofilm formation.

1.5 Significance of the Study

The incorporation of silver ions and HDTMA in kaolinite structure would provide a promising solution to develop a new generation of efficient anti-biofouling additive for maritime coatings with minimal environmental hazard compared to currently available metal-based coatings. Kaolinite controlled-release delivery system would reduce the release rates of silver from antifouling paints in marine waters hence, the load of copper to the environment could be substantially reduced. The new coating is useful to extend the life of marine structures and reduce the maintenance cost. The outcome of this study will be a steppingstone in producing a broad-spectrum effective environmental-friendly anti-biofouling paint which can be used primarily in marine industry.

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LIST OF PUBLICATION

Indexed Journal

1. **Amin, M. N. A.**, Wan Dagang, W. R. Z., Malek, N. A. N. N. and Jamaluddin, H. (2021) 'Isolation and identification of early marine biofilm-forming bacteria on commercial paint surface', *Malaysian Journal of Microbiology*, Vol. 7(2), pp. 130 - 142 **(Indexed by SCOPUS)**.