Surface bio-magnetism and its biophysical effects on bacterial cells

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Surface bio-magnetism and its biophysical effects on bacterial cells

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Abstract

Bacteria that attached to surfaces secrete a wall of three-dimensional (3-D) gelatinous matrix of extracellular polymers, called biofilms, to protect themselves against all kinds of man-made antimicrobial agents. The formation of conditioning films, which consists of organic molecules, are capable of neutralizing all forms of clean or specially treated surfaces and create favorable physiochemical conditions for bacteria adhesion.

A novel technique has been developed to influence bacterial adhesion, the subsequent formation of microbial biofilms, and the consequential effects on planktonic cell growth. This is achieved by using the properties of anisotropy surface energy and the phenomena of ordered electron spins to either channel the energy away or towards the open surfaces, which affects the biophysical interactions with the adhering bacteria. In addition, the magnetic field has been found to penetrate through the adhered bacteria unhindered and continued to influence the behavior of the bacterial cells even at the upper layers of the biofilm.

In this work, the changes in the surface free energy at the open surfaces of the perpendicularly polarized and non-polarized magnetic media were investigated and characterized by using the atomic force microscope's (AFM) surface topography, surface electric potential, and magnetic force microscopy. Contact angle measurements using the sessile water droplet method on perpendicularly polarized and non-polarized magnetic media were also done to observe the changes in the surface free energy of the magnetic media.

On the biophysical effects on the adhering bacterial cells, experiments were conducted using homogeneous bacterial cultures like *Pseudomonas fluorescens* (a spoilage

bacterium) and *Bacillus licheniformis* (a fermentative bacterium), and heterogeneous cultures from aquarium fish tank media.

Experimental results have consistently demonstrated that a perpendicularly polarized magnetic surface in the spin down state (south-polarized) was able to attract a relatively larger amount of bacterial cells to form larger quantities of biofilms as compared to non-polarized substrates. On the other hand, the magnetic substrate that was polarized in the spin up state (north-polarized) was able to reduce bacterial adhesion and the subsequent biofilm formation.

In addition, it was also observed that the adhering cells to a south-polarized substrate were in a more hydrated condition as compared to the adhered cells on the non-polarized substrate. AFM analyses have further found the presence of a larger quantity of extracellular polymeric substances (EPS) and proteins being synthesized by the adhered bacterial cells. On the contrary, the adhered cells on the north-polarized substrate were observed to be most dehydrated. There was also a marked reduction in the quantities of EPS and proteins secretion by the bacterial cells. This hydration phenomenon was similarly observed on the floppy disk itself when it was left exposed at room conditions after polarization.

Finally, it is suspected that the enhanced growth rate of the planktonic cells that were exposed to the south-polarized substrate was the result of the larger quantities of EPS and proteins secretion, which served as an additional food nutrient to the neighboring and planktonic cells.

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Contents

Abstract.....	i
Acknowledgement.....	iii
Table of Contents.....	iv
List of Tables & Figures.....	ix
List of Symbols.....	xiii

Chapter 1 INTRODUCTION

1.1 Microbial biofilms and its significance	2
1.2 Existing solutions and current problems	5
1.3 Objectives	7
1.4 Scope	7
1.5 Approach strategy	8
1.6 Outline of thesis	9

Chapter 2 LITERATURE REVIEW

2.1 Bacterial adhesion and biofilms formation	12
2.1.1 What are biofilms ?	12
2.1.2 Significance of biofilm	14
2.1.2.1 Negative effects of biofilms	
2.1.2.2 Positive effects of biofilms	
2.1.2.3 Summary on significance of biofilms	
2.1.3 Formation mechanism of biofilm	18
2.1.4 General theories of bacteria adhesion	19
2.1.4.1 Substratum surface properties	
2.1.4.2 Theoretical approach to bacterial adhesion	
2.1.5 Current remedial and prevention methods	27
2.1.5.1 Chemical biocides	
2.1.5.2 Physical treatments	
2.1.5.3 Other methods	
2.1.5.4 Summary of existing problems in biofilm control	

2.1.6	Concluding summary	32
2.2	Introduction to magnetism and surface magnetism	33
2.2.1	Bulk magnetism	33
2.2.2	Magnetic poles and spin directions	36
2.2.3	Magnetic anisotropy	38
2.2.4	Anisotropy energy and surface magnetism	41
2.2.5	Domain wall	42
2.2.6	Magnetization curves - hysteresis loop	43
2.2.7	Particulate films and thin films	46
2.2.8	Magnetic data storage media & recording technology	48
2.2.9	BioMagnetism and applications	51
2.2.10	Concluding summary	51
Chapter 3	NOVEL IDEA, CONCEPTS and APPROACH	
3.1	Novel idea and Concept	54
3.2	Approach plan	57
Chapter 4	METHODS AND MATERIALS	
4.1	Magnetic media and characterizations	59
4.1.1	Floppy Disk (FD)	61
4.1.2	Hard Disk (HD)	61
4.1.3	Magnetization (hysteresis) curve	62
4.1.4	Magnetic states	62
4.1.4.1	Lorentz microscopy	
4.1.4.2	Atomic Force Microscopy – Magnetic Force imaging	
4.1.5	Surface morphology using AFM	64
4.1.5.1	Atomic force microscopy (AFM) – in air	
4.1.5.2	Atomic force microscopy (AFM) – in liquid	
4.1.6	Surface energy	65
4.1.6.1	Contact angle measurement	
4.1.6.2	Surface electric force microscopy (EFM)	

4.2 Bacterial and biofilm culture	66
4.2.1 Biological safety standards	66
4.2.2 Homogeneous bacterial cell culture	67
4.2.2.1 <i>Pseudomonas fluorescens</i>	
4.2.2.2 <i>Bacillus licheniformis</i>	
4.2.3 Heterogeneous bacterial cell culture	68
4.2.4 Biofilm growth culture	69
4.2.4.1 Experimental setup and assay adhesion	
4.2.4.2 Growth medium for biofilm culture	
4.2.5 Planktonic cell growth culture	72
4.3 Measuring bacterial adhesion, biofilm growth and planktonic cells growth	73
4.3.1 Bacterial adhesion evaluation	73
4.3.2 Evaluation of biofilm growth on surfaces	76
4.3.2.1 Areal density method	
4.3.2.2 Cell mass method	
4.3.3 Evaluation on planktonic cell growth	76
4.4 Other comparative experiments	78
4.4.1 Electrified surface on planktonic cell growth	78
4.4.2 Bulk magnet test	79

Chapter 5 RESULTS AND DISCUSSION

5.1 Magnetic state and characterization	82
5.1.1 Lorentz microscopy	82
5.1.2 Magnetic Force Microscopy	83
5.1.3 Magnetization (hysteresis) curve	85
5.1.4 Summary on characterization of magnetic media	86
5.2 Polarization effects and surface free energy	86
5.2.1 Contact angle measurement on FD and HD	87

5.2.2	Co-relation between contact angle and work of adhesion	89
5.2.3	AFM analyses on surface topography and surface electric charge	90
5.2.4	Moisture adsorption phenomenon in FD	92
5.2.5	Test #1: FD in water	94
5.2.6	Test #2: Effects on surface free energy (contact angle)	95
5.2.7	Summary on polarized effects and surface free energy	96
5.3	Effect of anisotropy energy to bacterial adhesion	99
5.4	Bacterial adhesion and biofilms formation	101
5.4.1	Effect on <i>Pseudomonas fluorescens</i>	101
5.4.2	Effect on <i>Bacillus licheniformis</i>	105
5.4.3	Effect on Aquarium culture	107
5.4.4	Summary	109
5.5	Planktonic bacterial cell growth	111
5.5.1	Effect on <i>Pseudomonas fluorescens</i>	111
5.5.2	Effect on <i>Bacillus licheniformis</i>	118
5.5.3	Bulk magnetic effect on <i>B. licheniformis</i>	120
5.5.4	Surface electric effect on <i>B. licheniformis</i>	121
5.5.5	Summary	123
Chapter 6	CONCLUSIONS AND RECOMMENDATIONS	
6.1	Conclusions	125
6.2	Recommendations	128

REFERENCES

130

APPENDICES

Appendix A : Drawing of the flow cell reactor for biofilm culture 143

Appendix B : Chemical compositions of micronutrients and growth media for
P. fluorescens biofilm culture experiment 144

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Postgraduate courses undertaken

- (i) CV6531 - Environmental Biotechnology
- (ii) E6324 - Mass Storage Technology
- (iii) CV6521 - Air Quality Management
- (iv) M6426 - Management of Technology and Innovation

List of Tables

Table 1	List of systems that are affected by biofilms	15
Table 2	Summary of limitations encountered in present remedial and prevention methods	31
Table 3	Summary of physical and magnetic properties of floppy disk	48
Table 4	Summary of physical and magnetic properties of typical hard disk	49
Table 5	Co-relation between measured contact angles to work of adhesion	90
Table 6	AFM surface analyses on FD and HD	90
Table 7	Contact angle measurement on FD after 72hr exposure	95

List of Figures

Fig. 1	Conceptualized biofilm development and its dynamic behaviors	3
Fig. 2	Effects of biocides on planktonic bacteria	5
Fig. 3	Effectiveness of biofilm killing on various forms	5
Fig. 4	Outline structure of thesis	9
Fig. 5	A microstructure sample of a biofilm (SEM)	13
Fig. 6	Pictorial representation of the impact and potential applications in biofilms	17
Fig. 7	Biofilm formation mechanism	18
Fig. 8	A CSLM image (left) & a simplified model structure (right) of a typical biofilm	20
Fig. 9	Definition of contact angle and the interfacial forces	20
Fig. 10	Forces involved in attractive and repulsive interactions between a bacterium and a surface (HB = hydrophobic function groups)	22
Fig. 11	Current “top-down” anti-biofilm approach & its after effects	31
Fig. 12	Types of spin ordering	36
Fig. 13	A torroidal magnetic field	37
Fig. 14	Magneton : A spinning charged particle that generates a magnetic field of torroidal shape	38
Fig. 15	Axes of magnetization of a magnetic crystal	39
Fig. 16	Effects of applied tensile (+) and compressive (-) stress on the magnetization of nickel. Applied stress are in lb/in ²	40
Fig. 17	Spin directions at domain walls and transition wall	42
Fig. 18	Hysteresis loop in a magnet	44
Fig. 19	Hysteresis loop of a soft and hard magnetic material	45
Fig. 20	Multi- and single-domain particles	46
Fig. 21	Variation of coercivity with particle size	47

Fig. 22	Typical appearance of Co- γ -Fe ₂ O ₃ particles used in floppy disk	48
Fig. 23	Typical structure of a hard disk coating	49
Fig. 24	Configuration of a horizontal magnetic recording technology of a hard disk drive	50
Fig. 25	The transition area between a bit which gives rise to a perpendicular magnetic polarity – alternating dark-bright bars in MFM image demonstrates N-S changing polarity	50
Fig. 26	Inter-relation between bacterial biofilms, anisotropy energy and magnetism	55
Fig. 27	Conceptualized working mechanism of a biobarrier stages with (a) headings refer to magnetic-south polarization, whereas (b) indicates north- polarization	55
Fig. 28	Outline of approach to study, characterize, and evaluate the polarized magnetic surface on the biophysical influences on bacterial adhesion & growth	57
Fig. 29	Summary on materials and methods used	60
Fig. 30	A schematic illustration of the polarizing process of the magnetic media	61
Fig. 31	Vibrating sample magnetometer (LakeShore 7300) used for magnetization curve measurement (enlarged photo shows the holder for the magnetic samples)	62
Fig. 32	Experimental setup for the biofilm growth. Top figure illustrates the schematic diagram and the bottom figure shows the actual setup	70
Fig. 33	Top Figure: (A) A schematic overview of the parallel plate flow chamber system;(B) Schematic representation of the flow chamber with the magnetic sample attached to the bottom glass slide. Bottom Figure: Actual experimental setup for capturing the bacterial adhesion process	74
Fig. 34	Experimental setup for the surface electric test (a) illustrates the electrical circuit diagram for each beaker set, and (b) shows the method in securing the exposed wire to the aluminum foil. (c) illustrates the entire circuit diagram, with a complete set without any electric charge for reference	79
Fig. 35	Bulk magnetic effect test on planktonic cell growth	80
Fig. 36	Lorentz microscopy image at x 6 times on the magnetic north- and south-polarized FD and HD samples	83
Fig. 37	Representative MFM images on polarized FD and HD surfaces at 5 x 5 μm^2	84
Fig. 38	Distribution of MFM measurement on a 5 x 5 μm^2 south-, north- and non-polarized substrates (error bars denotes maxima and minima values)	84
Fig. 39	Hysteresis loops of FD and HD measured using VSM	85
Fig. 40	Contact angle measurement on perpendicularly polarized floppy disk surface	87
Fig. 41	Contact angle measurement on perpendicularly polarized hard disk surface	88
Fig. 42	Left figure: Alternating the magnetic polarization directions (N/S) repeatedly resulting in contact angle (CA) changes. Right figure illustrates the observed effect on sessile drops on the same sample surface but polarized at North (top) and South (bottom) direction	88

Fig. 43	Representative images of EFM with corresponding AFM data on polarized HD at $5 \times 5 \mu\text{m}^2$. Left image in each polarized state shows the EFM data, and right image shows the topographic data. The blotches seen in the images are surface irregularities.	91
Fig. 44	Contact mode AFM in ambient air @ $10 \times 10 \mu\text{m}^2$ resolution on different magnetized condition substrates (images rotated 100 and pitch angle 350) – circled white spots are swollen lumps	92
Fig. 45	Contact mode AFM in ambient air @ $1 \times 1 \mu\text{m}^2$ resolution on different magnetized condition substrates – Images (a,b,c) were obtained immediately after polarization. Images (d,e,f) were obtained after 72hr- humidity exposure	93
Fig. 46	Contact mode AFM in ambient air @ $10 \times 10 \mu\text{m}^2$ & $1 \times 1 \mu\text{m}^2$ resolution on different magnetized condition substrates (images rotated 10° and pitch angle 30°)	94
Fig. 47	Contact angle measurement on magnetic north and south polarized and non-polarized substrates (error bars denotes maxima and minima values)	95
Fig. 48	Effects of magnetic states on <i>P. fluorescens</i> adhesion with time	100
Fig. 49	Aerial photographic view of <i>P. fluorescens</i> biofilm growth on FD substrates	102
Fig. 50	Distribution of areal density measurement of biofilm growth on FD substrates	102
Fig. 51	AFM (tapping in liquid) on polarized and non-polarized substrates at $5 \times 5 \mu\text{m}^2$ in 3-D (upper row) and 2-D (lower row). 3-D images are rotated 100 and pitch angle 30°	104
Fig. 52	Measurement of biofilm cell mass on FD (the upper and lower bars represents the fluctuation range). Photos taken at 4X magnification illustrate corresponding biofilms formation	106
Fig. 53	Measurement of biofilm cell mass on HD (the upper and lower bars represents the fluctuation range). Photos taken at 4X magnification illustrate corresponding biofilms formation	106
Fig. 54	AFM imaging at $5 \times 5 \mu\text{m}^2$ on <i>B. licheniformis</i> biofilms on the polarized FD (top row) and HD (bottom row)	107
Fig. 55	Measurement of biofilm areal density on FD (the upper and lower bars represents the fluctuation range). Photos on right illustrate corresponding biofilms formation	108
Fig. 56	Magnetic polarity effects on relative cell growth rate of <i>P. fluorescens</i> w.r.t. non-polarized (error bars denotes maxima and minima values over three separate experiments)	112
Fig. 57	AFM analysis on <i>P. fluorescens</i> biofilms grown on north- and south-polarized magnetic surfaces	113
Fig. 58	AFM images with sectional analysis at $5 \times 5 \mu\text{m}^2$ on biofilms after 96hr incubation	115

Fig. 59	Width measurement on bacterial cells adhered to magnetic surfaces	116
Fig. 60	MFM analysis on fresh polarized FD and with biofilm growth	117
Fig. 61	Investigations of polarized FD on planktonic cell growth of <i>B. licheniformis</i>	118
Fig. 62	AFM imaging at $5 \times 5 \mu\text{m}^2$ on <i>B. licheniformis</i> on polarized FD. Top row (a ~ c) elucidates the cells after 2 hours incubation; Bottom row (d – f) elucidates the cells after 6 hours	119
Fig. 63	Bulk magnet effects on planktonic cell growth of <i>B. licheniformis</i>	121
Fig. 64	Effects of electrically charged Al foil on planktonic cell growth of <i>B. licheniformis</i>	122
Fig. 65	AFM imaging at $5 \times 5 \mu\text{m}^2$ on <i>B. licheniformis</i> on electrically charged Al foils	122

List of Symbols

Symbols	Meaning
Symbols for Magnetism	
H_c	Coercive field strength
H_{sat}	Saturation magnetization
M	Magnetization
H	Magnetizing field
M_R	Magnetic remanence
T_c	Curie temperature
Symbols for Free Surface Energies	
θ	Contact angle between 2 interfaces
γ_L	Surface tension within the liquid media
γ_S	Surface tension on the solid surface
γ_V	Surface tension within the vapor media
γ_{LS}	Interfacial surface tension bet. the liquid and solid interface
γ_{LV}	Interfacial surface tension bet. the liquid and vapor interface
γ_{SV}	Interfacial surface tension bet. the solid and vapor interface
A	Hamaker constant
r	Radius of bacterial cell
d	Separation distance between cell and substrate
κ	Reciprocal of Debye length

CHAPTER ONE

INTRODUCTION

1.1 Microbial biofilms and its significance

Bacteria are microbes or microorganisms and are essential to life. Among their many roles, decomposing is one of the most important jobs bacteria do. They are also used to produce food as well as pharmaceutical and industrial compounds. On the negative side, they are the cause of many diseases of plants and animals, and are responsible for the spoilage of food ¹. Bacteria are mostly single-celled microorganisms and their size range from 0.1 μm to 750 μm ². Although they may be very small in size, the thought and the very sight of bacteria have always brought fear in us because of their potent power to unleash damaging effects by their numbers through their exponential growth rate. Basically, humans fear what cannot be controlled, that is the growth rate.

For the first two centuries, these microorganisms were studied exclusively in planktonic form (freely floating in aquatic media). In the late 1970's, with the arrival of advanced microscopic technology, microbiologists were surprised to find that biofilms were the predominant form of bacterial growth in almost all-aquatic systems ^{3,4}. Since then, it has become apparent that microorganisms can behave very differently when living within a biofilm than when the same species is floating freely. The scientific community began to appreciate that bacteria organize themselves in elaborate ways, such as in biofilms.

In general, it is recognized that the first step to the development of biofilms begins with the adhesion of bacteria to solid surfaces ⁵. Once on the surfaces, the adhered cells will begin to colonize and secrete a three-dimension (3-D) gelatinous matrix of extracellular polymeric substances (EPS) that help to attach the cells tenaciously to the surface ^{3,6-11}. The role of the EPS is believed to provide structural functions and protects the cells from any harsh environment. These EPS are highly

hydrated and consist of a mixture of macromolecules such as proteins, polysaccharides, nuclei acids and lipids, which provide a source of nutrients for bacterial growth ³. In addition, these EPS also assist the attachment and provide the nutrients for the colonization and growth of other species in the aquatic environment ⁷. The stages of development of biofilms are illustrated in Fig. 1.

Fig. 1 Conceptualized biofilm development and its dynamic behaviors (adopted from CBE³)

In bacterial adhesion, the factors influencing the adhesion process are determined by the properties of all three phases involved, i.e. the suspending medium, adhering bacteria, and the substrata ⁸. Some of the factors in the suspending medium, such as the shear stress of the flowing medium, temperature, time of exposure, and bacterial concentration, will affect the bacterial adhesion process. The surface properties of bacteria, such as the surface hydrophobicity and the surface charge, are two important considerations. As for the substrata, the factors include chemical compositions, surface hydrophobicity, surface roughness, surface charge, and surface energy ^{3, 5, 9}.

It has been found that biofilms are not simply passive assemblages of cells that are stuck to surfaces, but are structurally and dynamically complex biological systems.

Cell-cell communication in bacteria through chemical signaling, known as “quorum sensing”, are believed to be released by biofilm bacteria into their local environment, where they can interact with neighboring cells ¹⁰. These chemical signals are found to be responsible for the release of extracellular enzymes and proteins that regulate the formation of EPS and planktonic cell growth ^{12, 11}. In addition, the bacteria growing in biofilms are found to require relatively high levels of energy substrate for growth. It has been reported that starved bacteria would tend to exhibit a size reduction and are also more adhesive than the well-fed bacteria ³.

The extracellular matrix in biofilms protects organisms within it from the grazing of larger protozoa such as amoeba, from antibodies or leucocytes of a host organism, or from antibiotics. Because of these many advantages, almost all microorganisms are capable of producing some amount of biofilm. The shedding of biofilm organisms into the bulk water serves to spread a given species from one region of the system to another. But once the species are widespread, the concentration of organisms in the water is merely a symptom of the amount of biofilm activity rather than a cause of biofilm formation ³. Consequently, planktonic bacteria counts can be misleading.

Researchers on biofilms ⁵ have found that using a biocide may kill a large percentage of the planktonic organisms while having little effect on anything but the outer surfaces of the biofilm. In this case, planktonic bacteria count may rise quickly after the biocide has left the system as shedding of organisms from the biofilm resumes (see Fig.2).

Fig. 2 Effects of biocides on planktonic bacteria (adopted from CBE³)

Beside the biofilm recovery phenomenon, it was also found in experiments³ that the growing half of the heterogeneous biofilm can be easily killed, but the non-growing (yet still viable) half is impervious to killing. The maximum kill that can be realized in the heterogeneous biofilm is approximately 50 percent, which corresponds to a logarithmic reduction of about 0.3 (see Fig.3).

Fig.3 Effectiveness of biofilm killing on various forms (adopted from CBE³)

1.2 Existing solutions and current problems

Scientists have constantly faced a difficult task in treating biofilms¹⁶. One main reason is the lack of understanding of the characteristics of the biofilm mode of growth. Understanding when and how to treat biofilm requires knowledge of whether the bacterial population is of one of several phenotypes. If separate phenotypes are shown

to exist, then anti-microbial treatment might not be effective against the entire community, leaving cells behind to re-colonize and grow again.

In response to increased awareness of biofilm, several new approaches^{12, 13} have come into use. New biocides and chemicals have been developed with increased rates of diffusion into biofilm. Another recent approach is the use of heterologous proteins on the surface of microorganisms that serve as carriers for foreign molecules to be displayed on the surface of cells in which the antibodies or vaccines will target^{14, 15}. This approach has been gaining popularity as recent evidences have found the biofilm bacteria produce proteins to increase its resistance to environmental conditions, including immune attack as well as the presence of antibiotics^{15,16}.

Other non-traditional methods like bio-electric¹⁶⁻²⁷, ultrasonics¹⁷ and bio-barriers^{18,19,20} have also been tested. However, practical tests have shown that none had been very successful so far^{21, 22}. The main reasons are that bacteria adhere to a wide variety of surfaces, especially wild bacteria in natural ecosystems⁵. The other reason is the formation of conditioning films by organic molecules that can be rapidly adsorbed onto clean surfaces, or even to specially treated surfaces that are intended to prevent bacteria adhesion. Once the surfaces are exposed to natural and *in-vitro* solutions containing organic molecules, the conditioning film will practically neutralize the anti-bacteria adhesion surface effects and create favorable physiochemical conditions for bacteria adhesion.

Presently, the common anti-biofilm approach used is to develop biocidal chemicals or genetically modified bacteria to target and kill the bacteria hiding within the extracellular matrix wall of polymers in the biofilm. This top-down approach, although in some way very effective against homogeneous cultures, its effectiveness

against heterogeneous cultures is limited. Moreover, this technique would only last for a period of time before the targeted bacteria mutated and developed into a more resistant strain, just like the case of using too much antibiotics to treat infections in human body. Moreover, there are also limitations to the amount of biocides that can be used without undesired side effects ^{18,23,24,33}. In general, whether it is a top-down or a bottom-up approach for bacterial and biofilm treatment, the effectiveness are limited.

1.3 Objectives

The objective of this research project is to develop a new biobarrier technique using the properties of anisotropy surface energy and the phenomena of ordered electron spins to influence and control bacterial adhesion and the subsequent formation of biofilms. For this purpose, a magnetic coated media would be used. The aim is to create the anisotropy energy from the perpendicular polarization and using the characteristics of ordered electron spin to channel it to the surface or away from it. The purpose of using an energy field is its ability to penetrate through living cells or any organic conditioning films, which will overcome the problems encountered by existing biobarrier techniques.

1.4 Scope

The magnetic media used in this research project will be the commercial data storage media. An atomic force microscope, AFM, would be used extensively to characterize and study the magnetic ^{25,26} and bacterial ⁴²⁻⁴⁷ surfaces. The bacterial experiments would be conducted under static and continuous flowing medium using two homogeneous cultured bacterial strains, *Pseudomonas fluorescens* and *Bacillus licheniformis*, under controlled conditions. One heterogeneous wild culture from the aquarium fish tank would also be used to test the samples under natural conditions.

These diversified tests are to provide a rigorous, realistic, and practical evaluation of the concept and its mechanisms.

1.5 Approach strategy

The intention in this research work is to develop a biobarrier that is able to generate an energy field that will not only alter the surface free energy, but can also penetrate through the layer of conditioning film, and also the layers of adhering bacterial in an aquatic environment. To the author's knowledge, this technique is a novelty, as it has not been reported elsewhere. To achieve this, one or more of the four forces of nature would be utilized. These forces are, by order of highest strength, the molecular atomic force, electromagnetic force, radioactive force, and potential/gravity force.

Here, the enhanced magnetic moments from the molecular spin of electrons at the surfaces and inter-surfaces of a magnetic media have been chosen over the other form of forces for its ability to penetrate through cells and organic materials easily. The evaluation and study of the biophysical effects of magnetic surfaces to bacterial and its adhesion behaviors will include the theoretical analyses of the mechanics involved. Essentially, this project will be multi-disciplinary and involves the application of applied physics in magnetism and the study and experimentation of the physical interactions between the magnetic surface and bacteria. In view of the wide scope involved, the experimental procedures and adhesion mechanisms would be adopted from established methods and theories. Given that the physical effects are generally macroscopic in nature, the microscopic and crystallography study of the magnetic materials will not be included. For bacterial adhesion and biofilm formation, the evaluation would be conducted using accepted ASTM²⁷ (American Society for Testing

and Materials) standard and publications from journals and organizations in the related fields.

1.6 Outline of the thesis

The thesis is organized into three major areas. The first is on magnetism and magnetic media. Next is on bacterial biofilms and its formation mechanism. And lastly is on the growth behavior of planktonic (free floating) cells. The outline of the thesis is illustrated in Fig. 4.

Fig.4 Outline structure of thesis

The literature review in Chapter Two is divided into two parts. The first part highlights the significance of biofilms, followed by the beneficial and detrimental impact to various industries. Thereafter, it discusses the formation mechanism of biofilms, and the three governing theories to bacterial adhesion, namely, the classical DLVO, extended DLVO, and the Thermodynamic models. The first part completes by introducing the existing solutions and recent works carried out on biofilms.

In the second half of the literature review, some background information on bulk magnetism, electron spins and magnetic anisotropy are discussed. This is followed by the characterization and applications of magnetic materials.

In Chapter Three, a novel idea has been conceptualized to develop a biobarrier that is based on energy generation and movement control to influence bacteria, namely the anisotropy energy and the phenomena of ordered electron spins. This is followed by a discussion on the approach plan to evaluate the concept.

In Chapter Four, the methods and materials used for magnetic media and its characterization, the handling and growing of bacteria cultures, and the conduct of biological experiments are given. This chapter also involves the methods used to verify the magnetic states of the magnetic media after polarization.

In Chapter Five, the results of the experimental and characterization are discussed. Summaries are also included at the end of each topic. In addition, a hypothesis has also been proposed in Section 5.2.7 to explain the working mechanism and the phenomena seen from the experiments. And finally, the last Chapter Six concludes the research work with recommendations for future considerations.

CHAPTER TWO

LITERATURE REVIEW

The literature review in this chapter contains two parts. The first part examines the significance and effects of bacterial biofilms, and the mechanism of biofilm formation. Various existing governing theories of bacterial adhesion to a solid surface are also introduced. A summary of the current remedial and preventive methods used is presented.

In the second part of the review, the focus is on bulk and surface magnetism. Given that these two areas contain a very large scope, only important and relevant portions pertaining to the research work will be highlighted.

2.1 Bacterial adhesion and biofilms formation

Understanding the mechanics of bacterial adhesion and biofilm formation are the first important steps to develop an effective bio-barrier technique, which would also subsequently influence the growth rate of the planktonic cells. In this aspect, the governing theories to bacterial adhesion to a solid surface and the surface characteristics of the solid surface would be studied. Although preventing bacterial adhesion and biofilm formation are preferred for a bio-barrier, promoting bacterial cell adhesion and biofilm formation is equally useful for biotechnology applications. Hence, the understanding of the positive and negative effects of bacterial adhesion is important, as it will subsequently influence the overall biofilm formation and the planktonic cell growth.

2.1.1 What are biofilms?

A biofilm is a population or community of microorganisms attaching to environmental surfaces. These collections of microorganisms, predominantly bacteria, also include fungi, yeasts and protozoa. They are encased within a three-dimension (3-

D) gelatinous matrix of extracellular polymers secreted by the microorganisms^{2, 6, 49-51}. Upon adhering to surfaces, the bacteria begin to excrete a slimy, glue-like substance that can anchor themselves to all kinds of materials^{52,53}; such as metals, plastics, soil particles, medical implants, and body tissues. Essentially, a biofilm may form on any surface exposed to bacteria and some amount of water. Once anchored, these microorganisms carry out a variety of detrimental or beneficial reactions (by human standards), depending on the surrounding environmental conditions.

The biofilm is composed of mineral particles, a variety of microorganisms, and a network of slime, or glycocalyx (indicated by the arrow in Fig. 5), that binds the microorganisms and particles together.

Fig. 5 A microstructure sample of a biofilm (SEM) – adopted from Ref⁵⁰

The microorganisms themselves may make up from 5 to 25 % of the volume of a biofilm. The remaining 75-95% of the volume, the biofilm matrix, is actually 95-99% water⁵¹. The dry weight consists primarily of acidic exopolysaccharides excreted by the organisms. Very close to the bacteria cells the biofilm matrix is more likely to consist of lipopolysaccharides (fatty carbohydrates), which are more hydrophobic than the exopolysaccharides.

2.1.2 Significance of biofilm

The biofilm matrix can protect organisms within it from the grazing of larger protozoa such as amoeba, from antibodies or leucocytes of a host organism, or from antibiotics. Because of these many advantages, almost all microorganisms are capable of producing some amount of biofilm. The effects of biofilms on humans and industries can be of negative or positive impact. The following sections summarize its significance and impact.

2.1.2.1 Negative effects of biofilms

(i) Biofilms and health

Given the protection by the biofilm against biocides and its ability to develop new resistant strains over time, the medical industry is constantly faced with a number of recurrent health problems^{56-58,75}, such as:

- Diseases (e.g. ear, artificial heart & valve, lung and respiratory infection).
- “Nosocomial” (hospital-acquired) infections (e.g. catheters, medical implants and medical devices).
- Illnesses (e.g. pathogenic organisms infection from unhygienic or ineffective cleaning of household items).

(ii) Biofilms and industry

The effects of biofilms in industry are very wide spread. The following items in Table 1 highlight the various impacts of biofilms on domestic and industrial systems.

System	Impact of Biofilm Formation
<ul style="list-style-type: none"> • Toilet bowls • Cooling water towers • Heat exchangers • Paper manufacture • Food processing • Semiconductor • Metalworking • Photo processing • Reverse osmosis 	<ul style="list-style-type: none"> • Cosmetic degradation • Reduced heat and mass transfer • Reduced heat transfer • Degradation of product quality • Contamination • Contamination • Degradation of metalworking fluid • Flawed prints, machine failure • Reduced membrane permeability, material degradation
<ul style="list-style-type: none"> • Swimming pools • Household drains • Process equipment • Drinking water pipes • Industrial water-based process • Water treatment and distribution 	<ul style="list-style-type: none"> • Health risks, cosmetic degradation • Slow draining sinks • Corrosion and biodeterioration • Health risks • Pipe plugging, corrosion & water contamination • Pipe plugging, corrosion & water contamination

Table 1 List of systems that are affected by biofilms (adopted from Ref²⁷)

2.1.2.2 Beneficial effects of biofilms

Besides the negative effects of biofilms, there are also several beneficial applications from biofilms. The extra-cellular polysaccharides (EPS) that are either secreted into the growth medium or remain attached to the bacterial cell wall have characteristic properties in rheology and physiological activity that are different from the natural gums and synthetic polymers. These EPS are susceptible to biodegradation in nature and less harmful to environmental pollution than synthetic polymers. For these reasons, some bacterial polysaccharides are produced in industrial scales and used as raw materials for food processing, medical, and industrial preparation ².

Polysaccharides can also serve as vicosifying, stabilizing, emulsifying, gelling, or water-binding agents in a variety of food product^{59,60}

(i) **Biofilms and biotechnology**

- **Wastewater treatment** uses selected strains of bacteria to consume the organic and inorganic matter found in sewerage (e.g. NEWATER) or industrial wastes. These bacteria will coagulate into flocs that will eventually settle down as the floc size increased. And the settled flocs will eventually be mechanically removed as sludge.
- Biofilms are also commonly used in the **biochemicals manufacturing industry** to help produce food additives, antibiotics and medicines. This is done through their biological activities in which enzymes and toxins are secreted by the selected strains of cultured bacteria.

(ii) **Biofilms and environment**

Bacteria are key elements that help evolve our ecosystem. They have the natural ability to adapt to all kinds of physical and chemical environment. They grow and collectively recycle all the carbon and nutrients that other organisms produce, and provide a balance within our environment. Examples of their usefulness are:

- Agricultural productivity (enhanced plant' roots growth)
- Soil bioremediation & biodegradation

2.1.2.3 Summary of significance of biofilms

Basically, the relationship between the free-floating bacteria and biofilms are inter-related. Controlling the bacteria adhesion rate will affect the rate at which biofilms grow (either retarding or enhancing them). A controlled growth of desired specific strains of bacteria and the subsequent formation of biofilms can have wide applications, ranging from environmental issues (e.g. soil, water and waste treatment), to pharmaceutical, and bio-engineering (medicines, antibiotics and food additives). However, in the case of uncontrolled growth of unwanted strains of bacteria, they can be harmful or pathogenic to our health and also to the environment.

In summary, the general significance and effects of biofilms can be illustrated in the pictorial representation as shown in Fig. 6. The usefulness is elucidated in item 3 and 5, whereas the adverse effects are shown in item 1, 2, 4 and 6.

*Fig. 6 Pictorial representation of the impact and potential applications in biofilms
(item 2,4,6 & 7 adopted from CBE³)*

2.1.3 Formation mechanism of biofilm

The formation of a biofilm is typically within minutes. Once the surfaces are exposed to natural or *in-vitro* solutions containing organic molecules, an organic film, consisting of polysaccharides or glycoproteins, can be rapidly adsorbed onto clean surfaces or even to specially treated surfaces that are intended to prevent bacteria adhesion^{3,9,49,50,61-65}. This organic film is commonly known as a conditioning film, and it will change the chemical and physical properties of the surface.

The stages of biofilm formation are illustrated in Fig. 7, whereby the free floating or planktonic bacteria first encounter the conditioned surface and form a reversible, sometimes transient attachment. This attachment, called adsorption, is influenced by electrical charges carried on the bacteria, by Van der Waals forces and/or by electrostatic attraction^{3,61,63}. Although the precise nature of the interaction is still a matter of intense debate (details to be discussed in section 2.1.4), if the association between the bacterium and its substrate persists long enough, other types of chemical and physical structures may be formed from a reversible adsorption to a permanent irreversible attachment.

Fig. 7 Biofilm formation mechanism (adopted from CBE³)

The final stage in the irreversible adhesion of a cell to an environmental surface will be the production of extracellular polymeric substances (EPS). Most of the

EPS of biofilms are polymers containing sugars such as glucose, galactose, mannose, fructose, rhamnose, and N-acetylglucosamine. This layer of EPS and bacteria is able to entrap particulate materials such as clay, organic materials, dead cells and precipitate minerals, and diversify the biofilm habitat^{64,65}. This growing biofilm can serve as the focus for the attachment and growth of other organisms, thereby increasing the biological diversity of the community.

Within the biofilms, the microbial inhabitants and microbial mats behave as multi-cellular assemblages. Far from being the homogeneous populations as usually assumed in planktonic pure cultures, the biofilms are complex and always heterogeneous in nature. At one time, most scientists⁵⁴ thought that biofilms consisted of bacteria randomly distributed within a uniform slimy matrix. However, when new imaging tools like the Confocal Scanning Laser Microscope (CSLM) was introduced, which allows viewing of hydrated living biofilms, scientists^{3,32} have found that biofilm structures exhibit a wide variety of forms, which depend on the age and growing conditions. This structural complexity allows bacteria deep within the biofilm to have access to nutrients carried by the convective flow of water. Some of the features typically seen in biofilms are labeled in Fig. 8.

2.1.4 General theories of bacteria adhesion

Many studies on mechanisms of attachment of aquatic bacteria have focused on the properties of the attachment substrata^{6,33,61-63}, primarily because of the desire to identify a nonstick surface. Microbial fouling is a serious problem on surfaces ranging from ship hulls, to heat exchangers, to drinking water pipelines. To inhibit microbial colonization by the use of material that resists adhesion would reduce the need for costly cleaning or replacement of heavily fouled structures.

Fig. 8 A CSLM image (left) & a simplified model structure (right) of a typical biofilm (adopted from CBE³)

2.1.4.1 Substratum surface properties

Studies^{63,66,67} have been made to relate bacterial attachment to the physico-chemical properties of substrata, such as net surface charge, surface free energy, surface tension, or work of adhesion for water. Values for most of these parameters are derived from the measurement of contact angle, θ , of liquids on the surfaces.

The concept of contact angle is based on the interfacial energies of three bulk phases: solid, liquid, and vapor; and three interfaces: solid-liquid, solid-vapor, and liquid-vapor⁵². The diagram in Fig. 9 illustrates the components of contact angle.

Fig. 9 Definition of contact angle and the interfacial forces

When a liquid droplet interacts with a solid surface, the shape of the droplet is determined by a combination of surface energy and gravity effects. Surface forces tend to make the droplet spherical, whereas gravity tends to elongate or flatten the sessile drop. At an equilibrium point, the droplet can be characterized by the angle formed at its edge where the liquid contacts the solid surface. This angle is called the contact angle.

Although the interfacial interactions between the three bulk surfaces are based on classical and surface thermodynamics, the behavior of the liquid's surface at the boundary edge in the equilibrium state can be described by the classical Young's equation:

$$\gamma_{SV} - \gamma_{SL} = \gamma_{LV} \cos \theta \quad \text{----- (1)}$$

where:

γ = surface tension

LV = surface free energy of the liquid

SL = interfacial free energy between the solid and liquid

SV = surface free energy of the solid

θ = contact angle between the liquid and solid interface

The surface tension, γ , is basically defined as the amount of infinitesimal work needed to increase the liquid surface by an infinitesimal area. It is an extra energy per unit surface area. The derivation of this equation assumes that the solid surface in contact with the liquid is smooth, homogeneous, isotropic and non-deformable. However, in most situations, the surface condition is usually not smooth and homogeneous. As a general guideline, Neumann has demonstrated that surface heterogeneity and roughness below approximately 0.1 μm would not influence the surface energy measurement⁶⁹.

2.1.4.2 Theoretical approach to bacterial adhesion

As a bacterium moves toward a solid surface, numerous factors come into play and affect the forces that determine whether adhesion will actually occur^{33,62,63,66,67}. The interaction forces of adhesion of a particle or bacterial at various distances from a solid surface are illustrated in Fig. 10.

Fig. 10 Forces involved in attractive and repulsive interactions between a bacterium and a surface (HB = hydrophobic function groups) – adopted from Ref⁵³

At separation distances of tens of nanometers, the interaction is a balance of attraction and repulsive forces between the two surfaces. Van der Waals interactions are usually attractive, which occur between all adjacent phases regardless of composition, and act over relatively long separation distances (> 50 nm), but they are relatively weak. Electrostatic forces are significant at closer separation distances (10 ~ 20 nm). If the opposing surfaces have opposite net surface charges, then electrostatic interactions are attractive^{62,63,70}. On the other hand, most often with bacteria and potential attachment surfaces, the net surface charges are negative, and therefore electrostatic repulsion occurs and may prevent the bacterium from getting close enough to the

surface for adhesion to occur. However, repulsion forces decrease with an increase in ionic strength of the medium, and many natural environments, such as seawater, which has sufficient electrolyte concentrations to eliminate the electrostatic repulsion barrier⁶⁶.

As the bacterium moves closer to the surface, another barrier to attachment is water that is absorbed to the bacterial or substratum surfaces. Displacement of absorbed water to allow closer approach of the two surfaces is energetically unfavorable⁶³. However, if either surface has non-polar groups or patches, these can assist the exclusion of water by hydrophobic interactions. Once the bacterium overcomes any water barrier and the separation distance is in the order of < 1.0 nm, additional adhesion interactions can come into effect, such as hydrogen bonding and cation bridging, as well as the receptor-ligand interactions that are characteristics of specific interactions between bacteria and plant or animal hosts.

Although the various types of interactions (e.g. electrostatic, hydrophobic) that can determine attachment are known, it is extremely difficult to evaluate these experimentally. However, three theoretical approaches derived from colloids and surface chemistry that incorporate attractive and repulsive interactions have been applied to studies of bacterial attachment in an attempt to evaluate and understand the interactions that control adhesion. These theoretical approaches^{9, 63, 71-74} are:

- (i) The classical DLVO (Derjaguin-Landau-Verwey-Overbeek) theory, which takes into account the attractive Van der Waals interactions and repulsive electrostatic interactions;
- (ii) The extended DLVO theory, which includes the short-range Lewis acid-base interactions into the classical DLVO approach, and

- (iii) The thermodynamic model, in which the adhesive interaction is treated as an equilibrium process, is described in terms of the surface free energies of the bacterium, substratum, and separating liquid.

- **Classical DLVO theory**

The classical DLVO theory predicts two distances of separation in which there is net attraction between the surface and an approaching bacterium. First, there is the "secondary minimum," the larger separation distance, at which the bacterium is weakly held near the surface and easily removed by shear forces. Closer approach is inhibited by electrostatic repulsion; however, if the bacterium overcomes this barrier, it may be bound at the closer separation distance, the "primary minimum," where attractive forces are strong and attachment is presumably irreversible. In short, the DLVO theory is used to describe the net interaction (V_{TOT}) between a cell and a flat surface (substratum) as a balance between two additive factors, V_A , that results from the Van der Waals (vdW) interactions (generally attractive) and repulsive interactions (V_R) from the overlap between the electrical double layer (dl) of the cell and the substratum (generally repulsive)^{53,72}. The net interaction can be described as:

$$V_{TOT} = V_A + V_R \quad \text{----- (2)}$$

And, V_A and V_R are defined as:

$$V_A \approx Ar / 12d \quad \text{----- (3)}$$

$$V_R \approx cr^2 e^{-\kappa d} / (2r + d) \kappa^2 \quad \text{----- (4)}$$

In equation (3), A is the Hamaker constant, d is the separation distance between the cell and the substratum, r is the radius of the cell, which are assumed to be

spherical. According to Hoogmoed, *et.al.*³³ and Hermansson⁷², the Hamaker constant is dependent on the amount of water absorbed in the surfaces. As in equation (4), c is a constant and κ is the reciprocal of the Debye length, which is the distance over which the electrostatic effect of a single charged particle can have an effect.

Laboratory studies^{63,70} have investigated the significance of the classical DLVO theory in bacterial adhesion by measuring the effects of net surface charges of bacteria and substratum, and by determining the influence of electrolyte concentration. Some studies^{63,66} have shown attachment numbers to increase with increase in ionic strength of the medium. It is likely that in situations where bacteria and surfaces bear significant negative surface charges that attachment is inhibited^{66,67,70}. However, there are also many experimental results that cannot be satisfactorily explained by classical DLVO theory. Hence, additional theoretical models have been explored, namely the Extended DLVO theory.

- **Extended DLVO theory**

The extended DLVO theory was developed to overcome the limitations in the classical DLVO theory, which includes the hydrophobic/hydrophilic interactions and osmotic interactions into the classical DLVO theory. The total change in the adhesion energy, given by the extended DLVO theory⁷²⁻⁷⁴, is expressed as:

$$\Delta G^{\text{adh}} = \Delta G^{\text{vdW}} + \Delta G^{\text{dl}} + \Delta G^{\text{AB}} \quad \text{-----} \quad (5)$$

where ΔG^{vdW} and ΔG^{dl} are the classical vdW and double layer interactions, and ΔG^{AB} relates to the acid-base interactions. The latter component, ΔG^{AB} , describes the attractive hydrophobic interactions and repulsive hydration effects. According to

Van Oss, it is generally 10~100 times stronger than the vdW interactions of surfaces in contact^{72,73}.

- **Thermodynamic theory**

In the thermodynamic theory, the adhesion forces that act between the surfaces of two different bodies in contact is expressed by the Dupré equation

$$\gamma_{12} = \gamma_1 + \gamma_2 - W_{12}$$

or,
$$W_{12} = \gamma_1 + \gamma_2 - \gamma_{12}$$

i.e.
$$W_{SL} = \gamma_{SV} + \gamma_{LV} - \gamma_{SL} \quad \text{-----} \quad (6)$$

where W_{SL} is the thermodynamic reversible work of adhesion, which is also the negative of the free energy of adhesion, $W = -\Delta G$. Assuming that the adsorption at the solid-vapor or liquid-vapor interface is negligible (i.e. $\gamma_{SV} = \gamma_S$ and $\gamma_{LV} = \gamma_L$), the Young's equation (1) becomes

$$\gamma_{SL} = \gamma_S - \gamma_L \cos \theta \quad \text{-----} \quad (7)$$

Substituting this equation (7) into Dupré equation (6), the results yield the Young- Dupré equation for reversible work of adhesion

$$W_{SL} = \gamma_L (1 + \cos \theta) \quad \text{-----} \quad (8)$$

Where subscripts SL and L denote the solid-liquid and liquid-vapor interfaces, respectively. Equation (8) relates the reversible work of adhesion for solid-liquid interaction to measurable quantities using the contact angle measurement. In other words, the higher the work of adhesion, W , or the more negative the free energy of adhesion, $-\Delta G$, the stronger will the interaction force of adhesion be, or a reduction

in the contact angle. This also implies that at high-energy surfaces would tend to reduce its energy by absorption from its immediate environment. Aside from the knowledge that the surface tension of bacteria would also affect the total free energy of adhesion between the bacteria and solid flat surface^{68,72-74}, a solid flat surface that either exhibits an increase or reduction in surface energy, is expected to attract or repel bacterial respectively.

2.1.5 Current remedial and prevention methods

Biofilms are presently removed or destroyed by chemical and physical treatments^{3,13,14,75-77}. Chemical biocides are divided into 2 major groups: oxidizing and non-oxidizing, whereas physical treatments include mechanical scrubbing and using hot water.

2.1.5.1 Chemical biocides

In order to enter biofilm bacteria cells, chemical species in the water would run the gauntlet of biopolymers that vary in properties from strongly anionic and hydrophilic to hydrophobic. This is exacerbated by the fact that many species will accelerate their production of EPS in response to stress, including the effects of biocides. As a result, there is a considerable amount of time and/or a high concentration of many biocides required for biocide molecules to diffuse into biofilm bacterial cells.

- **Oxidizing biocides**

Oxidizers such as chlorine, bromine and especially peroxide, are popularly used to break down the polymers making up the biofilm. The effect of an oxidizer is most efficient at high pH, where they are in the form of anions²⁷. It is in their neutral forms (hypochlorous acid, hypobromous acid and hydrogen peroxide) at lower pH level that they are able to diffuse across the membranes of cells and penetrate them. Within

the cells, each of these oxidizers causes damage by producing free radicals that destroy proteins and nucleic acids. The anions, however, are repelled by the negative charges of the biofilm polymers and only act superficially on the biofilm¹³. However, while planktonic organisms may not show increased resistance to oxidizers, biofilms do. Studies²⁷ have shown that with the continued use of chlorine, biofilms would display an increase in iron content. The iron acts as a reducing agent, thereby limiting the ability of chlorine to diffuse into the biofilm. In drinking water lines, investigations^{34,35} have also found that biofilms can continue to accumulate even at a continuous chlorine concentration of 0.8 mg/l. And with further increase in chlorine concentration, the risk of pipe corrosion would be significantly raised. In addition, it also creates halogenation, which produces carcinogenic halogenated organics and in some applications, unpleasant odors.

- **Non-oxidizing biocides**

In response to increased awareness of biofilm, several newer non-oxidizing biocides^{13,14,27} have also come into use, such as biodispersants. Biodispersants are usually nonionic molecules, which adsorb to metal surfaces more readily than biofilm polymers¹³. By reducing the size of the biofilm's points of contact with the surface, these materials cause biofilm to detach from the surface. However, in practice, since these materials do not actually destroy biofilm, the biofilm detaches from high-flow areas and accumulates in low-flow areas. In addition, the low solubility of these materials can also lead to fouling by the biodispersants themselves.

2.1.5.2 Physical treatments

When the growth of biofilms is too thick, chemicals are not be able to treat them efficiently and quickly. Hence, the traditional method of mechanical and thermal

treatment would still be the most efficient and fastest way to remove biofilms. Mechanical methods such as scrubbing, scrapping, or high pressure spraying are useful and efficient for biofilm removal. Harmful pathogenic bacteria can also be destroyed when subjected to heating at greater than 80 °C¹.

2.1.5.3 Other methods

Besides the direct chemical bio-killing method, other methods that are under research at present are:

(a) BioBarrier^{27,29,30}

Using chemicals that are easily absorbed at surfaces or interfaces would affect the physico-chemical properties of the interface. An example is coating a layer of biosurfactant on the substrata. This would reduce the size of the biofilm's points of contact with the surface, causing the biofilm to detach easily.

(b) BioElectric¹⁷⁻²⁶

Since a negatively charged surface has repelling properties against bacteria adhesion (refer to section 2.1.4.1), much research has been carried out by introducing an electrical charge to the test surface and evaluating the biofilm adhesion and growth characteristics. Results²²⁻²⁴ have found that the bioelectric effect required a current flow to influence bacterial adhesion whereas an electric potential could only cause a negligible effect. The reason reported was that the flow of the electric current across the biofilms would cause an electrolytic oxygen generation, which probably reached toxic levels to the bacterial cells.

(c) Ultrasonic

Experiments were conducted using ultrasonic wave energy on biofilms. The purpose is to break loose the biofilm so as to improve the efficacy of biofilm killing from the concurrent antibiotic treatments. This enhanced bacterial killing method was reported to depend on the ultrasound power density, frequency, and waveform^{78,79}. Although ultrasound alone was not significantly effective against biofilms, there was a substantial effect when ultrasound was used together with an antibiotic treatment. The amount of killing was observed to be between one and two orders of magnitude greater than the killing by antibiotic alone⁷⁸.

2.1.5.4 Summary of existing problems in biofilm control

As highlighted in section 2.1.1 and 2.1.5, since the discovery of biofilms two decades ago, much research has been done to prevent bacterial adhesion and the subsequent formation of biofilms on any man-made materials. Surface treatment to prevent bacteria adhesion, such as using organic surfactants in bio-barriers, or using bio-electric, which uses an electric charge, had been tested. However, practical tests have shown that none have been very successful so far^{32, 33}. The main reason is that bacteria adhere to a wide variety of surfaces, especially wild bacteria in natural ecosystems³. The other reason is the formation of conditioning films that can be rapidly adsorbed onto clean surfaces, or even to specially treated surfaces that are intended to prevent bacterial adhesion, once the surfaces are exposed to natural and in vitro solutions containing organic molecules. This conditioning film practically neutralizes the anti-bacterial adhesion surface effects and creates favorable physiochemical conditions for subsequent bacterial adhesion.

Presently, the common anti-biofilm approach used is to develop biocidal chemicals or genetically modified bacteria to target and kill the pathogenic bacteria hiding within the extracellular matrix wall of polymers in the biofilm. This top-down approach, although in some way very effective against homogeneous cultures, its effect against heterogeneous cultures is limited (refer to section 1.1). Moreover, this technique would only last for a period of time before the targeted bacteria mutate and developed into a more resistant strain, just like the case of using too much antibiotics to treat body infections (see illustrations in Fig. 11).

Fig. 11 Current "top-down" anti-biofilm approach & its after effects (adopted from Ref³)

In addition, there are also limitations in the amount of biocides that can be used without causing negative side effects. Table 2 summaries the limitations that are presently encountered.

Table 2. Summary of limitations encountered in present remedial and prevention methods

Actions	Methods	Main problems encountered
Remedial (to remove biofilm)	<ul style="list-style-type: none"> Oxidizing biocides (Note: → means from high to low effectiveness) (ozone → chlorine dioxide → chlorine → iodine → hydrogen peroxide) Non-oxidizing biocides (Quaternary ammonium, formaldehyde, anionic & non-ionic surfactants) 	<ul style="list-style-type: none"> Using chemicals to either destroy the extra-polymeric saccharides (EPS) or bacteria is never complete (max 50% effective). Moreover, concentration is limited by the side effects, such as corrosion. Surviving bacteria will develop new resistant strains or produce more slime to protect them.

Actions	Methods	Main problems encountered
Remedial (to remove biofilm)	<ul style="list-style-type: none"> • Genetic engineering (gene modification) • Mechanical treatment (heat and scrubbing) 	<ul style="list-style-type: none"> • Surviving bacteria will develop new resistant strains or produce more slime to protect them. • Limited accessibility and material weakness to heat and abrasion.
Prevention (to prevent adhesion)	<ul style="list-style-type: none"> • Bio-barrier (bio-surfactants) • Bio-electric (electric charge) • Ultrasonic (mechanical means) 	<ul style="list-style-type: none"> • Difficult to flush. Low solubility and fouling potential. • Low effectiveness and neutralized by conditioning film. • Low effectiveness & difficult to apply on non-assessable surfaces

2.1.6 Concluding summary

Bacteria are generally able to adhere to a solid surface by means of interfacial and surface energy interactions, or by electrostatic interactions. The mechanisms of bacterial adhesion to the solid surface are presently governed by three approaches, namely the classical DLVO, extended DLVO, and the thermodynamic model.

Upon the subsequent formation and growth of biofilms, many become undesirable, or harmful to human health and to its host surfaces. Scientists and researchers are presently carrying out on-going research to improve the efficacy in killing the bacteria living within the biofilms, or to prevent bacterial adhesion and biofilm formation. Thus, there is a need to find new methods or approaches to overcome the difficulties, especially from the neutralizing effects of the conditioning films and the diversity of bacterial species in an aquatic environment.

2.2 Introduction to magnetism and surface magnetism

The theories on magnetism and surface magnetism have long been established and applied in many fields. Although most of the applications of magnetism are presently focused on the semiconductor and data storage technology, on-going research is still in progress for biological applications. To the author's understanding, there are still no conclusive applications of magnetism to bacterial adhesion and biofilms formation.

In this literature review, the aim is to understand some basics on magnetism, particularly on magnetic materials used for data storage media, its characterization, and finally on the phenomenon of spins and anisotropy, which are the main tools for the development of the bio-barrier.

2.2.1 Bulk magnetism

Magnetism is the response of materials towards an applied magnetic field. Fundamentally, the magnetic properties of matter are the result of microscopic atomic currents, which produce magnetic moments in matter. The origin of this atomic current is the orbital and spin motion of electrons around the nucleus^{80,81,82}. This constant spin motion is a permanent source of energy and current. In the atom, there is no resistance to the motion of the electrons, and the induced current and magnetic moment generated will therefore persist indefinitely. Free atoms of some elements may have zero magnetic moment because the various orbital and spin components completely cancel each other. However, the magnetic properties of any material in bulk depend not only on the magnetic moment of the free atoms or molecules, but also on temperature and on the complicated inter-atomic forces in condensed matter. Apart from the electronic magnetic moment, the protons and neutrons (or nucleons) of atoms also have magnetic

moments associated with them, but these are small compared with those of electronic origin.

In magnetism, the orbital motion of electrons constitutes a magnetic dipole, an orbital dipole. However, experiments have shown that it is the electron spin and its associated magnetic moment, rather than the orbital motion, which give rise to the bulk magnetic properties of magnetic materials⁸³. Almost all electronic orbits are paired with orbits of equal and opposite angular momentum (clockwise and counterclockwise) so that their overall magnetic moments add up to zero. An increase in atomic number in the periodic table corresponds to an increase in the number of electrons in the shells surrounding a nucleus (and a corresponding increase of the number of protons in the nucleus). Usually, an inner shell is filled with the appropriate number of electrons before the next shell begins to get electrons. One of the important properties of filled shells is that the spins and the resulting magnetic moments of all the electrons in the shell, like their orbital angular moment, must add up to zero. The magnetic effects of different electrons tend to cancel each other, producing no net magnetization.

Some atoms, however, do have a magnetic moment. In the so-called transition elements, an outer shell begins to fill before an inner one is completed. The resultant electron spin in this partially filled inner shell can be non-zero, even though the orbital angular momenta are paired and have no orbital magnetic moment. It is this partially filled inner shell that can give rise to the magnetic moment of an atom^{84,85}. Thus, magnetic materials usually contain atoms of the transition elements iron (Fe), nickel (Ni), cobalt (Co), and manganese (Mn), or some of the rare earth elements.

The fact that the individual atoms in a solid have magnetic moments is not enough to make a big piece of the material a magnet. For bulk magnetic properties,

adjacent magnetic moments must somehow line up parallel. There must be an interaction between two adjacent atomic moments, which tends to make them line up parallel and not cancel each other out. There is just such a powerful coupling between the magnetic atoms in magnetic materials. The theoretical physicists call it exchange interaction. This is an extremely short-range quantum mechanical interaction between spins. In some systems it forces spins to be parallel, in others to be anti-parallel. The strength and the sign of the exchange interaction between two atoms depends on the distance between them and on the details of the quantum mechanical states within the solid. This study is a rather advanced topic in solid-state physics. Fortunately, we can observe experimentally the effects of exchange energy on the magnetic properties of a material.

The atomic magnetic moments in some magnetic materials are close enough to be affected by exchange interaction and to exert strong torques on each other. In the simplest case, this exchange interaction makes the energy lowest if adjacent spins are parallel. At very high temperatures, however, thermal disorder overrides this exchange interaction, resulting in no overall alignment of the spins (in thin films, it is known as super-paramagnetism). When the specimen is cooled below a special temperature (the Curie temperature, T_c , which varies with material), the exchange interaction suddenly dominates, and the spins in a large volume of the material lock into parallelism. We can think of a regular three-dimensional array of little arrows all parallel to each other (Fig. 12). The magnetization of the material is equal to the sum of the atomic magnetization. We say that the spins are "ordered" and that this ordering over long distances is a "cooperative" phenomenon. Where the spins are all parallel, the ordering is called "ferromagnetic".

Fig. 12 Types of spin ordering

In a ferromagnetic material, all the magnetic moments of the individual atoms or ions are aligned parallel to a particular direction. The strength of the internal magnetic field is known to be in the order of $10^6 \sim 10^7$ Gauss (i.e. 100T ~ 1000 Tesla) as estimated from the transition temperature, especially for ferromagnetic materials⁸⁶. And for iron, its internal magnetic field strength is as high as 103 Tesla. This level is extremely high as compared to the powerful magnets used in Magnetic Resonance Imaging (MRI) system, which is limited to 2 Tesla for safety reasons.

For the other two types of magnetic ordering (see Fig. 12), the antiferromagnetic materials are also considered as ordered but have no net moment, whereas the ferrimagnetic materials are in-between the two. Although the net magnetic strength in ferrimagnetic materials is lower than the ferromagnets, the magnetic properties are similar and are more widely used in magnetic recording applications.

2.2.2 Magnetic poles and spin directions

The “north-south” connotation in magnetism was established through historical association⁸⁴. The magnetic field of the earth is orientated in a manner that places the “magnetic poles” in close physical position with the geographic poles. In fact, a compass “north” needle would actually point towards the north geographic pole from many places on earth. However, these two entirely different concepts were unfortunately being merged into one. To avoid confusion, this report adopts the

compass “north” needle as a magnetic north pole and it will be attracted to a south-pole side of a permanent magnet. Based on scientific convention, the compass "north" needle points in the direction of the magnetic flux, that is, in an outward direction from a magnet's North pole end and inward at the magnet's South pole end. This configuration is consistent to the magnetometer instrument's and vibrating sample magnetometer's (VSM) positive forward direction.

On the direction of a magnetic field, it is determined according to the right hand rule; i.e. if the right hand is placed around a conductor with the thumb pointing in the direction of current flow, the fingers will point in the direction of the associated magnetic field. Similarly, if the right hand is placed around the solenoid of Fig. 13 with the fingers pointing in the direction of current flow, the thumb points in the direction of the magnetic field ⁸⁷.

*Fig. 13 A torroidal magnetic field
 (adopted from ⁸⁷)*

However, for an electron, or other negatively charged particle, it moves in the opposite direction from current flow (or positive particle movement), hence, the left hand is substituted for the right in determining field directions (refer to Fig. 14 for illustration).

Fig. 14 Magnetron : A spinning charged particle that generates a magnetic field of torroidal shape (adopted from ⁸⁷)

2.2.3 Magnetic anisotropy

The properties of a magnetic material depend on the direction of the magnetization vector with respect to the direction of a stress distribution. These are part of the general topic of magnetic anisotropy ^{84,86,117}. There are three kinds of anisotropy, namely, crystal anisotropy, shape anisotropy, and stress anisotropy

(i) Crystal anisotropy

Crystal anisotropy is an intrinsic property of magnetic materials. In films of most magnetic materials, the magnetization lies in various directions parallel to the surface. In most solid materials, except glasses, they are made up of little crystals in each of which the atoms are arranged in regular patterns. So, for example, in a crystal of nickel, less energy is required to magnetize the crystal in the diagonal path. This is said to be the easy axis, while a direction along the edge is a hard axis. The difference in the energy required to magnetize the crystal in an easy direction compared to a hard direction is a measure of anisotropy. In cubic crystals either the body diagonals, face

diagonal, or the cube edges can be easy directions, and the magnitude of anisotropy energy can vary widely in different materials.

The properties of the particular crystal structure and even the overall shape of a magnetic material give rise to anisotropy that determines the preferred direction of magnetization. A critical part of the manufacture of tapes, disks, and recording heads is the design of the little crystals of a material so that one can control the direction of magnetization and thus influence the amount of energy needed to magnetize it.

(ii) Shape anisotropy

When the magnetic crystal shape is non-spherical, it will be easier to magnetize it along the long axis than along a short axis. The reason for this difference is that the strength of shape anisotropy depends on the axial ratio of the magnetic crystal⁸⁴. The more elongated the crystal shape is, the higher the shape anisotropy is along the hard axis (see Fig. 15).

Fig. 15 Axes of magnetization of a magnetic crystal

(iii) Stress anisotropy (magnetostriction)

When a magnetic material is exposed to a magnetic field, its dimensions will be changed. This effect is called magnetostriction. This mechanical movement caused by

the external magnetic field will induce a stress into the crystal, and this in turn contributes to the magnetic anisotropy. The magnetostriction occurs during the magnetization process when the domain walls are in motion and rotation. The effects of magnetostriction on the magnetization curve (hysteresis loop) and the magnetic properties can be significant⁸⁸. In Fig. 16, the effects of an applied tensile and compressive stress on the magnetization (M-H) curve are demonstrated on nickel material.

Fig. 16 Effects of applied tensile (+) and compressive (-) stress on the magnetization of nickel. Applied stress are in lb/in² (adopted from Ref⁸⁸)

It is observed that the magnetization curve can be significantly shifted to either direction when a stress is applied to the magnetic material. Although the curve in Fig.16 shows an increase when the nickel was subjected to a compression (-) stress, and reduced upon a tensile (+) stress, the behavior can be complicated. For some other magnetic materials, such as 68 Permalloy (a family of Ni-Fe alloys), the behavior is the opposite. Nevertheless, it is important to note that the effects of magnetostriction (stress anisotropy) influence the properties of magnetic materials.

2.2.4 Anisotropy energy and surface magnetism

Anisotropy energy is by definition a part of the crystal energy that depends on the direction of magnetization ⁸⁹. When the magnetization vector deviates from the preferred easy direction, the anisotropy energy increases. At the magnetic surface, the atoms are known to present an electronic structure different from that found in the bulk ⁸⁶. This is due to the magnetic atoms having fewer neighbors and a lower coordination than the interior ones and as a result, a reduced symmetry occurs^{90, 91}. Based on research studies on ferromagnets, the surface magnetic moments from these un-paired electron spins at the free surfaces are enhanced by the narrowing of the electronic 3d-bands ⁹¹⁻⁹³. The electronic bands for the surface electrons in the spin up state is filled and has a higher density of d states (or surface energy), as compared to the spin down state, which contains holes and at a lower density of states ⁹⁴⁻⁹⁸. This phenomena leads to a uniaxial magnetic anisotropy that acts normal to the surface.

This magnetic surface anisotropy was first observed by Néel, who described the phenomenological surface anisotropy by a surface energy density equation ^{94,99,100}

$$\sigma = K_S \cos^2 \theta \quad \text{-----} (9)$$

where θ is the angle between spontaneous magnetization and normal surface. K_S is the magnetoelastic constant. From equation (9), the surface anisotropy energy increases to a maximum when the magnetization direction is exactly normal (90°) to the magnetic surface.

In addition, experiments (in vacuum state) on ferromagnetic surfaces in the spin down state were reported to exhibit strong hydrogen and oxygen molecule adsorption

into the surface ¹⁰¹⁻¹⁰³. These phenomena were explained by the lowering of energy state in the surface electrons, which contains more holes in the 3d shells.

2.2.5 Domain wall

Domain walls are interfaces between regions in which the spontaneous magnetization has different spin directions. Within the domain wall, it contains large exchange energy that is associated with spins in parallel and anti-parallel directions ^{83,92}. Figure 17 shows schematically how the spins would appear in the wall between two domains. At the wall, there is a 180-degree spin turn from the edge of one domain to another.

Fig. 17 Spin directions at domain walls and transition wall

Within the domain wall, there are spins that do not lie along the easy axis (see vertical arrow in Fig. 17), which is the anisotropy energy. There are adjacent spins that are not exactly parallel to each other, which is known as exchange energy. Making the wall thicker would reduce the change in spin direction between adjacent spins, and would thus reduce exchange energy. Making the wall thinner would reduce the number of spins not along an easy axis, and would reduce anisotropy energy. Thus the wall thickness is determined by a balance of the anisotropy and exchange energies, which also varies greatly from a material to another. Idealized wall thickness varies from a few hundred angstroms to a few thousand angstroms, but in actual fact, magnetic

materials usually break up into many such domains resulting in no large-scale magnetization.

2.2.6 Magnetization curves - hysteresis loop

The hysteresis loop is a series of curves that show the characteristics of a magnetic material. When a ferromagnetic material is magnetized in one direction, the magnetic material will not relax back to zero magnetization even when the imposed magnetizing field, H , is removed. It must be driven back to zero by a field in the opposite direction. If an alternating magnetic field is applied to the material, its magnetization in the material, M , will trace out a loop called a hysteresis loop. The lack of retraceability of the magnetization curve is the property called hysteresis and it is related to the existence of magnetic domains in the material. Once the magnetic domains are reoriented, it takes some energy to turn them back again. This property of ferromagnetic materials is useful as a magnetic "memory". Some compositions of ferromagnetic materials will retain an imposed magnetization indefinitely and are useful as "permanent magnets". An example of a hysteresis loop is illustrated in Fig. 18¹⁰⁵.

Investigations have found that an increase in the magnetizing field will cause the up domains to grow larger, whereas the down domains will decrease, and so the overall net magnetization increases. At some field level, all of the down domains are eliminated and the materials are considered saturated. This saturation field is known as H_{sat} , and fields above H_{sat} can no longer change the net magnetization. But when the magnetizing field is reversed or decreased, the net magnetization will begin to decrease after the applied magnetizing field is below the saturation level. Then the down domains will appear and gradually grow as the reversed magnetizing field continues to

increase in the opposite direction. However, the curve will not retrace back to the same curve as before, even when the magnetizing field is returned back to zero. Reason is that there is some magnetization left, which is called the remanent magnetization, M_R .

Fig. 18 Hysteresis loop in a magnet (adopted from ¹⁰⁵)

Upon further increase in the magnetizing field in the opposite direction, the magnetization will continue to decrease until zero (Amp-turn/m). The amount of magnetizing field to cause the magnetization to zero is known as the coercive field, $-H_C$. A further increase in the negative field will cause the magnetization to increase, but in the opposite direction, until the sample again becomes saturated when the field reaches $-H_{sat}$. However, when the positive field is again restored back, the up domains will reappear and eventually, the curve will trace back to the H_C , where the net magnetization is again at zero, but on the positive side. If the magnetizing field is continuously changed back and forth (applying alternating current to the coil), a loop known as a hysteresis loop can be traced out ^{83,84} (refer to Fig. 18).

Fig. 19 Hysteresis loop of a soft and hard magnetic material

Soft magnetic materials contain domains that move very easily, and are known to have low coercivity and low remanence, and will display narrow hysteresis loops. These materials tend to be mechanically soft and are easily deformable. An iron nail will bend, and it has a relatively thin hysteresis loop (see left - Fig. 19). In hard magnetic materials, they possess high coercivity and remanence and demonstrate wide hysteresis loops (see right - Fig. 19). Typically, these hard materials are mechanically hard and are often exceedingly brittle. A permanent magnet made of AlNiCo will break quite easily. Hard magnetic materials can be prepared by introducing nonmagnetic impurities to hinder the motion of domain walls. Another method to make a hard magnetic material is to divide it into particles so small that a domain wall will not fit within the particle, i.e. a thin film coating. If the film thickness is less than the domain wall size, there can be no domain wall and the magnetization will not be able to be reversed by the domain wall mechanism as previously described. Only when the magnetization field exceeds the anisotropy, the magnetization will then flip over. Thus, a thin film will provide a large anisotropy and high coercivity.

2.2.7 Particulate films and thin films

In magnetic data storage media, the most widely used particles are gamma-phase iron oxide, $\gamma\text{-Fe}_2\text{O}_3$. These particles are needles of less than a micron long and a length-to-width ratio of about 5-to-1. If the particles were very small such that they cannot support a domain wall, they would become single-domain particles (see Fig. 20).

Fig. 20 Multi- and single-domain particles

The magnetic particles will be magnetized along their length because of shape anisotropy, i.e. parallel to the horizontal surface. In order to reverse the direction of magnetization, it is necessary to apply a field high enough to exceed the coercivity value. However, in most modern applications, higher coercivity is a necessity, especially when recording data bits are placed close together for higher recording densities, where the demagnetizing effects from neighboring bits become large.

To achieve a higher coercivity, it has been discovered that the addition of cobalt to the iron oxide, either within the particles or near their surface, is able to produce particles with increased coercivities from originally 300 Oersteds (Oe) to as high as 600 to 800 Oe. However, the increase in coercivity from modified $\text{Co-}\gamma\text{-Fe}_2\text{O}_3$ is still insufficient for even higher recording densities that require the coercivity values to be as high as 1800 Oe. Such high coercivities can be achieved from thin films that are usually metallic layers deposited on the substrata in a high vacuum system. The films are commonly made of cobalt, nickel, and phosphorus. There are several methods for

producing these films, some of which give rise to needle-like metal particles aligned almost parallel to the surface. Others give rise to aligned crystallites with their easy axis of magnetization perpendicular to the surface (magneto-crystalline anisotropy). In these latter films, the information is stored in the form of domains magnetized in the up or down direction, perpendicular to the surface^{84,86,106-108}. This perpendicular recording scheme shows promise of providing much higher recording densities than the parallel recording method, which is still used in most applications. However, for thin film coating performance, there is an optimum film thickness for different magnetic materials^{84,109}. For example, for iron, the optimum film thickness for maximum coercivity is about 150 Å (refer to Fig. 21).

Fig. 21 Variation of coercivity with particle size (adopted from⁸⁴)

In principle, single domain particles must reverse their magnetization by spin rotation, which is a comparatively difficult process if the particle has a significant intrinsic magnetic anisotropy¹¹⁰. Consequently, single-domain particles have a high coercivity, which is the basis of most of their applications. The most important intrinsic factor determining the magnetic behavior of fine particles is their magnetization reversal mechanism. In very small (nanocrystalline) materials, this tends to approximate to spin rotation. However, larger particles, especially those approaching

the critical size for single domain behavior, reverse by complex “incoherent” mechanisms.

2.2.8 Magnetic data storage media and recording technology

- **Floppy disk structure**

The 3½” computer floppy disk (FD) is particulate film coated, consisting of 37~50% (by volume) of cobalt modified ferric oxide (Co- γ -Fe₂O₃), mixed in organic binders and resins to form the magnetic media (2 ~ 5 μ m thick) on the 12 ~ 36 μ m thick flexible polyethylene terephthalate (PET) substrate. It has a load bearing coating of either Al₂O₃ or Cr₂O₃, and an additional lubricant coating for wear resistance purposes ¹¹¹.

The magnetic particles are acicular in shape (see Fig. 22 for illustration) and the length-to-width ratio is about 5-to-1 ¹¹². The physical and magnetic properties of the floppy disk are summarized in Table 3.

Fig. 22 Typical appearance of Co- γ -Fe₂O₃ particles used in floppy disk

Table 3. Summary of physical and magnetic properties of floppy disk ^{111,113}

Particle length, μ m	Curie temperature, K	Density Kg / m ³	Coercivity Oe	Remanence magnetization Oe
0.3 ~ 0.6	793 ~ 870	4800	600 ~ 900	750

• **Hard disk structure**

In magnetic hard disks, the various film layers are mostly deposited by the chemical vapor deposition (CVD) or the sputtering process^{111,114,115}. The hard disk magnetic media is thin film sputtered-coated, which composes of (from top to bottom) an organic lubricant layer (0.5 ~ 4 nm), a protective overcoat layer (20 ~ 40 nm), the Ni-Co-Cr magnetic layer (25 ~ 100 nm thick), and the aluminum substrate (1.3 ~ 1.9 mm thick)¹¹¹. The structure of the hard disk coatings and the physical properties are illustrated in Fig. 23 and Table 4 respectively.

Fig. 23 Typical structure of a hard disk coating

Table 4. Summary of physical and magnetic properties of typical hard disk^{111,114}

Curie temperature, K	Density, Kg / m ³	Coercivity, Oe	Remanence magnetization, Oe
Approx. 1000	8000	400 ~ 2000	13,000

Although CVD or sputtering processes are able to produce uniform and thin coatings, the condensation of the evaporating atoms on the disk surface would induce high mechanical stress from the heat of condensation and the dissimilar coefficients of thermal expansion between the coating and target materials^{115,116}.

- **Magnetic recording technology**

Magnetic recording technology was first started in the late-twentieth century¹¹⁷ and has evolved into a highly sophisticated application using magnetic materials and various techniques. The most common recording technique used in data storage is illustrated in Fig. 24, which illustrates the schematic of components of a horizontal recording hard disk drive. On the magnetic recording medium, each data bit along the polarized track will exist a transition area that exhibit a near-perpendicularly magnetized condition at either north or south polarity^{118,119} (see Fig. 25). These perpendicularly polarized areas are not used in the data reading process but it can be observed to exist from the magnetic force microscopy (MFM) images, which is shown in Fig. 25.

Fig. 24 Configuration of a horizontal magnetic recording technology of a hard disk drive

Fig. 25 The transition area between a bit which gives rise to a perpendicular magnetic polarity – alternating dark-bright bars in MFM image demonstrates N-S changing polarity (adapted Ref

2.2.9 BioMagnetism and applications

Since ancient China time in 2000 B.C., the study of magnetic effects on biological living things, known as biomagnetism, had been known. It was claimed that the north magnetic pole of a magnet has a negative influence on biological activity¹²⁰, whereas the south pole of the magnet promotes biological activity¹²⁰⁻¹²³. The applications of biomagnetism, were later popularized in Europe by Mesmer¹²¹, an Austrain physician, in 1775 (called animal magnetism). In 1936, an American, Albert Davis, reported in details the dissimilar effects on biological life from the 2 poles of a magnet^{80,124}. However, to-date, these claims are still controversial¹²⁵. For example, the most common use of magnetic fields is in the treatment of pain, such as arthritis and rheumatism. In the practical sense, the use of this magnetic therapy has been reported to improve the condition¹²⁴, but due to the lack of strong scientific evidences, many scientists have doubted its capabilities.

In addition, much of its applications are based on using strong powerful magnets that produce large magnetic field strength, from kilo-gauss to mega-gauss, and they are used to treat living bodies, mainly in human bodies^{80,121,124}. The lowest magnetic strength used in research on biological cells and invertebrates¹²² is about 0.5 G, but using a 60 Hz pulse. To the author's knowledge, there are no documented research work on magnetic surface effects on biological cells and the adhesion properties.

2.2.10 Concluding summary

The origin of magnetism is from the ordering of the spin motion of electrons, and the exchange interaction that forces adjacent magnetic moments to align in parallel to give rise to a net magnetic field. In a magnetic data storage media, as long as the

external magnetizing field strength exceeds beyond the anisotropy and coercivity values, the magnetization direction can be retained even when the external magnetizing field ceased.

Magnetic anisotropy energy in a magnetic media, particularly from the shape and stress anisotropy, can be varied and increased by the angle and strength of the perpendicular magnetization field against a horizontally aligned media. In surface magnetism, it has long been established that the reduced symmetry of surface atoms will exhibit an enhanced magnetic moment from the un-paired electron spins. At the free surfaces, the electronic bands in the spin down state are in the lower energy of state and have more holes in the 3d shells. At this state (spin down), it was also reported to adsorb oxygen and hydrogen molecules into the magnetic surface.

From the literature review, it can be gathered that magnetic surface anisotropy energy is a source of high energy, especially when a horizontally aligned magnetic media is perpendicularly polarized. In addition, different spin directions have been demonstrated and explained by the change of energy state that can either be raised or reduced. Since energy and magnetic field propagates through most matter, it is plausible that a perpendicularly polarized magnetic data storage media may exhibit a change in the surface free energy when the energy of state changes at the surfaces and interfaces of the magnetic media. If this hypothesis is true, it would be possible to use the change of surface free energy to influence the bio-physical interaction with bacterial cells.

CHAPTER THREE

NOVEL IDEA, CONCEPTS

AND

APPROACH

3.1 Novel idea and Concept

The objective of this research project is to develop a new biobarrier technique using the properties of anisotropy surface energy and the phenomena of ordered electron spins to influence and control bacterial adhesion and the subsequent formation of biofilms. The aim is to create the anisotropy energy from the perpendicular polarization and using the characteristics of ordered electron spin to channel it to the surface or away from it. In addition, an energy field is preferred for its ability to penetrate through living cells or any organic conditioning films, which is to overcome the problems encountered by existing biobarrier techniques.

On the significance of the wild bacterial species that may overcome the influence of the energy field, the literature review in chapter 2 has found that all living cells at the cellular level respond to energy stimuli, and bacteria growing in biofilms require relatively high levels of energy substrate for growth. In addition, the adhesion process to a solid surface is also fundamentally governed by the thermodynamic reversible work of adhesion, which is also known as the free energy of adhesion.

Using these properties of ordered electron spins and the change of energy state as a channeling or switching mechanism, the induced energy from the anisotropic effects that act normal to the magnetic surface may probably be shifted according to the spin (or magnetization) directions. If this hypothesis is true, the surface free energy at the open surface can be altered and this in turn will influence the adhesion properties of the bacterial cells. Moreover, the change of energy level at the surface may also influence the growth behavior of the adhering cells. The inter-relation between the bacterial cells, anisotropy energy and electron spins are illustrated in Fig. 26. Figure 27 illustrates the conceptualized working mechanisms, which are described in the following four stages.

Fig. 26 Inter-relation between bacterial biofilms, anisotropy energy and magnetism

Fig. 27 Conceptualized working mechanism of a biobarrier (stages with (a) headings refer to magnetic-south polarization, whereas (b) indicates north-polarization)

At the first stage (Fig. 27 – stage 1a and 1b), an external magnetizing field is applied to the magnetic media to obtain a global perpendicular magnetization. Based on the phenomenological surface anisotropy equation (9), a perpendicular magnetization will induce a maximum surface anisotropy energy into the magnetic media.

At stage two (Fig. 27 – stage 2a and 2b), the anisotropy energy is channeled into or out from the magnetic media surface when the energy of state changes in response to the direction of electron spins (magnetization directions) in the up (north) or down (south) state.

In stage three (Fig. 27 – stage 3a and 3b), the surface free energy would be altered when the energy is either released to the surface or being absorbed (depending on the spin directions). Based on the thermodynamic reversible work of adhesion that is given in equation (8), bacterial adhesion would inevitably be affected.

And finally at the last stage (Fig. 27 – stage 4a and 4b), the change in the surface free energy at the open surfaces is expected to influence and affect the cell growth behavior. Knowing that cell-to-cell communication occurs through “quorum sensing” (refer to section 1.1), the conditions of the magnetically polarized surface would be communicated to the neighboring cells and may eventually affect the planktonic cell growth.

3.2 Approach plan

To verify the various stages of the concept, the work done is outlined in Fig. 28.

Fig. 28 Outline of approach to study, characterize, and evaluate the polarized magnetic surface on the biophysical influences on bacterial adhesion & growth

After achieving a perpendicular polarization of the magnetic media, the polarized substrates would be characterized for its magnetic states and surface changes, such as its topography, surface free energy and electric potential. This is followed by the biological experiments to evaluate its biophysical effects on bacterial adhesion and the subsequent biofilm formation. This includes the evaluation on the planktonic cell growth and a surface morphological study on the adhered cells in the biofilm.

CHAPTER FOUR

MATERIALS

AND

METHODS

This chapter describes the preparation method and processing for the magnetic substrates prior to the material and surface characterizations and biological experimentations. The chapter is divided into four topics, where the first topic describes the preparation of the magnetic substrates, followed by the methods and settings used to characterize the magnetic media.

The second topic focuses on the preparation of the bacterial cell cultures and the methods to grow the biofilms. On the third part, qualitative and quantitative analyses are discussed on the evaluation of the biofilm formation on the magnetic substrates and also on the effects to the planktonic cell growth.

And finally, the last topic presents the additional experiments that were conducted to compare the efficacy of the proposed mechanism against similar methods used in the industry.

Given that the scope and variety of the experiments is wide and numerous, a summary of the materials used, the experimental setup and the analyses methods adopted are given in Fig. 29.

4.1 Magnetic media and characterizations

Based on the Literature Review on magnetic anisotropy, the maximum anisotropy energy can be achieved by either polarizing a horizontally aligned magnetic media in the perpendicular direction, or a perpendicular media being horizontally polarized. Given the limitations from using a permanent magnet, it will be easier to polarize a horizontal media than a perpendicular media which requires the direction of the external magnetizing field to be along the media's plane surface. This would result in a less uniform polarization and induced anisotropic energy when the sample is large.

Moreover, perpendicular magnetic media are generally not easily available as compared to the horizontal type. Hence, perpendicular media will not be considered in the research.

For this research, the magnetic media used are the commercial horizontal magnetic data storage media, namely the 3½" floppy and hard disks. The commercial floppy disk was obtained from "Maxell 2 HD, 1.44 MB double density", whereas the hard disk was obtained from the "Western Digital – 85MB" hard drive.

Fig. 29 Summary on materials and methods used

Given that FD and HD have magnetic coatings on both sides of the disk, the direction of the electron spins and magnetic moments induced in both coatings would be the same. Hence, no magnetic interferences are expected from the unused side of the polarized

magnetic coating to the useful side at the opposite surface. In addition, all the experiments would be conducted on flat surfaces in which the unused side would be secured to the bottom surface of the container. This substrate mounting method would further ensure that the unused side of the coating does not interfere with the useful side.

4.1.1 Floppy Disk (FD)

The particulate film coated floppy disk (FD) was first extracted from the floppy disk assembly and subsequently cut into strips (using scissors) to the required sizes. This was followed by the perpendicular (001) magnetization to the film plane by placing it directly onto a permanent magnet block (NdFeB) with an external magnetizing field of 5000 Oe for a 30 min period. Figure 30 shows the schematic illustration for the magnetization process (the magnetic polarity is defined in previous Section 2.2.2).

Fig. 30 A schematic illustration of the polarizing process of the magnetic media

4.1.2 Hard Disk (HD)

Similarly for the 3½" hard disk, it was first extracted out from the hard drive assembly and subsequently cut to the required sizes using an industrial band saw cutter and deburred by grinding. Extra care was exercised during the cutting and grinding of the HD to prevent surface scratching. The magnetization method and duration for the HD samples were the same as the FD samples.

4.1.3 Magnetization (hysteresis) curve

The magnetization curves (hysteresis loop) of the FD and HD samples were obtained by the vibrating sample magnetometer (VSM LakeShore Model 7300). The FD and HD samples were affixed to the VSM holder (see Fig. 31) at perpendicular position to the magnetizing field path (see enlarged photo on the right in Fig. 31). At least 3 samples per magnetic state were used for this measurement.

Fig. 31 Vibrating sample magnetometer (LakeShore 7300) used for magnetization curve measurement (enlarged photo shows the holder for the magnetic samples)

4.1.4 Magnetic states

The magnetic states of the perpendicularly polarized substrates were determined first before conducting further experiments on the samples. These were done using the following two methods.

4.1.4.1 Lorentz Microscopy

The magnetic states of the polarized substrates were observed by the Lorentz microscopy, which was done in a scanning electron microscope, SEM^{126, 127}. The settings for the probe and filament current were set at 1.5 nA and 2.67 A respectively. The acceleration voltage used was 20 kV and the magnification was about 6 times. Sample quantity for each magnetized condition was more than 5.

4.1.4.2 Atomic Force Microscopy – Magnetic Force Imaging

Atomic Force Microscope (AFM) was first developed by Binnig *et.al.*¹²⁸ in 1986, and is presently an established instrument for imaging surfaces in their native environment at high resolutions^{129, 130}. The AFM consists of a flexible cantilever probe with a sharp tip on the end. The probe scans across a sample surface while maintaining a small constant force. There are two most commonly used modes of operation in AFM, which are the contact mode and the tapping mode. In addition, AFM imaging can be conducted either in air or liquid environment. The tapping mode is more suitable for imaging on soft, fragile, and adhesive surfaces without damaging them, which can be a drawback for the contact mode AFM^{131, 132}.

For the magnetic force imaging on the magnetic substrates, a commercial AFM (Nanoscope IIIa, Digital Instruments Inc) equipped with a J-type scanner and an Extender™ electronics module for frequency modulation was employed^{40,41,127}. A standard 225 μm long, 50 nm-thick Co alloy magnetic, etched silicon probe (MESP) was first magnetized using the south-pole side of the 1700 Oe permanent magnet. MFM imaging was carried out under the Interleave® and LiftMode® modes. Lift scan height used was 150 nm with various scan areas of 30 x 30 μm^2 , 10 x 10 μm^2 and 5 x 5 μm^2 , and scan lines of 256 were acquired. Drive frequency was 60 to 80 kHz (using the secondary resonance

frequency) with a scanning rate of 1 to 2 Hz. Sample quantities was at least 20 samples for every magnetized condition.

4.1.5 Surface morphology using AFM

In the study of the surface morphology of the magnetic samples and bacterial cell surfaces, two AFM modes of operations were employed. They were the tapping in air and in liquid.

4.1.5.1 Atomic force microscopy (AFM) – in air

For the surface morphological study ¹³³, the same AFM equipment (Nanoscope IIIa, Digital Instruments) that was used for MFM imaging was employed, except that the pre-magnetization of the probe tip was not necessary. The drive frequency used was 250 to 300 kHz (using the auto-tuned primary resonance frequency) with a scanning rate of 1 to 2 Hz. Images with scan areas of $10 \times 10 \mu\text{m}^2$, $5 \times 5 \mu\text{m}^2$ and $1 \times 1 \mu\text{m}^2$ using 256×256 pixels were acquired under the condition of tapping mode in air. More than 20 samples were collected for every magnetized condition.

4.1.5.2 Atomic force microscopy (AFM) – in liquid

For surface structure observation on soft and wet tissues or on microorganisms, direct observation under its natural hydrated state at ambient conditions was preferred than subjecting the samples to vacuum conditions (such as SEM), which will alter and dehydrate the substrates, including the possibility of destroying its original surface conditions. For this reason, the tapping mode AFM in a liquid environment was chosen, which is commonly used for surface morphological study on bacterial cell surfaces ^{134, 135}.

The topographic imaging in liquid was done with a $120 \mu\text{m}$ -long V-shaped cantilever with an oxide sharpened Si_3N_4 tip (Veeco Instruments), under the condition of

Tapping-in-liquid mode. The imaging was performed within a liquid media (distilled water) using a standard fluid cell without the o-ring seal. Prior to imaging, the sample was first affixed to a specially prepared 1 mm thick glass piece that was cut to a square of about 20 mm and glued onto a 1 mm teflon sheet with water insoluble epoxy glue. The teflon sheet (21 mm square) was made slightly larger than the 6 mm thick supporting steel disc, which was glued below the teflon sheet to act as a support base. Images with a scan area of $10 \times 10 \mu\text{m}^2$ and $5 \times 5 \mu\text{m}^2$, 256×256 pixels were acquired at a drive frequency of 8 to 8.4 kHz and a scanning rate of 1 Hz. More than 10 samples were collected for every magnetized condition.

4.1.6 Surface energy

Surface energy is defined by the disruption of chemical bonds that occurs when a surface is created. These surfaces will be intrinsically less energetically favorable than the bulk of a material. In the bulk material, all the bonding requirements of the constituent atoms of the material are filled. However, the exposed bonds at the surface are energetically favorable to react with the immediate environment. In this work, the surface energy is determined by the contact angle measurement and the electric force microscopy (EFM).

4.1.6.1 Contact angle measurement

The measurement of the surface free energy of the perpendicularly polarized magnetic media was done with the contact angle (CA) measurement instrument (First Ten Angstroms, FTÅ 200) at 25°C with a polar liquid (distilled water). Sessile drops of an average of $3.4 \mu\text{l}$ were deposited with a 0.1 mm ID gauge 34 needle (MicroFil MF34G). The surface hydrophobicity of the perpendicularly polarized magnetic samples and the bacterial cell surfaces were calculated using the sessile drop method⁶⁸. The CA measurement on bacterial cells was first obtained by filtering through a cellulose acetate

membrane filter (pore size 0.45 μm) by negative pressure¹³⁶. The average contact angles were determined after the sessile droplet on the filtered cells had attained a plateau drop shape^{137, 138, 145}. More than 5 measurements on the bacterial lawns and more than 20 for each polarized magnetic samples were obtained.

4.1.6.2 Surface electric force microscopy (EFM)

The method used to measure the surface electric potential was generally similar to the magnetic force imaging, except that the surface potential force was measured by detecting the deflection amplitude between the electrically charged probe and the surface¹³⁹. The voltage applied to the probe tip was determined by the drive amplitude parameter (a higher drive amplitude will increase the sensitivity of the probe tip), which was kept constant at 110 mV. In addition, the pre-magnetization of the standard 50 nm-thick Co alloy magnetic probe tip was also not necessary. The scanning frequency used for EFM was between 50 to 60 Hz, and the measurement quantity was repeated at least 20 times per magnetic state.

4.2 Bacterial and biofilm culture

The bacterial cell cultures used in this research involved two homogeneous cultures, namely the *P. fluorescens* and *B. licheniformis*, and one heterogeneous culture from the aquarium fish tank media. The methods to grow the bacterial cells and the biofilms on the magnetic substrates are discussed in the following.

4.2.1 Biological safety standards

Essentially, the guidelines for microbiological handling and the conduct of experiments in this project were based on the following:

- Standard guide for selecting of test methods on biofilm and related.

- *ASTM. E 1427-91* ⁴⁸.

- Guidelines for safe handling of microbiological cells

- *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* ¹⁴⁰

- *Microbiology, a laboratory manual* ¹⁴¹

- Basic equipment and microbiological techniques for studying bacterial adhesion

- *Handbook of bacteria adhesion* ¹⁴²,

- Jackson *et. al.* ¹⁴³: “Growing reproducible biofilms with respect to structure and viable cell counts”

4.2.2 Homogeneous bacterial cell culture

All homogeneous bacterial cultures were obtained from ATCC (American Type Culture Collection), and were contained in a vacuum-sealed vial and stored at -80°C . The deep-fried cells in a vial were then activated in the recommended growth medium and incubated. The duration for each regeneration of the cell cultures was determined by its stationary growth phase.

4.2.2.1 *Pseudomonas fluorescens*

The hydrophilic and flagellated *Pseudomonas fluorescens* bacteria were selected for its motility ^{144, 145}. It is a predominant and an opportunist organism that is able to grow readily in many environmental conditions ^{58, 146}, and produce large quantities of extra-cellular polymeric substances (EPS) ¹⁴⁷. The environmentally common bacteria are found in soil and water and also a common cause for food spoilage ¹⁴⁶. Moreover, it is a predominant and an opportunist organism that is able to grow readily in many environmental conditions

^{58, 147}

A frozen stock of pure culture of *P. fluorescens* (ATCC 700830) was grown in a 100 ml of Trypticase soy broth (Fisher® BBL 11768) at 30 °C with continuous shaking at 150 rpm for 24 hrs. Subsequent batches of *P. fluorescens* culture were grown by transferring 10 ml from the earlier cultured batch into another 100 ml of fresh trypticase soy broth. This was followed by continuous shaking at 150 rpm for 24 hrs. This process was repeated every 24 hrs for the period of the experiments. Purity of these strains was similarly checked by an optical microscope and an AFM. Cell concentration of the culture on R2A agar that was grown for 24 hrs in an incubator at 30 °C was between the range of $1.2 \sim 3.0 \times 10^9$ cfu/ml of solution.

4.2.2.2 *Bacillus licheniformis*

Bacillus licheniformis (ATCC # 37085) is a motile, rod-shaped, and a spore-forming mesophilic bacterium ¹⁴⁸. This bacterium is widely used in the brewery ¹⁴⁹ and pharmaceutical ¹⁵⁰ industries, and also for biosurfactants production ¹⁵¹.

A frozen stock of pure culture of *B. licheniformis* was activated and cultured in a 100 ml of Trypticase soy broth (30 g/l) and glucose (5 g/l) at 30 °C, with continuous shaking at 150 rpm for 24 hrs. Purity of these strains was similarly checked by an optical microscope and an AFM. Cell concentration of the culture on nutrient agar that was grown for 24 hrs in an incubator at 30 °C was in the range of 10^7 to 10^8 cfu/ml of solution.

4.2.3 Heterogeneous bacterial cell culture (aquarium fish medium)

To obtain a heterogeneous cell culture, the microorganisms grown in the aquarium medium were allowed to grow naturally in the aerated tank (using an air pump) containing several tropical aquarium fish that were fed with commercial-made nutrient food at room conditions (approximately 22 °C to 23 °C). Under such environment, multiple species of marine microorganisms would exist in the aquatic media, which were reported to

be more difficult to prevent against bacterial adhesion and subsequent formation of biofilms^{5, 152}.

4.2.4 Biofilm growth culture

The method adopted for biofilm culture was based on a continuous flow system in which a stream of suspended bacteria was channeled over the surfaces of the magnetic substrates using peristaltic pumps and an open channel flow reactor.

4.2.4.1 Experimental setup and assay adhesion

(a) Homogeneous culture test

The biofilms were grown on the 10 x 20 mm² polarized and non-polarized substrates (two pieces per magnetic state) that were randomly attached in series, leaving a gap of 5 mm between each sample and a clearance of 65 mm from the entrance and exit points of the channel. The open channel flow reactor, made of polycarbonate was 30 mm deep, 13 mm wide, and 280 mm long (refer to Fig. 32 and Appendix A for detailed drawing specifications) and was injected with either a 20 ml of *P. fluorescens* culture or a 1 ml of *B. licheniformis* via needle and syringe through the line in which the growth medium entered the reactor, and continuously circulated using peristaltic pumps. Fresh feed flow rate was 0.4 ± 0.1 ml/min and the recycle flow was 120 ml/min. The reactor was continuously aerated from the surface using filtered air at a rate of 50 ml/min. Twice a day, the reactor was flushed with sterile de-ionized water to remove suspended microorganisms, followed by 20 ml injection of fresh *P. fluorescens* culture or a 1 ml of *B. licheniformis*. Prior to inoculation, the sterile water in the reactor was replaced with freshly prepared sterile growth medium.

At the end of each experiment (total of four days for *P. fluorescens* and three days for *B. licheniformis* bacteria), the substrates were gently flushed at about 60 ml/min with

distilled water to remove the loosely adhered cells. The substrates were then removed and kept moist in a sterile petric dish containing a tissue damped with distilled water to avoid biofilm dehydration. After which, the biofilm analyses were carried out, and this process was repeated for a total of three times.

Fig. 32 Experimental setup for the biofilm growth. Top figure illustrates the schematic diagram and the bottom figure shows the actual setup

(b) For heterogeneous culture test

Similarly for the biofilm growth on the magnetic substrates using heterogeneous cell culture (aquarium fish media), the experimental setup was generally the same as the homogeneous cultures. The difference was that the growth media was obtained directly from the aquarium fish media. In addition, the overflow was also connected back to the aquarium tank instead of being discharged to the drain.

The flow rates used for the fresh feed and recycle flow were also similar as the homogeneous cultures that were at 0.4 ± 0.1 ml/min and 120 ml/min respectively. The experiment period was 5 days and the experiment was repeated for a total of three times.

4.2.4.2 Growth medium for biofilm culture

The preparation of the growth media for the three different cultures is as follow:

(a) *Pseudomonas fluorescens* culture

The preparation of the growth media for the biofilm experiment using *P. fluorescens* cells was adopted from Jackson, *et.al.*¹⁴³. It consisted of (reagents from Fisher®): Na_2HPO_4 (1.825 g/l), KH_2PO_4 (0.35 g/l), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.01 g/l), $(\text{NH}_4)_2\text{SO}_4$ (1.0 g/l), glucose (5 g/l) and yeast extract (0.1 g/l). The glucose and yeast were autoclaved individually at 121°C and then added to the autoclaved media. A 1 ml/l mixture of micro-nutrients: $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (527 mg/l), $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (228 mg/l), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (317 mg/l), $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ (231 mg/l), $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ (127 mg/l), ZnCl_2 (363 mg/l), CaCl_2 (3.7 g/l), and FeCl_3 (2.16 g/l), dissolved in 1 litre of 0.1M HCl, was then added to the media after sterilization. To prevent microbial contamination, the micronutrient solution was first autoclaved and then aseptically filtered using a $0.2\mu\text{m}$ syringe filter (Corning, New York) into the medium. The pH of the growth medium was measured at 7.2 ± 0.1 .

(b) *Bacillus licheniformis* culture

The growth medium that was prepared for *B. licheniformis* biofilm growth consisted of Trypticase soy broth (30 g/l) and glucose (5 g/l). The broth and glucose were mixed after autoclaving them separately at 121°C for 20 minutes. The pH of the growth medium was about 7.3 ± 0.1 .

(c) Aquarium fish media

The heterogeneous cell cultures that were obtained from the aquarium fish media were grown under natural (uncontrolled) conditions. The growth medium was derived from the commercial food nutrients that were used to feed the fish. This generally consisted of proteins, fats, carbohydrates and numerous minerals.

4.2.5 Planktonic cell growth culture (for homogeneous strain only)

As previously highlighted in Chapter 1, the growth of biofilms influences the growth rate of the planktonic cells through the detachment of cells in the biofilms into the bulk media ⁵ and also by providing nutrients for the colonization and growth of other species in the aquatic environment ¹². As such, the effects of the polarized magnetic media on bacterial adhesion and the subsequent formation of biofilms would inevitably influence the growth rate of the planktonic cells. Hence, by measuring the planktonic cells growth or cell density in the liquid media, the conditions of the biofilm could also be estimated.

Under such indirect observation of the biofilm conditions, a homogeneous cell culture under a static environment was necessary for control. The static condition was chosen to reduce additional factors that may arise from flow patterns, such as turbulent flow and boundary layer over flat surfaces. For a 4-day experiment, a twenty four-beaker set, inclusive of six beakers containing the magnetic coupons of the same polarity, was used. The polarized magnetic coupon was first cut to a 25 x 25 mm² size and then secured face up at the bottom of the empty 100 ml glass beaker using double-sided tape. After fixation, it was filled with 50 ml of Trypticase soy broth (30 g/l) and an additional glucose (5 g/l) for the case of *B. licheniformis* culture (only). And finally, a 2.5 ml of the cultured *P. fluorescens* bacterial or a 1 ml of *B. licheniformis* bacterial suspension was manually injected using a disposable syringe into each beaker. The openings of all beakers were sealed with a commercially available aluminum foil. Then incubated at 30 °C for a period of

1, 2, 3 or 4 days for *P. fluorescens* culture, and 1 to 6 hours for *B. licheniformis* culture. More than three sets of experiments were conducted for each bacteria type.

4.3 Measuring bacterial adhesion, biofilm growth and planktonic cells growth

4.3.1 Bacterial adhesion evaluation

Based on the recommendations for the study of bacterial cell adhesion^{153, 154}, two evaluation methods were adopted in this research. One was by using a static flow system^{155, 156}, whereby the bacterial adhesion process was captured on video and was subsequently analyzed by counting the number of attached bacteria to the solid surface by means of an image analysis software over a specific time period. The other method is by using a continuous flow system in which the streaming bacteria in a liquid suspension would adhere to the test sample surface and form a layer of biofilm over time^{143, 157}. This method has been described in detail in Section 4.2.4.1.

For the first evaluation method, a parallel plate flow chamber (internal space dimensions: $l \times b \times h = 20 \times 20 \times 0.4 \text{ mm}^3$) with an image capturing and analysis system was established to study the bacterial adhesion to the magnetic samples. A schematic representation of the flow cell is shown in Fig. 33. The flow chamber was an acrylic piece with an internal diameter of 90 mm. The chamber consisted of a 1 mm thick viewing window base coupled with silicon rubber inlet and outlet pipes at the two ends. A 100X magnification wide-field shop microscope (PEAK) was attached to the chamber with a 36 mm microscope extender tube and connected to a 1/4" 3-CCD color camera (JVC KY-F55B). The images from the CCD camera were displayed onto a 14" TV and captured using a VHS videocassette recorder. Using a 100 μm micrometer scale slide (Edmund Optics), the estimated field of view on the sample surface was 0.058 mm^2 (equivalent to 335X magnification).

Fig. 33 Top Figure: (A) A schematic overview of the parallel plate flow chamber system; (B) Schematic representation of the flow chamber with the magnetic sample attached to the bottom glass slide. Bottom Figure: Actual experimental setup for capturing the bacterial adhesion process

The polarized magnetic sample (of size $10 \times 15 \text{ mm}^2$) was first secured to the bottom side of the microscopic glass slide ($L75 \times B25 \times H1 \text{ mm}^3$) using a double-sided tape. Once the flow cell was assembled and further secured to the microscope support stand, it was filled with NaCl solution (at 0.01M) and finally a 2.5ml of *P. fluorescens* bacterial suspension, containing about 10^7 cells ml was manually injected (using a 5 ml disposable syringe) into the chamber. Video capturing was initiated when the flow was stationary. The

purpose of using a stagnant condition was to improve the observation of bacterial movements in association with the surface without interference from bacteria moving by the action of flow. The process of bacterial adhesion on the magnetic samples was recorded over a period of 14 min. The experiments were done in triplicate using the same bacterial culture.

After video recording, the recorded analog video images were converted to digital images and 10 images per every 2 min. interval were extracted for analyses. The digitized images were first converted to grey images prior to image enhancement, noise filtering and pixel (0~255 grey values) thresholding. The bacterial counting was based on the final processed image. As the initial background image of the magnetic sample appeared with uneven brightness, coupled with occasional movement of the sample during the 14 min. period, conducting a differential imaging for analysis¹⁵⁵ was difficult. To solve the problem, an image processing tool was used (Leica Qwin 97 software - V2.2a), which was able to uniformly threshold all relative bright grey areas from the neighboring darker background, regardless of the uneven background illumination. After the enumeration of the adhering bacteria in the images, the data was calculated in terms of number of bacteria adhering per unit area. To differentiate the absorbed and immobile cells, the final quantification of absorbed cells was based on the relative difference between the initial quantity at the beginning and the subsequent measured quantity. This relative approach was aimed to eliminate the immobile cells that were present at the beginning of the experiment. The ratio of the final data at each time interval was then calculated and plotted with reference to the data at the starting time.

4.3.2 Evaluation of biofilm growth on surfaces

The biofilms grown on the magnetic substrates were evaluated by the following methods:

4.3.2.1 Areal density method

This measurement method, which was one of the recommended methods for biofilms evaluation^{5, 48, 143}, determines the overall growth of biofilms on the samples from a global 2-dimensional aerial view. This was done with an optical microscope (Nikon SMZ-U) at magnification of 4 times. The image captured was subsequently digitized and pixel counted (using Leica Qwin 97 software). Areal density was calculated by the percentage ratio between the areas occupied by biofilm over the entire image area. At least 6 measurements were made on each captured image to obtain an averaged value. A total number of 10 samples per magnetized state were analyzed.

4.3.2.2 Cell mass method

Another method selected to determine the amount of biofilms grown on the substrate surface was by the dry cell mass measurement^{48, 59, 143}. After biofilm formation, the biofilm-stained substrates were first flushed at 60 ml/min with distilled water to remove the loosely adhered cells. Thereafter, the substrates were removed and oven-dried at 55°C for 12 hours. The weight of the biomass was subsequently determined by weighing the substrates with and without the biofilm, which was removed by ethanol cleaning.

4.3.3 Evaluation on planktonic cell growth

Continuing from Section 4.2.5, after each incubation period, the set of six beakers, with two containing the coupons of the same magnetic polarity, were removed from incubation. The magnetic coupons and broth were then aseptically removed for analyses. The coupon was soaked in distilled water in a sterile petric dish to remove

sedimented biomass and reversibly adhered bacteria, followed by AFM and MFM imaging. As for the broth, the amount or density of the planktonic cells grown was determined by aseptically transferring to an empty sterile 50 ml test tube for homogenizing, followed by either the viable cell count^{48, 143} (in colony forming unit, cfu, a robust but time-consuming technique for determining the relative amount of bacteria¹⁵⁸), or by the optical density measurement at 600 nm (OD₆₀₀), which used a UV-spectrophotometer to determine the cell concentration^{48, 60, 149, 151, 159, 160}

The viable cell count method was adopted for the experiments on the *P. fluorescens* bacteria. The viable cell count was determined by thoroughly mixing and diluting the bacteria through a series of sequential 1:10 dilution in sterile phosphate buffer solution (pH 7). Each of the 1:10 dilution tubes was mixed and 1.0 ml was plated onto separate R2A agar plates. A minimum of two agar plates was used for every dilution level. The diluted solution was spread over the surface of the plate using a disposable sterile bent rod. The plates were then incubated at 30 °C for 24 hr. Bacterial counts were determined by multiplying the number of colonies on a plate by the total dilution factor for that plate.

As each batch of bacterial culture had different numbers of viable cells at the beginning of every experiment, a direct comparison of viable counts between separate sets of experiments was difficult. Hence, the relative cell count was adopted instead. The relative cell count was determined by comparing the north or south-polarized condition to the non-polarized condition (reference) at each incubation period. This relative comparison at each incubation period improved the growth correlation between the three different states.

For the experiments on *B. licheniformis*, the planktonic cell growth was measured by the optical density measurement method. This method was chosen over the viable cell count method for its fast and direct measurement, which was necessary because of the rapid growth rate (exponential growth phase started within hours instead of days for *P.*

fluorescens). The cell mass was determined from the OD₆₀₀ measurements, through a calibration curve in which the biomass was first centrifuged, washed (twice with distilled water), oven dried for 12 hours at 55°C and subsequently weighed^{59, 161}, after each OD measurement.

For the optical density measurement, at least 3 measurements were obtained for every sample culture to obtain an average value.

4.4 Other comparative experiments

Given that the intended approach in using a perpendicularly polarized magnetic media was to alter the surface characteristics and subsequently influence the bacterial adhesion, a comparison on its efficacy against similar techniques would be useful.

4.4.1 Electrified surface on planktonic cell growth

For this experiment, a 25 x 25 mm² aluminum foil (using a 0.1 mm thick commercial type foil) was negatively charged at 9.0 V D.C. (using an ac-dc power adapter). The experimental setup for the electrical test is shown in Fig. 34.

Each aluminum foil was connected to a 10 MΩ resistor and the entire circuit was connected in a parallel system. Electric current flow from each Al foil was approximated to be 1.44×10^{-7} A/cm². The exposed end of the wire was taped to the back of the Al foil and insulated from water entry. The wired Al foil was then secured to the bottom of the glass beaker (using masking tape at the beaker rim and avoid in contact to bacterial culture), which was subsequently filled with 50 ml of the growth medium (refer to Section 4.2.4.2 for details). The entire electrical circuit diagram is illustrated in Fig.34(c). At each incubated period, the set of six beakers was removed from the 30 °C incubator and the wires

disconnected from the power source. Three sets of experiments were conducted for each bacteria strain.

Fig. 34 Experimental setup for the surface electric test.

(a) illustrates the electrical circuit diagram for each beaker set, and (b) shows the method in securing the exposed wire to the aluminum foil. (c) illustrates the entire circuit diagram, with a complete set without any electric charge for reference

4.4.2 Bulk magnet test

Besides comparing the magnetically polarized surfaces against the electrically charged surface, another important comparison made was to introduce a magnetic field using the bulk magnet itself. For this test, the experimental setup is illustrated in Fig. 35. The test was done by placing a L15 x B20 x H6 mm NdFeB permanent magnet, with an external magnetizing field strength of 3000 Oe, located below the glass beaker. Each beaker set, with a bulk magnet below, was then stacked vertically in the incubator at 30 °C. The same quantity of beakers without any bulk magnets was also used for comparative purposes.

*Fig. 35 Bulk magnetic effect
test on planktonic
cell growth*

CHAPTER FIVE

RESULTS & DISCUSSION

In this chapter five, the experimental and investigation results are presented and discussed. In the beginning part, the investigations on the magnetic states and surface characteristics of the perpendicularly polarized media are presented. Several discoveries were observed and a hypothesis has been proposed to explain the phenomena. In the following part, the effects of the perpendicularly polarized magnetic media on bacterial adhesion and the subsequent formation of biofilms are reported. During the course of the biofilm experiments, the effects on the planktonic cell growth rate and cell hydration were also discovered. These effects were found to have influenced the surface proteins secretion by the adhered bacterial cells on the magnetic media surfaces.

5.1 Magnetic state and characterization

After the completion of perpendicular polarization of the magnetic substrates, the magnetic states were verified using two techniques. One is by Lorentz microscopy and the other is by magnetic force microscopy. For the Lorentz microscopy, the technique provides a general aerial image from which two different polarities of the magnetized substrates would produce different contrast images. As for the magnetic force microscopy, the technique analyzes the magnetic states at micro-scales. Different magnetic polarity would cause the magnetized AFM probe to respond differently, i.e. either an attraction or repulsion against the magnetized surface.

5.1.1 Lorentz microscopy

The Lorentz microscopy results shown in Fig. 36 elucidate the magnetic states in the north- and south-polarized samples of the FD and HD. The image for the north polarized sample was observed to be darker in contrast as compared to that of the

south polarized substrate. The difference in contrast was due to the different deflection path of the emitted electrons from the polarized magnetic surfaces.

Fig.36 Lorentz microscopy image at x6 times on the magnetic north- and south-polarized FD and HD samples

5.1.2 Magnetic Force Microscopy

The MFM images on the magnetically polarized and non-polarized substrates were done at various scan areas of $30 \times 30 \mu\text{m}^2$, $10 \times 10 \mu\text{m}^2$, and $5 \times 5 \mu\text{m}^2$. Typical MFM images on the FD and HD are shown in Fig. 37 and the measured results for the $5 \times 5 \mu\text{m}^2$ are shown in Fig. 38. At the north-polarized state, the FD and HD samples were observed to exhibit similar weak pattern, whereas the south-polarized state demonstrates an increase. The bar-patterns seen in Fig. 37 are in good agreement with other MFM measurement done on magnetic media¹¹⁸. However, at the south-polarized state, the strength of the bar pattern of the HD was observed to be consistently weaker than the FD. This is probably due to the weaker induced magnetic moment as observed in the magnetization curves (to be discussed in Section 5.1.3).

Fig.37 Representative MFM images on polarized FD and HD surfaces at $5 \times 5 \mu\text{m}^2$

Fig. 38 Distribution of MFM measurement on a $5 \times 5 \mu\text{m}^2$ south-, north- and non-polarized substrates (error bars denotes maxima and minima values)

From the MFM images in Figs. 37 and 38, the different states of magnetization (north and south polarity) were also clearly identified. Using the non-polarized substrate as a reference, the interaction force between the north-polarity of the MFM tip to the south-polarized substrate showed an increase in attraction at 288

mV for FD and 83 mV for HD. Whereas the weak MFM tip interaction with the north-polarized substrate showed the repulsion effect at 95 mV for FD and 60 mV for HD.

5.1.3 Magnetization (hysteresis) curve

On the magnetization curve (hysteresis loop) of FD and HD, the hysteresis loops that were obtained from the VSM measurement are shown in Fig. 39. The VSM results showed that the external magnetizing field of 5000 Oe was sufficient to overcome its coercivity, H_c , at 920 Oe and 780 Oe respectively, but below its saturation values, H_{sat} , at 8400 Oe and 11800 Oe respectively. However, with the external magnetization of 5000 Oe, the HD was observed to exhibit a weaker induced magnetic moment from the saturation level at 85% as compared to the FD at 97%. Based on the hysteresis characteristics of the magnetic material as described in Section 2.2.6, the induced magnetic moment, after removing the external magnetizing field, is expected to be near its saturation value at 0.8 emu/gm for the FD (due to almost 100% saturation level) whereas the HD will likely to drop to its magnetic remanence, M_R , level at less than 0.01 emu/gm (see dotted lines in Fig. 39).

Fig. 39 Hysteresis loops of FD and HD measured using VSM.

5.1.4 Summary on characterization of magnetic media

The Lorentz microscopy results in Fig. 36 have shown that the magnetic states in the north- and south- polarized substrates were present. The image for the north polarized substrate was darker in contrast as compared to the south polarized substrate. The difference in contrast between the north- and south-polarized substrates has indicated a different deflection direction of the projected electron beam in the SEM, which also demonstrated the different magnetic states in the media.

The MFM analysis has also verified that the magnetic states were present in the north- and south-polarized FD and HD. However, the difference in the interaction force between the two states in the HD was lower than the FD. This can be explained by the weaker induced magnetic moment for the HD as compared with the FD.

With the external magnetizing field of 5000 Oe, it was observed from the magnetization curve that the FD was polarized to about 97% towards the saturation level, whereas the HD was only 85%. Due to the near saturation level achieved for the FD, the induced magnetic moment for the FD will likely to remain at the saturation point, H_{sat} , which is much higher than the HD where the induced magnetic moment will drop to the M_R point of about 0.1 emu/gm, as defined by the hysteresis characteristics. However, the external magnetizing field of 5000 Oe is sufficient to cause the magnetization direction in the FD and HD to change according to the external magnetization field direction.

5.2 Polarization effects and surface free energy

The purpose to perpendicularly polarize a horizontally aligned magnetic media was to obtain a maximum anisotropy surface energy. And depending on the polarization direction, the ordered electron spins was used to channel the induced

anisotropic energy either towards or away from the open surfaces of the magnetic substrates. The change in the surface free energy of the magnetic substrates can be determined from the contact angle (CA) measurement and the AFM's electric force measurement (EFM).

5.2.1 Contact angle measurement on FD and HD

The measured contact angles on FD that was perpendicularly polarized in the north/south states were compared against the non-polarized state (original condition), which are shown in Fig. 40. The FD surface in the north-polarized state was observed to exhibit an increase in the contact angle (CA) at an average of $106.7^\circ (\pm 0.4^\circ)$. The CA on the FD at the south-polarized state was observed to have reduced to $102.7^\circ (\pm 1.0^\circ)$ as compared to the non-polarized state at $105.5^\circ (\pm 0.6^\circ)$.

Fig.40 Co ntact angle measurement on perpendicularly polarized floppy disk surface

On the hard disk samples, the effect was more obvious than the floppy disk (see Fig. 41). The CA measured on the perpendicularly polarized HD in the north-polarized state was $88.3^\circ (\pm 2.2^\circ)$ as compared to the non-polarized state at $85.5^\circ (\pm 0.4^\circ)$. The CA on the HD at the south-polarized state was at $80.5^\circ (\pm 3.1^\circ)$. There was a significant difference between the north- and south-polarized states on the HD at approximately 8° as compared to the FD at approximately 4° .

Fig.41 Contact angle measurement on perpendicularly polarized hard disk surface

To further verify the effects of electron spin direction on the surface free energy, further tests were conducted using the same HD surface but polarized alternatively to different magnetization directions. Figure 42 illustrates the results from this experiment that was repeated more than three times using different HD samples. The results were almost similar in pattern; whereby the surface polarized to the north-polarized state would cause the CA to rise, and fall when polarized to the south-polarized state (see Fig.42 – right figure).

Fig.42 Left figure: Alternating the magnetic polarization directions (N/S) repeatedly resulting in contact angle (CA) changes. Right figure illustrates the observed effect on sessile drops on the same sample surface but polarized at North (top) and South (bottom) direction.

5.2.2 Co-relation between contact angle and work of adhesion

The relationship between the contact angles to the free energy of adhesion between a liquid (L) and a solid (S), which was previously explained by the thermodynamic reversible work of adhesion (W_{SL}) in Section 4.1.4.2, is expressed by the Young-Dupré equation (8):

$$W_{SL} = \gamma_L (1 + \cos \theta) \quad \text{----- (8)}$$

where subscript γ_L is the surface tension of the liquid (for water is 72.8 mJ/m²) and θ is the contact angle made between the solid and liquid media (θ_w for water contact angle).

Using the average results obtained in Fig. 40 and 41, the calculated work of adhesion is summarized in Table 5. The calculated results on W_{SL} have clearly shown that the surfaces of FD and HD that were perpendicularly polarized in the north-polarized state exhibited a reduction of adhesion work as compared to the non-polarized state. On the contrary, the surfaces in the south-polarized state exerted an increase in the adhesion work that resulted in the decrease of the contact angle (better adhesion).

Table 5. Co-relation between measured contact angles to work of adhesion

	water contact angle	W_{SL}
	θ_w (deg)	(mJ/m ²)
FD - N-polarized	106.7 (± 0.4)	51.9
FD - S-polarized	102.7 (± 1.0)	56.8
FD - Non-polarized (reference)	105.5 (± 0.6)	53.3
HD - N-polarized	88.3 (± 2.2)	75.0
HD - S-polarized	80.5 (± 3.1)	84.8
HD - Non-polarized (reference)	85.5 (± 0.4)	78.6

Note : ($\pm$) denotes one standard deviation.

W_{SL} is calculated from equation (8)

5.2.3 AFM analyses on surface topography and surface electric charge

On the surface roughness measurement by the AFM, the average R_a values shown in Table 6 have shown that the FD and HD for all three conditions were almost similar, at around 10 nm and 5~7 nm respectively. These observed values and differences in the surface conditions at different magnetization directions are not significant to cause any considerable impact to the contact angle measurement. Moreover, based on analytical studies made by Neumann⁶⁹, surface roughness that is below approximately 0.1 μm (≤ 100 nm) was reported to have no considerable influence on the surface energy measurement.

Table 6. AFM surface analyses on FD and HD (topography and electric)

	Surface roughness	Surface Electric Potential
	R_a (nm)	(mV)
FD - N-polarized	10.3 (± 0.8)	74.7 (± 9.2)
FD - S-polarized	10.1 (± 0.9)	118.5 (± 5.6)
FD - Non-polarized (reference)	10.4 (± 2.4)	103.2 (± 10.3)
HD - N-polarized	5.1 (± 0.9)	60.1 (± 11.7)
HD - S-polarized	7.2 (± 0.5)	141.8 (± 8.8)
HD - Non-polarized (reference)	4.8 (± 1.0)	75.1 (± 13.2)

Note : ($\pm$) denotes one standard deviation.

It was further observed in the EFM analysis that the surface electric potential on FD and HD that were polarized in the north-polarized direction, had shown a weaker surface potential as compared to the south-polarized and non-polarized conditions (refer to Table 6). Although it was difficult to judge the differences from the EFM

images between different polarized conditions, the AFM imaging was able to observe some changes to the surface structures. In Fig. 43, the representative photos of the EFM and the corresponding AFM images of the HD illustrate the observed differences. The HD' surfaces at the north-polarized state were seen to display a wavy pattern, whereas the pattern was absent at the south-polarized state. In addition, the non-polarized condition was also observed to be different from the polarized states. These differences in surface morphology were consistently observed during the AFM analyses, which were suspected to be caused by the effects of magnetostriction. However, no swelling lumps were seen in the HD as compared to the FD, which will be discussed in Section 5.2.4.

Fig. 43 Representative images of EFM with corresponding AFM data on polarized HD at 5 x 5 μm^2 . Left image in each polarized state shows the EFM data, and right image shows the topographic data. The blotches seen in the images are surface irregularities.

5.2.4 Moisture adsorption phenomenon in FD

The north and south polarized and the non-polarized magnetic substrates were first imaged at $10 \times 10 \mu\text{m}^2$ (see Fig. 44) under contact mode in ambient air, which was equivalent to 10,000 times in the SEM magnification. The 3D image of the south-polarized substrate (Fig. 44a) was observed to have numerous swollen lumps of about $0.5 \mu\text{m}$ or less over the entire surface (highlighted on circled white dots). Whereas in the north polarized substrate (Fig. 44b), there was no such observed phenomenon. For the non-polarized substrate (Fig. 44c), the 3D topographic image appeared in-between the north- and the south-polarized substrates. Generally, the lumps appeared fewer than the south polarized substrates (highlighted in circles).

Fig. 44 Contact mode AFM in ambient air @ $10 \times 10 \mu\text{m}^2$ resolution on different magnetized condition substrates (images rotated 10° and pitch angle 35°) – circled white spots are swollen lumps

Further AFM investigations on the swollen lumps were conducted immediately after polarizing the freshly prepared FD substrates. All the FD substrates were found to be similar in surface structure (see Fig. 45a for south-polarized, Fig. 45b for north-polarized and Fig. 45c for non-polarized). The surfaces of the substrates were observed to be generally flat, with the average step height of about 11.5 nm and an average surface roughness (R_a) of 5.4 nm.

However, after a 72 hr exposure in room conditions of 60 ~70% RH, the south-polarized substrate appeared more swollen (as indicated by the arrows in Fig. 45d). The swell size was approximately 0.2 μm , with the average swell height and R_a increased from 11.5 nm and 5.4 nm to about 18 nm and 7.4 nm respectively. For the north-polarized substrate, it had changed to a more dehydrated state (as indicated by the arrows in Fig. 45e). The average swell height and R_a was found to have reduced from 11.5 nm and 5.4 nm to about 7 nm and 5.1 nm respectively. As for the non-polarized substrate (see Fig. 45f), the topographic image appeared in-between the north and the south polarized condition. Its swell height increased slightly to about 14 nm, and R_a increased to 5.8 nm.

Fig. 45 Contact mode AFM in ambient air @ $1 \times 1 \mu\text{m}^2$ resolution on different magnetized condition substrates – Images (a,b,c) were obtained immediately after polarization. Images (d,e,f) were obtained after 72hr-humidity exposure

From the AFM topographic images in Figs. 44 and 45, the findings have discovered that the south- and north-polarized substrates reacted to moisture differently. To further verify these observations, additional tests were conducted as follow:

- (i) Conducting the AFM imaging under liquid environment (tapping in liquid)
- (ii) Measuring the contact angles of the sessile drop on the magnetically polarized and non-polarized substrates

5.2.5 Test #1: FD in water

The freshly prepared and polarized FD was soaked in distilled water for 24 hrs and a AFM measurement under liquid (distilled water) condition was conducted using scan areas of $10 \times 10 \mu\text{m}^2$ and $1 \times 1 \mu\text{m}^2$. The south-polarized substrate (Fig. 46a) was observed to be in a more swollen state over the entire surface as compared to the north polarized substrate (Fig. 46c). The $1 \times 1 \mu\text{m}^2$ scan area for the south-polarized (Fig. 46b) substrate had shown a substantial increase in swelling from the previous 18 nm swell height (seen in Fig. 45d), to about 24 nm. For the north-polarized substrate (Fig. 46d), the swelling was less serious than the south-polarized substrate at 13 nm.

Fig.46 Contact mode AFM in ambient air @ $10 \times 10 \mu\text{m}^2$ & $1 \times 1 \mu\text{m}^2$ resolution on different magnetized condition substrates (images rotated 10° and pitch angle 30°)

5.2.6 Test #2: Effects on surface free energy based on contact angle

The surface condition of the FD after the 72 hr environmental exposure was investigated by measuring the surface free energy through the contact angle measurement. Table 7 shows the results of the contact angles made between the distilled water droplet on substrates with different magnetic states. The average values of the contact angle for the south-polarized and north-polarized substrates are 96.55° and 102.67° respectively. These results have suggested a significant difference based on 1σ . In addition, the non-polarized substrate was observed with an average contact angle of 99.64° , which was in-between the two polarized states. Figure 47 shows the distribution of the contact angles made between the droplet and the substrate, which similarly demonstrates the differences were statistically significant.

Table 7. Contact angle measurement on FD after 72hr exposure

Substrate condition	Ave. water contact angle (deg)	Sample size, n
South field polarized (S)	96.55 ± 1.19 (based on 1σ)	22
North field polarized (N)	102.67 ± 1.05 (based on 1σ)	22
Difference (N-S)	6.12	--
Neutral Non-polarized	99.64 ± 1.38 (based on 1σ)	22
Data error (repeatability)	0.39	20

Fig. 47 *Contact angle measurement on magnetic north and south polarized and non-polarized substrates (error bars denotes maxima and minima values)*

5.2.7 Summary on polarized effects and surface free energy

The various findings obtained from the AFM and contact angle measurement are summarized.

(a) AFM measurement

Prior to the 72 hr environmental exposure after polarization, the surface structures of the substrates with different magnetized conditions did not reveal any differences. However, the surfaces after the humidity exposure changed significantly between the north and south polarized substrates. These differences have indicated that there was an interaction between the two magnetized states with the environmental moisture. The swollen lumps and the increase in the surface roughness seen in the magnetically south-polarized FD substrate (spin down state) have indicated a relatively higher surface energy, which explain the adsorption of water molecules into the organic-based polymers in the coating media. For the north polarized substrate FD (spin up state), the topographic images appeared to be in a dehydrated state as compared to the south-polarized and non-polarized substrates. This further suggested that the north-polarized surface exhibited a relatively lower surface free energy, which caused the moisture repulsion from the media.

To further validate the explanations, the FD was soaked for 24 hrs followed by the AFM imaging under liquid condition. The test results in the south-polarized substrate have shown that the minute lumps that appeared under ambient air have become enlarged when the substrates were soaked in water. Although the lumps that appeared on the north-polarized substrate had also showed similar swell behavior, the effects were noticeably less.

Hence, based on the findings and the investigation results, the swell lumps that were seen on the south-polarized substrate were very likely to be due to moisture absorption into the media. On the other hand, the north-polarized substrate showed a water repulsion behavior.

From the EFM measurements on the FD and HD (see Table 6), the results have demonstrated that the perpendicularly polarized magnetic media affected the surface electric charge. It was observed that the change in the surface electric potential was proportional to the change in the surface energy, whereby the south-polarized media that exhibited a relatively higher surface electrical potential has similarly shown a relative increase in the contact angle (higher work of adhesion) over the north- and non-polarized media (see Table 5). Vice versa for the north-polarized media.

(b) Contact angle measurement

The results on the contact angle measurement have found that the south-polarized substrate gave rise to a lower contact angle with water as compared to the north- and non-polarized substrates. This observation has indicated that the magnetically south-polarized substrate has a higher surface free energy (increase in adhesion work) as compared to the north- and non- polarized substrates. These results are re-enforced by the AFM analysis, which found that water was actually being retained and subsequently being absorbed into the magnetically south-polarized FD substrate. In other words, the magnetic south field from a magnetized FD is able to retain water molecules and hence causing the organic polymers to subsequently swell.

As for the north-polarized substrate and the non-polarized substrate, the contact angle difference has shown that the north-polarized substrate has a lower surface free energy (reduction in adhesion work) than the non-polarized substrate. This

finding is reaffirmed from the AFM results on the north-polarized substrate having the tendency to repel moisture. In addition, the series of reverse magnetizing polarity on the HD have demonstrated that the effects on the surface free energy are reversible.

(c) Concluding summary and hypothesis

The results of these experiments have clearly demonstrated that the perpendicular polarization of the magnetic media is able to influence the surface free energy. Although the non-magnetic coatings on the magnetic media of FD and HD would not respond to the magnetic field, the observed changes in the surface free energy, the electric potential, and the surface morphology, have all indicated that there was a change of state at the open surfaces. These phenomena may be explained by the effects of the perpendicular polarization, which increased the surface anisotropy energy that was observed from the surface deformations at different magnetization directions. In addition, the difference and the reversibility in the surface free energy between the north- and south-polarized conditions have further indicated that there was likely a change of state in the anisotropic energy, which has been proposed that it can be shifted by the direction of magnetization or spin.

At the north-polarized (spin up) state, the electrons were believed to have been shifted to a higher energy state that resulted in an adsorption of energy. Vice versa for the south-polarized state, the release of energy (potential) was believed to be caused by the electrons having moved to a lower state of energy. This energy reduction or increase in the magnetic media was then transmitted through the non-magnetic coatings to the open surfaces of the FD and HD, which was evident from the observed changes in the CA, EFM and AFM measurement. The larger differences seen in the contact angle and surface potential data between the north- and south-polarized states from the HD over the FD were probably due to the additional energy from the high thermal and

mechanical stresses induced into the HD's thin film coating, by nature of the sputter coating process¹¹⁵, and also probably due to the thinner non-magnetic coatings (refer to Section 2.2.8). Moreover, the moisture absorption phenomenon by the FD may also have caused the reduced effects in the FD.

In the south-polarized state, it was suspected that the relative increase in the surface electric potential over the non-polarized condition was attributed to the conversion of the released potential energy into electrical energy. The release of potential energy was also the most probable cause of the increase in the adhesion work (reduced contact angle). In addition, the observed effects on the contact angle from the alternating polarization test had further re-enforced the hypothesis, in which the surface free energy is reversible by the direction of magnetization (spin). Lastly, it was noted that different magnetization directions had created different surface deformations. This was probably caused by the magnetostriction effect. However, the magnitude of deformation was insufficient to influence the contact angle measurement.

5.3 Effect of anisotropy energy to bacterial adhesion

The effects of anisotropy energy induced in the magnetic media to the surface free energy are investigated. Based on the phenomenological surface anisotropy, the density of anisotropy energy increases with the strength of the magnetization field. Hence, the experiment was conducted with the FD polarized under various magnetizing field strengths of 600 Oe, 2000 Oe and 5000 Oe for a period of 10 minutes. Thereafter, the *P. fluorescens* bacteria were injected into the static flow cell and the adhesion process was recorded for 14 minutes and quantified at 2 minutes intervals (refer to Section 4.3.1 for details).

The relative number of *P. fluorescens* bacteria adhered to the south-polarized substrates was observed to be significantly higher than the north- and non-polarized samples. The south-polarized substrates that were magnetized at 5000 Oe had shown a significantly higher relative number of bacterial adhesions as compared to the 2000 Oe, with the lowest count for the 600 Oe magnetized substrates. At the end of the

Fig.48 Effects of magnetic states on *P. fluorescens* adhesion with time

the 14th minute period, the count ratio for the 5000 Oe sample has increased from 1.0 to 1.29 times (with reference to the start count). The count ratio for the 2000 Oe was lower at 1.18 times, and the 600 Oe was found to be the weakest at 1.12 times. However, the bacterial adhesions to the north- and non-polarized substrates were observed to be significantly lower for all levels of magnetization as compared to the south-polarized substrates. The initial count ratio for the north-polarized substrates was observed to be marginally lower than the non-polarized substrates, but both eventually saturated at around 1.085 after the 10th minute period.

From the results of the static-flow cell adhesion tests, the effect of varying anisotropy energy from different magnetizing field strengths was observed to affect the

P. fluorescens bacterial adhesion to its magnetic surface. The south-polarized substrates, especially the 5000 Oe magnetized substrates, were able to improve the bacterial adhesion significantly as compared to the 600 Oe and 2000 Oe magnetized substrates. On the contrary, the results of the north-polarized substrates demonstrated a mild bacterial repulsion effect as compared to the non-polarized condition.

Based on the bacterial adhesion results, it can be concluded that higher external magnetization strength in the south polarity would cause a higher number of bacterial adhesion. This can be explained by the higher induced magnetic moment from the stronger magnetizing field strength, which would produce a higher anisotropic energy.

5.4 Bacterial adhesion and biofilms formation

Given the observed changes in the surface free energy of the polarized magnetic substrates reported in Section 5.2, the influences on bacterial adhesion and subsequent biofilm formation are expected. The continuous flow experiments that were conducted to study the influences on biofilm formation are discussed in the following.

5.4.1 Effect on *Pseudomonas fluorescens*

The contact angles measured on the *P. fluorescens* bacterial lawn was 17.4° and the zeta potential was at -19.8 mV. The results have demonstrated that the *P. fluorescens* bacteria are generally hydrophilic and electro-negative charged, and these are in agreement with Valcarce, *et.al.*¹⁴⁵.

The bacteria that were injected into the open channel flow reactor for the continuous flow experiment have an average cell count (using agar plating method) of

2.0×10^9 cfu (colony forming unit). The pH of the growth media, which consists of micro-nutrients and reagents, was measured at 7.3.

After four days of continuous flow test, the biofilms that had grown on the FD substrates were removed and analyzed. The typical observed biofilm growth is given in Fig. 49, and the distribution of results on the areal density is shown in Fig. 50.

*Fig. 49 Aerial photographic view of *P. fluorescens* biofilm growth on FD substrates (x 4 times magnification)*

Fig. 50 Distribution of areal density measurement of biofilm growth on FD substrates (error bars denotes maxima and minima values)

The average values of the areal density on the south-polarized and north-polarized FD substrates were $77.1\% \pm 5.9$ and $25.5\% \pm 5.6$ ($\pm$ based on 1σ). The non-polarized FD substrate gives rise to an average areal density of $61.9\% \pm 6.0$. The results

have shown that the south-polarized substrate give rise to a relatively higher amount of biofilm growth as compared to the north-polarized and non-polarized samples.

Using the non-polarized substrate as a reference, the results have clearly shown that the north-polarized substrate was able to reduce the *P. fluorescens* bacteria from adhering to the surface. The reduction of biofilm growth was more than 50%. On the contrary, the results of the south-polarized substrate showed the opposite effect. The *P. fluorescens* bacteria adherence to the surface was enhanced, which resulted in approximately 25% increase in the biofilm growth. Based on the distribution chart in Fig. 49, the statistical differences between the north and south-polarized substrates were distinct enough to demonstrate that the polarity of the magnetic substrates has different effects on the bacteria adhesion and its biofilm growth.

The AFM measurement was carried out after the areal density measurement. Images were obtained under liquid condition using distilled water and the analyses were done at scan areas of $10 \times 10 \mu\text{m}^2$ and $5 \times 5 \mu\text{m}^2$. The typical AFM images are as shown in Fig.51.

From the AFM imaging, most of the *P. fluorescens* bacteria were observed to be approximately $1.0 \mu\text{m}$ in diameter and $2 \mu\text{m}$ in length. A comparison of the *P. fluorescens* bacteria is made on the south-polarized substrates against those on the north-polarized condition (see Fig. 51a vs. 51b). The surface morphology between the two was observed to be distinctly different. The bacteria adhered to the south-polarized substrates appeared less defined, and with large quantities of organic substances around them. However, on the north-polarized substrate, the *P. fluorescens* bacteria appeared bare in its individual shape. On the non-polarized substrate (Fig. 51c), the situation was somewhat in-between the north and south conditions.

From the AFM analyses under liquid conditions, the physical morphology of the *P. fluorescens* bacteria was observed in its natural environment. In Fig. 51, the bacteria were observed to be of rod shape with a diameter 1.0 μm and length 2 μm . These observed physical properties are in good agreement to descriptions reported in other AFM studies on *P. fluorescens*¹⁶².

Fig. 51 AFM (tapping in liquid) on polarized and non-polarized substrates at $5 \times 5 \mu\text{m}^2$ in 3-D (upper row) and 2-D (lower row). 3-D images are rotated 10° and pitch angle 30° .

The biofilm formed by the *P. fluorescens* bacteria on the south-polarized substrate was also found to be different from those seen on the north-polarized substrate. On the south-polarized substrate, the organic substances around the *P. fluorescens* bacteria was probably the extra-cellular substances (EPS), which by appearance, were similar in structure to those observed in other AFM studies on bacterial biofilms^{46, 47}. However, the bacteria on the north-polarized substrate appeared

physically bare, whereas the results appeared to be in-between the two on the non-polarized substrate.

5.4.2 Effect on *Bacillus licheniformis*

Similarly for the *B. licheniformis* bacteria, the contact angles measured on the *B. licheniformis* bacterial lawn was 36.04° and the zeta potential was at -20.5 mV. These results have demonstrated that the *B. licheniformis* bacteria are generally hydrophilic and electro-negative charged, and these are in good agreement with similar studies on *B. licheniformis*^{150, 159}.

For the experiments on bacterial adhesion, the results are shown in Fig. 52 for the FD and Fig. 53 for the HD, which also reveal the average biomass weight of the biofilms and the aerial images (taken at four times magnification). From these results, it was observed that the biofilms on the surfaces of FD and HD samples that were polarized in the N-polarized state are found to have reduced by more than two-fold as compared to the reference samples (non-polarized condition). On the contrary, the magnetic surfaces that were polarized to the S-polarized state exhibited an almost two-fold increase in cell mass. The pattern of the results is observed to be consistent throughout the three sets of experiments. Based on the data fluctuations in Figs. 52 and 53, the statistical differences between the N-polarized and S-polarized states are distinct enough to demonstrate that the polarity of the magnetic substrates has different effects on the bacteria adhesion and its biofilm growth.

Fig. 52 Measurement of biofilm cell mass on FD (the upper and lower bars represents the fluctuation range). Photos taken at 4x magnification illustrate corresponding biofilms formation

Fig. 53 Measurement of biofilm cell mass on HD (the upper and lower bars represents the fluctuation range). Photos taken at 4x magnification illustrate corresponding biofilms formation

For the AFM imaging in liquid on biofilm (see Fig. 54), significant differences on the morphological structures and sizes (length) of the *B. licheniformis* cells were observed after the end of three days of the experiment. The cells adhered to the FD and HD that was polarized in the N-polarized state (Fig. 54a and 54d) was

observed to have reduced in the overall size to less than 1 μm in width. On the contrary, the size of the cells on the FD and HD in the S-polarized state (Fig.54c and 54f) had remained relatively constant at slightly more than 1 μm . In addition, tiny macromolecules were also seen on the cells adhered to the HD (see arrow in Fig. 54f). For the non-polarized substrates, the conditions and sizes of he adhered cells were in-between the two different states (see Fig. 54b and 54e).

*Fig. 54 AFM imaging at $5 \times 5 \mu\text{m}^2$ on *B. licheniformis* biofilms on the polarized FD (top row) and HD (bottom row)*

5.4.3 Effect on Aquarium culture

For the heterogeneous biofilm culture tests, the growth media was replaced with the media in the aquarium fish tank. The water in the fish tank was pumped into the flow cell reactor and the return flow was connected back into the tank. This cycle was operated continuously for a period of 5 days. Throughout this period, the media in

the aquarium tank was neither replenished with fresh water nor the tank cleaned. However, the fish were fed with their food supplies, mainly containing proteins and fats. The purpose was to obtain a natural ecosystem that contains heterogeneous wild bacteria and other microorganisms.

At the end of the 5-day experiment, the test substrates were removed and photographed to obtain the areal image and also for the areal density calculation. This experiment was repeated for a total of three times. The typical observed biofilm growth is given at the right side of Fig. 55, and the distribution of results on the areal density is given at the left.

Fig.55 Measurement of biofilm areal density on FD (the upper and lower bars represents the fluctuation range). Photos on right illustrate corresponding biofilms formation

The average values of the areal density on the south-polarized and north-polarized FD substrates were $77.0\% \pm 7.4$ and $26.5\% \pm 5.2$ ($\pm$ based on 1σ). The non-polarized FD substrate gives rise to an average areal density of $52.2\% \pm 4.9$. These results have shown that the south-polarized substrate gave rise to a relatively higher

amount of biofilm growth as compared to the north-polarized and non-polarized samples.

Using the non-polarized substrate as a reference, the results have clearly shown that the north-polarized substrate was able to reduce the aquatic heterogeneous bacteria from adhering to the surface. The reduction of biofilm growth was more than 50%. On the contrary, the results of the south-polarized substrate showed the opposite effect. The heterogeneous bacteria attachment to the surface was enhanced, which resulted in approximately 50% increase in the biofilm growth. Based on the distribution chart in Fig. 55 the statistical differences between the north and south-polarized substrates were distinct enough to demonstrate that the polarity of the magnetic substrates has different effects on a heterogeneous bacteria adhesion and its biofilm growth.

5.4.4 Summary

Based on the experimental work on bacterial adhesion, the results have consistently demonstrated that a perpendicularly polarized magnetic surface in the south-polarized (spin down) state is able to attract relatively large amount of bacterial cells as compared to non-polarized samples. On the other hand, repulsion of bacterial cells occurred when the magnetic substrates are polarized in the north-polarized (spin up) state. In fact, these phenomena on surface free energy and adhesion work are found to concur with the observations made in the surface characterization of the polarized media in Section 5.2 and Section 5.2.7. Based on the DLVO and extended DLVO theories, a hydrated surface that were seen on the FD at the south-polarized state would result in a larger separation distance between the approaching bacteria and the solid surface; i.e. less bacteria adhesion to the solid surface. However, given that the results

have shown otherwise, the thermodynamic theory has shown to be a more suitable model to represent the observed phenomena.

In the previous Section 5.2.7 (c), it was proposed that when a magnetic media is perpendicularly polarized, the increase in the anisotropy energy would be channeled in the direction that depends on the magnetization directions. At the north-polarized state, the electrons would be shifted to a higher energy state that would require an adsorption of energy. Vice versa for the south-polarized state, it was proposed that there was a release of energy (potential) when the electrons move to a lower state of energy. This energy reduction or increase in the magnetic media would then be transmitted through the non-magnetic coatings to the open surfaces of the FD and HD to influence the surface free energy. Hence, the change in the surface free energy or the thermodynamic work of adhesion would eventually alter the biophysical interactions between the polarized magnetic surfaces and the bacteria.

Since it is known that energy is needed for any adhesion work to occur between two bodies, such as a bacteria and a solid surface, whether a cell culture is of homogeneous type or of a heterogeneous composition, the adhesion process will eventually be determined by the ability of the approaching cells to provide the necessary energy for the adhesion process to occur (assuming a constant surface energy on the solid surface). Hence, a solid surface with higher surface free energy would also provide the energy for cell adhesion and also possible cell growth.

Based on the AFM results, the bacterial that adhered to a north-polarized substrate was found to be in a weaker condition as compared to the non-polarized substrate. This was probably due to the reduced surface free energy that not only discouraged adhesion, but also a dehydrating effect that was reported in Section 5.2 and

5.2.4. On the south-polarized substrate, the situation was found to be opposite. This was probably due to the higher surface free energy, which encouraged adhesion and also gave a hydrating effect on the cells that are adhered to the magnetized surface.

5.5 Planktonic bacterial cell growth

It is reported that cells do communicate with one another through “quorum sensing” (refer to section 1.1) and the conditions of a solid surface would be communicated to the neighboring cells that may eventually affect the planktonic cell growth. Hence, it is believed that the change in the surface free energy at the open surfaces would also influence the cell growth behavior. In this work, the effect of a perpendicularly polarized FD on the planktonic cells growth is investigated in the following:

5.5.1 Effect on *Pseudomonas fluorescens*

The cell growth of *P. fluorescens* bacteria in the beakers containing the polarized magnetic substrates was measured via the total viable cell count (in $\log_{10}$) method, which is shown in Fig. 56. In the figure, the right vertical axis shows the actual cfu count (in $\log_{10}$), which were obtained from the non-polarized condition that acts as the reference against the north- and south-polarized conditions. The left vertical axis shows the relative cell growth rate from the beakers containing the north- or south-polarized coupons, with respect to the non-polarized condition.

Fig. 56 Magnetic polarity effects on relative cell growth rate of P. fluorescens w.r.t. non-polarized (error bars denotes maxima and minima values over three separate experiments)

From the results shown in Fig.56, the cells in the beakers containing the south-polarized magnetic coupons had shown a strong growth rate after 24 hours of incubation, but saturated after 72 hours. At the 96th hour of the incubation period, the average number of viable cells in the south-polarized condition was more than 10^5 times the non-polarized condition; i.e. the average cell count in the south-polarized condition was 10^{19} cfu/ml, as compared to 10^{14} cfu/ml in the non-polarized condition. However, the average cell growth in the beakers containing the north-polarized coupon was consistently about 5 times lower than the non-polarized condition throughout the incubation period.

As observed at each incubation period, the spread of the results for the north- and south-polarized conditions was statistically different. This statistical difference indicates reproducibility of the results.

From the AFM analysis on the biofilms at $5 \times 5 \mu\text{m}^2$, it was observed that after 24 hours of incubation, the adhered cells on the north-polarized substrate had

minimum quantity of organic substances around them (see representative images in Fig. 57). In Fig.57A, the 3-D AFM image shows the adhered *P. fluorescens* bacteria amassed together in the form of a biofilm. The macromolecules of EPS around the bacteria appeared very minimal (see arrows). A contrast enhancement to the Fig.57A image is shown in a 2-D form in Fig.57B, which clearly reveals the macromolecules secreted by the adhered bacteria (see corresponding circles). An enlarged image at $1 \times 1 \mu\text{m}^2$ further illustrates the minimal amount of the EPS secreted.

Fig. 57 AFM analysis on *P. fluorescens* biofilms grown on north- and south-polarized magnetic surfaces

However, the bacterial cells on the south-polarized substrates (as shown in Fig. 57D to 57F) had exhibited a large quantity of organic substances (see arrows and corresponding circles), which practically formed a film over the bacterial cells (see Fig.

57D and 57E). These organic substances on the bacterial cell surfaces, which can be clearly seen at $1 \times 1 \mu\text{m}^2$ in Fig. 57F, appeared in numerous clusters of macromolecules. The insert photo in Fig. 57F illustrates the physical appearance of a single macromolecule. AFM measurement on these individual macromolecules revealed its size of about 5 nm in height and 16 nm in diameter. The size of these macromolecules was observed to be consistent in all the test periods (24hr, 48hr, 72hr and 96hr), and they are generally similar to other AFM studies on proteins and its polymeric substances¹⁶³⁻¹⁶⁷.

By comparing the quantity of these protein-like macromolecules on the surfaces of the bacterial cells that adhered to the north- and south-polarized coupons, the difference has further suggested that these macromolecules were likely to be secreted by the adhered cells rather than from the surroundings. This assumption is plausible for two reasons. One is the planktonic condition for both were the same at the start point. The other is that the AFM analysis was done after soaking the magnetic coupons in distilled water. The soaking and removal from the distilled water will expose the biofilms to high shear forces that will dislodge much of the loose biomass and reversibly adhered bacteria²⁴. Hence, the bacterial remaining on the coupon surfaces were most likely the under-layers of the biofilm that mainly consisted of the irreversibly adhered bacteria.

In addition to the differences in secretion of organic substances by the adhered bacteria, the size of the adhered bacteria was observed to have reduced with incubation time (see Fig. 58). Representative AFM images of the bacterial cells on the north-polarized substrate at the 96th hour are shown in Fig. 58A. The topographic scan along the a-b direction illustrates the reduction of the physical size of the bacterial cells

as compared to the earlier 24th hour incubation period (refer to Fig. 57A and 57B). However, the size of the bacterial cells on the south-polarized coupon was observed to be significantly larger (see Fig. 58B). The line scan along a'-b' axis reveals the larger size of the adhered bacterial cells, with numerous humps on the bacterial cell surfaces, which are the macromolecules or the EPS.

Fig. 58 AFM images with sectional analysis at 5 x 5 μm^2 on biofilms after 96hr incubation

The chart in Fig. 59 further elucidates the change of the size of the bacterial cells in the biofilm over the test periods. Based on the AFM measurement, the approximate width of the bacterial cells on the north-polarized substrates was observed to have continuously reduced with incubation time from an average width of 1.30 μm ($\pm 0.10 \mu\text{m}$) at the beginning, to about 0.8 μm ($\pm 0.14 \mu\text{m}$) at the 96th hour stage. However, the size of the bacterial cells on the south-polarized substrates remained

stable at an average width of about $1.0 \mu\text{m}$ ($\pm 0.09 \mu\text{m}$) after the 48th hour stage. Although the changes in the sizes are not significant, the trend and phenomenon have suggested the physiological effect on the bacteria growth.

Fig. 59 Width measurement on bacterial cells adhered to magnetic surfaces

It has been reported in Section 1.2 that the formation of conditioning films and any adhering bacteria on the solid surface would mask the effects of the biobarrier, rendering it less effective. Here, the magnetic force discharged from the perpendicularly polarized FD at the freshly polarized state and on surfaces covered with biofilms at the 24th and 96th hour stage was measured using the MFM. The MFM results are shown in Fig. 60, which shows that the average magnetic force of the fresh south-polarized FD was 52.1 mV (indicated as “S-0hr” in Fig. 59). The MFM reading has remained almost constant at the 24th hour and 96th hour incubation period. Similarly for the MFM measurement on the north-polarized coupons, the average magnetic force has remained constant at about 120 mV for all the 3 test periods (indicated as “N-0hr”, “N-24hr” and “N-96hr”). As for the non-polarized substrate, which acted as a reference against the north- and south-polarized conditions, the average MFM reading was in-between the two conditions at 89.9mV.

Fig. 60 MFM analysis on fresh polarized FD and with biofilm growth

The results from the MFM measurement on the polarized magnetic substrates, from freshly polarized state to 96 hours of use in the bacteria culture, have implied that the magnetic force is able to penetrate through the bacteria cells and the layers of biofilms and still remain active, even up to 96 hours. This observation, coupled with the observed consistent effects on the EPS secretion on the north- and south-polarized magnetic substrates in all the test periods (24hr, 48hr, 72hr and 96hr), have indicated the efficacy of the magnetic effects on the bacteria has not been weakened in general.

In summary, it can be concluded from these investigations that the magnetic field is able to penetrate through the *P. fluorescens* bacteria cells and continue to exert its influence on the biofilms to cause the cells hydration, EPS secretion and also possibly proteins synthesis. In addition, given the presence of abundant EPS synthesis by the biofilm cells on the south-polarized substrates, these may provide an additional source of food nutrients to the neighboring cells in the biofilm and to the planktonic bacterial cells. Hence, the growth rate of the planktonic bacteria is expected to improve or enhanced. Similarly, the weaker EPS secretion would eventually create a nutrient-starved environment in the biofilm, which explains the observed slower planktonic

growth rate and the reduction of size of the bacterial cells that were adhered to the north-polarized surface.

5.5.2 Effect on *Bacillus licheniformis*

The effects of polarized FD and HD on the planktonic cell growth rate of *B. licheniformis* were monitored using the optical density measurement method (OD_{600}). The experiments were measured under static conditions with limited growth nutrients in a batch reactor. From the results shown in Fig. 61, it was noticed that the growth rates of the planktonic cells that were exposed to the FD samples polarized in the south-polarized state has consistently demonstrated an average of 33% higher growth during the exponential growth stage at the 5th hour as compared to the non-polarized samples. On the contrary, the relative growth with the FD in the north-polarized state has shown an average reduction of 8% at the same period.

Fig. 61 Investigations of polarized FD on planktonic cell growth of *B. licheniformis*

In the AFM imaging (in liquid) of the biofilm (see Fig. 62), significant differences on the morphological structures and sizes (length) of the *B. licheniformis* cells after 6 hours of incubation have been observed. The cells adhered to the FD that

was polarized in the spin up state (Fig. 62a and 62d) are observed to have reduced from about $4\mu\text{m}$ in length at the 2nd hour stage, to almost half the size at the 6th hour. The cells also appeared to be in collapsed, dehydrated and bare state as compared to the corresponding cells on the non-polarized FD (Fig. 62b and 62e). On the contrary, the size of the cells on the FD in the south-polarized state (Fig. 62c and 62f) has remained relatively constant for both, 2nd and 6th hour stages, but has appeared swollen with numerous macromolecules on its surfaces at the 6th hour. These macromolecules are approximately 10 nm in diameter and appeared to be extracellular protein secretion by the cells, which are similar in appearance and size as those seen on the earlier *P. fluorescens* experiments in Section 5.4.1.

Fig. 62 AFM imaging at $5 \times 5\mu\text{m}^2$ on *B. licheniformis* on polarized FD. Top row (a ~ c) elucidates the cells after 2 hours incubation; Bottom row (d – f) elucidates the cells after 6 hours.

From these experiments on *B. licheniformis*, it was observed that the FD in the south-polarized state was able to induce the adhered cells to secrete macromolecules on its surfaces after 6 hours of incubation, which was also at the on-set of the stationary growth stage. On the contrary, the opposite situation occurred for the cells that adhered to the FD in the north-polarized state. These behaviors can be explained from the observed hydration state of the adhered cells. It is known that cellular hydration acts as an anabolic proliferation signal and stimulates protein synthesis, whereas dehydrated or shrinking cells is catabolic and antiproliferative, which stimulates proteolysis and inhibit protein synthesis^{168, 169, 170}. Moreover, the macromolecules or extracellular protein globules are suspected to be responsible for the growth acceleration of the planktonic cells, possibly by releasing the secreted proteins into the surrounding nutrient medium as biocatalysts.

5.5.3 Bulk magnetic effect on *B. licheniformis*

Given that the surfaces of magnetic media was able to influence cell adhesion and subsequent indirect effects on the planktonic growth, the test with bulk magnets was also investigated. Based on the experimental setup as described in Section 4.4.2, the optical density measurement on the planktonic cells was recorded and shown in Fig. 63.

From the results seen in Fig. 63, it was noticed that there was no differences in the growth rates of the planktonic cells that were exposed to the magnetic north and south poles, as compared the reference that has no magnet below the beaker. These results were found to be consistent in all the three sets of experiments (Note: AFM imaging was not conducted as there were no substrates used in the experiment).

Fig. 63 Bulk magnet effects on planktonic cell growth of *B. licheniformis*

From the results of these experiments, it can be concluded that magnetic field alone (at 3000 Oe) does not influence the bacteria cells growth behavior.

5.5.4 Surface electric effect on *B. licheniformis*

Studies in using electrically charged surfaces have reported that an electric current flow from the solid surface was able to influence bacterial adhesion, whereas an electric potential could only cause a negligible effect. In this setup, an electric current flow at approximately $1.44 \times 10^{-7} \text{ A/cm}^2$ was applied to each Al foil from a 9V D.C. source. The electrical charge was applied to the Al foil from the positive and negative terminals of the D.C. electrical source (separate circuits and D.C. source used). The results from this test are illustrated in Fig. 64.

From the results seen in Fig. 64, there was no clear differences in the growth rates of the planktonic cells that were exposed to the Al foil with the electric current flowing from either a positive or a negative direction, as compared the reference that has no electrical charge applied to the Al foil. These results were found to be consistent in all the three sets of experiments.

Fig. 64 Effects of electrically charged Al foil on planktonic cell growth of B. licheniformis

Further analysis was conducted using the AFM on the bacteria that adhere to the Al foils. Figure 65 illustrates the typical AFM images seen on the bacterial cells on the different Al foils.

Fig. 65 AFM imaging at 5 x 5 μm² on B. licheniformis on electrically charged Al foils

From the AFM imaging on the *B. licheniformis* bacteria, there was also no clear differences seen on the bacteria on all three different types of electrically charged Al foils (+ve, -ve and no charge). These observations are similar for all the three sets of experiments and also concur with the results observed in the planktonic cell growth (Fig. 64).

Hence, the results of these experiments have shown that electrical current flow in either direction does not appear to have any influence to the bacteria cells growth behavior.

5.5.5 Summary

The growth rate of the planktonic bacterial cells of *P. fluorescens* and *B. licheniformis* was observed to be enhanced by the introduction of a perpendicularly polarized magnetic FD in the south polarity (spin down state) direction. In addition, the adhered cells were consistently observed to be more hydrated and also produced a larger quantity of polymeric substances and macromolecules surrounding the bacterial surfaces. Based on other works with AFM¹⁶³⁻¹⁶⁷, the polymeric substances and macromolecules are found to be the EPS and surface proteins respectively.

These EPS and proteins secreted by the cells in the biofilm are suspected to have served as an additional food nutrient to the neighboring and planktonic cells, which indirectly enhanced their growth rate.

Vice versa for the north-polarized FD, the adhered cells are observed to be dehydrated and there was also a marked reduction in the quantities of EPS and proteins secretion. The reduction in EPS and proteins has also created a nutrient-starved environment that may possibly have caused the weaker planktonic growth rate and also the size reduction of the bacterial cells in the biofilms.

CHAPTER SIX

CONCLUSIONS

&

RECOMMENDATIONS

6.1 Conclusions

From the experiments and surface characterizations of the FD and HD, the results have clearly demonstrated that the perpendicular polarization of the magnetic media is able to influence the surface free energy. Although the non-magnetic coatings on the magnetic media of FD and HD would not respond to the magnetic field, the observed changes in the surface free energy, the electric potential, and the surface morphology, have all indicated that there was a change of state at the open surfaces. These phenomena are explained by the effects of the perpendicular polarization, which would increase the surface anisotropy energy that was observed from the surface deformations at different magnetization (spin) states. In addition, the difference and the reversibility in the surface free energy between the north- and south-polarized conditions have further indicated that there was a change of state in the anisotropic energy, which can be shifted by the direction of spin.

At the north-polarized (spin up) state, the electrons are shifted to a higher energy state that would require an adsorption of energy. Vice versa for the south-polarized (spin down) state, there is a release of energy (potential) when the electrons move to a lower state of energy. This energy reduction or increase in the magnetic media is then transmitted through the non-magnetic coatings to the open surfaces of the FD and HD, which was evident from the observed changes in the CA, EFM and AFM measurements.

In the south-polarized state, the relative increase in the surface electric potential over the non-polarized condition is attributed from the conversion of the released potential energy into electrical energy. The release of potential energy has also caused the increase in the adhesion work that was demonstrated by the reduced contact angles between the sessile droplet and the south-polarized FD and HD. In addition, the

observed effects on the contact angle from the alternating polarization test has further re-enforced the hypothesis, whereby the surface free energy is reversible by the direction of spin.

Next, from the experiments on the biophysical effects on bacterial adhesion and subsequent biofilm formation, the experimental results have demonstrated that different polarity of a perpendicularly polarized magnetic media results in different bacterial interactions. The tests on homogeneous cultures like *P. fluorescens* and *B. licheniformis*, and heterogeneous cultures like aquarium fish tank media, have consistently demonstrated that a perpendicularly south-polarized magnetic surface (spin down state) is able to attract a relatively larger amount of bacterial cells to form larger quantities of biofilms as compared to non-polarized substrates. On the other hand, the magnetic substrates that are north-polarized (spin up state) is able to reduce bacterial adhesion and the subsequent biofilm formation. These phenomena are consistent with the observations made on the surface characterizations of the polarized FD and HD.

Moreover, the increase in the bacteria adhesion with stronger induced magnetization of the FD has further demonstrated the effects of anisotropy energy to the surface free energy. An increase of the anisotropy energy has been found to raise the bacterial adhesion power. The observed increase or reduction of bacterial adhesion to the polarized magnetic surfaces can be satisfactorily explained from the thermodynamic work of adhesion, which is given by the Young-Dupré equation.

Besides the effects on bacterial adhesion, the AFM analyses on the cells adhered to a north-polarized substrate are found to be in a weaker condition as compared to the non-polarized substrate. This is probably due to the reduced surface energy that discouraged adhesion, which also exhibits a dehydrating effect. It is

reported that cells dehydration is catabolic and antiproliferative, which stimulates proteolysis and inhibit protein synthesis. On the south-polarized substrate, the situation was found to be opposite. This is explained by the higher surface energy, which increases adhesion strength and also a hydrating effect on the adhered cells. It is also reported that cellular hydration acts as an anabolic proliferation signal and stimulates protein synthesis.

Lastly, the growth rate of the planktonic bacterial cells of *P. fluorescens* and *B. licheniformis* has been found to be enhanced by the introduction of a perpendicularly polarized magnetic FD in the south polarity (spin down state) direction. AFM analyses have found the presence of a larger quantity of EPS and proteins being synthesized by the adhered bacterial cells on the south-polarized substrates. These EPS and proteins secreted in the biofilms are suspected to have served as an additional food nutrient to the neighboring and planktonic cells, which would indirectly enhanced their growth rate.

On the other hand, the north-polarized FD gave rise to a marked reduction in the quantities of EPS and proteins secretion by the bacterial cells that adhered to the FD surface. The reduction in EPS and proteins may have created a nutrient-starved environment that possibly may have caused the weaker planktonic growth rate and also the size reduction of the bacterial cells in the biofilms.

Finally, the objective of this research project to develop a new biobarrier technique that uses the properties of anisotropy surface energy and the phenomena of ordered electron spins to influence and control bacterial adhesion and the subsequent formation of biofilms has been achieved. In addition, the purpose to use an energy field

to penetrate through living cells or any organic conditioning films that are reported to be an obstacle to its biobarrier efficacy has also been achieved.

6.2 Recommendations

The findings from this work are new discoveries, which present many potential applications in biotechnology, pharmaceutical and magnetic data storage industries. The following are some suggested applications.

(a) Biotechnology

- **Enhanced cells adhesion.** This is useful for biosensors whereby stronger cell adhesion and increased EPS and proteins secretion would help to improve the detection efficiency, especially for security and military use.
- **Accelerated planktonic cells growth** is also useful for bio-remediation and wastewater treatment.
- **Reversibility in surface free energy.** It is useful for “pick-and-place” applications for high energy objects, such as living cells and nano-size particles.

(b) Pharmaceuticals

- **Accelerated planktonic cell growth and proteins secretion.** This is important for drugs and medicines production.

(c) Magnetic data storage

- **Reversibility in the surface free energy** presents useful surface characterization of magnetic media, which also would affect the tribological factors in hard disk.

Apart from these commercial applications, this work has also presented a new perspective to the biophysics of cell adhesion and growth, and its physiological influences on cell hydration and protein synthesis. Further research into these may yield a better understanding on cellular behavior when it is subjected to a uni-direction magnetic field at the interfacial surfaces.

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APPENDICES

APPENDICES

APPENDIX A

DRAWING OF THE FLOW CELL REACTOR FOR BIOFILM CULTURE

APPENDIX B

Chemical compositions of micronutrients and growth media for *P. fluorescens* biofilm culture experiment

	S/N	Chemical name	Chemical formula	Mixing gram / litre	
			USE 1 Ltr D.I. WATER		
Reagent	1	Sodium Phosphate, Dibasic (Anhydrous)	Na ₂ HPO ₄	1.825	
	2	Potassium Phosphate, Monobasic	KH ₂ PO ₄	0.350	
	3	Magnesium Sulphate, 7 Hydrate	MgSO ₄ . 7H ₂ O	0.010	
	4	Ammonium Sulfate	(NH ₄) ₂ SO ₄	1.000	
	5	Glucose (Dextrose, Monohydrate)	C ₆ H ₁₂ O ₆ . H ₂ O	5.000	
	6	Yeast extract (Dehydrated), powder	nil	0.100	
			USE 1 Ltr 0.1M HCl	mg / 100ml	
Micro-Nutrients	1	Manganese (II) Chloride tetrahydrate	MnCl ₂ . 4H ₂ O	0.527	52.7
	2	Copper Chloride, Dihydrate	CuCl ₂ . 2H ₂ O	0.228	22.8
	3	Cobalt Chloride, 6 Hydrate	CoCl ₂ . 6H ₂ O	0.317	31.7
	4	Ammonium Molybdate, 4 Hydrate	(NH ₄) ₆ Mo ₇ O ₂₄ . 4H ₂ O	0.231	23.1
	5	Sodium Borate, 10 Hydrate	Na ₂ B ₄ O ₇ . 10H ₂ O	0.127	12.7
	6	Zinc Chloride		0.363	36.3
	7	Calcium Chloride, anhydrous powder	CaCl ₂	3.700	370
	8	Iron (III) Chloride, anhydrous	FeCl ₃	2.160	216
Others	1	Phosphate buffer solution, 1X soln.	---	X 1	
	2	Sodium Bicarbonate (pH adjutment soln)	NaHCO ₃	as per supplied	
	3	Hydrochloric Acid, 37% w/w aq. soln.	HCL (0.1M)	37%	