BIOLOGICAL MOTOR TO MOVE LOADS AT MICROSCALES: KINEMATIC ANALYSIS

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LIST OF ABBREVIATION

CR	*Chlamydomonas Reinhardtii*
HMW	high molecular weight
IONPs	iron oxide nano particles
LMW	low molecular weight
MEMS	microelectromechanical systems
PDDA	poly(diallyldimethylammonium chloride)

ABSTRACK

Kuasa magnet telah diperkenalkan sebagai salah satu kaedah untuk mengawal pengerakan mikroalga. Bagi membuat mikroalga menjadi magnetresponsif, polimer yang bercas positif , poli (diallyldimethylammonium klorida)) (PDDA) telah digunakan untuk menggalakkan lampiran berkesan zarah bersaiz nano besi oksida (IONPs) ke permukaan mikroalga, spesies *Clamydomonas reinhardtii* (spesies CR) melalui interaksi elektrostatik. Melalui eksperimen, didapati bahawa kelajuan daya berenang spesies CR yang bebas terletak antara 100 ụm/s hingga 128 ụm /s. Seterusnya, selepas pelekatan zarah bersaiz nano besi oksida kepada permukaan mikroalga, kelajuan daya berenang spesies CR dikurangkan secara drastic kepada 9 ụm /s hingga 29 ụm /s . Kelajun daya berenang dikurangkan lebih kurang 75.23% hingga 91.79% daripada purata kelajuan berenang spesies CR yang bergerak bebas. Ini disebabkan oleh pergerakan flagella telah diganggu oleh zarah bersaiz nano besi oksida yang melekat kepada flagella ataupun persekitaran flagella. Mengikut hukum pemuliharaan momentum, apabila IONPs melekat kepada mikroalga, peningkatan dalam jumlah jisim menyebabkan, pengurangan kelajuan renang spesies CR. Akhir sekali, apabila daya magnet digunakan, kelajuan renang spesies CR meningkat dengan kenaikan yang berbeza-beza dari 64.65% kepada 334.83% daripada kelajuan renang asalnya. Pergerakan mikroalga telah diarahkan ke arah sumber kuasa magnet.

ABSTRACT

Magnetic force was introduced as one of the method to control the motion of microalgae. To make the microalgae become magnetically responsive, the cation polymer binder, poly (diallyldimethylammonium chloride) (PDDA) was used to promote the effective attachment of iron oxide nano particles (IONPs) onto the surface of microalgae, *Clamydomonas Reinhardtii* species (CR species) through electrostatic interaction. From the experiment, it was found that the swimming velocity of fresh CR species species is within 100 um/s to 128 um/s. Next, after the attachment of PDDA functionalized IONPs, the swimming velocity of CR species reduced drastically to only about 9 um/s to 29 um/s which around 75.23% to 91.79% of reduction from the average velocity of free moving CR species. This is because motion of the flagella is being distorted by PDDA functionalized IONPs. According to law of conservation of momentum, when IONPs attached to the microalgae, the increase in total mass caused its swimming velocity reduced. Lastly, when magnetic force is applied, the swimming velocity of targeted CR species with PDDA functionalized IONPs increased significantly with an increment vary from 64.65% to 334.83% of its original swimming velocity. The movement of microalgae is directed toward the source of magnetic force.

CHAPTER ONE

INTRODUCTION

1.1 Research Background

The idea of molecular-scale mechanical nano machine was firstly suggested by Richard Feynman, a Nobel Laureate in physics in his famous speech in the 1959 Meeting of the American Society of Physics entitled "There is plenty of room at the bottom." He stated: "So I want to build a billion tiny factories, models of each other, which are manufacturing simultaneously, drilling holes, stamping parts, and so on". This idea had introduced the world to nanotechnology and driven the development and research in nanotechnology field (Wang, 2013).

The study of the mechanical movement and behaviour at nano scale become important and critical in order to develop practical nano machines for widely application in diverse fields especially for medical purpose. The molecular motors that found in living cells have the attribute to convert chemical energy into mechanical work. These tiny bio motor introduced as micro/nano bio motor due to their size and complexity of their process.

There are many examples of bio motors in nano size that occur naturally. Inside the cells, the linear motors, DNA, RNA, kinesin microtubule and myosin actin-based system have gracefully specific binding to corresponding complementary filaments to perform mechanical work (Hess & Bachand, 2005). In eukaryotic mitochondria, the direct efficiently conversion of chemical energy from ATP in ATP synthase is one of nature's miracles. Outside the cell, the ciliary dyneins contributed

to the moving of eukaryotic cilia and flagella (Hess & Bachand, 2005; Weibel et al., 2005) . In bacteria, the energy stored within proton gradients that are generated across a phospholipid membrane is utilized for the rotary movement of flagella that enable bacteria to move toward source of nutrition(Vogel, 2005).

The sophisticated operation of tiny bio motors shows extraordinary motion capabilities with an advanced directional movement and speed regulations. Understanding the remarkable underlying principles of nature bio motors provide researchers the fundamental knowledge and new ideas for the design and development of new artificial nano machines. Bio mechatronics is the combination of biological components with artificial devices, in which the biological component provide significant functional capability to the system and the artificial component provides specific cellular and tissue interfaces that serve as the maintenance and functional adaptation of the biological component (Herr & Dennis, 2004; Wang, 2013).

Artificial nano machine can perform various tasks similar to bio motors in living cells including transportation of micro/nano loads and facilitating chemical reactions. Synthetic nano machines promise a wide range of future technological applications such as bio medical while providing unlimited possibilities base on one's creative and innovative. The development of such powerful nano machine can greatly improve our quality of life (Wang, 2013).

The key factor to success in the development of artificial nano machines is the clear and deep understanding about the working principle within nature's bio motors. However, the most significant bottleneck for further development of micro/nano machine is the miniaturization of on-board actuators and power sources provided for the mobility. The utilization of bio motor as micro actuator would be the best solution.

As compare to artificial micro actuators, the bio motor actuator is much smaller and capable to perform more complicated motion with the highly energy conversion efficiency of chemical energy into mechanical works (Bahareh Behkam, 2007).

Quite a number of research has been conducted in order to exploit the potential of living cells as micro machine to perform mechanical works in micro scale. For example, unicellular photosynthetic algae, *Chlamydomonas Reinhardtii* (CR species) as cargo to transport microloads (Weibel et al., 2005), cardiomyocytes as micro actuator to oscillate micro pillars (Morishima et al., 2006), gliding bacterium Mycoplasma mobile as transporter along microtracks (Hiratsuka et al., 2005), control of the direction of kinesin microtubule along micro track (Hiratsuka et al., 2001), animal–derived muscle tissue as embedded microcontroller for micro swimming robot (Herr & Dennis, 2004), and also the employment of protozoa as micro manipulator with negative galvano taxis (Itoh, 2000). These researches clearly showed the possibility to harvest and utilize the power generated by living cells power to perform mechanical work in micro/nano scale at high efficiently.

1.2 Problem Statements

The mechanical behaviour of micro algal cells under magnetophoretic phenomena is important to exploit the potential on utilizing magetophoretic phenomenon as a method to control the movement and direction of bio motor. Hence, it is the aim of this study to investigate the motion of micro algal cells at micro scale to determine how the application of magnetic force affects the motion of micro algal cells.

1.3 Research Objectives

1. To kinematically study the motion of free CR species and CR species with PDDA functionalized IONPs under the effect of magnetophoresis.
2. To study the effect of IONPs attachment toward the motion of CR species.

1.4 Scope of Study

In this research, whole intact cells was employed as the bio motor to perform mechanical works by using magneto taxis to control the motion of the bio motor. Surface functionalized iron oxide nano particles (IONPs) act as the modifier that attaches to the living cells, *Chlamydomonas Reinhardtii* (CR species) so that the CR species will respond toward magnetic force. The application of magneto taxis provides an alternative way to control the direction and motion of the cells. Kinematic analysis is performed so that the mechanical behaviour of CR species under the effect of magnetophoretic would be investigated and identified.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bio motor

Bio molecular motor can efficiently convert chemical energy, source from light, molecules and electric field into mechanical works (Hess & Bachand, 2005). This attribute mostly come from the respond of cells toward external stimuli. There are plenty of living cells have the ability to perform specific functions as they respond to external stimuli. Different functionalized cells perform different respond toward stimuli.

Inside the cells, the linear motors, DNA, RNA, kinesin microtubule and myosin actin-based system have specific binding to certain complementary filaments in order to perform mechanical work. In eukaryotic mitochondria, the direct efficient conversion of chemical energy from ATP in ATP synthase is one of such nature's miracles. Outside the cell, the ciliary dyneins contribute to the moving of eukaryotic cilia and flagella (Hess & Bachand, 2005; Weibel et al., 2005). In bacteria, the energy stored within proton gradients that are generated across a phospholipid membrane is utilized for the rotary movement of flagella that enable bacteria to move toward source of nutrition (Vogel, 2005).

Basically, the effective method to harvest the power generated by bio motor do not exist yet mainly due to the unstable of such molecules under artificial environment (Hiratsuka et al., 2005). Here comes the interest to exploit the whole microorganism as the biological motor for transportation purpose in micro scale. By

introducing intact cell as the biological motor, there are a few eye-catching features (Weibel et al., 2005):

I. Genetic engineering or protein purification is not necessitating.
II. The bio motor no need to be reconstituted in vitro since they are already integral.
III. Utilization of the whole cell surface for the attachment of micro loads.
IV. Phenotypes can be exploited to steer cells' movement.
V. The cells itself is the power source; no external power source required.
VI. The bio motor can be easily duplicated, just simply by growing the new cells.

The major disadvantage is the cells itself as living organism is fragile. They can simply dead just because the changes in surrounding artificial environment. However, the potential advantages of bio motor still attract researchers to further develop and explore the possibilities to utilize bio motor as micro transporters or micro motives as micro/nano scale.

2.1.1 Motor Protein

The development and exploration of bio motors technology would have been impossible without the pioneering work of biophysicists and cell biologists in the designing motor proteins (Hess & Bachand, 2005). The development of bio motor comes with the study of motility of cells. The models of kinesin microtubule and myosin actin-based system had been study to understand the mechanism of motility (Vale & Milligan, 2000). Comparison of these 2 motors comes out with the discovery of common principles to convert chemical energy into mechanical works. This has deepened our understanding of the interaction between physical properties of

biological components and design of synthetic elements (Hess & Bachand 2005). Eventually, this knowledge will contribute as fundamental and basic knowledge for the further development and exploration in nanotechnology especially the design and operation of artificial synthetic nano machine.

2.1.2 Microchip

Microchip or lab-on-chip is the integration of various chemical reactions with complex operations into a single chip. This micro device provides several advantages such as reduction in reagent consumption, space requirement and analysis time (Morishima et al., 2006). Over the past few years, the reduced in the size of microchips attracted researchers to harness the power generated by biological cells and integrated biological cells into an synthetic environment, the microchip. This is mainly due to the micro scale liquid environment in microchips become suitable for biological cells accommodation (El-Ali, Sorger & Jensen, 2006).

2.1.2 Bio Actuators

The most important use of biological cells in microchip is the cell-based actuators that harness and exploit the mechanical ability of cells. These bio actuators used the micro-fabricating technology and utilize living organisms including microorganism, skeletal muscle cells and cardiomyocytes to delivery micro scales loads and rotate microstructure (Tanaka et al., 2007). These cell-based actuators has the benefits of self-actuated, require no external energy sources and wireless. However, these bio-based micro machines also have the shortage of short life span and low tolerance to surrounding environment change (Hess & Bachand, 2005).

Control of the direction of bio motor can perform with various methods. This including by using photo taxis, magneto taxis, potential gradient and also modification of artificial device that accommodate the bio motor.

2.2 Phototaxis

A study on the biological propulsion of micro loads by unicellular photosynthetic algae *Chlamydomonas reinhardtii* (CR) was carried out by researchers to illustrate a method to exploit the mechanical motion generated by biological motor (Weibel et al., 2005). The CR species act as the transporter to deliver micro loads from one point to the other. The researchers make use of the nature of photo taxis of CR species to control and steer the moving direction of swimming CR species in micro fluidic channels. The CR species showed negative photo taxis toward high intensity of light, moving away from the light source and vice versa (Weibel et al., 2005). For the micro loads, photo chemistry was applied to unburden the micro loads. An 80-W mercury lamp used as light source to photo-cleavage the beads from CR species.

From this study, the researchers claimed that the bead concentration to cells concentration ratio influenced the amount of beads attach to cells. The location of beads attached onto cells appeared to be random and influenced cells velocity. Lastly, the utilization of intact cell as bio motor would be the more practical way rather than extract the bio motor protein from living organism and use them solely (Weibel et al., 2005).

2.3 Magnetotaxis

This method utilizes the phenomena of magneto taxis in controlling the motion direction of living cells. In a paper, a method to create artificially magnetotactic organism was demonstrated. A type of ciliate protozoan, T. *pyriformis* was chosen and internalization of iron oxide particles in the cells through digestion of magnetite particles of the cells. The figure 2.1 showed the images of *T. pyriformis* at normal status, after internalization of iron oxide particles and after magnetization of internalization particles:

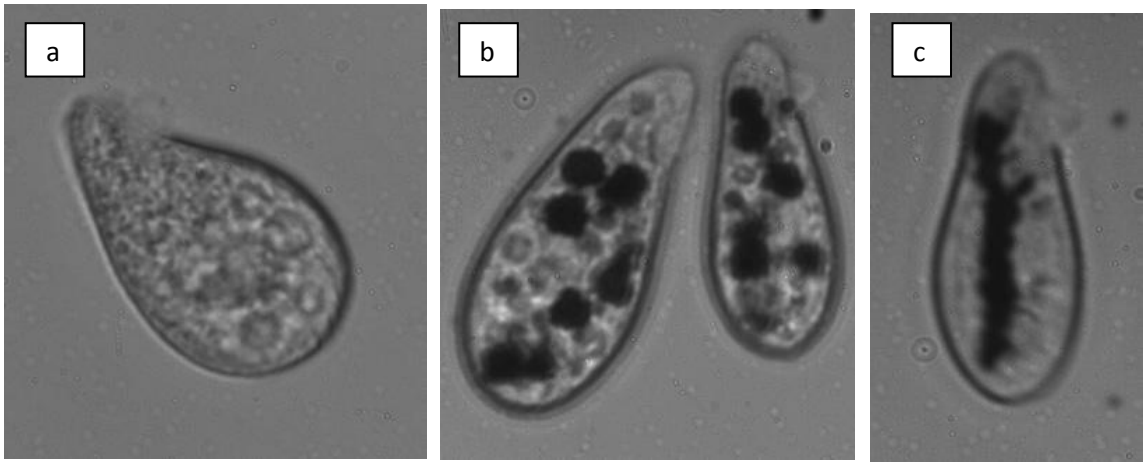

Figure 2.1: a) Images of T. pyriformis a) normal status b) after internalization of iron oxide particles c) after magnetization of internalization particles (Dal Hyung Kim, 2010)

A simple feedback control algorithm was designed to control the movement of cells toward specific location through the manipulation of magnetic field around the cells. This controller combined with two sets of approximate Helmholtz coils on x and y-axis succeeds to drive the cells to perform movement among five points repeatedly (Dal Hyung Kim et al., 2010). Another similar study was done but involving three dimensional monitoring. The vertical motion along z-axis is determined through the intensity difference (Dal Hyung Kim et al., 2012). These

researches show the possibility to use micro algal cells as highly specific actuator that function as propulsion and sensor for micro robot in micro fluidic environment.

2.4 Potential Gradient

As illustrated by Itoh and co-workers (Itoh, 2000), the application to use the protozoa as micromachines orintelligent micro actuators for microelectromechanical systems (MEMS) was studied. Electrical potential gradient was used to control the motion of protozoa (Itoh, 2000). The specimen used *paramecium caudatum* species showed negative galvano taxis as it swam toward negative electrode in the present of electrical potential gradient. A sudden break in the applied of electric field would cause the rapid stop of moving paramecium. The figure 2.2 showed the block diagram for the experiment conducted by Itoh and co-workers:

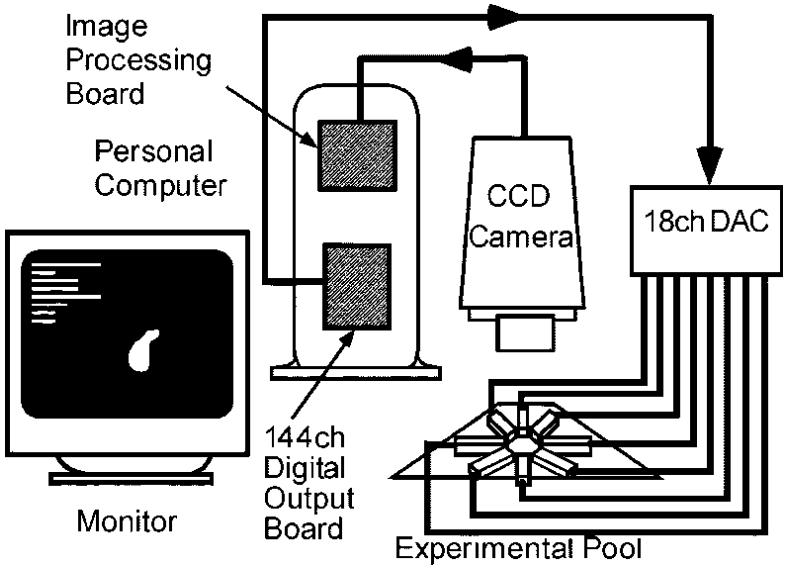

Figure 2.2: Block Diagram of the Experimental Apparatus. (Itoh, 2000)

In this experiment, the *paramecium caudatum* species was succeeding to rotate turn the micro pillar. This shows the possibility of protozoa as micro actuator in

bio-MEMS system coordinate by using negative galvano taxis. However, only motion direction of paramecium is able to be control but not the speed (Itoh, 2000).

2.5 Modification of Artificial Device

In another study, the researchers focus on the utilization of on-board cells that directly convert chemical energy into mechanical work. The cardiomyocyte cells were attached to hydro gel micro pillars to measure the contracting force generated by the cardiomyocyte cells. The number and location of cardiomyocyte attaching the micro pillars greatly affect the oscillation of micro pillars.

They found that the force generated by single or a few cardiomyocyte cells was more than a few nano newtons within an appropriate volume. This is much larger than other micro/nano actuator such as molecular motors and laser tweezers that typically generate force up to a few pico newtons. With this, a conclusion had make in which cardiomyocytes could be a more suitable material as micro/nano actuator to transport object or fluids within micro space. The design of micropillar play an important role as a motion guiding tool in harvesting the power generated by cardiomyocyte cells (Morishima et al., 2006). The figure 2.3 showed the Design and actuating principle of hydro gels micro pillars powered by cultured cardiomyoctyes:

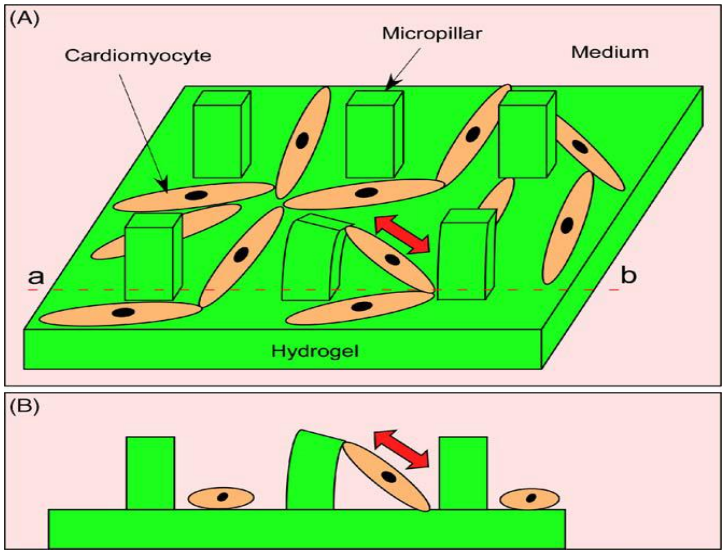

Figure 2.3: Design and Actuating Principle of Hydro Gels Micro Pillars Powered by Cultured Cardiomyoctyes (Morishima et al., 2006)

Morishima and coworkers found that the force generated by single or a few cardiomyocyte cells was more than a few nano newtons within an appropriate volume in which much larger than other micro/nano actuator such as molecular motors and laser tweezers that typically generate force up to a few pico newtons. With this, a conclusion had make in which cardiomyocytes could be a more suitable material as micro/nano actuator to transport object or fluids within micro space (Morishima et al., 2006).

The utilization of living cells come with one unavoidable problem which is the motion of living cells always is not in desired the direction. In most circumstances, the cells appear to move randomly and freely at the absence of external influence. Effort had been dedicated to restrict the motion of motor proteins, kinesin-drive microtubules along linear micro tracks for only unidirectional rather than bidirectional on two-dimensional surfaces. A rectifier had been introduced that enable to efficiently and stably limit the kinesin-driven movement of microtubules in one direction (Hiratsuka et al., 2001).

By doing so, the mechanical force generate by motor proteins can restricted to one direction and enabled actively transporting of materials at the micro scale. This shows how the creative and innovative design of artificial device can efficiently harvest and utilize the power generate by bio motor to perform specific tasks.

Besides, some microorganism exhibit nteresting mechano-behaviour that has the potential to become useful method to perform mechanical work. One of the behaviour is gliding. In a research, the gliding attribute of bacteria, *Mycoplasma mobile* had been studied. The *mycoplasma mobile* together with streptavidin coated polystyrene beads attached put on patterned lithographic substrates. The tall walls design of the platform result in the unidirectional movement of *mycoplasma mobile* at the bottom edge of the walls (Hiratsuka et al., 2005). This is another example that shows the important of appropriate and suitable design of the artificial device to fully utilize the potential of living cells as bio motor.

2.6 Magnetophoresis of Microalgal Cells

The first microorganism been discovered that has the magnetite particle, Fe_3O_4 deposited within the cells is Anisonema platysomum Skuja. The biomineralizes magnetite particles arranged in chains that act as magnetic dipole moment and contributed to the magnetotactic response (de Araujo et al., 1986; Safarik et al., 2016).

However, most of the living cells do not present such special properties and show no respond to applied magnetic force. Here comes the interest to apply magnetic modification of micro algae to harvest culture algae from pond as bio fuel as the solution for energy crisis. Micro algae can act as environmental friendly source of renewable energy to meet the global energy demand (Parmar et al., 2011). Besides, removal of harmful algae and removal of algal bloom in fresh water also are

important (Xu et al., 2011). This also can be done through the magnetic modification of targeted micro algal cells follows by magnetic separation (Bitton et al., 1975).

Recently, many studies been carried out on the magnetic separation of algal cells. For example, harvesting of marine algae, *Nannochloropsis maritime* by the adsorption of naked Fe_3O_4 onto the algal cells through electrostatic attraction (Hu et al., 2013), in situ magnetic separation of *Botryococcus raunii* and *Chlorella ellipsoidea* that can achieved very high efficiency of cell separation (Xu et al., 2011), and also the harvest of *chlorella ellipsoidea* by using a magnetic separator consisted of permanent magnetic drum, separation chamber and scraper blade (Hu et al., 2014).

The attachment of magnetic particles onto micro algal cells would turn these living cells become magnetic responsive (Toh et al., 2012). There are two main method of magnetic labelling micro algal cells' surface, adsorption based attachment and electrostatic mediated attachment (Toh et al., 2014). The figure 2.4 showed the illustration of IONPs attachment strategies onto microalgal cells:

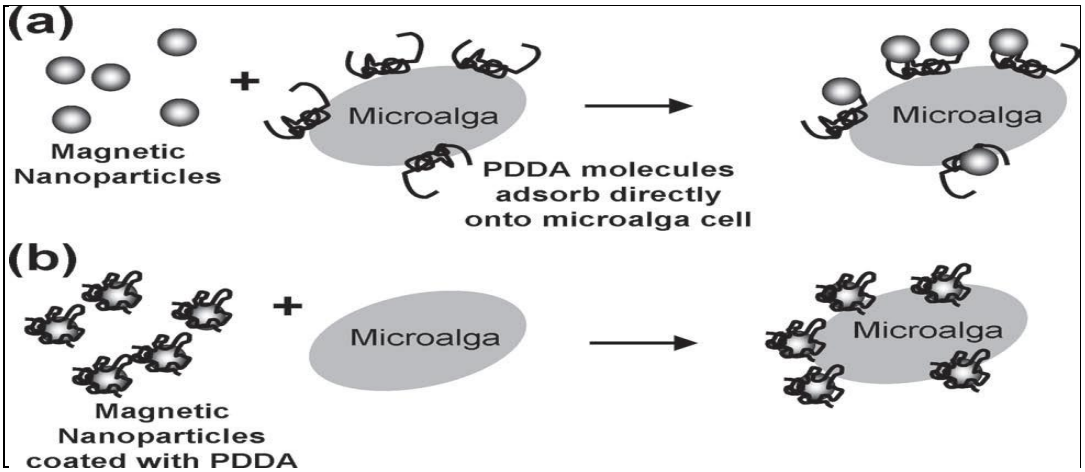

Figure 2.4: Two Strategies employed to encourage the Attachment of IONPs onto Microalgal Cells: a) 'attach to' Strategy b) 'immobilized on' Strategy (Lim et al., 2012)

The electrostatic mediated attachment can further divided into ‘attach to’ and ‘immobilized on’ strategies. ‘attach to’ method is the algal cells firstly bond with PDDA follow by the attachment of IONPs while for ‘immobilized on’ method, the IONPs coated with PDDA then attach to algal cells (Lim et al., 2012) .

2.7 Surface Functionalized Iron Oxide Nanoparticles

With the introduction of cationic polyelectrolyte as a binding agent to promote the immobilization of IONPS onto micro algal cells through electrostatic mediated attachment, the rapid magnetophoretic separation of cells could be happened. Basically, poly (diallyldimethylammonium chloride) (PDDA) would be more suitable to be the binding agent for all type of microalgae medium compare to other types of cationic binders . This is due to its properties of pH independent. This means that PDDA can use for different pH conditions of cell medium while promising high cell separation performance (Toh et al., 2014).

For ‘immobilized on’ method of IONPs modification, low molecular weight (LMW) PDDA is suggested rather than high molecular weight (HMW) PDDA. HMW PDDA is not suitable to promote the attachment of IONPs onto micro algal cells because it caused the irreversible flocculation of the IONPs prior to use. In addition, LMW PDDA has much broader flocculation curve leading to a higher degree of packing efficiency on surfaces of micro algal cells and its narrow capture range to promote bridging between cells (Lim et al., 2012).

Recently, the utilization of magnetic functionalized micro algal cells as biosensor had been studied. Micro algal cells, *C.pyrenoidosa* species were coated with poly (allamine hydrochloride) functionalized biocompatible magnetite nano particles and these cells used to construct amperometric biosensor that able to detect the

triazine herbicides, inhibitors of photosynthetic activity (Zamaleeva et al., 2011). For the application of magnetophoresis, the magnetic particles use for magnetic labelling must possess good colloidal stability to promote effective tagging with target (Leong et al., 2016).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Cultivation of Algae

The *Chlamydomonas* species used in this research is provided by Prof.Chunzhao Liu group from Chinese Academy of Science. The cultivation medium, Bristol solution's composition is shown in table 3.1:

Table 3.1: Components of Culture Medium of CR species

	Chemical	Quantity	Quantity for dilution
1	$NaNO_3$	1 mL/L	25 g/100 mL dH_2O
2	K_2HPO_4	1 mL/L	7.5 g/100 mL dH_2O
3	$MgSO_4 \cdot 7H_2O$	1 mL/L	7.5 g/100 mL dH_2O
4	$CaCl_2 \cdot 2H_2O$	1 mL/L	2.5 g/100 mL dH_2O
5	KH_2PO_4	1 mL/L	17.5 g/100 mL dH_2O
6	NaCl	1 mL/L	2.5 g/100 mL dH_2O
7	$FeCl_3 \cdot 6H_2O$	1ml/L	0.5 g/100 mL dH_2O
8	EDTA-Fe solution[1]	1 mL/L	
9	A5 （Trace metal solution)[2]	1ml/L	
10	Soil Extract[3]	40ml/L	

EDTA-Fe Solution

(Solution A) Added 4.1 mL of concentrate HCl into 50 mL of distilled water

(Solution B) Dissolved 0.9306 g of EDTA-Na 2 into 50 mL of distilled water

(Solution C) Dissolved 0.901 g of $FeCl_3.6H_2O$ into 10 mL of Solution A above then added in 10 mL of solution B

(Final solution) Dilute solution C with distilled water up to 1000 Ml

A5 (Trace metal solution)

Table 3.2: Components of A5 trace Metal Solution

	Chemical	Quantity	Quantity for dilution
1	H_3BO_3	2.86 g/L dH_2O	0.286 g/100mL dH_2O
2	$MnCl_2$ ·4H2O	1.86 g/L dH_2O	0.186 g/100mL dH_2O
3	$ZnSO_4$ ·7H_2O	0.22 g/L dH_2O	0.022 g/100mL dH_2O
4	$Na_2MoO_4.2H_2O$	0.39 g/L dH_2O	0.039 g/100mL dH_2O
5	$CuSO_4$. $5H_2O$	0.08 g/L dH_2O	0.008 g/100mL dH_2O
6	$Co(NO_3)_2$.6 H_2O	0.05 g/L dH_2O	0.005 g/100mL dH_2O

Firstly, 100 ml of EDTA solution and A5 trace metal solution were prepared according to procedure and table 3.2 for EDTA solution and A5 trace metal solution respectively. Then, culture medium was prepared according to ratio of components stated in table 3.1. The solution was left to autoclaved before carried out the cultivation process to remove and kill all foreign particles and microorganisms.

3.1.2 Preparation of PDDA Functionalized IONPs

Firstly, IONPs at 1000mg/L (1000ppm) was prepared. This was done by adding 30mg of IONPs into 30mL of deionised water and sonicated for an hour in a Fisherbrand Ultrasonic Analog SRH Baths to evenly distribute the IONPs within the solution. Meanwhile, PDDA solution was prepared by adding 5 mL of PDDA solution

into 30mL of deionised water and put on end-to-end rotator at 40rpm for an hour to produce a well mix PDDA solution.

Then, 10mL of IONPs with concentration of 1000ppm was added drop by drop into the PDDA solution and left on end-to-end rotator at 40rpm for 2 hours to promote complete surface coating of the IONPs with PDDA polyelectrolyte. To verify the success of IONPs coating, the zeta potential of the PDDA functionalized IONPs solution was checked and will be discuss in Chapter 4.

3.1.3 Attachment of PDDA functionalized IONPs to CR species

The PDDA functionalized IONPs was added into the CR species sample with a ratio of 1:5. Then, the mixture was left on end-to-end rotator at 5 rpm for 10 minutes to promote better attachment of PDDA functionalized IONPs onto the cells.

3.2 Experiment Procedure

3.2.1 Use of Microscope and Software

The Olympus BX53 microscope was used in this study together with the software CellZens Dimensions for the capture and analyse of the motion of CR species. The video and images were captured and analysis was carried out with this software or another software, ImegJ.

3.2.1.1 Modification of Glass Slide.

To have a better observation of sample and prevent the PDDA functionalized IONPs and CR species stick on the glass slide, some modification was made on the glass slide. Some barriers made from tape were added on the glass slide to provide

sufficient space for the CR species to move and ease the observation of the motion of CR species.

3.2.1.2 Study of Magnetophoretic Effect

Firstly, 1 micro meter of the CR species without modification was put on the glass slide to obtain data for kinematic study of free CR species. Next, the free CR species was replaced with CR species with PDDA functionalized IONPs for the kinematic study of magnetophoretic effect.

The magnet used in this study for the purpose of providing magnetic attraction is NdFeB magnet. This magnet was place at 1cm direction away from the glass slide which contain sample. The video was start recorded before locating the magnet. After 30 seconds, the magnetic force was applied while the video recording was continued. By doing so, the movement of CR species with PDDA functionalized IONPs attached with and without magnetophoretic effect can be recorded and compared.

3.2.2 Analysis of Video Frames and Images

The video frames recorded are useful for the kinematic analysis of CR species under magnetophoresis effect. The coordinate of the CR species in each frame was determined and recorded in Microsoft Excel. Then, these data use for the calculation of moving velocity and energy of the CR species under vary conditions and the results can be compared for the free CR species and CR species with PDDA functionalized IONPs with and without magnetophoretic effect. The sample of calculation was showed in Appendices.

3.3 Summary of Chemicals and Equipments Used

The tables below summarized the chemicals and equipments used in this experiment.

Table 3.3: Chemicals used in the Experiment

Nutrient medium	Sodium Nitrate
	Potassium Hydrogen Phosphate
	Magnesium Sulfate Heptahydrate
	Calcium Chloride Dihydrate
	Potassium Dihydrogen Phosphate
	Sodium Chloride
	Iron Chloride Hexahydrate
	Hydrochloric acid
	Ethylenediaminetetraacetic acid disodium salt dihydrate
Cationic poly-binder	Poly (diallyldimethylammonium chloride) (PDDA)
pH adjustment	Sodium Hydroxide
	Hydrochloric acid
Iron oxide nano-particles	

Table 3.4: Equipments used in the Experiment

Equipment	Purpose
Olympus BX53 microscope	To observe and analyze the motion of CR species
End- end rotator	To disperse and yield uniform solution
FB15050 Fisherbrand Ultrasonic Analog SRH Baths (2.75 L)	To sonificate the iron oxide nano particles solution for evenly distribution of IONPs within the solution
Malvern Instruments Nanosizer ZS	To measure the zeta potential and hydrodynamic sizes of IONPs
Cylindrical shaped N50-graded Neodymium Boron Ferrite (NdBFe)	To induce magnetic force to perform magnetophoretic separation
DR 5000 UV–Vis spectrophotometer	To measure the changes in microalgae culture concentration.

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter presents the results and discussion that make up of two sections. The first section discusses the properties of surface functionalized IONPs. The next section focus on the kinematic analysis of magnetophoresis effect of microalgae which involve the free moving microalgae, microalgae with PDDA functionalized IONPs and lastly the magnetophoretic process.

4.1 Properties of Surface Functionalized IONPs

For successful coating of IONPs with PDDA polymer binder, the zeta potential of IONPs should be negative value to promote the electrostatic interaction between IONPs with the PDDA cationic binder. In aqueous solution, the zeta potential of naked IONPs is depend on the pH of the solution. The surface of metal oxide nano particles may undergo protonation or deprotonation as per the following reaction (Nassar, 2010; Roonasi & Holmgren, 2009):

$$H_2O + MO\text{-}O^- \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} MO\text{-}OH \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} MO\text{-}OH_2^+$$

where MO refers to metal oxide, H+ and OH- refer to the potential determining ions. According to the equation, as the pH increase, negatively charged sites increase and the positively charged sites decrease (Xu et al., 2011).

The figure 4.1 showed how the pH of solution affects the zeta potential of IONPs t which involve the use of magnetic separation to harvest algae:

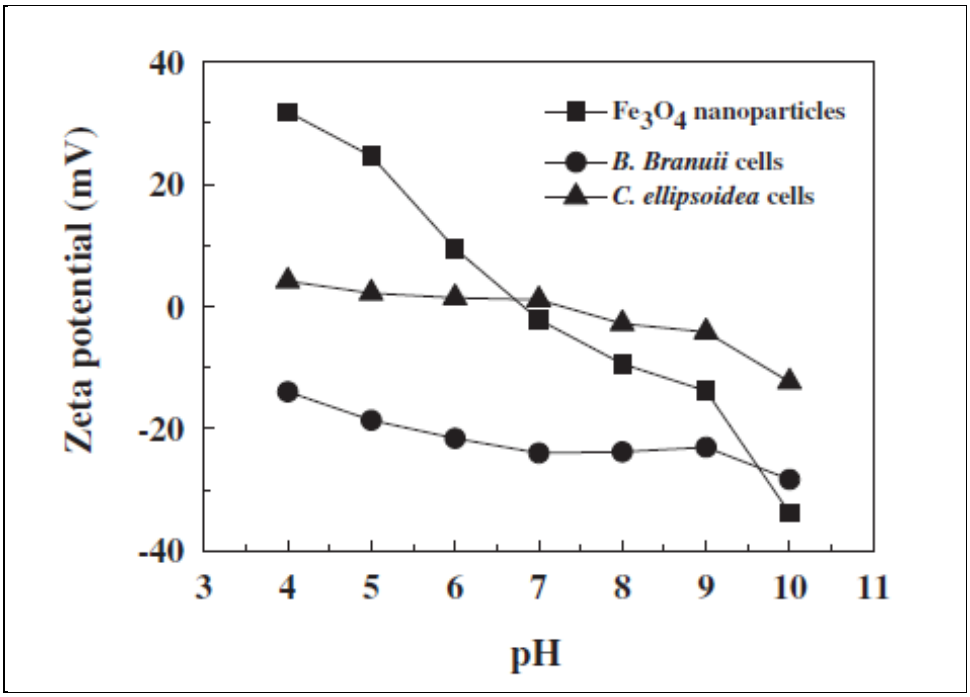

Figure 4.1: Zeta Potential of Fe3O4 Nano Particles, B. braunii and C. ellipsoidea Cells at Different pH Values (Xu et al., 2011).

From the figure 4.1, we can see that the zeta potential of IONPs turned into negative value once the pH values exceed 7. According to Toh, for the naked IONPs, its isoelectric point is at pH 5.4 (Toh et al., 2014). The IONPs will be protonated and form IO-OH_2^+ at pH < 5.4 and will deprotonated and form IO-O^- at pH >5.4 (Foster & Smyth, 1980; Toh et al., 2014; Xu et al., 2011). Hence, we can conclude that the suitable pH value for the IONPs solution would be within basic range.

In the preparation of IONPs solution, deionised water was used to prevent the occurrence of foreign ions either positive or negative charged ions that would greatly affect the surface charge of IONPs. However, the deionised water that used for IONPs dispersion is within the range of 5.0 to 5.8. Hence, pH adjustment had to be done on deionised water to ensure it is within basic range so that the surface charge of IONPs is negative value. The pH value was adjusted to pH 8.0-8.1 as the zeta potential measured for this pH is greater than -30 mV. The zeta potential > |30 mV| is an indication of good colloidal stability. The table 4.1 showed the zeta potential of naked IONPs at different pH value:

Table 4.1: Zeta Potential of Naked IONPs at Different pH Value

Zeta Potential of Naked IONPs at Different pH Value				
	Zeta Potential (mV)			
	1	2	3	Average
pH 8.05	-31.6	-31.7	-30.8	-31.37