

**CADMIUM REMOVAL BY IMMOBILIZED AND FREE CELL
OF CYANOBACTERIA IN A BATCH SYSTEM**

The seal of Mahidol University is a circular emblem. It features a central blue field with a golden chedi (a traditional Thai Buddhist stupa) and a flame-like motif below it. The seal is surrounded by a golden border containing Thai script. The text 'SUDARAT CHAICHALEARM' is superimposed over the central blue field.

SUDARAT CHAICHALEARM

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(ENVIRONMENTAL SANITATION)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY**

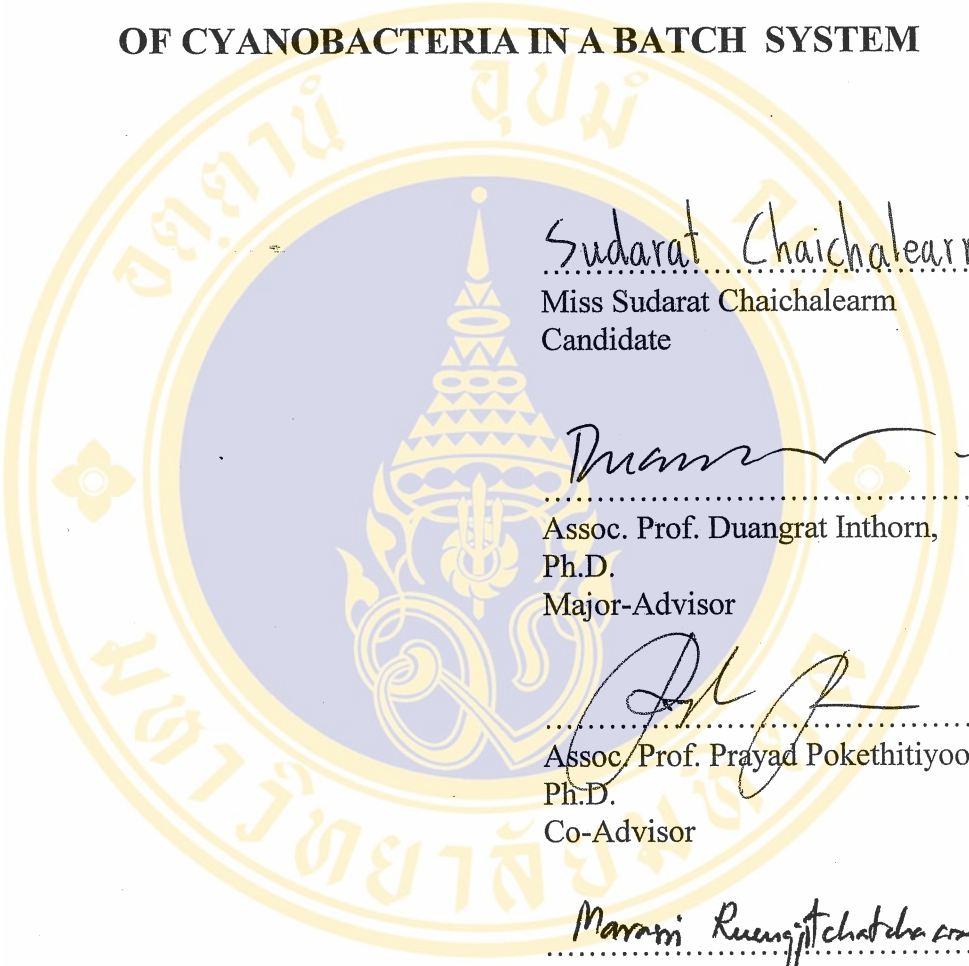
2006

ISBN 974-04-6995-7

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Thesis
entitled

**CADMIUM REMOVAL BY IMMOBILIZED AND FREE CELL
OF CYANOBACTERIA IN A BATCH SYSTEM**



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was submitted to the Faculty of Graduate Studies, Mahidol University
for the degree of Master of Science (Environmental Sanitation)

on

April 7, 2006



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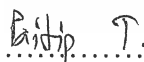
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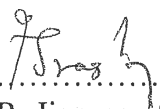
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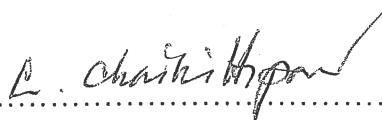
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ACKNOWLEDGEMENT

The success of this thesis can be attributed to the extensive support and assistance from my major advisor, Assoc. Prof. Duangrat Inthorn and my co-advisor, Assoc. Prof. Prayad Pokethitiyook and Asst. Prof. Marasri Ruengjitchatchawalya. I deeply thank them for their valuable advises and guidance during my study.

My appreciation is also expressed to Assoc. Prof. Pisit Vatanasomboon and Assoc. Prof. Udom Kompayak for their valuable discussion and suggestion. Special thanks are expressed also to Assoc. Prof. Paitip Thiravetyan the external examiner, for her comments, and useful suggestion, which significantly improved the quality of this research.

I would like to express grateful thanks to Prof. Akiko Mimura for providing immobilized material and valuable suggestion for my study. Sincere thanks to BIOTEC Culture Collection (BCC) of the National Science and Technology Development Agency (NSTDA), The Ministry of Science and Technology, Thailand for providing algal strains for my study.

I wish to thank staff of Central Instrument Facilities, Faculty of Sciences, Mahidol University for their instruction and assistance for the use of the flame Atomic absorption spectrophotometer. Special thanks to Department of Parasitology and staff, for the permission of the use of microscope and any assistance for the cyanobacteria photography.

I would like to grateful thanks to staff of Scientific and Technological Research Equipment Centre, Chulalongkorn University for suggestion and taking picture under Scanning electron microscope and staff of Department of Tropical Pathology, Faculty of Tropical Medicine, Mahidol University for her instruction, assistant and valuable suggestion and taking picture under Transmission electron microscope.

I would like to grateful thanks to Faculty of Graduate Studies, Mahidol University for research grant in this study and equally grateful to all the lecturers, staff, my friends at the Environmental Health Sciences Department, Faculty of Public Health for their valuable advices and their kind supports.

Finally, I am grateful to my beloved family for their financial support, entirely care and good understanding throughout my study.

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CADMIUM REMOVAL BY IMMOBILIZED AND FREE CELL OF CYANOBACTERIA IN A BATCH SYSTEM

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ABSTRACT

Cadmium removal from aqueous solution (1 mg/l) was investigated using four strains of filamentous cyanobacteria, *Scytonema* sp., *Oscillatoria agardhi*, *Mastigocladus* sp. and *Hapalosiphon hibernicus* immobilized on eight types of immobilized material such as loofa sponge (LS), delicate duty scour pad (DP), synthetic fiber (SF), polyethylene terephthalate (PET), polyethylene terephthalate (PET non-woven fabric), polyethylene (PE), polypropylene (PP) and polyurethane (PU). The results showed that immobilized cells on SF and free cell of *Scytonema* sp. and *H. hibernicus* had the highest cadmium removal at 93.31%, 94.80% and 85.14%, 98.32%, respectively. Immobilized ability and dry weight per surface area (DW/SA) were highest at 96.00%, 0.0033 g cell/cm² and 97.45%, 0.0023 g cell/cm² for *Scytonema* sp. immobilized on PET and *H. hibernicus* immobilized on SF, respectively. Initial cell concentration influencing cadmium uptake at 0.015, 0.030, 0.045 and 0.060 g dry wt. were studied. The optimum cell at 0.015 g dry wt. was selected for extended experimentation because it had the highest uptake at 4.24, 7.64 and 3.56 mg Cd/g dry wt. for immobilized cells on SF, PET and free cell of *Scytonema* sp., respectively. While immobilized cells on SF, PET and free cells of *H. hibernicus* which were the highest at 5.51, 7.03 and 5.87 mg Cd/g dry wt., respectively. The Langmuir isotherm could explain the cadmium adsorption. Immobilized cells on SF, PET and free cells of *Scytonema* sp. had the maximum adsorption capacity (q_{max}) at 26.74, 37.74 and 31.95 mg/g dry wt. and binding constant (k_b) at 6.45, 2.14 and 2.70 l/mg, respectively when $R^2 > 0.96$ while immobilized cells on SF, PET and free cells of *H. hibernicus* had q_{max} at 57.80, 64.52 and 59.17 mg/g dry wt. and k_b at 1.57, 2.07 and 1.50 l/mg, respectively when $R^2 > 0.99$. The uptake of cadmium was affected by cell ages at 5, 10, 15 and 20 days. Immobilized cells on SF, PET and free cells at age 15 days of *Scytonema* sp. had the highest uptake at 5.64, 5.71, 5.67 mg Cd/g dry wt., respectively. While immobilized cells on SF, PET and free cell of *H. hibernicus* were 9.05, 9.96 and 8.46 mg Cd/g dry wt., respectively. The ultrastructure changes of both strains exposed to 1 mg/l of cadmium were observed under transmission electron microscope. Cells were damaged and convoluted in cell surface and intracellular organelle. Electron dense particles deposited in the cell wall and internal organelle of *H. hibernicus* but indistinctly found in the cell of *Scytonema* sp. indicated that cadmium uptake was probably mechanism with adsorption in cell membrane and accumulated in the intracellular organelle. Therefore, immobilized cells on SF and PET of *Scytonema* sp. and *H. hibernicus* have the potential to remove cadmium in wide spread low cadmium contaminated natural water as in rivers or channels and can be applied in shrimp ponds.

KEY WORDS: CYANOBACTERIA / SCYTONEMA / HAPALOSIPHON
HIBERNICUS / CADMIUM REMOVAL / IMMOBILIZED CELL

191 P. ISBN 974-04-6995-7

การกำจัดแคดเมียมโดยใช้ไซยาโนแบคทีเรียที่ผ่านการตรึงเซลล์และเซลล์อิสระในระบบการเลี้ยงแบบกะ (CADMIUM REMOVAL BY IMMOBILIZED AND FREE CELL OF CYANOBACTERIA IN A BATCH SYSTEM)

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บทคัดย่อ

ศึกษาการกำจัดแคดเมียม 1 มิลลิกรัมต่อลิตร ในไซยาโนแบคทีเรีย 4 สายพันธุ์คือ *Scytonema* sp., *Oscillatoria agardhi*, *Mastigocladus* sp. และ *Hapalosiphon hibernicus* โดยการตรึงเซลล์ด้วยวัสดุตรึง 8 ชนิด คือ โยวบ, สก็อตไบรท์, ตัวกรองอาหารในตู้ปลา, โพลีเอธิลีนเทอเรฟทาเรต, โพลีเอธิลีนเทอเรฟทาเรต (ชนิดไม่มีการจัดเรียงเส้นใย), โพลีเอธิลีน, โพลีโพรพิลีนและโพลียูรีเทน พบว่าเซลล์ตรึงบนตัวกรองอาหารในตู้ปลาและเซลล์อิสระของ *Scytonema* sp. และ *H. hibernicus* มีค่าการกำจัดแคดเมียมได้สูงสุดที่ 93.31%, 94.80% และ 85.14%, 98.32% ตามลำดับ โดย *Scytonema* sp. ตรึงบนโพลีเอธิลีนเทอเรฟทาเรตมีความสามารถในการตรึงเซลล์และสัดส่วนของน้ำหนักแห้งต่อพื้นที่ผิววัสดุตรึงสูงสุดเท่ากับ 96.00% และ 0.0033 กรัมเซลล์ต่อตารางเซนติเมตร และ *H. hibernicus* ที่ตรึงบนตัวกรองอาหารในตู้ปลามีความสามารถในการตรึงเซลล์และสัดส่วนของน้ำหนักแห้งต่อพื้นที่ผิววัสดุตรึงสูงสุดเท่ากับ 97.45% และ 0.0023 กรัมเซลล์ต่อตารางเซนติเมตร ตามลำดับ จากการศึกษาความเข้มข้นของเซลล์เริ่มต้นที่ 0.015, 0.030, 0.045 และ 0.060 กรัม/น้ำหนักรวม พบว่าความเข้มข้นของเซลล์เริ่มต้นที่ 0.015 กรัม/น้ำหนักรวมมีความเหมาะสมในการนำมาใช้ในการทดลองเนื่องจาก *Scytonema* sp. ตรึงบนตัวกรองอาหารในตู้ปลา, โพลีเอธิลีนเทอเรฟทาเรต และเซลล์อิสระมีความสามารถในการดูดซับแคดเมียมสูงสุดเท่ากับ 4.24, 7.64 และ 3.56 มิลลิกรัมแคดเมียมต่อกรัมน้ำหนักรวม ตามลำดับ ในขณะที่ *H. hibernicus* ตรึงบนตัวกรองอาหารในตู้ปลา, โพลีเอธิลีนเทอเรฟทาเรต และเซลล์อิสระมีความสามารถในการดูดซับแคดเมียมสูงสุดเท่ากับ 5.51, 7.03 และ 5.87 มิลลิกรัมแคดเมียมต่อกรัมน้ำหนักรวม ตามลำดับ

การใช้แสงเมอริโอโซโทมสามารถอธิบายถึงการดูดซับสารละลายแคดเมียม โดยค่าการดูดซับสูงสุด (q_{max}) ของ *Scytonema* sp. ตรึงบนตัวกรองอาหารในตู้ปลา โพลีเอธิลีนเทอเรฟทาเรต และเซลล์อิสระเท่ากับ 26.74, 37.74 และ 31.95 มิลลิกรัมแคดเมียมต่อกรัมน้ำหนักรวม และมีค่าสัมประสิทธิ์ของการดูดซับ (k_p) เท่ากับ 6.45, 2.14 และ 2.70 ลิตรต่อมิลลิกรัม ตามลำดับเมื่อ $R^2 > 0.96$ ในขณะที่ *H. hibernicus* ตรึงบนตัวกรองอาหารในตู้ปลา โพลีเอธิลีนเทอเรฟทาเรต และเซลล์อิสระมีค่า q_{max} เท่ากับ 57.80, 64.52 และ 59.17 มิลลิกรัมแคดเมียมต่อกรัมน้ำหนักรวม และมีค่า k_p เท่ากับ 1.57, 2.07 และ 1.50 ลิตรต่อมิลลิกรัม ตามลำดับ เมื่อ $R^2 > 0.99$ จากการศึกษาอายุของเซลล์ที่ 5, 10, 15 และ 20 วันมีผลต่อการดูดซับแคดเมียม พบว่า *Scytonema* sp. ตรึงบนตัวกรองอาหารในตู้ปลา โพลีเอธิลีนเทอเรฟทาเรต และเซลล์อิสระที่อายุเซลล์ 15 วันมีความสามารถในการดูดซับแคดเมียมสูงสุดเท่ากับ 5.64, 5.71 และ 5.67 มิลลิกรัมแคดเมียมต่อกรัมน้ำหนักรวม ตามลำดับ ในขณะที่ *H. hibernicus* ตรึงบนตัวกรองอาหารในตู้ปลา โพลีเอธิลีนเทอเรฟทาเรต และเซลล์อิสระมีความสามารถในการดูดซับแคดเมียมสูงสุดเท่ากับ 9.05, 9.96 และ 8.46 มิลลิกรัมแคดเมียมต่อกรัมน้ำหนักรวม ตามลำดับ จากการศึกษาภายใต้สภาวะการตรึงเซลล์ที่ต่างกันถึงผลของแคดเมียมต่อเซลล์พบว่าเซลล์มีความผิดปกติเกิดขึ้นที่บริเวณผนังเซลล์และภายในเซลล์ของ *H. hibernicus* นอกจากนี้พบการที่บวมของอเล็กตรอนที่บริเวณผนังเซลล์และภายในเซลล์ของ *H. hibernicus* ส่วนเซลล์ของ *Scytonema* sp. ไม่สามารถเห็นได้ชัดเจน ดังนั้นในการศึกษาครั้งนี้จึงมีความเป็นไปได้ว่ากลไกการดูดซับแคดเมียมจะเกิดขึ้นที่บริเวณผิวเซลล์และสามารถเข้าไปสู่ภายในเซลล์ของไซยาโนแบคทีเรีย การตรึงเซลล์บนตัวกรองอาหารในตู้ปลาและโพลีเอธิลีนเทอเรฟทาเรต ของ *Scytonema* sp. และ *H. hibernicus* จึงเป็นทางเลือกที่น่าสนใจในการนำไซยาโนแบคทีเรียมาประยุกต์ใช้จริงในแหล่งน้ำที่มีการปนเปื้อนของแคดเมียมปริมาณต่ำ เช่น ในแหล่งน้ำตามธรรมชาติและในบ่อเลี้ยง เนื่องจากสามารถแยกไซยาโนแบคทีเรียออกจากระบบได้ง่ายหลังจากการบำบัดน้ำเสีย

191หน้า. ISBN 974-04-6995-7

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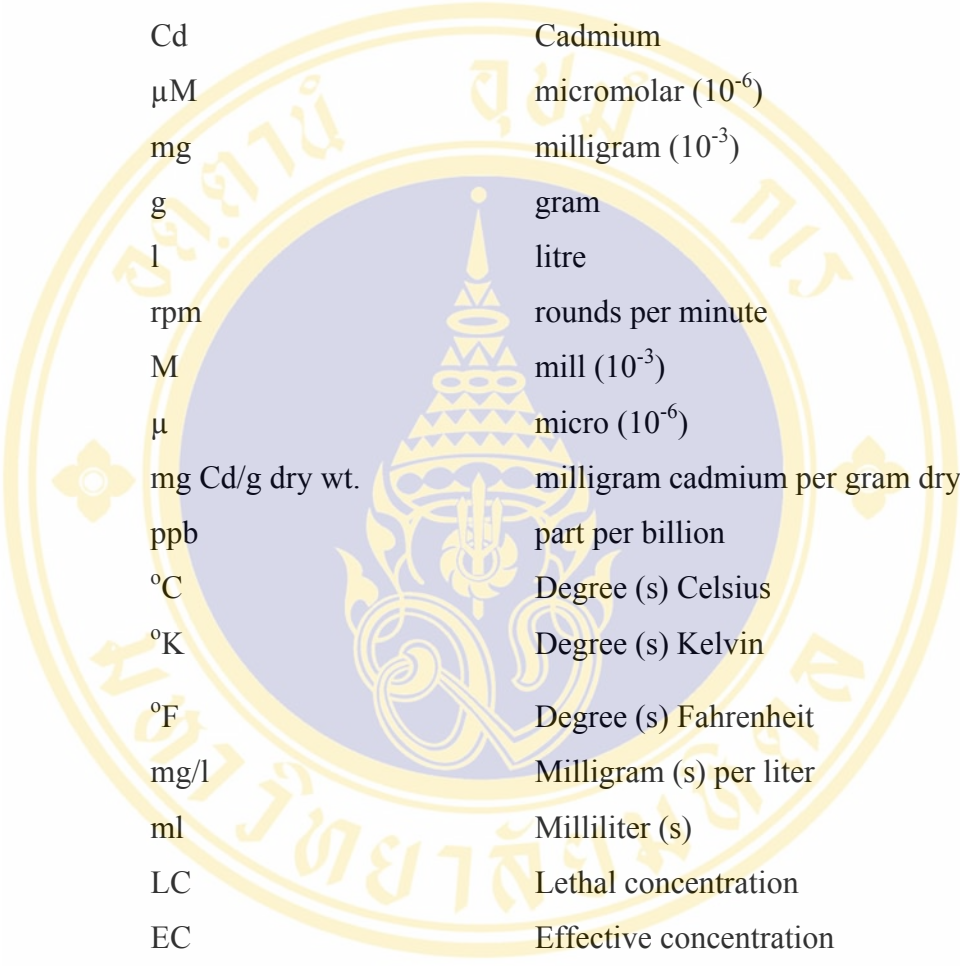
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LIST OF ABBREVIATIONS



Cd	Cadmium
μM	micromolar (10^{-6})
mg	milligram (10^{-3})
g	gram
l	litre
rpm	rounds per minute
M	mill (10^{-3})
μ	micro (10^{-6})
mg Cd/g dry wt.	milligram cadmium per gram dry weight
ppb	part per billion
$^{\circ}\text{C}$	Degree (s) Celsius
$^{\circ}\text{K}$	Degree (s) Kelvin
$^{\circ}\text{F}$	Degree (s) Fahrenheit
mg/l	Milligram (s) per liter
ml	Milliliter (s)
LC	Lethal concentration
EC	Effective concentration
IC	Growth Inhibit concentration

CHAPTER I

INTRODUCTION

1.1 Statement of problem

Environmental contamination by heavy metal is a serious problem due to its incremental accumulation in the food chain and continued persistence in the ecosystem (1, 2). Cadmium as one of the big three group of heavy metals which posing great hazard to human health. The cases of severe metal poisoning occurred in Japan that people suffered painful Itai-Itai in contaminated soil irrigated by river polluted with cadmium (3, 4, 5). Cadmium accumulated readily in living system that have been in placated as the cause of renal disturbances, lung insufficiency, bone lesions, cancer and hypertension (6). General sources of expose to cadmium including air, water and food from various industries such as electronic, plating, battery, pigment, production of television tube, nuclear reactor shields and rods, stabilizers ammunition manufacture, zinc mining and refining (3, 7). United States Environmental Protection Agency has set the maximum contaminant level for Cd (II) cations in domestic water supplies to be 5 µg/l (1). In Thailand, the Department of Pollution Control, Ministry of Natural Resources and Environment has set a standard for allowable effluent of cadmium concentration at 0.03 mg/l (8).

Physico-chemical processes usually achieve the removal of heavy metals from a metal-bearing wastewater. Before discharging into natural water, the effluent was sequently pretreated by ion exchange, activated carbon adsorption, precipitation and evaporative recovery. However, these process have significant disadvantage, insufficient metal removal, high reagent or energy requirements and generally very expensive, especially application for removal heavy metal at low concentrations typically less than 100 mg/l (9, 10, 11, 12).

Bioremoval treatment process are the current acceptable technologies that can reduce heavy metal concentrations to environmentally acceptable levels and use of inexpensive biosorbent materials (13, 14, 15). Bacteria, fungi, sphagnum peat moss,

yeast, algae and plant root have been successfully used as adsorbing agent for heavy metal (16, 17). Interaction between metals and microbial cells can occur through adsorption to cell surfaces, through metabolically assisted accumulation within the cell or as metal complexes with extracellular microbial metabolite (18, 19, 20). Microalgae have received more attention than other microorganism because of their effective in removing heavy metal at extremely low level (parts per trillion) due to their large surface area and have very high affinity to metal ion (6, 21, 22).

Cyanobacteria are attractive photosynthetic prokaryotes which have remarkable affinity for heavy metal. Some species are easily to separate from a solution by filtration or self-immobilized as a biofilm on porous support. In addition, cyanobacteria can be cultivated under outdoor or in large-scale laboratory cultures at low cost. They are capable of growing under low nutrient and nitrogen fixation as well as adapt to the temperature and chemical changes in the medium (22, 23, 24).

Immobilized cell system is an useful technique that could provide additional advantages over freely suspended cells. Because of they can avoid the biomass separation from liquid requirement and prevent the independent movement of cell during the aqueous phase of the system. The advantage of immobilized algae on suitable support increased the retention time of biomass in contaminated water (25, 26, 27). A variety of matrices either natural biopolymer or synthetic polymer such as alginate, carrageenan and polyacrylamide have been used for algal cells, but restricted diffusion to their closed embedding structure with low mechanical strength and high cost (28, 29).

This research has focused on the cadmium removal ability by immobilized cell on selected immobilized material which screened from various natural and synthetic polymer such as loofa sponge, delicate duty scour pad, synthetic fiber, polyethylene terephthalate (PET), polyethylene terephthalate (PET non-woven fabric), polyethylene, polypropylene and polyurethane were compared with the free cell of selected cyanobacteria strains. Furthermore, the effects of initial cell concentration, contact time, initial cadmium concentration and cell age in a batch system were investigated.

1.2 Objectives of study

1.2.1 General objective

To screen for strains of high cadmium removal ability and high immobilized ability on suitable immobilized material and comparative to free cell at optimal condition

1.2.2 Specific objectives

- (1) To screen for cyanobacterial strains of high cadmium removal and high immobilized ability on immobilized materials in different immobilized materials and comparative to free cell
- (2) To study the effect of initial cell concentration and contact time on cadmium uptake between immobilized and free cell of selected cyanobacteria
- (3) To study the effect of initial cadmium concentration on cadmium uptake between immobilized and free cell of selected cyanobacteria
- (4) To study the effect of cell ages on cadmium uptake between immobilized and free cell of selected cyanobacteria

1.3 Research definition

1.3.1 Cyanobacteria are the fresh blue green algal strains obtained from BIOTEC Culture Collection (BCC) at the National Science and Technology Development Agency (NSTDA), the Ministry of Science, Technology and Environment, Thailand.

1.3.2 Cadmium removal is the percentage of cadmium removal by immobilized and free cell of cyanobacteria defined as;

$$\text{Cadmium removal ability (\%)} = \frac{(C_i - C_f) \times 100}{C_i}$$

Where C_i is the initial cadmium concentration (mg/l)

C_f is the equilibrium cadmium concentration (mg/l)

1.3.3 Immobilized ability is the percentage of cyanobacteria that are immobilized on immobilized material define as ;

$$\text{Immobilized ability (\%)} = \frac{(W_f - W_i) \times 100}{W_{ni} + (W_f - W_i)}$$

Where W_i is the initial dry weight of immobilized material (g)

W_f is the final dry weight of immobilized material (g)

W_{ni} is the final dry weight of non – immobilized cell in solution (g)

1.3.4 Cadmium uptake (q) is the cadmium uptake in cyanobacteria cell and calculated for the construction of biosorption isotherm as follows;

$$q = \frac{V(C_i - C_f)}{M}$$

Where q is cadmium adsorption (mg of cadmium/g of biosorbent)

V is volume of cadmium solution (l)

C_i is initial concentration of cadmium in the solution (mg/l)

C_f is final (equilibrium) concentration of cadmium in the solution (mg/l)

M is dry weight of biomass (g dry wt.)

1.3.5 Maximum adsorption capacity (q_{max}) and binding constant (k_b) were calculated as follow the Langmuir adsorption equation as follow;

$$q = \frac{q_{max} C_f}{k_b + C_f}$$

Where q is the cadmium adsorbed to the solid phase (mg/g dry wt.)

q_{max} is the maximum adsorption capacity (mg/g dry wt.)

k_b is the binding constant (mg/l)

C_f is the final (equilibrium) concentration of heavy metal cadmium in solution (mg/l)

1.3.7 Immobilized cell is cyanobacterial cell that immobilized on selected immobilized material.

1.3.8 Non-immobilized cell is cyanobacterial cell that could not immobilized on immobilized material and left as free cell in the solution.

1.3.9 Free cell is the suspend cell in the aqueous solution in the system without immobilized material.

1.3.10 Type of immobilized material is synthetic polymer and natural polymer such as loofa sponge, delicate duty scour pad, synthetic fiber, polyethylene terephthalate (PET), polyethylene terephthalate (PET non-woven fabric), polyethylene, polypropylene and polyurethane.

1.3.11 Selected cyanobacteria strains were strains of cyanobacteria attachment on immobilized material that have high cadmium removal ability and high immobilized ability.

1.3.12 Selected immobilized materials were immobilized material for immobilized cell which screened in different immobilized materials that have high cadmium removal ability and high immobilized ability.

1.4 Scope of study

In this study, filamentous cyanobacterial strains were screened in different immobilized materials for the selected cyanobacteria immobilized on selected immobilized materials in comparative to free cells were high cadmium removal ability and immobilized ability. Cadmium uptake were investigated in various parameters as, initial cell concentration, contact time, initial cadmium concentration and cell age in batch experiment.

1.5 Limitation of study

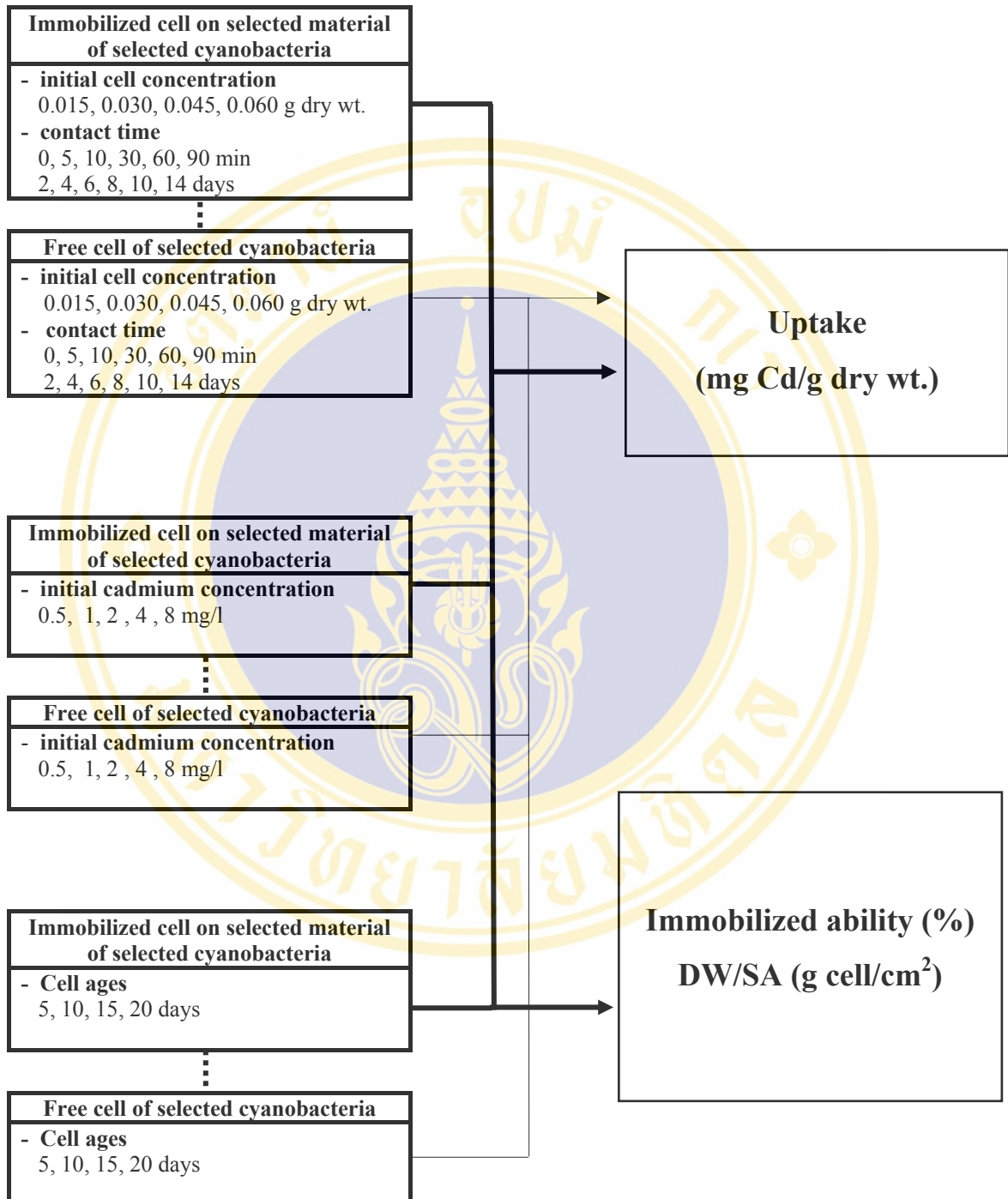
The experiment had performed in synthetic wastewater as cadmium aqueous solution with Medium 18 (see appendix A) and all experiment conditions had performed in laboratory scale.

1.6 Expected outcome

1.6.1. The high cadmium removal efficiency and high immobilized ability of cyanobacteria strains with suitable to immobilized materials which can be applied to use in real treatment condition

1.6.2. An attractive alternative and beneficial way for treatment cadmium in wide spread low cadmium contamination water in natural water as in river or channel.

1.7 Conceptual framework



CHAPTER II

LITERATURE REVIEWS

2.1 Cadmium

Cadmium is the transition element in II b group of the Periodic Table which can mainly be found in the earth's crust at an average concentration of 0.2 mg/kg. It always occurs in combination with zinc and consists in the industries as an inevitable by-product of zinc, lead and copper extraction (30, 31, 32). Cadmium is one of the non-essential trace elements but it is also highly toxic to most organism that having a toxicity of 2-20 times higher than many other heavy metals (33). Cadmium content in soil and water has been dramatically increased from anthropogenic sources including smelters and agriculture applications of fertilizer and sewage sludge. Since cadmium in soil and water is available for plant uptake and mobilization, its high toxic effects to humans and the environment subsequently human uptake, cadmium to be involved in environmental pose a significant health risk (34, 35).

2.1.1 Physical and chemical properties

Cadmium is a silver-white metal with a faint bluish tinge, soft malleable, ductile, flexible and resistant to corrosion (30). The basic information of cadmium physical properties are shown in Table 1.

Table 1 Basic information of cadmium physical properties

Name:	Cadmium
Symbol:	Cd
Atomic Number:	48
Atomic weight:	112.411 (8) g
Melting Point:	320.9 °C (594.05 °K, 609.62 °F)
Boiling Point:	765.0 °C (1038.15 °K, 1409.0 °F)
Number of Protons/Electrons:	48
Number of Neutrons:	64
Classification:	Transition Metal
Crystal Structure:	Hexagonal
Density at 293 °K	8.65 g/cm ³
Color	Silvery Grey Metallic
Solubility:	Insoluble 5 mg/l in water

Source: Department of Pollution Control (35)

Cadmium is insoluble in water but soluble in acid, particularly nitric acid. It forms water-soluble and inorganic compounds such as cadmium sulfate, cadmium chloride and cadmium nitrate. Cadmium is chemically classified as a soft acid and forms complex with sulfides. Additionally, it also forms complexes with other substances, including cyanides, amines and alkyl-group (6)

2.1.2 Industrial uses

Cadmium is a rare element usually found as an impurity in ores of other metals. It is obtained as a by-product in the extraction and refining of these metals (36). Cadmium has been produced since the 19th century and the world production of refined cadmium increased from 6,000 tons/year in 1950 to approximately 19,000 tons/year in 1991 (37). The leading cadmium producers are Japan, USA, Canada, Belgium, Germany, Australia and in many countries. In Thailand, cadmium has been produced since 1990, both transformed and non-transformed cadmium is imported from abroad for using in Thailand. Application of cadmium in industries were developed based on unique chemical and physical properties. The primary uses are in

electroplating other metals or alloys for protection against corrosion and in the manufacture of cadmium-nickel battery. Cadmium compounds are well known pigments in paints, plastics, glasses, ceramics and enamels. Furthermore, cadmium is utilized in nuclear reaction, some solders, graphics and some biocides (6, 30, 38).

2.1.3 Sources of cadmium

(1) Natural sources

The natural occurrence of cadmium in the environment results mainly from the weathering of crusted materials. Volcanic aerosols and the vast amount of matter discharged from volcanic eruption also transport cadmium into the ecosystem. Other natural sources include forest fires release from terrestrial vegetation (39). However, cadmium is not usually found in the natural sources resulted in high cadmium concentration in the environment.

(2) Anthropogenic sources

The presence of elevated concentrations of cadmium associated with human activities. Non-ferrous metal mines can be a significant source of local cadmium contamination, particularly those mines, which exploit zinc and lead ores. Agriculture indicated as an important non-point source of cadmium by impurities in fertilizer such as phosphate fertilizer, composts and manure, sewage sludge and corrosion of metal objects, such as galvanized metal roofs and wire fences (40).

The main sources of cadmium from waste disposal are leachates from landfills which polluted soil, groundwater and fumes from incinerators. Sewage sludge also contains many metals as well as cadmium. Food crops planting in soils added with sludge can uptake cadmium easily. Other significant sources of cadmium are manufactures and disposals, such as batteries, alloys, pigments and paints, printing and graphics and polymer stabilizers. In addition, fossil fuel combustion also disperses cadmium into the environment (30, 40).

2.1.4 Fate and distribution of cadmium in environmental

(1) Cadmium in atmosphere

Cadmium is forms of dust, fumes and aerosols emitted into the atmosphere from many sources, including non-ferrous metals production, fossil fuel combustion, iron and steel facilities, refuse incineration, phosphate fertilizers, cement production, and mobile sources. Median global emission of cadmium to the atmosphere was 7,570

tons/year in 1983 (41). The aerosol particles of more than 10 μm in diameter tend to settle out rather quickly under gravity, but the smaller ones remain in the atmosphere for 10-30 days. The particles are removed by wash out, settlement, impaction, and in the case of small particles ($< 0.3 \mu\text{m}$) will diffuse and deposit easily. From these phenomena, cadmium can be transferred to the aquatic environment and soils (40).

(2) Cadmium in the aquatic environment

The average cadmium content in the world's ocean has variously been reported as low as $< 5 \text{ ng/l}$ (6). Higher levels have been noted around certain coastal areas and variations of cadmium concentration with the ocean depth. The effluent from non-ferrous metals production are the main source of cadmium release into the aquatic ecosystem. The indirect inputs are atmosphere fall out, leachates from landfill, run-off from waste dump sites and wash-off from agricultural areas. In water, cadmium ions react with either constituents of water or sediments. Moreover, cadmium solution not only can be absorbed into aquatic plants and animals but also cause toxic effects on them when its concentration is sufficiently high (42).

(3) Cadmium in soil and sediment

Cadmium in soils is derived from a wide variety of industrial wastes than natural sources, nevertheless, the contributions of cadmium to soils emitted and deposited onto the soils assumed that 70% has generally decreased significantly from the atmosphere (41). The waste associated with agricultural and food production can also affect cadmium budget of many soils. Cadmium in the soils is persistent due to the relatively strong adsorption onto the humic and clay colloids, and it uptaken and accumulated by plants, particularly edible plants, eventually it can accumulate throughout in the food chain (30, 40, 41, 42, 43).

Cadmium contaminated in sediments is mostly transferred from the water-bearing cadmium, and then is uptake by benthic invertebrate (41). Lee *et al.* found that the concentration of metals, including cadmium in benthic invertebrate tissue was correlated with the metal concentrations extracted from sediments (30).

2.1.5 Toxicity of cadmium

(1) Toxic effects to plants and microorganism

Cadmium is highly toxic to both higher plants and several microorganisms. Boonyapookana reported that the EC_{50} of cadmium (II) for *Wolffia globosa* is only 1.53 mg/l compared with 18.21 mg/l for chromium at 9 days of exposure (44). Rangsayatorn reported that the cadmium concentration both to IC_{50} at 14.68 mg/l and 18.35 mg/l for *Spirulina (Arthospina) platensis* TISTR 8217. It changed the ultrastructural and the alterations induced by cadmium were disintegration and disorganization of thylakoid membranes (45). Kapland reported that the growth of *Chlorella* sp. was severely inhibited at 10 μ M Cd^{2+} with LD_{50} of 3 μ M (46). Similarly, Devi reported that EC_{50} for *Lemna gibba* was only 0.8 mg/l (47). Acute and chronic toxicity to plants is ameliorated by change of pH in water or soil, the plant genotype and the concomitant effect on availability of the free cadmium ion. The excess of cadmium concentration in plants also causes biochemical effects, such as changes in the permeability of cell membranes chlorosis and inhibition enzymes (30).

(2) Toxic effects to invertebrates

The acute toxicity of cadmium to aquatic invertebrates varies enormous among the different species. The LC_{50} for *Daphnia magna*, one of the most sensitive species are in the range of 34 to 60 μ g/l (48), whereas the LC_{50} for the clam *Mya arenaria* is 150 μ g/l. Adverse effects to aquatic animals are documented at relatively low cadmium concentrations. Exposure to cadmium for 26 days reduced the growth and survival of the snail at the range of 4.79 to 7.63 μ g/l. In general, cadmium is less toxic in hard water, the elevated temperatures and any other factors, that may increase the metabolic rate of aquatic animals, usually increase both the uptake and toxicity of cadmium (30, 34, 48).

(3) Toxic effects to humans

While the workers in industries using cadmium, it is estimated that 1.5 million U.S. workers are potentially exposed to cadmium at the work place (48). Cadmium dusts are generated by mechanical processes whereas cadmium fumes can be generated by heating during metal smelting and processing or during electroplating. Concentration of less than 10 ppb in water and in aquatic organisms are considered safe for human ingestion (47). Dietary intake represents the largest source of cadmium

exposure in general population, in meat especially liver and kidney, and seafood tend to be higher in cadmium accumulation than other foodstuffs. Tobacco is an additional source in smokers since it contains cadmium, in general corresponding to 1-2 µg per cigarette. The body burden of cadmium is about 1.5 to 2 times greater in smokers than in nonsmokers of the same age. Cadmium acts as accumulative poison in soft tissue. For instance, cadmium can lead to liver and kidney problems and can cause disorders in the respiratory, renal, skeletal, and vascular systems. Acute cadmium poisoning may occur among occupationally exposed workers via the respiratory route or among general population via accidental oral intake in food or the ingestion of cadmium for the purpose of suicide. The symptoms of acute cadmium from food poisoning are nausea, vomiting, salivation, diarrhea, and muscular cramp. The different symptoms of acute toxicity which depend on levels of exposure, such as irritation in the upper respiratory tract, chest pain, headache, dizziness, coughing, dyspnoea, nausea, vomiting, chills, exhaustion and diarrhea. In addition, pneumonitis characterized by edema and pulmonary epithelium necrosis from exposure to cadmium dust or fumes. The chronic toxicity of cadmium are the long-term exposure to dose of cadmium causes harmful effects to human health. The symptoms are characterized by impairment of the respiratory system, renal tubular dysfunction and bone damage, besides that, cadmium also causes carcinogenic effects (30, 39, 49).

2.2 Heavy metal treatment process (50, 51)

There are many treatment processes of heavy metals from wastewater such as physical-chemical treatment process and biological treatment process. In fact, all methods are only methods for concentrating the metal into a smaller volume of liquid or solid.

2.2.1 The physical-chemical treatment process

The physical-chemical treatment process is being given more consideration to design and operating parameters that specifically improve heavy metal removal in the part of wastewater treatment. These conventional treatment process usually achieve the removal of heavy metal from wastewater before discharging the effluent into a natural water body. There are many treatment methods of the physical-chemical

process were effectively remove heavy metal such as precipitation, reverse osmosis, ion exchange and activated carbon adsorption.

(1) Precipitation

The usual method for removal of heavy metal is chemical precipitation. from wastewater, including Cr, Cu, Zn, Pb, Cd, Mg and Hg. Precipitation of heavy metal is depended on pH, the optimum pH is unique for each metal. Furthermore, carbonates and sulfides are used to precipitate heavy metal. Sodium sulfide (Na_2S) and sodium bisulfide (NaSH), popular sulfide chemicals, are widely added to precipitate heavy metals. The chemical method is used to raise the pH and cause precipitation such as hydrated lime ($\text{Ca}(\text{OH})_2$), quicklime (CaO), soda ash (Na_2CO_3), caustic soda (50% NaOH) and sodium sulphide.

(2) Reverse osmosis

Osmosis is a process which a solvents flows through a semipermeable membrane from a dilute to a more concentrated solution. In general, the solvent flows in the direction that will reduce the concentration of solution. The osmotic pressure of the solution is applied to the solution will just prevent the passage of the solvent through the semipermeable membrane. In reverse osmosis, a differential pressure that exceeds the osmotic pressure is applied to the membrane, causing the solvent to the flow from the stronger to the weaker solution.

(3) Ion exchange

Ion exchange is a reversible exchange of ions between liquid and solid phase. Ions held by electrostatic forces to charge functional groups on the surface of an insoluble solid are replaced by similar ions charge in a solution. Ion exchange materials should have ion active site in whole structure, high capacity, selectivity for ionic species, capability of regeneration, chemical and physical stability and low solubility.

(4) Adsorption

Activated carbon adsorption is a popular method in both water and wastewater treatment to remove soluble heavy metal ions, for example, the removal of copper and chromium from electroplating wastewaters. Activated carbon, amorphous form of carbon, is made from different raw materials, for example, coal, wood, coconut shells, pulp mill residues, petroleum base residues and char from sewage

sludge pyrolysis. Activated carbon is not a pure carbon and has high affinity to adsorb heavy metals and other compounds.

(5) Evaporation

Evaporation of liquid is applied in heavy metal management. This method is dependent on a vapor pressure. At high vapour pressure, evaporation will be readily, but at low vapor pressure, evaporation is more slowly. The boiling temperature of pure liquid has been reached when the vapor pressure of liquid equals the atmospheric pressure. Soluble salts and other compounds elevate the boiling point. Evaporation are used in single and multi-effect evaporation, to conserve energy and enhance separation of volatile wastes from liquids, a multi-effect evaporator may be used.

(6) Ozone treatment

Metal removal by ozone treatment effective from both the oxidation of organometallic complexes, which maintain a significant portion of the metal in solution and the oxidation of the metal to a higher.

(7) Cementation

Cementation can also effect to metal removal by dissolved metal contacts a more active metal such as iron or zinc and becomes cemented to it.

(8) Anaerobic filter

The effectiveness in bench-scale tests of an anaerobic filter by remove metals from a leachate with a high organic content from landfill site. Aerobic bacteria are attached to plastic filter medium in a column. Precipitation the metals solution which passing slowly up thoroughly the column under the anaerobic conditions as sulfide, carbonates and hydroxides.

(9) Starch-based products

Starch can very effectively scavenge uncomplexed metals from a wastewater when converted to an insoluble starch xanthate (ISX). Strach is both abundant from agricultural crops and relatively inexpensive.

(10) Ferrite process

The ferrite process has been reported that to be widely used in Japan to remove metals from laboratory wastewaters in universities and institutes. The process relies upon the formation of magnetically susceptible Fe_3O_4 particles (0.2-0.3 μm in

size). These result from the oxidation of $\text{Fe}(\text{OH})_2$, derived from ferrous sulfate added to the wastewater.

Other options available but of limits significance including liquid-liquid extraction, electrolysis, evaporation or electro dialysis. Conventional treatment technologies, such as precipitation and coagulation become less effective and more expensive when the effluent have high volumes and low metal concentrations (typically less than 100 mg/l).

2.2.2 Biological treatment process

Environmental pollution caused by toxic heavy metal in industrial effluents is one of the most pressing problem in many densely populated cities worldwide (15). Biological treatment process which the method for removal of heavy metal that has received attention increasingly in recent years because of its potential in environment protection. Bioremoval is the accumulation and concentration of pollutants from aqueous solutions by the uses of biological materials base on plant biomass or animal polymers. Bioremoval studies typically have use microorganism in termed “Biosorption” (2, 50) which a new technology for uses of biologically inactive microorganism for the passive absorptive binding of metal in aqueous solution (15). Biosorption may involve one or more of several process depending on physicochemical conditions and the origin and physiological state of the biomass and mainly involves cell surface complexation, ion exchange and microprecipitation (20, 52). This technology is based on metal ions binding to various functional groups of biomass cell wall through the passive mechanism. The functional groups on microbial cell wall biopolymers, such as $-\text{COOH}$, $-\text{NH}_2$, $-\text{OH}$, and $-\text{SH}$, provide the binding sites for interaction of metal ions. The advantage of biosorption are described below.

- (1) Metals at low concentrations can be selectively removed.
- (2) Biosorbents can be cheaply produced and some of them have higher uptake capacities than those ion exchange resin.
- (3) This method is able to operate over the wide ranges of pH, temperature and presence of other ions including Ca^{2+} and Mg^{2+} .
- (4) The capital investment and operation costs are low.
- (5) Cost and environmental liability of toxic sludge disposal are minimized by recovery of metals and biosorbent for reuse in industry.

- (6) Ability to treat large volumes of wastewater due to rapid kinetics.
- (7) Ability to handle multiple heavy metal and mixed waste.
- (8) High affinity and reducing residual metals to below 1 ppb in many cases ;
- (9) Greatly improved recovery of bound heavy metals from the biomass and greatly reduced volume of hazardous waste produced (2, 5, 15, 30, 50, 52).

Therefore, this method has been widely studied and also recognized as a promisingly alternative for removal of heavy metals in metal-bearing wastewater.

(1) Mechanisms of heavy metals biosorption

The uptake of metal ions by microorganisms has been described to occur in two stages. The first stage is a passive uptake involving metal cations binding to the cell surface termed biosorption. In the second stage, the metal ions adsorbed onto the cytoplasm called bioaccumulation. This stage is slow process that depends on the metabolic activity, thereby it only takes place in living cells (30, 52).

Biosorption, which is defined as the passive metal sequestering capability of cell, is caused by a number of different physicochemical mechanism. First is often rapid metabolism-independent uptake, reversible and usually complete in 5-10 min. Biosorption is generally not influenced by light, temperature, or the presence of metabolic inhibitor (49). Typically, biosorption may involved two phase depend on the location where the metal removed from the solution is found. First, the metal ions diffuse and bind to sites on the microbial cell surface and interskeletal space with some chemical affinity. These steps may consist of passive accumulation process, adsorption, ion exchange, coordination, complexation, chelation and microprecipitation. Dealing with dispersed cell subsequently metal ion is bound often irreversibly, the exact relationship can be affected by other cations, especially proton which the metals adsorb to algal surfaces by an ion exchange based on electrostatic attraction to negative charged sites. It is associated with the presence of Van der Waals's force (13, 30). For the phenomenon microorganisms have been demonstrated as potential biosorbent of metals. Özer reported that metal ions could be electrostatically bonded to carboxyl and sulphate anions on the algal cell wall (30, 55). Physical adsorption has been found to be responsible for cadmium biosorption by marine algae as well as for the lead and zinc biosorption by *Streptovercillium cinnamoneum* waste biomass (30, 56). The cell walls of microorganisms contain

different polysaccharides as basic building blocks. The ion exchange properties of natural polysaccharides have been well established that bivalent metal ions exchange with counter ions of the polysaccharides. For example, alginates of marine algae usually take place as natural salts of K^+ , Na^+ , Ca^{2+} and/or Mg^{2+} . The metallic ions such as Co^{2+} , Cu^{2+} , Cd^{2+} and Zn^{2+} can substitute the counter ions as presented in the following reaction (13, 30).



This mechanism also results in the biosorptive uptake of metals. Prakasham *et al.* indicated that chromium biosorption by *Rhizopus arrhizus* biomass is attributed to the surface adsorption because of the action of ion exchange with the participation of the carboxyl groups of uronic acids (57). The other mechanisms of cell surface sorption are coordination and complexation. The metal removal from solution may also take place through complex formation on the cell surface after interaction between the metal and active groups (13). Second, extracellular process including excreting exopolymers and/or compounds, which favor the precipitation process to entrap metal ions and one of microbial responses to the toxic metals present in the surroundings (30). The extracellular compounds can be generated by only living cells since extracellular processes are metabolism – dependent. On the contrary, the action of entrapping metal ions of these compound do not relate to the cell metabolism (13).

In activated sludge, the bacterium *Zoogloea ramigera* is important in flocculation owing to its extensive exopolysaccharide production, these negatively charged polysaccharides form matrix around the cells for the attachment of metallic cations (55). Scott and Palmer concluded that naturally produced exopolysaccharides provide enhanced cadmium biosorption by *Arthrobacter viscosus*. Other bacterial polymers that have received attention in relation to metal binding are polysaccharides from capsulate *Klebsiella aerogenes* and *Pseudomonas* sp. (30). Holan *et al.* also proposed that an additional mechanism, such as entrapment of metals in the form of insoluble microdeposits can greatly contribute to cadmium biosorption by the biomass of marine algae. By the way, the precipitation of metals can actually occur both in solution and on the cell surface for this reason, there may be deposition of the metal in a chemically altered form from the solution (34). Most metal accumulated this way is easily

removed by washing algae with distilled water alone or with a chelator (EDTA) (48, 50).

Bioaccumulation is a slower metabolism-dependent uptake lasting hours or days, usually referred to as active uptake, is a slower intracellular bioaccumulation, is often irreversible and is related to the metabolic activity, such as inhibited by low temperatures and absence of energy sources, light, metabolic inhibitors and uncouples involving the transport of ions across the cell membrane toward the cytoplasm occurring only in living cells (19). The important route for intracellular uptake by passive diffusion owing to increased permeability of cell membrane from stress.

The site of accumulation may be adsorption or biosorption at the surface, wall, membrane or external mucilage or internal called absorption to organelles, cytoplasmic ligands and cytoplasmic structure (47). For example, *Chlorella vulgaris* appears to have more adsorption sites for zinc than cadmium with carboxyl group being the major sites of metal adsorption to the cell wall and with amine groups playing some role. Rijstenbil *et al.* noted that stress may increase surface area and roughness of algal cell walls so that metal adsorption (30). Once inorganic stressors are inside the cell, the ions may bind to intracellular components or precipitate. Biological macromolecules and enzymes with appropriate functional groups or metal cofactors are impacted by metal activity, for example the distribution of Sn in *Ankistrodesmus falcatus* was about 85 % in the cellular polysaccharide fraction, 15% in the protein fraction, and 0.2 % in the lipid and low-molecular weight metabolite fraction. Metals may be detoxified by accumulating in polyphosphate bodies and to intracellular metal – binding proteins of blue – green algae and eukaryotic algae. Accumulation of metals may damage cellular ultrastructure. The presence of one inorganic chemical can change the distribution of others among cellular components. For example, mixtures of cadmium and chromium influenced each other's concentration and distribution among membrane, cell wall, soluble and miscellaneous fractions in cells of *Chlorella ellipsoida* (49).

The summarizes the route of uptake for an algal cell and with seven possible mechanism or sites where tolerance mechanisms could operation. The metal normally has to be in ionic or “free” state for uptake to operate. The ion then has to pass through or become absorbed upon the cell wall. Exclusion could operate at this point if the

cell wall or the sheath were able to take up the metal ion and thus prevent its encountering the cell membrane (1). At the membrane, permeability may be controlled, such that the ion does not move into the cell proper or moves in very slowly (2). Subsequently, mechanism (3), (4) and (5) refer to the binding of cations to non-sensitive sites inside the cells, and mechanism (6) requires the production of extracellular ligands, normally in response to the metal which either transport the metal outside or more probably bind external metal and thus prevent further entry. Mechanism (7) is conceived as some type of alternative biochemical pathway or shunt by which the need for the metal-sensitive enzymes is avoided as shown in Fig 1

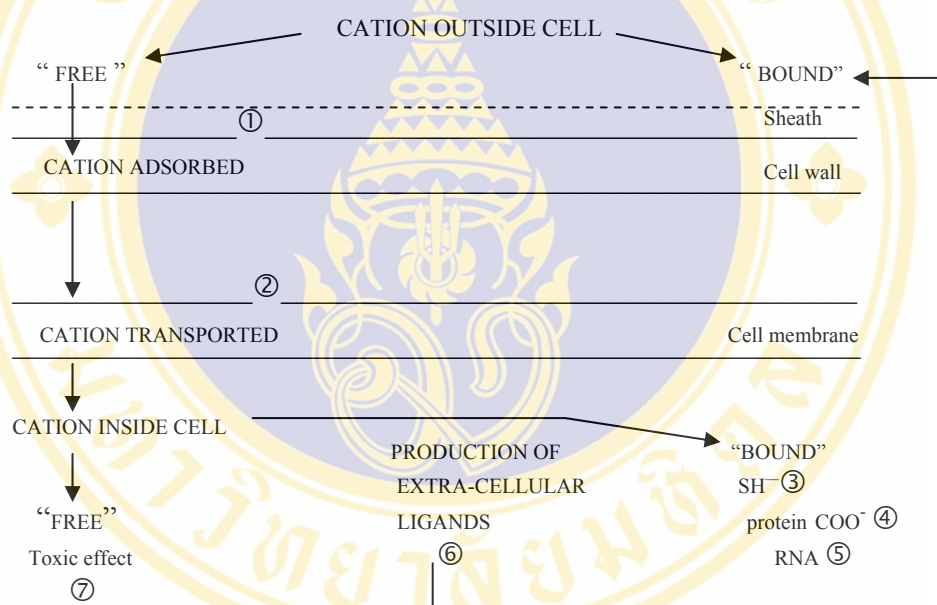


Figure 1 Route of uptake for potentially toxic cations (49, 58).

For application purpose, the advantage of the high rates of biosorption is due to the fast kinetics of the sorption reactions. Because of mass transfer of the sorption rate, which is more than controlled by mass transfer of the sorbed solute metal to the active reaction site on the cell wall could have more efficiencies to control the overall sorption rate. Although not all of the potentially applicable biosorbents have yet been systematically examined, substantial evidence identifies ion exchange as the main mechanism of metal biosorption (53, 59).

2.2.3 Biosorption isotherms

The sorption process considered here involved a solid phase (sorbate) and a liquid phase (solvent) containing a dissolved species to be sorbed. Due to the higher affinity of the sorbent for the sorbate species, the latter is attracted into the solid and bound there by different mechanism. This process takes place until an equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in solution at a residual that final or equilibrium concentration. The degree of the sorbent affinity for the sorbate determine its distribution between the solid and liquid phases. The equilibrium of the process is often described by fitting experimental data with models usually used for the representation of biosorption isotherm. There are two widely used models, which were proposed by Langmuir and Freundlich.

The Langmuir isotherm is widely accepted and easily linearized isotherm model. This model derived a relationship for q and C_{eq} based on several assumptions as follow (22, 26, 30).

- (1) The surface consists of adsorption sites.
- (2) All adsorbed species interact only with a site and not with each other.
- (3) Adsorption is limited to a monolayer.
- (4) Adsorption energy of all sites is identical and independent of the presence of adsorbed species on neighboring sites

The general form of Langmuir adsorption isotherm is represented by:

$$q_e = \frac{q_{max}bC_e}{1 + bC_e}$$

Where q_e is the metal uptake (mg/g), b is the binding constant (mg/l), and C_e is the equilibrium (final) cadmium concentration.

This equation can be rearranged to linear equation:

$$\frac{C_e}{q_e} = \frac{1}{q_{max}b} + \frac{C_e}{q_{max}} \quad (1)$$

$$\frac{1}{q_e} = \frac{1}{q_{max}bC_e} + \frac{1}{q_{max}} \quad (2)$$

Where q_{max} is the maximum amount of the metal ion per unit weight of algae to form a complete monolayer on the surface bound at high C_e and b is a constant related to the affinity of the binding sites. The q_{max} corresponds to the surface

concentration at monolayer coverage and represents the maximum value of q_e that can be achieved as C_e is increased. The constant b is related to the energy of adsorption and increases as the strength of the adsorption bond increases. The value of q_{max} and b can be determined from the linear plot of C_e/q vs C_e usually yield a straight line with the slope $1/q_{max}$ and the intercept $1/bq_{max}$.

The Langmuir isotherm is being widely used as it incorporates two easily interpretable constants, q_{max} is the highest possible metal uptake sorbent and b is another constant which reflects the strength or affinity of the sorbent for the sorbate (13, 26, 30, 50, 56).

2.3 Algae

There are several naturally biomaterials using in biosorption including fungus, bacteria, algae, yeast, fruit juice, plant tissue and plant root tissue. In the early 1990s, several proprietary biosorption processes such as AlgaSORBTM and AMT-BioclainTM were developed and commercialized (50).

Algae have received more attention than other microorganism because of the active uptake can reduce the residual metal concentration to very low (15). Algae are photosynthetic, non vascular plants, contain chlorophyll a and have simple reproductive structures. In addition, algae are ubiquitous that occur in most habitats ranging from marine and freshwater to desert sands and from hot boiling springs to snow and ice (60). Algae consist of a vary diverse group of organism, some algae resemble animal and others resemble higher plants. The algae include a heterogeneous assemblage of simple plant of great diversity in size, pigment, reproductive mechanism and habitat (50). They are vary from small, single-celled forms to complex multicellular form, such as the giant kelps of eastern Pacific that grow to more than 60 meters in length and form dense main forests. Algae are found in fossil record dating back to approximately three billion years in the Precambrian. They are many range in size of algae from single cells as small as one micrometer to large seaweed that may grow to over 50 μm (58). Algae are the major primary producers of organic compounds, and play a central role as the base of the food chain in aquatic system.

Besides forming the basic food source they also form the oxygen necessary for the metabolism of the consumer organisms. Uses of algae, such as human food,

fertilizer, manure, fodder, aquaculture, medicine, textile, paper and paint industries. Algae are also important surface binding agent which reduce erosion and can be used for wastewater treatment (58, 60). Various species of algae require for manganese, zinc, calcium, boron and possibly copper. Immobilized microalgae biomass is already being used in the only present commercial bioremoval processes and living microalgae have been used in the clean-up of mining effluents.

2.3.1 Cyanobacteria

The cyanobacteria are morphologically a heterogeneous mixture of bacteria. They come in five different types. Firstly they are either unicellular or filamentous, unicellular species come in two forms depending on whether they divide by binary fission or multiple fission, they latter tending to be colonial. The non-branching types are divided into two groups depending on the presence or absence of special nitrogen fixing heterocysts. Each individual cell typically has thick, gelatinous cell wall, which has a gram-negative strain (60, 61, 62). They lack flagella, but may move about by gliding along surface. Cyanobacteria are group of aquatic bacteria that obtain their energy through photosynthetic organisms. Photosynthesis in cyanobacteria generally uses water as an electron donor and produces oxygen as a by-product, though some may also use hydrogen sulfide as occurs among other photosynthetic bacteria. Carbon dioxide is reduced to form carbohydrate via the Calvin cycle (50, 60). In most forms the photosynthetic machinery is embedded into folds of the cell membrane, called thylakoids. There are two main sorts of pigmentation, most cyanobacteria contain chlorophyll a, together with various proteins called phycobilins, which give the cells a typical blue-green to grayish-brown colour. A few genera, however, lack phycobilins and have chlorophyll b as well as, giving them a bright green colour. Chloroplasts most likely represent reduced endosymbiotic cyanobacteria. This endosymbiotic hypothesis is supported by various structural and genetic similarities. Primary chloroplasts are found among the green plants, where they contain chlorophyll b, and among the red algae and glaucophytes, where they contain phycobilins (63, 64, 65).

Cyanobacteria can be found in many habitat, such as soil, on rocks, in fresh water and salt water. They can also be found in the desert where they remain dormant for most of the time, taking advantage of the occasional rains. In aquatic environments

they often form thick mats. Like many bacteria they have a higher tolerance of heat and low pH values than green plants, so they are often the main autotroph in hot springs. Finally, a number of cyanobacteria have formed symbioses with other organisms such as liverworts, ferns and cycads, however, their best known symbiosis is with various fungi to form numerous lichens. Cyanobacteria were probably the first organisms on earth to release oxygen into the atmosphere, in this way they would have played a major role in making the planet suitable for animals like ourselves (61, 62).

Cyanophyceae are in Phylum Cyanophyta (Myxophyceae) including unicellular, colonial or filamentous that divided into four orders (50, 58, 62, 66)

I. Heterocyst and hormogonia lacking (only rarely indistinctly filamentous) such as ;

- **Chroococcales** (*Aphanothece*; *Coccochloris*, *Aphanocapsa*, *Chroococcus*, *Coelosphaerium*, *Entophysalis*, *Eucapsis*, *Gloeocapsa*, *Gomphosphaeria*, *Holopedia*, *Merismopedia*, *Microcystis*, *Synechococcus*, *Synechocystis*)

- **Chamaesiphonales** or Dermocarpales (*Chamaesiphon*, *Dermocarpal*)

- **Pleurocapsales** (*Hyella*, *Oncobyrsa*, *Pleurocapsa*, *Xennococcus*)

II Distinctly filamentous; heterocysts, hormogonia and akinates sometime present

- **Hormogonales or Oscillatoriales**, There are two suborder;

- 1) **Nostocinales** is filaments simple (*Anabaena*, *Calothrix*, *Cylindrospermum*, *Gleotrichia*, *Lynbya*, *Nostoc*, *Oscillatoria*, *Phormidium*, *Rivularia*, *Scytonema*, *Spirulina*, *Tolypothrix*, etc.).

- 2) **Stigonematales** is filaments heterotrichous, with connecting pits (*Fischerella*, *Haplosiphon*, *Mastigocoleus*, *Mastigocladus*, *Stigonema*).

2.3.2 Growth curve of cyanobacteria (34, 67, 68)

For an entirely autotrophic cyanobacteria, all that is needed for growth is light, CO₂, H₂O, nutrients and trace element. Photosynthesis attributed the cyanobacteria that are able to synthesize the biochemical compound necessary for growth. The cyanobacteria cells can be cultured in limited volume with finite resources. When the resources present in the culture medium are abundant, the cyanobacteria population cell density increases constantly until the exhaustion of some limiting factors, while other nutrient component of the culture medium decreased over

time. Any products produced by the cells during growth also increase in concentration in the culture medium. Batch culture systems are highly dynamic and population show a typical pattern of growth. These growth phase of cyanobacteria are characterized by variations in a sigmoidal growth rate. However, the growth rate will decline and eventually become zero due to the accumulation of cyanobacterial metabolic wastes, starvation and stress from high population density because the resource have been utilized by the cells, the cultures die unless supplied with new medium. The growth curve can be divided into several distinct phase as shown in Figure 2.

(1) Lag phase

When cells are inoculated into a new medium that unable to adapt cell and will not grow but they remain viable and can represent a potential source of contamination. No increase in cell numbers or mass. This is a time of adaptation of the inoculum cells to the medium and is dependent upon the age and state of the inoculum cells. The cells are metabolically-active and may be synthesizing essential metabolites which may be depleted in old inoculum cells, such as ATP, cofactors and different enzymes to utilize different nutrients. A lag is also observed when a population is transferred from a rich culture medium to poorer one. This happened because for growth to occur in a particular culture medium the cells must have a complete complement of enzyme for synthesis of the essential metabolites not present in that medium. On transfer to a new medium which time is required for synthesis of the new enzymes.

(2) Exponential phase

In the lag growth phase also called exponential phase and the cell components such as protein, nucleic acid etc. that produced in balanced amounts represent active cells of uniform composition for biochemical studies. Cell divided to form two cells, each of which also divides to form two more cells and cells in exponential growth are usually in their healthiest state, and thus, cells in “mid-exponential phase” are often desirable for studied of enzymes or other cell components. Most cell grow exponentially, but the rates of exponential growth vary greatly. The rate of this phase is influenced by environmental conditions (temperature, composition of the culture medium) as well as by genetic characteristics of the organism itself. The exponential phase is also constant growth rate and the slope of the exponential phase is indicative

of the growth rate with increasing, the generation or doubling time decreases and the maximal and metabolism at its peak.

(3) Stationary phase

In this phase, cell may undertaken a different form of metabolism, using endogenous reserves as nutrient sources, which growth usually does not occur in the stationary phase, but many cell function may continue, including energy metabolism and some biosynthetic processes. In some organisms that slow growth, whereas others die, the two processes balancing out so that no net increase or decrease in cell number occurs.

(4) Death phase

Eventually the number of viable cell begins to decline, the kinetics of cell death same those the growth in exponential phase because of the death phase represents the result of the ability of the cell to carry out further reproduction. The rate of death phase can be equal to the rate of growth during the exponential phase. The rate of death is proportional to the number of survivors. The environmental conditions can alter the rate of exponential growth and death rate of cell.

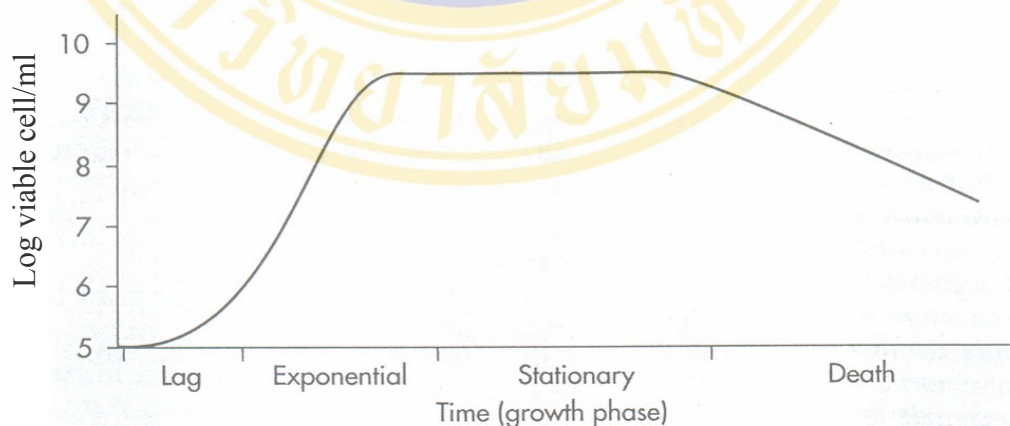


Figure 2 Growth curve of cyanobacteria (68)

2.3.3 Heavy metal removal by cyanobacteria

For the sewage treatment plants, many plants were not large enough to manage increased capacities. They might not have been able to take care of flow during all parts of the year and providing only minimal treatment. Cyanobacteria are photosynthetic prokaryote that have remarkable affinity for heavy metal. These cell have developed natural methods of responding to metals such as copper, lead and cadmium through passive accumulation in cells and through surface binding to various functional groups.

From the biomass that *Spirulina* sp., *Tolypothrix tenuis*, *Nostoc mucorum*, *Anabaena subindrica*, *Phormidium laminosum* and *Oscillatoria angustissima* were shown to contain detectable levels of heavy metal due to the algal cell wall contain biopolymeric substances that could provide binding groups for metal sorption such as amino, carboxyl, phosphate, and sulphate anions acting as a cationic resin. The nitrogen and oxygen from amino and carboxyl groups in the peptide could be available for characteristic coordination bonding with metallic ions. Live algae possess intracellular polyphosphate which participate in metal sequestration as well as algal extracellular polysaccharide that serve to chelate or bind metal ion (22, 23, 26).

In accordance to the effect of extracellular polypeptides in reducing the toxicity of the heavy metals was found in the freshwater cyanobacteria, *Anabaena cylindrica*. Subsequently, the complex capacity of extracellular organic ligands, including organic acids and polypeptides has been demonstrated in other cyanobacteria and in eukaryotic algae (34). Tien *et al.* found algal surface composed of cell wall and mucilage containing different functional groups in four freshwater algae including with different cell surface characteristics to investigate metal binding activities in an effort to examine the role of mucilage in the biogeochemical cycle of metal ions in the natural environment and the potential of using such material for wastewater treatment (69). EL-Enany *et al.* found that *Nostoc linkia* and *Nostoc rivularis* can be accumulate Zn and Cd by adsorption on the pellet and through sequestration via metal-binding protein that same as strains of *Synechocystis* spp. have been shown to develop a thickened calyx when exposed to copper-stressed growth conditions. *Synechococcus* spp. was found to possess a copper-transporting P-type ATPase in the thylakoid membrane (70). The important mechanism may be the production of metallothioneins which are low

molecular weight proteins or polypeptides that presence of Cd-binding material in *Anacystis nidulans* and *Anabaena dolilolum* in response to Cd and this protein may play a role in metal tolerance. Addition function of metallothioneins include control of intracellular redox potential, cellular repair processes, growth and differentiation, where are likely to serve as the source of Zn for newly synthesized apoenzyme as well as regular molecule in gene expression (23). Moreover, cyanobacteria are easy separated from aqueous solution by filtration of nylon sieve, can grow under low conditions compared with other microorganisms because they are capable of photosynthesis and nitrogen fixation and growth under high pH conditions for prevent the contamination from other organisms, also can be cultivated in open ponds or in large-scale laboratory culture at low costs (22).

2.4 Immobilization

The studies of immobilized cell were initiated by immobilized enzyme, however, recently much attention has been directed toward use of immobilized viable cell with more complicated or conjugated reaction. Immobilized cells have been employed for the production of useful compound by various type of bioreactor.

The several advantage of immobilized cell include the following: application to multistep enzyme reaction, operational stability is generally high, high cell density and can be expected to be maintained over the long time, immobilized cells appear to be less susceptible to microbial contamination. In addition, allow high cell density and column operation. The immobilization of microorganisms can be divided into two main immobilization process (13, 71, 72).

1. The self – attachment of microorganisms to the filter bedding material, which is defined as attached growth or biofilm.

2. The artificial immobilization of microorganisms to the bedding material, there are several methods for artificial immobilization of microorganisms to a support material.

2.4.1 Attached growth

The spontaneous adsorption of microbial cells to different types of carrier gives natural immobilized cell systems in which cells are attached to their support by weak (non-covalent), generally non-specific interactions, such as electrostatic

interactions. In suitable environmental conditions, this initial adsorption step may be followed by colonization of the support, leading to the formation of a biofilm in which microorganisms are entrapped within a matrix of extracellular polymers (73).

(1) The advantages of attached microbial film compared to suspended microorganisms in the degradation of fluid pollutants

- Higher biomass concentrations

In natural and artificial aquatic ecosystem, the concentration of microorganisms upon submerged surfaces is generally higher than the suspended microorganism, a higher microbial concentration could be maintained within systems treating fluids with attached microorganisms.

- Higher metabolic activity

A higher metabolic activity was measured within attached growth treatment systems compared to that of the suspended growth systems, the explanation for this increased biodegradation activity might originate in the higher amount of active biomass concentration within the attached growth systems. This higher activity has also been attributed to the high concentration of nutrients around attached biofilm. Due to its slimy nature, the biofilm traps particulate matter from the treated fluid, so the nutrient concentration around biofilms is usually higher than the free fluid. This high nutrient concentration may increase the microbial growth rate and enhance the degradation activity. Another explanation for this increased biodegradation activity is a physiological difference between attached and suspended microorganisms (72). It has been suggested that with the attachment, the switching on of different genes occur, which leads to a physiological difference between the attached and the suspended microorganisms. This difference finds expression in a faster growth rate, increased metabolic activity and greater resistance to toxicity (74).

- Greater resistance to toxicity

A greater resistance to toxicity is usually seen within attached biofilms compared to suspended microorganisms that increased concentration of nutrients around biofilms help the microorganisms to survive in the higher concentration of toxic compounds.

- Better sludge properties

Free microorganism being immersed in a fluid have a relatively small diffusion barrier around themselves compared to a biofilm, which is surrounded by a diffusion resistant extracellular matrix. The diffusion restriction of the biofilm might reduce oxygen penetration into a thick biofilm and limit the amount of aerobic decomposition .

- (2) The structure and properties of biofilm

- The biofilm thickness

The biofilm thickness is influenced by several factors. These include the rate of flow through the biofilter, the bedding material construction and the different designs. Generally, a rapid flow through the biofilter will limit the growth of bacterial films to small thicknesses. Microorganisms form thinner layers upon smooth surfaces in comparison to microbial films upon porous material

- The extracellular matrix

Within biofilms the total cell volume is only a small part of the total volume. The extracellular matrix, a polymer matrix in which cells and colonies are embedded, accounts for the majority of the biofilm volume. The extracellular matrix structure and composition varies between biofilms, but generally polysaccharides predominate the extracellular matrix (EPS) and represent up to 65% of this material, while proteins usually comprise up to 10-15 % of its total biomass.

- The mechanisms and forces responsible for the attachment of microorganisms to a surface

- I. Microbial structures

Microorganisms possess a variety of structures which are used for attachment. These include fimbria (pilli), capsules (glycocalyx), various holdfast structures, stalks, cell wall components and slimes (68). It is believed that the main microbial structure which is involved in attachment is the glycocalyx. The glycocalyx consists of extracellular polysaccharides, which were found to have no other special properties beside their participation in the attachment process (65). Besides the glycocalyx, any extension from the bacterial cell often comes into contact with surfaces and is involved in attachment.

II. Forces governing microbial attachment

Several forces are involved in microbial attachment to a surface. The strength of the attachment and the composition of the forces, which govern it change with different environmental conditions, different microbial species, different surface properties and with different fluid properties

i Electrostatic interactions.

The electrostatic interactions involved in microbial attachment are mostly of ionic and hydrogen bonding which, although being individually weak when compared to covalent bonds, nevertheless capable of producing relatively firm binding, if the number of bonds is sufficiently large. The electrostatic forces are highly significant in the initial stages of adsorption. Since the surface charge of almost all microorganisms is negative and in many cases the bedding material is also negatively charged, an electrostatic repulsive force can prevent attachment. Usually other stronger forces overcome this repulsive barrier and negatively charged cells do attach to negatively charged material, such as glass and ceramics. In other cases, where the attachment surface is positively charged, the electrostatic forces can dominate the attachment process.

ii Covalent bond formation.

The outer surfaces of microbial cells and cell walls contain large quantities in various of reactive groups. Covalent bonds can form those ligands on the cell surface and specific groups, which are located on the bedding material surface. Usually covalent bonds are formed between organic groups on the bedding material and the microbial ligands (72).

iii Hydrophobic interactions.

Since the attachment of microorganisms to the bedding material takes place in a liquid environment, hydrophobic interactions between the microorganism and the bedding material are very important. Hydrophobic groups on the microbial surface can interact with hydrophobic groups on the bedding material, while removing the water molecules which separate the microorganisms from the surface.

iv Partial covalent bond formation between microorganisms and hydroxyl groups on surfaces.

Because of the microbial immobilization is performed in solution, hydroxides are formed instead of different metal oxides incorporated in the bedding material (70). Suitable amino or carboxyl groups on the cell surface can replace those hydroxyl groups. As a result, partial covalent bonds form between the microbial cells and the bedding material.

- The influence of fluid properties on microbial attachment

I. pH

Marcipar *et al.* studied the adsorption of microorganisms to an inorganic ceramic bedding material as a function of pH 4 and 6. They found that the rate of adsorption was rather specific for each microbial strain, but at the lower pH, the percentage of microorganisms adsorbed was higher. A possible explanation for this higher attachment of microorganisms at lower pH arises from the influence of pH on the microbial surface charge. Most microbes have a dipolar character, which can function as a cation or anion depending on the pH of the solution. It is reasonable that at low pH values, the negative charge of the microbial surface is reduced, which leads to a reduction in the electrostatic repulsion between the microorganism and negatively charged bedding material. This reduction in the electrostatic repulsion might be the cause of the greater microbial attachment at low pH values (72).

II. Salt content

Meadows has reported that he was able to detach microbial cells from sand by washing with distilled water, but when salt was present in the water the microbial surface. Kolot found that an increase in Ca-ion concentration. In cases, where the microorganisms and the bedding material are negatively charged, the divalent positively charged ions reduce the electrostatic repulsion between the microorganisms and the bedding material and may even act as bridges between those two negatively charged surfaces (70).

2.4.2. The artificial immobilization (13, 72, 73, 75)

The method of artificial immobilization of microorganisms can be divided as below;

(1) Active immobilization consisted of

- Crosslinking

This type of immobilization is support - free and involves joining the cell or the enzymes to each other to form a large, three-dimensional complex structure, and can be achieved by chemical or physical methods (76). Chemical methods of crosslinking normally involved covalent bond formation between the cells by means of a bi- or multifunction reagent, such as glutaraldehyde and toluene diisocyanate. However, the toxicity of such reagent is a limiting factor in applying this method to living cells and many enzymes. The addition of a cross-linker leads to the formation of stable cellular aggregates (77). Flocculating agents, such as polyamines, polyethyleneimine, polystyrene sulfonates, formaldehyde, glutaric dialdehyde, divinylsulfone, formaldehyde-urea mixtures and various phosphates, have been used extensively and are well characterized. Crosslinking is rarely used as the only means of immobilization because the absence of mechanical properties and poor stability are severe limitations. Therefore, crosslinking is most often used to enhance other methods of immobilization, normally by reducing cell leakage in other systems (78).

- Covalent bonding

This popular method of immobilization involves the formation of a covalent bond between the enzyme or cell and a support material (79, 80, 81). The bond is normally formed between functional groups present on the surface of the support and functional groups belonging to amino acid residues on the surface of the enzyme. A number of amino acid functional groups are suitable for participation in covalent bond formation. Many varied support materials are available reflects the fact that no ideal support exists. Therefore, the advantage and disadvantage of support must be taken into account when considering possible procedure for a given enzyme immobilization (82, 83, 84). Many factors may influence the selection of a particular support, and research work has shown that hydrophilicity is the most important.

- Entrapment

Entrapment methods are based on the confinement of the cells in a three-dimensional gel lattice. The cells are free within their compartment and the pores in the material allow substrate and product to diffuse to and from the cells. Entrapment is by far the most frequently used method in laboratory experiments and there are also some examples of industrial processes based on entrapped cells (83, 85). The method of entrapment has restricted the use of substrates to those having a low molecular weight. Immobilization by entrapment differs from adsorption and covalent binding in that enzyme molecules are free in solution, but restricted in movement by the lattice structure of a gel (86, 87, 88). The porosity of the gel lattice is controlled to ensure that the structure is tight enough to prevent leakage of enzyme or cells, at the same time allow free movement of substrate and product. Inevitably, the support will act as a barrier to mass transfer, and although this can have serious implications for reaction kinetics, it can have useful advantages since harmful cells, proteins, and enzymes are prevented from interaction with the immobilized biocatalyst (87, 88).

Several polymers have been utilized for the entrapment of cells. The most commonly used synthetic polymer are the acryl polymers, calcium alginate, polyacrylamide, polysulfone, polyethylenimine and polyhydroxoethylmethacrylate. The materials obtained from the immobilization in calcium alginate and polyacrylamide are in the form of gel particles, and those obtained from immobilization in polysulfone and polyethylenimine proved to be the strongest. Fukui and co-workers have applied the technology to use polymers in entrapment of *Nocardia rhodocrons*, that was used for the modification of steroids. Organic solvents were used because of the low solubility of the substrate as well as the product. The hydrophobicity of the polymer markedly influenced the catalytic efficiency, due to a close correlation with the partition coefficient of the substrate between the bulk phase and the polymer.

Entrapment in natural polymer consisted of agar and agarose, cellulose that has been used for the covalent coupling of enzymes, such as DEAE and CM-cellulose (13, 85). Temperature change is a simple method of gelation by phase transition using 1-4% solutions of agarose or gelatin. However, the gel formed are soft and unstable. A significant development in this area has been the introduction of K-carragenan

polymers that can form gels by ionotropic gelation and by temperature-induced phase transition, which has introduced a greater degree of flexibility in gelation systems for immobilization.

Alternatively, it is possible to mix the enzyme with chemical monomers that are then polymerized to form a crosslinked polymeric network, trapping the enzyme in the interstitial spaces of the lattice.

(2) Passive Immobilization consisted of

- Adsorption

Immobilization by adsorption is the simplest method and involves reversible surface interactions between enzyme or cell and support material (88, 89). The forces involved are mostly electrostatic, such as van der Waals forces, ionic and hydrogen bonding interactions, although hydrophobic bonding can be significant. These forces are very weak, but sufficiently large in number to enable reasonable binding. The adsorption process can be studied with respect to properties of the support or those of the cells. Important factors that effect cell properties are cell wall composition, charge and age. Composition of the carrier, surface charge, surface area, and pore size are some of the factors that have to be taken into account, so the properties of the support for the adsorption process. The great advantage with adsorption methods is in their operational simplicity, cell loading is often carried out by simply pumping a cell suspension through a bed of adsorbent, little or no damage to enzyme or cells, no chemical changes to support or enzyme or cell, reversible to allow regeneration with fresh enzymes or cells (77). And the other principle, for example;

- Encapsulation

Encapsulation of enzymes and or cells can be achieved by enveloping the biological components within various forms of semipermeable membranes. It is similar to entrapment in that the enzymes or cells are free in solution, but restricted in space. The large proteins or enzymes cannot pass out of or into the capsule, but small substrates and products can pass across the semipermeable membrane. Many materials have been used to construct microcapsules varying from 10-100 μm in diameter; for example, nylon and cellulose nitrate have proven popular. The problems associated with diffusion are more acute and may result in rupture of the membrane if products from a reaction accumulate rapidly. A further problem is that of the bulk solution with

consequent problems in reactor configuration, flow dynamics, and so on. It is also possible to use biological cells as capsules, and a notable example of this is the use of erythrocytes or red blood cells. The distinct advantage of this method is coimmobilization with cell and/or enzymes may be immobilized in any desired combination to suitable particular applications (90, 91, 92).

2.5 Factors affecting biosorption performance

The biosorption potential of microbial biomass depends on many factors. Another conditions during the actual biosorption process, such as contact time, biosorbent concentration, type of biosorbent and concentration of metal are all the important parameters affecting the performance of a biosorbent.

2.5.1 Contact time

The contact time between microbial cells and metal ions is one of physical factor. It is necessary to provide enough exposure time for biosorption process to reach equilibrium. Various periods of contact time are needed for different types of biosorbents. The removal of Cd and Pb by *Nostoc paludosum* and *Phormidium angustissimum* were adsorption kinetics that has a rapid metabolism-independent phase with binding to cell walls and external surface during the initial 30 min of sorbent contact. The Cd removal by *Tolypothrix tenuis* slowing equilibrium in 30 min, and also for the blue green algae *Chroococcus parvulus* which accumulated Cd within 1 min. The *Chlorella vulgaris* had fast adsorption of Cd at the equilibrium after less than 5 min, whereas Conway and Williams reported that two fresh water diatom *Asterionella formosa*. *Formosa* and *Fragilaria crotonensis* showed rapid Cd sorption during the first 5-10 min of exposure (22, 34, 50). In general, there was a rapid accumulation of Cd in the first 2 days of 6 day growth period. At the higher concentration, the accumulation continued and a maximum was recorded on the fifth day of the culture. Thus, the response of cells to metal stress depends upon the ambient concentration of metal (93).

2.5.2 Biosorbent concentration

In several research, biosorbent concentration could effect on sorption capacity. Their result demonstrated that an increase in biosorbent concentration generally increased the adsorbed metal concentration on account of an increasing

adsorption surface area whereas the metal uptake capacity (q) at equilibrium decreased by increasing the biosorbent quantity. The decreased in q value with increase in biosorbent concentration because of complex interactions of several factors. One of the proposed explanations takes into consideration that it may be due to aggregate formation by high biosorbent concentration leading to the reduction of the effective sorption area and decrease in the ratio of metal uptake per unit amount of biosorbent.

2.5.3 Types and initial concentrations of metal

The types and initial concentrations of heavy metals also influence biosorption of microorganisms. Apparently, each type of biomass may be better for only certain types of metals. The attractive feature of biosorption is a certain spectificity of the biosorbent for the metal cations. The reason for these different sorption efficiencies might be the electrochemical properties of the metal ions and the sequestration properties of microbial cell wall. The initial concentrations of heavy metal can affect to removal ability. Kitjaharn reported that the rates of lead accumulation by *Aphanothece halophytica* depended on metal concentration in solution but were saturated when the metal was in excess. Blanco *et al.* reported that the biosorption of heavy metals to immobilized *Phormidium laminosum* biomass increased when initial metal concentration rose as long as binding sites were still unsaturated (30, 94, 95).

2.5.4 Aging effect

The effect of culture age has been shown to be important for determining the ability of biosorption to remove metals from solution. The ability of lead accumulation by *Aphanothece halophytica* was decreased when the culture age of cell increasing up to 14 days (95). Becker reported that the accumulation factors and growth rates in different models, in static system the retention time of metal concentration by 50% requires 14 day while in a dynamic model at retention time of about 19 days (50). In accordance with Sombatjinda found that cell of 14-day cultured had higher uptake cadmium removal than cell of 10 days-cultured (96).

2.6 Consideration of immobilized material

Table 2 Fundamental consideration in selecting a support and method of immobilization

Property	Points for consideration
Physical	Strength, non-compression of particles, available surface area, shape/form(bead/sheets/fibers), degree of porosity, pore volume, permeability, density, space for increased biomass, flow rate and pressure drop
Chemical	Hydrophilicity (water binding by the support), inertness toward enzyme activity, maintenance of cell viability and mechanical stability of support material
Stability	Storage, residual enzyme activity, cell productivity, regeneration of enzyme activity, maintenance of cell viability, and mechanical stability of support material
Resistance	Bacterial/fungal attack, disruption by chemicals, pH, temperature, organic solvents, proteases, and cell defense mechanisms (proteins/cell)
Safety	Biocompatibility (involves an immune response), toxicity of component reagents, health and safety for processes workers and end product users, specification of immobilized preparation for food, pharmaceutical, and medical applications
Economic	Availability and cost of support, chemicals, special equipment, reagents, technical skill required, environmental impact, industrial-scale chemical preparation, feasibility for scale-up, continuous processing, effective working life, reusable support, and zero contamination (enzyme/cell-free product)
Reaction	Flow rate, enzyme/cell loading and catalytic productivity, reaction kinetics, side reactions, multiple enzyme and/or cell systems, batch and so on ; diffusion limitation on mass transfer of cofactors, substrates, and products

Source: Immobilization of enzymes and cells (76)

2.7 The related research

Blanco *et al.* (94) reported that the capacity to biosorption Cu (II), Fe (II), Ni (II) and Zn (II) by non-viable biomass of the cyanobacterium *Phormidium Luminosum* that entrapped in polysulfone and epoxy resin bead, based on the wetting of biomass beads, the rate of metal biosorption was rapidly during first hour and then continuous of slower rate. A decrease in the immobilized biomass bead size led to an increase in the rate of metal biosorption. The amount of metal biosorbed increased with the biomass and the amount of metal and the biosorbed metal was completely, desorbed from biomass beads by washing with 0.1 M HCl. The polysulfone biomass can be reused for at least ten consecutive biosorption and/or desorption cycles without apparent loss of efficiency after its reconditioning with 0.1 M NaOH.

Akhtar *et al.* (29) reported that a new sorption system of microalgal cells immobilized on the biostructural matrix of *Luffa cylindrical* for sequestering cadmium by free and immobilized *Chlorella sorokiniana* removed cadmium from 10 mg/l solution at the efficiency of 92.17% and 97.90% respectively. Maximum cadmium sorption was 39.2 mg/g at equilibrium concentration of 112.8 mg/l by immobilized microalgal biomass from initial concentration of 150 mg/l. The cadmium sorption capacity of immobilized *C.sorokiniana* at saturation 192.0 mg/g biomass from 5 mg/l Cd²⁺ solution which was 73.2% of the total metal passed in 51.5 liters.

Rangsayatorn *et al.* (26) reported that the biosorption of cadmium by immobilized *Spirulina platensis* on alginate gel and silica gel, the maximum biosorption capacities for alginate immobilized cell and silica immobilized cell were 70.92 and 36.63 mg Cd/g biomass and sorption occurred in a wide pH range 3-8. The highest adsorption of alginate immobilized cell was at pH 6 while silica immobilized cell adsorption was not affected by pH between 4 and 7. The effect of temperature on cadmium removal did not affect cadmium removal of alginate immobilized cell, but temperature significantly affected cadmium adsorption capacity of silica immobilized cell.

Hakawati *et al.* (97) reported that *Rhizopus oryzae* was immobilized by inclusion in six different polymer, such as polyvinylformal, polysulfone, polyurethane, alginate, polyacrylamide, k-carrageenan, polyethyleneimine. The biomass/polymer matrices were form equal size and were used to uptake copper at 2 mg/l from a laboratory-

formulated copper solution in shake flask experiments at room temperature at 2 initially neutral pH. Results showed that the copper uptake capacity of immobilized biomass was either equal to or less than that of free biomass. Biomass immobilized in polyurethane gave a capacity equal to the free cell (77% and 86%), while the other matrices hindered the uptake to different degrees. Whereas the k-carrageenan matrix proved to be unstable in the copper solution and dissolved during the experiment releasing the biomass and leading to an erroneous result.

Yalcinkaya *et al.* (7) reported that the biosorption of Cd (II) ions on carboxymethylcellulose and both immobilized live and heat-killed fungal mycelia of *Trametes versicolor* was studied from aqueous solutions in the concentration range of 30-70 mg/l. Maximum biosorption capacity for immobilized live and heat-inactivated fungal mycelia of *T. versicolor* was found as 124 and 153 mg Cd (II)/g respectively whereas the amount of equilibrium were established in about 1 hr and correlation regression coefficients can be well defined by Langmuir equation. The temperature change between 15 and 45 °C did not affect the biosorption capacity. The effect of pH was also investigated and the maximum adsorption of Cd (II) ions on the carboxymethylcellulose and both live and heat-inactivated immobilized fungal mycelia were observed at pH 6.0.

Akhtar *et al.* (91) reported that the biosorption process for the removal of nickel (II) by loofa sponge-immobilized biomass of *Chlorella sorokiniana* (LIBCS) was studied compare with free biomass of *C. sorokiniana* (FBCS). Effects of environmental factors on metal uptake capacity of LIBCS was found to be influence by pH of the solution, initial metal concentration and biomass concentration. The biosorption of nickel (II) ions by both LIBCS and FBCS increased as the initial concentration of nickel (II) ions increased in the medium. No loss of biosorption capacity of LIBCS was found as 48.08 and 60.38 mg nickel (II)/g, respectively, whereas the amount of nickel (II) ions adsorbed on the plain loofa sponge was 61 mg/g. The nickel (II) removal was reached at equilibrium in about 15 min for LIBCS and 20 min for FBCS. The biosorption capacities were found to be solution pH dependent and the maximum adsorption was found at a solution pH 4-5.

Sombatjinda (96) reported that screening for cadmium removal and immobilized ability in aqueous solution by using 5 strains of cyanobacteria: *Nostoc paludosum*,

Phormidium angustissimum, *Nostoc commune*, *Nostoc micropicum* and *Rivularia* sp. Results showed that *N. paludosum* and *Rivularia* sp. were selected for further studies because they had highest cadmium removal and high growth rate. Cadmium removal was 87.56%, 85.52% and 98.64%, 97.12% for immobilized and free cell of *N. paludosum* and *Rivularia* sp. respectively. The initial cell concentration at 0.05 g dry wt./l was selected for extended experiment because it has high cadmium removal at 85.42%, 79.58% and 93.75%, 96.65%, respectively, and it had highest uptake at 1.14, 0.93 and 1.66, 1.59 mg/g dry wt. for immobilized and free cell of *N. paludosum* and *Rivularia* sp., respectively. The cadmium adsorption capacity (q_{max}) was 0.45 and 1.07 mg/g dry wt. for immobilized and free cell of *N. paludosum* and *Rivularia* sp., respectively.

Iqbal *et al.* (99) reported that the performance of fungi biomass immobilized within a loofa sponge (FBILS) to remove heavy metal ions such as Pb (II), Cu (II) and Zn (II) from aqueous solution with selectivity in the order of Pb (II)>Cu (II)>Zn (II). The biosorption of metal ions by FBILS increased as the initial concentration of metal ions adsorbed was found to be 135.3, 102.8, 50.9 mg/g FBILS for Pb (II), Cu (II) and Zn (II), respectively. The maximum uptake of metal was obtained at pH 6.0. Temperature over the range 10 – 50 °C had no effect on the biosorption capacity of FBILS. The adsorption of all three metal ions followed the Langmuir isotherm. Competitive adsorption of Pb (II), Cu (II) and Zn (II) was also studied. FBILS was regenerated by washing with 50 mM solution of HCl.

Bayramoglu *et al.* (100) reported that entrapment of *Lentinus sajor-caju* into alginate gel via a liquid curing method in the presence of Ca (II) ions for biosorption of cadmium. The entrapped cell live and dead fungal biomass has been studied in the effect of initial cadmium concentration, pH and temperature. The maximum experimental biosorption capacities for entrapped live and dead fungal mycelia of *L. sajor-caju* were found to be 104.8 ± 2.7 mg Cd (II)g⁻¹ and 123.5 mg Cd(II)g⁻¹, respectively. The kinetics of cadmium biosorption was fast and approximately 85% of biosorption taking place within 30 min.

Bai *et al.* (101) studied the Cr (VI) biosorption potential of immobilized *Rhizopus nigricans* and to screen a variety of non-toxic desorbing agents. The biomass was immobilized by various mechanisms and evaluated for removal of Cr (VI) from

aqueous solution, mechanical stability to desorbents and reuse in successive cycles. The different combinations (%w/v) of biomass dose compared for each matrix, 8% (calcium alginate), 6% (polyacrylamide and PVA), 12 % (polyisoprene), and 10% (polysulfone) were found to be the optimum. The results of Cr sorption capacity (mg Cr/g sorbent) of all immobilized biomass was lesser than native, powdered biomass. The Cr sorption capacity were highest at free biomass (119.2 mg Cr/g sorbent) and followed by polysulfone entrapped (101.5 mg Cr/g sorbent), polyisoprene immobilized (98.76 mg Cr/g sorbent), PVA immobilized (96.69 mg Cr/g sorbent), calcium alginate entrapped (84.29 mg Cr/g sorbent) and polyacrylamide (45.56 mg Cr/g sorbent), respectively at 500 mg/l concentration of Cr(VI).

Veglio *et al.* (102) found that *Arthobacter* sp. was entrapped inside a macro-and microporous matrix build up with poly(hydroxiethyl methacrylate)(Poly-HEMA) cross-linked with trimethylopropanetrime-thacrylate (TMPTM). The resin-biomass complex (RBC) was prepared with different characteristics according to a factorial experiment. The results indicated an essentially monodispersed structure of resins, with lower crosslinker weight fractions and a bidispersed structure of the resin with the highest cross-linking degree. A maximum copper specific uptake of about 0.6 mg Cu g⁻¹ RBC (7 mg Cu g⁻¹ biomass dry weight.) has been studied in the case of a RBC with the following characteristics 2% w cross-linker concentration, 8% w biomass concentration at 425-750 µm granulometry.

CHAPTER III

MATERIALS AND METHODS

3.1 Research design

The experiments were performed in aqueous cadmium solution in a batch system. The aim of this study is to screen for high cadmium removal and high immobilized ability cyanobacterial strains in different immobilized materials. The effect of initial cell concentrations, contact time, initial cadmium concentrations and cell ages were studied. The supernatants were sampled and measured for cadmium concentration by using Atomic Adsorption Spectrophotometer (AAS).

3.2 The place of experiment

The experiments were performed in the laboratory at the Department of Environment Health Sciences, Faculty of Public Health, Mahidol University.

3.3 Cyanobacterial strains

The cyanobacterial strains obtained from BIOTEC Culture Collection (BCC), the National Science and Technology Development Agency (NSTDA), The Ministry of Science, Technology and Environment, Thailand.

Cyanobacteria strains consist of;

1. *Scytonema* sp. (BCC 83)
2. *Oscillatoria agardhi* (BCC 52)
3. *Hapalosiphon hibernicus* (BCC 27)
4. *Mastigocladus* sp. (BCC 43)

3.4 Immobilized materials

Immobilized material using in this experiment were natural and synthetic polymer such as loofa sponge, delicate duty scour pad, synthetic fiber, polyethylene terephthalate, polyethylene terephthalate (non-woven fabric), polyethylene, polypropylene and polyurethane.

3.5 Equipment and chemical reagent

3.5.1 Equipment

1. pH-meter, Eutech Cybernetics pH scan I, Singapore
2. Laminar air flow, RFC 120000, Australia
3. Autoclave, HA-240M, Hirayama, Japan
4. Hot air oven, Mammert, Germany
5. Desiccator, Auto-C series, Sanplatec, Japan
6. Shaker
7. Centrifuge, D-7200, Hitch universal II , Japan
8. Cool white fluorescence lamp
9. Atomic Adsorption Spectrophotometer, Z-8200 Polarized Zeeman, Hitachi, Japan

3.5.2 Glassware

1. Erlenmeyer flask size 125, 250, 500, 1000 and 2000 ml
2. Volumetric flask size 100 and 1000 ml
3. Test tubes
4. Cultivation tubes 200 ml
5. Pipette, 1, 5 and 10 ml
6. Funnel
7. Micropipette 200, 1000 and 5000 μ l, Gilson, France
8. Pipette tips
9. Inoculate loop and Needle
10. Alcohol lamp
11. Nylon sieve (50 μ m)
12. Aluminum foil

3.5.3 Chemical reagents

1. Cadmium nitrate ($\text{Cd}(\text{NO}_3)_2$), Unilab, Australia
2. Nitric acid (HNO_3) 69 %, BDH Laboratory Supplies, England
3. NaOH, Merck, Germany
4. Methyl alcohol (CH_3OH), the Government Pharmaceutical Organization, Thailand
5. Ethyl alcohol ($\text{C}_2\text{H}_5\text{OH}$), the Government Pharmaceutical Organization, Thailand
6. NaNO_3 , Carlo erba, Italy
7. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, Merck, Germany
8. $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, Merck, Germany
9. NaCl, Riedel-de HaenAG Seeize-Hannover, Germany
10. K_2HPO_4 , Merck, Germany
11. $\text{Fe}_2(\text{SO}_4)_3 \cdot n\text{H}_2\text{O}$, May and Baker Ltd., Dagenham, England
12. $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, May and Baker Ltd., Dagenham, England
13. H_3BO_3 , May and Baker Ltd., Dagenham, England
14. $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, May and Baker Ltd., Dagenham, England
15. $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, BDH Laboratory Supplies, England
16. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, May and Baker Ltd., Dagenham, England
17. $\text{COCl}_2 \cdot 6\text{H}_2\text{O}$, BDH Laboratory Supplies, England
18. Milli-Q water, Milli-Q UF Plas, France
19. Distilled water

3.6 Experimental procedure

3.6.1 Cultivation system

The cyanobacteria were cultivated in 125 ml of Medium 18 (See Appendix A) in Erlenmeyer flask size 250 ml at room temperature (25-28°C) with light intensity of 4,000 lux by illumination of cool white fluorescence lamp at 12 hour light and 12 hours dark interval for 10 days as a stock culture as shown in Figure 3.

3.6.2 Glassware preparation

After cleaning, glassware used in this experimental were immersed in 20% HNO₃ for overnight. The glassware were rinsed with distilled water and were rinsed again with Milli-Q water twice. The glassware were dried in hot air oven at 103 °C before using in this experiment.

3.6.3 Preparation of cadmium solution

Cadmium solution were prepared as 100 ml stock solution of Cd(NO₃)₂ (See appendix B) in 100 ml of Milli-Q water and were kept in refrigerator at 4°C.

3.6.4 Preparation of immobilized material

Eight types of immobilized material were prepared as size 2x3 cm and were dried in the hot air oven at 103°C until their weight become constant, cool down in desiccator for 30 min and dry weight were measured. Immobilized materials were wrapped with aluminums foil and autoclaved before using in this experiment.

3.6.5 Dry weight determination

The cyanobacteria cells were dried in the hot air oven at 103°C until their weight became constant, cool down in desiccator for 30 min and dry weight were measured.

3.6.6 Atomic absorption spectrophotometer analysis

The samples solutions were measured by Atomic Absorption Spectrophotometer by using flameless method of graphite system at wave length 228.8 nm for cadmium. The sample solutions were fixed with 65% HNO₃ before measurement.

3.6.7 Screening for high cadmium removal and high immobilized ability cyanobacteria strains in different immobilized materials

Cyanobacterial strains were cultivated in Medium 18 for 10 days. In free cell system, cell were centrifuged and 0.060 ±1 g dry wt. of cell were added in 130 ml of 1 mg/l cadmium solution at pH 7. In the immobilized cell system, each immobilized material (size 2X3 cm) were submerged into the system at the same condition as in free cell system. Both cyanobacteria in free cell system and immobilized cell system were shaken in rotary shaker at 100 rpm for 10 days. The supernatant were sampled for 1 ml at 2, 4, 6, 8 and 10 days for cadmium concentration measurement by using Atomic Absorption Spectrophotometer. After that immobilized cyanobacteria were

separated by filtration with nylon sieve pore size 50 μm . Immobilized cell, non-immobilized cell and free cell were filtrated and dried in hot air oven at 103°C. The weight of immobilized material, non-immobilized and free cell of cyanobacteria were measured by using dry weight determination. The immobilized ability (%), cadmium removal ability (%), dry weight per surface area (DW/SA), cadmium uptake (mg/g dry wt.) were calculated as;

$$\text{Cadmium removal ability (\%)} = \frac{(C_i - C_f) \times 100}{C_i}$$

Where C_i is the initial cadmium concentration (mg/l)

C_f is final (equilibrium) cadmium concentrations (mg/l)

$$\text{Immobilized ability (\%)} = \frac{(W_f - W_i) \times 100}{W_{ni} + (W_f - W_i)}$$

Where W_i is the initial dry weight of immobilized material (g)

W_f is the final dry weight of immobilized material (g)

W_{ni} is the final dry weight of non-immobilized cell in solution (g)

$$\text{Cadmium uptake (q)} = \frac{V(C_i - C_f)}{M}$$

Where q is cadmium uptake (mg of cadmium/g dry wt.)

V is volume of cadmium solution (l)

C_i is initial concentration of cadmium in the solution (mg/l)

C_f is final (equilibrium) concentration of cadmium in the solution, (mg/l)

M is amount of biosorbent in the solution (g dry wt.)

Dry weight per surface area (DW/SA) ratio

DW is the increased of dry weight on immobilized material (g dry wt.)

SA is the surface area of immobilized material (cm^2) and calculated at 16 cm^2

3.6.8 Effect of initial cell concentrations and contact time on cadmium uptake between immobilized cell and free cell cyanobacteria

The selected cyanobacterial strains at 0.015, 0.030, 0.045 and 0.060 ±1 g dry wt. were centrifuged and cell were suspended in 130 ml of 1 mg/l cadmium solution at pH 7 in both immobilized and free cell system. The cyanobacteria were shaken in the rotary shaker 100 rpm for 14 days. The cell-free supernatant were sampled at 0, 5, 10, 30, 60, 90 min and 2, 4, 6, 8, 10 and 14 days. The immobilized cell, non-immobilized cell and free cell of cyanobacteria were separated by filtration with nylon sieve pore size 50 µm. Cadmium uptake (mg Cd/g dry wt.), immobilized ability (%) and dry weight per surface area (DW/SA) were calculated as described previously.

3.6.9 Effect of initial cadmium concentrations on cadmium uptake between immobilized cell and free cell cyanobacteria

The selected cyanobacterial strains at selected cell concentration (from section 3.6.8) were centrifuged and suspended in 130 ml at pH 7 in various initial cadmium concentrations at 0.5, 1, 2, 4 and 8 mg/l in both immobilized cell system and free cell system. The experiment were performed the same as in section 3.6.7. Cadmium uptake (mg Cd/g dry wt.), immobilized ability (%) and dry weight per surface area (DW/SA) were determined as described previously, the maximum adsorption capacity (q_{max}) and binding constant (k_b) were calculated by using Langmuir equation as follows :

$$q = \frac{q_{max} C_f}{k_b + C_f}$$

- Where q is the cadmium adsorbed to the solid phase (mg/g dry wt.)
 q_{max} is the maximum adsorption capacity (mg/g dry wt.)
 k_b is the binding constant (mg/l)
 C_f is the final (equilibrium) concentration of cadmium in solution (mg/l)

3.6.11 Effect of cell ages on cadmium uptake between immobilized cell and free cell cyanobacteria

The selected cyanobacterial strains were harvested for cell ages at 5, 10, 15, 20 days. The optimum cell concentration (from section 3.6.8) were suspended in 130 ml of optimum cadmium concentration (from section 3.6.9) at pH 7 in the immobilized cell system and free cell system. The experiment were performed the same as in section 3.6.7. Cadmium uptake (mg Cd/g dry wt.), immobilized ability (%) and dry weight per surface area (DW/SA) were calculated as described previously.



Figure 3 Cultivation systems of cyanobacteria in 125 ml of Medium 18 in Erlenmeyer flask size 250 ml in rotary shaker 100 rpm at room temperature with light intensity of 4,000 lux by illumination of with cool white fluorescence lamp at 12 hours dark and 12 hours light interval.

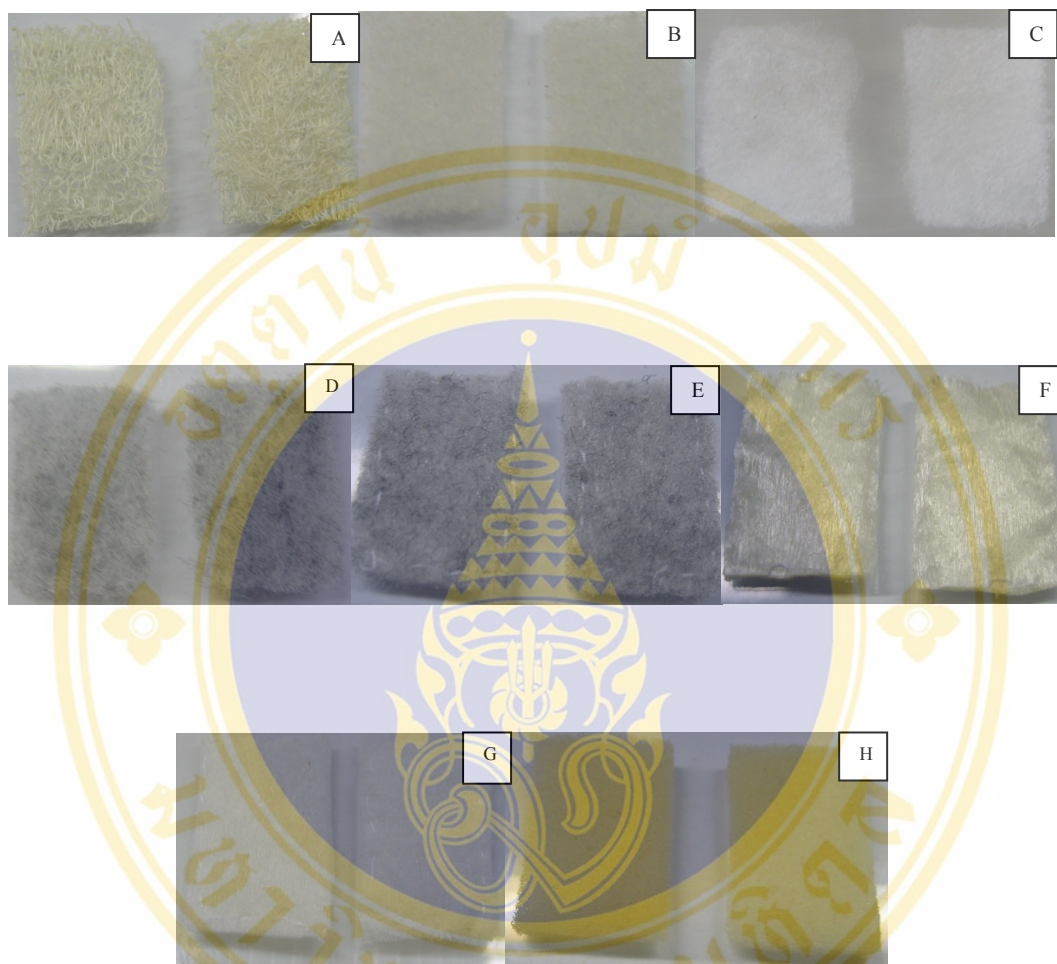
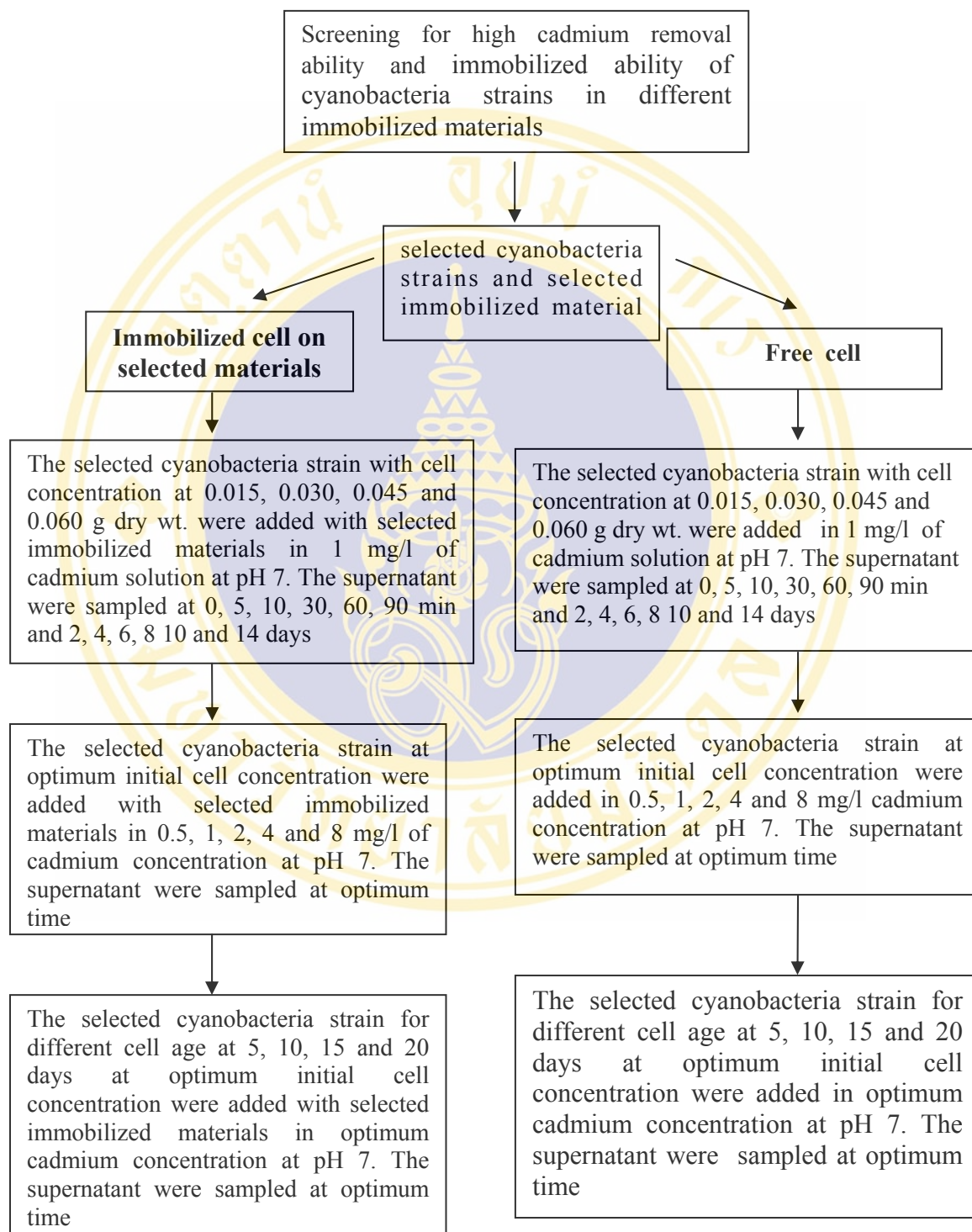


Figure 4 Characteristics of immobilized materials using in this experiments such as loofa sponge (A), delicate duty scour pad (B), synthetic fiber (C), polyethylene terephthalate (D), polyethylene terephthalate (non-woven fabric) (E), polyethylene (F), polypropylene (G) and polyurethane (H).

3.7. Research diagram



3.8 Statistic analysis

This research used descriptive statistic for data analysis as mean, percentage and standard deviation for calculating cadmium removal (%), immobilized ability (%), cadmium uptake (mg Cd/g dry wt.) and cell growth.



CHAPTER IV

RESULTS

4.1 Screening for high cadmium removal and high immobilized ability cyanobacterial strains in different immobilized materials

4.1.1 Cadmium removal (%)

In this study, four strains of cyanobacteria were tested for cadmium removal at 1 mg/l of initial cadmium concentration as shown in Figure 5. From the results found that immobilized cell on loofa sponge (LS), delicate duty scour pad (DP), synthetic fiber (SF), polyethylene terephthalate (PET), polyethylene terephthalate (PET non-woven fabric), polyethylene (PE), polypropylene (PP), polyurethane (PU) and free cell of *Scytonema* sp. had cadmium removal at $62.17 \pm 3.17\%$, $79.43 \pm 4.03\%$, $93.31 \pm 0.21\%$, $81.93 \pm 2.02\%$, $91.77 \pm 1.13\%$, $69.14 \pm 3.36\%$, $74.79 \pm 7.08\%$, $71.33 \pm 6.41\%$ and $94.80 \pm 3.08\%$, respectively. While the immobilized cell on LS, DP, SF, PET, PET non-woven fabric, PE, PP, PU and free cell of *Oscillatoria agardhi* had cadmium removal at $54.14 \pm 2.36\%$, $69.54 \pm 4.10\%$, $74.43 \pm 4.69\%$, $72.56 \pm 1.90\%$, $70.34 \pm 2.93\%$, $63.30 \pm 3.67\%$, $57.69 \pm 6.18\%$, $57.30 \pm 5.64\%$ and $95.30 \pm 2.19\%$, respectively.

The immobilized cell on LS, DP, SF, PET, PET non-woven fabric, PE, PP, PU and free cell of *Mastigocladus* sp. had cadmium removal at $31.25 \pm 4.32\%$, $67.90 \pm 7.67\%$, $81.34 \pm 6.95\%$, $80.02 \pm 9.49\%$, $76.37 \pm 2.68\%$, $56.30 \pm 6.20\%$, $49.46 \pm 1.73\%$, $66.03 \pm 6.47\%$ and $75.35 \pm 1.43\%$, respectively. While the immobilized cell on LS, DP, SF, PET, PET non-woven fabric, PE, PP, PU and free cell of *Hapalosiphon hibernicus* had cadmium removal at $51.63 \pm 1.73\%$, $75.71 \pm 3.33\%$, $85.14 \pm 1.76\%$, $83.42 \pm 3.03\%$, $77.69 \pm 6.22\%$, $75.66 \pm 1.34\%$, $71.74 \pm 2.91\%$, $66.75 \pm 6.45\%$ and $98.32 \pm 0.20\%$, respectively. In four strains of cyanobacteria, immobilized cell on SF of *Scytonema* sp. and free cell of *H. hibernicus* had the highest cadmium removal and followed by free cell of *O. agardhi* and immobilized cell on PET of *Scytonema* sp., respectively. While immobilized cell on LS of *Mastigocladus* sp. had the lowest cadmium removal.

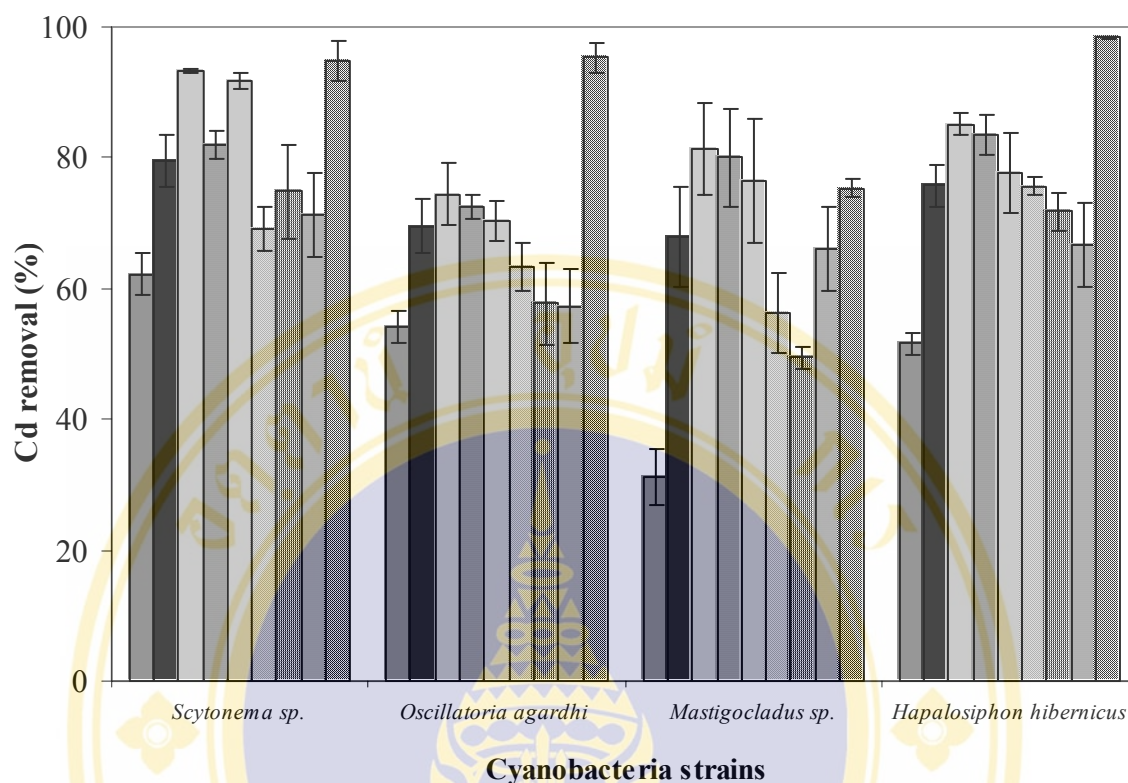


Figure 5 Cadmium removal between immobilized cell on loofa sponge (■), delicate duty scour pad (■), synthetic fiber (■), polyethylene terephthalate (■), polyethylene terephthalate (non-woven fabric) (■), polyethylene (■), polypropylene (■), polyurethane (■) and free cell (■) in four cyanobacteria strains. Cell were suspended in 130 ml of 1 mg/l cadmium solution and mixed slowly in rotary shaker at 100 rpm. The supernatant were sampled for 1 ml in 10 days cultivation.

4.1.2 Immobilized ability and dry weight per surface area (DW/SA)

Immobilized ability of immobilized cell on eight types of immobilized materials were shown in Table 3. The results found that immobilized ability on LS was the highest at 96.17% and followed by immobilized ability on PET, SF, DP, PET non-woven fabric, PP, PU, and PE of *Scytonema sp.* at 96.00%, 94.79%, 94.51%, 92.37%, 85.71%, 82.58.% and 62.53%, respectively. While immobilized cell on PP had highest immobilized ability at 96.46% and followed by SF, DP, PU, PET, PET non-woven fabric, PE and LS of *O. agardhi* at 94.91%, 94.77%, 89.98%, 81.24%, 69.92%, 60.33%, and 48.15%, respectively.

Table 3 Immobilized ability of immobilized cell in various immobilized materials in four cyanobacterial strains

Types of immobilized materials	Immobilized ability (%)			
	<i>Scytonema</i> sp.	<i>O. agardhi</i>	<i>Mastigocladus</i> sp.	<i>H.hibernicus</i>
Loofa sponge (LS)	96.17	48.15	68.99	57.38
Delicate duty scour pad (DP)	94.51	94.77	86.11	94.35
Synthetic fiber (SF)	94.79	94.91	92.02	97.45
Polyethylene terephthalate (PET)	96.00	81.24	90.99	86.80
Polyethylene terephthalate (PET non-woven fabric)	92.37	69.92	89.38	88.97
Polyethylene (PE)	62.53	60.33	85.12	56.36
Polypropylene (PP)	85.71	96.46	90.52	92.22
Polyurethane (PU)	82.58	89.98	81.62	89.69

Immobilized cell on SF had the highest immobilized ability at 92.02% and followed by PET, PP, PET non-woven fabric, DP, PE, PU and LS of *Mastigocladus* sp. at 90.99%, 90.52%, 89.38%, 86.11%, 85.12%, 81.62% and 68.99%, respectively. While immobilized cell on SF had the highest immobilized ability at 97.45% and followed by DP, PP, PU, PET non-woven fabric, PET, LS and PE of *H. hibernicus* had immobilized ability at 94.35%, 92.22%, 89.69%, 88.97%, 86.80%, 57.38% and 56.36%, respectively.

The dry weight per surface area ratio (DW/SA) of immobilized cell on eight types of immobilized materials in four strains of cyanobacteria were shown in Table 4. From the result found that dry weight per surface area of immobilized cell on LS, DP, SF, PET, PET non-woven fabric, PE, PP and PU of *Scytonema* sp., *O. agardhi*, *Mastigocladus* sp. and *H. hibernicus* were similar ratio.

Table 4 Dry weight per surface area ratio (DW/SA) in various immobilized cell materials of four strains cyanobacterial strains

Types of immobilized materials	DW/SA (gcell/cm ²)			
	<i>Scytonema</i> sp.	<i>O. agardhi</i>	<i>Mastigocladus</i> sp.	<i>H. hibernicus</i>
Loofa sponge (LS)	0.0061	0.0010	0.0009	0.0008
Delicate duty scour pad (DP)	0.0027	0.0018	0.0010	0.0015
Synthetic fiber (SF)	0.0023	0.0018	0.0019	0.0023
Polyethylene terephthalate (PET)	0.0033	0.0025	0.0015	0.0017
Polyethylene terephthalate (PET non-woven fabric)	0.0032	0.0020	0.0016	0.0021
Polyethylene (PE)	0.0018	0.0008	0.0009	0.0014
Polypropylene (PP)	0.0034	0.0019	0.0014	0.0021
Polyurethane (PU)	0.0015	0.0015	0.0010	0.0023

The immobilized cell on synthetic fiber (SF) and polyethylene terephthalate (PET) of *Scytonema* sp. and *H. hibernicus* were selected for further study because of had high cadmium removal, immobilized ability and dry weight per surface area. Moreover, *Scytonema* sp. and *H. hibernicus* were filamentous cyanobacteria which had high growth rate, easy to separate from solution and suitable for application in outdoor cultivation system (Figure 6). The immobilized material as synthetic fiber (SF) and polyethylene terephthalate (PET) were suitable because of low cost sustainable sourcing and high porosity from the observation by scanning electron microscope (SEM) (Figure 7 and Figure 34-35).

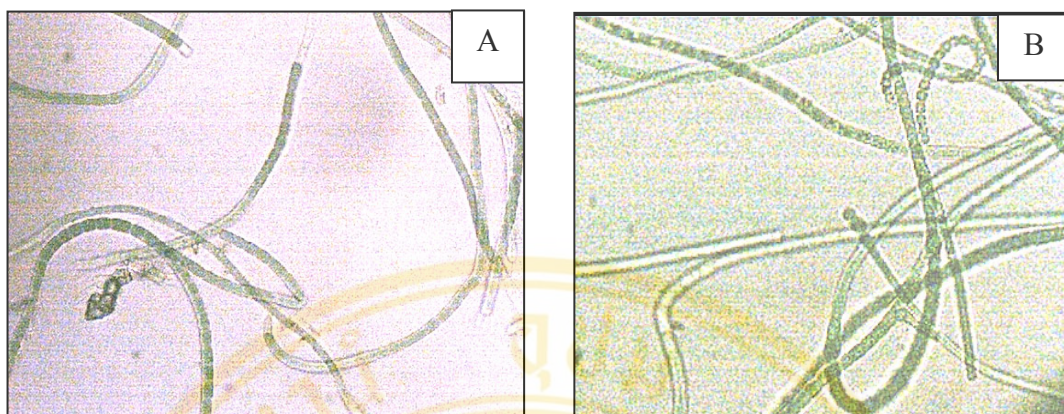


Figure 6 Morphology of *Scytonema* sp. and *H. hibernicus* cultivated in Medium 18 for 10 days under microscope (magnification X 40)

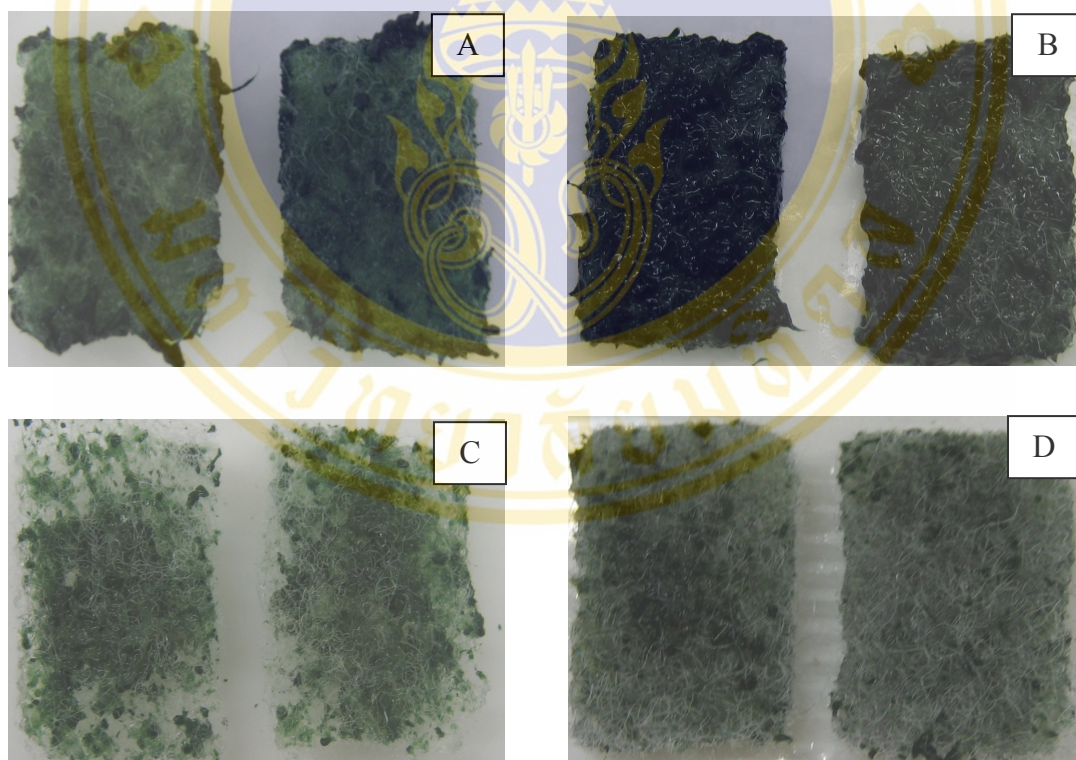


Figure 7 Characteristics of immobilized cell on synthetic fiber (SF) and polyethylene terephthalate (PET) of *Scytonema* sp. (A and B) and *H. hibernicus* (C and D)

4.2 Effect of initial cell concentrations and contact time on cadmium uptake between immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus*

4.2.1 Cadmium uptake in *Scytonema* sp.

Time course experiment in initial cell concentration at 0.015, 0.030, 0.045 and 0.060 ± 1 g dry wt. were studied in short period. Immobilized cell on SF of *Scytonema* sp. had rapidly increased cadmium uptake in 15 min and became constant after 30 min. Initial cell concentration at 0.030 g dry wt. had highest cadmium uptake at 2.37 mg Cd/g dry wt. and followed by initial cell concentration at 0.015, 0.060 and 0.045 g dry wt. at 2.09 mg Cd/g dry wt., 1.85 mg Cd/g dry wt. and 1.66 mg Cd/g dry wt., respectively as shown in Figure 8.

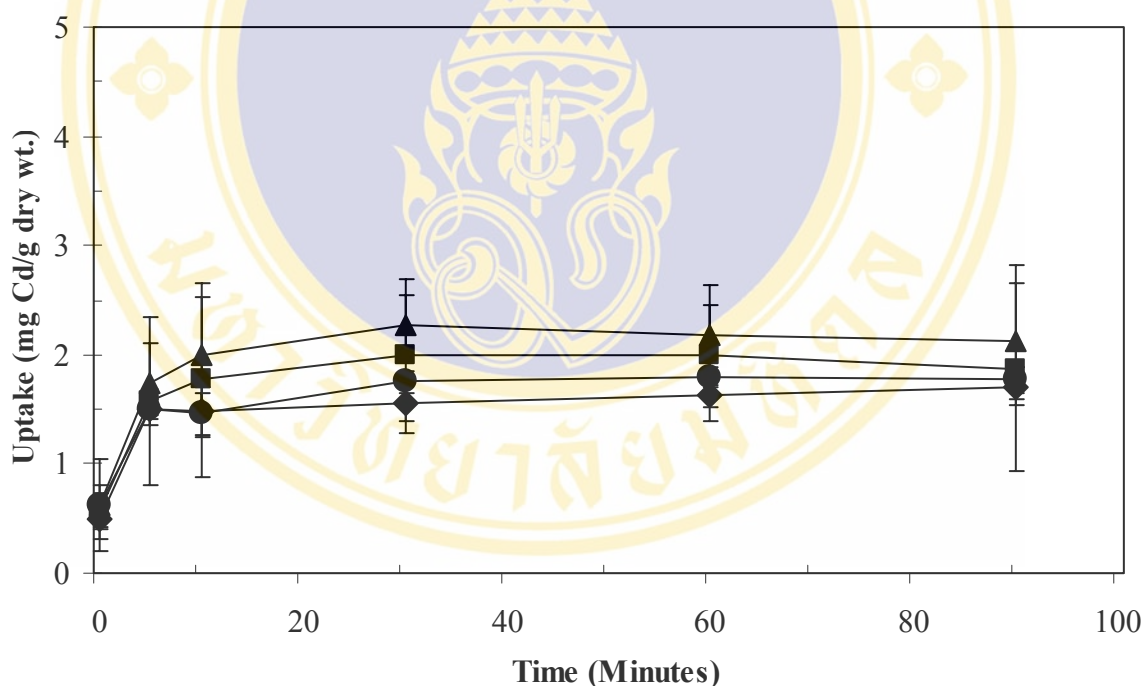


Figure 8 Time course of cadmium uptake in immobilized cell on synthetic fiber (SF) of *Scytonema* sp. at cell concentration 0.015 g dry wt. (■), cell concentration 0.030 g dry wt. (▲), cell concentration 0.045 g dry wt. (◆) and cell concentration 0.060 g dry wt. (●). Cell were suspended in 130 ml of 1 mg/l cadmium solution and mixed slowly in rotary shaker at 100 rpm. The supernatant was sampled for 1 ml at 0, 5, 10, 30, 60 and 90 min.

Immobilized cell on PET of *Scytonema* sp. in all initial cell concentrations had rapidly increased cadmium uptake in 15 min and became constant after 30 min. Initial cell concentration at 0.015 g dry wt. had highest cadmium uptake at 3.67 mg Cd/g dry wt. and followed by initial cell concentration at 0.030, 0.045 and 0.060 g dry wt. at 2.12 mg Cd/g dry wt., 1.89 mg Cd/g dry wt. and 1.62 mg Cd/g dry wt., respectively as shown in Figure 9.

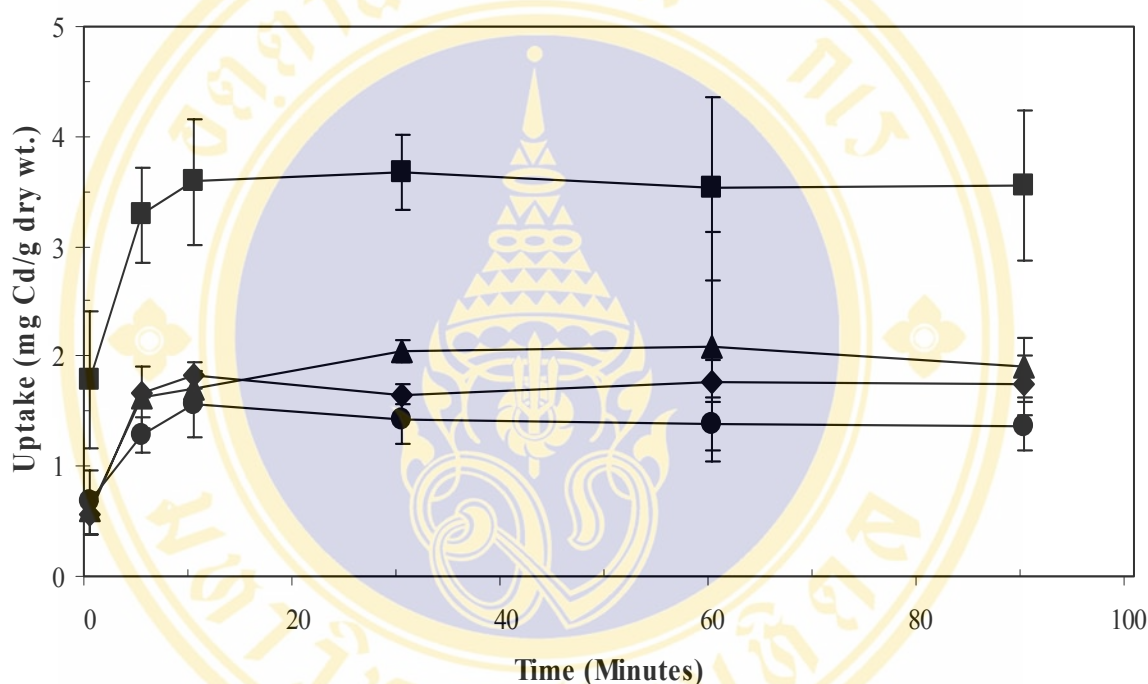


Figure 9 Time course of cadmium uptake in immobilized cell on polyethylene teraphthalate (PET) of *Scytonema* sp. at cell concentration 0.015 g dry wt. (■), cell concentration 0.030 g dry wt. (▲), cell concentration 0.045 g dry wt. (◆) and cell concentration 0.060 g dry wt. (●). Cell were suspended in 130 ml of 1 mg/l cadmium solution and mixed slowly in rotary shaker at 100 rpm. The supernatant was sampled for 1 ml at 0, 5, 10, 30, 60 and 90 minutes.

Free cell of *Scytonema* sp. in all initial cell concentration had rapidly increased cadmium uptake in 15 min and became constant after 30 min. Initial cell concentration at 0.015 g dry wt. had highest cadmium uptake at 1.45 mg Cd/g dry wt. and followed by initial cell concentration at 0.030, 0.045 and 0.060 g dry wt. at 1.28 mg Cd/g dry

wt., 1.17 mg Cd/g dry wt. and 1.12 mg Cd/g dry wt, respectively after that 90 min as shown in Figure 10.

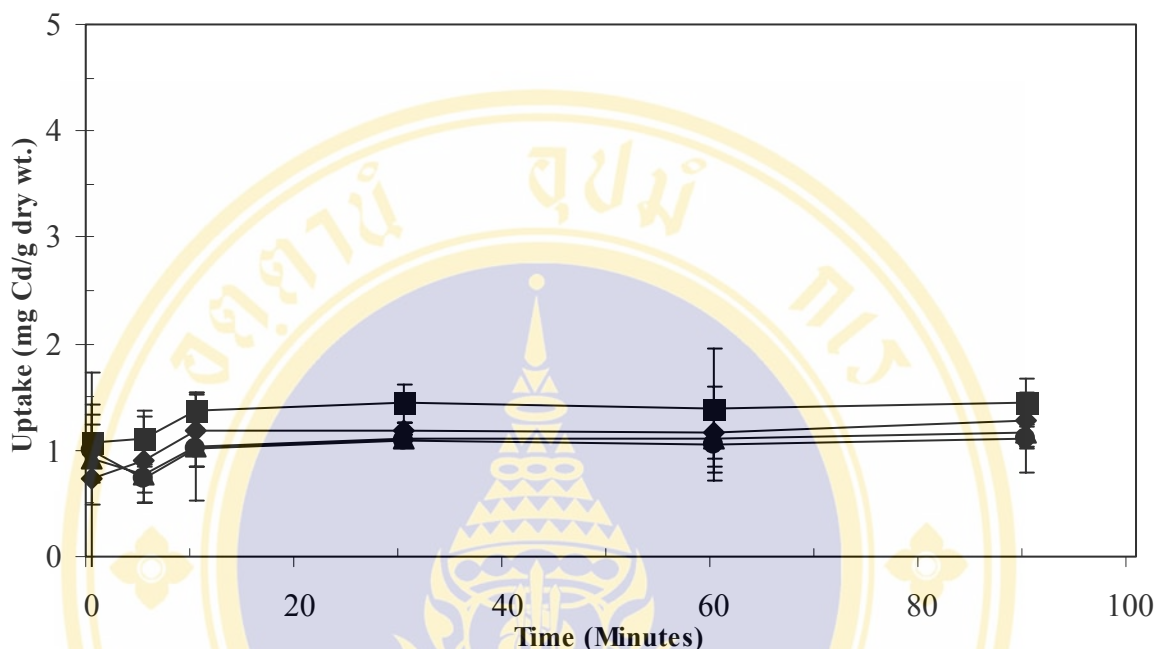


Figure 10 Time course of cadmium uptake in free cell of *Scytonema* sp. at cell concentration 0.015 g dry wt. (-■-), cell concentration 0.030 g dry wt. (-▲-), cell concentration 0.045 g dry wt. (-◆-) and cell concentration 0.060 g dry wt. (-●-). Cell were suspended in 130 ml of 1 mg/l cadmium solution and mixed slowly in rotary shaker at 100 rpm. The supernatant was sampled for 1 ml at 0, 5, 10, 30, 60 and 90 minutes.

After continue growing two strains of cyanobacteria until 14 day, cadmium uptake in immobilized cell on SF of *Scytonema* sp. was increased from 3.24 mg Cd/g dry wt. to 4.24 mg Cd/g dry wt. and from 3.24 mg Cd/g dry wt. to 3.64 mg Cd/g dry wt. from 2 day until 6 day and became constant at initial cell concentration 0.015 g dry wt. and 0.030 g dry wt., respectively. While the cadmium uptake was slightly increased and became constant after 2 day at 2.26 mg Cd/g dry wt. and 2.16 mg Cd/g dry wt. at initial cell concentration 0.045 g dry wt. and 0.060 g dry wt., respectively as shown in Figure 11.

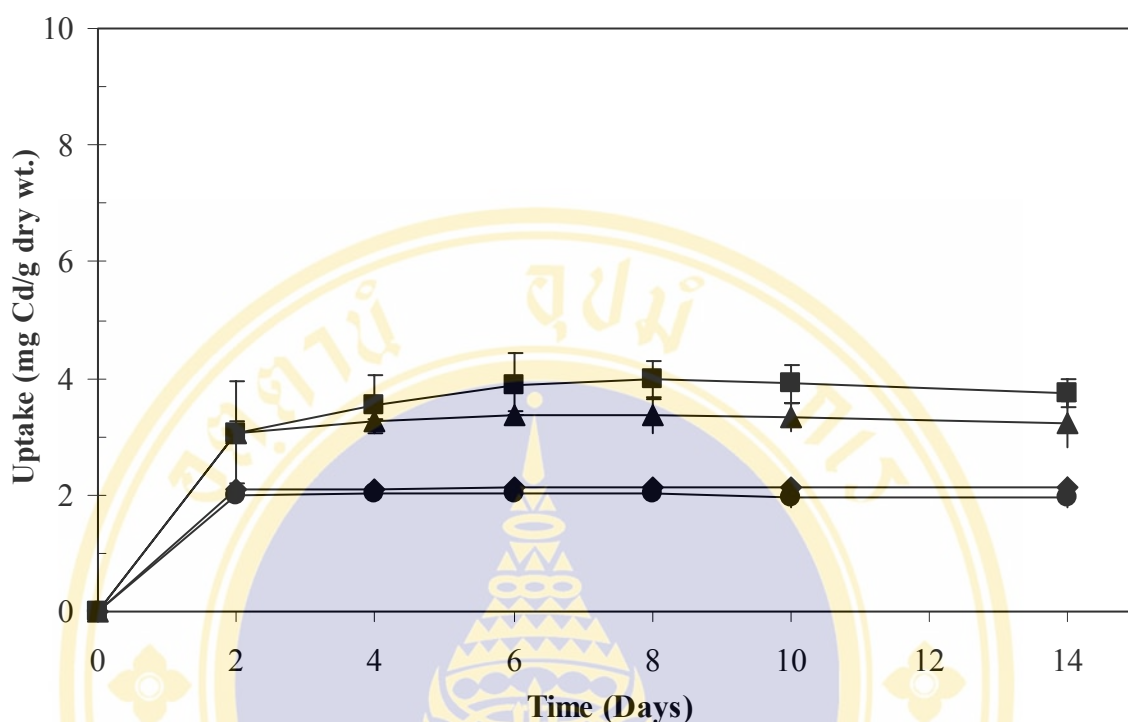


Figure 11 Time course of cadmium uptake in immobilized cell on synthetic fiber (SF) of *Scytonema* sp. at cell concentration 0.015 g dry wt. (■), cell concentration 0.030 g dry wt. (▲), cell concentration 0.045 g dry wt. (◆) and cell concentration 0.060 g dry wt. (●). Cell were suspended in 130 ml of 1 mg/l cadmium solution and mixed slowly by rotary shaker at 100 rpm. The supernatant was sampled for 1 ml at 2, 4, 6, 8, 10 and 14 days.

Cadmium uptake in immobilized cell of *Scytonema* sp. on PET was increased from 7.29 mg Cd/g dry wt. to 7.46 mg Cd/g dry wt., 2.88 mg Cd/g dry wt. to 3.33 mg Cd/g dry wt., 2.24 mg Cd/g dry wt. to 2.54 mg Cd/g dry wt. and 1.83 mg Cd/g dry wt. to 1.97 mg Cd/g dry wt. from 2 day until 6 day and became constant at initial cell concentrations 0.015, 0.030, 0.045 and 0.060 g dry wt., respectively as shown in Figure 12.

In free cell of *Scytonema* sp. had the same profile of cadmium uptake as immobilized cell on SF and PET. The cadmium uptake was slightly increased from 2.73 mg Cd/g dry wt. to 3.55 mg Cd/g dry wt., 2.36 mg Cd/g dry wt. to 2.77 mg Cd/g dry wt., 1.90 mg Cd/g dry wt. to 2.37 mg Cd/g dry wt. and 1.75 mg Cd/g dry wt. to 1.95 mg Cd/g dry wt. from 2 day until 6 day and became constant at initial cell concentration 0.015, 0.030, 0.045 and 0.060 g dry wt., respectively as shown in Figure 13.

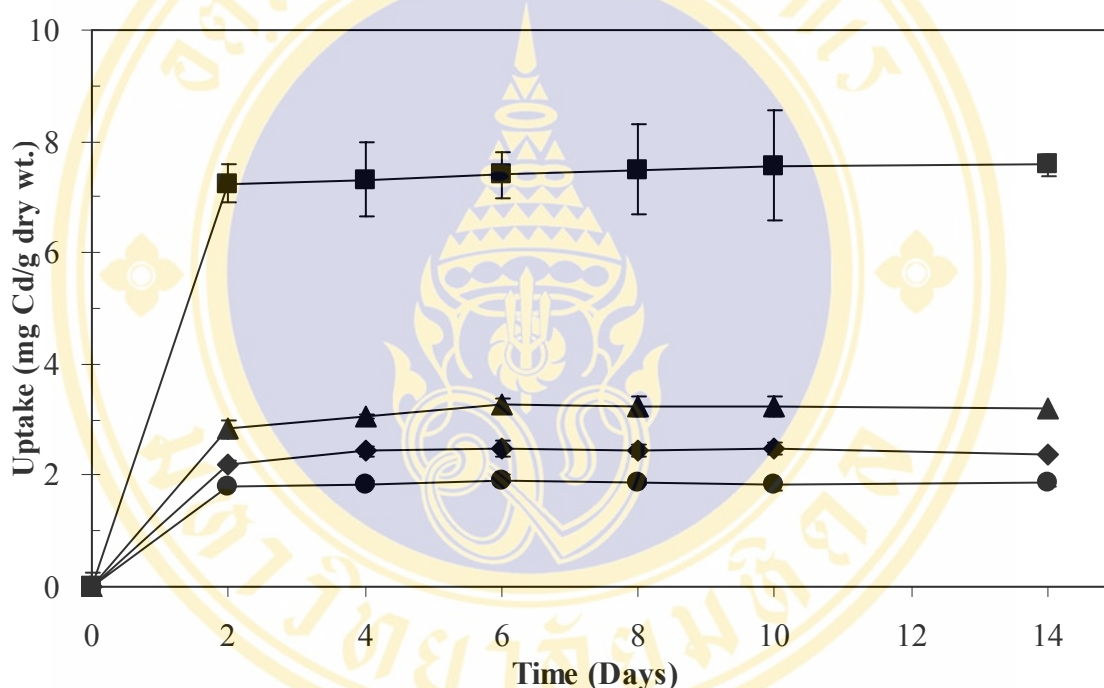


Figure 12 Time course of cadmium uptake in immobilized cell on polyethylene teraphthalate (PET) of *Scytonema* sp. at cell concentration 0.015 g dry wt. (■), cell concentration 0.030 g dry wt. (▲), cell concentration 0.045 g dry wt. (◆) and cell concentration 0.060 g dry wt. (●). Cell were suspended in 130 ml of 1 mg/l cadmium solution and mixed slowly in rotary shaker at 100 rpm. The supernatant was sampled for 1 ml at 2, 4, 6, 8, 10 and 14 days.

In 14 day cultured, the cadmium uptake was decreased when the initial cell concentration increased. Immobilized cell of *Scytonema* sp. on PET had highest cadmium uptake at 7.64 mg Cd/g dry wt. and followed by immobilized cell on SF and free cell were 4.05 mg Cd/g dry wt., 3.56 mg Cd/g dry wt. at initial cell concentration 0.015 g dry wt., respectively. Immobilized cell on SF and PET had higher cadmium uptake than free cell at 3.53 mg Cd/g dry wt., 3.26 mg Cd/g dry wt., 2.84 mg Cd/g dry wt. at initial cell concentration 0.030 g dry wt. and 2.47 mg Cd/g dry wt., 2.43 mg Cd/g dry wt., 2.33 mg Cd/g dry wt. at initial cell concentration 0.045 g dry wt. and 2.25 mg Cd/g dry wt., 1.93 mg Cd/g dry wt., 1.82 mg Cd/g dry wt. at initial cell concentration 0.060 g dry wt., respectively are shown in Figure 14.

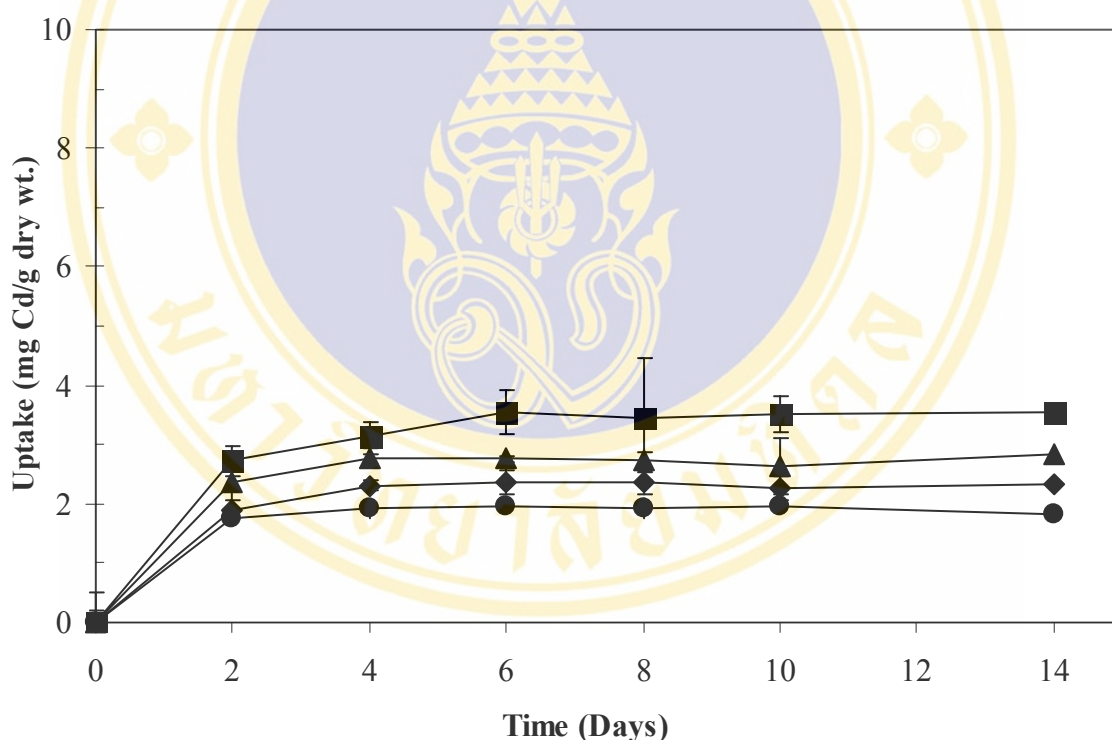


Figure 13 Time course of cadmium uptake in free cell of *Scytonema* sp. at cell concentration 0.015 g dry wt. (■-), cell concentration 0.030 g dry wt. (▲-), cell concentration 0.045 g dry wt. (◆-) and cell concentration 0.060 g dry wt. (●-) Cell were suspended in 130 ml of 1 mg/l cadmium solution and mixed slowly by rotary shaker at 100 rpm. The supernatant was sampled for 1 ml at 2, 4, 6, 8, 10 and 14 days.

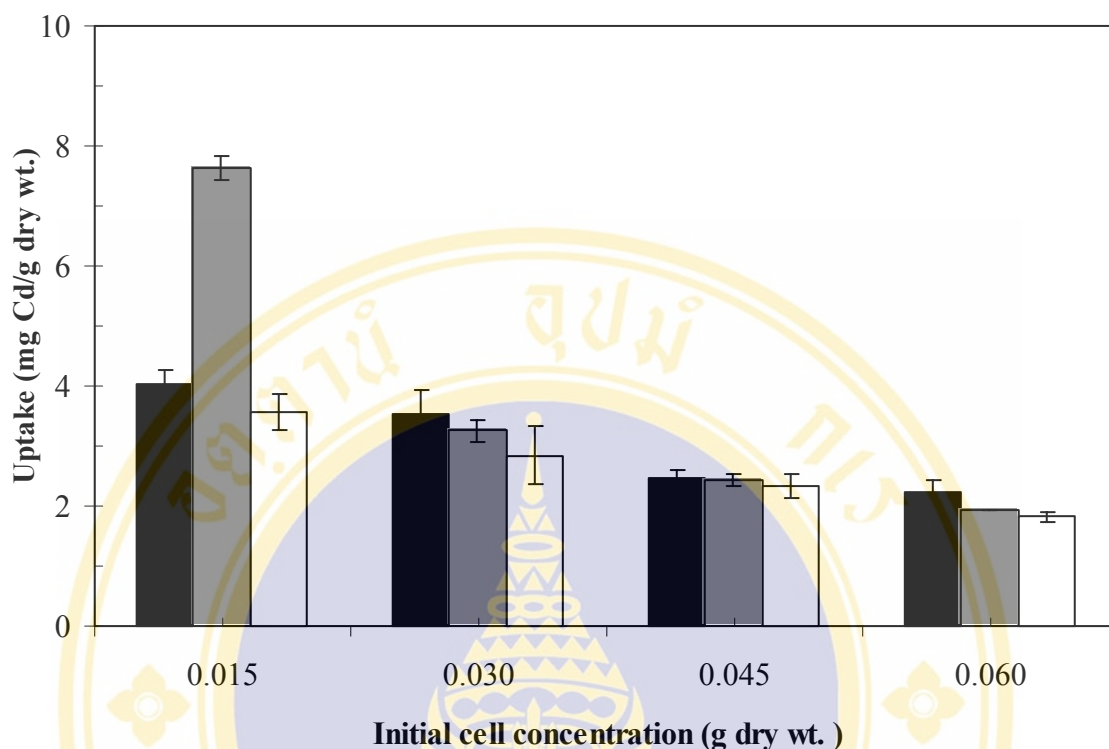


Figure 14 Cadmium uptake between immobilized cell on synthetic fiber (SF) (■), polyethylene terephthalate (PET) (▨) and free cell (□) of *Scytonema* sp. at cell concentration 0.015, 0.030, 0.045 and 0.060 g dry wt. Cell were suspended in 130 ml of 1 mg/l of cadmium solution mixed slowly in rotary shaker at 100 rpm for 14 days.

4.2.2 Cadmium uptake in *H. hibernicus*

The time course experiment were studied in the short period, initial cell concentrations at 0.015, 0.030, 0.045 and 0.060±1 g dry wt. in immobilized cell on SF of *H. hibernicus* had rapidly increased cadmium uptake in 15 min and became constant after 90 min. Initial cell concentration at 0.0015g dry wt. had the highest cadmium uptake at 4.18 mg Cd/g dry wt. and followed by initial cell concentration at 0.045, 0.030 and 0.060 g dry wt. at 3.26 mg Cd/g dry wt., 3.08 mg Cd/g dry wt. and 2.43 mg Cd/g dry wt., respectively are shown in Figure 15.

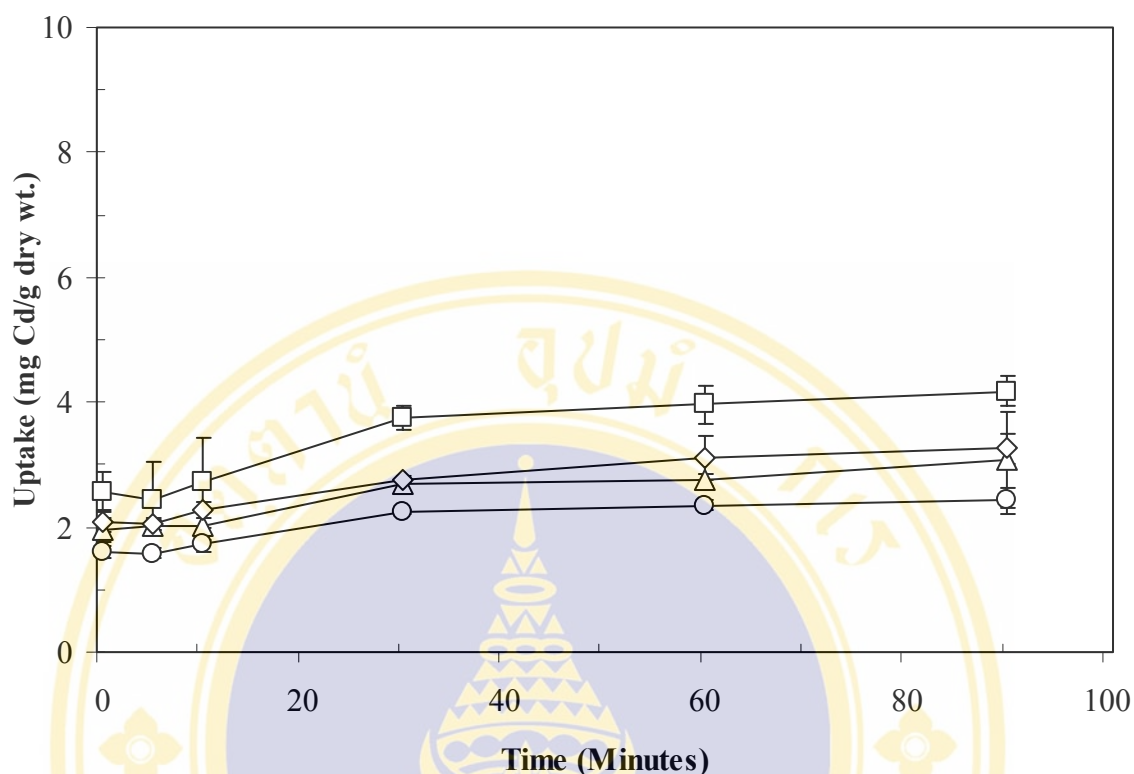


Figure 15 Time course of cadmium uptake in immobilized cell on synthetic fiber (SF) of *H. hibernicus* at cell concentration 0.015 g dry wt. (□), cell concentration 0.030 g dry wt. (△), cell concentration 0.045 g dry wt. (◇), cell concentration 0.060 g dry wt. (○). Cell were suspended in 130 ml of 1 mg/l cadmium solution and mixed slowly in rotary shaker at 100 rpm. The supernatant was sampled for 1 ml at 0, 5, 10, 30, 60 and 90 minutes.

Immobilized cell on PET of *H. hibernicus* in all initial cell concentrations had rapidly increased cadmium uptake in 15 min and continuously increased until became constant after 90 min. At initial cell concentration 0.015 g dry wt. had highest cadmium uptake at 4.38 mg Cd/g dry wt. and followed by initial cell concentration at 0.045, 0.030 and 0.060 g dry wt. at 3.07 mg Cd/g dry wt., 2.92 mg Cd/g dry wt. and 2.19 mg Cd/g dry wt., respectively as shown in Figure 16.

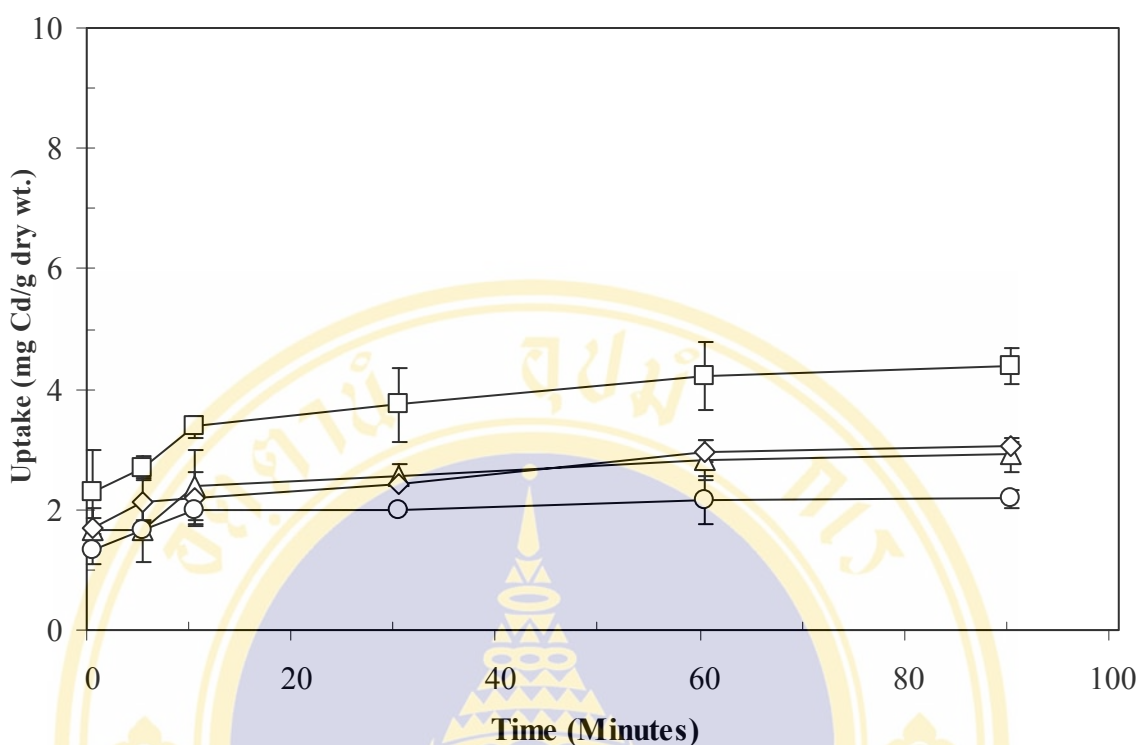


Figure 16 Time course of cadmium uptake in immobilized cell on polyethylene terephthalate (PET) of *H. hibernicus* at cell concentration 0.015 g dry wt. (-□-), cell concentration 0.030 g dry wt. (-△-), cell concentration 0.045 g dry wt. (-◇-), cell concentration 0.060 g dry wt. (-○-). Cell were suspended in 130 ml of 1 mg/l cadmium solution and mixed slowly in rotary shaker at 100 rpm. The supernatant was sampled for 1 ml at 0, 5, 10, 30, 60 and 90 minutes.

Free cell of *H. hibernicus* had the same profile of cadmium uptake as immobilized cell on SF and PET. In all initial cell concentration had rapidly increased cadmium uptake in 15 min and continuously increased until became constant after 90 min. Initial cell concentration at 0.015 g dry wt. had the highest cadmium uptake at 3.62 mg Cd/g dry wt. and followed by initial cell concentration at 0.030, 0.045 and 0.060 g dry wt. at 2.80 mg Cd/g dry wt., 2.72 mg Cd/g dry wt., and 2.37 mg Cd/g dry wt., respectively as shown in Figure 17.

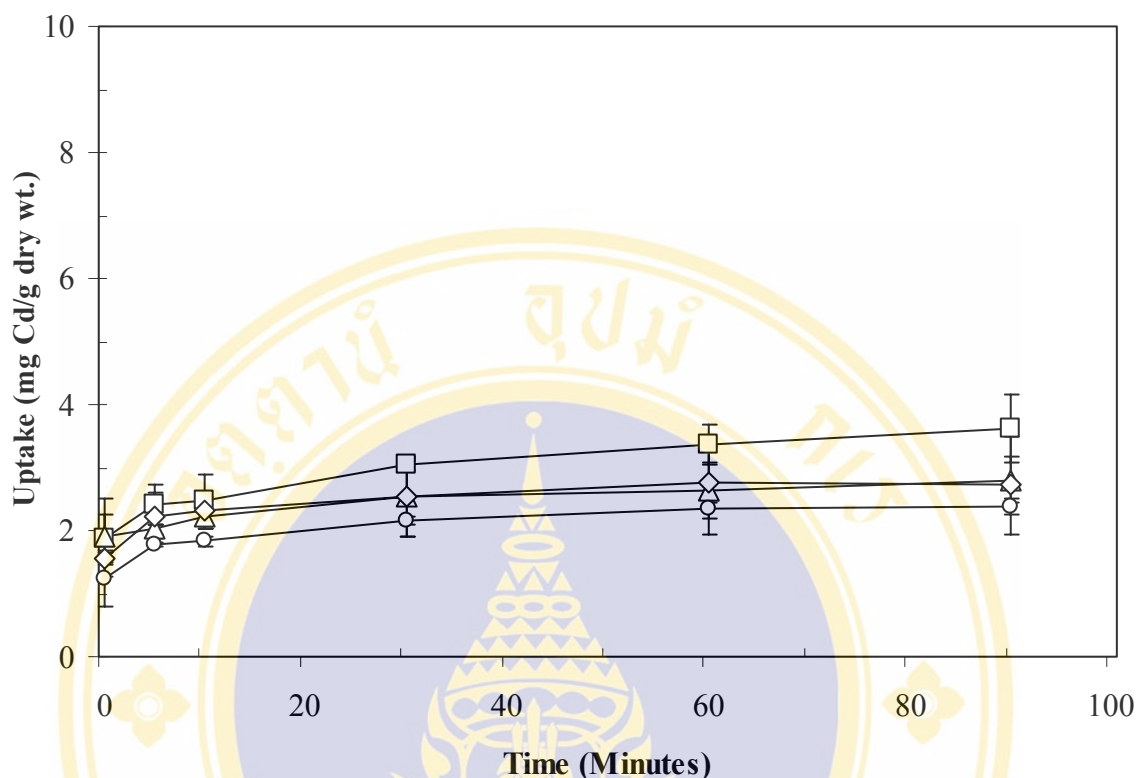


Figure 17 Time course of cadmium uptake in free cell of *H. hibernicus*. at cell concentration 0.015 g dry wt. (-□-), cell concentration 0.030 g dry wt. (-△-), cell concentration 0.045 g dry wt. (-◇-), cell concentration 0.060 g dry wt. (-○-). Cell were suspended in 130 ml of 1 mg/l cadmium solution and mixed slowly in rotary shaker at 100 rpm. The supernatant was sampled for 1 ml at 0, 5, 10, 30, 60 and 90 min.

After continued growing two strains of cyanobacteria until 14 day, cadmium uptake in immobilized cell on SF of *H. hibernicus* was increased from 4.89 mg Cd/g dry wt. to 5.51 mg Cd/g dry wt. and from 4.10 mg Cd/g dry wt. to 4.32 mg Cd/g dry wt. from 2 day until 6 day and became constant at initial cell concentration 0.015 g dry wt. and 0.030 g dry wt., respectively. They had slightly increased and became constant after 2 day at 4.53 mg Cd/g dry wt. and 3.26 mg Cd/g dry wt. at initial cell concentration 0.045 g dry wt. and 0.060 g dry wt., respectively as shown in Figure 18.

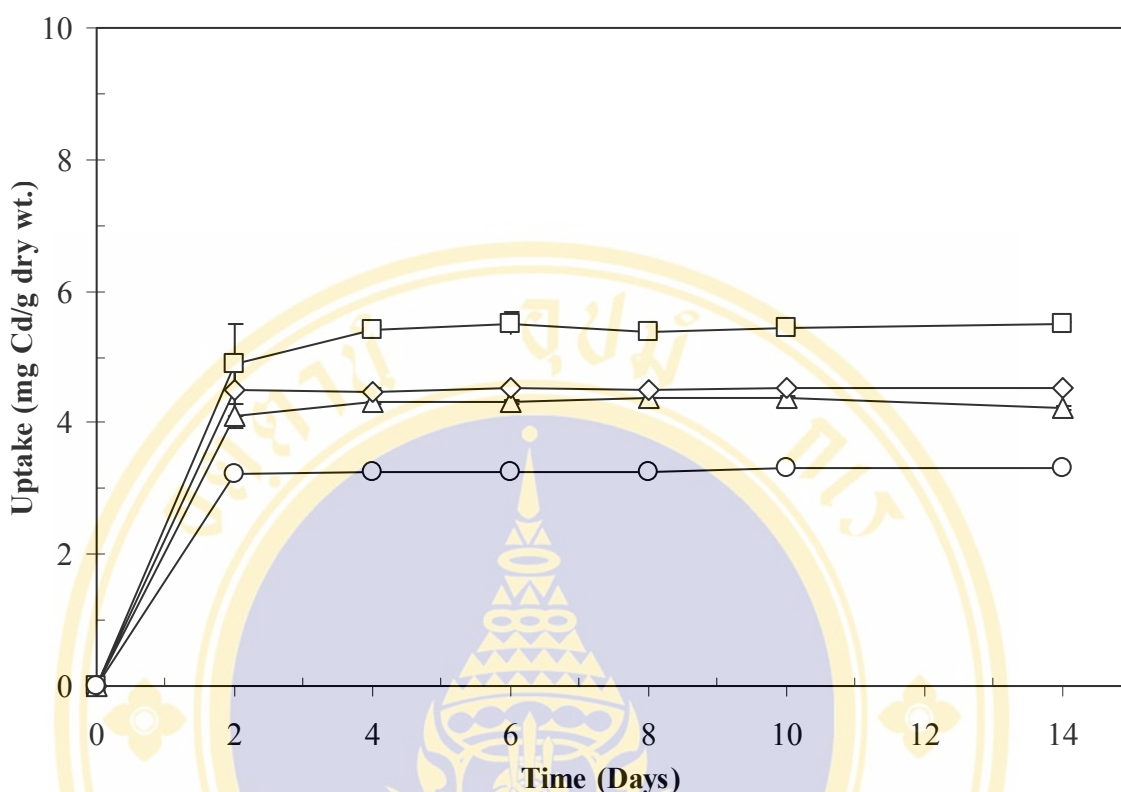


Figure 18 Time course of cadmium uptake in immobilized cell on synthetic fiber (SF) of *H. hibernicus* at cell concentration 0.015 g dry wt. (□-), cell concentration 0.030 g dry wt. (△-), cell concentration 0.045 g dry wt. (◇-), cell concentration 0.060 g dry wt. (○-). Cells were suspended in 130 ml of 1 mg/l cadmium solution and mixed slowly in rotary shaker at 100 rpm. The supernatant was sampled for 1 ml at 2, 4, 6, 8, 10 and 14 days.

Cadmium uptake in immobilized cells on PET of *H. hibernicus* was increased from 6.62 mg Cd/g dry wt. to 7.00 mg Cd/g dry wt., 4.28 mg Cd/g dry wt. to 4.44 mg Cd/g dry wt. from 2 days until 6 days and became constant at initial cell concentrations 0.015 and 0.030 g dry wt., respectively, and slightly increased and became constant after 2 days at 4.07 mg Cd/g dry wt. and 3.15 mg Cd/g dry wt. at initial cell concentrations 0.045 and 0.060 g dry wt., respectively, as shown in Figure 19.

In free cells of *H. hibernicus*, cadmium uptake had the same pattern in the immobilized cells on SF and PET. The cadmium uptake had increased from 5.78 mg Cd/g dry wt. to 5.87 mg Cd/g dry wt. and from 4.09 mg Cd/g dry wt. to 4.35 mg Cd/g dry wt. from 2 days until 6 days and became constant at initial cell concentration 0.015

and 0.030 g dry wt., respectively and slightly increased and became constant after 2 day at 3.93 mg Cd/g dry wt. and 3.22 mg Cd/g dry wt. at initial cell concentration 0.045 and 0.060 g dry wt., respectively as shown in Figure 20.

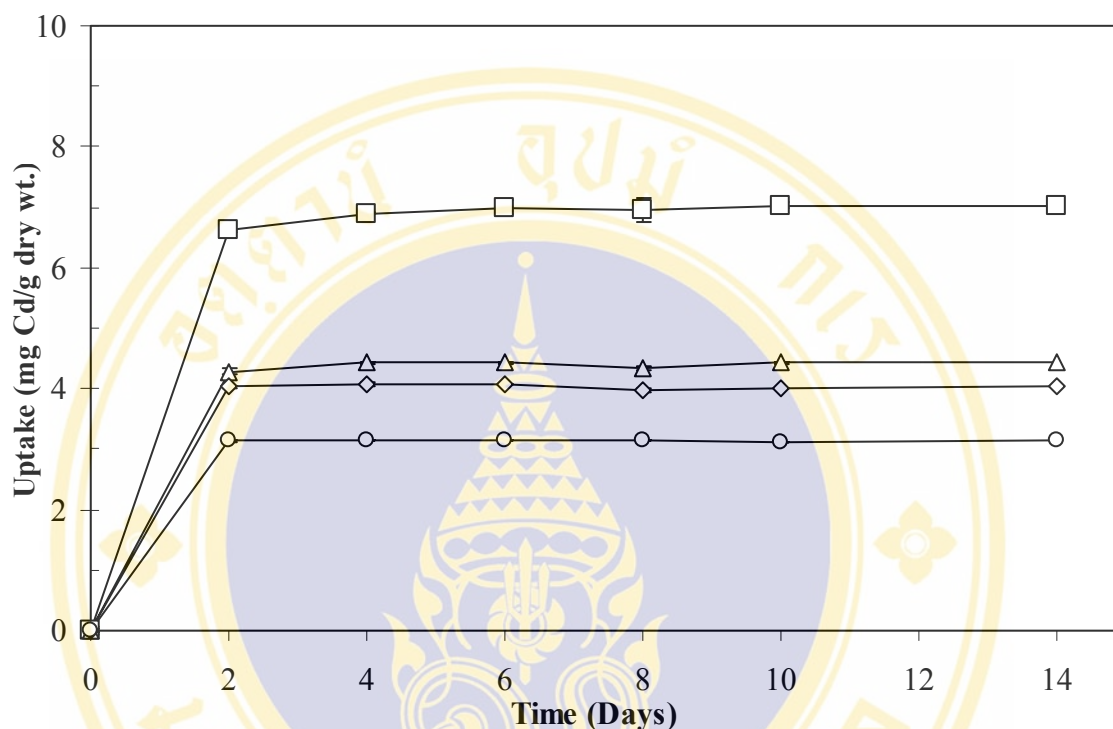


Figure 19 Time course of cadmium uptake in immobilized cell on polyethylene teraphthalate (PET) of *H. hibernicus* at cell concentration 0.015 g dry wt. (-□-), cell concentration 0.030 g dry wt. (-△-), cell concentration 0.045 g dry wt. (-◇-), cell concentration 0.060 g dry wt. (-○-). Cell were suspended in 130 ml of 1 mg/l cadmium solution and mixed slowly in rotary shaker at 100 rpm. The supernatant was sampled for 1 ml at 0, 5, 10, 30, 60 and 90 min.

In 14 day cultured, the cadmium uptake was decreased when the initial cell concentration increased. Immobilized cell on PET had highest cadmium uptake as 7.02 mg Cd/g dry wt. and followed by free cell and immobilized cell on SF were 5.72 mg Cd/g dry wt. and 5.51 mg Cd/g dry wt. at initial cell concentration 0.015 g dry wt., respectively. Immobilized cell on PET had slightly higher cadmium uptake than free cell and immobilized cell on SF at 4.45 mg Cd/g dry wt., 4.36 mg Cd/g dry wt. and 4.22 mg Cd/g dry wt. at initial cell concentration 0.030 g dry wt., respectively while immobilized cell of SF had slightly higher cadmium uptake than immobilized cell on

PET and free cell at 4.52 mg Cd/g dry wt., 4.03 mg Cd/g dry wt., 3.88 mg Cd/g dry wt. at initial cell concentration 0.045 g dry wt. and 3.32 mg Cd/g dry wt., 3.14 mg Cd/g dry wt., 3.14 mg Cd/g dry wt. at initial cell concentration 0.060 g dry wt., respectively as shown in Figure 21.

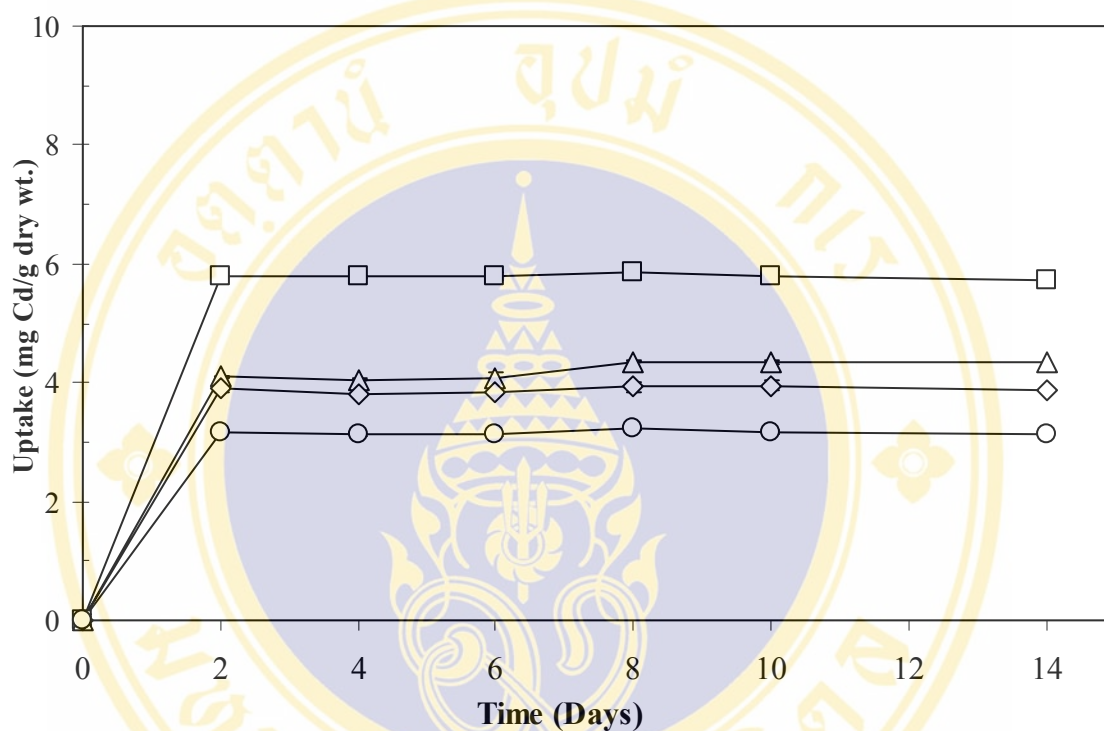


Figure 20 Time course of cadmium uptake in free cell of *H. hibernicus* at cell concentration 0.015 g dry wt. (□), cell concentration 0.030 g dry wt. (△), cell concentration 0.045 g dry wt. (◇), cell concentration 0.060 g dry wt. (○). Cell were suspended in 130 ml of 1 mg/l cadmium solution and mixed slowly in rotary shaker at 100 rpm. The supernatant was sampled for 1 ml at 2, 4, 6, 8, 10 and 14 days.

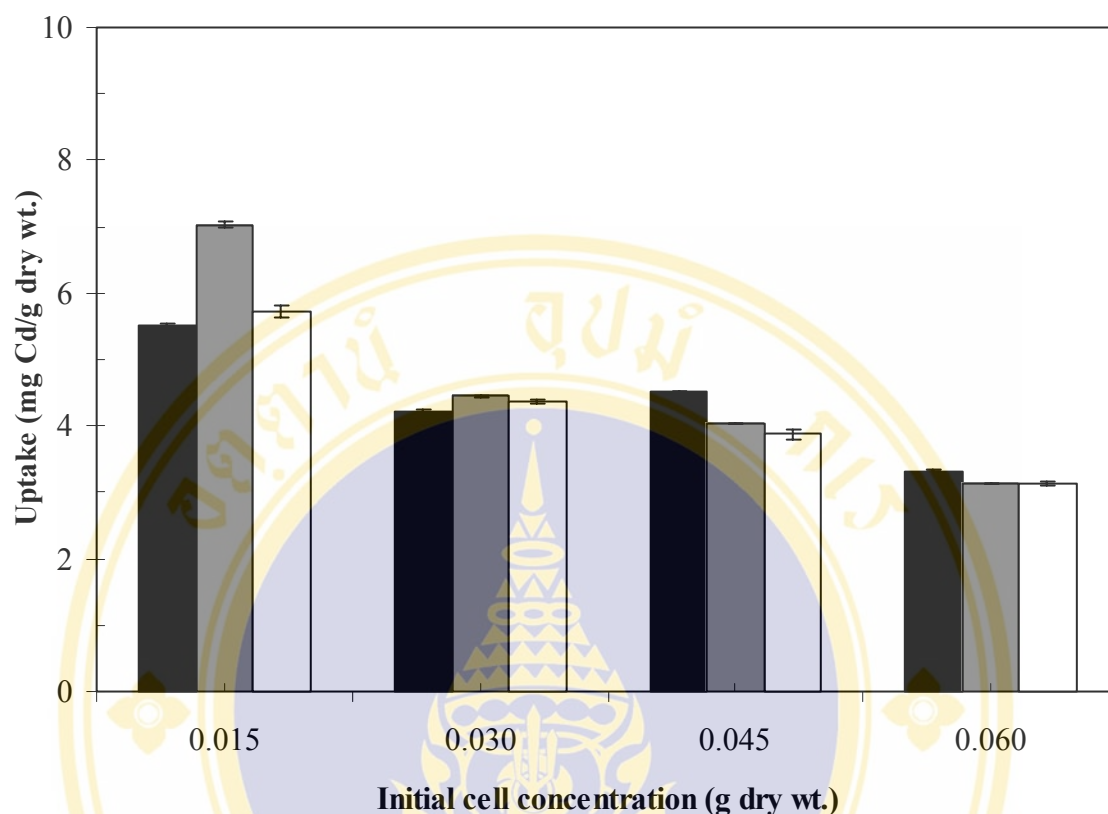


Figure 21 Cadmium uptake between immobilized cell on synthetic fiber (SF) (■), polyethylene terephthalate (PET) (▨) and free cell (□) of *H. hibernicus* at initial cell concentration 0.015, 0.030, 0.045 and 0.060 g dry wt. Cell were suspended in 130 ml of 1 mg/l cadmium solution mixed slowly in rotary shaker at 100 rpm for 14 days.

4.2.3 Immobilized ability and dry weight per surface area (DW/SA)

At initial cell concentration 0.015 g dry wt., immobilized cell on SF and PET of *Scytonema* sp. had the highest immobilized ability at 97.38% and 96.49%, respectively. Immobilized cell on SF had immobilized ability at 94.01%, 94.94% and 96.31% at initial cell concentration 0.030, 0.045 and 0.060 g dry wt., respectively. Immobilized cell on PET had immobilized ability at 93.88%, 95.46%, and 90.95% at initial cell concentration 0.030, 0.045 and 0.060 g dry wt., respectively. In *H. hibernicus*, the immobilized ability of immobilized cell on SF and PET had the highest immobilized ability at 90.91% and 89.06% at 0.060 g dry wt., respectively. Immobilized ability at initial cell concentration 0.015, 0.030 and 0.045 g dry wt. of immobilized cell on SF were 89.31%, 90.00%, 86.13% and immobilized cell on PET

were 81.72%, 86.41%, 84.66%, respectively. Increasing of initial cell concentration also increased the DW/SA ratio in immobilized cell on SF and PET of *Scytonema* sp. and *H. hibernicus*. Initial cell concentration at 0.060 g dry wt. had the highest DW/SA at 0.0037 and 0.0044 g cell/cm² in immobilized cell on SF and PET of *Scytonema* sp., respectively. While immobilized cell on SF had the DW/SA at 0.0018 g cell/cm², 0.0023 g cell/cm² and 0.0036 g cell/cm² at initial cell concentration 0.015, 0.030 and 0.045 g dry wt., respectively. Immobilized cell on PET had the DW/SA at 0.0009 g cell/cm², 0.0026 g cell/cm² and 0.0033 g cell/cm² at initial cell concentration 0.015, 0.030 and 0.045 g dry wt., respectively. In *H. hibernicus*, DW/SA ratio of immobilized cell on SF and PET at initial cell concentration 0.015, 0.045 and 0.060 g dry wt. were 0.0009 g cell/cm² and 0.0007 g cell/cm², 0.0014 g cell/cm² and 0.0016 g cell/cm², 0.0021 g cell/cm² and 0.0022 g cell/cm², respectively. In both immobilized material had the same DW/SA at 0.0014 g cell/cm² at initial cell concentration 0.030 g dry wt. Immobilized cell on SF and PET of *Scytonema* sp. had both higher immobilized ability and DW/SA than *H. hibernicus* as shown in Table 5.

Table 5 Immobilized ability and DW/SA between immobilized cell on synthetic fiber (SF) and polyethylene terephthalate (PET) of *Scytonema* sp. and *H. hibernicus* at various initial cell concentrations in 14-day-cultured

Type of immobilized material	Initial cell concentration (g dry wt.)	DW/SA (g cell/cm ²)		Immobilized ability (%)	
		<i>Scytonema</i> sp.	<i>H. hibernicus</i>	<i>Scytonema</i> sp.	<i>H. hibernicus</i>
Synthetic fibre (SF)	0.015	0.0018	0.0009	97.38	89.31
	0.030	0.0023	0.0014	94.01	90.00
	0.045	0.0036	0.0014	94.94	86.13
	0.060	0.0037	0.0021	96.31	90.91
Polyethylene terephthalate (PET)	0.015	0.0009	0.0007	96.49	81.72
	0.030	0.0026	0.0014	93.88	86.41
	0.045	0.0033	0.0016	95.46	84.66
	0.060	0.0044	0.0022	90.95	89.06

From the result initial cell concentration at 0.015 g dry wt. was selected for future experiment because of the highest cadmium uptake and high immobilized ability.

4.3 Effect of initial cadmium concentration on cadmium uptake between immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus*

4.3.1 Cadmium uptake in *Scytonema* sp. and *H. hibernicus*

Adsorption isotherm was studied to evaluate biosorption performance of biosorbent and typical cadmium biosorption isotherm plotting uptake capacity (q) vs equilibrium cadmium concentration (C_f). The uptake of cadmium in immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* at initial cadmium concentration 0.5, 1, 2, 4 and 8 mg/l were studied in 14 days and the data were fitted to Langmuir isotherm as showed in Figure 22 and Figure 23, respectively.

The cadmium uptake in immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* increased as increasing cadmium concentration. The maximum adsorption ability (q_{max}) and the binding constant (k_b) of cadmium adsorption were shown in Table 6. Immobilized cell on SF, PET and free cell of *Scytonema* sp. had q_{max} at 26.74 mg/g dry wt., 37.74 mg/g dry wt. and 31.95 mg/g dry wt., respectively and had k_b at 6.45 l/mg, 2.14 l/mg and 2.70 l/mg, respectively when $R^2 > 0.96$. While immobilized cell on SF, PET and free cell of *H. hibernicus* had q_{max} at 57.80 mg/g dry wt., 64.52 mg/g dry wt. and 59.17 mg/g dry wt., respectively and had k_b at 1.57 l/mg, 2.07 l/mg and 1.50 l/mg, respectively when $R^2 > 0.99$.

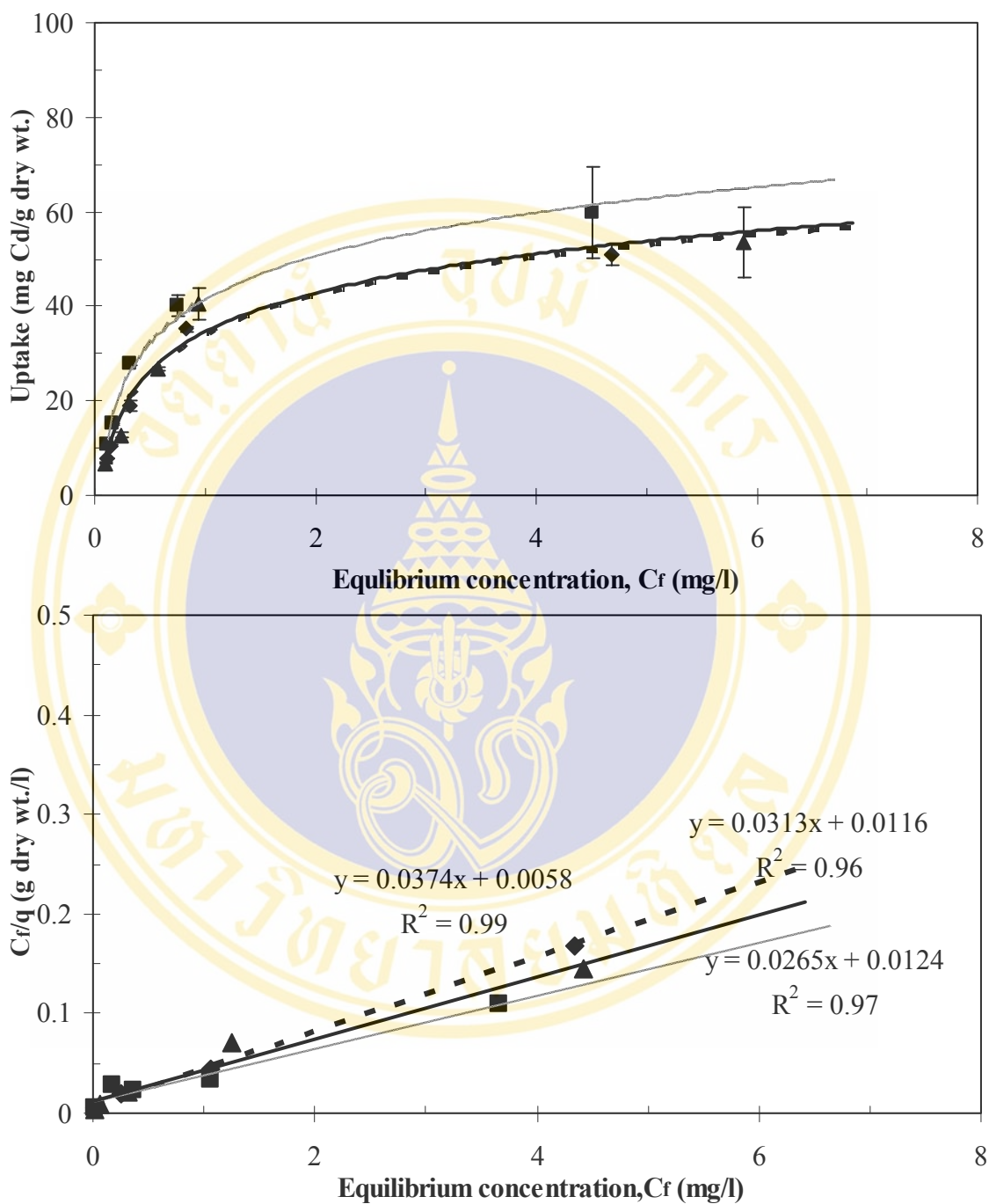


Figure 22 Langmuir adsorption isotherm of cadmium uptake by immobilized cell on synthetic fiber (SF) (-◆-), polyethylene terephthalate (PET) (-■-) and free cell (-▲-) of *Scytonema* sp. Cell were suspended in 130 ml of cadmium concentration at 0.5, 1, 2, 4 and 8 mg/l for 14 days.

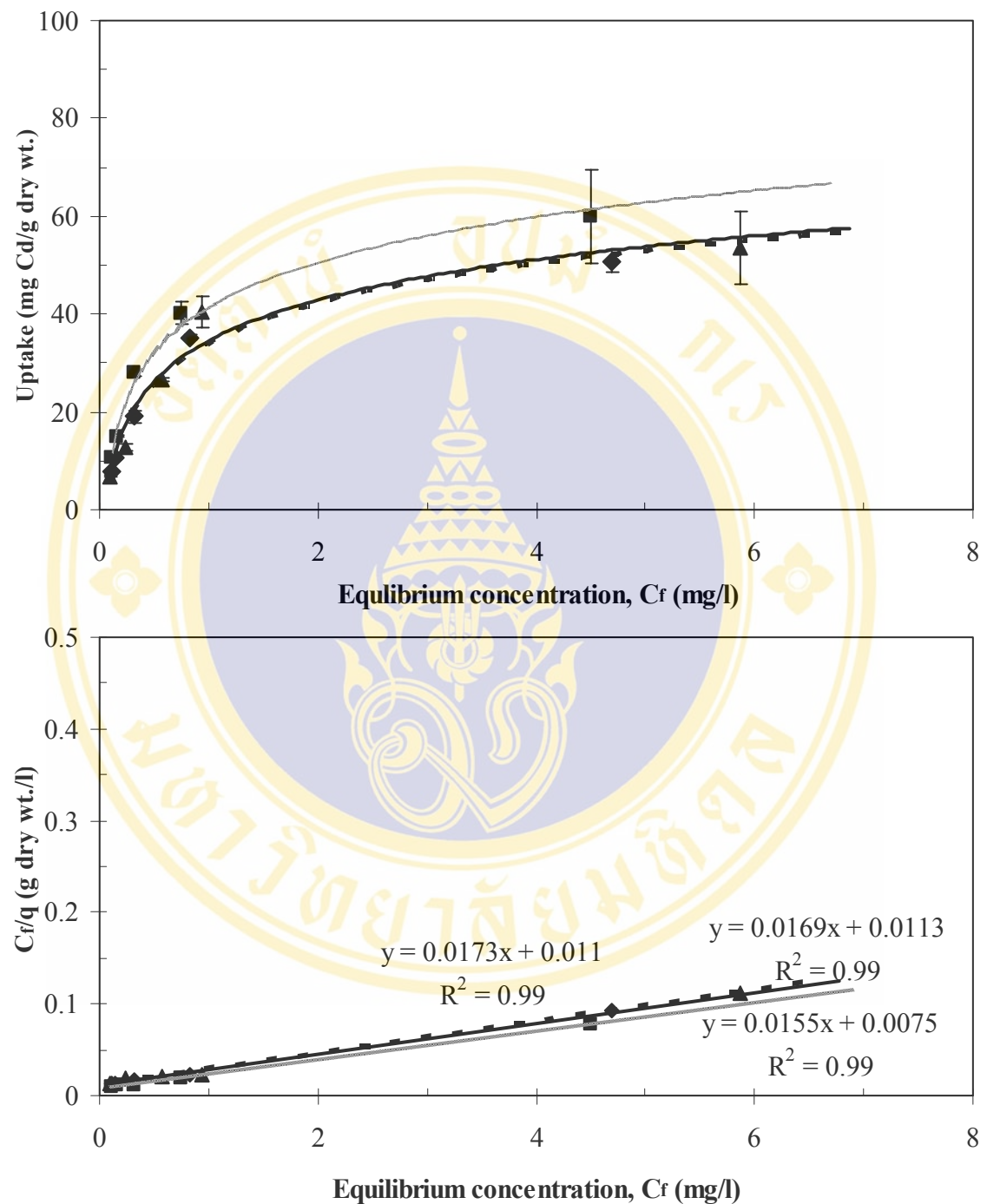


Figure 23 Langmuir adsorption isotherm of cadmium uptake by immobilized cell on synthetic fiber (SF) (-◆-), and polyethylene terephthalate (PET) (-■-) and free cell (-▲-) of *H. hibernicus*. Cell were suspended in 130 ml of Cd concentration at 0.5, 1, 2, 4 and 8 mg/l for 14 days.

Table 6 The maximum adsorption ability (q_{max}) and the binding constant value (k_b) of cadmium uptake in immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus* at 14-day-cultured

Characteristics of Cyanobacteria	q_{max} (mg/g dry wt.)	k_b (l/mg)
<i>Scytonema</i> sp.		
Immobilized cell on SF	26.74	6.45
Immobilized cell on PET	37.74	2.14
Free cell	31.95	2.70
<i>H. hibernicus</i>		
Immobilized cell on SF	57.80	1.57
Immobilized cell on PET	64.52	2.07
Free cell	59.17	1.50

4.3.2 Immobilized ability and DW/SA ratio

Immobilized cell on SF and PET of *Scytonema* sp. had the highest immobilized ability at 95.80% and 94.58% at initial cadmium concentration 0.5 mg/l, respectively. Immobilized cell on SF had immobilized ability at 94.76%, 94.45%, 90.56% and 88.86% at initial cadmium concentration 1, 2, 4 and 8 mg/l, respectively. Immobilized cell on PET had immobilized ability at 91.58%, 91.82%, 90.24% and 88.91% at initial cadmium concentration 1, 2, 4 and 8 mg/l, respectively. In *H. hibernicus*, had the same pattern of immobilized ability as *Scytonema* sp. Immobilized ability of immobilized cell on SF and PET had the highest at 93.98% and 93.14% at initial cadmium concentration 0.5 mg/l, respectively. Immobilized ability of immobilized cell on SF were 89.92%, 89.71%, 86.57% and 85.17% at initial cadmium concentration 1, 2, 4 and 8 mg/l, respectively. While immobilized ability of immobilized cell on PET were 90.23%, 89.33%, 89.02% and 82.05% at initial cadmium concentration 1, 2, 4 and 8 mg/l, respectively. Increasing of initial cadmium concentration had the similar DW/SA ratio in immobilized cell on SF and PET of *Scytonema* sp. and *H. hibernicus*. Immobilized cell on SF of *Scytonema* sp. had the same DW/SA at 0.0014 g cell/cm² at 0.5 and 1 mg/l cadmium concentration and the same DW/SA at 0.0012 g cell/cm² at 2 and 8 mg/l cadmium concentration. While the

DW/SA was 0.0011 g cell/cm² at 4 mg/l cadmium concentration, respectively. Immobilized cell on PET had the DW/SA at 0.0012 g cell/cm², 0.0010 g cell/cm² and 0.0011 g cell/cm² at initial cadmium concentration 0.5, 1 and 8 mg/l, respectively. Immobilized cell on PET had the same DW/SA at 0.0009 g cell/cm² at 2 and 4 mg/l cadmium concentration, respectively. DW/SA ratio in immobilized cell on SF and PET of *H. hibernicus* at initial cadmium concentration 0.5, 1, and 2 mg/l were 0.0008 g cell/cm² and 0.0005 g cell/cm², 0.0007 g cell/cm² and 0.0005 g cell/cm², 0.0008 g cell/cm² and 0.0006 g cell/cm², respectively. In both immobilized material had the same DW/SA ratio at 0.0006 g cell/cm² at 4 and 8 mg/l of cadmium concentration, respectively. Immobilized cell on SF and PET of *Scytonema* sp. had both higher immobilized ability and DW/SA than *H. hibernicus* as shown in Table 7.

Table 7 Immobilized ability and DW/SA between immobilized cell on synthetic fiber (SF) and polyethylene terephthalate (PET) of *Scytonema* sp. and *H. hibernicus* at initial cadmium concentration 0.5, 1, 2, 4 and 8 mg/l at 14-day-cultured

Type of immobilized material	Initial cadmium concentration (mg/l)	DW/SA (g cell/cm ²)		Immobilized ability (%)	
		<i>Scytonema</i> sp.	<i>H. hibernicus</i>	<i>Scytonema</i> sp.	<i>H. hibernicus</i>
Synthetic fiber (SF)	0.5	0.0014	0.0008	95.80	93.98
	1	0.0014	0.0007	94.76	89.92
	2	0.0012	0.0008	94.45	89.71
	4	0.0011	0.0006	90.56	86.57
	8	0.0012	0.0006	88.86	85.71
Polyethylene terephthalate (PET)	0.5	0.0012	0.0005	94.58	93.14
	1	0.0010	0.0005	91.58	90.23
	2	0.0009	0.0006	91.82	89.33
	4	0.0009	0.0006	90.24	89.02
	8	0.0011	0.0006	88.91	82.05

From this result, cadmium concentration at 1 mg/l were selected as a model for further study because of the contamination of cadmium in effluent from industrial source and natural water were normally not more than 1 mg/l. The industrial effluent standard permission had set cadmium concentration not more than 0.03 mg/l and this system can be applied in real condition treatment with low cadmium concentration.

4.4 Growth rate of *Scytonema* sp. and *H. hibernicus*

Immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* were cultivated in Medium18 without cadmium and were shaken in rotary shaker system. The growth rate were measured by dry weight determination as shown in Figure 24 and Figure 25.

The growth curve of immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* were followed by the typical growth curves. From first day to 6 days the dry weight was slightly increased and the acceleration in growth was observed during day 6 to day 15. The dry weight was increased and reach to the maximum level at day 15. From day 15 to day 21, the dry weight became constant and started to decline which effect to reduction the growth rate. The growth curve profile of immobilized cell on SF and PET were not difference to free cell of *Scytonema* sp. and *H. hibernicus*.

The specific growth rate (k) was decided and found that immobilized cell on SF, PET and free cell of *Scytonema* sp. without cadmium had k at 0.0940, 0.0938 and 0.0865 day⁻¹, respectively (Table 8 and Appendix G) while the specific growth rate (k) in immobilized cell on SF, PET and free cell of *H. hibernicus* without cadmium were 0.0738, 0.0740 and 0.0694 day⁻¹, respectively. (Table 8 and Appendix G)

Table 8 Growth rate (day⁻¹) of *Scytonema* sp. and *H. hibernicus* in Medium 18 without cadmium

Characteristics of Cyanobacteria	Growth rate (day ⁻¹)	R ²
<i>Scytonema</i> sp.		
Immobilized cell on SF	0.0940	0.92
Immobilized cell on PET	0.0938	0.92
Free cell	0.0865	0.90
<i>H. hibernicus</i>		
Immobilized cell on SF	0.0738	0.75
Immobilized cell on PET	0.0740	0.76
Free cell	0.0694	0.63

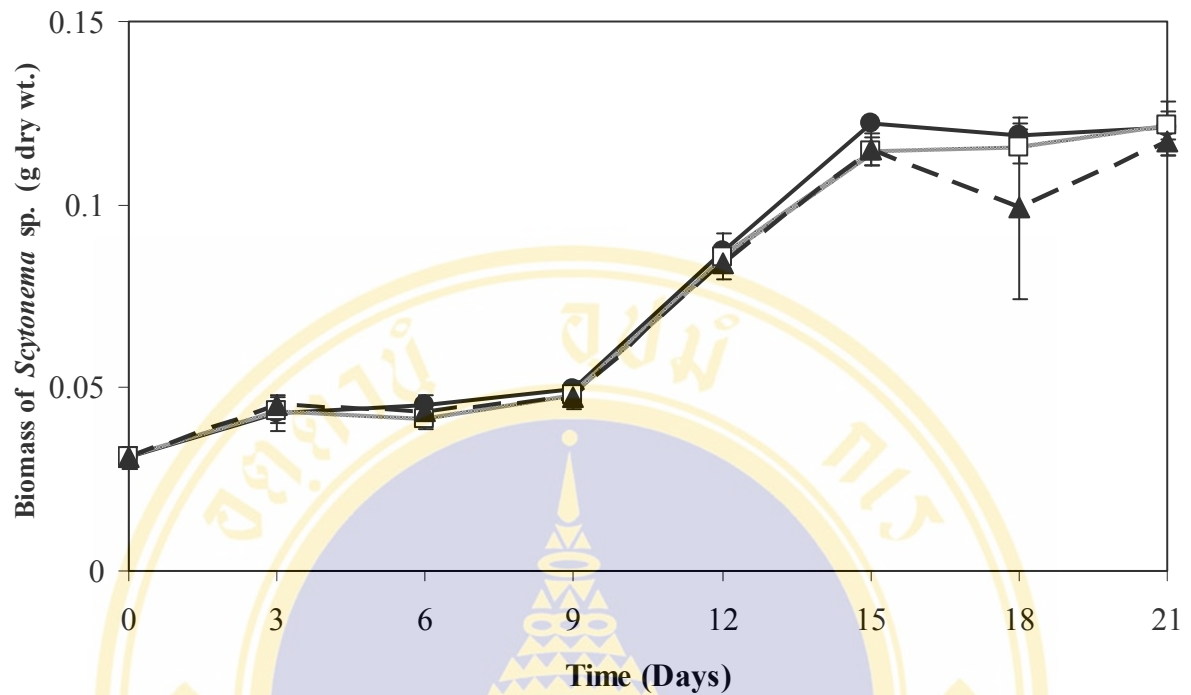


Figure 24 The growth curve of immobilized cell on synthetic fiber (SF) (●-), polyethylene terephthalate (PET) (□-) and free cell (▲-) of *Scytonema* sp. for 21 days.

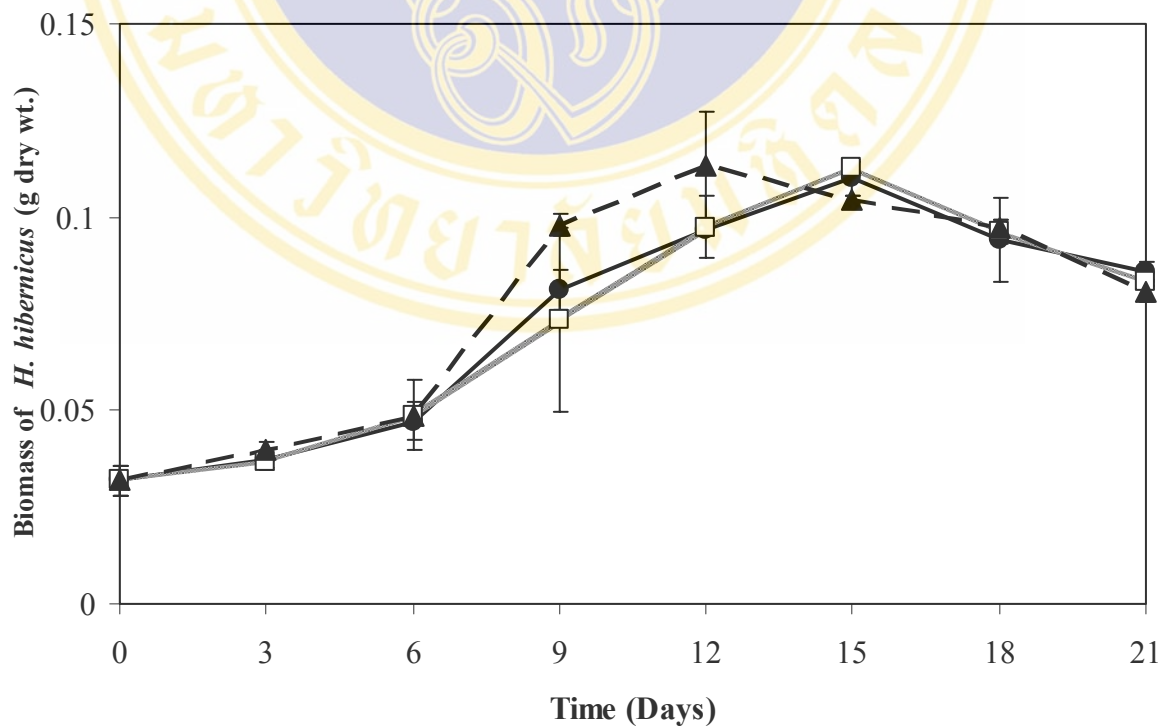


Figure 25 The growth curve of immobilized cell on synthetic fiber (SF) (●-), polyethylene terephthalate (PET) (□-) and free cell (▲-) of *H. hibernicus* for 21 days.

4.5 Effect of cell ages on cadmium uptake between immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus*

4.5.1 Cadmium uptake in *Scytonema* sp. and *H. hibernicus*

Cadmium uptake in immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* in various cell ages at 5, 10, 15 and 20 days were studied at 6-day-cultured as shown in Figure 26 and Figure 27.

The uptake of cadmium in immobilized cell on SF, PET and free cell of *Scytonema* sp. for 6 days were highest at 4.82 mg Cd/g dry wt., 4.90 mg Cd/g dry wt. and 4.51 mg Cd/g dry wt. at 15 day cell ages, respectively. Immobilized cell on SF had cadmium uptake at 1.30 mg Cd/g dry wt., 4.26 mg Cd/g dry wt. and 3.79 mg Cd/g dry wt. at 5, 10 and 20 day cell ages, respectively. Immobilized cell on PET had cadmium uptake at 1.20 mg Cd/g dry wt., 4.45 mg Cd/g dry wt and 3.75 mg Cd/g dry wt. at 5, 10 and 20 day cell ages, respectively. While cadmium uptake of free cell were 1.07 mg Cd/g dry wt., 4.11 mg Cd/g dry wt. and 3.54 mg Cd/g dry wt. at 5, 10 and 20 day cell ages, respectively.

In *H. hibernicus*, the uptake of cadmium in immobilized cell on SF, PET and free cell for 6 days were highest at 8.35 mg Cd/g dry wt., 8.83 mg Cd/g dry wt. and 7.77 mg Cd/g dry wt. at 15 day cell ages, respectively. Immobilized cell on SF had cadmium uptake at 6.68 mg Cd/g dry wt., 7.78 mg Cd/g dry wt. and 6.46 mg Cd/g dry wt. at 5, 10 and 20 day cell ages, respectively. Immobilized cell on PET had cadmium uptake at 8.03 mg Cd/g dry wt., 8.44 mg Cd/g dry wt. and 8.00 mg Cd/g dry wt. at 5, 10 and 20 day cell ages, respectively. While free cell had cadmium uptake at 4.95 mg Cd/g dry wt., 7.55 mg Cd/g dry wt. and 7.20 mg Cd/g dry wt. at 5, 10 and 20 day cell ages, respectively.

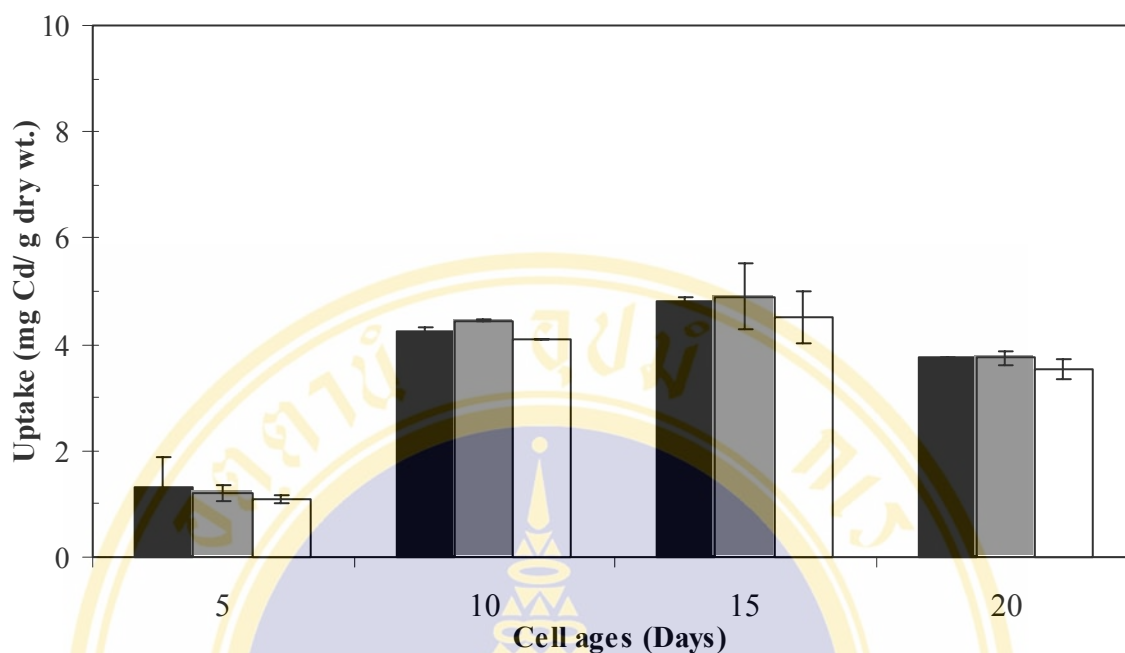


Figure 26 Effect of cell ages on cadmium uptake in immobilized cell on synthetic fiber (SF) (■), polyethylene terephthalate (PET) (▨) and free cell (□) of *Scytonema* sp. Cell ages were suspended in 130 ml of 1 mg/l of cadmium solution mixed slowly in rotary shaker at 100 rpm for 6 days.

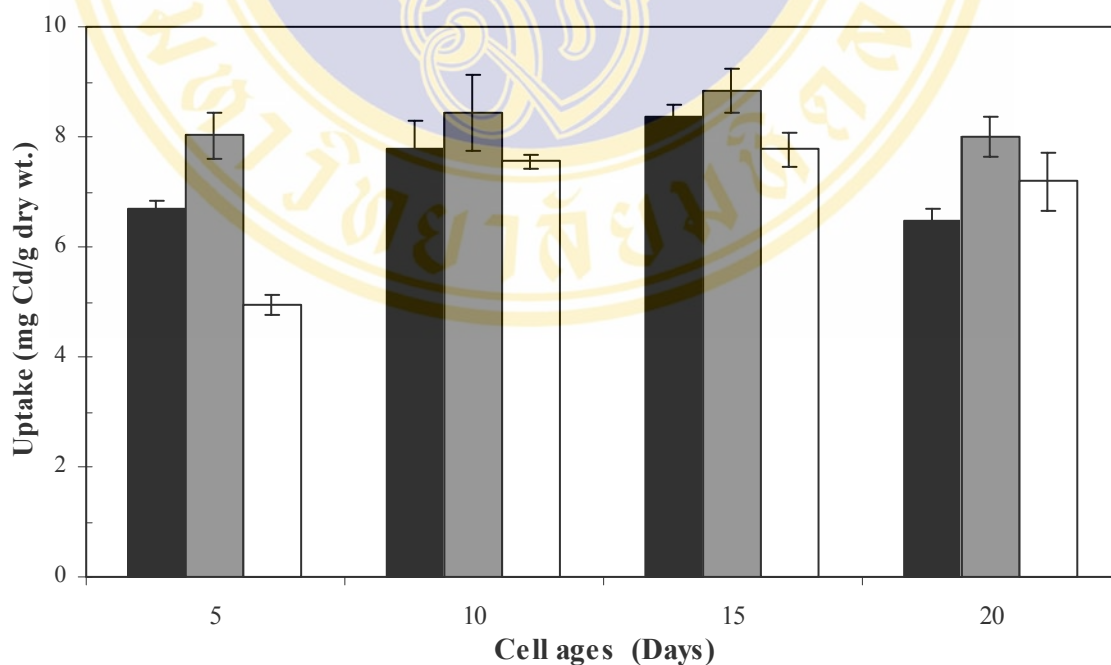


Figure 27 Effect of cell ages on cadmium uptake in immobilized cell on synthetic fiber (SF) (■), polyethylene terephthalate (PET) (▨) and free cell (□) of *H. hibernicus*. Cell ages at 5, 10, 15 and 20 days were suspended in 130 ml of 1 mg/l of cadmium solution mixed slowly in rotary shaker at 100 rpm for 6 days.

Cadmium uptake in immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* in various cell ages at 5, 10, 15 and 20 days were studied at 14-day-culture and had similar profile at 6-day-culture as shown in Figure 28 and Figure 29. However, cadmium uptake were similar at 5.55 mg Cd/g dry wt., 5.64 mg Cd/g dry wt. and 5.35 mg Cd/g dry wt. in immobilized cell on SF and cadmium uptake were 5.49 mg Cd/g dry wt., 5.71 mg Cd/g dry wt. and 5.41 mg Cd/g dry wt. in immobilized cell on PET at 10, 15 and 20 day cell ages of *Scytonema* sp., respectively. While free cell had cadmium uptake at 5.67 mg Cd/g dry wt., 5.36 mg Cd/g dry wt. and 5.01 mg Cd/g dry wt. at 10, 15 and 20 day cell ages of *Scytonema* sp., respectively. The lowest cadmium uptake at 1.59, 1.55 and 1.35 mg Cd/g dry wt. were observed at 5 day cell age in immobilized cell on SF, PET and free cell, respectively. The uptake of cadmium in immobilized cell on SF, PET were not difference at 10, 15 and 20 day cell ages and the lowest uptake at 5 day cell age.

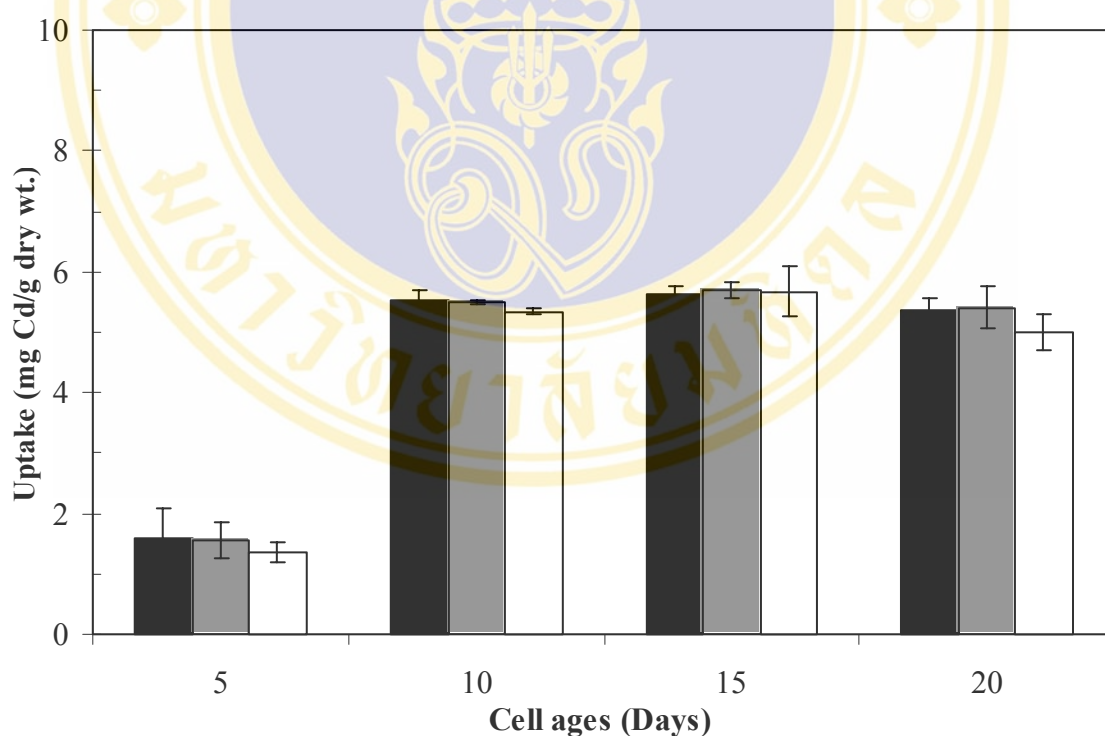


Figure 28 Effect of cell ages on cadmium uptake in immobilized cell on synthetic fiber (SF) (■), polyethylene terephthalate (PET) (▨) and free cell (□) of *Scytonema* sp. Cell ages at 5, 10, 15 and 20 days were suspended in 130 ml of 1 mg/l of cadmium solution mixed slowly in rotary shaker at 100 rpm for 14 days.

In *H. hibernicus*, the similar cadmium uptake pattern between 6-day-cultured and 14-day-cultured was observed. The uptake of cadmium in immobilized cell on SF, PET and free cell of *H. hibernicus* were highest at 9.05 mg Cd/g dry wt, 9.96 mg Cd/g dry wt. and 8.46 mg Cd/g dry wt. at 15 day cell age, respectively. Immobilized cell on SF had cadmium uptake at 7.28 mg Cd/g dry wt., 8.44 mg Cd/g dry wt. and 7.63 mg Cd/g dry wt. and immobilized cell on PET had cadmium uptake at 8.85 mg Cd/g dry wt., 9.56 mg Cd/g dry wt. and 9.30 mg Cd/g dry wt. while free cell had cadmium uptake at 5.56 mg Cd/g dry wt., 8.33 mg Cd/g dry wt. and 8.22 mg Cd/g dry wt. at 5, 10 and 20 day cell ages, respectively.

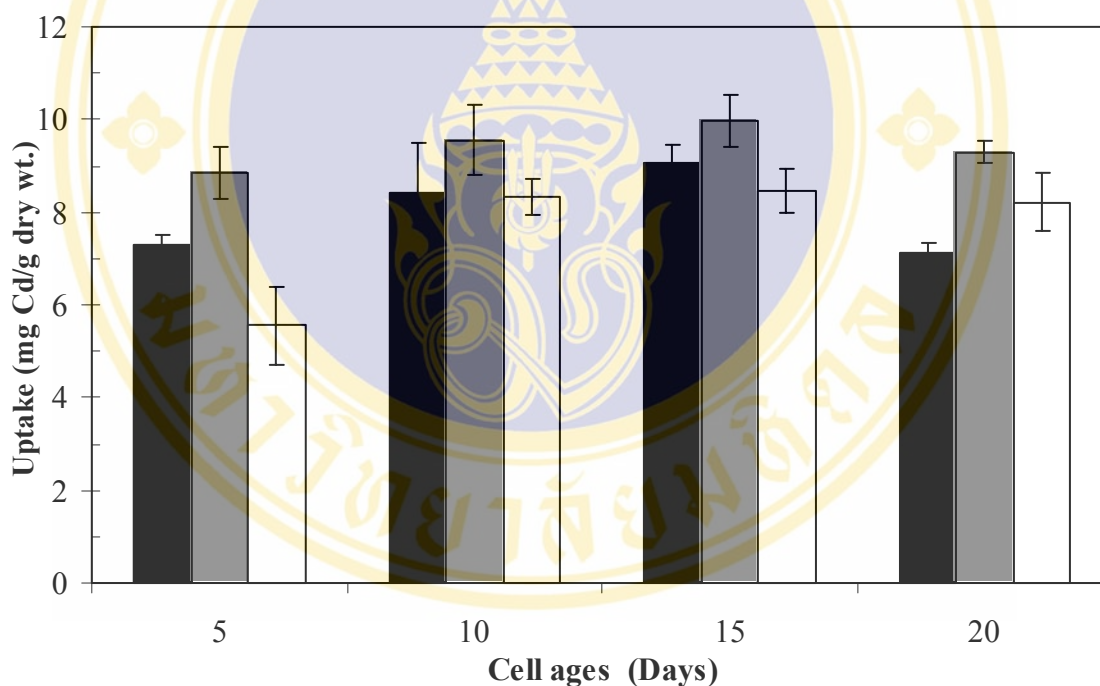


Figure 29 Effect of cell ages on cadmium uptake in immobilized cell on synthetic fiber (SF) (■), polyethylene terephthalate (PET) (▨) and free cell (□) of *H. hibernicus*. Cell ages at 5, 10, 15 and 20 days were suspended in 130 ml of 1 mg/l of cadmium solution mixed slowly in rotary shaker at 100 rpm for 14 days.

4.5.2 Comparison of biosorption isotherm between 6-day-cultured and 14-day- cultured in immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus*

Adsorption isotherm was studied to evaluate biosorption performance of biosorbent and typical cadmium adsorption isotherm plotting uptake capacity (q) vs equilibrium cadmium concentration (C_f). The uptake of cadmium by immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* were studied in 6 days and 14 days cultivation. The data were fitted to Langmuir isotherm as showed in Figure 30-31 at 6-day-cultured and Figure 32-33 at 14-day-cultured, respectively.

The maximum adsorption capacity (q_{max}) and the binding constant (k_b) of cadmium adsorption as shown in Table 9. The immobilized cell on SF, PET and free cell of *Scytonema* sp. had q_{max} at 24.57 mg/g dry wt., 36.23 mg/g dry wt. and 26.88 mg/g dry wt. and had k_b at 2.73 l/mg, 1.27 l/mg and 1.75 l/mg, respectively when $R^2 > 0.99$. Immobilized cell on SF, PET and free cell of *H. hibernicus* had q_{max} at 47.85 mg/g dry wt., 58.82 mg/g dry wt. and 56.12 mg/g dry wt. and had k_b at 1.22 l/mg, 1.36 l/mg, and 1.05 l/mg, respectively when $R^2 > 0.99$ at 6-day-cultured.

While immobilized cell on SF, PET and free cell of *Scytonema* sp. had q_{max} at 26.74 mg/g dry wt., 37.74 mg/g dry wt. and 31.95 mg/g dry wt. and had k_b at 6.45 l/mg, 2.14 l/mg and 2.70 l/mg, respectively when $R^2 > 0.96$. Immobilized cell on SF, PET and free cell of *H. hibernicus* had q_{max} at 57.80 mg/g dry wt., 64.52 mg/g dry wt. and 59.17 mg/g dry wt. and had k_b at 1.57 l/mg, 2.07 l/mg and 1.50 l/mg, respectively when $R^2 > 0.99$ at 14-day-cultured.

The result found that immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* at 14-day-cultured had slightly higher the maximum adsorption capacity (q_{max}) and the binding constant (k_b) of cadmium adsorption than at 6-day-cultured.

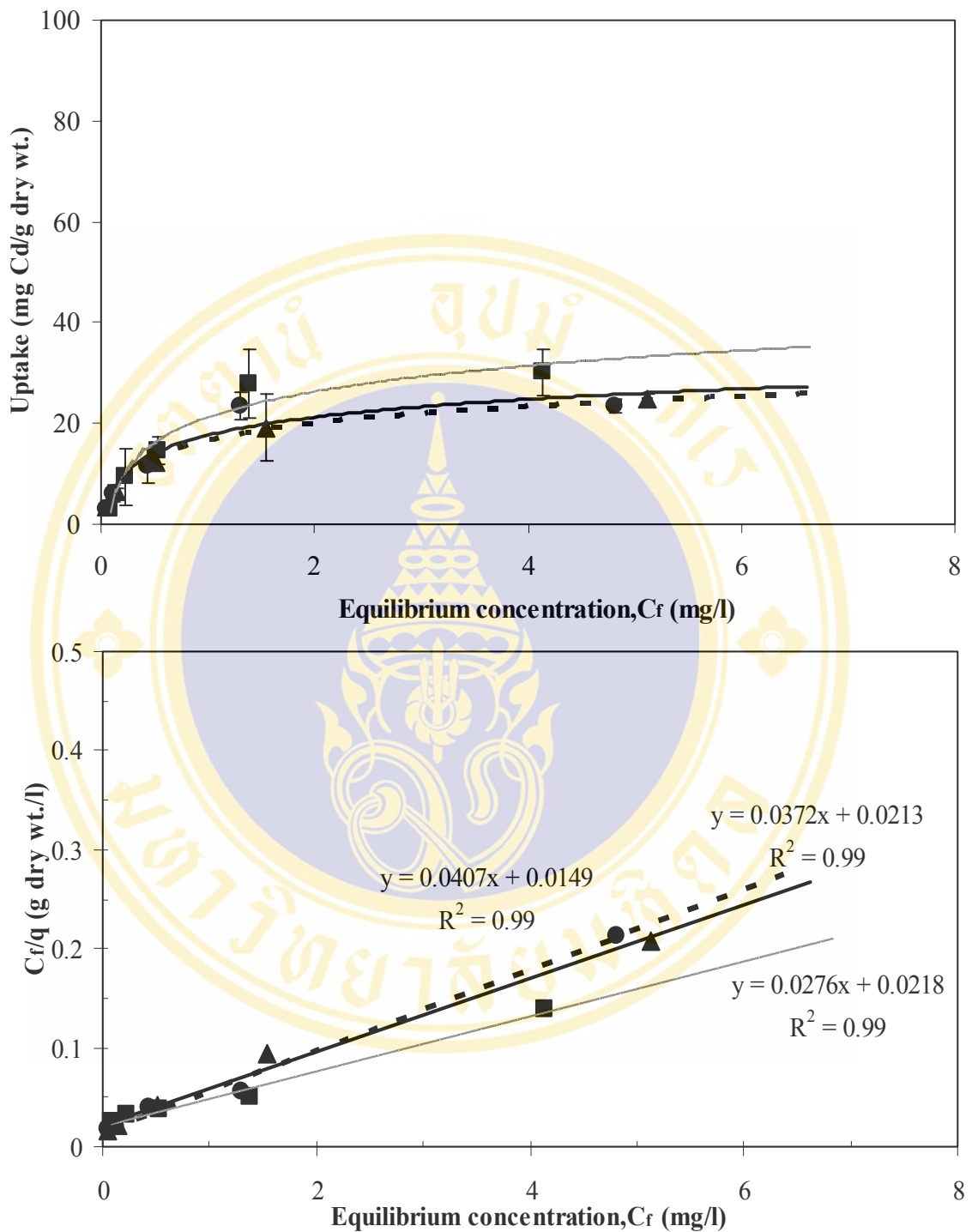


Figure 30 Langmuir adsorption isotherm of cadmium uptake by immobilized cell on synthetic fiber (SF) (-●-), polyethylene terephthalate (PET) (-■-) and free cell (-▲-) of *Scytonema* sp. Cell were suspended in 130 ml of 0.5, 1, 2, 4 and 8 mg/l of cadmium concentration and cultivated for 6 days.

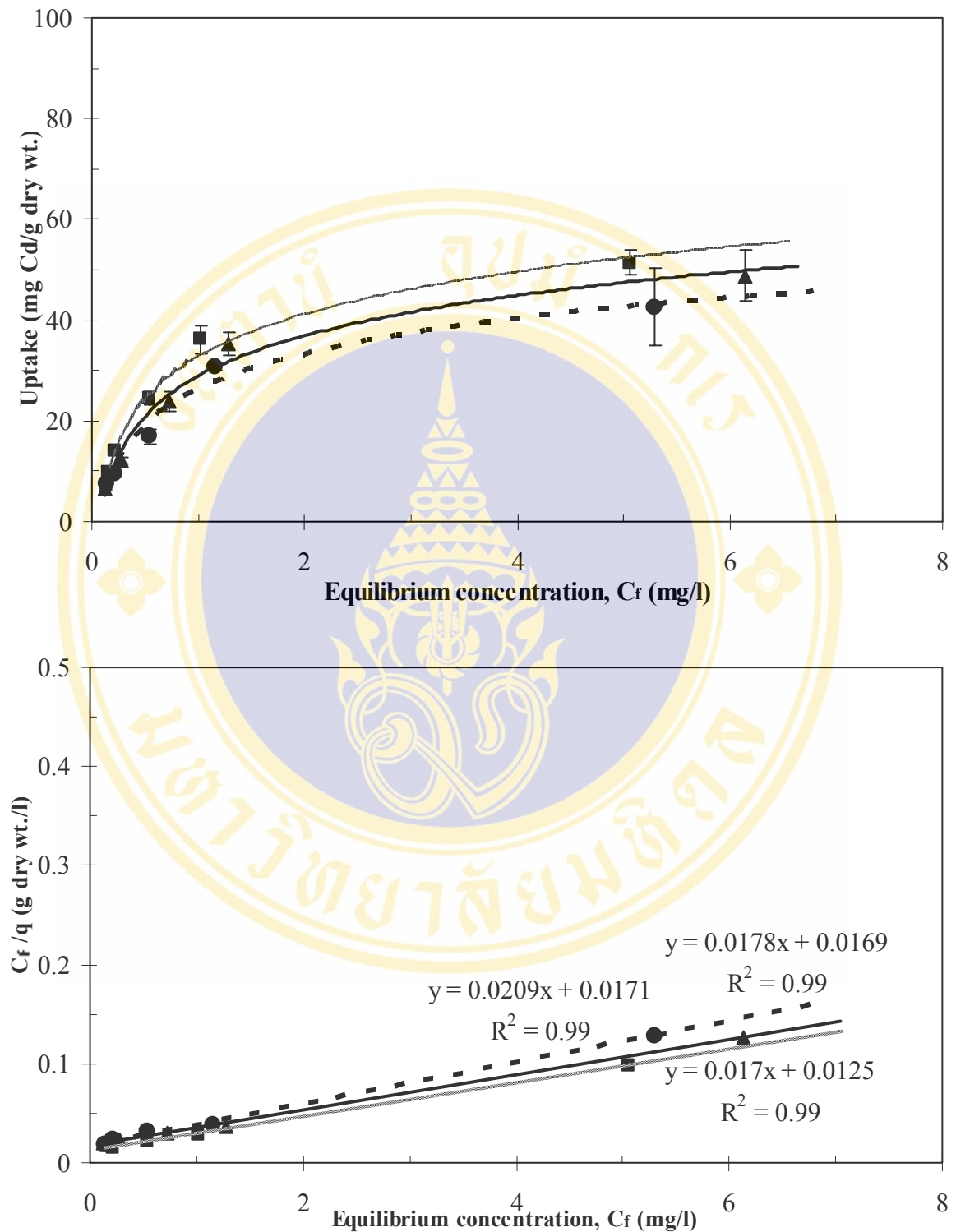


Figure 31 Langmuir adsorption isotherm of cadmium uptake by immobilized cell on synthetic fiber (SF) (●-), polyethylene terephthalate (PET) (■-) and free cell (▲-) of *H. hibernicus*. Cell were suspended in 130 ml of 0.5, 1, 2, 4 and 8 mg/l of cadmium concentration and cultivated for 6 days.

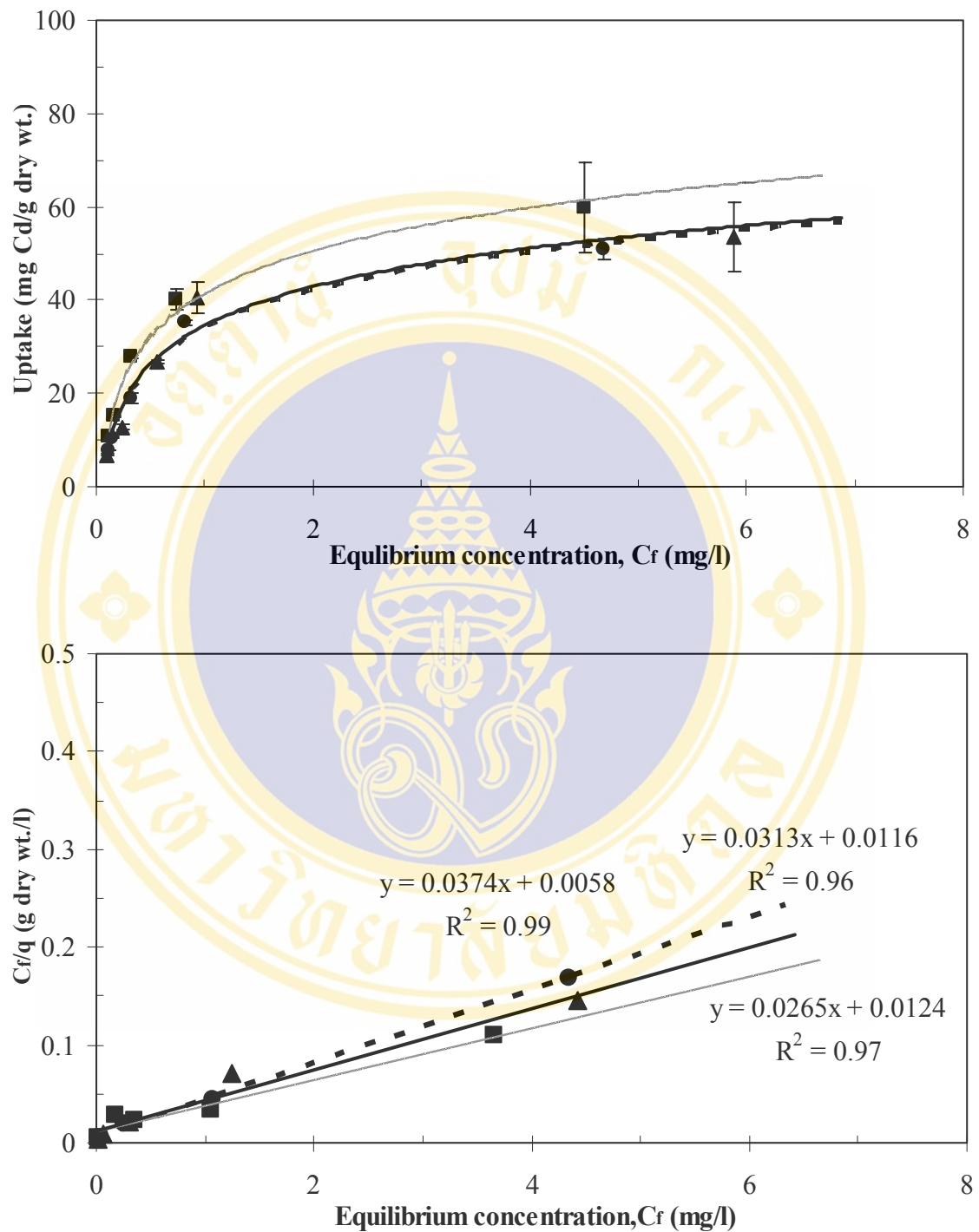


Figure 32 Langmuir adsorption isotherm of cadmium uptake by immobilized cell on synthetic fiber (SF) (-●-), polyethylene terephthalate (PET) (-■-) and free cell (-▲-) of *Scytonema* sp. Cell were suspended in 130 ml of 0.5, 1, 2, 4 and 8 mg/l of cadmium concentration and cultivated for 14 days.

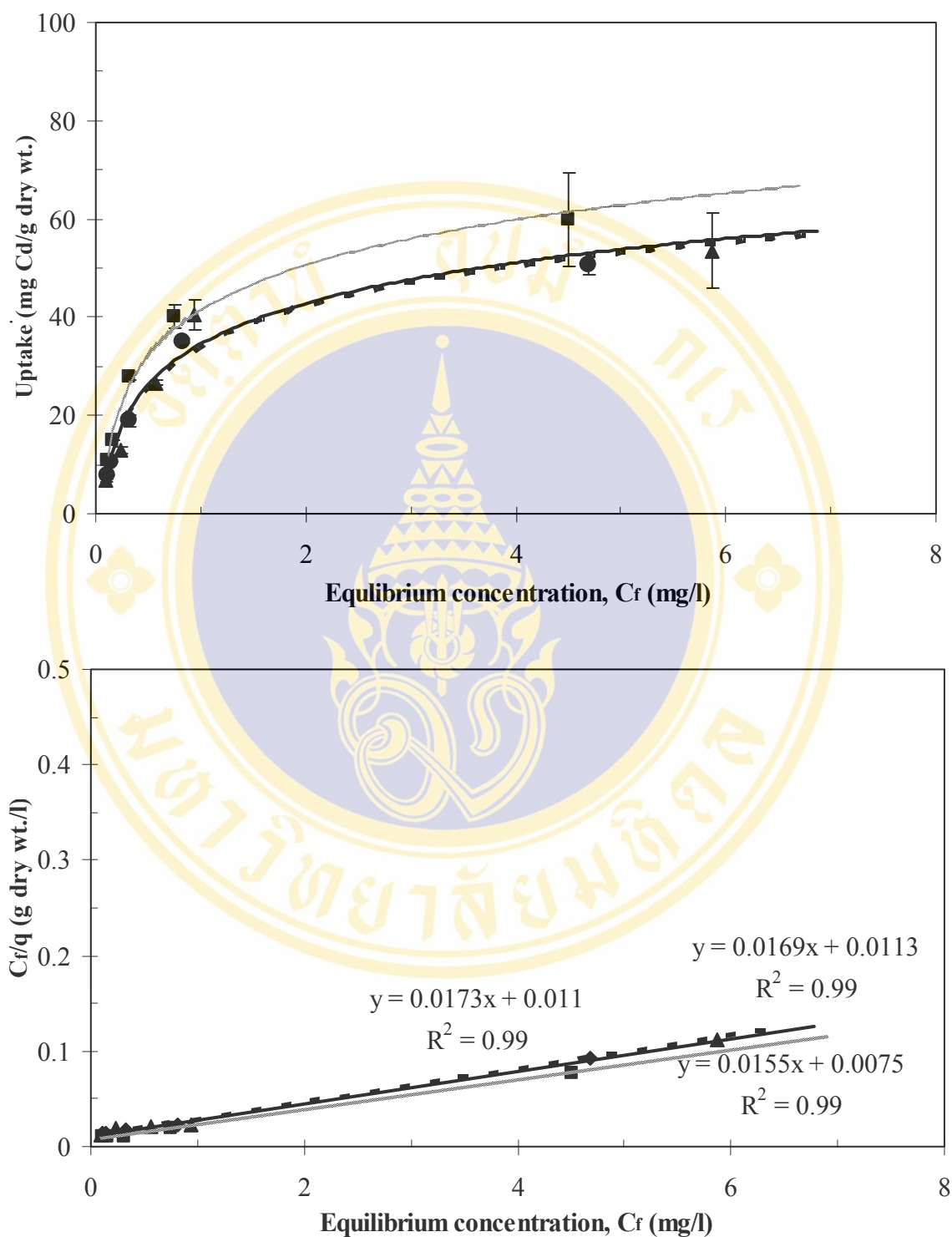


Figure 33 Langmuir adsorption isotherm of cadmium uptake by immobilized cell on synthetic fiber (SF) (-●-), and polyethylene terephthalate (PET) (-■-) and free cell (-▲-) of *H. hibernicus*. Cell were suspended in 130 ml of 0.5, 1, 2, 4 and 8 mg/l of cadmium concentration and cultivated for 14 days.

Table 9 The maximum adsorption ability (q_{max}) and the binding constant (k_b) of cadmium uptake by immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* at 6-day-cultured and 14-days-cultured

Characteristics of Cyanobacteria	$q_{max}(\text{mg/g dry wt.})$		$k_b(\text{l/mg})$	
	6 - day - cultured	14 - day - cultured	6 - day - cultured	14 - day - cultured
<i>Scytonema</i> sp.				
Immobilized cell on SF	24.57	26.74	2.73	6.45
Immobilized cell on PET	36.23	37.74	1.27	2.14
Free cell	26.88	31.95	1.75	2.70
<i>H. hibernicus</i>				
Immobilized cell on SF	47.85	57.80	1.22	1.57
Immobilized cell on PET	58.82	64.52	1.36	2.07
Free cell	56.18	59.17	1.05	1.50

4.5.3 Immobilized ability and DW/SA ratio

Immobilized cell on SF and PET of *Scytonema* sp. had similar immobilized ability in various cell ages and had the highest immobilized ability at 95.47% and 97.52%, respectively at 15 days cell ages. Immobilized cell on SF had immobilized ability at 92.53%, 91.48% and 94.68% and immobilized cell on PET had immobilized ability at 95.13%, 97.38% and 96.63% at 5, 10 and 20 day cell ages, respectively. In *H. hibernicus*, immobilized ability of immobilized cell on SF and PET were highest at 96.06% and 95.52% at 15 and 5 days cell ages, respectively. Immobilized cell on SF and PET had immobilized ability at 94.98% and 92.78% at 5 and 15 days cell ages, respectively. While immobilized cell on SF and PET had immobilized ability at 93.95 % and 92.71 %, 93.43 % and 92.17% at 10 and 20 day cell ages, respectively. Increasing of cell ages had no difference the DW/SA ratio in immobilized cell on SF and PET of *Scytonema* sp. and *H. hibernicus*. Cell ages at 5, 10, 15 and 20 days had the same DW/SA at 0.0013 gcell/cm² in immobilized cell on SF and PET of *Scytonema* sp., respectively. While immobilized cell on SF and PET of *H. hibernicus* had DW/SA ratio at 0.0010 gcell/cm² and 0.0008 gcell/cm², 0.0008 gcell/cm² and 0.0007 gcell/cm², 0.0011gcell/cm² and 0.0009 gcell/cm², at 5, 10 and 20 day cell ages, respectively. In both immobilized material at 15 days cell ages had the

same DW/SA ratio at 0.0008 gcell/cm², respectively. Immobilized cell on SF and PET of *Scytonema* sp. had the both higher immobilized ability and DW/SA than *H. hibernicus* as shown in Table 10.

Table 10 Immobilized ability and DW/SA between immobilized cell on synthetic fiber (SF) and polyethylene terephthalate (PET) of *Scytonema* sp. and *H. hibernicus* at 5, 10 15 and 20 days cell ages at 14-day-cultured

Type of immobilized material	Cell ages (days)	DW/SA (g cell/cm ²)		Immobilized ability (%)	
		<i>Scytonema</i> sp.	<i>H. hibernicus</i>	<i>Scytonema</i> sp.	<i>H. hibernicus</i>
Synthetic fibre (SF)	5	0.0013	0.0010	92.53	94.98
	10	0.0013	0.0008	91.48	93.95
	15	0.0013	0.0008	95.47	96.06
	20	0.0013	0.0011	94.68	93.43
Polyethylene terephthalate (PET)	5	0.0013	0.0008	95.13	95.52
	10	0.0013	0.0007	97.38	92.71
	15	0.0013	0.0008	97.52	92.78
	20	0.0013	0.0009	96.63	92.17

4.6 Characteristics of immobilized *Scytonema* sp. and *H. hibernicus* on synthetic fiber (SF) and polyethylene terephthalate (PET) under Scanning electron microscope (SEM)

The structure of immobilized cell on SF and PET of *Scytonema* sp. were evaluated by coated under vacuum with a thin layer of gold and examined by scanning electron microscope as shown in Figure 34.

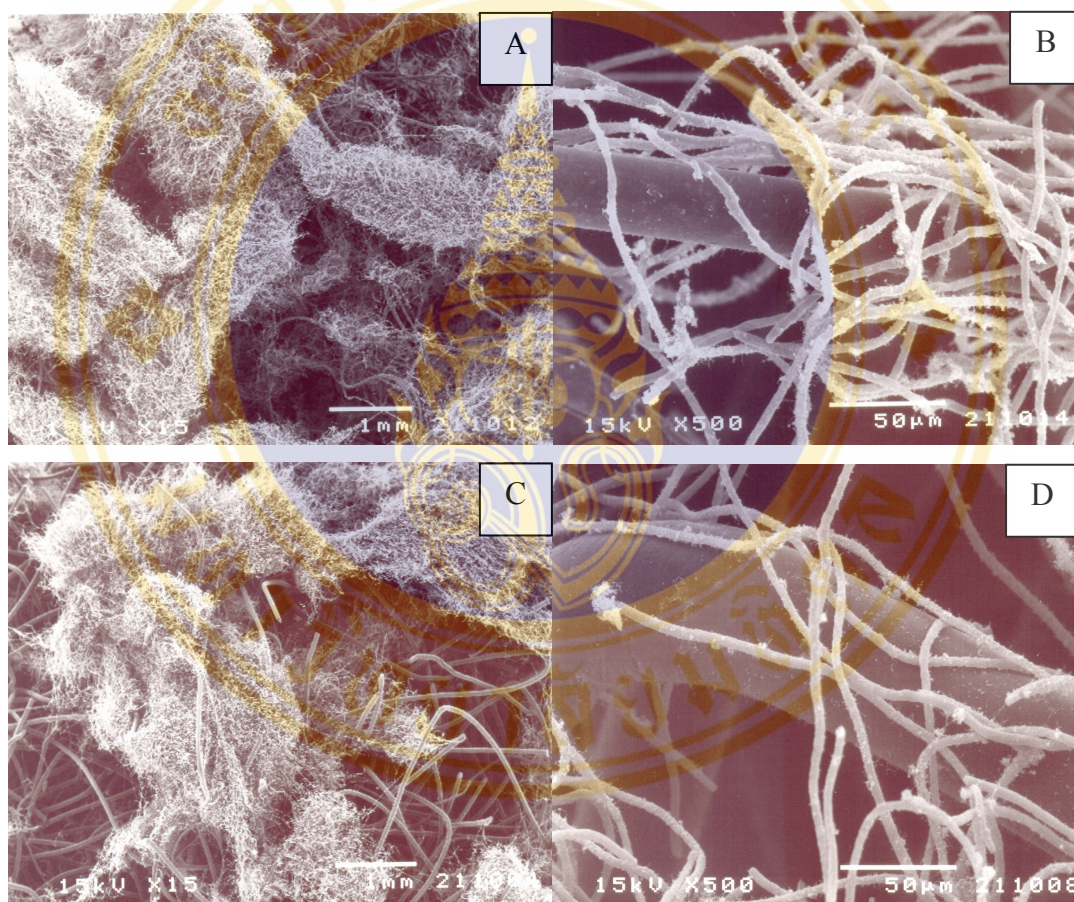


Figure 34 Scanning electron microscope (SEM) of immobilized cell on synthetic fiber (SF) and polyethylene terephthalate (PET) of *Scytonnema* sp. for 14 days ; immobilized cell on SF (magnification 15X) (A), immobilized cell on SF (magnification 500X) (B), immobilized cell on PET (magnification 15X) (C) and immobilized cell on PET (magnification 500X) (D).

The SEM micrograph revealed that immobilized cell on SF and PET were completely covered and penetrated into porous of immobilized material as shown in Figure 34 A and C. Cell of *Scytonema* sp. were completely covered and penetrate along the single surface of fibrous network of SF and PET as shown in Figure 34 B and D

The structure of immobilized cell on SF and PET of *H. hibernicus* were evaluated by scanning electron microscope (SEM) same *Scytonema* sp. as shown in Figure 35.

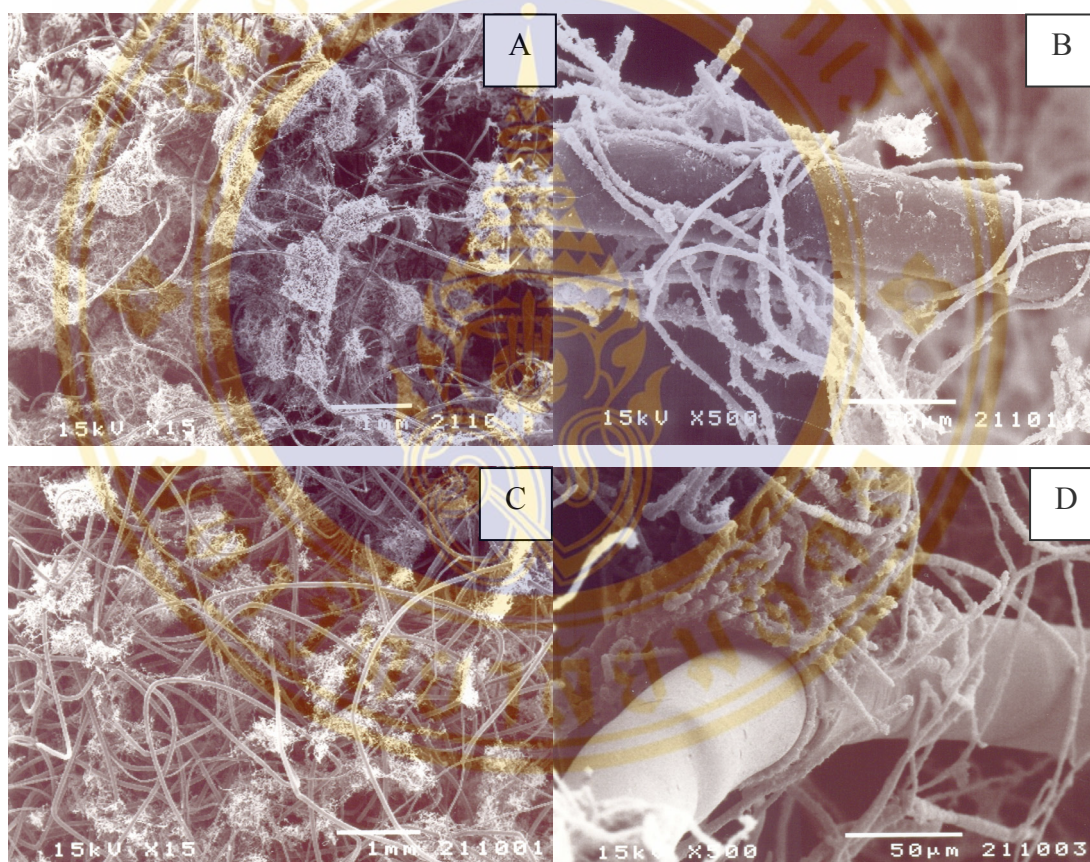


Figure 35 Scanning electron microscope (SEM) of immobilized cell on synthetic fiber (SF) and polyethylene terephthalate (PET) of *H. hibernicus* for 14 days; immobilized cell on SF (magnification 15X) (A), immobilized cell on SF (magnification 500X) (B), immobilized cell on PET (magnification 15X) (C) and immobilized cell on PET (magnification 500X) (D).

The SEM micrograph revealed that immobilized cell on SF and PET of *H. hibernicus* were completely covered into porous of immobilized material as shown in Figure 35 A and C. Cell of *H. hibernicus* were completely penetrate along the single

surface of fibrous network of SF and PET as shown in Figure 35 C and D. Immobilized cell had higher tightly packed on SF more than immobilized cell on PET of both *Scytonema* sp. and *H. hibernicus*. While immobilized cell of *Scytonema* sp. had higher amount of cell on fibrous network of SF and PET than immobilized cell of *H. hibernicus*.

4.7 Characteristics of *Scytonema* sp. and *H. hibernicus* under Transmission electron microscope (TEM)

The ultrastructure morphology of *Scytonema* sp. and *H. hibernicus* under transmission electron microscope in *Scytonema* sp. without cadmium (control) and exposed to cadmium 1 mg/l for 6 days as shown in Figure 36 and Figure 37.

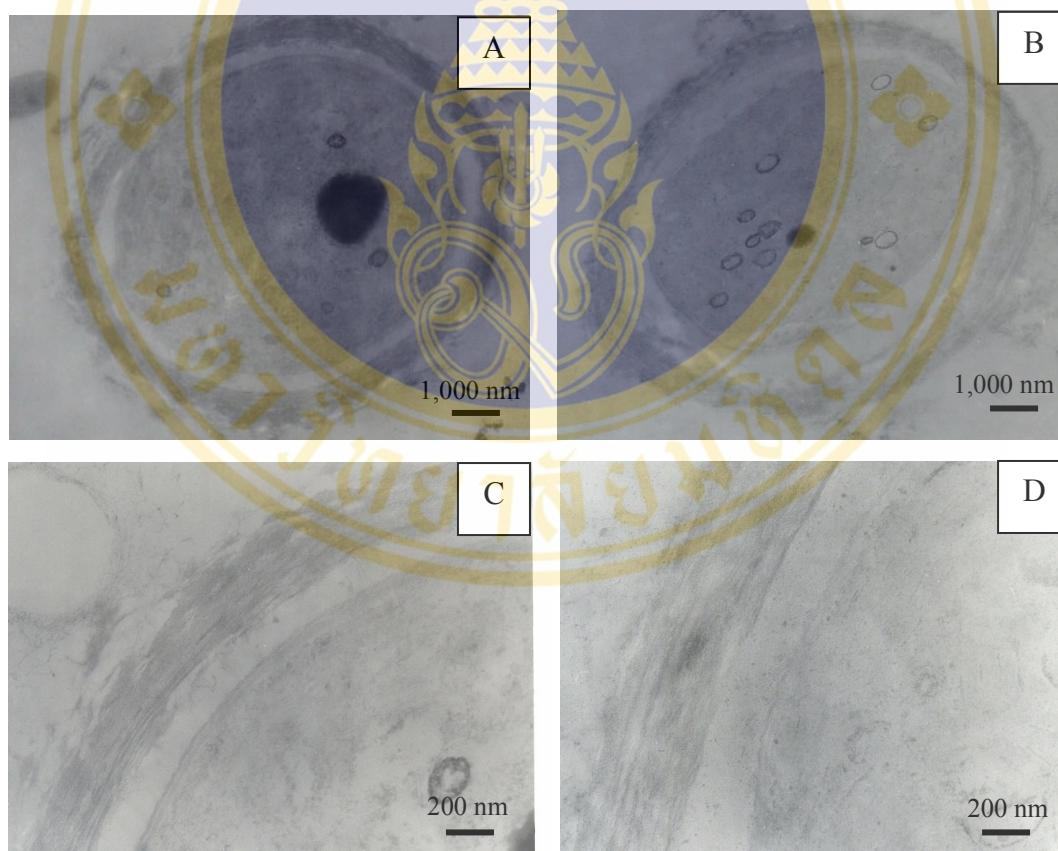


Figure 36 Transmission electron microscope (TEM) of *Scytonema* sp. cell without cadmium (control) (A, C) and cell treated with 1 mg/l of cadmium concentration for 6 days (B, D) ; non-treated cell (control) (magnification x 10,000) and treated cell (magnification x 60,000).

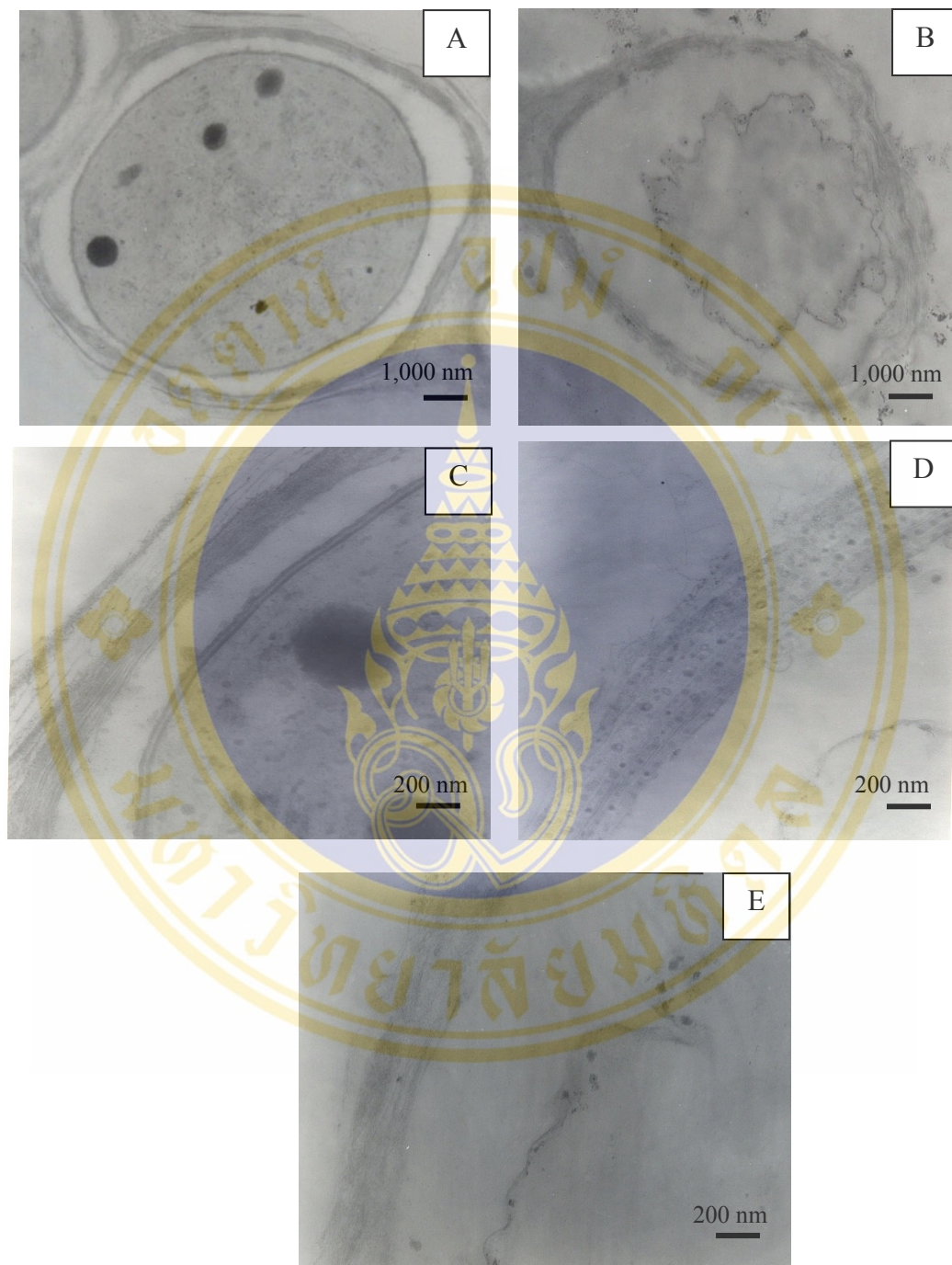


Figure 37 Transmission electron microscope of *H. hibernicus* cell without cadmium (control) (A, C) and cell treated with 1 mg/l of cadmium concentration for 6 days (B, D, E); non-treated cell (control) (magnification x 10,000) and treated cell (magnification x 60,000).

From the TEM micrograph demonstrated that cell of *Scytonema* sp. and *H. hibernicus* were cultivated in medium without cadmium. They were retained in the typical of cyanobacteria cell condition which is very similar to other species of the order Nostocales and Stigonemalales. The cell wall of *H. hibernicus* were more thickness than *Scytonema* sp. and cell membrane space was enclosed surround by the cell wall. The thickness of outer cell wall were observed in the dark region and inner cell wall in the inner light periphery of the photograph. Cyanobacterial cell was surrounded by a multilayered cell wall and consisted of an outer membrane with the unit membrane structure and peptidoglycan layer. The other structure of cyanobacteria such as thylakoid membrane, starch granule, cytoplasmic membrane, polyphosphate bodies and vacuole may be also present but could not clearly observed in photograph. After culturing *Scytonema* sp. in 1 mg/l of cadmium found that cell was slightly affected by cadmium. The first observed in the cell membrane which became irregular and damaged. Cyanobacterial cell had the space between cell wall and cell membrane (periplasma) while the intracellular organelles were trivially damaged. The ultrastructure appearance of *H. hibernicus* cells changed considerably when cell treated with 1 mg/l of cadmium. The first observed in periplasma space between cell wall and cell membrane were increased. The second observed in the mucilaginous sheath were secreted and slightly detached around the cell wall in the photograph. The main damages were noticed in the internal membrane which were irregular and convoluted when compared with control cell. In addition to the accumulation electron dense clearly deposit in the form of free granules of various size and large complexes. The abundant electron dense were located in cell membrane and in some parts were deposited in the intracellular organelle near the portion between cell wall and internal membrane of *H. hibernicus*. While the electron dense were not clearly observed in the extra and intracellular membrane of *Scytonema* sp.

CHAPTER V

DISCUSSIONS

5.1 Screening for high cadmium removal and high immobilized ability of cyanobacterial strains in different immobilized materials

The filamentous cyanobacteria were screened for both high cadmium removal and high immobilized ability in this study because of some filamentous cyanobacteria had high growth rate. The other advantage of filamentous cyanobacteria is easy to separate from solution after treatment and in addition to self-immobilized as a biofilm on porous supports (22). Moreover, cyanobacteria are suitable organisms for application in outdoor cultivation system because of the weather in Thailand have sunshine for the whole year. Immobilization provided high cell density and easy to separation of treated water from biomass. A good support material should be rigid and inert matrices that should bind cell firmly and had high loading capacity (96, 97). The immobilized cell on a natural and synthetic polymer matrix such as loofa sponge (LS), delicate duty scour pad (DP), synthetic fiber (SF), polyethylene terephthalate (PET), polyethylene terephthalate (PET non-woven fabric), polyethylene (PE), polypropylene (PP), polyurethane (PU) were used to immobilized cell in this study due to low cost, easy to find out and convenient for retain cells by attachment in pore size matrices. Immobilized material which invented in Thailand and Japan were compared in order to find out suitable matrix for high immobilized ability and high cadmium removal efficiency.

From this results, *Scytonema* sp. and *H. hibernicus* were selected for immobilized cell on SF and PET in the further study because of they had the highest cadmium removal at 93.31% in *Scytonema* sp. immobilized on SF and had the highest immobilized ability at 97.45 % in *H. hibernicus* immobilized on SF. In addition, SF and PET were low cost sustainable sourcing and high porosity from observation with scanning electron microscope (SEM). Many researchers reported the several polymeric

can be used for biomass immobilization for removed of cadmium, such as *Spirulina platensis* immobilized on alginate and silica gel for biosorption of cadmium more than 95% (26). Aghtar *et al.* found that immobilized *Chlorella sorokiniana* on loofa sponge could removed cadmium at 97.9% (29). Cadmium removal capacity of immobilized *Rivularia* sp. on PET was 99% (96). In our study had lower cadmium removal than the previous studied. Immobilized cell of *Scytonema* sp. and *H. hibernicus* on SF and PET had higher cadmium removal than DP, PET non woven fabric, PP, PU, PE and LS when compare to another strains. Because of some reports noticed that the extracellular polymeric substance which is anionic in nature on account of the presence uronic acid to bind metal ions selectively and bind metal cations by form capsules or loose aggregates around cell (20). Moreover, the functional group of cell wall composed of carboxyl, amine, imidazole, phosphate, sulhydry, sulfate and hydroxyl are the major binding site that cause significant in the amount of metal ion binding to cyanobacteria. The mass transfer of the metal ions from aqueous phase to the solid biosorbent sites is dependent on the porosity of the biosorbent and matrices. In addition to the condition of moderate agitation had the binding site freely exposed to the sorbate influencing to heavy metal removal capability (97). This supported by Al-Hakawati *et al.* who found that *Rhizopus oryzae* immobilized in polyurethane, polysulfone, polyacrylamide, alginate, PEI and polyvinylformal showed copper removals at 77, 32, 31, 25, 22, 11 and 86%, respectively (97). Similar studied by Bai *et al.* who reported that the variation of Cr (VI) adsorption percentage of immobilized cell on Ca-alginate, PVA, polysulfone, polyisoprene, polyacrylamide, polyurethane and coconut coir fiber of *Rhizopus nigricans* at 70.07%, 81.78%, 89.61%, 89.98%, 41.76%, 76.84% and 80.77%, respectively (101).

Immobilized cell on eight types of immobilized material demonstrated the same or lower cadmium removal than the free cell in all four strains of cyanobacteria except *Mastigocladus* sp. The result indicated that the mass transfer limitation due to restricted porosity of the immobilization matrices and masking active sites of biomaterial in this study. In accordance with Al -Hakawati *et al.* found that *Rhizopus oryzae* strain grown in reticulated polyurethane foam had the same heavy metal adsorption capacity as the free biomass (105). Similar study to Prakasham *et al.* who

reported that immobilized cell on alginate beads had slightly lower adsorption of chromium than free cell at 46.50% and 50.63 %, respectively (57).

From the results found that immobilized cell on eight types of immobilized material showed the same DW/SA ratio because of both cyanobacteria strain had the same culture age. This supported by Sombatjinda who found that in 5 strains of cyanobacteria immobilized on PET had the same DW/SA at 0.01 g cell/cm² within 10 days. Charoensombat who found that at 15 day cultured had higher DW/SA ratio than 1 day cultured due to the older cell had more exopolysaccharide which act as the immobilized enhancer (96, 106). The immobilized ability had difference in eight types of immobilized material and four strains of cyanobacteria. While immobilized cell of *H. hibernicus* on SF had the highest immobilized ability at 97.45 % but immobilized cell of *Oscillatoria agardhi* on LS had the lowest immobilized ability at 48.15 %. Because of the properties and porosity of SF is controlled to ensure that the structure is tight enough to prevent leakage of cells including the mass transfer in the matrices must be sufficiently porous to enable diffusion of the sorbate to the sorbent surface (107). In addition to the synthesis of extracellular investment of polysaccharide nature in each species of cyanobacteria and different environmental condition were generally considered to attachment cell. In addition to the morphology of some filamentous cyanobacteria could well more immobilized on immobilized material than suspended cell. (108, 109). Moreover, Al-Hakawati *et al.* found that biomass leaching from the biomass in polyurethane and PEI with about 20 % and 4 % respectively. The loss in weight at the end of the experiment as a result of the flask shaking of the biomass on polymeric matrix to copper adsorption and would probably not occur in a static packed bed reactor (97).

5.2 Effect of initial cell concentration and contact time on cadmium uptake between immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus*

From the result demonstrated that initial cell concentration at 0.015 g dry wt. was selected for further experiment because of the highest cadmium uptake and high immobilized ability.

Generally, the cadmium uptake has often been observed to occur in two stage. The first stage, biosorption is rapid due to surface adsorption on the extracellular surface of algal cell. This biosorption process occurs in both living and dead cells and metabolism-independent binding. The second stage, bioaccumulation is slow process involving an active transport of metal across the cell membrane into the intracellular organelles (110, 111). This process occurs only in living cell and metabolism-dependent binding and may be completed in hours or days (15). In the first stage, the uptake of cadmium by immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. became equilibrium after 30 minute in all cell concentration while immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* became equilibrium after 90 min. There were no difference in cadmium uptake of immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of both strain because of during the first stage in 90 min the immobilized cell of both strains were not occurred and the cell in immobilized system were still free cell in solution (96).

In the second stage demonstrated that cadmium uptake of immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* became equilibrium after 2 day because of the binding site at cell wall was saturated. The result on the adsorption rate in accordance with Matsunaga *et al.* who found that the cadmium adsorption of *Chlorella* sp. reached saturation value within 2 day and intracellular accumulation was observed to increase slowly in 8 day (112). While Inthorn *et al.* found that more than 90% of cadmium was removed from the solution within 10 min by *Tolypothrix tenuis*. Lu *et al.* reported that immobilized yeast had metal biosorption more than 90% within 3 hour and reached equilibrium after 24 hour (113). Similar studied to the cadmium biosorption of *Chlamydomonas reinhardtii* and immobilized *Phanerochaete chrysosporium* on loofa sponge were gradually obtained within 60 min. (99). There are several parameter determining the biosorption rate, such as structural properties of both the support and biosorbent (e.g., protein and carbohydrate composition and surface charge density, topography and surface area) including amount of sorbent, properties of the ions, initial concentration of ionic species and the presence of other metal ions which may compete with the ionic species of interest for the active

biosorption sites (7). However, the adsorption rate in this study was fast at 30 min which immobilized cell of both strains were appropriate for cadmium adsorbent.

From the result found that immobilized cell on SF, PET and free cell of both strain of cyanobacteria had decreased cadmium uptake when increasing initial cell concentration from 0.015 to 0.06 g dry wt. due to the dependence of adsorption on cell density could reduce cell distances and thus less sites were available for metal binding. It has also been suggested that the aggregation cell during biosorption and able to reduction of total surface area. Similar result to the specific uptake of zinc decreased when the biomass concentration was increased from 0.04 to 0.02 mg dry wt/ml (114, 115).

As refer to *Phormidium laminosum* immobilized on polysulfone and epoxy resin while the concentration of biomass was varied from 1 to 5 mg/l found that the lowering the biomass concentration at a given metal concentration enhanced the metal/biosorption ratio and thus increased metal biosorption per gram of biomass as long as the latter is not saturated (95). Immobilized cell on PET had higher cadmium uptake than immobilized cell on SF and free cell of both algae at initial cell concentration 0.015 g dry wt. While immobilized cell on PET and SF had slightly reduction of cadmium uptake in the same profile to free cell of *Scytonema* sp. and *H. hibernicus* at initial cell concentration 0.030 g dry wt., 0.045 g dry wt. and 0.060 g dry wt. Because of cell immobilization on the surface of the fibrous thread little or no interaction with other immobilized cells. However, immobilized cell were no clumping on the reticulated open network of immobilized matrix contributed to enhanced the surface area and free access of the metal to adsorption site (98). This was also in immobilized *Chlorella sorokiniana* on biomatrix had higher cadmium removal than free cell at 97.9% and 92.7%, respectively. Aksu *et al.* found that Ca-alginate immobilized *Chlorella vulgaris* were lower than the removal of Cu (II) by agarose-immobilized *Chlorella vulgaris*. Similar studied to Blanco who noted that metal diffusion rate was slower when *Phormidium laminosum* was entrapped in epoxy resin than polysulfone matrix because of diffusion in the limiting step in the biosorption of metal ion. Owing to polysulfone bead interior was porous and epoxy resin bead was more homogeneous and biomass aggregates appear completely embedded into the resin (94). While immobilized cell had slightly reduction of cadmium adsorption to

free cell. This supported by Mehta *et al.* who found that immobilized *Chlorella vulgaris* on alginate and free cell at lower biomass concentration were more efficient in removing copper but at biomass concentration above 100 mg/l. Free cell were more efficient than the immobilized cell because of the sorption of Cu per unit of dry wt. of biomass rapidly decreased with rise in biomass concentration of free cell as well as immobilized cell (116).

From the result found that increased initial cell concentration had increased DW/SA ratio due to increased initial cell concentration had decreased the nutrient in aqueous solution. The cyanobacteria formed cell aggregation had decreased the nutrient and energy. Similar study to Nardpiriyarad who suggested that when cell had rare nutrient, they would aggregate to each other by produced exopolysaccharide to attach on immobilized matrix (96, 117).

5.3 Effect of initial cadmium concentration on cadmium uptake between immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus*

The uptake of cadmium in immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* increased which increasing cadmium concentration when initial cadmium concentrations were 0.5, 1, 2, 4 and 8 mg/l due to the number of ions adsorbed from solutions of higher concentration are more than removed from less concentrated solutions. In addition to increased electrostatic interaction that relative to covalent interaction, involving sites of progressively lower affinity for metal ion (99). The maximum adsorption ability (q_{max}) and the binding constant (k_b) of cadmium adsorption were fitted and calculated by the Langmuir isotherm. According to the Langmuir model based on several assumptions as follow, sorption occurs uniformly on the active site and no further sorption can take place at this site.

The result showed that the concentration of cadmium is a significant factor considered for the sorbent/sorbate equilibrium of the system. Initial concentration of ions influenced on the rate of adsorption. The q_{max} is inversely to k_b revealed that the affinity for heavy metals adsorption of cyanobacteria were represented by the highest of q_{max} with the lowest of k_b which means to the higher affinity of cyanobacteria for

binding with heavy metal even if lower concentration. The equilibrium data on cadmium adsorption by immobilized cell on SF, PET and free cell of both strains were fitted into the Langmuir isotherms. The adsorption data fitted well with the isotherm showed correlation coefficient value ranges 0.96 to 0.99 which supported that monolayer sorption onto the cell surface of mechanism. The immobilized cell on PET had higher q_{max} than free cell and immobilized cell on SF of both *Scytonema* sp. and *H. hibernicus*. While immobilized cell on SF had higher k_b than free cell and immobilized cell on PET of *Scytonema* sp. but immobilized cell on SF had nearly the similar k_b to immobilized cell on PET and free cell of *H. hibernicus*. In accordance with Iqbal *et al.* who found that the q_{max} and k_b of Cd (II) adsorbed of immobilized *Phanerochaete chrysosporium* on loofa sponge had higher than free cell at 85.98 (mg/g biosorbent) and 0.064 l/mg, 70.38 (mg/g biosorbent) and 0.043 l/mg, respectively (118). Rangsayatorn *et al.* reported that the q_{max} of alginate immobilized cell was 70.92 mg Cd/g dry weight and the k_b was 0.071 l/mg but the q_{max} of silica immobilized cell was 36.63 mg Cd/g dry weight and k_b was 0.196 l/mg. The q_{max} of alginate immobilized cell had higher than silica immobilized cell and they were lower than free cell of *Spirulina platensis* at 98.04 mg Cd/g dry weight because of changes in the structure of the cell during the immobilization and part of the cell surface might be shielded by the gel matrix and thus would not be available for metal binding (26). The immobilized cell on SF, PET and free cell of *H. hibernicus* had higher q_{max} than *Scytonema* sp. whereas immobilized cell on SF, PET and free cell of *H. hibernicus* had lower k_b than *Scytonema* sp. Because of the different sorbent having a wide range of adsorption capacity for heavy metal ion. Changes in metal ion binding capacity may also due to the properties of the metal sorbate such as ionic size, atomic weight or reduction potential of the metal. In addition to the properties of the algae such as structure, function groups and surface area depending on the algal division, genera and species are the factor causing changes in metal ion binding capacity (50). In other studies reported that the q_{max} and k_b for cadmium uptake by *Rhodotorula rubra* were 5.8 mg/g and 0.21 l/mg, respectively (12). The q_{max} of *Rhizopus arrhizus* was determined as 17.67 mg/g at 75 mg/l initial cadmium concentration whereas *Aspergillus niger* was 2.91 mg/g at 25 mg/l initial cadmium concentration (119). The fungal biomass of white rot fungus *Phanerochaete chrysosporium* used for heavy metal

from artificial wastewater had cadmium removal capacity of the dry fungal biomass of 23.4 mg Cd (II)/g dry wt. (100). The q_{max} of living cell of *Actinomyces* strain AK61 and JL322 were 22.81 mg/g dry wt., 22.02 mg /g dry wt, while the k_b were 0.29 l/mg, 0.23 l/mg, respectively (120). The q_{max} and binding constant (k_b) of cadmium for *Tolypothrix tenuis* was 19.6 mg/g dry wt. and 3.0 mg/l, respectively (22). Immobilized cell on SF, PET and free cell of both strains had q_{max} and k_b more than this previous studied. Moreover, similar observations were reported that other biomass including cyanobacteria can be attributed to a variety of resistance mechanism include extracellular complexation with metal binding proteins. Phytochelatins which are proteins that contain large amounts cysteine and bind heavy metal ions and in addition to the efficient pumping out metal ions from the living cell (52, 70, 121). Therefore, immobilized cell on SF and PET of *Scytonema* sp. and *H. hibernicus* are attractive biosorption material for use in wastewater treatment systems due to more efficient and cheaper than the quaternary treatment (15).

5.4 Growth rate of *Scytonema* sp. and *H. hibernicus*

The growth curve of both strains can be divided into 4 phase as lag phase, log phase, stationary phase and decline phase. The specific growth rate (k) of immobilized cell on SF, PET and free cell of *Scytonema* sp. without cadmium were 0.0940, 0.0938 and 0.0865 day⁻¹, respectively. While the specific growth rate (k) in immobilized cell on SF, PET and free cell of *H. hibernicus* without cadmium were 0.0738, 0.0740 and 0.0694 day⁻¹, respectively. Immobilized cell on SF, PET and free cell of both stains were no difference in growth rate. In contrast to the studied of Iqbal *et al.* found that immobilization has a positive influence on the growth of *Phanerochaete chrysosporium* with 21% increase in biomass compared with freely growing hyphae. The mean amount of immobilized fungal biomass was 355±14 mg/g (118). This is threefold higher than for the same fungi immobilized within Ca-alginate bead indicated that the superiority of the open structure over the polymeric matrices with simply to made by inoculation sponge discs with an appropriate suspension in suitable growth medium (28, 118). Moreover, when decided the specific growth rate (day⁻¹), it was found that *Scytonema* sp. had higher growth rate than *H. hibernicus*. While Torres *et al.* found that *Phaeodactylum tricornutum* without cadmium had growth rate (day⁻¹)

at 0.658 day^{-1} (122). Sombatjinda reported that *Nostoc paludoasum* and *Rivularia* sp. had growth rate at 0.0094 day^{-1} and 0.0069 day^{-1} in solution without cadmium. While the growth rate of *Nostoc paludoasum* and *Rivularia* sp. in solution with cadmium were reduced to 2.61-3.15% because the photosynthetic structure is the target of cadmium to inhibit the cell of cyanobacteria (96). The growth rate was inhibited by the low activity the photosynthetic. Fernandez-Pinas found that photosynthetic organelle of *Nostoc* sp. is cellular target of cadmium which inhibited the photosynthesis and deterioration of thylakoid membranes in photosynthetic lamellae. The damage of thylakoid distinctly indicates a decreased in photosynthetic activity and growth (123). The different algal species had different growth rate because the ability to survive of algae and reproduce in metal-polluted habitats may depend on genetic adaptation over the time periods by mutations, genetic exchange, selection or changes in physiology resulting from metal exposure (96).

5.5 Effect of cell ages on cadmium uptake between immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus*

The growth curve of *Scytonema* sp. and *H. hibernicus* can be divided in four phase that consisted of lag phase, early log phase, late log phase and stationary phase. Similar studied to Charoensombat who can be divided age of *Spirulina platensis* in lag phase (0 - 20 h), early log phase (24 h), mid log phase (120 h), late log phase (200 h) and stationary phase (240 – 363 h) (106). From the result found that the uptake of cadmium by immobilized cell on SF, PET and free cell of both cyanobacteria strains had highest at 15 day of cell ages in during late log phase or early stationary phase. On account of the adsorption of cadmium influence of growth phase that was mainly on the number of adsorption site for cadmium rather than the affinity of cadmium to the cells. The exponential phase exhibited higher cadmium adsorption capacity because of the binding site of cadmium with biomass was probably facilitated by membrane-bound product that are growth associated (124). This supported by Sombatjinda who found that *Nostoc paludosum* and *Rivularia* sp. immobilized cell on PET at 14-day cultured had higher cadmium removal than 10- day culture due to they had more cell surface

area to sorb cadmium (96). Immobilized cell on SF, PET and free cell of both strains at 15 days cell ages had slightly higher cadmium uptake than 5, 10 and 20 days cell ages. In several previous studies reported that heavy metal adsorption capacity of microorganism cells of various cell ages were different and the variation related to the cell surface components and structure (125). Cyanobacteria envelope were similar structure and phylogenetically coherent group of gram-negative bacteria that consists of three major structural components. The first, outer membrane which is mostly composed of lipopolysaccharides, proteins and phospholipids. The second, the inner membrane which is mostly composed of phospholipids and proteins and third, the peptidoglycan (PEG) layer. The cell envelope of cyanobacteria consists mainly of polysaccharide that are negatively charged and uronic acid exhibited high metal binding capacity (126, 127, 128). In accordance with Wang *et al.* who found that growth curve of *Pseudomonas putida* can be classified into four phase, such as lag phase, log phase, stationary phase and death phase. The adsorption capacity of the cell envelope decreased along with cell age and the lowest point of adsorption capacity appeared at about 18 h. thus finally reached the highest point at about 36 h. The content peaked at 36-38 h at the end of stationary phase with PEG layer had the relationship of the content of cell age was similar to the relationship of the cell envelope adsorbent capacity to cell growth (129). However, immobilized cell on SF, PET and free cell had the similar cadmium uptake of *Scytonema* sp. at 5, 10, 15 and 20 days cell ages. While the cadmium uptake had slightly higher increased in immobilized cell on PET than immobilized cell on SF and free cell of *H. hibernicus* at 5, 10, 15 and 20 days cell ages. Moreover, they had slightly increased and stayed relatively unchanged after 14 days cultivation. Similar studied to Kitjaharn who found that the accumulation of lead by *Spirulina platensis* increased up to 8 days and became constant afterward. Similar to the age of *Aphanothece halophytica* up to 20 days not influenced on the ability of cells to accumulate zinc (95). In contrast to Sombatjinda who found that free cell of 14 day-culture had lower cadmium removal than 10 day-culture that agree with Volesky who reported that uranium biosorption by cell of *Saccharomyces cerevisiae* was affected by culture age. Similar studied to Simmond and Singleton who demonstrated silver biosorption and the younger biomass could remove from solution more than older cell and in accordance with the 5-day old cultures were twice as effective as 15 day-old

culture that explanation that change in the cell-surface chemistry and morphology with age contributed to higher uptake (59, 96).

5.6 Characteristic of immobilized *Scytonema* sp. and *H. hibernicus* on synthetic fiber (SF) and polyethylene terephthalate (PET) under Scanning electron microscope (SEM)

The SEM micrograph revealed that immobilized cell on SF and PET of both cyanobacteria strain were completely penetrated along the surface of fibrous network. The completely embedded of cell on immobilized material was important criterion for the proper biosorption of heavy metals on the whole surface area of the immobilized cell. However, immobilized cell on SF of *Scytonema* sp. and *H. hibernicus* were more clearly covered cell than PET because of the structure of SF made from modified polyester that had higher pore size than PET which made from combined between terephthalic acid and ethylene glycol (recycled polyester) (130, 131, 132, 133). According to the degree of porosity of the bedding material is an important factor in the adsorption of microorganism to matrix (72, 74). The structure of SF and PET should be sufficient porous to provide sufficient channels for gas and nutrients transfer which enables to immobilized biomass (134). In addition to the microbial structure which were various structure that used for attachment including fimbria (pilli), capsules (glycocalyx) cellular component and slime (23, 135, 136). The glycocalyx consists of extracellular polysaccharides which the main structure for attachment. Besides the glycocalyx, any extension from the cell often comes into contact with surfaces and involved in attachment. The force governing microbial attachment such as electrostatic interaction, covalent bond formation and hydrophobic interaction were affected to immobilized cell (72). Even through the immobilized cell were packed tightly but there were still sufficient porosity and void volume for free movement of solute was important criterion for the increasing biosorption of heavy metal on the surface area of the immobilized cell. Thus, immobilized cell can also provide additional advantages over the freely suspended cell (98, 118).

5.7 Characteristic of *Scytonema* sp. and *H. hibernicus* under Transmission electron microscope (TEM)

The ultrastructure alterations of *Scytonema* sp. and *H. hibernicus* which exposed 1 mg/l of cadmium for 6 days were observed to and compared with control. The first observed response occurred in the cell wall which became irregular and slightly damaged in *Scytonema* sp. while internal organelle of *H. hibernicus* were severely damaged and convoluted. The intracellular of *H. hibernicus* had the large portion between cell wall and internal membrane. The alternation of cyanobacteria morphology were observed in the light periphery in the photograph surround the thickness of outer cell wall in the dark region in the photograph that resulted from secretion of extracellular polymer substance. From the different alternation in the structure of the cell membrane between *Scytonema* sp. and *H. hibernicus* due to the thickness of membrane layer, the formation rate of the cell wall during cultivation, the ultrastructure of cytoplasm, heavy metal concentration and microbial species (137, 138). The cell wall of *H. hibernicus* were thickening due to increase of polysaccharide component that provided charged group binding to cadmium ion (139). Similar studied to Park *et al.* reported that the thickness of outer cell wall and inner cell wall of *Saccharomyces cerevisiae* ATCC834 was larger than *Saccharomyces cerevisiae* ATCC 24858 that cadmium adsorption occurred only on the cell wall and the thickness of the cell wall obstructed cadmium accumulation inside the cell (137). The tolerance and resistance of metal exposure can be affected to survival and reproduction in different algae species. In accordance with Tobaski *et al.* found that *Monodictys pelagica* was still growing in 10 mg/l of cadmium and the growth was totally depressed at 50 mg/l while *Corollospora lacera* could grown under all cadmium concentration up to 500 mg/l with suggest that this species is resistant to cadmium toxicity. While Joho *et al.* reported that cadmium - sensitive and cadmium - resistant strain of *Saccharomyces cerevisiae* differed in the distribution of cadmium after exposure because of the cadmium – resistant strain was found to contain metal mostly in the intracellular soluble fraction while the cadmium – sensitive metal was associated predominantly with the insoluble intra and extracellular fraction (140).

The TEM micrograph shown electron dense particle in the form granules or large complexes were clearly observed within cell surface and slightly distribution

within internal organelle of *H. hibernicus* but not clearly found in the internal membrane of *Scytonema* sp. Previous reports have demonstrated that the heavy metals may exist in solution in the form of free cations (Me^{2+}) or hydrated forms which are toxic and complexes most probably of the type of salt form which decrease the concentration of the toxic form (137, 140). Cadmium is one of the non-essential heavy metal which has been considered as an extremely significant pollution due to its high toxicity and great solubility in water (141). These metals are often found as deposits in various cellular organelles such as mitochondria, vacuoles, chloroplast, nuclei, polyphosphate bodies, cell wall and even in eyespots (49). They are generally considered affect to cell wall due to the biomass were mainly formed polysaccharide related to the increased cell wall which can be provided charged group binding to Cd^{2+} . In addition to the cell wall may have a role in cell ionic regulation by the chemical adaptability of it polysaccharides besides preventing toxic ion entering to the cell (139). Nagase *et al.* found that Cd^{2+} distribution was localized on the surface of non – treated *Tolypothrix tenuis* cells (142). Similar studied to Shu *et al.* who insisted that cadmium was adsorbed on the cell surface of *Saccharomyces cerevisiae* and Taboski *et al.* who reported that cadmium was bound to the cell wall of *Pleurotus ostreatus* (140). While Aguilera *et al.* reported that the ultrastructural of *Chlamydomonas* sp. cells changed when exposed to cadmium. The periplasma space increased greatly due to the presence of the large vacuoles and decrease nucleus, pyrenoid and starch granules (143). Similar dense granules have been visualized in other microalgae species treated with heavy metals. This supported by Diannelidis *et al.* who reported that the accumulation of heavy metal to the *Ceramium* carpospores were mainly bound to the cell wall and surrounding mucilage with some amount taken into the cytosol. While electron dense area were also found at the outer surface layers of *Ectocarpus siliculosus* after cadmium adsorption and heavy metal caused electron dense deposits in the cell wall of *Cystoseira* (144). Many researcher have reported that electron dense deposit were from polyphosphate bodies which have the ability to accumulate metals and also to protect cyanobacteria cells from metal toxicity. This supported by Rangsayatorn who found that the increase number of polyphosphate bodies which have been suggested that the site of metal sorption cyanobacteria (45). The strongly negative surface charge of the polyphosphate in the polyphosphate

bodies and the lipids and proteins also found in the polyphosphate bodies role to adsorb the metal. Similarly to Nishikawa *et al.* who found that polyphosphate hydrolyzed forming shorter-chain species or orthophosphates and combined with cadmium in vacuoles of *Chlamydomonas acidophila* (145). From the fine structure changes are consistent with physical data reported in the literature for location to heavy metal deposit of the cell wall polysaccharides and within internal organelle such as vacuole and polyphosphate bodies. The mechanism of cadmium detoxification is accomplish by several mechanism with adsorption in cell membrane by the ion-exchange reaction and cell membrane which plays a role in transmitting metal and destroy for heavy metal to accumulate in the cell. The metal accumulated internally within living cell including phytochelatin were probably mechanism to uptake of cadmium in *Scytonema* sp. and *H. hibernicus* (140).

CHAPTER VI

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

6.1.1 Screening for high cadmium removal and immobilized ability of cyanobacteria strains in different immobilized materials

The living cells of cyanobacteria in both immobilized and free cell had high potential to adsorb and accumulate the large amount in low cadmium concentration.

Immobilized cell of *Scytonema* sp. and *H. hibernicus* on SF and PET were selected for further study because they had high cadmium removal and high immobilized ability.

Immobilized cell on SF, PET and free cell of *Scytonema* sp. had cadmium removal at $93.31 \pm 0.21\%$, $81.93 \pm 2.02\%$ and $94.80 \pm 3.08\%$, immobilized ability were 94.79%, 96.00% and DW/SA were 0.0023 g/cm^2 , 0.0033 g/cm^2 , respectively.

Immobilized cell on SF, PET and free cell of *H. hibernicus* had cadmium removal at $85.14 \pm 1.76\%$, $83.42 \pm 3.03\%$ and $98.32 \pm 0.20\%$, immobilized ability were 97.45% and 86.80 % and DW/SA were 0.0023 g/cm^2 and 0.0017 g/cm^2 , respectively.

6.1.2 Effect of initial cell concentration and contact time on cadmium uptake between immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus*

The initial cell concentration at 0.015 g dry wt. was selected because they had highest cadmium uptake, high cadmium removal, immobilized ability and DW/SA ratio.

The uptake of cadmium in short time (0 - 90 min) by immobilized cell on SF, PET and free cell of *Scytonema* sp. became equilibrium after 30 minute in all cell concentration while immobilized cell on SF, PET and free cell of *H. hibernicus* became equilibrium after 90 min.

The uptake of cadmium in long time (2 -14 days) by immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* became equilibrium after 2 day

Cadmium uptake was decreased when the initial cell concentration increased. Immobilized cell on SF, PET and free cell of *Scytonema* sp. had highest cadmium uptake at 4.24 mg Cd/g dry wt., 7.64 mg Cd/g dry wt. and 3.56 mg Cd/g dry wt., respectively. Immobilized cell on SF and PET had immobilized ability at 97.38% and 96.49 %, DW/SA were 0.0018 g cell/cm² and 0.0009 g cell/cm², respectively.

Immobilized cell on SF, PET and free cell of *H. hibernicus* had highest cadmium uptake at 5.51 mg Cd/g dry wt., 7.03 mg Cd/g dry wt. and 5.87 mg Cd/g dry wt., respectively. Immobilized ability of immobilized cell on SF and PET were 89.31% and 81.72%, DW/SA were 0.0009 gcell/cm² and 0.0007 gcell/cm², respectively.

6.1.3 Effect of initial cadmium concentration on cadmium uptake between immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus*

The cadmium uptake in immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* increased as increasing cadmium concentration. Biosorption isotherm could be explained by Langmuir isotherm equation

The immobilized cell on SF, PET and free cell of *Scytonema* sp. had q_{max} at 26.74 mg/g dry wt., 37.74 mg/g dry wt. and 31.95 mg/g dry wt. and had k_b at 6.45 l/mg 2.14 l/mg and 2.70 l/mg, respectively when $R^2 > 0.96$. Immobilized cell on SF and PET of *Scytonema* sp. had the highest immobilized ability at 95.80% and 94.58%, DW/SA were 0.0014 gcell/cm² and 0.0012 gcell/cm² at initial cadmium concentration 0.5 mg/l, respectively.

Immobilized cell on SF, PET and free cell of *H. hibernicus* had q_{max} at 57.80 mg/g dry wt., 64.52 mg/g dry wt. and 59.17 mg/g dry wt. and had k_b at 1.57 l/mg, 2.07 l/mg and 1.50 l/mg, respectively when $R^2 > 0.99$. Immobilized ability of immobilized cell on SF and PET had the highest at 93.98% and 93.14%, DW/SA were 0.0008 gcell/cm² and 0.0005 gcell/cm² at initial cadmium concentration 0.5 mg/l, respectively.

6.1.4 Effect of cell ages on cadmium uptake between immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus*

Cadmium uptake in immobilized cell on SF, PET and free cell of *Scytonema* sp. and *H. hibernicus* at 5, 10, 15 and 20 day cell ages were slightly different between 6 day - cultured and 14 day - cultured.

The uptake of cadmium in immobilized cell on SF, PET and free cell of *Scytonema* sp. were the highest at 5.64 mg Cd/g dry wt., 5.71 mg Cd/g dry wt. and 5.67 mg Cd/g dry wt. at 15 day cell ages, respectively.

The uptake of cadmium in immobilized cell on SF, PET and free cell of *H. hibernicus* were the highest at 9.05 mg Cd/ g dry wt, 9.96 mg Cd/g dry wt. and 8.46 mg Cd/g dry wt. at 15 day cell ages, respectively.

Immobilized cell on SF and PET of *Scytonema* sp. and *H.hibernicus* had slightly difference immobilized ability and DW/SA ratio in various cell ages at 5, 10, 15 and 20 days.

6.1.5 Characteristic of *Scytonema* sp. and *H. hibernicus* under scanning electron microscope (SEM) and transmission electron microscope (TEM)

The SEM micrograph elucidated that SF and PET had high porosity and suitable to immobilized material for immobilized cell of *Scytonema* sp. and *H. hibernicus*.

The TEM micrograph demonstrated that ultrastructure alterations of *Scytonema* sp. and *H. hibernicus* treated with 1 mg/l of cadmium were damaged and disorganization cell membrane and internal organelles.

Electron dense particle were deposited in the cell wall and internal organelle of *H. hibernicus* but not clearly found in the cell of *Scytonema* sp. indicated that cadmium uptake were probably to accomplish by several mechanism with adsorption in cell membrane and accumulated in the intracellular organelle.

6.2 Recommendation

6.2.1 Recommendation for application

Immobilized cell on synthetic SF and PET of *Scytonema* sp. and *H. hibernicus* have the potential to removed cadmium in wide spread low cadmium contaminated natural water as in rivers or channels and can be applied in shrimp pond at low cost.

6.2.2 Recommendation for further study

Immobilized cell on synthetic SF and PET of *Scytonema* sp. and *H. hibernicus* are interesting to study in other system including continuous system in packed bed column and in combination of multi-heavy metal system. Moreover, they could be reused and regenerated in multiple adsorption-desorption cycles.

There should be a further confirm in the mechanism of cadmium uptake by using X-ray microanalysis to confirm with TEM and SEM analysis in order to evaluated the localization of metal deposit within cell and supported the mechanism of heavy metal biosorption.

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APPENDIX A

Culture medium

Medium 18 consisting of : (per liter)

NaNO_3	1.5 g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	380 mg
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	110 mg
NaCl	70 mg
K_2HPO_4	120 mg
$\text{Fe}_2(\text{SO}_4)_3 \cdot n\text{H}_2\text{O}$ (n=11~14),	10 mg
$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	9 mg
H_3BO_3	3 mg
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	2 mg
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.3 mg
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.08 mg
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.04 mg

The pH was adjusted to 7.5 with 0.03 N NaOH and 0.03 N NH_4OH

APPENDIX B

Cadmium (Cd^{2+}) 1 mg/l was prepared from $\text{Cd}(\text{NO}_3)_2$

Molecular Weight of $\text{Cd}(\text{NO}_3)_2$	=	308.47
$\text{Cd}(\text{NO}_3)_2$ 1 mol/l	=	308.47 g/l
	=	$308.47 \times 10^3 \text{ mg/l}$
$\text{Cd}(\text{NO}_3)_2$ mol/l or 1M	=	$3.0847 \times 10^5 \text{ mg/l (ppm)}$
$\text{Cd}(\text{NO}_3)_2$ $3.0847 \times 10^5 \text{ mg/l}$	=	1 mol/L
$\text{Cd}(\text{NO}_3)_2$ 1 mg/l	=	$\frac{1 \times 1}{3.0847 \times 10^5}$
	=	$3.2418 \times 10^{-6} \text{ mol/l}$
$\text{Cd}(\text{NO}_3)_2$ 1 mg/l	=	$3.2418 \times 10^{-6} \text{ mol/l}$
Molecular weight of Cd^{2+}	=	112.41
Cd^{2+} 1 mol/l	=	112.41 g/l
	=	$112.41 \times 10^3 \text{ mg/l}$
Cd^{2+} 1 mol/l or 1 M	=	$1.1241 \times 10^5 \text{ mg/l (ppm)}$
Cd^{2+} $1.1241 \times 10^5 \text{ mg/l}$	=	1 mol/l
Cd^{2+} 1 mg/l	=	$\frac{1 \times 1}{3.0847 \times 10^5}$
	=	8.896×10^{-6}
Cd^{2+} 1 mg/l	=	0.0089 mM
$\text{Cd}(\text{NO}_3)_2$ 1M = $3.0847 \times 10^5 \text{ mg/l}$ has Cd^{2+}	=	$1/1241 \times 10^5 \text{ mol/l}$
$\text{Cd}(\text{NO}_3)_2$ 1mg/l has Cd^{2+}	=	0.3644 mg/l
Cd^{2+} 1 mg/l, $\text{Cd}(\text{NO}_3)_2$	=	$\frac{1}{0.644} = 2.74 \text{ mg/l}$
Stock of Cd^{2+} 1000 mg/l 100 ml was prepared Cd^{2+} 1		
mg/l used $\text{Cd}(\text{NO}_3)_2$	=	2.74 mg/l
Cd^{2+} 1000 mg/l used $\text{Cd}(\text{NO}_3)_2$	=	2740 mg/l
Cd^{2+} 1000 mg/l volumn 1000 ml used $\text{Cd}(\text{NO}_3)_2$	=	2.740 g/l
Cd^{2+} 1000 mg/l volumn 100 ml used $\text{Cd}(\text{NO}_3)_2$	=	0.274 g/l

APPENDIX C

Characteristics of immobilized material

1. Loofa sponge (LS)

Loofa sponge was obtained from the ripened dried fruit of *Luffa cylindrical* (29, 118).

2. Delicate duty scour pad (DP)

Delicate duty scour pad was obtained from available pad of Scotch-brite from 3M company, Thailand and were analyzed by National Metal and Materials Technology Center (M-TEC) which was performed by using FT-IR spectra. The result found that the delicate duty scour pad was resemblance to standard polyester and standard magnesium silicate (MgSiO_3). Therefore, it is possible that the main chemical structures of DP is similar to polyester mixed with other compound, which is expected to be magnesium silicate (MgSiO_3) and the other functional group.

3. Synthetic fiber (SF)

Synthetic fiber was obtained from filter which available in the market produced from aquarium company and were analyzed by National Metal and Materials Technology Center (M-TEC) which was performed by using FT-IR spectra. The result found that the synthetic fiber was resemblance to standard polyester. Therefore, it is possible that the main chemical structures of SF is similar to polyester in the IR-Spectrum.

4. Polyethylene terephthalate (PET and PET non-woven fabric)

Polyethylene terephthalate (PET) is the most important example of the class of polymers known as chain-growth or condensation polymers. This group also includes nylon and virtually all of the naturally occurring polymers except rubber (e.g., cellulose, silk, etc.) PET make by simply heating two different kinds of molecules together, such as phthalic acid and ethylene glycol. Each of these monomers has two of the same functional group (phthalic acid has two carboxylic acid groups) while ethylene glycol has two alcohol groups (Figure 38). Another functional group that called esters, is usually formed from an alcohol and carboxylic acid. If the process is carried out using double-barreled monomers such as phthalic acid and ethylene glycol, the product is left still has one carboxylic acid and one alcohol left. Therefore, each of

these can react again to form two more esters, and there are still two reactive functional groups at each end. This process can continue for a very long time, giving large polymer molecules made up of repeating ester units. That is the particular class of polymers is also called polyesters and assume that any item of clothing contains a certain percentage of polyester probably has PET in it (130, 131, 132).

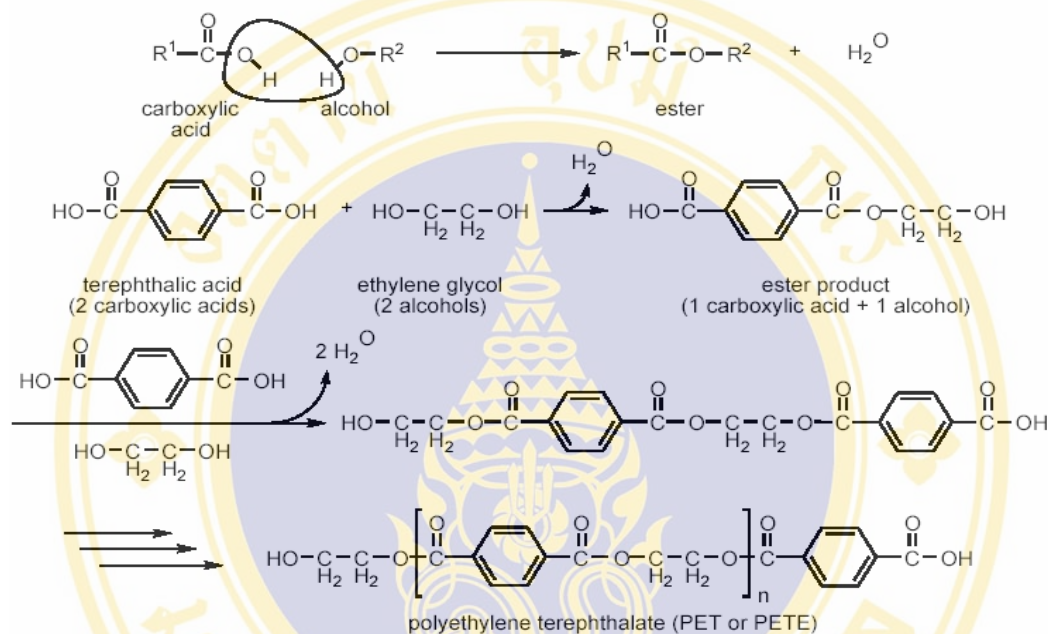


Figure 38 Polyethylene terephthalate from pthalic acid and ethylene glycol (133).

Recycled polyethylene terephthalate from old drinks bottles and of sources is now being used to make the heating, ventilation and air conditioning duct vent doors. These components, which mix and direct air from heating and cooling systems into the passenger compartment, were normally made from 45% glass-filled polyester, mainly virgin material. PET molecules are highly aligned, creating a strong and abrasion-resistant material that is used to produce films and polyester fibers. This plastic is used to make the trademarked textiles Dacron, Fibre V, Fortrel, and Kodel and transparent PET films. Non woven fabric are porous, textile material that the fibers are processed directly into a planar sheet like fabric structure by passing the intermediate one-dimensional yarn state and bonded chemically to achieve a cohesive fabric. The type of non woven fabrics that is produced depends largely on fiber type and method of manufacture such as air-laid and wet-laid non woven fabrics (131, 132).

5. Polyethylene (PE)

Polyethylene was obtained from available plastic which were polymerized from the organic compound ethylene. Ethylene is the old name for ethane that simplest example of an alkane. Polyethylene is a chain-growth polymer, which the double bond of the alkene monomer gets broken a new bonds are formed between monomer molecules. There are two main types of polyethylene, high density polyethylene (HDPE) and low density polyethylene (LDPE). High density polyethylene is harder and stronger than LDPE, it is used for gas tanks, heavy duty pipes, appliance cabinets and in other situations requiring great strength. LDPE have encountered include plastic wrap, grocery bags and disposable diaper liners (131, 132, 133).

6. Polypropylene(PP)

Polypropylene was obtained from available plastic which were polymerized from the organic compound propylene which similar in structure to polyethylene. Propylene is like ethylene, except with a methyl group (CH_3) replacing one of the hydrogen. However, polypropylene is made only using catalysts like the ones used for HDPE and lead to a very regular arrangement of the methyl groups on alternating carbon atoms. Polypropylene is fairly versatile polymer which used as a plastic and fiber. One of the most common uses of polypropylene fibers is in indoor-outdoor carpeting. It is also used in thermal underwear (131, 132, 133).

7. Polyurethane (PU)

Polyurethane was obtained from available plastic which were typically made from two different monomer between isocyanate group and alcohol group. Urethane is like a cross between an amide and an ester which contain a carbonyl with both a nitrogen and an oxygen attached to the carbonyl carbon. There are many examples of polyurethanes, foam rubber make up one of the parts of the unusual material known as spandex. Polyurethane foams are used to make seat cushions, mattresses, and packing and hard polyurethane foam which are used as insulation in refrigerators, freezers and home (131, 132, 133).

Appendix D

Part I: Screening for high cadmium removal and immobilized ability of cyanobacterial strains in different immobilized materials

Table D-1 Results from screening for high cadmium removal of *Scytonema* sp. in different immobilized materials

Types of immobilized material	Characteristics of system	Cd removal (%)				
		Rep 1	Rep 2	Rep 3	Avg	SD
Loofa sponge (LS)	Control	37.35	30.19	31.28	32.94	3.86
Loofa sponge (LS)	Immobilized cell	58.52	63.74	64.25	62.17	3.17
Delicate duty scour pad (DP)	Control	4.47	11.64	16.02	10.71	5.83
Delicate duty scour pad(DP)	Immobilized cell	84.06	77.49	76.73	79.43	4.03
Synthetic fiber (SF)	Control	5.14	6.91	6.07	6.04	0.89
Synthetic fiber (SF)	Immobilized cell	93.51	93.09	93.34	93.31	0.21
Polyethylene terephthalate(PET)	Control	14.67	16.61	15.77	15.68	0.97
Polyethylene terephthalate(PET)	Immobilized cell	83.98	79.93	81.87	81.93	2.02
Polyethylene teraphthalate (PET non-woven fabric)	Control	5.56	5.56	7.25	6.13	0.97
Polyethylene terephthalate (PET non-wovenfabric)	Immobilized cell	92.24	92.58	90.47	91.77	1.13
Polyethylene (PE)	Control	23.52	26.73	21.75	24.00	2.52
Polyethylene (PE)	Immobilized cell	71.16	65.26	70.99	69.14	3.36
Polypropylene (PP)	Control	24.79	16.02	27.57	22.79	6.03
Polypropylene (PP)	Immobilized cell	71.75	82.88	69.73	74.79	7.08
Polyurethane (PU)	Control	32.80	28.58	19.48	26.95	6.81
Polyurethane (PU)	Immobilized cell	65.94	69.65	78.41	71.33	6.41
	Free cell	97.39	91.40	95.62	94.80	3.08

Table D-2 Results from screening for high cadmium removal of *O. agardhi* in different immobilized materials

Types of immobilized material	Characteristics of system	Cd removal (%)				
		Rep 1	Rep 2	Rep 3	Avg	SD
Loofa sponge (LS)	Control	40.36	40.30	40.17	40.28	0.09
Loofa sponge (LS)	Immobilized cell	55.08	55.88	51.45	54.14	2.36
Delicate duty scour pad (DP)	Control	27.23	19.41	22.06	22.90	3.98
Delicate duty scour pad(DP)	Immobilized cell	64.82	72.09	71.72	69.54	4.10
Synthetic fiber(SF)	Control	13.56	17.38	21.44	17.46	3.94
Synthetic fiber(SF)	Immobilized cell	79.79	71.10	72.40	74.43	4.69
Polyethylene terephthalate (PET)	Control	20.83	10.22	21.07	20.38	1.00
Polyethylene terephthalate(PET)	Immobilized cell	71.28	74.74	71.66	72.56	1.90
Polyethylene terephthalate (PET non-wovenfabric)	Control	27.97	19.90	23.35	23.74	4.05
Polyethylene terephthalate (PET non-wovenfabric)	Immobilized cell	69.62	73.57	67.84	70.34	2.93
Polyethylene (PE)	Control	25.32	33.03	25.26	27.87	4.46
Polyethylene (PE)	Immobilized cell	66.30	59.21	64.39	63.30	3.67
Polypropylene(PP)	Control	40.48	30.31	42.33	37.71	6.47
Polypropylene(PP)	Immobilized cell	55.02	64.76	53.30	57.69	6.18
Polyurethane (PU)	Control	41.96	39.56	30.25	37.26	6.18
Polyurethane (PU)	Immobilized cell	52.68	55.64	63.59	57.30	5.64
	Free cell	93.47	94.70	97.72	95.30	2.19

Table D-3 Results from screening for high cadmium removal of *Mastigocladus* sp. in different immobilized materials

Types of immobilized material	Characteristics of system	Cd removal (%)				
		Rep 1	Rep 2	Rep 3	Avg	SD
Loofa sponge (LS)	Control	54.07	56.87	50.81	53.92	3.03
Loofa sponge (LS)	Immobilized cell	36.21	29.24	28.31	31.25	4.32
Delicate duty scour pad (DP)	Control	10.43	13.20	9.76	11.13	1.82
Delicate duty scour pad (DP)	Immobilized cell	76.37	65.95	61.39	67.90	7.67
Synthetic fiber (SF)	Control	10.49	12.21	9.76	10.82	1.25
Synthetic fiber (SF)	Immobilized cell	85.08	73.33	85.62	81.34	6.95
Polyethylene terephthalate (PET)	Control	0.54	4.97	2.53	2.68	2.22
Polyethylene terephthalate (PET)	Immobilized cell	90.69	72.51	76.85	80.02	9.49
Polyethylene terephthalate (PET non-wovenfabric)	Control	12.30	7.78	6.69	8.92	2.97
Polyethylene terephthalate (PET non-wovenfabric)	Immobilized cell	78.66	77.03	73.42	76.37	2.68
Polyethylene (PE)	Control	19.26	12.03	8.05	13.11	5.68
Polyethylene (PE)	Immobilized cell	49.19	60.58	59.13	56.30	6.20
Polypropylene (PP)	Control	36.26	22.06	22.42	26.91	8.09
Polypropylene (PP)	Immobilized cell	48.37	48.55	51.45	49.46	1.73
Polyurethane (PU)	Control	6.96	17.18	19.26	14.47	6.58
Polyurethane (PU)	Immobilized cell	71.43	67.81	58.86	66.03	6.47
	Free cell	76.84	73.99	75.22	75.35	1.43

Table D-4 Results from screening for high cadmium removal of *H. hibernicus* in different immobilized materials

Types of immobilized material	Characteristics of system	Cd removal (%)				
		Rep 1	Rep 2	Rep 3	Avg	SD
Loofa spong(LS)	Control	21.16	33.31	31.88	28.78	6.64
Loofa spong(LS)	Immobilized cell	53.44	49.99	51.46	51.63	1.73
Delicate duty scour pad (DP)	Control	13.62	19.58	16.14	16.45	2.99
Delicate duty scour pad (DP)	Immobilized cell	79.23	72.62	75.26	75.71	3.33
Syntheticfiber (SF)	Control	11.11	10.32	12.17	11.20	0.93
Synthetic fiber(SF)	Immobilized cell	84.13	87.17	84.13	85.14	1.76
Polyethylene terephthalate (PET)	Control	9.79	14.29	11.64	11.90	2.26
Polyethylene terephthalate (PET)	Immobilized cell	86.90	82.01	81.35	83.42	3.03
Polyethylene terephthalate (PET non-wovenfabric)	Control	12.70	26.19	19.58	19.49	6.75
Polyethylene terephthalate (PET non-wovenfabric)	Immobilized cell	83.86	71.43	77.78	77.69	6.22
Polyethylene (PE)	Control	16.53	15.48	14.29	15.43	1.12
Polyethylene (PE)	Immobilized cell	75.93	74.21	76.85	75.66	1.34
Polypropylene(PP)	Control	25.89	25.75	18.99	23.54	3.94
Polypropylene(PP)	Immobilized cell	69.61	70.55	75.05	71.74	2.91
Polyurethane (PU)	Control	31.35	36.51	24.60	30.82	5.97
Polyurethane (PU)	Immobilized cell	66.01	60.71	73.54	66.75	6.45
	Free cell	98.28	98.15	98.54	98.32	0.20

Table D-5 Results from screening for high immobilized ability and DW/SA (g cell/cm²) of *Scytonema* sp. in different immobilized materials

Type of immobilized material	Replicate	Immobilized cell (W _f -W _i)	Non-immobilized cell (W _{ni})	Immobilized ability (%)	DW/SA (g cell/cm ²)
Loofa sponge (LS)	1	0.1347	0.0027	98.03	0.0084
	2	0.1342	0.0036	97.39	0.0084
	3	0.0256	0.0019	93.09	0.0016
	avg	0.0982	0.0027	96.17	0.0061
	SD	0.0628	0.0009	2.69	0.0039
Delicate duty scour pad (DP)	1	0.0302	0.0033	90.15	0.0019
	2	0.0295	0.0011	96.41	0.0018
	3	0.0706	0.0022	96.98	0.0044
	avg	0.0434	0.0022	94.51	0.0027
	SD	0.0235	0.0011	3.79	0.0015
Synthetic fiber (SF)	1	0.0347	0.0016	95.59	0.0022
	2	0.0527	0.0003	99.43	0.0033
	3	0.0235	0.0028	89.35	0.0015
	avg	0.0370	0.0016	94.79	0.0023
	SD	0.0147	0.0013	5.09	0.0009
Polyethylene terephthalate (PET)	1	0.0644	0.0005	99.23	0.0040
	2	0.0452	0.0031	93.58	0.0028
	3	0.0496	0.0025	95.20	0.0031
	avg	0.0531	0.0020	96.00	0.0033
	SD	0.0101	0.0014	2.91	0.0006
Polyethylene terephthalate (PET non-woven fabric)	1	0.0554	0.0029	95.03	0.0035
	2	0.0516	0.0024	95.56	0.0032
	3	0.0482	0.0075	86.54	0.0030
	avg	0.0517	0.0043	92.37	0.0032
	SD	0.0036	0.0028	5.06	0.0002
Polyethylene (PE)	1	0.0365	0.0164	69.00	0.0023
	2	0.0237	0.0183	56.43	0.0015
	3	0.0271	0.0165	62.16	0.0017
	avg	0.0291	0.0171	62.53	0.0018
	SD	0.0066	0.0011	6.29	0.0004
Polypropylene (PP)	1	0.0453	0.0045	90.96	0.0028
	2	0.0631	0.0121	83.91	0.0039
	3	0.0565	0.0122	82.24	0.0035
	avg	0.0550	0.0096	85.71	0.0034
	SD	0.0090	0.0044	4.63	0.0006
Polyurethane (PU)	1	0.0237	0.0056	80.89	0.0015
	2	0.0184	0.0014	92.93	0.0012
	3	0.0289	0.0102	73.91	0.0018
	avg	0.0237	0.0057	82.58	0.0015
	SD	0.0053	0.0044	9.62	0.0003

Table D-6 Results from screening for high immobilized ability and DW/SA ratio (g cell/cm²) of *O. agardhi* in different immobilized materials

Type of immobilized material	Replicate	Immobilized cell (W _r -W _i)	Non-immobilized cell (W _{ni})	Immobilized ability (%)	DW/SA (g cell/cm ²)
Loofa sponge (LS)	1	0.0155	0.0117	56.99	0.0010
	2	0.0124	0.0184	40.26	0.0008
	3	0.0195	0.0218	47.22	0.0012
	avg	0.0158	0.0173	48.15	0.0010
	SD	0.0036	0.0051	8.40	0.0002
Delicate duty scour pad (DP)	1	0.0262	0.0018	93.57	0.0016
	2	0.0266	0.0016	94.33	0.0017
	3	0.0323	0.0012	96.42	0.0020
	avg	0.0284	0.0015	94.77	0.0018
	SD	0.0034	0.0003	1.47	0.0002
Synthetic fiber (SF)	1	0.0345	0.0041	89.38	0.0022
	2	0.0179	0.0005	97.28	0.0011
	3	0.0356	0.0007	98.07	0.0022
	avg	0.0293	0.0018	94.91	0.0018
	SD	0.0099	0.0020	4.81	0.0006
Polyethylene terephthalate (PET)	1	0.0333	0.0072	82.22	0.0021
	2	0.0492	0.0114	81.19	0.0031
	3	0.0359	0.0088	80.31	0.0022
	avg	0.0395	0.0091	81.24	0.0025
	SD	0.0085	0.0021	0.96	0.0005
Polyethylene terephthalate (PET non-woven fabric)	1	0.0522	0.0155	77.10	0.0033
	2	0.0170	0.0107	61.37	0.0011
	3	0.0278	0.0112	71.28	0.0017
	avg	0.0323	0.0125	69.92	0.0020
	SS	0.0180	0.0026	7.95	0.0011
Polyethylene (PE)	1	0.0116	0.0067	63.39	0.0007
	2	0.0289	0.0167	63.38	0.0018
	3	0.0064	0.0054	54.24	0.0004
	avg	0.0156	0.0096	60.33	0.0010
	SD	0.0118	0.0062	5.28	0.0007
Polypropylene (PP)	1	0.0357	0.0014	96.23	0.0022
	2	0.0253	0.0004	98.44	0.0016
	3	0.0305	0.0017	94.72	0.0019
	avg	0.0305	0.0012	96.46	0.0019
	SD	0.0052	0.0007	1.87	0.0003
Polyurethane (PU)	1	0.0231	0.0023	90.94	0.0014
	2	0.0320	0.0024	93.02	0.0020
	3	0.0184	0.0030	85.98	0.0012
	avg	0.0245	0.0026	89.98	0.0015
	SD	0.0069	0.0004	3.62	0.0004

Table D-7 Results from screening for high immobilized ability and DW/SA ratio (g cell/cm²) of *Mastigocladus* sp. in different immobilized materials

Type of immobilized material	Replicate	Immobilized cell (W _r -W _i)	Non-immobilized cell (W _{ni})	Immobilized ability (%)	DW/SA (g cell/cm ²)
Loofa sponge (LS)	1	0.0222	0.0063	77.89	0.0014
	2	0.0109	0.0073	59.89	0.0007
	3	0.0119	0.0053	69.19	0.0007
	avg	0.0150	0.0063	68.99	0.0009
	SD	0.0063	0.0010	9.00	0.0004
Delicate duty scour pad (DP)	1	0.0109	0.0026	80.74	0.0007
	2	0.0245	0.0013	94.96	0.0015
	3	0.0119	0.0025	82.64	0.0007
	avg	0.0158	0.0021	86.11	0.0010
	SD	0.0076	0.0007	7.72	0.0005
Synthetic fiber (SF)	1	0.0359	0.0014	96.25	0.0022
	2	0.0240	0.0033	87.91	0.0015
	3	0.0306	0.0027	91.89	0.0019
	avg	0.0302	0.0025	92.02	0.0019
	SD	0.0060	0.0010	4.17	0.0004
Polyethylene terephthalate (PET)	1	0.0309	0.0023	93.07	0.0019
	2	0.0230	0.0001	99.57	0.0014
	3	0.0188	0.0046	80.34	0.0012
	avg	0.0242	0.0023	90.99	0.0015
	SD	0.0061	0.0023	9.78	0.0004
Polyethylene terephthalate (PET non-woven fabric)	1	0.0220	0.0026	89.43	0.0014
	2	0.0236	0.0055	81.10	0.0015
	3	0.0326	0.0008	97.60	0.0020
	avg	0.0261	0.0030	89.38	0.0016
	SD	0.0057	0.0024	8.25	0.0004
Polyethylene (PE)	1	0.0163	0.0054	75.12	0.0010
	2	0.0111	0.0009	92.50	0.0007
	3	0.0143	0.0020	87.73	0.0009
	avg	0.0139	0.0028	85.12	0.0009
	SD	0.0026	0.0023	8.98	0.0002
Polypropylene (PP)	1	0.0261	0.0031	89.38	0.0016
	2	0.0225	0.0018	92.59	0.0014
	3	0.0198	0.0023	89.59	0.0012
	avg	0.0228	0.0024	90.52	0.0014
	SD	0.0032	0.0007	1.80	0.0002
Polyurethane (PU)	1	0.0201	0.0046	81.38	0.0013
	2	0.0236	0.0024	90.77	0.0015
	3	0.0040	0.0015	72.73	0.0003
	avg	0.0159	0.0028	81.62	0.0010
	SD	0.0105	0.0016	9.02	0.0007

Table D-8 Results from screening for high immobilized ability and DW/SA ratio (g cell/cm²) of *H. hibernicus* in different immobilized materials

Type of immobilized material	Replicate	Immobilized cell (W _f -W _i)	Non-immobilized cell (W _{ni})	Immobilize ability (%)	DW/SA
Loofa sponge (LS)	1	0.0204	0.0150	57.63	0.0013
	2	0.0012	0.0007	63.16	0.0001
	3	0.0169	0.0160	51.37	0.0011
	avg	0.0128	0.0106	57.38	0.0008
	SD	0.0102	0.0086	5.90	0.0006
Delicate duty scour pad (DP)	1	0.0249	0.0016	93.96	0.0016
	2	0.0286	0.0022	92.86	0.0018
	3	0.0178	0.0007	96.22	0.0011
	avg	0.0238	0.0015	94.35	0.0015
	SD	0.0055	0.0008	1.71	0.0003
Synthetic fiber (SF)	1	0.0342	0.0018	95.00	0.0021
	2	0.0372	0.0002	99.47	0.0023
	3	0.0371	0.0008	97.89	0.0023
	avg	0.0362	0.0009	97.45	0.0023
	SD	0.0017	0.0008	2.26	0.0001
Polyethylene terephthalate (PET)	1	0.0291	0.0038	88.45	0.0018
	2	0.0289	0.0044	86.79	0.0018
	3	0.0218	0.0038	85.16	0.0014
	avg	0.0266	0.0040	86.80	0.0017
	SD	0.0042	0.0003	1.65	0.0003
Polyethylene terephthalate (PET non-woven fabric)	1	0.0298	0.0036	89.22	0.0019
	2	0.0339	0.0049	87.37	0.0021
	3	0.0354	0.0038	90.31	0.0022
	avg	0.0330	0.0041	88.97	0.0021
	SD	0.0029	0.0007	1.48	0.0002
Polyethylene (PE)	1	0.0237	0.0157	60.15	0.0015
	2	0.0213	0.0160	57.10	0.0013
	3	0.0198	0.0184	51.83	0.0012
	avg	0.0216	0.0167	56.36	0.0014
	SD	0.0020	0.0015	4.21	0.0001
Polypropylene (PP)	1	0.0343	0.0027	92.70	0.0021
	2	0.0356	0.0035	91.05	0.0022
	3	0.0314	0.0024	92.90	0.0020
	avg	0.0338	0.0029	92.22	0.0021
	SD	0.0022	0.0006	1.02	0.0001
Polyurethane (PU)	1	0.0383	0.0022	94.57	0.0024
	2	0.0365	0.0044	89.24	0.0023
	3	0.0374	0.0063	85.58	0.0023
	avg	0.0374	0.0043	89.69	0.0023
	SD	0.0009	0.0021	4.52	0.0001

Appendix E

Part II: Effect of initial cell concentrations and contact time on cadmium uptake between immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus*

Table E-1 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 0 minutes

Time (min)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
0	Synthetic fiber (SF)		control	0.05	0.22	0.13	0.13	0.09
		0.015	Immobilized cell	0.55	0.88	0.71	0.71	0.16
		0.030	Immobilized cell	0.69	0.36	1.21	0.75	0.43
		0.045	Immobilized cell	0.42	0.77	0.69	0.63	0.18
		0.060	Immobilized cell	0.94	0.56	0.75	0.75	0.19
	Polyethylene terephthalate (PET)		control	0.04	0.03	0.05	0.04	0.01
		0.015	Immobilized cell	1.42	2.54	1.50	1.82	0.62
		0.030	Immobilized cell	0.69	0.55	0.70	0.64	0.08
		0.045	Immobilized cell	0.43	0.57	0.81	0.60	0.19
		0.060	Immobilized cell	0.54	0.54	1.05	0.71	0.29
		0.015	Free cell	1.15	0.66	1.38	1.06	0.37
		0.030	Free cell	1.02	0.58	0.57	0.73	0.26
		0.045	Free cell	1.07	0.75	0.93	0.92	0.16
		0.060	Free cell	1.12	0.79	1.06	0.99	0.18

Table E - 2 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control , immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 5 minutes

Time (min)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
5	Synthetic fiber (SF)		control	0.37	0.21	0.07	0.22	0.15
		0.015	Immobilized cell	1.37	2.69	1.34	1.80	0.77
		0.030	Immobilized cell	2.30	1.55	2.01	1.95	0.38
		0.045	Immobilized cell	1.74	1.67	1.77	1.72	0.05
		0.060	Immobilized cell	1.71	1.63	1.81	1.72	0.09
	Polyethylene terephthalate (PET)		control	0.05	0.06	0.04	0.05	0.01
		0.015	Immobilized cell	3.84	3.08	3.08	3.33	0.44
		0.030	Immobilized cell	1.92	1.37	1.73	1.67	0.28
		0.045	Immobilized cell	1.75	1.70	1.68	1.71	0.04
		0.060	Immobilized cell	1.23	1.25	1.52	1.33	0.16
		0.015	Free cell	1.12	0.11	2.10	1.11	1.00
		0.030	Free cell	0.91	1.31	0.51	0.91	0.40
		0.045	Free cell	0.48	0.70	1.15	0.78	0.34
		0.060	Free cell	0.64	0.77	0.76	0.72	0.08

Table E -3 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 10 minutes

Time (min)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
10	Synthetic fiber (SF)		control	0.34	0.23	0.23	0.27	0.06
		0.015	Immobilized cell	1.83	3.01	1.27	2.04	0.89
		0.030	Immobilized cell	2.79	2.28	1.70	2.26	0.54
		0.045	Immobilized cell	1.80	1.52	1.92	1.75	0.21
		0.060	Immobilized cell	1.62	2.00	1.60	1.74	0.23
	Polyethylene terephthalate (PET)		control	0.04	0.06	0.06	0.05	0.01
		0.015	Immobilized cell	3.45	4.21	3.10	3.59	0.57
		0.030	Immobilized cell	1.93	1.59	1.76	1.76	0.17
		0.045	Immobilized cell	1.77	2.01	1.89	1.89	0.12
		0.060	Immobilized cell	1.38	1.96	1.53	1.62	0.30
		0.015	Free cell	0.99	1.84	1.29	1.37	0.43
		0.030	Free cell	1.46	0.91	1.19	1.19	0.28
		0.045	Free cell	0.46	1.42	1.22	1.03	0.51
		0.060	Free cell	1.04	0.99	1.00	1.01	0.03

Table E -4 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control , immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 30 minutes

Time (min)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
30	Synthetic fiber (SF)		control	0.04	0.02	0.38	0.15	0.20
		0.015	Immobilized cell	1.66	2.90	1.71	2.09	0.70
		0.030	Immobilized cell	2.67	2.13	2.31	2.37	0.27
		0.045	Immobilized cell	1.63	1.51	1.85	1.66	0.17
		0.060	Immobilized cell	1.86	1.74	1.95	1.85	0.10
	Polyethylene terephthalate (PET)		control	0.05	0.02	0.01	0.03	0.02
		0.015	Immobilized cell	3.92	3.29	3.81	3.67	0.34
		0.030	Immobilized cell	2.19	1.99	2.06	2.08	0.10
		0.045	Immobilized cell	1.71	1.58	1.75	1.68	0.09
		0.060	Immobilized cell	1.26	1.38	1.69	1.45	0.22
		0.015	Free cell	1.44	1.19	1.68	1.44	0.25
		0.030	Free cell	1.04	1.27	1.24	1.19	0.12
		0.045	Free cell	1.18	0.93	1.22	1.11	0.15
		0.060	Free cell	1.15	1.07	1.04	1.09	0.06

Table E -5 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 60 minutes

Time (min)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
60	Synthetic fiber (SF)		control	0.07	0.07	0.08	0.07	0.01
		0.015	Immobilized cell	2.17	2.47	1.55	2.06	0.47
		0.030	Immobilized cell	2.59	1.72	2.45	2.25	0.46
		0.045	Immobilized cell	1.57	1.56	1.95	1.69	0.22
		0.060	Immobilized cell	1.88	1.78	1.96	1.87	0.09
	Polyethylene terephthalate (PET)		control	0.03	0.02	0.02	0.02	0.00
		0.015	Immobilized cell	2.70	4.37	3.58	3.55	0.84
		0.030	Immobilized cell	1.76	1.30	3.29	2.12	1.04
		0.045	Immobilized cell	1.72	1.66	2.02	1.80	0.19
		0.060	Immobilized cell	1.57	1.54	1.14	1.41	0.24
		0.015	Free cell	1.41	1.42	1.36	1.40	0.03
		0.030	Free cell	1.33	1.08	1.06	1.16	0.15
		0.045	Free cell	1.11	1.06	1.13	1.10	0.04
		0.060	Free cell	1.03	1.08	1.04	1.05	0.03

Table E -6 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 90 minutes

Time (min)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
90	Synthetic fiber (SF)		control	0.06	0.03	0.05	0.05	0.01
		0.015	Immobilized cell	1.54	2.99	1.20	1.91	0.95
		0.030	Immobilized cell	2.76	1.74	1.96	2.15	0.54
		0.045	Immobilized cell	1.65	1.63	1.95	1.74	0.18
		0.060	Immobilized cell	1.84	1.68	1.90	1.80	0.12
	Polyethylene terephthalate (PET)		control	0.01	0.05	0.02	0.02	0.02
		0.015	Immobilized cell	3.30	4.36	3.08	3.58	0.68
		0.030	Immobilized cell	2.23	1.70	1.86	1.93	0.27
		0.045	Immobilized cell	1.72	1.52	2.05	1.76	0.27
		0.060	Immobilized cell	1.63	1.21	1.34	1.39	0.22
		0.015	Free cell	1.52	1.41	1.42	1.45	0.06
		0.030	Free cell	1.55	1.16	1.13	1.28	0.23
		0.045	Free cell	1.35	0.95	1.20	1.17	0.20
		0.060	Free cell	1.10	1.14	1.11	1.12	0.02

Table E -7 Effect of initial cell concentration on cadmium uptake mg (Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 2 days

Time (days)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
2	Synthetic fiber (SF)		control	0.08	0.22	0.20	0.17	0.07
		0.015	Immobilized cell	2.83	4.25	2.63	3.24	0.88
		0.030	Immobilized cell	3.48	3.13	3.12	3.24	0.20
		0.045	Immobilized cell	2.17	2.24	2.37	2.26	0.10
		0.060	Immobilized cell	2.15	2.10	2.24	2.16	0.07
	Polyethylene terephthalate (PET)		control	0.03	0.03	0.06	0.04	0.01
		0.015	Immobilized cell	7.01	7.68	7.16	7.29	0.35
		0.030	Immobilized cell	3.10	2.94	2.60	2.88	0.26
		0.045	Immobilized cell	2.15	2.20	2.38	2.24	0.12
		0.060	Immobilized cell	1.84	1.79	1.85	1.83	0.03
		0.015	Free cell	2.85	2.62	2.72	2.73	0.12
		0.030	Free cell	2.77	1.80	2.50	2.36	0.50
		0.045	Free cell	2.08	1.66	1.95	1.90	0.21
		0.060	Free cell	1.67	1.80	1.78	1.75	0.07

Table E - 8 Effect of initial cell concentration on cadmium uptake mg (Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 4 days

Time (days)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
4	Synthetic fiber (SF)		control	0.13	0.20	0.23	0.19	0.05
		0.015	Immobilized cell	3.37	4.48	3.37	3.74	0.64
		0.030	Immobilized cell	3.40	3.48	3.48	3.45	0.04
		0.045	Immobilized cell	2.24	2.29	2.32	2.28	0.04
		0.060	Immobilized cell	2.24	2.28	2.14	2.22	0.07
	Polyethylene terephthalate (PET)		control	0.05	0.04	0.02	0.04	0.02
		0.015	Immobilized cell	7.36	7.86	6.86	7.36	0.50
		0.030	Immobilized cell	3.07	2.94	3.25	3.09	0.16
		0.045	Immobilized cell	2.51	2.41	2.50	2.47	0.06
		0.060	Immobilized cell	1.88	1.91	1.87	1.89	0.02
		0.015	Free cell	3.09	2.94	3.43	3.15	0.25
		0.030	Free cell	3.03	2.23	3.01	2.76	0.46
		0.045	Free cell	2.42	2.14	2.38	2.31	0.15
		0.060	Free cell	1.97	1.94	1.84	1.92	0.06

Table E -9 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 6 days

Time (days)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
6	Synthetic fiber (SF)		control	0.20	0.30	0.31	0.27	0.06
		0.015	Immobilized cell	3.89	4.79	3.82	4.16	0.54
		0.030	Immobilized cell	3.65	3.71	3.56	3.64	0.07
		0.045	Immobilized cell	2.37	2.34	2.44	2.38	0.05
		0.060	Immobilized cell	2.29	2.29	2.26	2.28	0.02
	Polyethylene terephthalate (PET)		control	0.06	0.07	0.07	0.06	0.01
		0.015	Immobilized cell	7.09	7.91	7.38	7.46	0.41
		0.030	Immobilized cell	3.35	3.29	3.36	3.33	0.04
		0.045	Immobilized cell	2.59	2.58	2.45	2.54	0.08
		0.060	Immobilized cell	2.01	2.00	1.91	1.97	0.05
		0.015	Free cell	3.36	3.49	3.79	3.55	0.22
		0.030	Free cell	2.81	2.76	2.73	2.77	0.04
		0.045	Free cell	2.47	2.38	2.27	2.37	0.10
		0.060	Free cell	1.76	2.03	2.04	1.95	0.16

Table E -10 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 8 days

Time (days)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
8	Synthetic fiber (SF)		control	0.07	0.38	0.40	0.28	0.18
		0.015	Immobilized cell	4.14	4.60	3.99	4.24	0.32
		0.030	Immobilized cell	3.27	3.75	3.81	3.61	0.29
		0.045	Immobilized cell	2.39	2.39	2.38	2.38	0.00
		0.060	Immobilized cell	2.30	2.35	2.18	2.28	0.09
	Polyethylene teraphthalate (PET)		control	0.07	0.05	0.07	0.06	0.01
		0.015	Immobilized cell	8.41	6.80	7.43	7.55	0.81
		0.030	Immobilized cell	3.23	3.25	3.43	3.30	0.11
		0.045	Immobilized cell	2.67	2.43	2.38	2.49	0.15
		0.060	Immobilized cell	1.97	1.81	2.02	1.93	0.11
		0.015	Free cell	3.82	3.40	3.08	3.43	0.37
		0.030	Free cell	2.73	2.67	2.77	2.72	0.05
		0.045	Free cell	2.59	2.24	2.23	2.35	0.21
		0.060	Free cell	1.82	1.97	1.99	1.93	0.09

Table E -11 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 10 days

Time (days)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
10	Synthetic fiber (SF)		control	0.28	0.23	0.27	0.26	0.03
		0.015	Immobilized cell	4.24	4.44	3.82	4.17	0.32
		0.030	Immobilized cell	3.79	3.69	3.33	3.60	0.24
		0.045	Immobilized cell	2.53	2.27	2.38	2.39	0.13
		0.060	Immobilized cell	2.43	2.08	2.16	2.23	0.18
	Polyethylene terephthalate (PET)		control	0.03	0.07	0.10	0.07	0.03
		0.015	Immobilized cell	7.40	8.71	6.77	7.63	0.99
		0.030	Immobilized cell	3.21	3.50	3.18	3.30	0.17
		0.045	Immobilized cell	2.66	2.45	2.59	2.57	0.11
		0.060	Immobilized cell	1.93	1.97	1.85	1.91	0.06
		0.015	Free cell	2.43	3.63	4.50	3.52	1.04
		0.030	Free cell	2.72	2.74	2.45	2.64	0.16
		0.045	Free cell	2.08	2.46	2.24	2.26	0.19
		0.060	Free cell	1.90	2.14	1.83	1.96	0.16

Table E - 12 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 14 days

Time (days)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
14	Synthetic fiber (SF)		control	0.26	0.38	0.27	0.30	0.07
		0.015	Immobilized cell	3.90	4.32	3.94	4.05	0.23
		0.030	Immobilized cell	3.28	3.31	3.99	3.53	0.40
		0.045	Immobilized cell	2.50	2.39	2.51	2.47	0.06
		0.060	Immobilized cell	2.24	2.43	2.08	2.25	0.17
	Polyethylene terephthalate (PET)		control	0.08	0.09	0.04	0.07	0.03
		0.015	Immobilized cell	7.41	7.74	7.78	7.64	0.20
		0.030	Immobilized cell	3.04	3.36	3.37	3.26	0.19
		0.045	Immobilized cell	2.54	2.44	2.32	2.43	0.11
		0.060	Immobilized cell	1.92	1.94	1.93	1.93	0.01
		0.015	Free cell	3.24	3.65	3.81	3.56	0.29
		0.030	Free cell	3.30	2.87	2.34	2.84	0.48
		0.045	Free cell	2.43	2.45	2.11	2.33	0.19
		0.060	Free cell	1.76	1.78	1.92	1.82	0.09

Table E-13 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 0 minutes

Time (min)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
0	Synthetic fiber (SF)		control	0.25	0.12	0.13	0.17	0.08
		0.015	Immobilized cell	2.81	2.66	2.20	2.56	0.32
		0.030	Immobilized cell	1.84	1.84	2.17	1.95	0.19
		0.045	Immobilized cell	2.26	2.09	1.90	2.08	0.18
		0.060	Immobilized cell	1.72	1.58	1.51	1.61	0.11
	Polyethylene terephthalate (PET)		control	0.01	0.04	0.05	0.03	0.02
		0.015	Immobilized cell	2.46	2.89	1.51	2.29	0.70
		0.030	Immobilized cell	1.77	1.81	1.45	1.68	0.19
		0.045	Immobilized cell	1.30	1.88	1.86	1.68	0.33
		0.060	Immobilized cell	1.57	1.14	1.29	1.33	0.22
		0.015	Free cell	1.52	2.31	1.75	1.86	0.40
		0.030	Free cell	1.30	1.85	2.52	1.89	0.61
		0.045	Free cell	1.48	1.61	1.62	1.57	0.08
		0.060	Free cell	1.74	0.90	1.10	1.25	0.44

Table E-14 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control , immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 5 minutes

Time (min)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
5	Synthetic fiber (SF)		control	0.19	0.18	0.14	0.17	0.03
		0.015	Immobilized cell	2.48	3.01	1.83	2.44	0.59
		0.030	Immobilized cell	2.00	2.13	1.97	2.03	0.09
		0.045	Immobilized cell	2.17	1.96	2.02	2.05	0.11
		0.060	Immobilized cell	1.50	1.59	1.67	1.59	0.08
	Polyethylene terephthalate (PET)		control	0.03	0.06	0.05	0.05	0.02
		0.015	Immobilized cell	2.91	2.55	2.66	2.71	0.19
		0.030	Immobilized cell	1.66	1.52	1.83	1.67	0.15
		0.045	Immobilized cell	1.94	1.96	2.53	2.14	0.34
		0.060	Immobilized cell	1.36	1.33	2.25	1.65	0.52
		0.015	Free cell	2.76	2.13	2.33	2.40	0.32
		0.030	Free cell	2.06	2.06	1.92	2.02	0.08
		0.045	Free cell	1.85	2.55	2.30	2.23	0.36
		0.060	Free cell	1.80	1.75	1.78	1.78	0.03

Table E-15 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF) , polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 10 minutes

Time (min)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
10	Synthetic fiber (SF)		control	0.14	0.12	0.19	0.15	0.04
		0.015	Immobilized cell	3.41	2.75	1.98	2.71	0.72
		0.030	Immobilized cell	1.90	2.28	1.86	2.02	0.23
		0.045	Immobilized cell	2.40	2.15	2.26	2.27	0.12
		0.060	Immobilized cell	1.65	1.68	1.86	1.73	0.11
	Polyethylene terephthalate (PET)		control	0.05	0.02	0.06	0.04	0.02
		0.015	Immobilized cell	3.46	3.17	3.49	3.37	0.18
		0.030	Immobilized cell	1.70	2.84	2.60	2.38	0.60
		0.045	Immobilized cell	2.69	1.84	2.03	2.19	0.45
		0.060	Immobilized cell	2.22	1.91	1.90	2.01	0.18
		0.015	Free cell	2.94	2.35	2.11	2.46	0.43
		0.030	Free cell	2.41	2.05	2.20	2.22	0.18
		0.045	Free cell	2.21	2.18	2.58	2.32	0.22
		0.060	Free cell	1.78	1.92	1.81	1.83	0.08

Table E-16 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 30 minutes

Time (min)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
30	Synthetic fiber (SF)		control	0.11	0.19	0.21	0.17	0.05
		0.015	Immobilized cell	3.96	3.70	3.60	3.75	0.19
		0.030	Immobilized cell	2.81	2.71	2.52	2.68	0.15
		0.045	Immobilized cell	2.76	2.71	2.77	2.75	0.03
		0.060	Immobilized cell	2.21	2.27	2.22	2.24	0.03
	Polyethylene terephthalate (PET)		control	0.03	0.05	0.05	0.04	0.01
		0.015	Immobilized cell	4.23	3.06	3.94	3.74	0.61
		0.030	Immobilized cell	2.66	2.35	2.67	2.56	0.18
		0.045	Immobilized cell	2.39	2.41	2.43	2.41	0.02
		0.060	Immobilized cell	2.00	2.02	1.97	2.00	0.02
		0.015	Free cell	3.05	2.96	3.12	3.05	0.08
		0.030	Free cell	3.18	2.56	1.89	2.54	0.64
		0.045	Free cell	2.54	1.90	3.14	2.53	0.62
		0.060	Free cell	2.17	2.11	2.22	2.17	0.06

Table E-17 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 60 minutes

Time (min)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
60	Synthetic fiber (SF)		control	0.17	0.08	0.27	0.17	0.09
		0.015	Immobilized cell	4.23	3.61	4.04	3.96	0.32
		0.030	Immobilized cell	2.86	2.75	2.67	2.76	0.10
		0.045	Immobilized cell	3.52	2.84	2.95	3.10	0.36
		0.060	Immobilized cell	2.39	2.38	2.25	2.34	0.08
	Polyethylene terephthalate (PET)		control	0.00	0.05	0.05	0.03	0.03
		0.015	Immobilized cell	4.20	3.65	4.77	4.21	0.56
		0.030	Immobilized cell	2.87	2.46	3.11	2.81	0.33
		0.045	Immobilized cell	2.93	2.82	3.17	2.97	0.18
		0.060	Immobilized cell	2.56	1.76	2.16	2.16	0.40
		0.015	Free cell	3.50	3.01	3.61	3.37	0.32
		0.030	Free cell	1.86	3.23	2.81	2.64	0.70
		0.045	Free cell	2.73	2.48	3.10	2.77	0.31
		0.060	Free cell	2.16	2.43	2.44	2.34	0.16

Table E-18 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 90 minutes

Time (min)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
90	Synthetic fiber (SF)		control	0.17	0.19	0.13	0.16	0.03
		0.015	Immobilized cell	4.46	4.09	4.00	4.18	0.24
		0.030	Immobilized cell	3.93	2.85	2.44	3.08	0.77
		0.045	Immobilized cell	3.13	3.54	3.10	3.26	0.25
		0.060	Immobilized cell	2.55	2.19	2.55	2.43	0.21
	Polyethylene terephthalate (PET)		control	0.03	0.05	0.03	0.04	0.01
		0.015	Immobilized cell	4.56	4.03	4.54	4.38	0.30
		0.030	Immobilized cell	2.77	2.74	3.25	2.92	0.29
		0.045	Immobilized cell	3.01	3.09	3.12	3.07	0.06
		0.060	Immobilized cell	2.04	2.34	2.18	2.19	0.15
		0.015	Free cell	3.07	3.61	4.18	3.62	0.55
		0.030	Free cell	3.22	2.57	2.61	2.80	0.36
		0.045	Free cell	2.79	1.91	3.47	2.72	0.78
		0.060	Free cell	2.26	2.36	2.50	2.37	0.12

Table E-19 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 2 days

Time (days)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
2	Synthetic fiber (SF)		control	0.17	0.04	0.20	0.14	0.08
		0.015	Immobilized cell	5.57	4.37	4.72	4.89	0.61
		0.030	Immobilized cell	4.30	4.03	3.98	4.10	0.17
		0.045	Immobilized cell	4.51	4.09	4.88	4.49	0.39
		0.060	Immobilized cell	3.13	3.28	3.21	3.21	0.07
	Polyethylene terephthalate (PET)		control	0.04	0.06	0.03	0.04	0.02
		0.015	Immobilized cell	7.14	5.85	6.86	6.62	0.68
		0.030	Immobilized cell	4.27	4.29	4.28	4.28	0.01
		0.045	Immobilized cell	4.13	3.95	4.02	4.03	0.09
		0.060	Immobilized cell	3.20	3.16	3.04	3.13	0.08
		0.015	Free cell	5.68	5.83	5.83	5.78	0.09
		0.030	Free cell	3.71	4.38	4.19	4.09	0.35
		0.045	Free cell	4.09	3.36	4.26	3.90	0.48
		0.060	Free cell	3.22	3.21	3.10	3.18	0.07

Table E-20 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 4 days

Time (days)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
4	Synthetic fiber (SF)		control	0.23	0.15	0.13	0.17	0.05
		0.015	Immobilized cell	5.91	4.87	5.49	5.42	0.53
		0.030	Immobilized cell	4.38	4.22	4.32	4.31	0.08
		0.045	Immobilized cell	4.74	4.24	4.44	4.48	0.25
		0.060	Immobilized cell	3.29	3.23	3.29	3.27	0.03
	Polyethylene terephthalate (PET)		control	0.04	0.05	0.08	0.06	0.02
		0.015	Immobilized cell	7.04	6.88	6.77	6.90	0.14
		0.030	Immobilized cell	4.37	4.77	4.15	4.43	0.32
		0.045	Immobilized cell	4.05	4.08	4.09	4.07	0.02
		0.060	Immobilized cell	3.19	3.11	3.13	3.15	0.04
		0.015	Free cell	5.81	5.80	5.74	5.78	0.04
		0.030	Free cell	3.95	4.16	4.02	4.05	0.11
		0.045	Free cell	3.86	3.43	4.14	3.81	0.36
		0.060	Free cell	3.09	3.15	3.13	3.12	0.03

Table E-21 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 6 days

Time (days)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
6	Synthetic fiber (SF)		control	0.16	0.20	0.18	0.18	0.02
		0.015	Immobilized cell	5.50	5.36	5.68	5.51	0.16
		0.030	Immobilized cell	4.34	4.32	4.29	4.32	0.03
		0.045	Immobilized cell	4.54	4.53	4.52	4.53	0.01
		0.060	Immobilized cell	3.27	3.24	3.26	3.26	0.02
	Polyethylene terephthalate (PET)		control	0.08	0.04	0.04	0.05	0.02
		0.015	Immobilized cell	6.88	7.04	7.07	7.00	0.10
		0.030	Immobilized cell	4.37	4.46	4.48	4.44	0.06
		0.045	Immobilized cell	4.08	4.11	4.03	4.07	0.04
		0.060	Immobilized cell	3.14	3.16	3.15	3.15	0.01
		0.015	Free cell	6.05	5.58	5.75	5.79	0.24
		0.030	Free cell	4.20	4.06	3.96	4.07	0.12
		0.045	Free cell	3.75	3.63	4.17	3.85	0.29
		0.060	Free cell	3.03	3.18	3.15	3.12	0.08

Table E-22 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 8 days

Time (days)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
8	Synthetic fiber (SF)		control	0.07	0.27	0.27	0.20	0.12
		0.015	Immobilized cell	5.93	4.46	5.77	5.39	0.80
		0.030	Immobilized cell	4.40	4.36	4.40	4.38	0.02
		0.045	Immobilized cell	4.53	4.50	4.49	4.51	0.02
		0.060	Immobilized cell	3.28	3.26	3.23	3.26	0.02
	Polyethylene terephthalate (PET)		control	0.06	0.07	0.03	0.05	0.02
		0.015	Immobilized cell	7.07	5.62	8.17	6.95	1.28
		0.030	Immobilized cell	4.27	4.32	4.45	4.35	0.09
		0.045	Immobilized cell	4.00	3.97	3.99	3.98	0.02
		0.060	Immobilized cell	3.16	3.12	3.13	3.14	0.02
		0.015	Free cell	6.27	5.28	6.05	5.87	0.52
		0.030	Free cell	5.04	4.24	3.76	4.35	0.65
		0.045	Free cell	3.77	4.14	3.87	3.93	0.19
		0.060	Free cell	3.17	3.15	3.34	3.22	0.11

Table E-23 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 10 days

Time (days)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
10	Synthetic fiber (SF)		control	0.24	0.14	0.22	0.20	0.05
		0.015	Immobilized cell	5.60	5.02	5.65	5.43	0.35
		0.030	Immobilized cell	4.34	4.34	4.48	4.38	0.08
		0.045	Immobilized cell	4.51	4.49	4.54	4.51	0.03
		0.060	Immobilized cell	3.33	3.28	3.34	3.32	0.03
	Polyethylene terephthalate (PET)		control	0.02	0.06	0.07	0.05	0.02
		0.015	Immobilized cell	7.05	6.81	7.23	7.03	0.21
		0.030	Immobilized cell	4.62	4.22	4.43	4.42	0.20
		0.045	Immobilized cell	4.15	3.79	4.10	4.02	0.20
		0.060	Immobilized cell	3.14	3.09	3.07	3.10	0.04
		0.015	Free cell	6.29	5.52	5.60	5.80	0.42
		0.030	Free cell	4.19	4.38	4.43	4.33	0.13
		0.045	Free cell	4.06	3.50	4.31	3.96	0.42
		0.060	Free cell	3.14	3.19	3.17	3.16	0.02

Table E-24 Effect of initial cell concentration on cadmium uptake (mg Cd/g dry wt.) in control, immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 14 days

Time (days)	Types of immobilized material	Initial cell (g dry wt.)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
				Rep 1	Rep 2	Rep 3	Avg	SD
14	Synthetic fiber (SF)		control	0.13	0.22	0.21	0.19	0.05
		0.015	Immobilized cell	5.81	5.43	5.29	5.51	0.27
		0.030	Immobilized cell	4.41	3.98	4.27	4.22	0.22
		0.045	Immobilized cell	4.56	4.54	4.47	4.52	0.05
		0.060	Immobilized cell	3.34	3.33	3.29	3.32	0.03
	Polyethylene terephthalate (PET)		control	0.08	0.04	0.03	0.05	0.03
		0.015	Immobilized cell	7.05	7.04	6.97	7.02	0.05
		0.030	Immobilized cell	4.38	4.49	4.48	4.45	0.06
		0.045	Immobilized cell	4.02	4.04	4.02	4.03	0.01
		0.060	Immobilized cell	3.13	3.14	3.14	3.14	0.01
		0.015	Free cell	6.80	5.26	5.10	5.72	0.94
		0.030	Free cell	4.15	4.61	4.32	4.36	0.23
		0.045	Free cell	4.07	3.41	4.16	3.88	0.41
		0.060	Free cell	3.13	3.04	3.23	3.14	0.10

Table E-25 Effect of initial cell concentration on immobilized ability and DW/SA in immobilized cell on synthetic fiber (SF) of *Scytonema* sp.

Type of immobilized material	Initial cell (g dry wt.)	Replicate	Immobilized cell ($W_f - W_i$)	Non-immobilized cell (W_{ni})	Immobilized ability (%)	DW/SA (g dry wt./cm ²)
Synthetic fiber (SF)	0.015	1	0.0203	0.0012	94.42	0.0013
		2	0.0507	0.0002	99.61	0.0032
		3	0.0155	0.0003	98.10	0.0010
		average	0.0288	0.0006	97.38	0.0018
		SD	0.0191	0.0006	2.67	0.0012
	0.03	1	0.0344	0.0035	90.77	0.0022
		2	0.0388	0.0032	92.38	0.0024
		3	0.0359	0.0004	98.90	0.0022
		average	0.0364	0.0024	94.01	0.0023
		SD	0.0022	0.0017	4.31	0.0001
	0.045	1	0.0469	0.0042	91.78	0.0029
		2	0.0554	0.0003	99.46	0.0035
		3	0.0684	0.0047	93.57	0.0043
		average	0.0569	0.0031	94.94	0.0036
		SD	0.0108	0.0024	4.02	0.0007
	0.06	1	0.0601	0.0015	97.56	0.0038
		2	0.0578	0.0004	99.31	0.0036
		3	0.0615	0.0053	92.07	0.0038
		average	0.0598	0.0024	96.31	0.0037
		SD	0.0019	0.0026	3.78	0.0001

Table E-26 Effect of initial cell concentration on immobilize ability and DW/SA in immobilized cell on polyethylene terephthalate (PET) of *Scytonema* sp.

Type of immobilized material	Initial cell (g dry wt.)	replicate	Immobilized cell ($W_f - W_i$)	Non-immobilized cell (W_{ni})	Immobilize ability (%)	DW/SA (g dry wt./cm ²)
Polyethylene terephthalate (PET))	0.015	1	0.0103	0.0008	92.75	0.0006
		2	0.0201	0.0004	98.05	0.0013
		3	0.0143	0.0002	98.62	0.0009
		average	0.0149	0.0007	96.49	0.0009
		SD	0.0049	0.0003	3.21	0.0003
	0.03	1	0.0412	0.0022	94.93	0.0026
		2	0.0391	0.0031	92.65	0.0024
		3	0.0442	0.0028	94.04	0.0028
		average	0.0415	0.0027	93.88	0.0026
		SD	0.0026	0.0005	1.15	0.0002
	0.045	1	0.0514	0.0020	96.25	0.0032
		2	0.0435	0.0019	95.81	0.0027
		3	0.0631	0.0038	94.32	0.0039
		average	0.0527	0.0026	95.46	0.0033
		SD	0.0099	0.0011	1.01	0.0006
	0.06	1	0.0668	0.0111	85.75	0.0042
		2	0.1042	0.0041	96.21	0.0065
		3	0.0399	0.0040	90.89	0.0025
		average	0.0703	0.0064	90.95	0.0044
		SD	0.0323	0.0041	5.23	0.0020

Table E-27 Effect of initial cell concentration on immobilized ability and DW/SA in immobilized cell on synthetic fiber (SF) of *H. hibernicus*

Type of immobilized material	Initial cell (g dry wt.)	Replicate	Immobilized cell ($W_f - W_i$)	Non-immobilized cell (W_{ni})	Immobilized ability (%)	DW/SA (g dry wt./cm ²)
Synthetic fiber (SF)	0.015	1	0.0155	0.0044	77.89	0.0010
		2	0.0156	0.0013	92.31	0.0010
		3	0.0130	0.0003	97.74	0.0008
		average	0.0147	0.0020	89.31	0.0009
		SD	0.0015	0.0021	10.26	0.0001
	0.03	1	0.0351	0.0022	94.10	0.0022
		2	0.0194	0.0020	90.65	0.0012
		3	0.0133	0.0023	85.26	0.0008
		average	0.0226	0.0022	90.00	0.0014
		SD	0.0112	0.0002	4.46	0.0007
	0.045	1	0.0190	0.0050	79.17	0.0012
		2	0.0246	0.0026	90.44	0.0015
		3	0.0253	0.0032	88.77	0.0016
		average	0.0230	0.0036	86.13	0.0014
		SD	0.0035	0.0012	6.08	0.0002
	0.06	1	0.0376	0.0027	93.30	0.0024
		2	0.0354	0.0021	94.40	0.0022
		3	0.0261	0.0046	85.02	0.0016
		average	0.0330	0.0031	90.91	0.0021
		SD	0.0061	0.0013	5.13	0.0004

Table E-28 Effect of initial cell concentration on immobilize ability and DW/SA in immobilized cell on polyethylene terephthalate (PET) of *H. hibernicus*

Type of immobilized material	Initial cell (g dry wt.)	Replicate	Immobilized cell ($W_f - W_i$)	Non-immobilized cell (W_{ni})	Immobilized ability (%)	DW/SA (g dry wt./cm ²)
Polyethylene terephthalate (PET)	0.015	1	0.0112	0.0043	72.26	0.0007
		2	0.0073	0.0017	81.11	0.0005
		3	0.0168	0.0015	91.80	0.0011
		average	0.0118	0.0025	81.72	0.0007
		SD	0.0048	0.0016	9.79	0.0003
	0.03	1	0.0220	0.0041	84.29	0.0014
		2	0.0292	0.0029	90.97	0.0018
		3	0.0152	0.0029	83.98	0.0010
		average	0.0221	0.0033	86.41	0.0014
		SD	0.0070	0.0007	3.95	0.0004
	0.045	1	0.0266	0.0047	84.98	0.0017
		2	0.0259	0.0042	86.05	0.0016
		3	0.0248	0.0051	82.94	0.0016
		average	0.0258	0.0047	84.66	0.0016
		SD	0.0009	0.0005	1.58	0.0001
	0.06	1	0.0340	0.0040	89.47	0.0021
		2	0.0372	0.0041	90.07	0.0023
		3	0.0361	0.0051	87.62	0.0023
		average	0.0358	0.0044	89.06	0.0022
		SD	0.0016	0.0006	1.28	0.0001

Appendix F

Part III: Effect of initial cadmium concentrations on cadmium uptake between immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus*

Table F-1A Effect of initial cadmium concentration on cadmium uptake (mg Cd/g dry wt.) in immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. for 14 days

Types of immobilized material	Cadmium concentration (mg/l)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
			Rep 1	Rep 2	Rep 3	Avg	SD
Synthetic fiber (SF)	0.5	Immobilized cell	4.11	2.73	2.86	3.23	0.76
	1.0	Immobilized cell	7.81	4.53	7.96	6.77	1.94
	2.0	Immobilized cell	11.12	10.89	16.32	12.78	3.07
	4.0	Immobilized cell	28.72	22.12	25.54	25.46	3.30
	8.0	Immobilized cell	24.88	25.25	27.81	25.98	1.59
Polyethylene terephthalate (PET)	0.5	Immobilized cell	4.14	3.73	2.90	3.59	0.63
	1.0	Immobilized cell	4.09	9.56	17.30	10.32	6.64
	2.0	Immobilized cell	13.18	18.64	17.35	16.39	2.86
	4.0	Immobilized cell	24.93	39.04	28.86	30.95	7.28
	8.0	Immobilized cell	30.80	35.12	34.10	33.34	2.26
	0.5	Free cell	2.73	3.20	4.85	3.59	1.11
	1.0	Free cell	7.04	5.94	7.82	6.93	0.94
	2.0	Free cell	14.22	15.38	12.22	13.94	1.60
	4.0	Free cell	12.78	26.07	24.88	21.24	7.35
	8.0	Free cell	29.32	32.26	30.02	30.53	1.54

Table F-1B Effect of initial cadmium concentration on cadmium uptake (mg Cd/g dry wt.) in immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. for 14 days

Types of immobilized material	Cadmium concentration (mg/l)	Characteristics of system	C _r (mg/l)				
			Rep 1	Rep 2	Rep 3	Avg	SD
Synthetic fiber (SF)	0.5	Immobilized cell	0.014	0.015	0.016	0.015	0.001
	1.0	Immobilized cell	0.025	0.042	0.040	0.036	0.009
	2.0	Immobilized cell	0.260	0.200	0.295	0.252	0.048
	4.0	Immobilized cell	0.672	1.329	1.200	1.067	0.348
	8.0	Immobilized cell	4.560	4.900	3.565	4.342	0.694
Polyethylene terephthalate (PET)	0.5	Immobilized cell	0.023	0.024	0.011	0.019	0.007
	1.0	Immobilized cell	0.249	0.192	0.062	0.168	0.096
	2.0	Immobilized cell	0.442	0.346	0.286	0.358	0.079
	4.0	Immobilized cell	1.125	1.383	0.654	1.054	0.370
	8.0	Immobilized cell	3.760	2.810	4.404	3.658	0.802
	0.5	Free cell	0.006	0.004	0.025	0.012	0.012
	1.0	Free cell	0.055	0.039	0.082	0.059	0.022
	2.0	Free cell	0.408	0.340	0.180	0.309	0.117
	4.0	Free cell	1.614	1.176	0.966	1.252	0.331
	8.0	Free cell	4.230	4.795	4.225	4.417	0.328

Table F-1C Effect of initial cadmium concentration on cadmium uptake (mg Cd/g dry wt.) in immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. for 14 days

Types of immobilized material	Cadmium concentration (mg/l)	Characteristics of system	C _f /q (g dry wt./l)				
			Rep 1	Rep 2	Rep 3	Avg	SD
Synthetic fiber (SF)	0.5	Immobilized cell	0.003	0.005	0.006	0.005	0.001
	1.0	Immobilized cell	0.003	0.009	0.005	0.006	0.003
	2.0	Immobilized cell	0.023	0.018	0.018	0.020	0.003
	4.0	Immobilized cell	0.023	0.060	0.047	0.043	0.019
	8.0	Immobilized cell	0.183	0.194	0.128	0.169	0.035
Polyethylene terephthalate (PET)	0.5	Immobilized cell	0.006	0.006	0.004	0.005	0.001
	1.0	Immobilized cell	0.061	0.020	0.004	0.028	0.029
	2.0	Immobilized cell	0.034	0.019	0.016	0.023	0.009
	4.0	Immobilized cell	0.045	0.035	0.023	0.034	0.011
	8.0	Immobilized cell	0.122	0.080	0.129	0.110	0.027
	0.5	Free cell	0.002	0.001	0.005	0.003	0.002
	1.0	Free cell	0.008	0.007	0.010	0.008	0.002
	2.0	Free cell	0.029	0.022	0.015	0.022	0.007
	4.0	Free cell	0.126	0.045	0.039	0.070	0.049
	8.0	Free cell	0.144	0.149	0.141	0.145	0.004

Table F-2A Effect of initial cadmium concentration on cadmium uptake (mg Cd/g dry wt.) immobilized cell on synthetic fiber(SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* for 14 days

Types of immobilized material	Cadmium concentration (mg/l)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
			Rep 1	Rep 2	Rep 3	Avg	SD
Synthetic fiber (SF)	0.5	Immobilized cell	7.77	7.72	7.99	7.83	0.14
	1.0	Immobilized cell	10.44	10.60	10.56	10.53	0.08
	2.0	Immobilized cell	19.37	17.70	19.97	19.02	1.18
	4.0	Immobilized cell	35.70	35.30	34.58	35.19	0.56
	8.0	Immobilized cell	53.46	49.19	49.82	50.82	2.30
Polyethylene terephthalate (PET)	0.5	Immobilized cell	10.86	10.55	10.74	10.72	0.16
	1.0	Immobilized cell	15.07	15.12	15.00	15.06	0.06
	2.0	Immobilized cell	27.93	28.32	27.33	27.86	0.50
	4.0	Immobilized cell	42.00	40.85	37.55	40.13	2.31
	8.0	Immobilized cell	70.23	58.02	51.29	59.85	9.60
	0.5	Free cell	6.59	7.09	6.86	6.84	0.25
	1.0	Free cell	12.04	13.47	12.92	12.81	0.72
	2.0	Free cell	27.13	26.47	26.40	26.67	0.40
	4.0	Free cell	43.49	37.13	40.90	40.51	3.20
	8.0	Free cell	48.03	62.15	50.36	53.51	7.57

Table F-2B Effect of initial cadmium concentration on cadmium uptake (mg Cd/g dry wt.) immobilized cell on synthetic fiber(SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* for 14 days

Types of immobilized material	Cadmium concentration (mg/l)	Characteristics of system	C _f (mg/l)				
			Rep 1	Rep 2	Rep 3	Avg	SD
Synthetic fiber (SF)	0.5	Immobilized cell	0.109	0.114	0.090	0.104	0.013
	1.0	Immobilized cell	0.153	0.139	0.143	0.145	0.007
	2.0	Immobilized cell	0.290	0.452	0.232	0.325	0.114
	4.0	Immobilized cell	0.783	0.813	0.867	0.821	0.043
	8.0	Immobilized cell	4.490	4.806	4.760	4.685	0.171
Polyethylene terephthalate (PET)	0.5	Immobilized cell	0.100	0.120	0.108	0.109	0.010
	1.0	Immobilized cell	0.157	0.154	0.161	0.157	0.004
	2.0	Immobilized cell	0.308	0.282	0.348	0.313	0.033
	4.0	Immobilized cell	0.618	0.696	0.921	0.745	0.157
	8.0	Immobilized cell	3.820	4.625	5.069	4.505	0.633
	0.5	Free cell	0.085	0.117	0.065	0.089	0.026
	1.0	Free cell	0.216	0.292	0.202	0.237	0.048
	2.0	Free cell	0.616	0.522	0.562	0.567	0.047
	4.0	Free cell	0.861	0.774	1.170	0.935	0.208
	8.0	Free cell	6.360	5.995	5.274	5.876	0.553

Table F-2C Effect of initial cadmium concentration on cadmium uptake (mg Cd/g dry wt.) immobilized cell on synthetic fiber(SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* for 14 days

Types of immobilized material	Cadmium concentration (mg/l)	Characteristics of system	C _f /q (g dry wt./l)				
			Rep 1	Rep 2	Rep 3	Avg	SD
Synthetic fiber (SF)	0.5	Immobilized cell	0.014	0.015	0.011	0.013	0.002
	1.0	Immobilized cell	0.015	0.013	0.014	0.014	0.001
	2.0	Immobilized cell	0.015	0.026	0.012	0.017	0.007
	4.0	Immobilized cell	0.022	0.023	0.025	0.023	0.002
	8.0	Immobilized cell	0.084	0.098	0.096	0.092	0.007
Polyethylene terephthalate (PET)	0.5	Immobilized cell	0.009	0.011	0.010	0.010	0.001
	1.0	Immobilized cell	0.010	0.010	0.011	0.010	0.000
	2.0	Immobilized cell	0.011	0.010	0.013	0.011	0.001
	4.0	Immobilized cell	0.015	0.017	0.025	0.019	0.005
	8.0	Immobilized cell	0.054	0.080	0.099	0.078	0.022
	0.5	Free cell	0.013	0.017	0.009	0.013	0.004
	1.0	Free cell	0.018	0.022	0.016	0.018	0.003
	2.0	Free cell	0.023	0.020	0.021	0.021	0.001
	4.0	Free cell	0.020	0.021	0.029	0.023	0.005
	8.0	Free cell	0.132	0.096	0.105	0.111	0.019

Table F-3 Effect of initial cadmium concentration on immobilized ability and DW/SA in immobilized cell on synthetic fiber (SF) of *Scytonema* sp.

Type of immobilized material	Cadmium concentration (mg/l)	Replicate	Immobilized cell ($W_f - W_i$)	Non-immobilized cell (W_{ni})	Immobilize ability (%)	DW/SA (g dry wt./cm ²)
Synthetic fiber (SF)	0.5	1	0.0171	0.0012	93.44	0.0011
		2	0.0237	0.0011	95.56	0.0015
		3	0.0245	0.0004	98.39	0.0015
		average	0.0218	0.0009	95.80	0.0014
		SD	0.0041	0.0004	2.48	0.0003
	1	1	0.0188	0.0003	98.43	0.0012
		2	0.0319	0.0024	93.00	0.0020
		3	0.0182	0.0014	92.86	0.0011
		average	0.0230	0.0014	94.76	0.0014
		SD	0.0077	0.0012	2.98	0.0005
	2	1	0.0220	0.0012	94.83	0.0014
		2	0.0232	0.0011	95.47	0.0015
		3	0.0147	0.0011	93.04	0.0009
		average	0.0200	0.0011	94.45	0.0012
		SD	0.0046	0.0001	1.26	0.0003
	4	1	0.0168	0.0031	84.42	0.0011
		2	0.0178	0.0004	97.80	0.0011
		3	0.0161	0.0019	89.44	0.0010
		average	0.0169	0.0018	90.56	0.0011
		SD	0.0009	0.0014	6.76	0.0001
	8	1	0.0193	0.0027	87.73	0.0012
		2	0.0172	0.0028	86.00	0.0011
		3	0.0221	0.0017	92.86	0.0014
		average	0.0195	0.0024	88.86	0.0012
		SD	0.0025	0.0006	3.57	0.0002

Table F-4 Effect of initial cadmium concentration on immobilized ability and DW/SA in immobilized on polyethylene teraphthalate (PET) of *Scytonema* sp.

Type of immobilized material	Cadmium concentration (mg/l)	Replicate	Immobilized cell ($W_f - W_i$)	Non-immobilized cell (W_{ni})	Immobilize ability (%)	DW/SA (g dry wt./cm ²)
Synthetic fiber (SF)	0.5	1	0.0167	0.0019	89.78	0.0010
		2	0.0185	0.0004	97.88	0.0012
		3	0.0244	0.0010	96.06	0.0015
		average	0.0199	0.0011	94.58	0.0012
		SD	0.0040	0.0008	4.25	0.0003
	1	1	0.0285	0.0014	95.32	0.0018
		2	0.0130	0.0011	92.20	0.0008
		3	0.0082	0.0012	87.23	0.0005
		average	0.0166	0.0012	91.58	0.0010
		SD	0.0106	0.0002	4.08	0.0007
	2	1	0.0167	0.0007	95.98	0.0010
		2	0.0125	0.0019	86.81	0.0008
		3	0.0139	0.0011	92.67	0.0009
		average	0.0144	0.0012	91.82	0.0009
		SD	0.0021	0.0006	4.64	0.0001
	4	1	0.0169	0.0022	88.48	0.0011
		2	0.0099	0.0013	88.39	0.0006
		3	0.0168	0.0011	93.85	0.0011
		average	0.0145	0.0015	90.24	0.0009
		SD	0.0040	0.0006	3.13	0.0003
	8	1	0.0191	0.0031	86.04	0.0012
		2	0.0204	0.0020	91.07	0.0013
		3	0.0147	0.0017	89.63	0.0009
		average	0.0181	0.0023	88.91	0.0011
		SD	0.0030	0.0007	2.59	0.0002

Table F-5 Effect of initial cadmium concentration on immobilize ability and DW/SA in immobilized cell on synthetic fiber (SF) of *H. hibernicus*

Type of immobilized material	Cadmium concentration (mg/l)	Replicate	Immobilized cell ($W_f - W_i$)	Non-immobilized cell (W_{ni})	Immobilize ability (%)	DW/SA (g dry wt./cm ²)
Synthetic fiber (SF)	0.5	1	0.0116	0.0002	98.31	0.0007
		2	0.0109	0.0004	96.46	0.0007
		3	0.0136	0.0020	87.18	0.0009
		average	0.0120	0.0009	93.98	0.0008
		SD	0.0014	0.0010	5.96	0.0001
	1	1	0.0109	0.0020	84.50	0.0007
		2	0.0121	0.0006	95.28	0.0008
		3	0.0117	0.0013	90.00	0.0007
		average	0.0116	0.0013	89.92	0.0007
		SD	0.0006	0.0007	5.39	0.0000
	2	1	0.0132	0.0012	91.67	0.0008
		2	0.0120	0.0002	98.36	0.0008
		3	0.0140	0.0037	79.10	0.0009
		average	0.0131	0.0017	89.71	0.0008
		SD	0.0010	0.0018	9.78	0.0001
	4	1	0.0116	0.0016	87.88	0.0007
		2	0.0095	0.0015	86.36	0.0006
		3	0.0094	0.0016	85.45	0.0006
		average	0.0102	0.0016	86.57	0.0006
		SD	0.0012	0.0001	1.22	0.0001
	8	1	0.0130	0.0022	85.53	0.0008
		2	0.0078	0.0013	85.71	0.0005
		3	0.0091	0.0017	84.26	0.0006
		average	0.0100	0.0017	85.17	0.0006
		SD	0.0027	0.0005	0.79	0.0002

Table F-6 Effect of initial cadmium concentration on immobilize ability and DW/SA in immobilized cell on polyethylene terephthalate (PET) of *H. hibernicus*

Type of immobilized material	Cadmium concentration (mg/l)	Replicate	Immobilized cell ($W_f - W_i$)	Non - immobilized cell (W_{ni})	Immobilize ability (%)	DW/SA (g dry wt./cm ²)
Polyethylene terephthalate (PET)	0.5	1	0.0093	0.0011	89.42	0.0006
		2	0.0061	0.0003	95.31	0.0004
		3	0.0107	0.0006	94.69	0.0007
		average	0.0087	0.0007	93.14	0.0005
		SD	0.0024	0.0004	3.24	0.0001
	1	1	0.0091	0.0009	91.00	0.0006
		2	0.0080	0.0010	88.89	0.0005
		3	0.0069	0.0007	90.79	0.0004
		average	0.0080	0.0009	90.23	0.0005
		SD	0.0011	0.0002	1.16	0.0001
	2	1	0.0099	0.0010	90.83	0.0006
		2	0.0098	0.0008	92.45	0.0006
		3	0.0072	0.0013	84.71	0.0005
		average	0.0090	0.0010	89.33	0.0006
		SD	0.0015	0.0003	4.08	0.0001
	4	1	0.0094	0.0002	97.92	0.0006
		2	0.0092	0.0011	89.32	0.0006
		3	0.0091	0.0023	79.82	0.0006
		average	0.0092	0.0012	89.02	0.0006
		SD	0.0002	0.0011	9.05	0.0000
	8	1	0.0123	0.0009	93.18	0.0008
		2	0.0039	0.0017	69.64	0.0002
		3	0.0105	0.0021	83.33	0.0007
		average	0.0089	0.0016	82.05	0.0006
		SD	0.0044	0.0006	11.82	0.0003

Appendix G

Part IV: Effect of cell ages on cadmium uptake between immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. and *H. hibernicus*

Table G-1 Biomass in immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp.

Day	Characteristics of system	Biomass (g dry wt.) $\pm$ SD
0	Immobilized cell on SF	0.0309 $\pm$ 0.0030
	Immobilized cell on PET	0.0309 $\pm$ 0.0030
	Free cell	0.0309 $\pm$ 0.0030
3	Immobilized cell on SF	0.0429 $\pm$ 0.0048
	Immobilized cell on PET	0.0434 $\pm$ 0.0028
	Free cell	0.0452 $\pm$ 0.0028
6	Immobilized cell on SF	0.0453 $\pm$ 0.0028
	Immobilized cell on PET	0.0414 $\pm$ 0.0024
	Free cell	0.0437 $\pm$ 0.0018
9	Immobilized cell on SF	0.0497 $\pm$ 0.0010
	Immobilized cell on PET	0.0479 $\pm$ 0.0039
	Free cell	0.0474 $\pm$ 0.0020
12	Immobilized cell on SF	0.0874 $\pm$ 0.0049
	Immobilized cell on PET	0.0857 $\pm$ 0.0008
	Free cell	0.0841 $\pm$ 0.0043
15	Immobilized cell on SF	0.1222 $\pm$ 0.0008
	Immobilized cell on PET	0.1146 $\pm$ 0.0040
	Free cell	0.1151 $\pm$ 0.0044
18	Immobilized cell on SF	0.1189 $\pm$ 0.0032
	Immobilized cell on PET	0.1158 $\pm$ 0.0045
	Free cell	0.0991 $\pm$ 0.0248
21	Immobilized cell on SF	0.1208 $\pm$ 0.0073
	Immobilized cell on PET	0.1217 $\pm$ 0.0037
	Free cell	0.1170 $\pm$ 0.0034

Table G-2 Biomass in immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus*

Day	Characteristics of system	Biomass (g dry wt.) $\pm$ SD
0	Immobilized cell on SF	0.0319 ± 0.0040
	Immobilized cell on PET	0.0319 ± 0.0040
	Free cell	0.0319 ± 0.0040
3	Immobilized cell on SF	0.0373 ± 0.0024
	Immobilized cell on PET	0.0367 ± 0.0013
	Free cell	0.0397 ± 0.0020
6	Immobilized cell on SF	0.0472 ± 0.0049
	Immobilized cell on PET	0.0489 ± 0.0090
	Free cell	0.0486 ± 0.0020
9	Immobilized cell on SF	0.0812 ± 0.0051
	Immobilized cell on PET	0.0733 ± 0.0237
	Free cell	0.0980 ± 0.0027
12	Immobilized cell on SF	0.0966 ± 0.0016
	Immobilized cell on PET	0.0974 ± 0.0079
	Free cell	0.1131 ± 0.0143
15	Immobilized cell on SF	0.1102 ± 0.0045
	Immobilized cell on PET	0.1130 ± 0.0011
	Free cell	0.1043 ± 0.0010
18	Immobilized cell on SF	0.0940 ± 0.0536
	Immobilized cell on PET	0.0960 ± 0.0580
	Free cell	0.0973 ± 0.0567
21	Immobilized cell on SF	0.0859 ± 0.0025
	Immobilized cell on PET	0.0831 ± 0.0008
	Free cell	0.0809 ± 0.0027

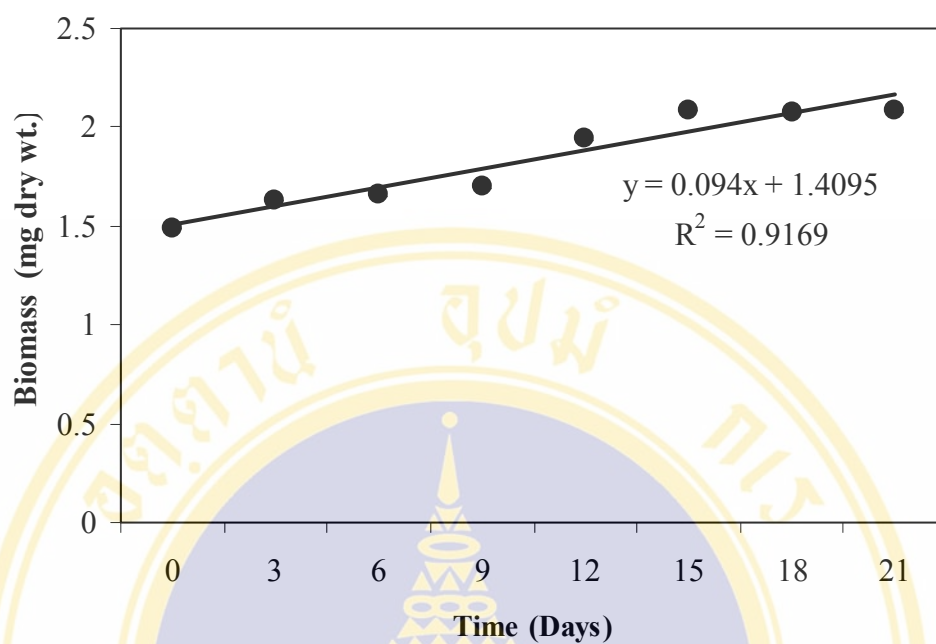


Figure G – 1 Growth rate (day^{-1}) of immobilized cell on synthetic fiber (SF) of *Scytonema* sp.

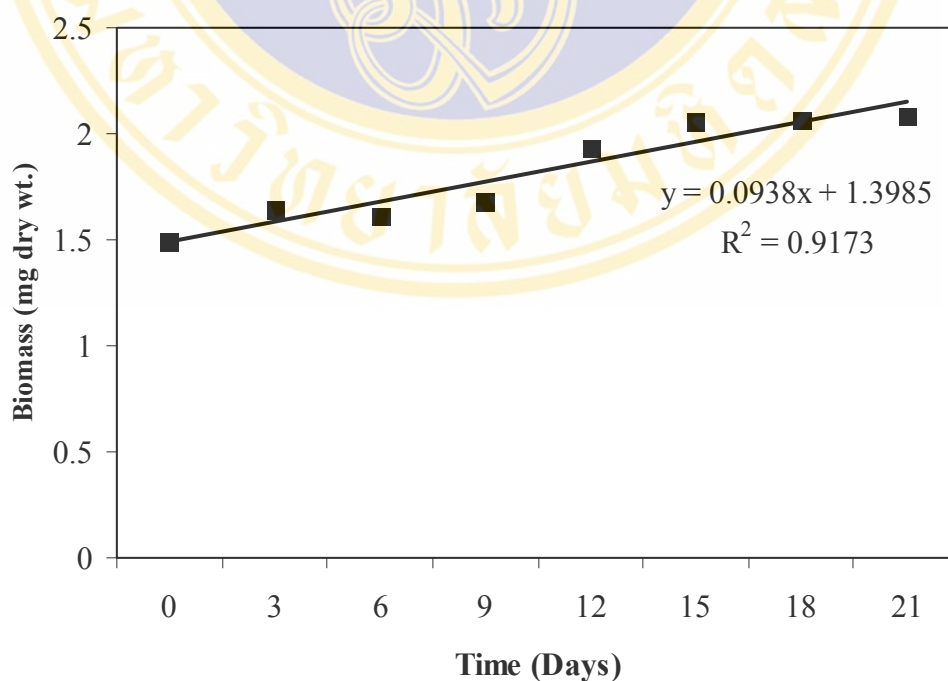


Figure G – 2 Growth rate (day^{-1}) of immobilized cell on polyethylene terephthalate (PET) of *Scytonema* sp.

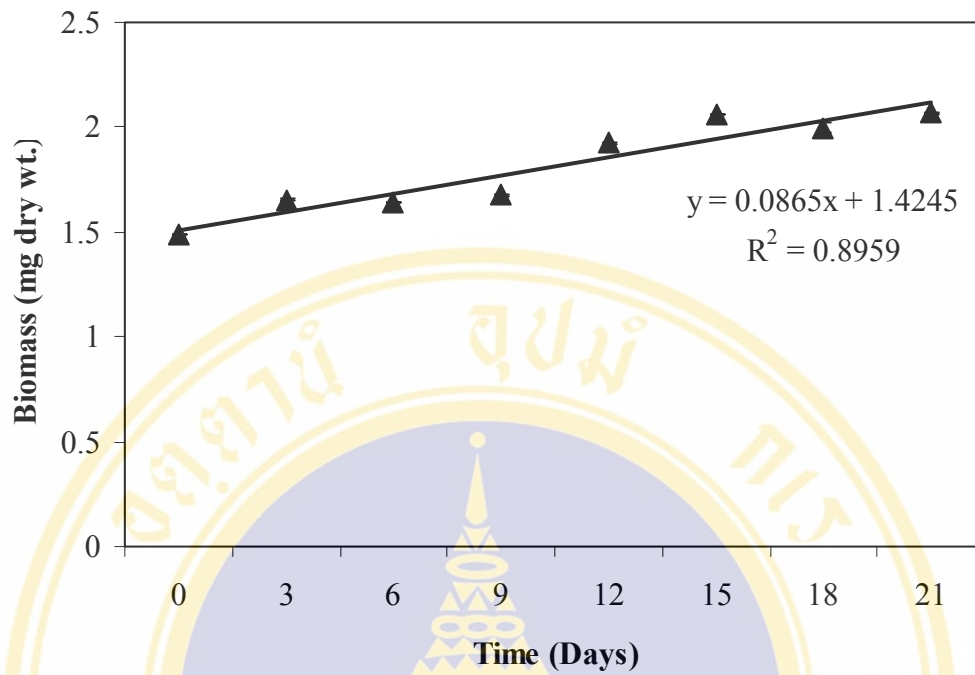


Figure G – 3 Growth rate (day^{-1}) of free cell of *Scytonema* sp.

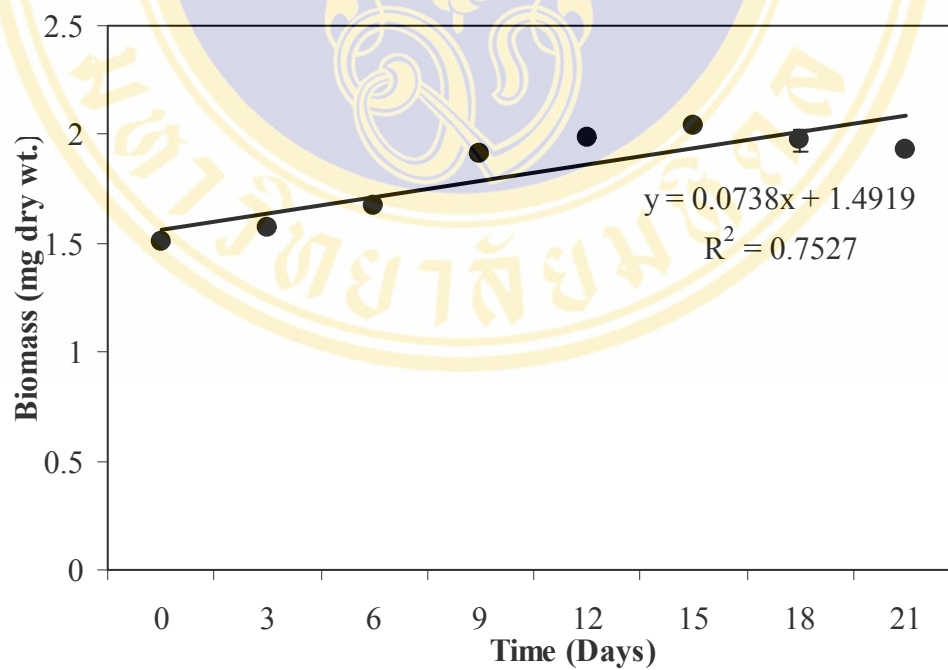


Figure G – 4 Growth rate (day^{-1}) of immobilized cell on synthetic fiber (SF) of *H. hibernicus*

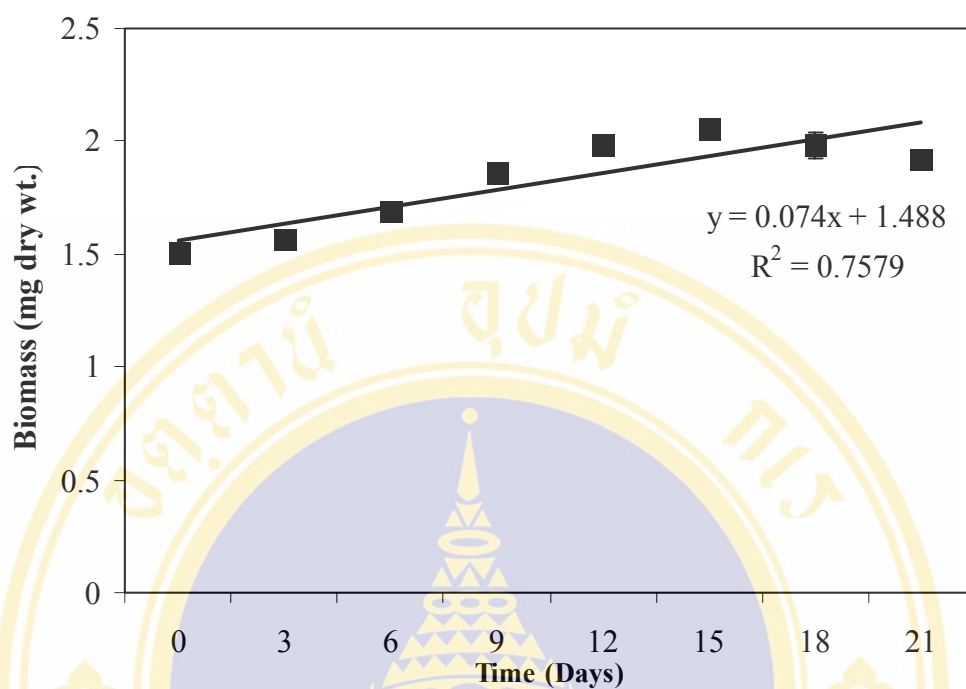


Figure G – 5 Growth rate (day^{-1}) of immobilized cell on polyethylene terephthalate (PET) of *H. hibernicus*

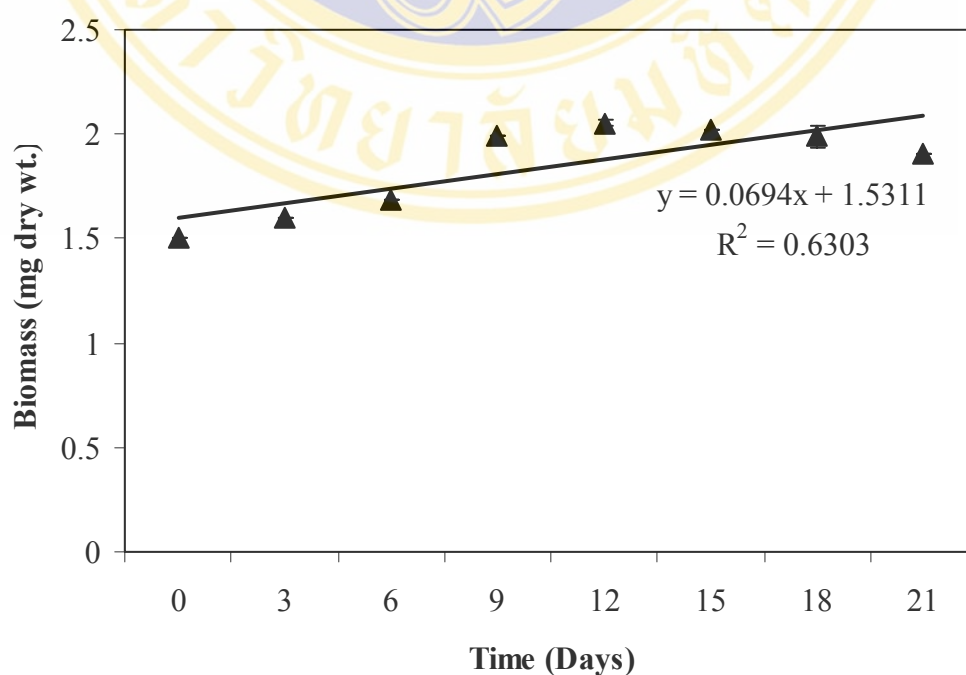


Figure G – 6 Growth rate (day^{-1}) of free cell of *H. hibernicus*

Table G-3 Effect of cell ages on cadmium uptake (mg Cd/g dry wt.) in immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 6 - day - cultured

Types of immobilized material	Cell ages (days)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
			Rep 1	Rep 2	Rep 3	Avg	SD
Synthetic fiber (SF)	5	Immobilized cell	0.85	1.93	1.14	1.30	0.56
	10	Immobilized cell	4.22	4.21	4.34	4.26	0.07
	15	Immobilized cell	4.88	4.74	4.83	4.82	0.07
	20	Immobilized cell	3.78	3.82	3.76	3.79	0.03
Polyethylene terephthalate (PET)	5	Immobilized cell	1.33	1.21	1.05	1.20	0.14
	10	Immobilized cell	4.48	4.44	4.43	4.45	0.02
	15	Immobilized cell	4.18	5.16	4.04	4.90	0.61
	20	Immobilized cell	3.79	3.86	3.59	3.75	0.14
	5	Free cell	0.99	1.08	1.15	1.07	0.08
	10	Free cell	4.10	4.12	4.10	4.11	0.01
	15	Free cell	4.00	4.55	5.00	4.51	0.50
	20	Free cell	3.43	3.42	3.76	3.54	0.19

Table G-4 Effect of cell ages on cadmium uptake (mg Cd/g dry wt.) in immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *Scytonema* sp. at 14- day - cultured

Types of immobilized material	Cell ages (days)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
			Rep 1	Rep 2	Rep 3	Avg	SD
Synthetic fiber (SF)	5	Immobilized cell	1.44	2.14	1.20	1.59	0.49
	10	Immobilized cell	5.64	5.36	5.64	5.55	0.16
	15	Immobilized cell	5.78	5.61	5.53	5.64	0.13
	20	Immobilized cell	5.31	5.57	5.19	5.36	0.20
Polyethylene terephthalate (PET)	5	Immobilized cell	1.68	1.22	1.76	1.55	0.29
	10	Immobilized cell	5.53	5.45	5.49	5.49	0.04
	15	Immobilized cell	5.70	5.57	5.85	5.71	0.14
	20	Immobilized cell	5.00	5.64	5.59	5.41	0.35
	5	Free cell	1.34	1.19	1.51	1.35	0.16
	10	Free cell	5.40	5.32	5.32	5.35	0.05
	15	Free cell	5.96	5.86	5.20	5.67	0.41
	20	Free cell	4.66	5.14	5.22	5.01	0.30

Table G-5 Effect of cell ages on cadmium uptake (mg Cd/g dry wt.) in immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 6 - day - cultured

Types of immobilized material	Cell ages (days)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
			Rep 1	Rep 2	Rep 3	Avg	SD
Synthetic fiber (SF)	5	Immobilized cell	6.47	6.77	6.78	6.68	0.17
	10	Immobilized cell	7.46	7.54	8.36	7.78	0.50
	15	Immobilized cell	8.53	8.08	8.46	8.35	0.24
	20	Immobilized cell	6.52	6.21	6.64	6.46	0.22
Polyethylene terephthalate (PET)	5	Immobilized cell	7.58	8.40	8.10	8.03	0.41
	10	Immobilized cell	9.03	8.62	7.66	8.44	0.70
	15	Immobilized cell	8.78	9.24	8.46	8.83	0.39
	20	Immobilized cell	8.41	7.79	7.80	8.00	0.35
	5	Free cell	5.11	5.01	4.74	4.95	0.19
	10	Free cell	7.57	7.67	7.42	7.55	0.13
	15	Free cell	7.56	7.64	8.12	7.77	0.30
	20	Free cell	7.40	7.59	6.60	7.20	0.53

Table G-6 Effect of cell ages on cadmium uptake (mg Cd/g dry wt.) in immobilized cell on synthetic fiber (SF), polyethylene terephthalate (PET) and free cell of *H. hibernicus* at 14 - day - cultured

Types of immobilized material	Cell ages (days)	Characteristics of system	Uptake (mg Cd/g dry wt.)				
			Rep 1	Rep 2	Rep 3	Avg	SD
Synthetic fiber (SF)	5	Immobilized cell	7.07	7.50	7.27	7.28	0.22
	10	Immobilized cell	8.39	7.42	9.51	8.44	1.05
	15	Immobilized cell	9.28	9.30	8.59	9.05	0.40
	20	Immobilized cell	7.78	7.69	7.41	7.63	0.20
Polyethylene terephthalate (PET)	5	Immobilized cell	8.21	9.33	9.01	8.85	0.58
	10	Immobilized cell	9.94	10.06	8.68	9.56	0.77
	15	Immobilized cell	9.94	10.53	9.41	9.96	0.56
	20	Immobilized cell	9.53	9.04	9.33	9.30	0.25
	5	Free cell	5.34	6.47	4.85	5.55	0.83
	10	Free cell	8.38	7.92	8.71	8.33	0.39
	15	Free cell	7.93	8.59	8.86	8.46	0.48
	20	Free cell	8.47	8.67	7.53	8.22	0.61

Table G-7 Effect of cell ages on immobilized ability and DW/SA in immobilized cell on synthetic fiber (SF) of *Scytonema* sp.

Type of immobilized material	Cell ages (days)	Replicate	Immobilized cell ($W_f - W_i$)	Non-immobilized cell (W_{ni})	Immobilized ability (%)	DW/SA (g dry wt./cm ²)
Synthetic fiber (SF)	5	1	0.0158	0.0012	92.94	0.0010
		2	0.0230	0.0025	90.20	0.0014
		3	0.0239	0.0014	94.47	0.0015
		average	0.0209	0.0017	92.53	0.0013
		SD	0.0044	0.0007	2.16	0.0003
	10	1	0.0182	0.0014	92.86	0.0011
		2	0.0229	0.0025	90.16	0.0014
		3	0.0192	0.0018	91.43	0.0012
		average	0.0201	0.0019	91.48	0.0013
		SD	0.0025	0.0006	1.35	0.0002
	15	1	0.0193	0.0010	95.07	0.0012
		2	0.0222	0.0007	96.94	0.0014
		3	0.0202	0.0012	94.39	0.0013
		average	0.0206	0.0010	95.47	0.0013
		SD	0.0015	0.0003	1.32	0.0001
	20	1	0.0198	0.0010	95.19	0.0012
		2	0.0208	0.0010	95.41	0.0013
		3	0.0199	0.0014	93.43	0.0012
		average	0.0202	0.0011	94.68	0.0013
		SD	0.0006	0.0002	1.09	0.0000

Table G-8 Effect of cell ages on immobilize ability and DW/SA of immobilized cell on polyethylene terephthalate (PET) of *Scytonema* sp.

Type of immobilized material	Cell ages (days)	Replicate	Immobilized cell ($W_f - W_i$)	Non-immobilized cell (W_{ni})	Immobilized ability (%)	DW/SA (g dry wt./cm ²)
Polyethylene terephthalate (PET)	5	1	0.0129	0.0012	91.49	0.0008
		2	0.0155	0.0007	95.68	0.0010
		3	0.0332	0.0006	98.22	0.0021
		average	0.0205	0.0008	95.13	0.0013
		SD	0.0110	0.0003	3.40	0.0007
	10	1	0.0208	0.0010	95.41	0.0013
		2	0.0210	0.0005	97.67	0.0013
		3	0.0212	0.0002	99.07	0.0013
		average	0.0210	0.0006	97.38	0.0013
		SD	0.0002	0.0004	1.84	0.0000
	15	1	0.0240	0.0004	98.36	0.0015
		2	0.0213	0.0006	97.26	0.0013
		3	0.0158	0.0005	96.93	0.0010
		average	0.0204	0.0005	97.52	0.0013
		SD	0.0042	0.0001	0.75	0.0003
	20	1	0.0205	0.0006	97.16	0.0013
		2	0.0201	0.0008	96.17	0.0013
		3	0.0197	0.0007	96.57	0.0012
		average	0.0201	0.0007	96.63	0.0013
		SD	0.0004	0.0001	0.50	0.0000

Table G-9 Effect of cell ages on immobilize ability and DW/SA of immobilized cell on synthetic fibre (SF) of *H. hibernicus*

Type of immobilized material	Cell ages (days)	Replicate	Immobilized cell ($W_r - W_i$)	Non-immobilized cell (W_{ni})	Immobilized ability (%)	DW/SA (g dry wt./cm ²)
Synthetic fiber (SF)	5	1	0.0192	0.0025	88.48	0.0012
		2	0.0143	0.0002	98.62	0.0009
		3	0.0136	0.0003	97.84	0.0009
		average	0.0157	0.0010	94.98	0.0010
		SD	0.0031	0.0013	5.64	0.0002
	10	1	0.0137	0.0009	93.84	0.0009
		2	0.0134	0.0002	98.53	0.0008
		3	0.0136	0.0016	89.47	0.0009
		average	0.0136	0.0009	93.95	0.0008
		SD	0.0002	0.0007	4.53	0.0000
	15	1	0.0140	0.0003	97.90	0.0009
		2	0.0059	0.0006	90.77	0.0004
		3	0.0200	0.0001	99.50	0.0013
		average	0.0133	0.0003	96.06	0.0008
		SD	0.0071	0.0003	4.65	0.0004
	20	1	0.0184	0.0003	98.40	0.0012
		2	0.0170	0.0002	98.84	0.0011
		3	0.0196	0.0040	83.05	0.0012
		average	0.0183	0.0015	93.43	0.0011
		SD	0.0013	0.0022	8.99	0.0001

Table G-10 Effect of cell ages on immobilize ability and DW/SA of immobilized on polyethylene terephthalate (PET) of *H. hibernicus*

Type of immobilized material	Cell ages (days)	Replicate	Immobilized cell ($W_f - W_i$)	Non-immobilized cell (W_{ni})	Immobilized ability (%)	DW/SA (g dry wt./cm ²)
Polyethylene terephthalate (PET)	5	1	0.0159	0.0002	98.76	0.0010
		2	0.0113	0.0012	90.40	0.0007
		3	0.0112	0.0003	97.39	0.0007
		average	0.0128	0.0006	95.52	0.0008
		SD	0.0027	0.0006	4.48	0.0002
	10	1	0.0113	0.0012	90.40	0.0007
		2	0.0118	0.0007	94.40	0.0007
		3	0.0126	0.0009	93.33	0.0008
		average	0.0119	0.0009	92.71	0.0007
		SD	0.0007	0.0003	2.07	0.0000
	15	1	0.0125	0.0011	91.91	0.0008
		2	0.0124	0.0008	93.94	0.0008
		3	0.0123	0.0010	92.48	0.0008
		average	0.0124	0.0010	92.78	0.0008
		SD	0.0001	0.0002	1.05	0.0000
	20	1	0.0137	0.0018	88.39	0.0009
		2	0.0153	0.0008	95.03	0.0010
		3	0.0121	0.0009	93.08	0.0008
		average	0.0137	0.0012	92.17	0.0009
		SD	0.0016	0.0006	3.41	0.0001

Appendix H

Industrial Effluent Standard

Parameter	Standard values
1. pH value	5.5 - 9.0
2. Total Dissolved Solids (TDS)	- not more than 3,000 mg/l depending on receiving water or type of industry under consideration of PCC but not exceed 5,000 mg/l - not more than 5,000 mg/l exceed TDS of receiving water having salinity of more than 2,000 mg/L or TDS of sea if discharge to sea
3. Suspended solids (SS)	- not more than 50 mg/l depending on receiving water or type of industry or wastewater treatment system under consideration of PCC but not exceed 150 mg/L
4. Temperature	- not more than 40°C
5. Color and Odor	- not objectionable
6. Sulphide as H ₂ S	- not more than 1.0 mg/l
7. Cyanide as HCN	- not more than 0.2 mg/l
8. Fat, Oil & Grease (FOG)	- not more than 5.0 mg/l depending of receiving water or type of industry under consideration of PCC but not exceed 15.0 mg/l
9. Formaldehyde	- not more than 1.0 mg/l
10. Phenols	- not more than 1.0 mg/l
11. Free Chlorine	- not more than 1.0 mg/l
12. Pesticides	- not detectable
13. Biochemical Oxygen Demand (BOD)	- not more than 20 mg/l depending on receiving water or type of industry under consideration of PCC but not exceed 60 mg/l
14. Total Kjeldahl Nitrogen (TKN)	- not more than 100 mg/l depending on receiving water or type of industry under consideration of PCC but not exceed 200 mg/l

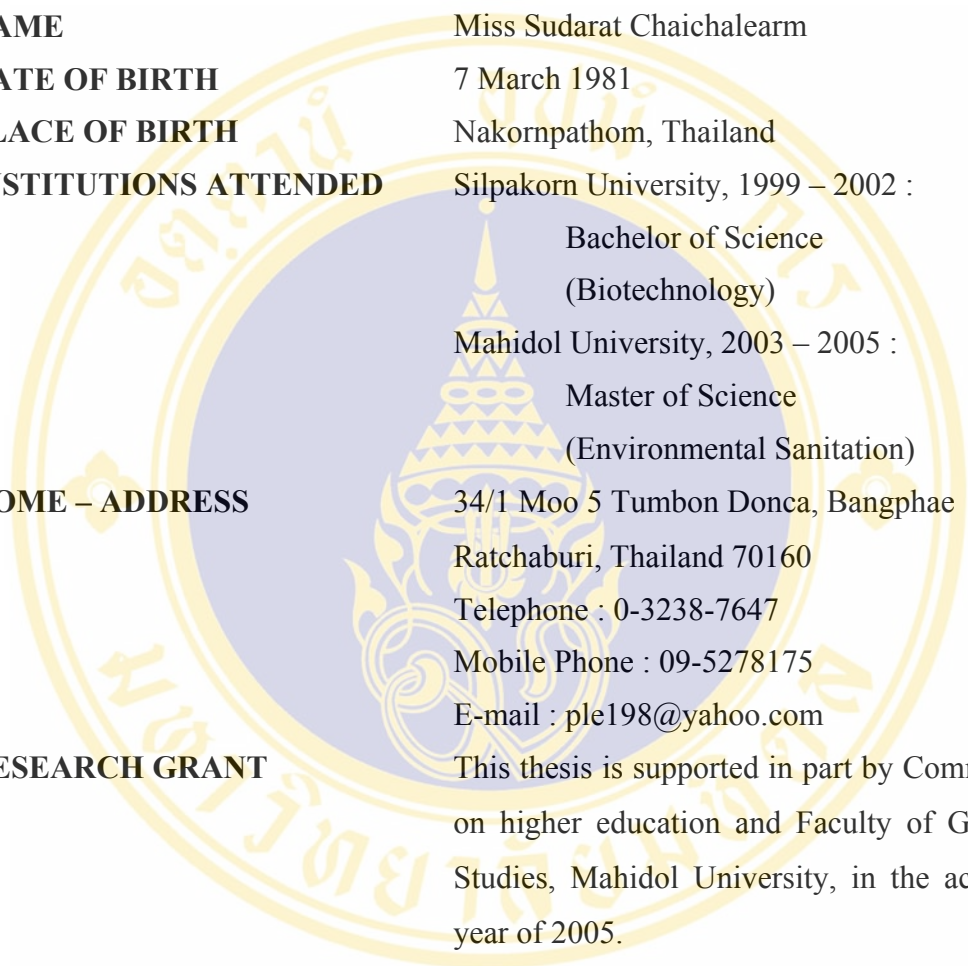
Industrial Effluent Standard (Continued)

Parameter	Standard values
15. Chemical Oxygen Demand (COD)	– not more than 120 mg/l depending on receiving water or type of industry under consideration of PCC but not exceed 400 mg/l
16. Heavy metals	
Zinc (Zn)	– not more than 5.0 mg/l
Chromium (Hexavalent)	– not more than 0.25 mg/l
Chromium (Trivalent)	– not more than 0.75 mg/l
Copper (Cu)	– not more than 2.0 mg/l
Cadmium (Cd)	– not more than 0.03 mg/l
Barium (Ba)	– not more than 1.0 mg/l
Lead (Pb)	– not more than 0.2 mg/l
Nickel (Ni)	– not more than 1.0 mg/l
Manganese (Mn)	– not more than 5.0 mg/l
Arsenic (As)	– not more than 0.25 mg/l
Selenium (Se)	– not more than 0.02 mg/l
Mercury (Hg)	– not more than 0.005 mg/l

Remark: 1. PCC: Pollution Control Committee

2. The standards were summarized from the Notification of the Ministry of Science, Technology and Environment, No. 3, B.E. 2539 (1996) and it specifies that pollution sources that the above standards are to be applied are factories group 11 and III issues under the Factory Act B.E. 2535 (1992) and every kind of industrial estates (8).

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