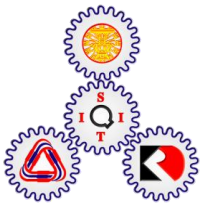

INVESTIGATION OF OPTIMAL CONDITIONS FOR MICROBIAL CULTIVATION AND ITS IMMOBILIZATION ON MICROBIAL FUEL CELL ELECTRODES

BY

PRUETSAJI WINAIKIJ

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENGINEERING (ENGINEERING TECHNOLOGY)

SIRINDHORN INTERNATIONAL INSTITUTE OF TECHNOLOGY

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A Thesis Presented

By

PRUETSAJI WINAIKIJ

Submitted to

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Approved as to style and content by

Advisor and Chairperson of Thesis Committee _____

(Asst. Prof. Dr. Paiboon Sreearunothai)

Committee Member and
Chairperson of Examination Committee _____

(Assoc. Prof. Dr. Rachnarin Nitisoravut)

Committee Member _____

(Dr. Korakot Sombatmankhong)

Committee Member _____

(Prof. Dr. Mina Okochi)

DECEMBER 2016

Abstract

INVESTIGATION OF OPTIMAL CONDITIONS FOR MICROBIAL CULTIVATION AND ITS IMMOBILIZATION ON MICROBIAL FUEL CELL ELECTRODES

by

PRUETSAJI WINAIKIJ

Bachelor of Public Health, Thammasat University, 2014

Microbial fuel cell (MFCs) is a biological device that harvests chemical energy from microbial catalytic abilities of organic compounds in wastewater which is then converted to electrical energy. It is known that electrochemically active microorganisms that are effective in electron transfer are also a key factor to the MFC performance. In the study of microbe-electrode immobilization, the microorganisms collected from an up-flow anaerobic sludge blanket at Cho-heng rice vermicelli industry were employed as inoculum. The optimal conditions for microbial cultivation were initially investigated to identify the period of various growth phases (i.e. lag, log, stationary and death phases) under different cultivation temperatures such as 25, 30 and 35°C. The effect of agitation including horizontal and turbulence shaking was also explored. It was found that the microorganisms cultivated at 35°C using a horizontal shaking provided the fastest growth rate as the dry weight of microorganisms went up to 1.82 g/L and reached the log phase within 6 hours. The interactions between microorganisms at various growth phases were studied by cyclic voltammetry (CV). The mechanism of electron transfer in term of direct (DET) or indirect (IDET) electron transfer was also investigated using three electrolyte solutions: microbe-suspended MLB media, microbe-suspended PBS media and the used MLB media without microbe. The results suggested that the main process of electron transfer was the IDET via the endogenous or exogenous mediator. In addition, electrodes of MFCs were studied through the microbe-electrode

immobilization processes which were assessed through electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM). Electrodes used in this present work were stainless steel (SS), granular activated carbon (GAC) and graphite plate. The results of EIS show that the resistance is greatly influenced by the immobilization time of microbes to form the biofilm on the electrode surface, the longer the time the smaller the resistance. The morphology of microorganisms immobilized on electrode surface showed a vast network of electrochemically active bacteria biofilm. For the first time, this work experimentally demonstrated the benefits of biofilm formation on the electrode surface using electrochemical techniques. The electrical connections through a formation of biofilm exhibited an improved electrochemical activity of the microorganisms.

Keywords: Microbial fuel cell, Growth rate, Electrochemical activities, Immobilization

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Table of Contents

Table of Contents

Table of Contents

List of Tables

Tables **Page**

List of Figures

List of Figures

Figures **Page**

List of Figures

Figures **Page**

List of Figures

Figures **Page**

Chapter 1
Introduction

1.1 Concept and Significance

A bio-electrochemical device that converts chemical energy into electrical energy using the activity of microorganisms is called the Microbial Fuel Cells (MFC). At the anode, microorganisms can oxidize organic compound in the wastewater and electrons are transferred through the external circuit to cathode producing electricity. Electron transferred may be classified into two types: (i) direct electron transfer (DET) by redox outer membrane proteins or putatively conductive nanowire or bacteria surface and (ii) indirect electron transfer (IDET) by exogenous or endogenous mediators [1] with an electrochemical active property. DET is more preferred for MFC applications because of their higher efficiency [2]. Not all microorganisms can produce electrons and transfer to the electrode but electrochemically active bacteria can perform such phenomenon, for example Shewanella putrefaciens [3], Geobacter sulfurreducens [4], Pseudomonas aeruginosa [5] and Escherichia coli [6]. It has been reported that the inoculum in terms of mixed culture provided greater power densities and easy to handle compared to the pure culture [7] because a mixture of various interdependent bacteria helped to degrade the organic matter at a faster rate in MFCs.

Generally, the growth rate of bacterial population depends on the phases of the growth which are the lag, log, stationary, and death phase. In the lag phase, bacteria initially adapt to the new environment and the cell size increased slowly. During log phase; bacteria grow and divide rapidly; their metabolic activity are at maximum rate. After that, the bacteria enter stationary phase in which the bacteria still develop at the similar growth rate but with the depletion of nutrients lead to the reproduction rate was retarded, the number of cell division is equal to the number of death cell. At the final stage “death phase”, bacteria completely lose their ability to reproduce due to the depletion of nutrients and the accumulation of toxic compounds[1]. This information is believed that the use of microorganisms in the log phase probably will reduces the cultivation time, the immobilization period, and then the start-up time for MFCs to

reach its open circuit potential of about 0.7 V. Moreover, the maximum current and power may be generated while the electrochemically active microbes are under exponential growth phase.

There are several parameters that strongly affect the growth of microorganisms such as temperature, osmotic pressure, pH, and types of media [8]. Among them, cultivation temperature is the most. For instance, the optimal temperature for anaerobic digestion is 37.7°C [9]. When the cultivation temperature is optimal, microorganisms can grow rapidly and then release high quantity of mediator to transfer electrons during the electrochemical processes. It has also been reported that both single and double chamber of MFCs exhibited the highest chemical oxygen demand removal and the maximum current density at the operating temperature of 35°C as compared with 4, 8, 15, 20, 25 and 30°C [10]. Microorganisms were formed on the electrode surface as biofilm. This formation requires the adhesion force between microorganisms and the substrate surface. It takes several days to create a stable biofilm [11]. The best way to reduce the resistance between electrons that transfer from microorganisms and the electrode to immobilized microorganisms onto the electrode surface [12].

Electricity that generated from MFCs depends also on the inoculum, substrate and reactor [13] and electrode materials [14]. To improve the performance of MFCs, the inoculum is investigated in terms of the optimal condition for microbial cultivation, and also the immobilization time of the microorganisms on the electrodes.

For this study, the sample was collected from the up-flow anaerobic sludge blanket system of rice vermicelli industry and used as mixed culture to investigate the growth rate of microorganisms and their electrochemical activity. In addition, immobilization of microorganisms on the electrodes which are stainless steel type 304 and 316, granular activated carbon (GAC) and graphite plate. The resistance and morphology of electrodes were investigated by electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM) respectively.

Moreover, to ensure the performance of MFC that obtains from the all results double chamber microbial fuel cell was operated using microorganisms at different growth phases to investigate the value of open circuit voltage (OCV).

1.2 Objectives of the study

(1) Investigate the growth rate of microorganisms in different temperatures and different agitation systems.

(2) Investigate the correlation of the growth rate of microorganisms and their electrochemical activity.

(3) Investigate morphology of microorganisms that immobilized on the electrodes at different phases of growth rate.

(4) Investigate open circuit voltage (OCV) of MFC that operated using microorganisms at different phases of growth rate.

1.3 Scopes of the study

(1) Inoculated microorganisms in the media of Modified Lysogeny broth (MLB) with horizontal and turbulence shaking.

(2) The temperatures at 25, 30 and 35 degree Celsius are investigated.

(3) The growth rate of microorganisms in each phase are investigated.

(4) Stainless steel type 304 and 316, granular activated carbon (GAC) and graphite plate are used as electrode.

(5) Electrochemical impedance spectroscopy (EIS) was used to study the resistance of electrodes.

(6) Scanning electron microscopy (SEM) was used to study the morphology of microorganisms.

Chapter 2
Literature Review

2.1 Design of microbial fuel cells (MFCs)

Many different configurations of MFCs are shown in **Figure 2.1.1.** The easiest structure is shown in **Figure 2.1A**, it has two containers that were linking by a salt bridge. The next configuration (**Figure 2.1B**) is a type of four batch MFCs that have membrane to separated four chambers and linking them by bolts. And configuration C (**Figure 2.1C**) is the same **Figure 2.1B** but it has anode with continuous flow and close cathode-anode placement. **Figure 2.1D** is MFCs that use bacteria to oxidized organics matters under anaerobic condition and a light, which was call photoheterotrophic MFC. And the next configuration was single chamber of MFCs (**Figure 2.1E**) with a tube shape. The last configuration is "H" shape of two chamber MFCs with low-cost design that was normally use. It consist of two chambers connecting by separator that is cation exchange membrane (CEM) in **Figure 2.1F** [2].

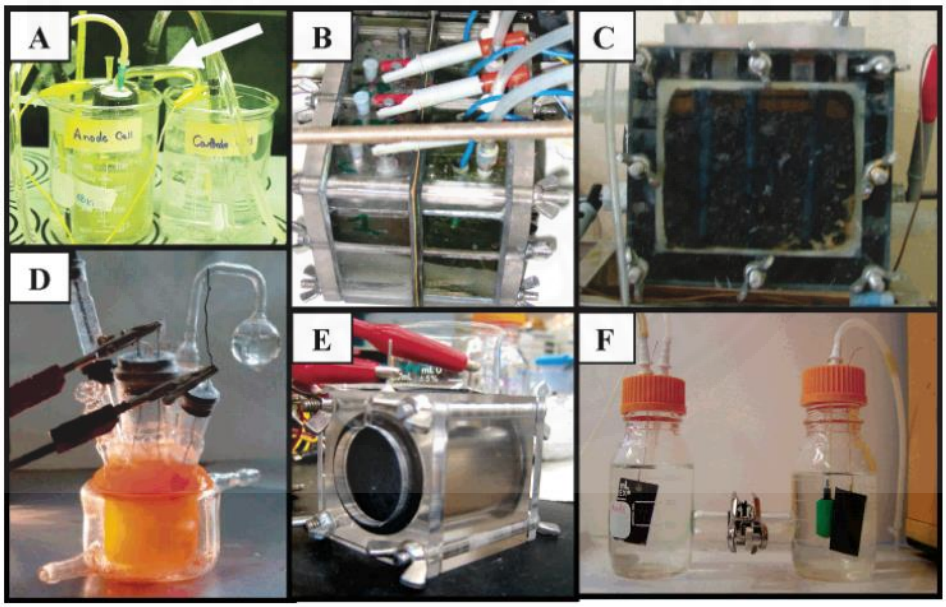

Figure 2.1 Different configurations of MFCs [2].

Although MFCs have many different configurations, the normally basic components of MFCs are shown in **Table 2.1**.

Table 2.1 Basic component of MFCs.

Component	Remark
Anode	Necessary
Cathode	Necessary
Proton exchange system	Necessary
Anode chamber	Necessary
Cathode chamber	Optional
Electrode catalyst	Optional

2.1.1 Anode

Electrons that generated from microorganisms on the anode transfer through an external circuit to the cathode. The structure and material of the anode affect to microbial adhesion or immobilization, some case of direct substrate oxidation and electron transfer [3]. An effective material should have the following characterization, (1) strong biocompatibility (2) good electrical conductivity and low resistance (3) large surface area (4) chemical stability (5) anti-corrosion and (6) strength and toughness. The useful anode material including graphite fiber brush, graphite, carbon paper, carbon felt, carbon cloth, and reticulated vitreous carbon (RVC) [4] are shown in **Figure 2.2**.

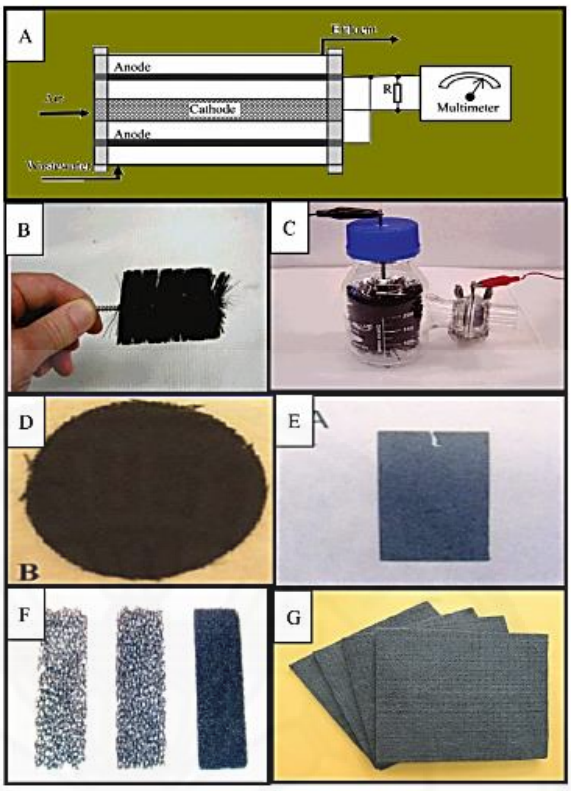

Figure 2.2. Anode materials: A) graphite anode, B) graphite brush, C) single-chamber MFC with graphite brush, D) carbon cloth, E) carbon paper, F) reticulated vitreous carbon (RVC), and G) carbon felt [4].

2.1.1.1 Graphite

Graphite is the most commonly used as anode in MFCs because it was a good chemical stabilization, good electrical conductivity, low price and simple to operate. However, graphite's application was restrictive due to it was low surface area and low porosity for microbial attachment.

2.1.1.2 Graphite brush

Bind graphite fiber around metal wires that was conductive and anti-corrosion to made graphite fiber brush. It provides a low electrode resistance and also provides high surface area.

2.1.1.3 Carbon cloth and carbon paper

Carbon cloth and carbon paper used as flat-plate electrodes. There are physically flexible. Moreover, it can reduce the distance between two electrodes.

2.1.1.4 Reticulated Vitreous Carbon (RVC)

RAC is a useful electrode material, it provides high current densities and good electrical conductivities but it has large resistance and easy to fragile.

2.1.1.5 Granular Activated Carbon (GAC)

GAC is produce from organic materials that have high value of carbon compound (coal or wood). GAC was a commonly used by packing to the system for wastewater and water treatment. It was low-priced and lasting material with great surface area (1000 m^2/g at lowest), that should to improve microbial attachment for anode in MFCs [5].

2.1.1.6 Metal anode

Metal electrodes such as platinum, gold are quite rare and very expensive. Stainless steel is the one electrode that low price and suitable for use because it is anti-corrosion and large surface area.

The performance of different systems with the different anode is shown in **Figure 2.3**. The highest maximum power density (6,000 mW/m^2) is the system of single chamber that used platinum and polyanilineco-modified as anode and Escherichia coli as bacteria.

Substrate	Anode	Bacteria	System configuration	Maximum power density (mW/m^2)
Glucose	Carbon paper	*GeobacterSPP* (Firmicutes)	Two-chamber	40.3 ± 3.9
Glucose	Graphite	*Saccharomyces cerevisiae*	Two-chamber	16
Acetate	Carbon paper	*G. sulfurreducens*	Two-chamber	48.4 ± 0.3
Lactate	Carbon paper	*Geobacter SPP*	Two-chamber	52 ± 4.7
Ethanol	_	*Betaproteo bacterium*	Two-chamber	40 ± 2
Cyctenin	Carbon paper	*Gammaproteo* and *shewanellaaffinis* (KMM3586)	Two-chamber	36
Marine sediment reached in acetate	Graphite	*Deltaproteo bacterium*	Two-chamber	14
Marine sediment	Noncorroding graphite	*Desulfurmonas SPP and ...*	Two-chamber	25.4_26.6
Sewage sludge	Graphite with Mn^{4+}	*Escherichia coli*	Single chamber	91
Sewage sludge	Graphite with neutral red (NR)	*Escherichia coli*	Single chamber	152
Sewage sludge	Platinum and polyanilineco-modified	*Escherichia coli*	Single chamber	6000
Glucose	Composite electrode (graphite/PTFE)	*Escherichia coli*	Single chamber	760
Glucose	Teflon treated carbon fiber paper	Electrochemically active bacteria	Two chamber (H-tyape MFC)	15.2
Lactose	"	"	"	17.2
Cellulose	Non-wet-prof carbon paper	Cellulose derading bacteria	"	188
Glucose	Graphite plates	Mixed culture	2-chamber air-cathode MFC	283
Glucose	Carbon paper with PPY-CNTs	*Escherichia coli*	DCMFC	228

Figure 2.3 The performance of different systems with different anode material. [6]

2.1.2 Cathode

Protons transfer into the cathode pass through the proton exchange membrane which completes the electrical circuit in the anode chamber. The electrons that generated at the anode transfer to cathode chamber and convey onto oxygen [6]. Due to the performance of MFCs, cathode material is another key parameter to effect because it has high redox potential and readily to capture protons. Common cathode materials are carbon paper, carbon cloth and graphite thanks to cost and performance.

The performance of different cathode is shown in **Figure 2.4**. The cathode that provides the highest maximum power density (667 W/m^3) is ACFF granule (1 cm.).

Cathode	Max power density	Max current density	Max Voltage
Activated carbon fiber felt (ACFF)	315 mW/m^2 (0.7 W/m^3)	1.67 * 10^{-3} mA/m^2	679 mV
Air–cathode with graphite	283 mW/m^2	1210 mA m^{-2}	440 mV
Carbon felt	77 mW/m^2 (0.2 $W/m^{3)}$	6 * 10^{-3} mA/m^2	575 mV
Plain carbon	67 mW/m^2 (0.1 $W/m^{3)}$	1.5 mA/m^2	598 mV
Pt-coated carbon paper	0.3 W/m^3	4.69 mA/m^2	644 mV
Tubular ACFF	784 mW/m^2	3.17 A/m^2	716 mV
ACFF granules (1 cm)	667 W/m^3	3.34 A/m^2	658 mV
Biocathode	19.53 W/m^3	41.78 A/m^3	432 mV
Graphite felt	539 mW/m^2	3145 mA/m^2	742.3 mV
Parallel sheets of carbon paper secured by carbon fiber coated with pt	7.29 W/m^3	13.16 A/m^3	553 mV
Air–cathode with Carbon cloth	50 W/m^3	363 A/m^3	710 mV

Figure 2.4 The performance of different cathode in MFCs [6].

2.1.3 Anode chamber

In anodic chamber, type of substrate, feed rate, and concentration are significant factors that affect the performance of MFCs because microorganisms grow in this place. The increase in fuel concentration lead to the increase of power density [7].

2.1.4 Cathode chamber

The normal electron acceptor that used in MFCs is oxygen in cathodic reaction. To reduce the value of coulomb yield, oxygen should diffuse through cation membrane. And the concentration value of electron acceptor effect to power output of MFCs.

2.1.5 Proton exchange system

Proton exchange system intensely effects internal resistance of MFCs and also effect to power output. Nafion is the most extensive because it was durable and greatly selective permeability of proton.

2.1.6 Electrode catalyst

The buffer that used in MFCs change the pH of the system (pH is difference) result in the increase of proton driving force from anode to cathode chamber [8].

2.2 Microorganisms in microbial fuel cell

Microorganisms in anode compartment, first there oxidize organic compound in metabolism process. Protons, electrons, and cations carry to substrate through many mechanisms. For example, directly contact via nanowire or indirect contact via mediators. Electrons from anode arrive at cathode through external circuit to generated electricity. Respiration of the microorganisms occurs in the presence of heterotrophic (For the metabolism, require complex organic compounds of nitrogen and carbon). The highest energy that gains for microorganisms and glucose oxidation process is aerobic respiration, while anaerobic respiration microorganisms using carbon dioxide, nitrates, fumarate, metal ions or sulfates as terminal electron acceptors. This process provides lower energy because of small positive redox potential compared with oxygen.

Electrons transfer processes were developed by different microorganisms for fulfilling respiration process thru extracellular route by giving or receiving electrons in metabolism.

There are two main pathways of electron transfer. The first is direct electron transfer (DET) via bacteria surface, outer membrane proteins (cytochromes) or conductive nano-wire **(Figure 2.5a)**. It has been shown that some microorganisms develop conducting pili (nanowires) to establish physical connections indirectly contact with the surface **(Figure 2.5b)**. Cytochrome was connected to pili that allow electron transfer to the outside of microorganisms. The second is indirect electron transfer (IDET) through exogenous or endogenous mediators **(Figure 2.6)**. Electron mediator should be (1) electrochemically active, (2) capable to contact physically to surface of electrode and (3) redox potential of the substrate should have closely to standard potential. When microorganisms produced shuttles as secondary metabolism in mediation process, exogenous mediators are not necessary.

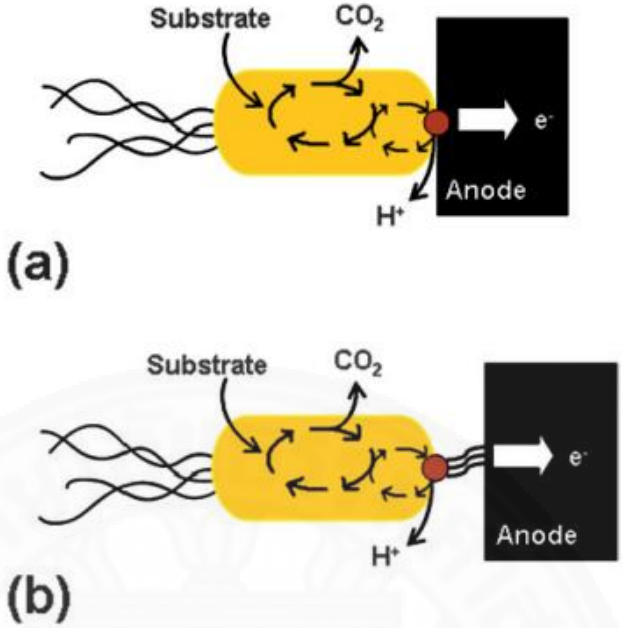

Figure 2.5 DET via (a) outer membrane proteins (cytochromes) and (b) via conductive nanowires (pili) [9].

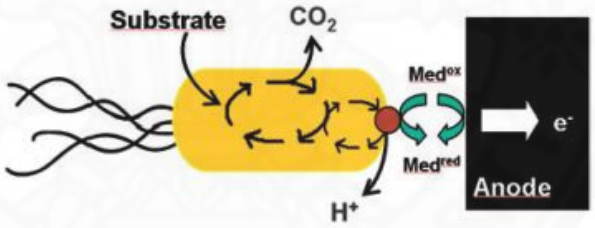

Figure 2.6 IDET via exogenous or endogenous electron shuttles [9].

There are two key interesting concepts about MFC biocatalysts.

(1) When the sophisticated substrate was used, pure culture should not trust because a mixed culture or a group of microorganisms provides good dependable performances.

(2) DET and IDET pathways may cohabit in an MFC. [9]

Pure culture provides lower power densities and hardly to operated compared to the mixed culture [10]. The reason was the good collaboration among the interdependent and competing bacteria species of mixed cultures that help to degrade

organic matter in MFCs. The first group of fermentation microorganisms **(Figure 2.7)** disrupting sophisticated compound into energy-rich reduced metabolites that proper for anaerobic bacteria in second group. For the last group, when provided with suitable anode substrate, microorganisms were carrying extracellular respiration. And the last ones take benefit of cohabiting adjuvant microorganisms [9].

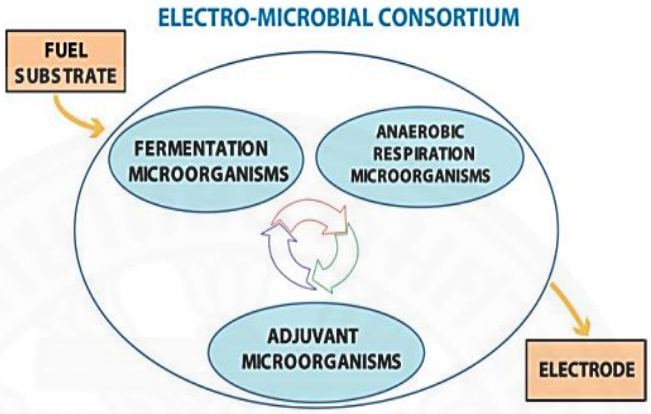

Figure 2.7 Microbial consortium (mixed culture): three types of microorganisms collaborate in substrate degradation and electron transfer [9].

Not all microorganisms can produce electrons and transfer to the electrode but some microorganisms can do this. There are call "electrochemically active bacteria". Microorganisms that used in MFCs are shown in **Table 2.2**.

Table 2.2 Pure culture and mixed culture that use in MFCs [11], [8]**.**

Microorganisms	**Substrate**
Pure culture	
Actinobacillus succinogenes	Glucose
Aeromonas hydrophila	Acetate
Alcaligenes faecalis, Enterococcus gallinarum, Pseudomonas aeruginosa	Glucose
Pure culture	
Clostridium beijerinckii	Starch, glucose, lactate, molasses
Clostridium butyricum	Starch, glucose, lactate, molasses

Microorganisms	Substrate
Desulfovibrio desulfuricans	Sucrose
Erwinia dissolven	Glucose
Escherichia coli	Glucose, sucrose
Geobacter metallireducens	Acetate
Geobacter sulfurreducens	Acetate
Gluconobacter oxydans	Glucose
Klebsiella pneumoniae	Glucose
Lactobacillus plantarum	Glucose
Proteus mirabilis	Glucose
Pseudomonas aeruginosa	Glucose
Rhodoferax ferrireducens	Glucose, xylose, sucrose, maltose
Shewanella oneidensis	Lactate
Shewanella putrefaciens	Lactate, pyruvate, acetate, glucose
Streptococcus lactis	Glucose
Mixed culture	
Mixed, saltwater	Acetate
Mixed consortium, batch	Glucose
Mixed consortium, continuous	Sucrose, Glucose, Acetate, Butyrate
Activated sludge	Wastewater, Lactate, Glucose

Microorganisms that use in this study collected from an up-flow anaerobic sludge blanket (UASB) system of rice vermicelli industry and used UASB granule as an inoculum. In UASB system, the ability of the reactors to accumulate large amounts of biomass is due to the adhesion of bacterial cells to each other [12]. The degradation of sophisticated substrates into carbon dioxide and methane during anaerobic digestion involve the collaboration of at least three metabolic groups. The first group of fermentative bacteria, the acidogenic bacteria, conducts the initial degradation of biopolymers. The acids and alcohols so produced are utilized by the second group of organisms called the acetogenic bacteria. The third group of bacteria is the

methanogens. Located at the end of the nutrient cascade, methanogens convert CO_2 and H_2, acetate, and a few other simple compounds to methane. The steps of anaerobic digestion are shown in **Figure 2.8.**

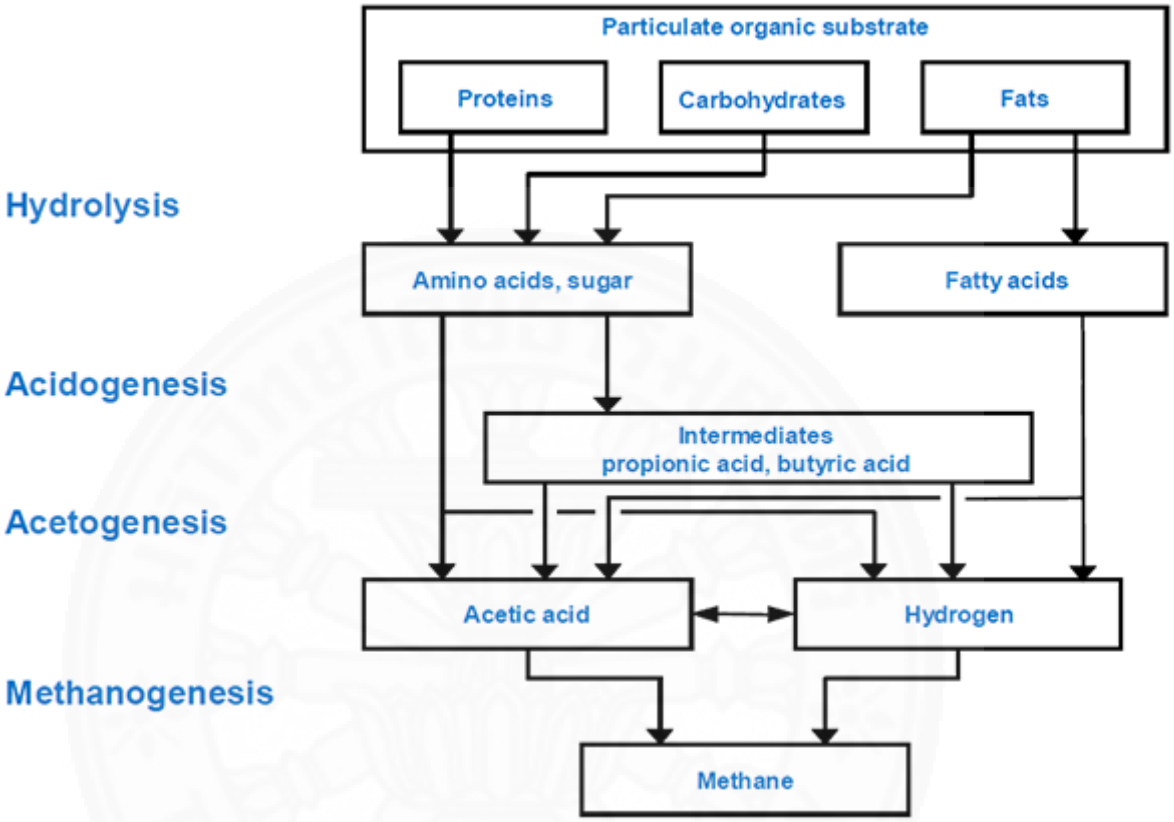

Figure 2.8 Steps of degradation in anaerobic digestion process [13].

The microorganisms were different in three group of anaerobic bacteria **(Figure 2.9)**. The first group is acidogenic bacteria. The second is acetogenic bacteria. And the last group is methanogenic bacteria.

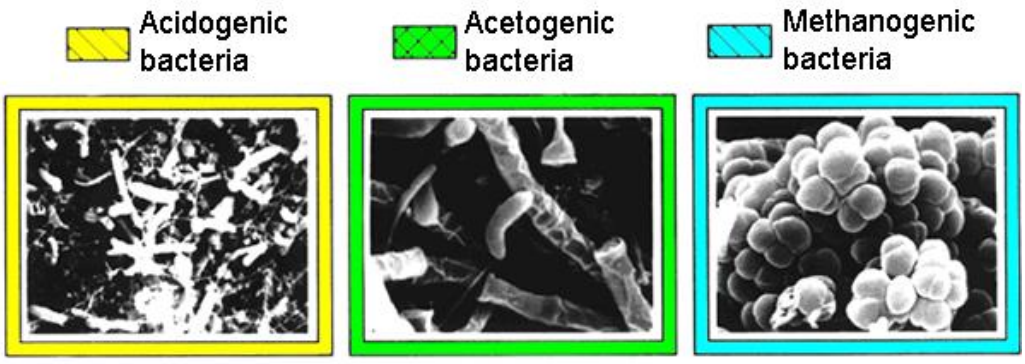

Figure 2.9 The microorganisms in three groups of anaerobic bacteria [13].

Operation temperature influences the digestion rate. The suitable temperature was at mesophillic range around 35 to 40.5 °C with an optimum of 37.7 °C [14].

The morphology of surface granule from UASB system, the granule surface is colonized by a mixed population that includes rods, coccus, and filaments [12] is shown in **Figure 2.10.** The granular of UASB system have three layered is shown in **Figure 2.11.**

(1) The outer layer consists of heterogeneous species which included bacillus, coccus, and filamentous in various sizes.

(2) The second layer contains bacillus. That was predominant in this layer.

(3) The inner layer formed the core in granules. This core contained a big group of *Methanothrix* [12].

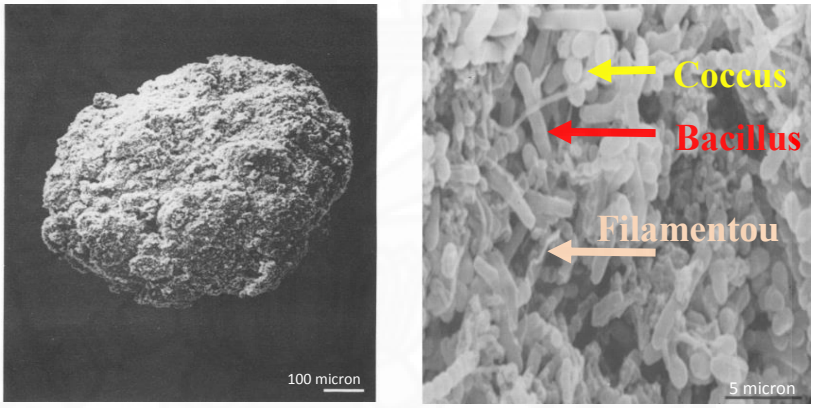

Figure 2.10 Scanning electron micrograph showing the granule surface of UASB system [12].

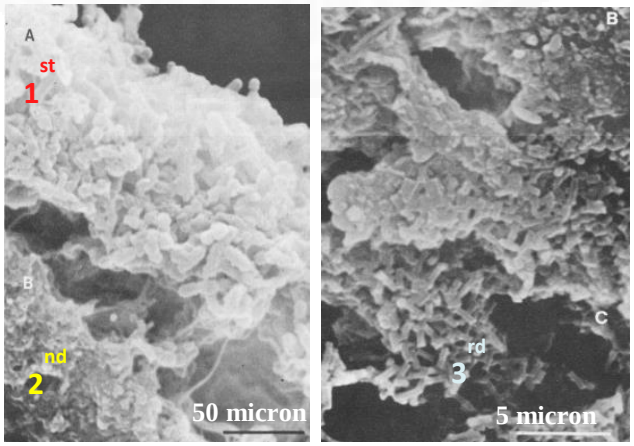

Figure 2.11 Scanning electron micrograph showing the three layered structure of the granule [12].

2.2.1 The growth of bacteria population

The increase in the cell mass and cell size during the evaluation of microorganisms is termed like growth. The population of microorganisms will grow like the graph in **Figure 2.12** when a media or substrate is inoculated with cell.

The first phase is lag phase, microorganisms take little time to adapt to a new environment when there inoculated in a fresh media. The size of cell is increase but cell mass not increase because bacteria cell cannot replicate. The growth conditions depend directly on the length of lag phase. Microorganisms will start to establish the essential substance such as vitamins, proteins, and co-enzymes that imperative for the growth. If microorganisms cultivated in excess nutrient in the media, microorganisms can adapt to a new environment in a short time. Therefore, it may have a less lag phase or it may disappear.

The second phase is logarithmic or exponential (log) phase. In this state, microorganisms are rapidly growing and dividing. The cell replicated by binary fission. Microorganisms reach the maximum growth rate and bacteria population increases exponentially. The length of this state depends on the type of microorganisms.

The third phase is stationary phase, the nutrients in the media are used up during microorganisms continue to grow and lead to the storing of toxic compound. This situation change pH and temperature of media result in unfavorable environment for the bacteria growth. The rate of reproduction was slow down, the number of live cells equal to the number of death cell. Finally, cell completely loss its ability to reproduce. Therefore, the cell number is not increase lead to the growth rate is stabilized. If cells gain a more nutrient, cells can move to log phase again.

The last phase is death or decline phase, the lack of nutrients and the accumulation of toxic compound in the media created unfavorable environment lead to the cell die. During this phase, the number of live cells less than the number of dead cells. Therefore, the growth curve was drop

down. Only bacteria that can produce endospores can survive in this environment [1].

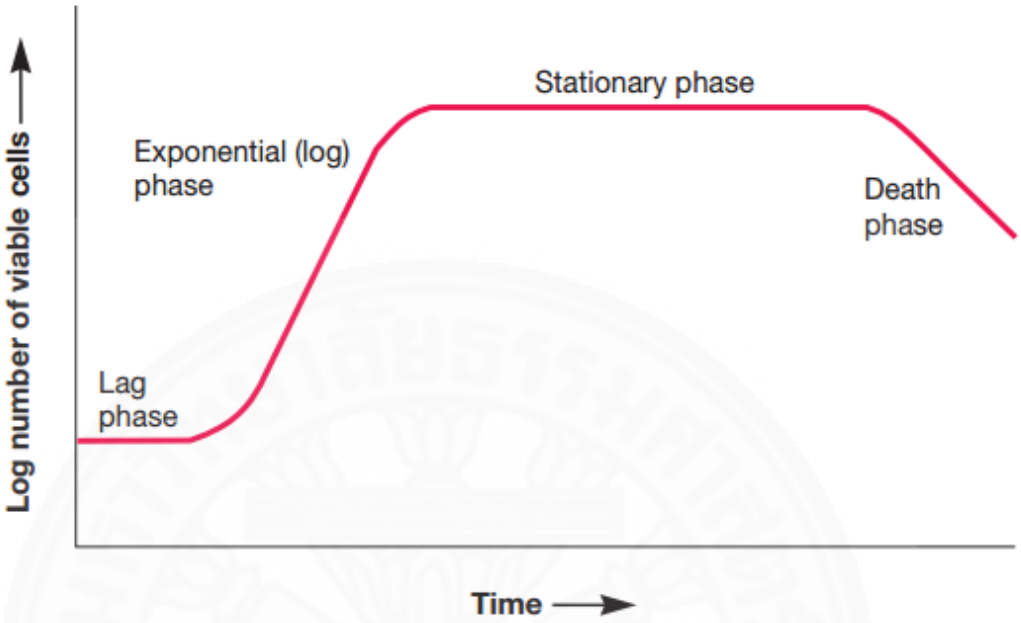

Figure 2.12 Growth phases of microorganisms [1].

2.2.2 Factors that effect to the growth of microorganisms

They have several parameters that effect to the development of microorganisms. The first is chemical parameter [15].

(1) Carbon (C): Dry weight of organism contains 50 % of carbon.

(2) Nitrogen (N): Dry weight of organism contains 14 % of nitrogen. It using for form DNA, RNA, and amino acids.

(3) Sulfur (S): It using for form some vitamins (biotin and thiamin) and proteins.

(4) Phosphorus (P): It using for form phospholipids, RNA, ATP, and DNA.

(5) Elements: Calcium (Ca), potassium (K) and magnesium (Mg) are used as enzyme cofactors.

(6) Trace Elements: Copper (Cu), Iron (Fe), Zinc (Zn) and Molybdenum (Mo) are applied as enzyme cofactors.

(7) Oxygen (O_2): Microorganisms use oxygen for their respiration. Aerobic bacteria produce higher energy than anaerobic bacteria. Oxygen demand

was different in microorganisms, it can classify into 5 types (arrange in order from the most requirement to least requirement); obligate aerobes (such as *Pseudomonas*), facultative anaerobes (such as *E. coli*), obligate anaerobes(such as *Clostridium*), aerotolerant anaerobes (such as *Lactobacillus*) and microaerophiles (such as *Campylobacter*).

And the second is physical parameter;

(1) pH: Most bacteria prefer neutral pH around 6.5 -7.5. Microorganisms can classify into 3 types depend on pH that suitable for their growth; acidophiles (grow at pH 0.1-5.4), neutrophiles (grow at pH 5.4-8.5) and alkaliphiles (grow at pH 7-12 up).

(2) Osmotic Pressure: Microorganisms contain 80-90% of water.

(3) Temperature: If temperature not suitable for the growth, microorganisms will grow slowly but not to die. It can classify into 3 types depend on temperature that their preferred; psychrophiles (grow at 0°C), mesophiles (grow at 25-40°C and optimal temperature was at 37°C) and thermophiles (grow at >45°C). Temperature plays role important to effect the growth of microorganisms.

2.2.3 Effect of temperature on the performance of microbial fuel cells

Temperature is key parameter to affect the performance of MFCs, this research study at temperature 4-35°C under the same conditions. The experiment performed with single chamber (use carbon cloth as cathode) and double chamber under load of 1 kΩ. The results that obtained in this study were maximum voltage (V), maximum current density (i), maximum accumulated charge (Q), maximum chemical oxygen demand removal (COD_R) and maximum columbic efficiency (Y_Q) are shown in **Figure 2.13**.

T (°C)	MFC type	Max voltage (V)	Max i (mA m^{-2})	Max Q (C)	Max COD_{rem} (%)	Max Y_q
4	Double	0.003	2.35	0.94	42.29	0.08
	Single	0.029	23.11	9.07	58.03	0.77
8	Double	0.002	1.30	0.54	66.02	0.05
	Single	0.041	32.87	15.17	57.60	1.34
15	Double	0.002	1.71	0.50	73.12	0.03
	Single	0.074	58.72	27.73	88.24	1.58
20	Double	0.036	28.77	9.90	77.23	0.69
	Single	0.075	59.64	27.97	90.56	1.42
25	Double	0.045	35.97	17.73	82.08	0.67
	Single	0.093	73.99	49.36	91.01	1.41
30	Double	0.052	41.73	24.77	74.76	1.10
	Single	0.109	86.71	41.84	95.11	1.65
35	Double	0.096	76.15	40.40	74.94	1.78
	Single	0.118	93.87	57.65	94.50	1.76

Figure 2.13 The results of MFCs that operated under temperature of 4-35 °C [16].

From the results, single chamber provides greater value than double chamber in all results. However, the effect of temperature was more significant than the configuration of MFCs. The performance of both MFCs increase (V, i, Q, COD_R, Y_Q) with the temperature increase from 4 to 35°C [16].

2.3 The immobilization of microorganisms on the electrode

Microbial biofilms consist of bacterial cells that have assembled and attached to a surface via excreted adhesive polysaccharides. These polysaccharides form a sticky, protective envelope around the cells. The cells benefit from this configuration by having a defense from physical or chemical forces, having the ability to remain in a favorable niche (for example, nutrient-rich surface), and allowing them to live in close association with one another to encourage genetic exchange and cell-cell communication [10].

There are 5 stages of biofilm formation **(Figure 2.14)**.

(1) Cell reversibly attach to the surface. Extracellular organs were used for attaching to surface of microorganisms such as pili, flagella, and outer membrane proteins.

(2) Cell irreversibly attach to the surface. Extracellular polymeric substance (EPS) that excrete from the cell containing proteins, DNA, lipids and lipopolysaccharides that supported adhesion force between cells and surfaces.

(3) Cells replicate adsorbed on surfaces and develop to micro-colonies with diameter around 10-100 microns. EPS turn into encapsulation in a layer of hydrogel that form barrier between inner and outer environment.

(4) Cell maturates to biofilm and grows in three-dimension structure. Biofilm sticks together by EPS that withstand mechanic stress.

(5) Some cells dislocate from biofilm and disperse to fluid. In this place, cells can adsorb on surfaces and form biofilms in new place [17].

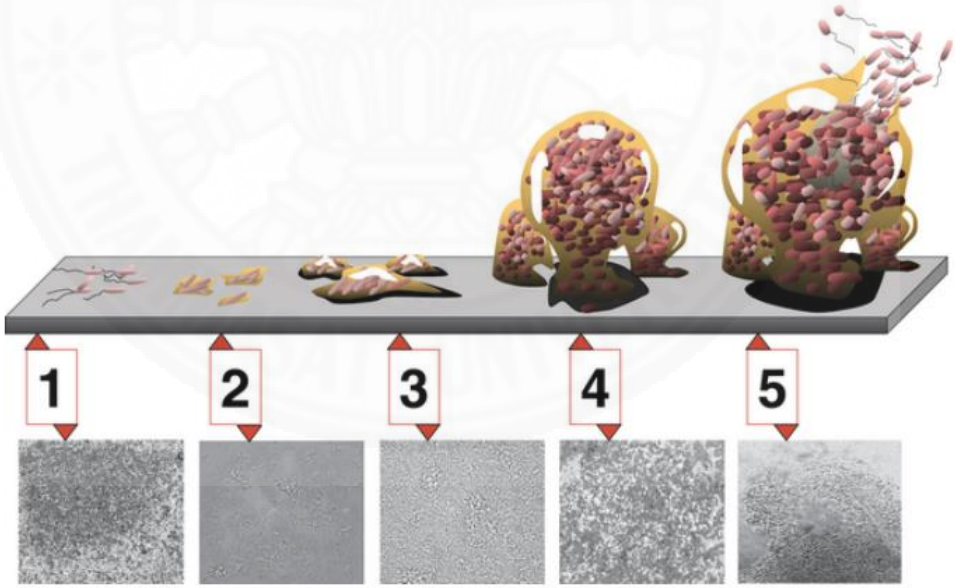

Figure 2.14 5 Stages of Biofilm Development. Photomicrographs of each stage in developing *P. aeruginosa* biofilm can be seen below each step [18].

2.4 Morphology of microorganisms

For the morphology of microorganisms that attach in the porous of granular activated carbon (GAC) is shown in **Figure 2.15** Structure of GAC allows microorganisms to attach on the porous surface [19]. Before MFC was operated, no microorganisms attach on the surface of GAC but after MFCs was run, it has many microorganisms attach on the porous of GAC. Morphology of microorganisms was coccus.

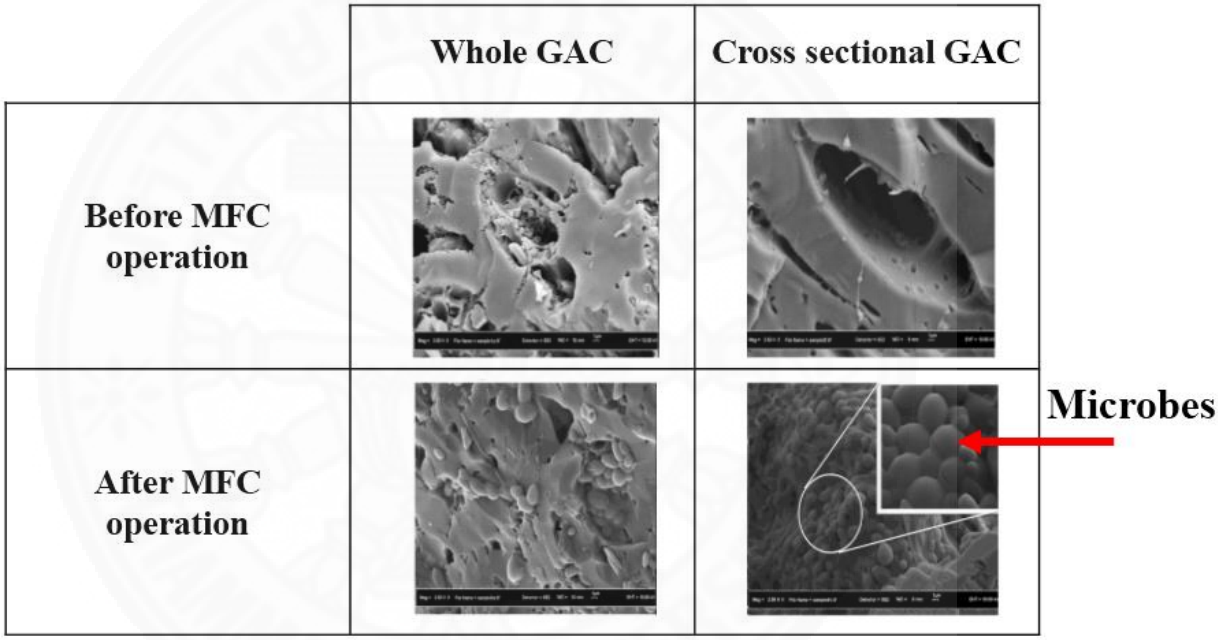

Figure 2.15 Morphology of microorganisms that immobilized on the porous of GAC before and after MFC was operated [19].

And morphology of microorganisms that attach on the surface of graphite is shown in **Figure 2.16**. The shape of microorganisms was bacillus that adheres to the roughly surface of graphite. And morphology of microorganisms that attach to the surface of stainless steel was coccus **(Figure 2.17)**.

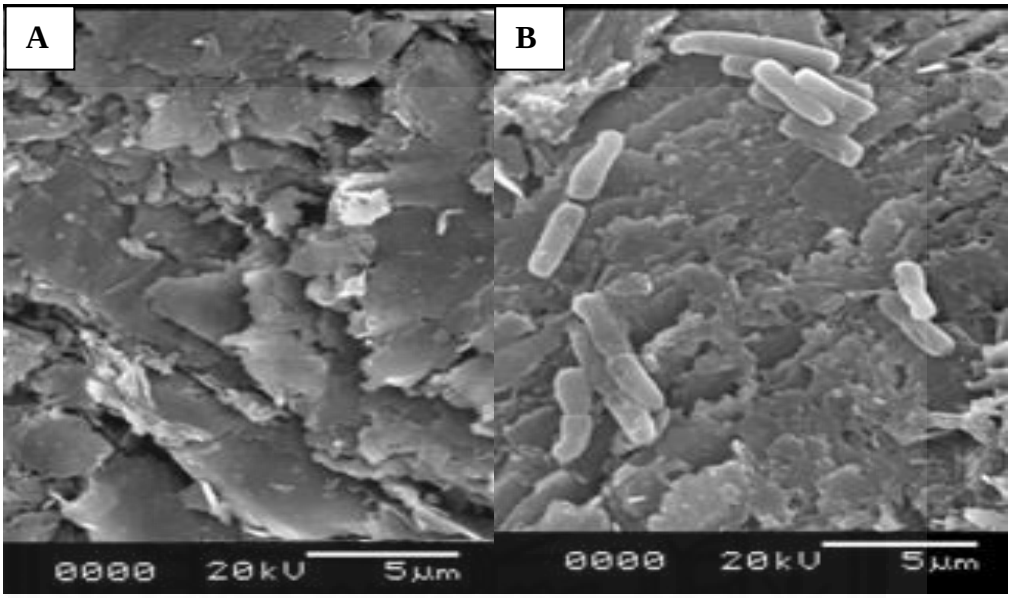

Figure 2.16 Morphology of microorganisms that immobilized on the graphite before (A) and after (B) MFC was operated [20].

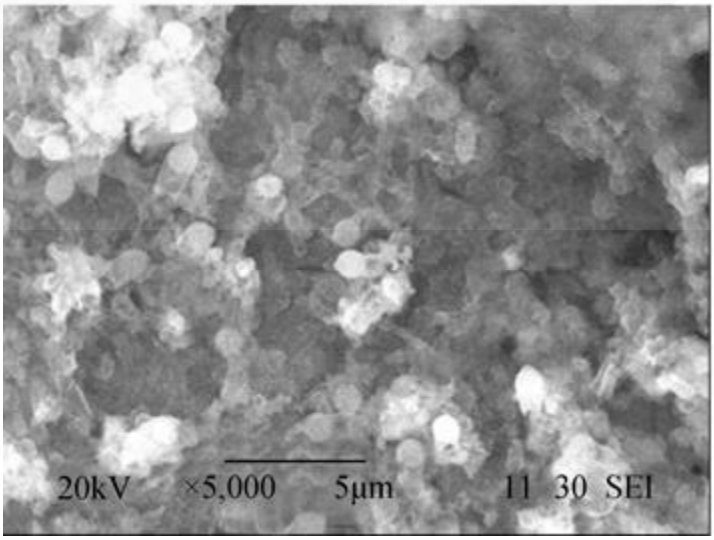

Figure 2.17 Morphology of microorganisms that immobilized on the stainless steel [21].

2.5 Resistance of electrode in MFCs

Instrument that uses for analyzing internal resistance of electrodes in MFCs was electrochemical impedance spectroscopy (EIS). The results in the different anode and cathode materials with *Shewanella oneidensis MR-1* in a two-chamber MFC are shown in **Figure 2.18**.

When cultivated microorganisms with electrode in a period of time, microorganisms will produce biofilm cover the surface of electrode.

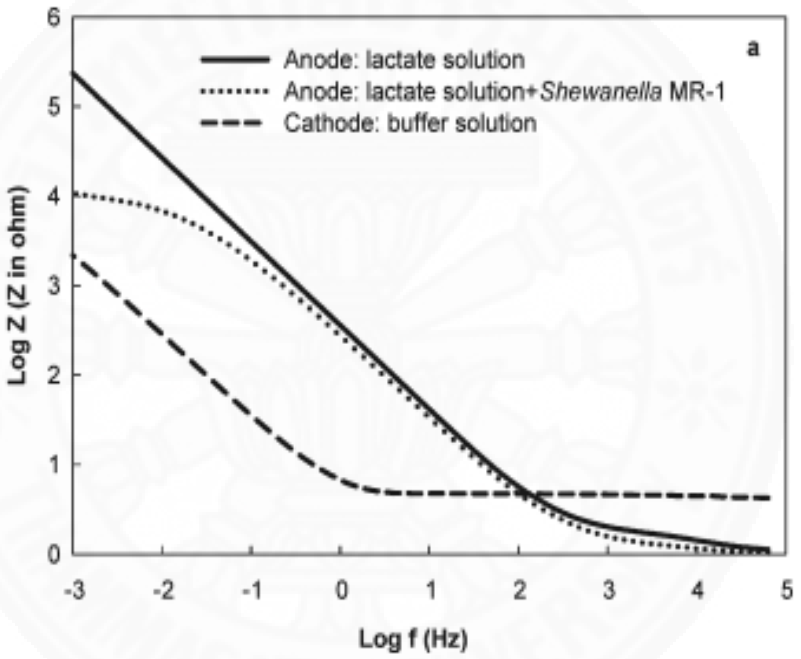

Figure 2.18 The impedance of the anode and cathode [22].

A graph in dot line demonstrate anode with bacteria and in contrast, a graph in straight line demonstrate anode without bacteria. The results show that anodic polarization resistance in straight line was smaller than dot line. It can be concluded that biofilm on the surface of the electrode that establishes from bacteria and their metabolism using lactate reduced the resistance [22].

2.6 Open circuit voltage of microbial fuel cell (OCV)

The value of voltage in long term was investigated by OCV. It connects to anode and cathode of chamber and record data with data logger.

The results of open circuit voltage of single chambered microbial fuel cell (SCMFC) are shown in **Figure 2.19**. The higher OCV values of electrode with microorganisms suggest that the immobilization of cell provide the close contact and lead to in this system have more electrons transfer [19].

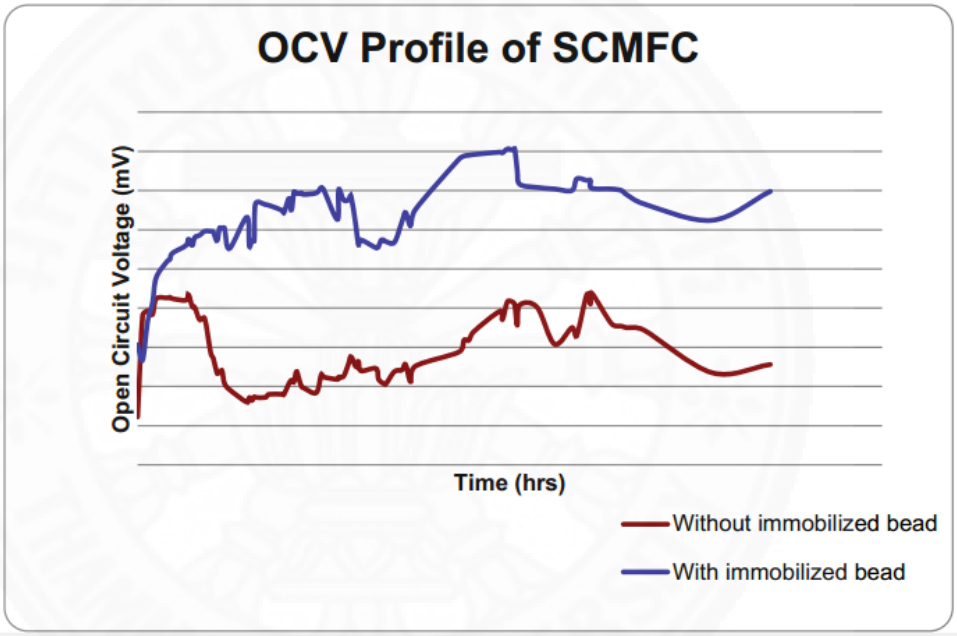

Figure 2.19 Open circuit voltage (OCV) of single chambered microbial fuel cell (SCMFC) [19].

Chapter 3
Materials and Methods

3.1 Chemicals

The media that was used to cultivated microorganisms was a modified lysogeny broth (MLB). It was composed of following (per liter of distilled water): sodium chloride; (NaCl) 10 grams (>98%, Daejung), phosphate buffer saline (PBS) 10 tablets (biotechnology grade, Amresco), yeast extract 5 grams (biotechnology grade, Becton Dickinson and company) and tryptone 10 grams (biotechnology grade, Becton Dickinson and company).

The solution that was used for pre-treatment the electrodes was acetone (analytical reagent grade, Fisher scientific) and ethanol absolute (analytical reagent grade, Liquor distillery organization).

3.2 Growth rate measurement

The microorganisms used UASB granules that were collected from an up-flow anaerobic sludge blanket (UASB) system of cho-heng rice vermicelli industry in Nakhon Pathom, Thailand. These microorganisms were used as an inoculum in terms of mixed culture. The media was used modified lysogeny broth (MLB) and synthetic wastewater which was purged with oxygen-free N_2 for 15 minutes and was then sterilized using autoclave (at 121°C, 15 minutes). The microorganisms were cleaned with PBS solution for 5 times and then homogenized with homogenizer before use as inoculum. 10 grams of inoculum were cultivated in 100 mL of MLB media. All experiments performed under anaerobic conditions. The cultivation temperature was varied at 25, 30, and 35 °C for 0-96 hours in two different agitation systems: (1) turbulence shaking, 500 rpm and (2) horizontal shaking, 130 rpm. At the end up of cultivation time, microorganisms were separated from the MLB media using a centrifuge at 2,000 rpm for 30 minutes. Then, pull out the media and was pulled out microorganisms cleaned with PBS solution. After that, it was centrifuge again at 2,000 rpm for 15 minutes. Finally, microorganisms were died at 105°C using a vacuum oven for 24 hours. The dry weight of microorganisms was determined to

evaluate the growth rate of the microorganisms by recording the weight increase of the microorganisms as Y axis and the cultivation time as X axis.

3.3 Immobilized cell on the electrodes

Granular activated carbon (GAC) 2.5 grams, stainless steel gauze type 316 (20 mesh plain-woven from 0.28 mm dia wire) and stainless steel gauze type 304 (20 mesh plain-woven from 0.30mm dia wire) in size 10×40 mm. and graphite in size 20×6 mm. were used as electrodes for immobilization.

First, cultivate microorganisms at optimal condition was obtained from previous experiment. The selected cultivation times were used to cultivated microorganisms.

(1) 0 hour: microorganisms that has not been cultivated.
(2) 3 hours: middle of log phase.
(3) 6 hours: start of stationary phase.
(4) 24 hours: middle of stationary phase.
(5) 72 and 96 hours: death phase.

At the end of cultivation time, electrodes were immersed in the microorganisms for 7 days for immobilization. Finally, the samples were taken to analyze with electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM).

3.4 Morphology of microorganisms

Scanning electron microscopy (SEM) was used to investigate morphology of microorganisms. To prepare the sample for analysis, clean the samples (immobilized-SS, immobilized-GAC and immobilized-graphite) by rinsing with PBS solution pull up and down around the samples 5 minutes/time, 3 times and fixed cell with 4% paraformaldehyde at 4°C, 24 hours. After that, dehydrate the cell with ethanol 10, 30, 50, 70 and 100% respectively, 5 minutes/time for 3 times. Finally, dried the samples until the moisture was removed using oven at 35°C and coated with gold for 30 seconds before analyze is using SEM Model HITACHI SU5000 operated at beam energy of 15.0 kV.

3.5 Electrochemical activity measurement

3.5.1 Cyclic voltammetry (CV): electrochemical system and the way microorganisms transfer electrons were investigated by electrochemical technique using three different electrolyte solutions: microbe suspended MLB media, microbe-suspended PBS media and used MLB media without microbe. Glassy carbon, platinum, and Ag/AgCl electrode were used as working, counter and reference electrode in an electrochemical cell, respectively. The samples were purged with the oxygen-free N_2 during the measurement. All experiments were performed at the scanning rate of 0.1 V/s with a potential range from -1 to 1 V.

3.5.2 Electrochemical impedance spectroscopy (EIS): was used to investigated the resistance of electrodes. The samples were performed with an autolab potentiostat (model PGSTAT 204) using the method of FRA impedance potentiostatic at the open circuit voltage with amplitude of 0.01 V and a frequency range of 1×10^6 Hz to 0.01 Hz for SS, and a frequency range of 1×10^5 Hz to 10 Hz for GAC and graphite.

3.6 Open circuit voltage measurement

The maximum voltage can be obtained by open circuit voltage (OCV) of double chambered microbial fuel. OCV was operated without the presence of current. It demonstrated the electrons motive force of MFC. The media (fuel) was MLB and operated at 35°C. Electrodes of anode and cathode were the same type but not the same size, For anode; SS 304 (20 mesh plain-woven from 0.30mm dia wire) in size 14.15×8.7 mm. and GAC 60 g. For cathode; SS 304 (20 mesh plain-woven from 0.30mm dia wire) in size 14.15×5.0 mm. and GAC 30 g. Three cultivation times were selected to use as inoculum in the MFC including

(1) 0 hour: microorganisms that has not been cultivated.
(2) 3 hours: middle of log phase.
(3) 24 hours: middle of stationary phase.

Chapter 4

Results and Discussions

4.1 Growth rate of microorganisms

The cultivation conditions for the growth of microorganism

(1) Temperatures at 25, 30 and 35 °C.

(2) Media was modified lysogeny broth (MLB).

(3) Agitation systems were horizontal (130 rpm) and turbulence shaking (500 rpm).

Growth curve of microorganisms is a graph that plot between dry weight of microorganisms that was increased from dry weight of microorganisms which has not been cultivated as Y axis and cultivation time as X axis. The results of growth rate measurement are shown in the graph below **(Figure 4.1)**.

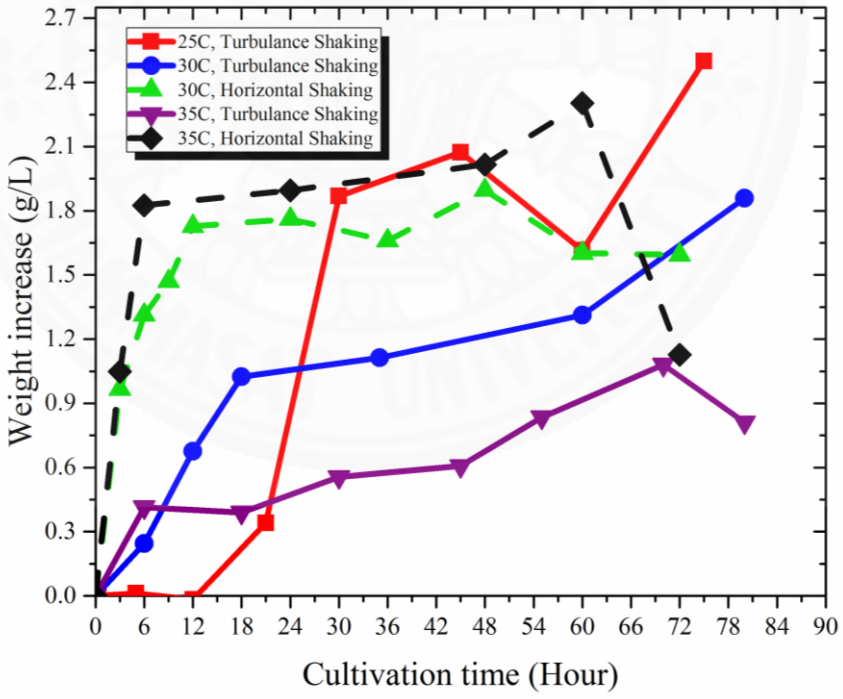

Figure 4.1 Weight increase of dry cell cultivated in different temperature (25, 30, and 35°C) and agitation systems: horizontal shaking (dash line) and turbulence shaking (solid line). The media is MLB.

The growth of bacterial populations could be classified to two terms, the first is the increase in the cell size and the second is the increase in the cell mass during the development of microorganisms. The growth rate of microorganisms in MLB at different temperature and agitation systems are shown in Figure 2. When considering the dependence of the weight increase with agitation systems, horizontal shaking (dash line) enabled the microorganisms to grow at the faster rate than that of turbulence shaking (solid line). The growth of microorganisms and also the production of metabolic were decreased with increasing agitation speed to 150 and 200 rpm [23]. The reproduction process of microorganisms at 30 and 35°C with turbulence shaking may be intercepted by shear force caused by turbulence shaking around 500 rpm.

Moreover, cultivation temperature is another key parameter that influences the growth of microorganisms. Microorganisms cultivated at 30°C (green line) underwent the log phase within 12 hours with the weight increase of around 1.72 g/L while the microorganisms cultivated at 35°C (purple line) reached the log phase within 6 hours with the weight increase of around 1.82 g/L and reached stationary phase because of the toxic compound are accumulated and the nutrient are used up resulting in the cells stop dividing completely till 60 hours before entering the dead phase. Microorganisms that inoculated at 35°C approach to log phase more quickly than microorganisms that inoculated at 30°C. The cells are dividing regularly by binary fission and the population increase exponentially lead to the dividing rate of the cells at 35°C are faster than at 30°C. It can demonstrate that the temperature at 35°C is suitable for the growth of the cell. This was consistent with the optimal cultivating temperature for anaerobic digestion usually occurring at 37.7°C [14].

Therefore the optimal cultivation conditions were at the temperature of 35°C with horizontal shaking. As mentioned before, it was assumed that the electrochemical activity of microorganisms could be different at different growth phases. The interested cultivation times were selected including t=3 hours (i.e. middle of log phase), t=6 hours (i.e. end of log phase), t=9, 12 hours (i.e. start of stationary phase), t=24 hours (i.e. middle of stationary phase) and t =72, 96 hours (i.e. death phase) as detailed in the next section.

4.2 Electron transfers in microorganisms

In theory, microorganisms can directly transfer electrons in electrochemical system. Such microorganisms are so-called electrode-oxidizing bacteria or electrochemically active bacteria, similar to iron-oxidizing, sulfur-oxidizing or methane-oxidizing microbes [24].

The electron transfer mechanism of microorganisms in different growth phases could be classified as DET or IDET which in this work was investigated by cyclic voltammetry. In IDET, microorganisms excrete soluble redox-active mediators into the media to shuttle electrons from the electron transport chain to the insoluble electron acceptor. In contrast, bacteria cell demonstrated the direct electron transfer (i.e. DET) through the direct contacts using redox outer membrane proteins or putatively conductive nanowire. The combination between IDET and DET could possibly occur at the same time.

At 0 hour **(Figure 4.2)**, the CVs of fresh MLB media were similar to that of microbe-suspended MLB media at which the oxidation and reduction peaks appeared at –0.25 and 0.25 V respectively.

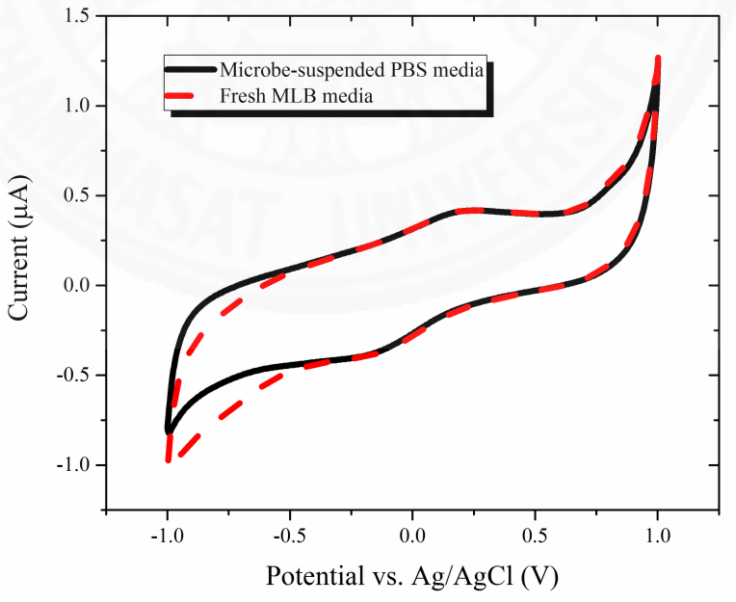

Figure 4.2 Cyclic voltammograms of microbe-suspended MLB media (black line) and fresh MLB media (red line) at t=0 hour (has not been cultivated) using the scanning rate of 0.05 V/s.

At 3 hours cultivation time (**Figure 4.3A**), electrochemical activity of the used MLB media without microbe was slightly different from microbe-suspended PBS media. It can be implied that the microorganisms cultivated at 3 hours were at a transition state to transform the types of cell growth from an increase in cell size to cell mass, leading to a few excretion of redox components. This means electrons that transfer in this state come from two pathway; the organ of microorganisms as the microbe-suspended PBS media and the mediator that cell was excreted as used MLB media without microbe. Consequently, both IDET and DET possibly occurred in this state.

Furthermore, cyclic voltammograms (**Figure 4.3A-4.4D**) showed that the microbe-suspended MLB media (green line) provided the highest magnitude of current compared to the microbe-suspended PBS media (red line) or the used MLB media without microbe (blue line) in all cultivation times. Interestingly, electrochemical activities of the microbe-suspended MLB media were much higher than that of microbe-suspended PBS media and used MLB media without microbe especially at 6, 9 and 12 hours of cultivation time. This indicated that the electron transfer at stationary state was mainly relied on IDET using mediators.

In **Figure 4.4**, the higher current response with increasing cultivation period from 6 to 12 hours verified that the microorganisms were highly electrochemically active and also excreted large amount of soluble redox species that can undergo electrochemical activity in the stationary state. From the best of our knowledge, the redox compounds produced from the microorganisms can be quinones, hydroquinones, pyocyanine, and phenazine-1-carboxamide [25]. These may act as endogenous redox mediators to help transfer electrons to and from the electrode surface.

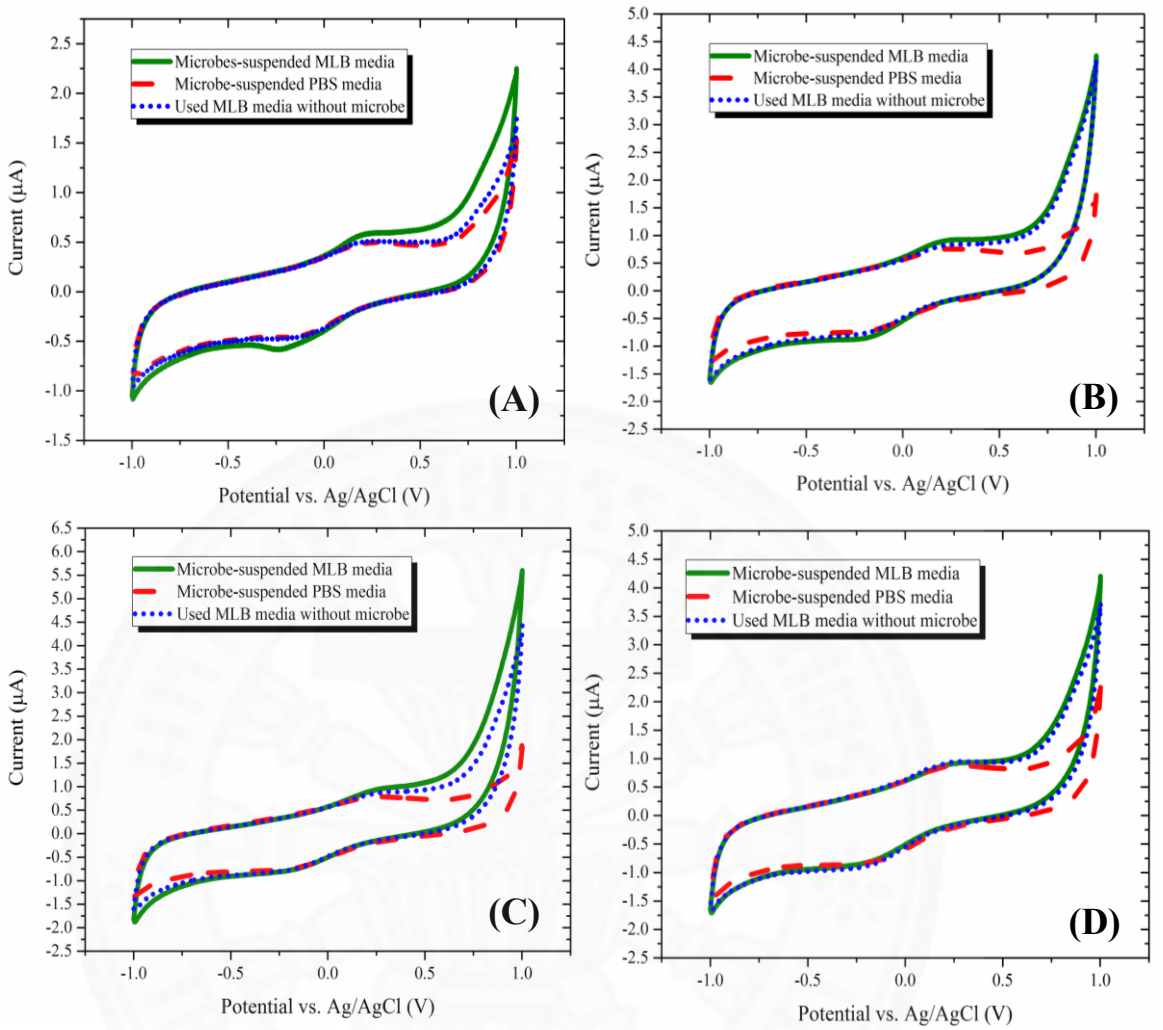

Figure 4.3 Cyclic voltammetric measurements of microbe-suspended MLB media (green line), microbe-suspended PBS media (red line), used MLB media without microbe (blue line) at 3, 6, 9, and 12 hours (A to D respectively). At scanning rate 0.1 V/s.

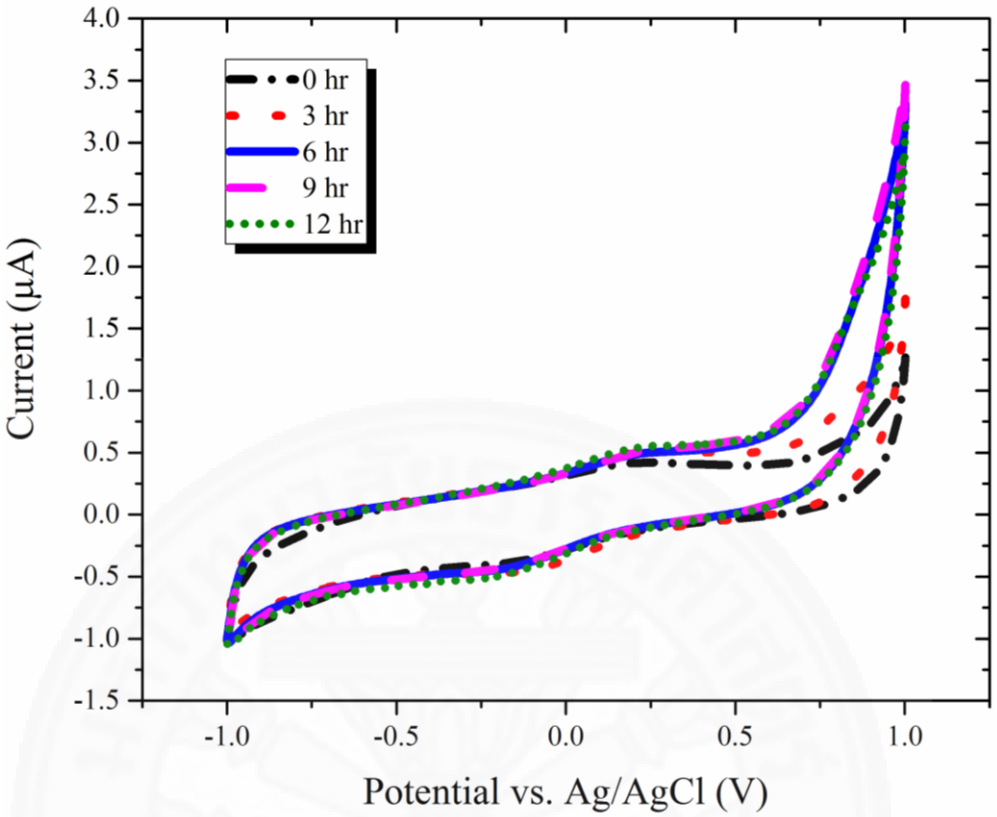

Figure 4.4 Cyclic voltammetric measurements of used MLB media without microbe at 0 hours (black line), 3 hours (red line), 6 hours (blue line), 9 hours (pink line), and 12 hours (green line). Scanning rate 0.1 V/s.

4.3 The immobilization of microorganisms on electrodes

Microbial biofilms consist of bacteria cells that attached to a surface by excreted the solution that was polysaccharides. These polysaccharides form a sticky, protective around the cells. This biofilm can remain a favorable surface and allow microbes to live in close association with others to advocate genetic exchange and cell-cell communication.

Microorganisms that has not been cultivated (0 hour) and cultivated at 3 hours (i.e. middle of the log phase), 6 hours (i.e. start of the stationary phase), 24 hours (i.e. middle of the stationary phase), 72 and 96 hours (i.e. the death phase) were selected to investigate. All microorganisms were attached on the electrodes after cultivation and immobilization for 7 days.

Electrode materials that were used in this study are;

(1) Stainless steel gauze type 316 (20 mesh plain-woven from 0.28 mm dia wire) and stainless steel gauze type 304 (20 mesh plain-woven from 0.30mm dia wire). It has low prices and provides the good electrochemical activity anti-corrosion and large surface area.

(2) Granular activated carbon (GAC) which is cheap and durable with a high surface area (1000 m^2/g at minimum), that help to improve microorganisms attach (adhesion force) on electrode in MFCs [5].

(3) Graphite is the most commonly used as anode in MFCs because it is a good electrical conductivity, good chemical stability, inexpensive and also easy to handle.

There have 7 types of bacteria according to their shapes; spherical (that called cocci), rod (that called bacilli), spiral (that called spirilla), filamentous, box-shaped or square-shaped, and appendaged and pleomorphic. They can live in single cells, in pairs, in chains, and in a big group [26]. They have many name of bacteria shape based on their arrangement. For the spherical shape, individual cell was called coccus, arrange in pairs was called diplococcus, arranged in chains was called streptococcus, arranged in group of four cells was called tetrads and arranged in big group with irregular cell in three plains was called staphylococcus. For the rod shape, individual cell was called bacillus, arrange in pairs was called diplobacillus, arranged in chains was called streptobacillus, the cells that stubby and short looks like both coccus and bacillus was called coccobacillus, the cells like a comma shape with twist was called vibrios and the rod shape that has flagella was called flagellate rods. For spiral shape, the cells have more than one twist in the cell, the cells that do not have and have sheath and endoflagella were called spirillum and spirochetes, respectively. For Filamentous bacteria, they have long thin filament. For Box-shaped or Square-shaped Bacteria, they are plain and flexible with 90° angles like a box. For appendaged bacteria, they have prolongation of the cells with long tubes or buds. For pleomorphic bacteria, these bacteria do not have identity shape, they can change to the other shapes [27].

In this study, It has 7 types of bacteria; coccus, staphylococcus, coccobacillus, bacillus, diplobacillus, flagella rods, and flagellate cocci.

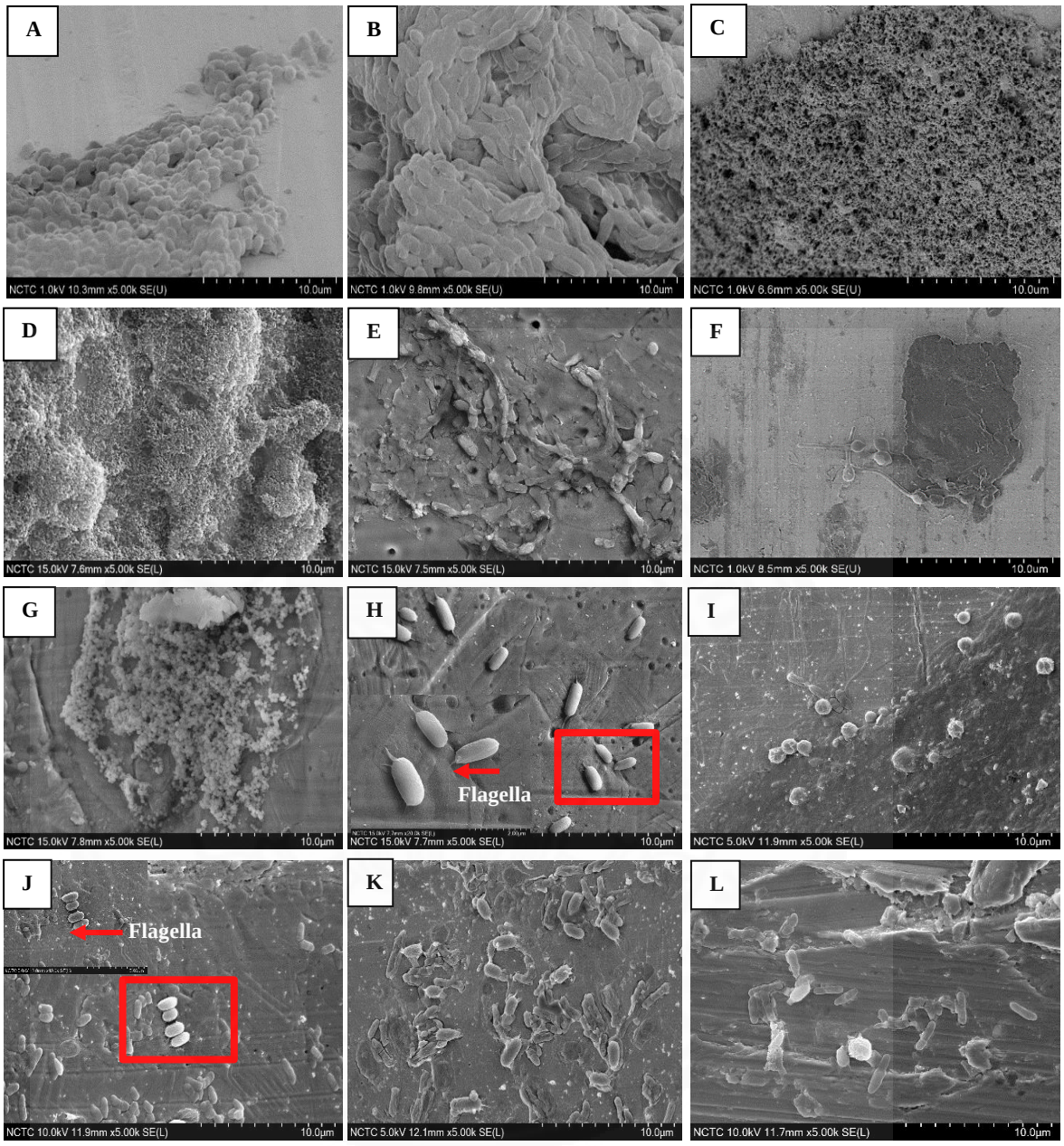

Figure 4.5 The shape of microorganisms in each cultivation time that formed biofilm and were attached on the surface of stainless steel at magnification 5,000x. At 0 hour: (A) and (B). At 3 hours: (C). At 6 hours; the (D), (E) and (F). At 24 hours: (G) and (H). At 72 hours: (I) and (J). At 96 hours: (K) and (L).

Microorganisms that attached on the surface of stainless steel in each cultivation time were quite different. The shape of microorganisms that has not been cultivated they were staphylococcus and coccobacillus (**Figure 4.5A-4.5B** respectively). And microorganisms that were cultivated for 3 hours they were very small coccus (in **Figure 4.5C**). For microorganisms that were cultivated for 6 hours, they were very small coccus, bacillus and the coccus that have long flagella look like clostridium tetani in **Figure 4.5D-4.5F** respectively. For microorganisms that were cultivated for 24 hours, they were small coccus (**Figure 4.5G**) and flagellate rods (**Figure 4.5H**). For microorganisms that were cultivated for 72 hours, they were coccus (**Figure 4.5I**) and flagellate rods (in **Figure 4.5J**). Finally, microorganisms that were cultivated for 96 hours, they were coccobacillus (in **Figure 4.5K**) and bacillus (in **Figure 4.5L**). The surface of stainless steel is shown in **Figure 4.6**.

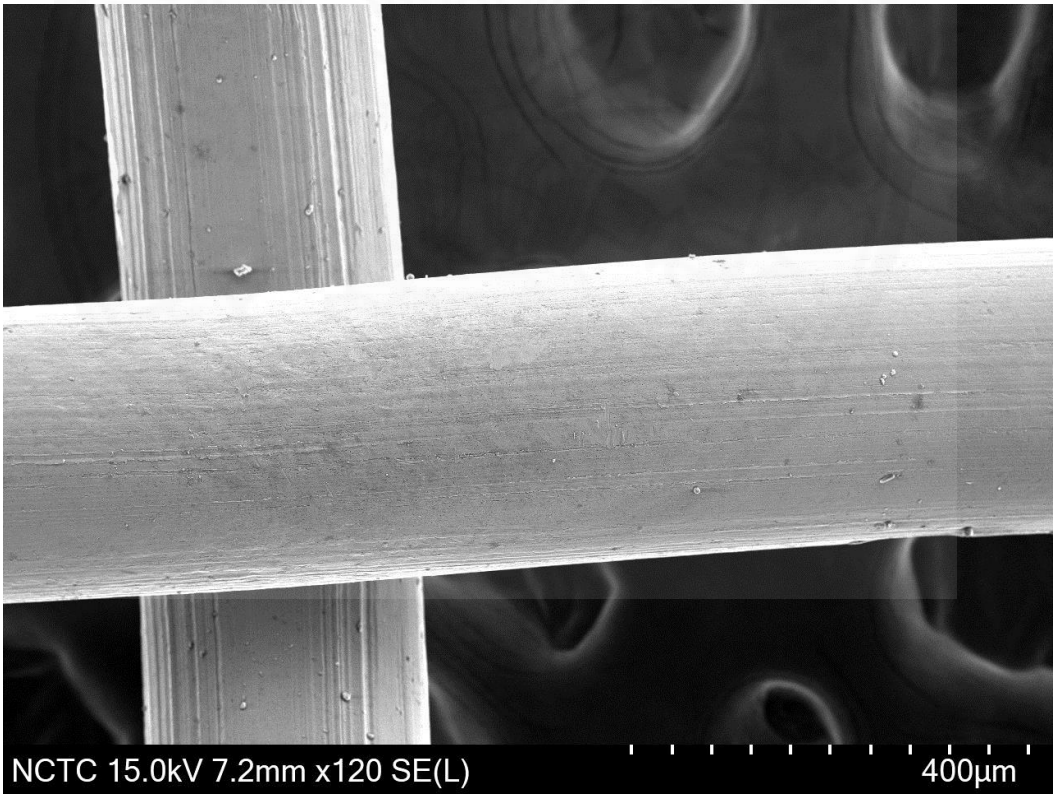

Figure 4.6 Surface area of stainless steel at magnification 120x.

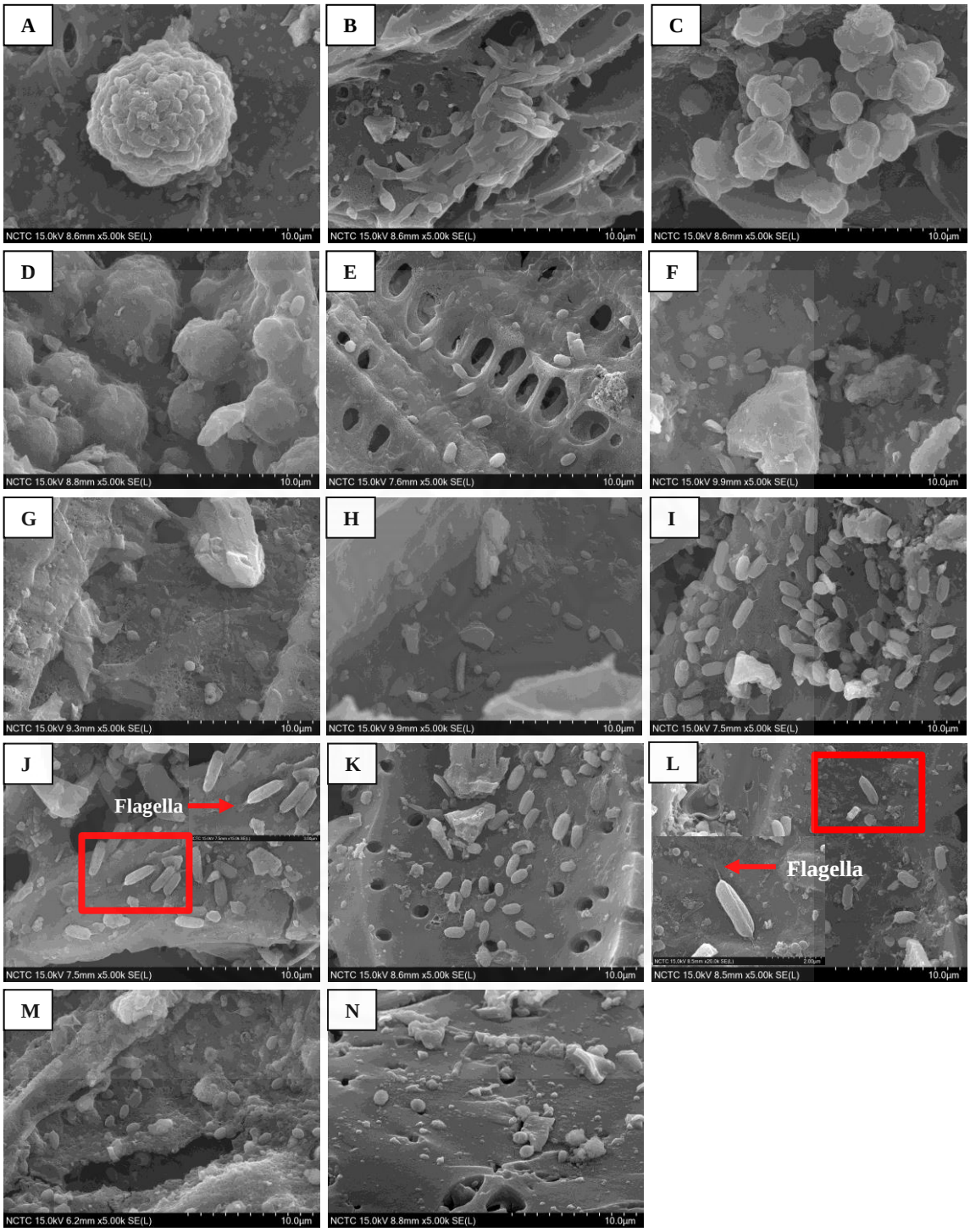

Figure 4.7 The shape of microorganisms in each cultivation time that formed biofilm and were attached on the surface of GAC at magnification 5,000x. At 0 hour: (A), (B) and (C). At 3 hours: (D) and (E). At 6 hours: (F), (G) and (H). At 24 hours: (I) and (J). At 72 hours: (K) and (L). At 96 hours: (M) and (N).

The morphology of microorganisms provides many kinds of shape but these shapes also occur in stainless steel. Microorganisms that attached on the surface of GAC in each cultivation time were small different. The shape of microorganisms that has not been cultivated they were coccobacillus form in a big group, coccobacillus, and coccus (**Figure 4.7A-4.7C** respectively). And microorganisms that were cultivated for 3 hours they were coccus and coccobacillus (in **Figure 4.7D and 4.7E**). For microorganisms that were cultivated for 6 hours, they were coccobacillus, coccus, and bacillus (**Figure 4.7F-4.7H** respectively). For microorganisms that were cultivated for 24 hours, they were bacillus and coccobacillus (**Figure 4.7I**) and flagellate rods (**Figure 4.7J**). For microorganisms that were cultivated for 72 hours, they were bacillus and coccobacillus (**Figure 4.8K**) and flagellate rods (**Figure 4.8L**). Finally, microorganisms that were cultivated for 96 hours, they were coccobacillus and coccus (**Figure 4.7M and 4.7N**). The surface area of GAC has many porous (**Figure 4.8**)

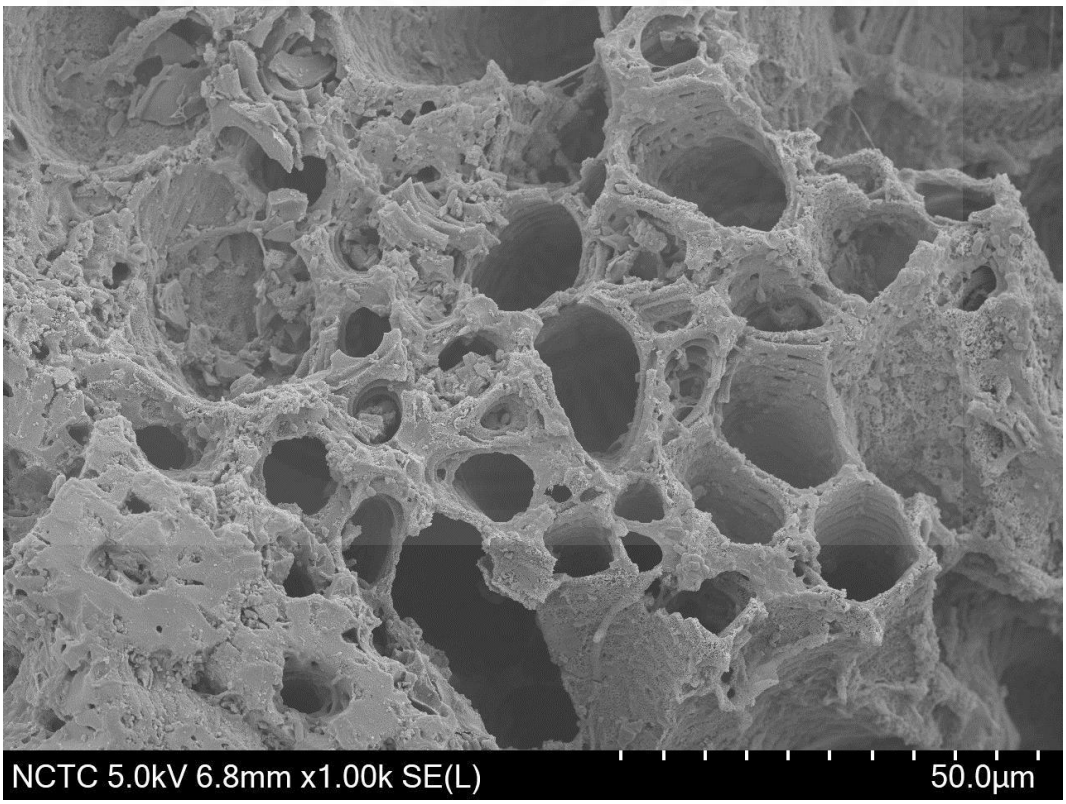

Figure 4.8 Surface area of GAC at magnification 1,000x.

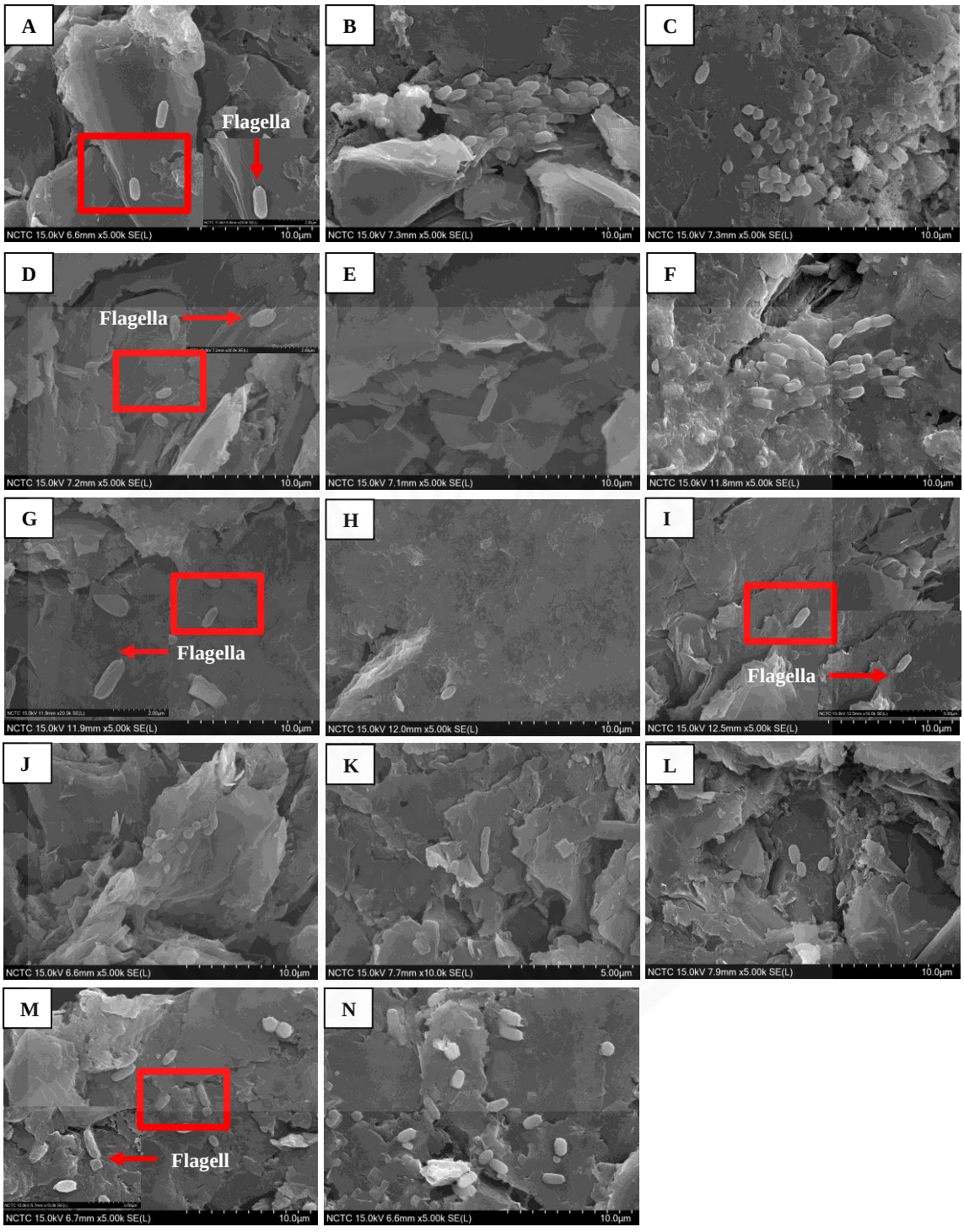

Figure 4.9 The shape of microorganisms in each cultivation time that formed biofilm and were attached on the surface of graphite at magnification 5,000x. At 0 hour: (A), (B) and (C). At 3 hours: (D) and (E). At 6 hours: (F) and (G). At 24 hours: (H), (I) and (J). At 72 hours: (K) and (L). At 96 hours: (M) and (N).

The morphology of microorganisms provides many kinds of shape but these shapes also occur in stainless steel and GAC. Microorganisms that attached on the surface of graphite in each cultivation time were small different. The shape of microorganisms that has not been cultivated they were flagellate rods, coccobacillus and clostridium tetani (**Figure 4.9A-4.9C** respectively). And microorganisms that were cultivated for 3 hours they were flagellate rods and bacillus (**Figure 4.9D and 4.9E**). For microorganisms that were cultivated for 6 hours, they were coccobacillus and flagellate rods in **Figure 4.9F-4.9G** respectively. For microorganisms that were cultivated for 24 hours, they were coccobacillus, flagellate rods, and clostridium tetani (in **Figure 4.9H and 4.9J**). For microorganisms that were cultivated for 72 hours, they were diplobacillus and coccobacillus (in **Figure 4.9K and 4.9L**). Finally, microorganisms that were cultivated for 96 hours, they were flagellate rods and coccobacillus (in **Figure 4.9M and 4.9N**). The surface area of graphite was rough (**Figure 4.10**).

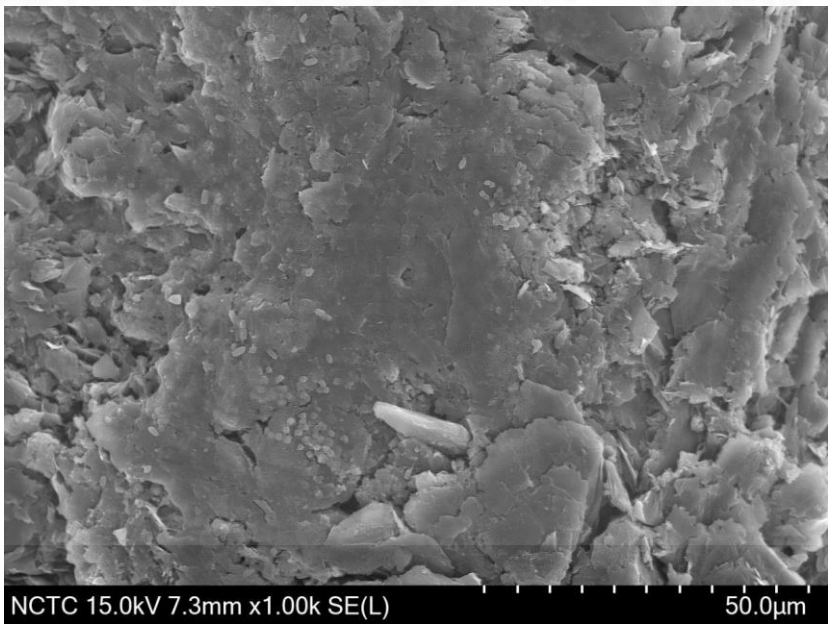

Figure 4.10 Surface area of graphite at magnification 1,000x.

Coccobacillus was found in cultivated 96 hours of all electrodes. This bacteria can survive in longer cultivation time and can grow in different surface of electrodes. This microorganisms are predominant of this cultivation times and may be the main microorganisms to degrades the substrate and form biofilm.

4.4 The resistance of electrodes

After the immobilization, the resistance of electrodes was examined by electrochemical impedance spectroscopy (EIS).

There are two graphs of EIS, (1) Nyquist plot and (2) Bode plot. The Nyquist plots the imaginary portion versus the real portion of impedance. Nyquist plots are useful in determining the resistances and characterizing the physical processes occurring in the system. Bode plots are used for representing all the three values i.e., impedance, phase angle, and frequency.

Figure 4.11 show Nyquist and Bode plots obtained from SS304 at different cultivation time of microbes-coated electrodes.

The magnitude of the Nyquist curve of cultivated 96 h/SS304 was smallest when compared to microbes-coated at the other cultivation times. In addition, in **Figure 4.12** the magnitude of the Nyquist curve of cultivated 96 h/SS316 also smallest when compared to microbes-coated at the other cultivation times.

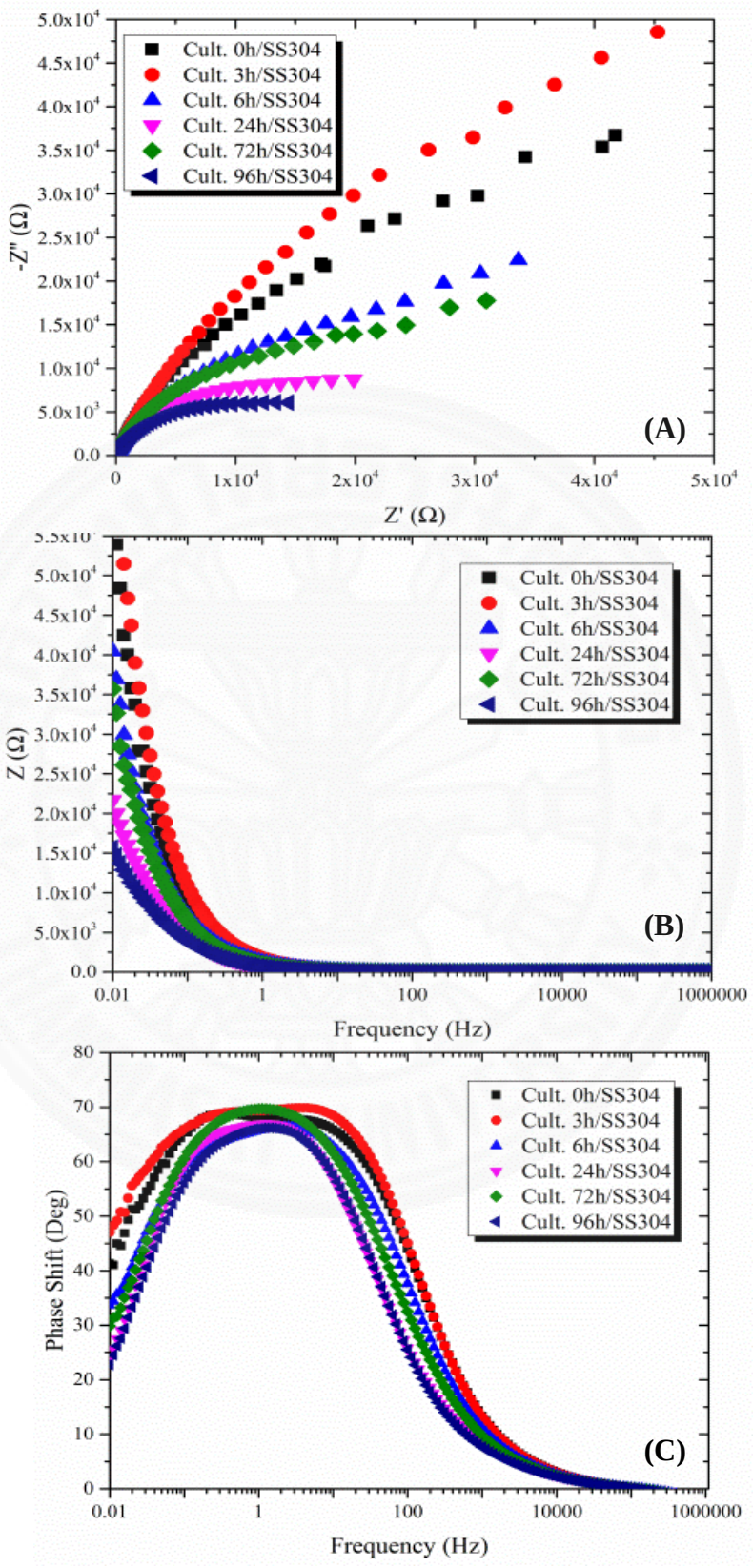

Figure 4.11 A: Nyquist, B: Bode and C: Bode-phase plots of cultivated/SS304 at different cultivation time.

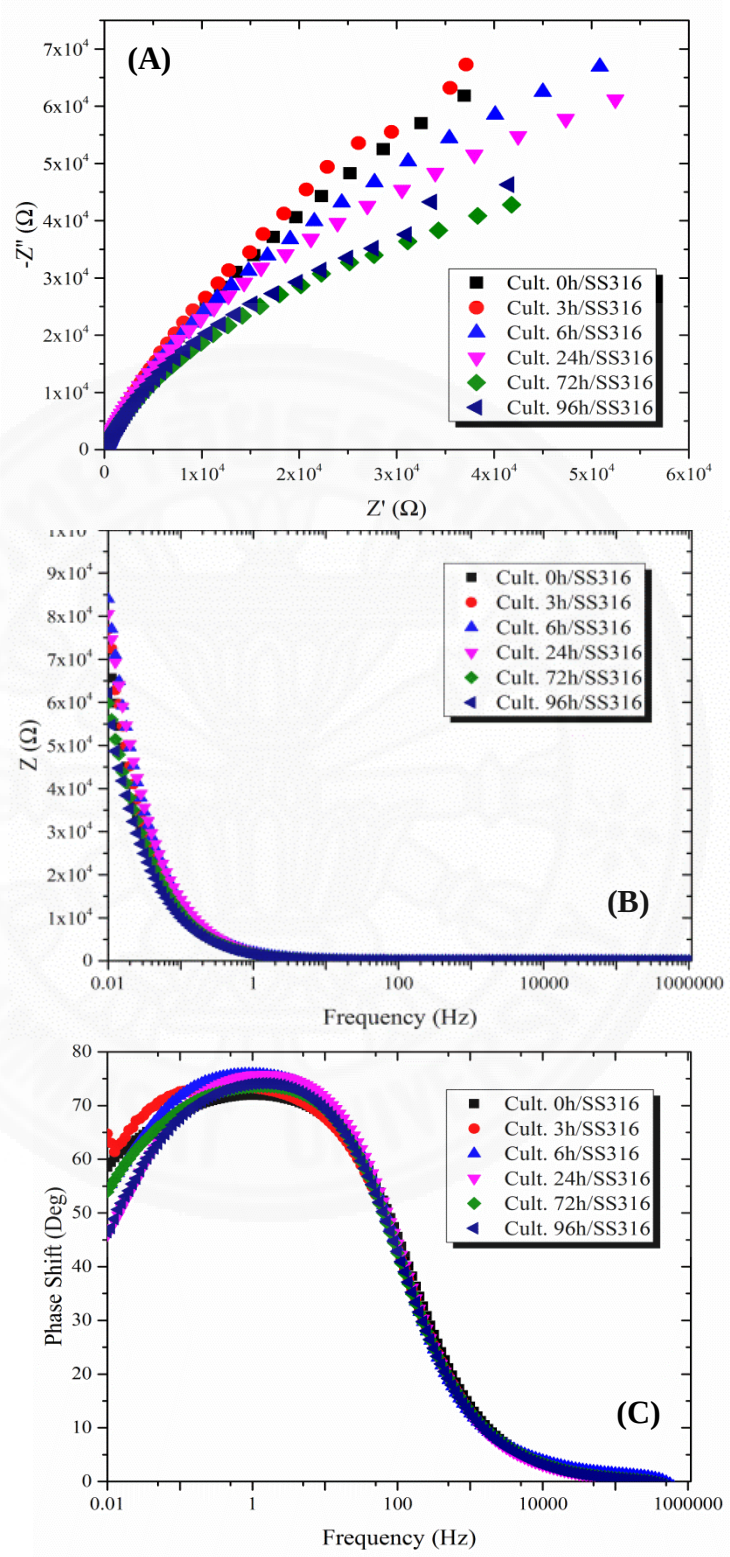

Figure 4.12 A: Nyquist, B: Bode and C: Bode-phase plots of cultivated/SS316 at different cultivation time.

The impedance data usually fit to an electrical equivalent circuit for estimating the resistance and capacitance are shown in **Figure 4.13**.

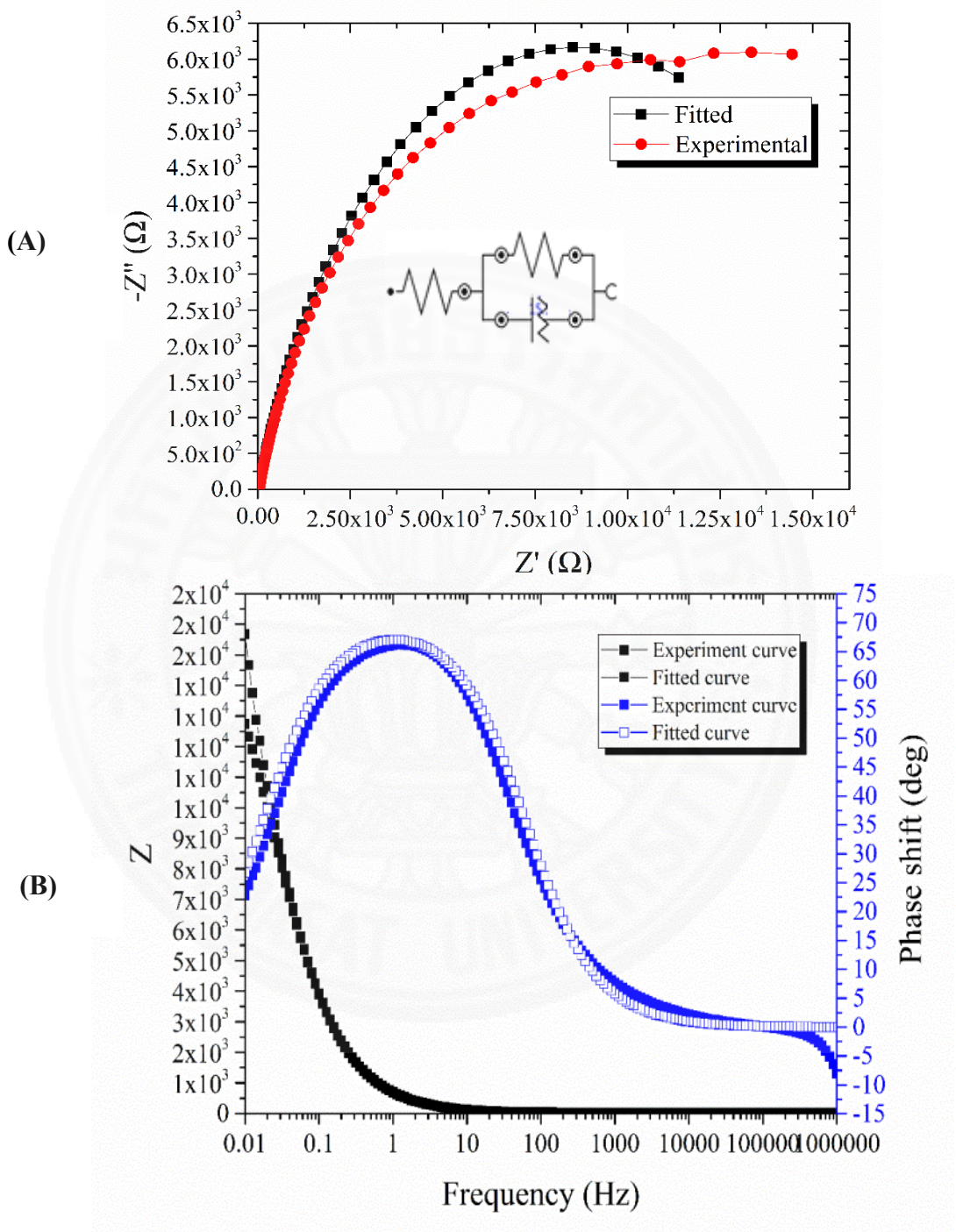

Figure 4.13. Overlaid experimental and fitted of A: Nyquist, B: Bode-phase plots with equivalent circuit of cultivated 96 h/SS304.

The resistance of stainless steel type 304 and 316 in different cultivation time are shown in **Figure 4.14**. The bar graph of cultivated 3 h/SS (red bar) that obtain microorganisms from the log phase, microorganisms in this phase were very actives and their metabolic activity was increased result in microorganisms reaches to the maximum growth rate. And the bar graph of cultivated 96 h/SS (navy bar) that obtain microorganisms from death phase, microorganisms in this phase completely lose its ability to reproduce but the value of resistance was lower than microorganisms in the log phase-coated SS. This result indicated that the immobilization time is key parameter that effects to microbes-coated SS in term of the formation of biofilm that excretes from microorganisms.

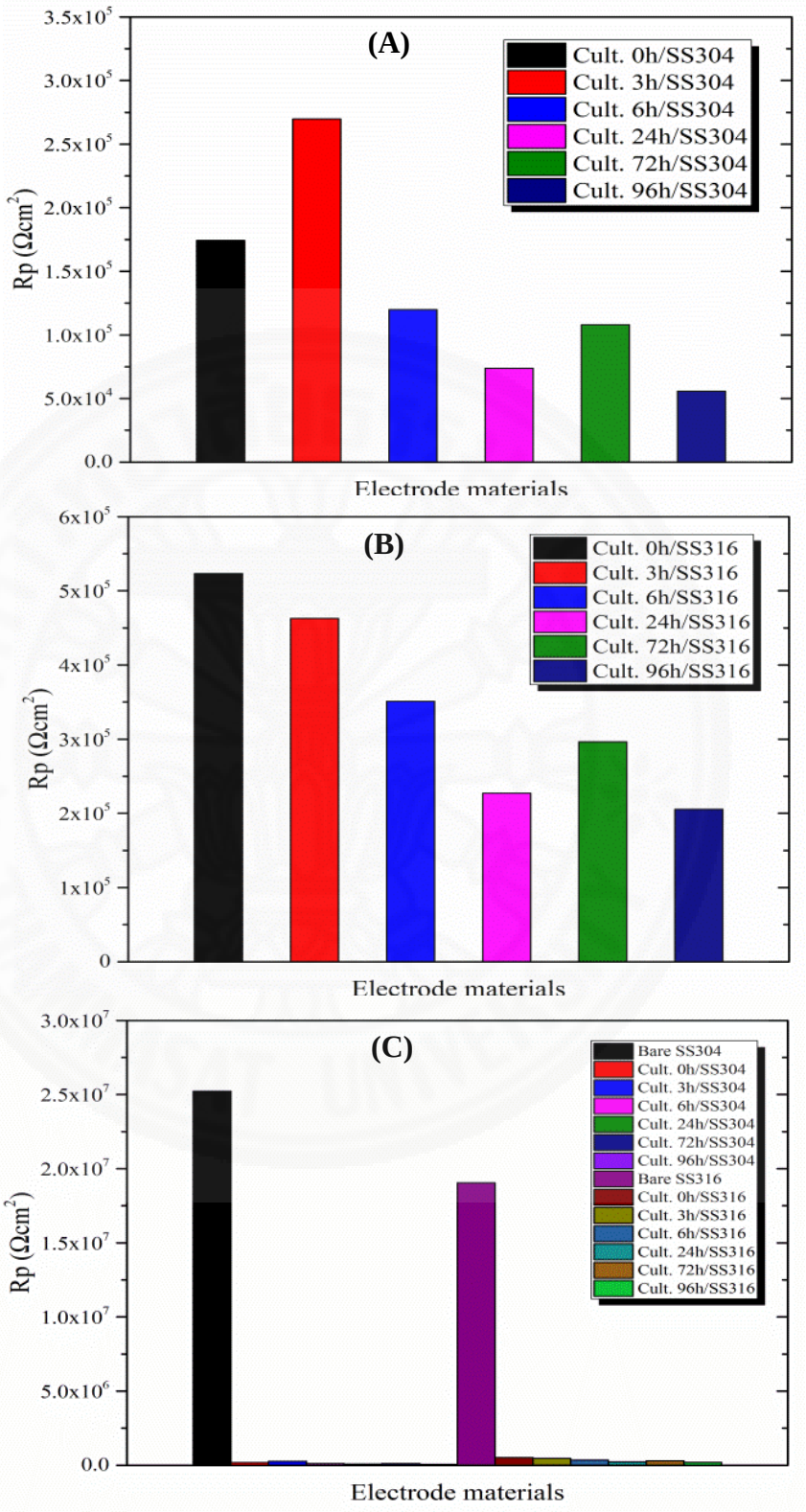

Figure 4.14 The electrode material resistance of A: Bare SS compared with cultivated SS, B: SS304 and C: SS316.

For the resistance of GAC, the magnitude of the Nyquist curve of cultivated 96 h/GAC was smallest when compared to microbes-coated at the other cultivation times are shown in **Figure 4.15**.

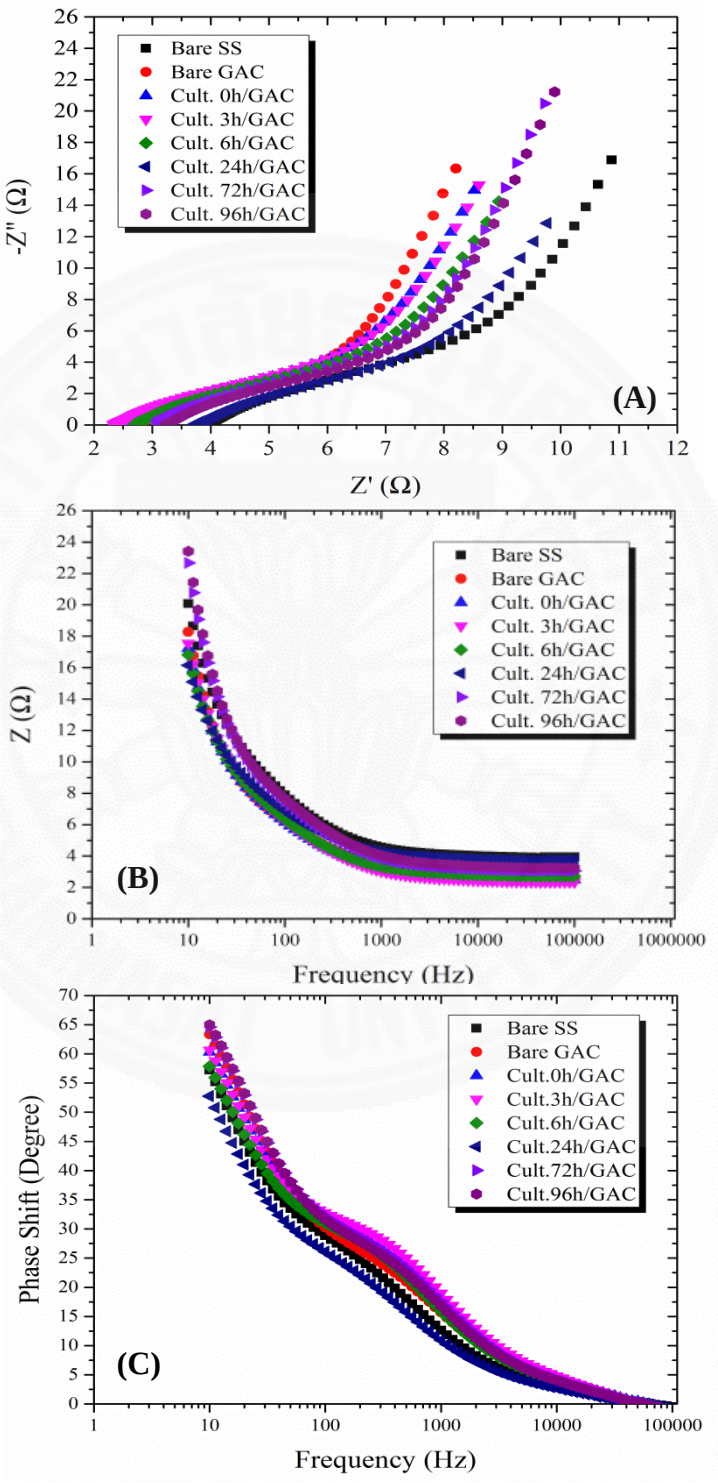

Figure 4.15 A: Nyquist, B: Bode and C: Bode-phase plots of cultivated/GAC at different cultivation time.

The impedance data usually fit to an electrical equivalent circuit for estimating the resistance and capacitance are shown in **Figure 4.16**.

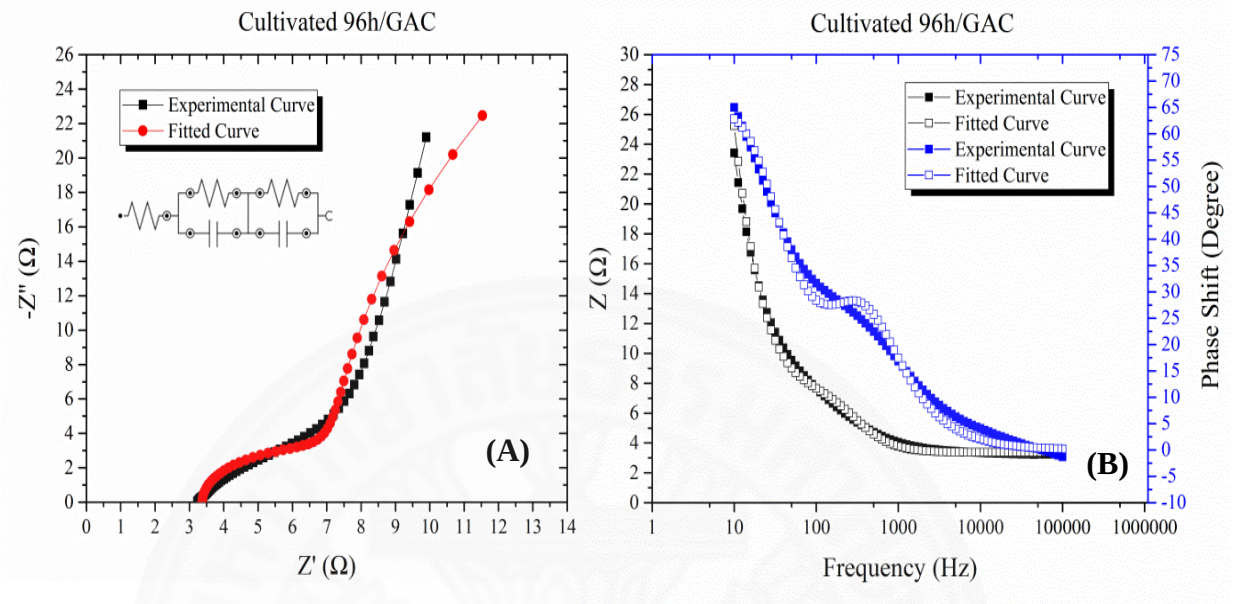

Figure 4.16 Overlaid experimental and fitted of A: Nyquist, B: Bode-phase plots with equivalent circuit of cultivated 96 h/GAC.

The resistance of cultivated/GAC in different cultivation time are shown in **Figure 4.17**. From the results, the resistance decreases when cultivation time was increase. The smallest resistance was 14.13 Ωcm^2 that obtained from cultivated 96 h/GAC.

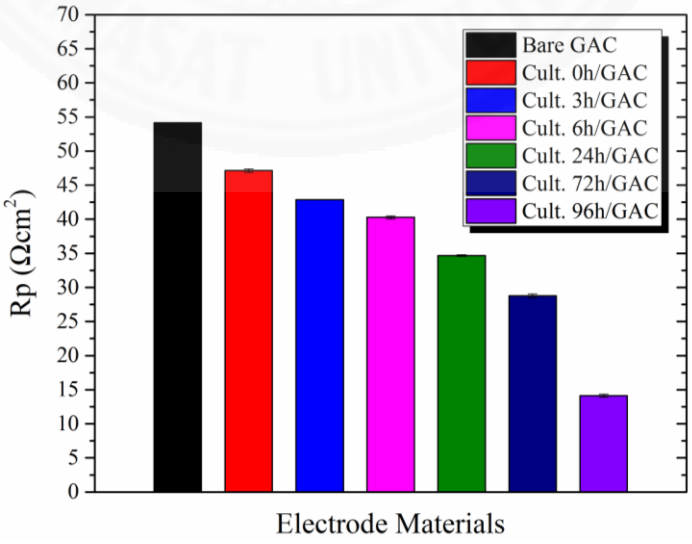

Figure 4.17 The electrode material resistance of GAC.

For the resistance of graphite. The magnitude of the Nyquist curve of cultivated 96 h/Graphite was smallest when compared to microbes-coated at the other cultivation times are shown in **Figure 4.18**.

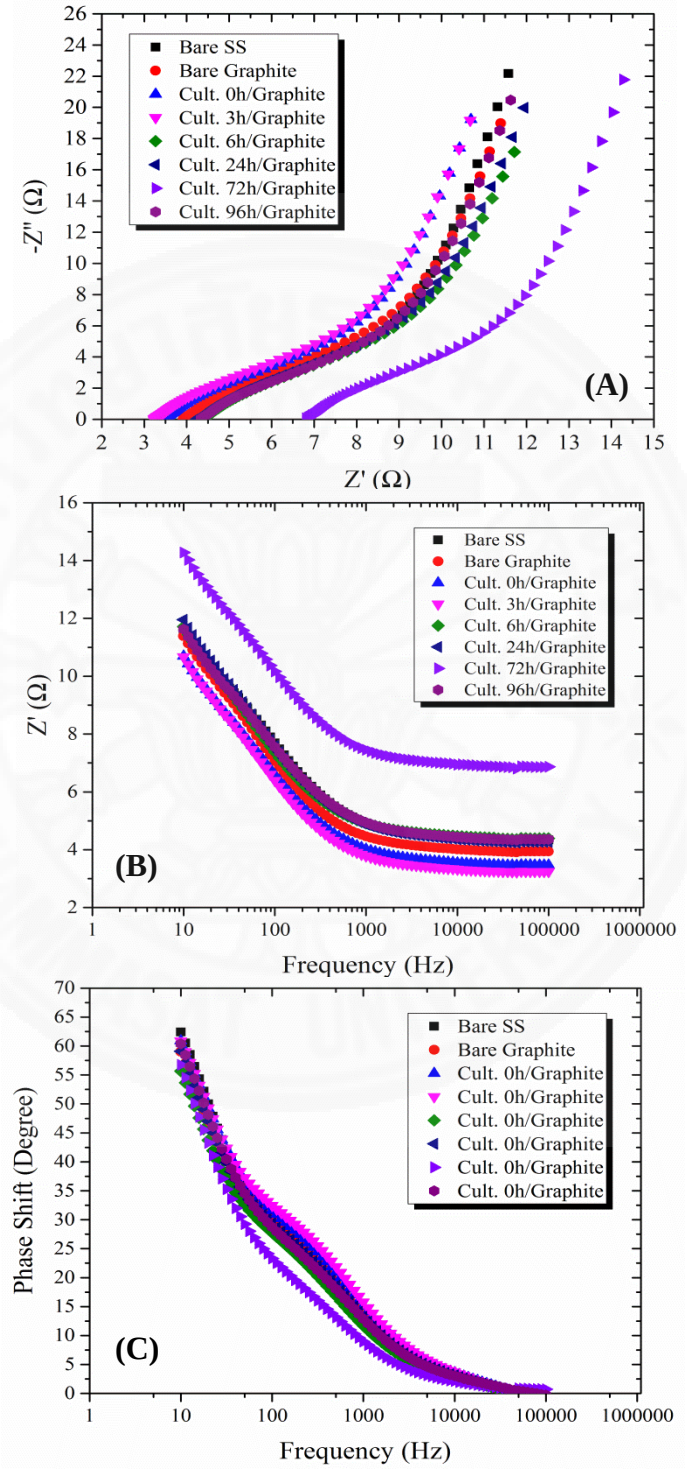

Figure 4.18 A: Nyquist, B: Bode and C: Bode-phase plots of cultivated/Graphite at different cultivation time.

The impedance data usually fit to an electrical equivalent circuit for estimating the resistance and capacitance are shown in **Figure 4.19**

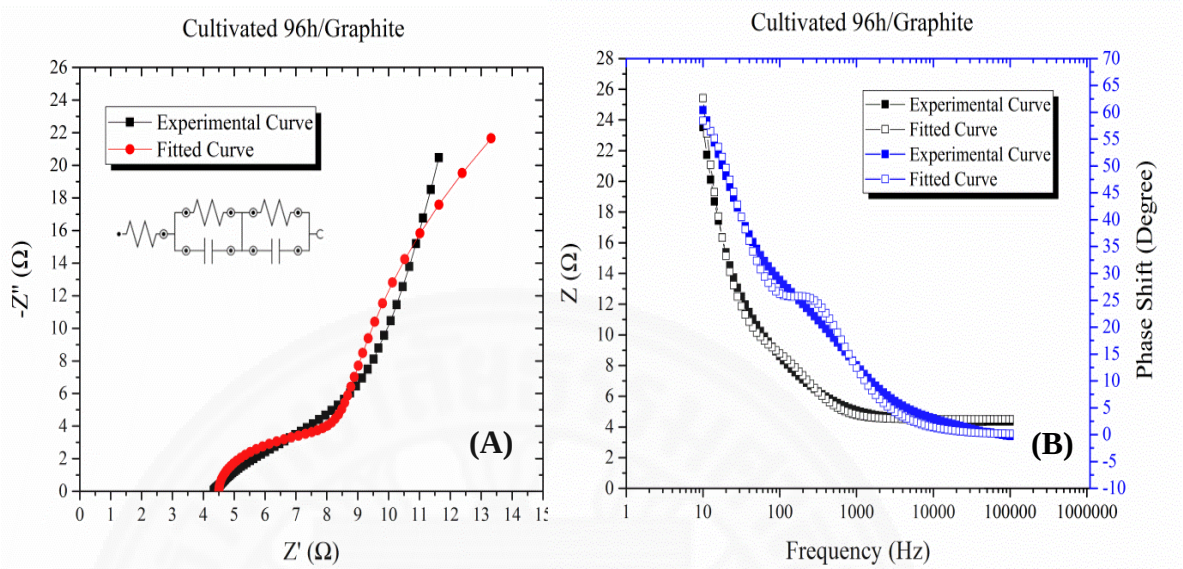

Figure 4.19 Overlaid experimental and fitted of A: Nyquist, B: Bode-phase plots with equivalent circuit of cultivated 96 h/Graphite.

The resistance of cultivated/graphite in different cultivation time are shown in **Figure 4.20**. From the results, the resistance decreases when cultivation time was increase. The smallest resistance was 17.79 Ωcm^2 that obtained from cultivated 96 h/Graphite.

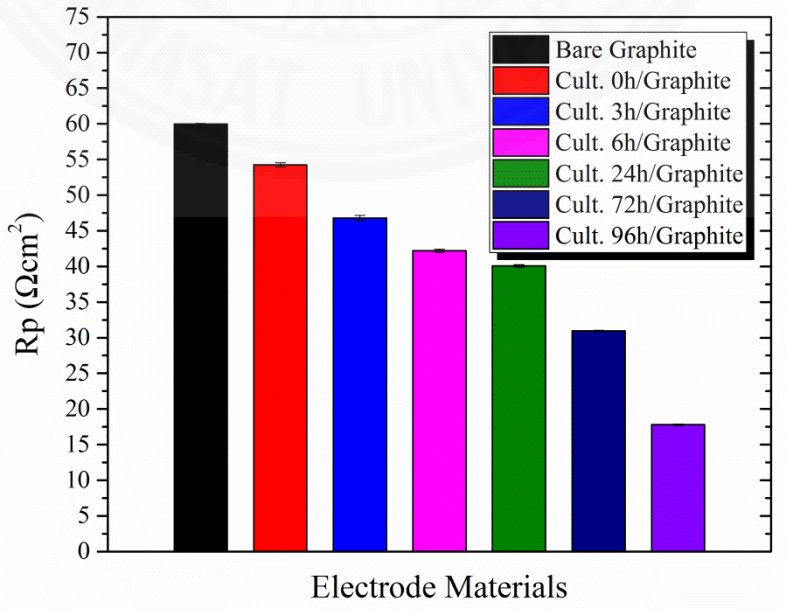

Figure 4.20 The electrode material resistance of graphite.

The resistance of whole materials in this study in **Table 4.1** shows that bare electrodes provide the highest resistance compared to other electrodes that have microorganisms attach. In addition, the results in each material indicated that cultivated 96h/Electrodes provide the smallest resistance. It can be concluded that the formation of microorganisms on the electrodes can reduce the resistance [10]. Because of the increasing of biofilm formation with the increasing of cultivation time

Table 4.1 The resistance of whole materials.

Electrode Materials	Resistance (Ωcm^2)		
	Experimental	Fitted	Resistance decrease from bare materials
Bare SS304	2.52E+07	1.93E+06	-
Cultivated 0h/SS304	1.74E+05	6.49E+04	2.50E+07
Cultivated 3h/SS304	2.70E+05	7.64E+04	2.49E+07
Cultivated 6h/SS304	1.20E+05	4.08E+04	2.51E+07
Cultivated 24h/SS304	7.38E+04	3.88E+04	2.51E+07
Cultivated 72h/SS304	1.08E+05	4.25E+04	2.51E+07
Cultivated 96h/SS304	5.57E+04	2.46E+04	2.51E+07
Bare SS316	1.91E+07	2.72E+06	-
Cultivated 0h/SS316	5.23E+05	9.37E+04	1.86E+07
Cultivated 3h/SS316	4.63E+05	1.22E+05	1.86E+07
Cultivated 6h/SS316	3.51E+05	1.26E+05	1.87E+07
Cultivated 24h/SS316	2.27E+05	1.00E+05	1.89E+07
Cultivated 72h/SS316	2.96E+05	8.11E+04	1.88E+07
Cultivated 96h/SS316	2.06E+05	8.10E+04	1.89E+07
Bare GAC	5.41E+01	3.38E+02	-
Cultivated 0h/GAC	4.71E+01	2.95E+02	7.00E+00
Cultivated 3h/GAC	4.29E+01	2.68E+02	1.13E+01
Cultivated 6h/GAC	4.03E+01	2.52E+02	1.38E+01
Cultivated 24h/GAC	3.47E+01	2.17E+02	1.95E+01
Cultivated 72h/GAC	2.88E+01	1.80E+02	2.53E+01
Cultivated 96h/GAC	1.41E+01	8.83E+01	4.00E+01
Bare Graphite	6.00E+01	1.44E+02	-
Cultivated 0h/Graphite	5.42E+01	1.30E+02	5.74E+00
Cultivated 3h/Graphite	4.68E+01	1.12E+02	1.32E+01
Cultivated 6h/Graphite	4.22E+01	1.01E+02	1.78E+01
Cultivated 24h/Graphite	4.01E+01	9.62E+01	1.99E+01
Cultivated 72h/Graphite	3.10E+01	7.43E+01	2.90E+01
Cultivated 96h/Graphite	1.78E+01	4.27E+01	4.22E+01

The position and the size of electrodes and the electrolyte solution that were used to analyze by EIS play role important to effect the resistance of electrodes [26]. The impedance results were a function of the reference electrode position [27]. In addition, different microorganisms that immobilized on the electrodes provides different magnitude of resistance. Therefore, it was difficult to compare the values of resistance to the other literature.

The position of stainless steel in this study is shown in **Figure 4.21**

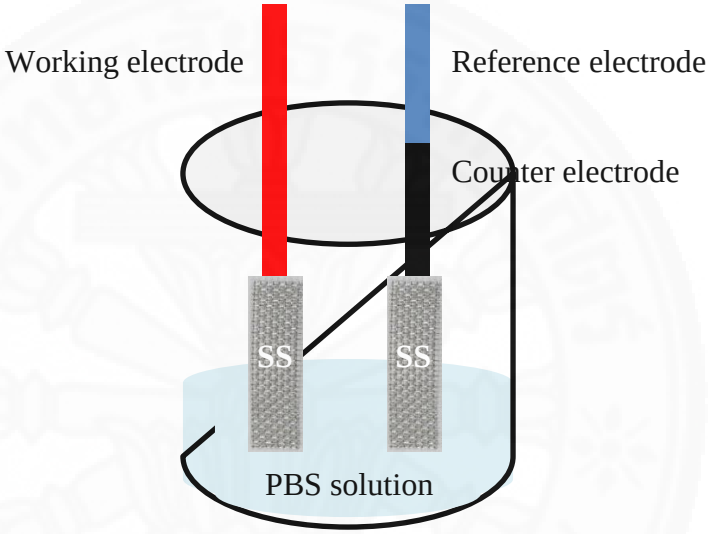

Figure 4.21 The position of stainless steel to analyze by EIS.

For SS304, the lowest value of resistance was 5.57×10^{4} Ωcm^{2} that obtain from cultivated 96 h/SS304. And the resistance decrease from bare SS304 ($R=2.52\times10^{7}$ Ωcm^{2}) around 2.51×10^{7} Ωcm^{2}.

For SS316, the lowest value of resistance was 2.06×10^{5} Ωcm^{2} from cultivated 96 h/SS316. And the resistance decrease from bare SS304 ($R=1.91\times10^{7}$ Ωcm^{2}) around 1.89×10^{7} Ωcm^{2}.

The position of GAC to analyze by EIS is shown in **Figure 4.22**. GAC in two sides was the same

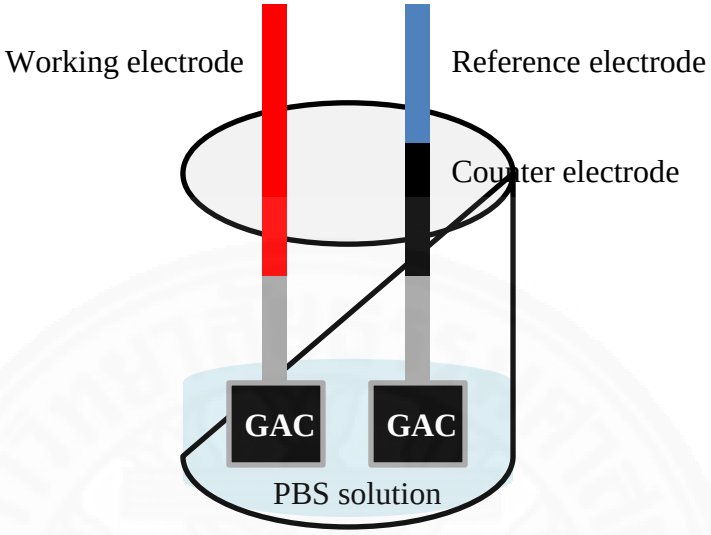

Figure 4.22 The position of GAC to analyze by EIS.

Cultivated 96h/GAC provides the lowest resistance around 14.1 Ωcm^2. And the resistance decrease from bare GAC (R=54.1 Ωcm^2) around 40 Ωcm^2.

The position of graphite to analyze by EIS is shown in **Figure 4.23**. Graphite in two sides was the same

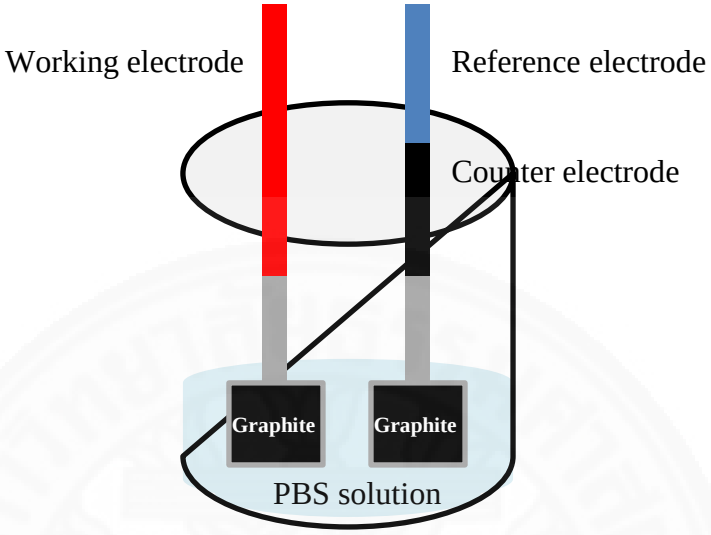

Figure 4.23 The position of graphite to analyze by EIS.

Cultivated 96h/Graphite provides the lowest resistance around 17.8 Ωcm^2. And the resistance decrease from bare GAC (R=60 Ωcm^2) around 42.2 Ωcm^2.

4.5 Open circuit voltage (OCV) of double chamber microbial fuel cell

In this study, stainless steel type 304 and GAC were used as electrode for both anode and cathode in double chamber microbial fuel cell (**Figure 4.24**) and operated under anaerobic condition at 35°C. The media (MLB) was adding when it was used up by microorganism. The voltage was increase when adding the media into the chamber. Data logger was used for record the voltage of the systems every 1 minute. Microorganisms that form biofilm on the electrode oxidized organic compound and transfer electrons to the cathode. Electrons that transfer was recorded in term of electrons motive force of OCV.

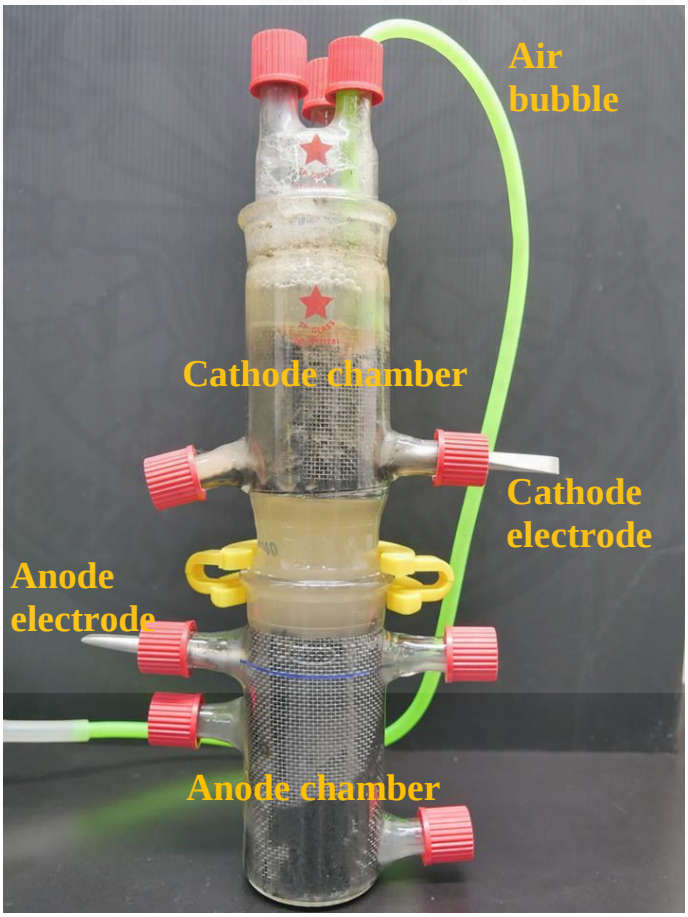

Figure 4.24 Double chamber microbial fuel cell.

The values of OCV are shown in **Figure 4.24**. The maximum voltage around 423.8 mV was obtained from double chamber microbial fuel cell with microorganisms that were cultivated for 24 hours. The maximum voltage of double chamber microbial fuel cell with microorganisms that has not been cultivated and cultivated for 6 hours were small different, the voltage was around 248.4 and 258.5 mV respectively, that was almost 2 times smaller when compared with microorganisms that were cultivated for 24 hours.

Anode material at open circuit affects bacterial communities that attach on surface as biofilm, while at closed circuit the growth of microorganisms was violated by the effect of a current flow [28]. The different communities that grow on the anode materials at open circuit were significant. This means the biofilm formation that was obtained from microorganisms that were cultivated in different cultivation time was also important. Double chamber microbial fuel cell with microorganisms that were cultivated for 24 hours provides the highest values compared to the other systems with smaller cultivation time. This result related to the previous experiment, the longer cultivation time provides the higher performance.

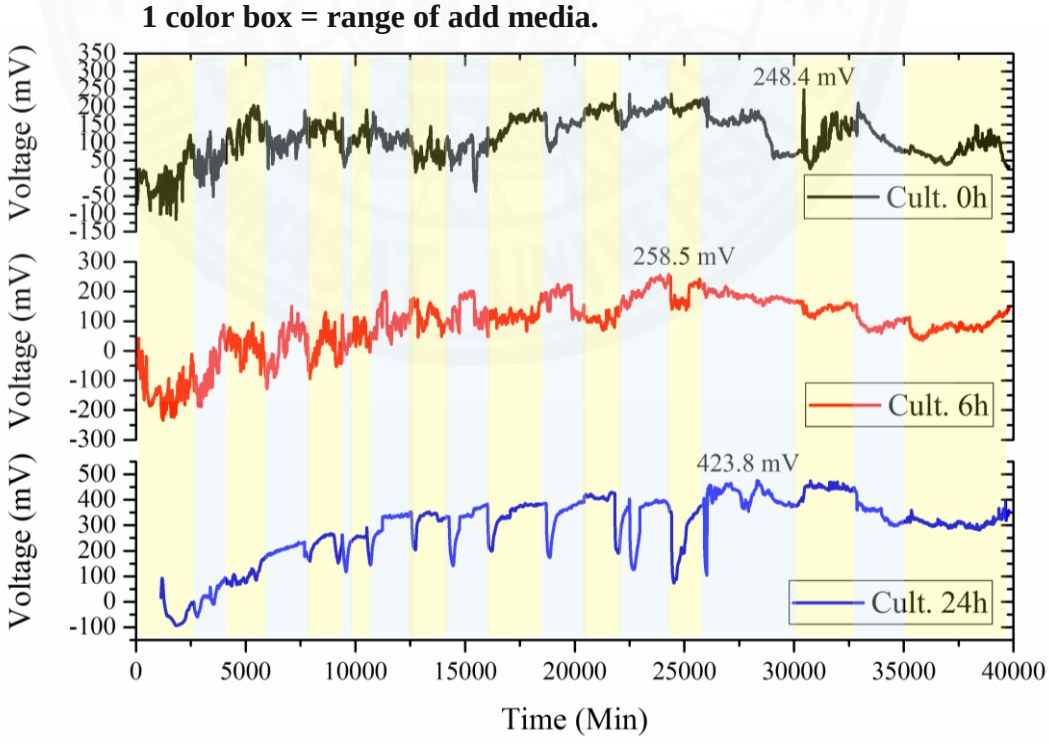

Figure 4.25 OCV of double chamber microbial fuel cell with three cultivation time: t=0h (black line), t=6h (red line), and t=24h (blue line).

Chapter 5
Conclusions and Recommendations

5.1 Conclusions

The optimal temperature for microbial cultivation was 35°C with horizontal. It was also found that in stationary phase microorganisms were highly active at longer cultivation time from 6 to 12 hours as the highest anodic current of the used MLB media without microbes was obtained when compared to the anodic current of microbe-suspended PBS media. This result indicated that the main process of electrons transfer occurring in MFC system was indirect electron transfer through endogenous or exogenous redox mediators.

For the immobilization, microorganisms are formed on the electrode surface in term of biofilm. This immobilization can reduce the resistance between electrons that transfer from microorganisms and electrode but the resistance was not affected by the ability of microorganisms in each phase. The longer cultivation time provides the lower resistance of electrode. The resistance of cultivated 96h/GAC provides the lowest resistance compared to other electrodes. Moreover, the resistance of cultivated/Graphite is small different from cultivated/GAC. But, the resistance of cultivated/SS provides the highest value. These result indicated that resistance decreases from the beginning as a result of the development of biofilm on the electrode surface. All these studies confirmed the fact that the resistance is greatly influenced by the microbial growing on the electrode surface as the biofilm.

Due to the attachment of microorganisms, there was many kinds of shapes such as coccus, staphylococcus, coccobacillus, bacillus, diplobacillus, flagellate rods, and flagellate cocci. These microorganisms excreted large amount of mediator which helps to transfer electrons that were related to the previous experiment that reports the main pathway to transfer electrons was IDET.

The double chamber microbial fuel cell with microorganisms that was cultivated for 24 hours provides the value of OCV around 400mV that was highest. This result related to all results in this study and it can conclude that the longer cultivation time leads to the higher performance of MFC.

5.2 Recommendations

5.2.1 This study is preliminary experiment to use microorganism from Choheng rice vermicelli industry. The other rice vermicelli industry may be applied.

5.2.2 The concentration of inoculum should be more than 5% of the media for the growth rate measurement.

5.2.3 The microorganisms may be investigated by DNA and RNA based methods to identified species of microorganism.

5.2.4 This study use SS and GAC as electrode in double chamber MFC, the combination of other electrodes may be applied in MFC for the investigation of OCV.

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Appendices

Appendix A

The immobilization of microorganisms

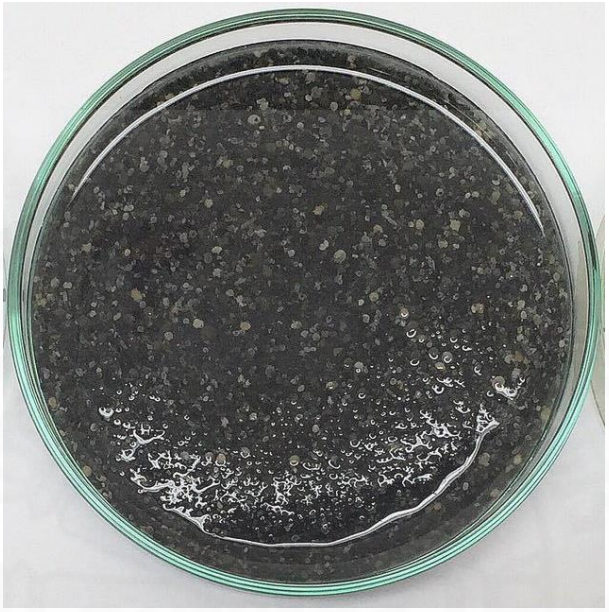

Figure A-1 UASB granule from Cho-heng rice vermicelli industry.

Figure A-2 Homogenized microorganisms with homogenizer.

Figure A-3 MLB media for the cultivation.

Figure A-4 Immobilized microorganisms in MLB media with electrodes (GAC) for 7 days.

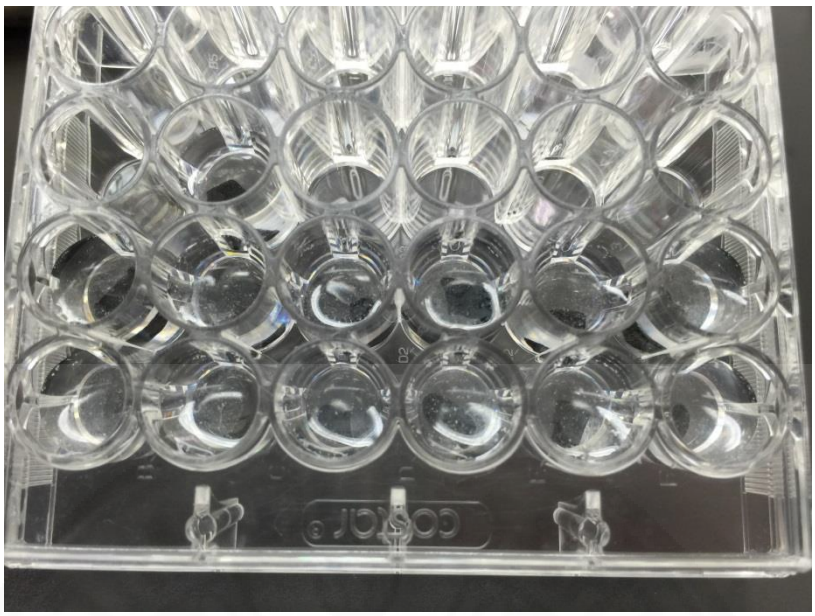

Figure A-5 Dehydrated the immobilized-GAC with ethanol (ethanol sequence) in the well.

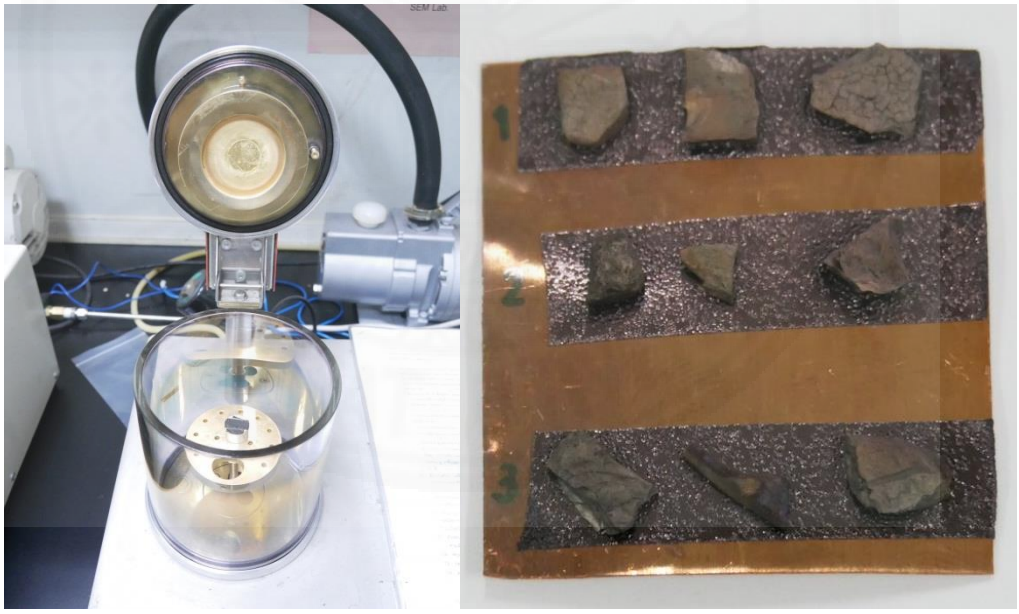

Figure A-6 Coated the immobilized-GAC with gold.