SELECTION OF BACTERIA FOR USE IN MICROBIAL FUEL CELLS BY ELECTRIC CURRENT

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ได้คัดเลือกแบคทีเรียด้วยกระแสไฟฟ้าค่าต่างๆ และนำมาทดสอบในเซลล์เชื้อเพลิงชีวภาพที่ปราศจากตัวนำพาอิเล็กตรอน แบคทีเรียที่คัดเลือกได้ทั้งหมด 72 ไอโซเลต คัดแยกมาจาก 3 แหล่งดังนี้ 28 ไอโซเลต แยกจากตะกอนใต้บ่อพักน้ำเสีย ด้านหน้าตึกฟิสิกส์ 1 จุฬาลงกรณ์มหาวิทยาลัย 40 ไอโซเลต คัดแยกจากตะกอนใต้ดินจากเกาะล้าน จังหวัดชลบุรี และ 4 ไอโซเลต จากดินที่อุทยานแห่งชาติภูเรือ จังหวัดเลย ทุกไอโซเลตถูกนำมาทดสอบความสามารถในการรีดิวซ์ไอออนของเหล็กภายใต้ภาวะที่ไม่มีอากาศ จากการทดลองเบื้องต้น พบว่าการใช้โมเดลอะคริลิกไม่เหมาะสมต่อการนำมาใช้ในงานระบบเซลล์เชื้อเพลิงชีวภาพที่ปราศจากตัวนำพาอิเล็กตรอน ต่อมาได้ออกแบบและประดิษฐ์ โมเดล Glass I และ Glass II ที่ทำให้ปราศจากเชื้อเพื่อใช้เลี้ยงจุลินทรีย์ เมื่อนำแบคทีเรียบริสุทธิ์ 40 ไอโซเลต แยกจากเกาะล้านมาทดสอบความสามารถในการส่งผ่านอิเล็กตรอนด้วยตัวเองในโมเดล Glass I ทำให้สามารถสรุปได้ว่าความสามารถในการรีดิวซ์ไอออนของเหล็ก เป็นปัจจัยที่สำคัญต่อการผลิตกระแสไฟฟ้าของแบคทีเรียในเซลล์เชื้อเพลิงชีวภาพมากกว่าความสามารถในการทนต่อกระแสไฟฟ้า ในโมเดล Glass I แบคทีเรียแกรมลบที่แยกจากเกาะล้าน 12 ไอโซเลต มีลักษณะโคโลนีแผ่ สามารถรีดิวซ์ไอออนของเหล็ก ให้ความหนาแน่นกระแสไฟฟ้า ประมาณ 11-13 มิลลิแอมแปร์ต่อตารางเมตร หลังจากนั้นเมื่อนำมาทดสอบในโมเดล Glass II พบว่า ไอโซเลต KL22 ให้ความหนาแน่นกระแสไฟฟ้าและความหนาแน่นกำลัง ไฟฟ้าสูงสุด เท่ากับ 18.57 มิลลิแอมแปร์ต่อตารางเมตรและ 0.62 มิลลิวัตต์ต่อตารางเมตร ตามลำดับ ซึ่งมีค่าเพิ่มขึ้นจากเดิมเมื่อเทียบกับค่าความหนาแน่นกระแสไฟฟ้าและความหนาแน่นกำลังไฟฟ้าสูงสุดที่ได้จากโมเดล Glass I เท่ากับ 67 และ 179 เปอร์เซ็นต์ ตามลำดับ จากผลดังกล่าวทำให้ทราบว่า ในภาวะที่ไม่มีอากาศ ช่วยทำให้ อิเล็กตรอนถ่ายโอนไปที่ขั้วไฟฟ้าได้ดีขึ้นส่งผลให้ได้ไฟฟ้าเพิ่มมากขึ้น เมื่อวิเคราะห์ลำดับของ16S rDNA และทดสอบด้วยชุด API 20E สามารถพิสูจน์เอกลักษณ์ของ ไอโซเลต KL22 เป็น *Proteus vulgaris* ผลการศึกษานี้เป็นรายงานแรกที่แสดงความสำเร็จของ *Proteus vulgaris* ในการคะตะไลซ์ในเซลล์เชื้อเพลิงชีวภาพที่ปราศจากตัวนำพาอิเล็กตรอนได้ อย่างไรก็ตาม ควรศึกษาหาภาวะที่เหมาะสมของพารามิเตอร์ทางกายภาพและทางเคมีที่มีผลต่อเซลล์เชื้อเพลิงชีวภาพ ทั้งนี้เพื่อความยั่งยืนของการพัฒนาพลังงานทางเลือกในอีกรูปแบบหนึ่ง

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KAMOL RODYOU : SELECTION OF BACTERIA FOR USE IN MICROBIAL FUEL CELLS BY ELECTRIC CURRENT. THESIS ADVISOR : ASSOC. PROF. SIRIRAT RENGPIPAT, Ph.D., THESIS CO-ADVISOR : ASSOC. PROF. MANA SRIYUDTHSAK, D.ENG., 82 pp.

Bacteria enriched under various electricity currents, were selected for tested in mediator-less microbial fuel cell. Total of 72 electricity enriched bacteria were isolated from three sources including, 28 isolates from sediment of pond in front of Physic I building, Chulalongkorn University, 40 and 4 isolates from sub-sediment from Koh Larn, Chonburi and soil from Phu Rua, Loei, respectively. Ferric reduction activity of all isolates under anaerobic condition was also characterized. Preliminary experiment found that acrylic model was not suitable for operating mediator-less MFC. Glass I and Glass II models were later designed and constructed for sterile system that suitable for microbiological aseptic techniques. Pure culture of 40 isolates of electricity enriched bacteria from Koh Larn were determined the ability of their self-mediate electron transfer in Glass I model. It can be concluded that ferric reduction activity has more impact than electricity current that used for selection and enrichment on the electricity generation of isolates in mediator-less MFC. In Glass I model, 12 isolates of Gram's negative, ferric reducing, swarming bacteria from Koh Larn gave high current density ~11-13 mA m^{-2}. After tested in Glass II, the highest of 18.57 mA m^{-2} and 0.62 mW m^{-2} for current density and power density, respectively were generated by KL22. These indicated that the increase of current density and power density more than those of Glass I, 67 % and 179%, respectively. As the results, anaerobic condition in anodic compartment enhanced electron transfer to anode electrode led to the increasing of electricity output. KL22 was identified as *Proteus vulgaris* by using 16S rDNA analysis and rapid identification kit API 20E. *Proteus vulgaris* can biocatalyse successfully in mediator-less MFC system from this study is firstly reported. Further improvements by optimizing the physical and chemical parameters of microbial fuel cells for the sustainable alternative energy in the future are required.

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CHAPTER I

INTRODUCTION

Hydrogen fuel cell, an electrochemical energy conversion device is believed to be an alternative and renewable energy source for sustainable future energy needs. However, hydrogen production through either electro-catalysis requires electrical energy to produce, or fermentation process which latter has only 15% hydrogen recovery [1] from biomass. Hence, the effectiveness of overall process has to be considered by cost and energy balance. In recent years, microbial fuel cell (MFC) is a new fuel cell technology that quickly attracted the attention of several researchers because it can directly convert biomass into electricity by catalytic activity of microorganisms [2]. Microbes play an important role in this system that it uses biomass as an electron donor in their catabolic pathway and transfers electrons to the electrode. But the direct activity of microbe's electron transfer usually occurred in very low efficiency when using *Escherichia coli* [2], *Saccharomyces cerevisiae* [2, 3] and other microorganisms [4] that lack of ability to facilitate electron transfer to the anode electrode. The electron transfer of the microbes in MFC can be enhanced by electron mediators [5], redox compounds, which have an electrochemical activity and act as an electron shuttle between the microbes and the anode electrode. The use of mediators such as neutral red have an advantage in electron transport of *E. coli* that resulted in 10-fold increasing of electricity current [6]. On the other hand, the continuous addition of the artificial mediators adds expensive cost and the ones are used in previous studies are toxic to human and could not be released into the environment without responsibility.

Mediator-less microbial fuel cell [7], the artificial mediator free system was proposed by Kim *et al*. (2002). The system was catalyzed by ferric reducing bacterium, *Shewanella putrefaciens* that can self-mediate electron transfer towards the anode electrode [7]. Many of ferric-reducing bacteria (FRB), which have the ability to use soluble and insoluble Fe(III) ion as an electron acceptor, express electrochemical activity by using graphite electrode of

mediator-less MFC as an electron acceptor [8-12]. Electrochemical activity of bacteria can be determined by cyclic voltammetry [8] which use to characterize oxidation and reduction of redox protein including cytochromes on bacterial cell membrane.

The performance of the MFC can also be enhanced by the reduction of overpotentials or internal resistance of the system. To minimize the overpotentials [13] in MFC, parameters including physical, chemical and biological parameters needs to be controlled due to their affect to the electron transfer.

In our research group, many physical parameters (i.e., electrode materials, electrode surface area, Proton exchange (PEM) surface area and some chemical parameters (i.e. various of electron mediator, electron mediator concentration, cathode electron acceptor concentration) that affect the electron transfer have been analyzed by Ouitrakul, S. (2007), electrical engineer [14]. Previously, they constructed MFCs using baker yeasts, *Saccharomyces cerevisiae,* as a biological component. The results of their studies showed promising of the decrease in overpotentials in overall system [14]. Then, the biological part of MFC should be investigated in terms of microbiological aspect. As a result, this thesis works on the assumption of bacteria that can resist and survive in electrical field. These bacteria should be electrochemically active bacteria that directly transfer electron towards the electrode. Electric current enrichment bacteria from sub-sediment and sediment were isolated and determined ferric reduction capacity under an anaerobic condition, and also will be compared with baker yeasts in mediator-less MFC of their ability to facilitate electron transfer.

Objectives of this research are listed as follows:

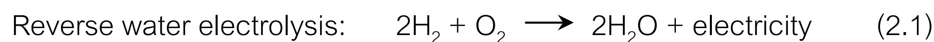
1. Isolation of electric current enriched bacteria from soil, sub–sediments and sediments, collected from different areas
2. Selected bacteria were tested in mediator-less MFCs
3. Identification of the selected bacteria

CHAPTER II

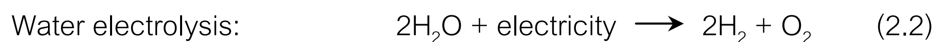
THEORY AND LITERATURE SURVEY

2.1 Fuel cell

Fuel cell, an electrochemical device that transforms chemical energy to electrical energy was discovered and developed by Sir William R. Grove in 1839 [15]. Gas battery or fuel cell concept which is based on the principle of reverse water electrolysis (as shown in Equation 2.1) occurred in the accident of Sir William Grove's water electrolysis (as shown in Equation 2.2) experiment.



Reverse water electrolysis: $2H_2 + O_2 \longrightarrow 2H_2O$ + electricity (2.1)



Water electrolysis: $2H_2O$ + electricity $\longrightarrow 2H_2 + O_2$ (2.2)

Afterwards, significant researches on fuel cells began again in the 1930s, by Francis T. Bacon and successfully produced the first practical fuel cell, an alkaline-type, in the 1950s. In the early 1960s, General Electric (GE) chemist, Thomas Grubb and Leonard Niedrach, invented and developed the first polymer electrolyte membrane (PEM) fuel cell — also call proton exchange membrane fuel cell — that use sulfonated polystyrene ion-exchange membrane to separate anode from cathode part. Platinum was coated onto the electrodes, which served as catalyst for the necessary hydrogen oxidation and oxygen reduction reactions. In the 1960s, fuel cell was developed as a power plant for the Apollo spacecraft [16]. Hydrogen gas was used as a fuel in combustion engines and used to produce both electricity as well as drinking water for the astronauts on their journey to the moon. That is why this system was called as "Fuel cell" up to now.

In recent years, PEM fuel cells were continuously developed with a significant increase in power density while reducing the amount of platinum required which was suitable for portable and residential applications. The PEM fuel cell is consisted of a proton

exchange membrane (PEM) as electrolyte which divided fuel cell into anode and cathode side. Hydrogen gas submitted into anodic compartment is oxidized and catalyzed by platinum at electrode, and yield protons as byproduct that move across through the PEM. At the same time electron will move via external load into cathode side. Oxygen gas is injected into the cathodic compartment to oxidize electrons and protons from anode to form water. Basic physical structure and the principle of PEM fuel cell was shown in figure 2.1. The reaction taking place in the anode and cathode, and the overall reactions were shown in Eqs. 2.3, 2.4 and 2.5, respectively.

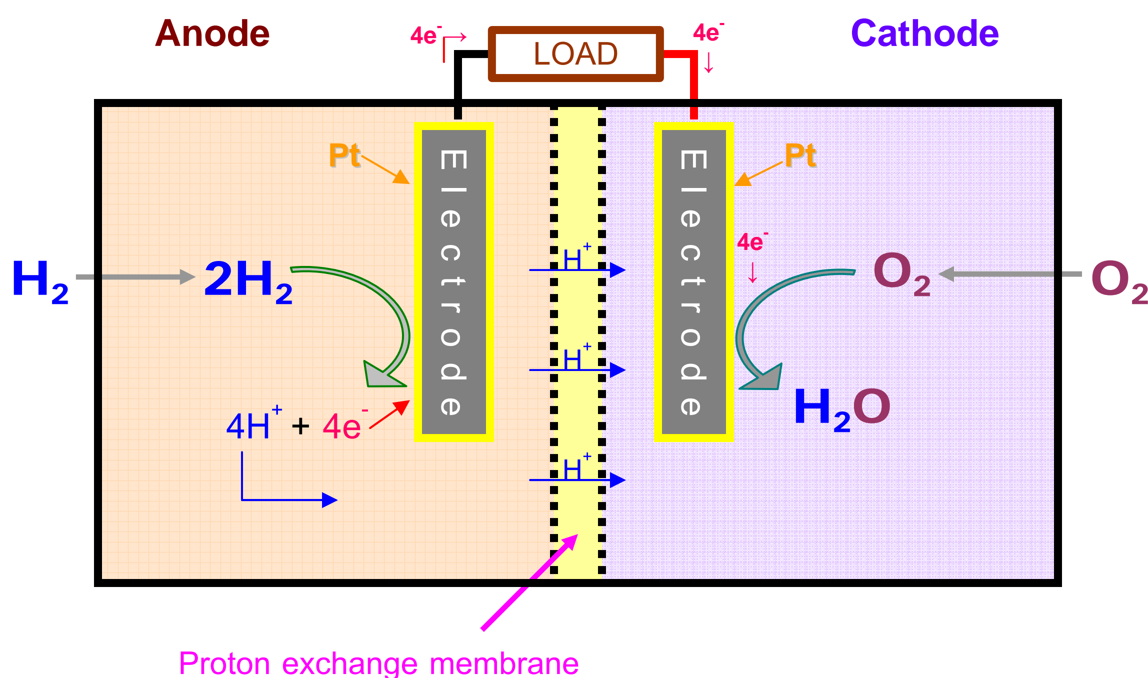
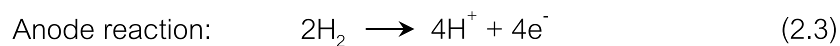
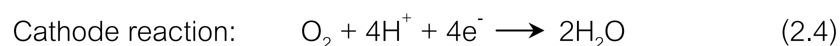


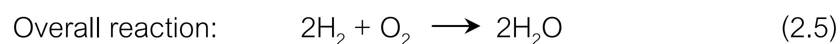
Figure 2.1 Schematic diagram of a PEM fuel cell



Anode reaction: $2H_2 \longrightarrow 4H^+ + 4e^-$ (2.3)



Cathode reaction: $O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$ (2.4)



Overall reaction: $2H_2 + O_2 \longrightarrow 2H_2O$ (2.5)

Hydrogen fuel cell that based on the principle of PEM fuel cell has been recognized as a future energy source for electric engine of automobile. It has a big advantage over the petrol engine that used fossil fuel but hydrogen fuel cell provides less emission of green house gas, energy saves and low noise pollution, etc. However, hydrogen production which was produced by electro-catalysis of water or fermentation process from biomass paid a lot of cost and energy consumption. Moreover, hydrogen was recovered only 15 % from fermentation process [1].

2.2 Microbial fuel cell (MFC)

Microbial fuel cell (MFC) is a bio-electrochemical device that can directly convert biomass or organic compounds into electricity through the catalytic activity of microorganisms. It was firstly proposed by Potter in 1911 [2]. Microorganisms including yeast, *Saccharomyces cerevisiae* and bacteria, *Escherichia coli*, were used in his fuel cell experiment. They could perform oxidizing ability on glucose, starch and cane sugar which could later be changed into electricity. In 1931, Barnet Cohen drew more attention in this area when he created a series-connected of microbial fuel cells that were capable of producing over 35 volts, but only current of 2 milliamperes was achieved [17].

In generally, MFC is consisted of two separated compartments, anode and cathode, which are partitioned by the PEM. Microorganisms are used in the anodic compartment to oxidize biomass or organic compounds and generate electrons, hydrogen ions and other fermentation products. The electrons or fermentation product are reduced at anode electrode, and electrons move from the anode to the cathode through the external load including a resistor, a capacitor or some other electrical device, while the hydrogen ions transported from the anodic across through the PEM into the cathodic compartment to combine electron with oxygen to form water. The schematic diagram of fuel cell and MFC are shown in Fig. 2.2.

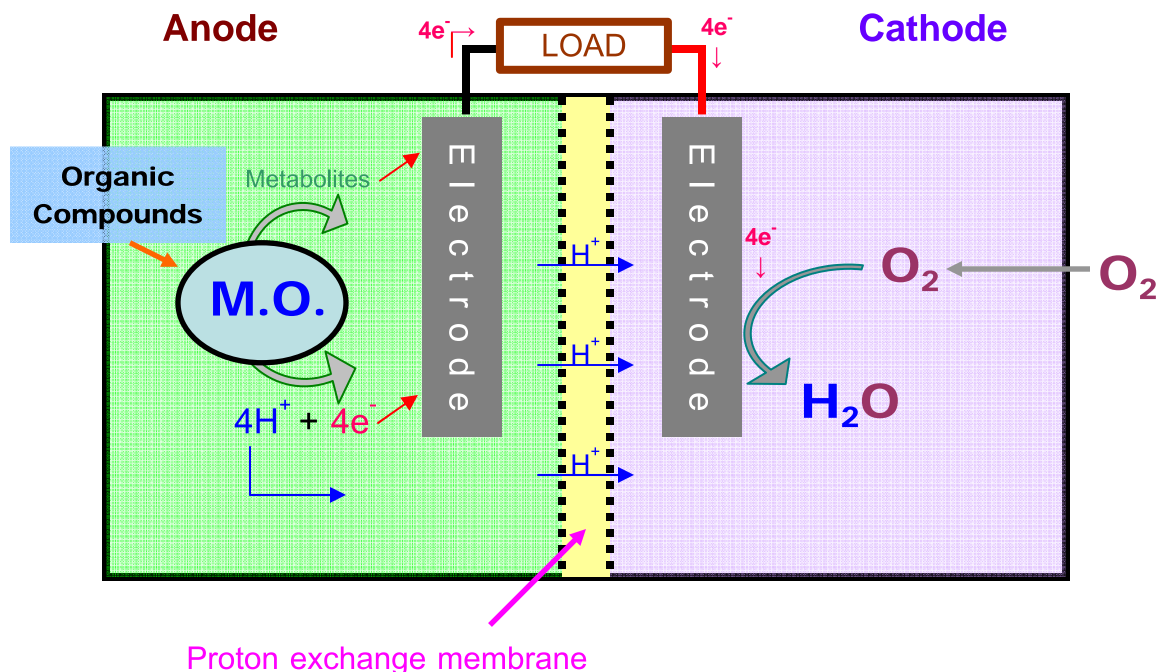


Figure 2.2 Schematic diagram of a MFC

However, the electron transfer to the anode electrode of microbes and their metabolite end products occurred in very low efficiency that was reflected in low electric current generation. The electron transfer of microbes to anode electrode in MFC can be enhanced by electron mediators [5]. Electron mediators are redox compounds which are electrochemically active and act as an electron shuttle between the microbes and the anode electrode [6]. Mechanism for electron transferring of mediator is that oxidized-form mediators diffuse into cytoplasmic membrane of microorganisms to accept electron and then reduced-form mediator diffuse back across the membrane to reduce electron at anode electrode as shown in Fig. 2.3. There are many reports of using mediators such as thionine [18, 21], ferric chelate compounds [18, 19], redox dyes [6, 20-22] and quinone compounds [23] in MFC that operated with microbes including *E. coli* [6, 18, 19, 22], *Actinobacillus succinogenes* [6], *S. cerevisiae* [3, 22], *Proteus vulgaris* [21], *Clostridium butyricum* [22], *Staphylococcus aureus* [22], *Synechococcus* sp. [23], *Lactobacillus plantarum* [24],

Streptococcus lactis [24], and *Erwinia dissolvens* [24]. Especially, the use of neutral red as mediator have an advantage in electron transport of *E. coli* that resulted in about 10-fold increasing of electricity current [6]. On the other hand, the continuous addition of the artificial mediators adds expensive cost and the ones are used in previous studies are toxic to human and could not be released into the environment without responsibility.

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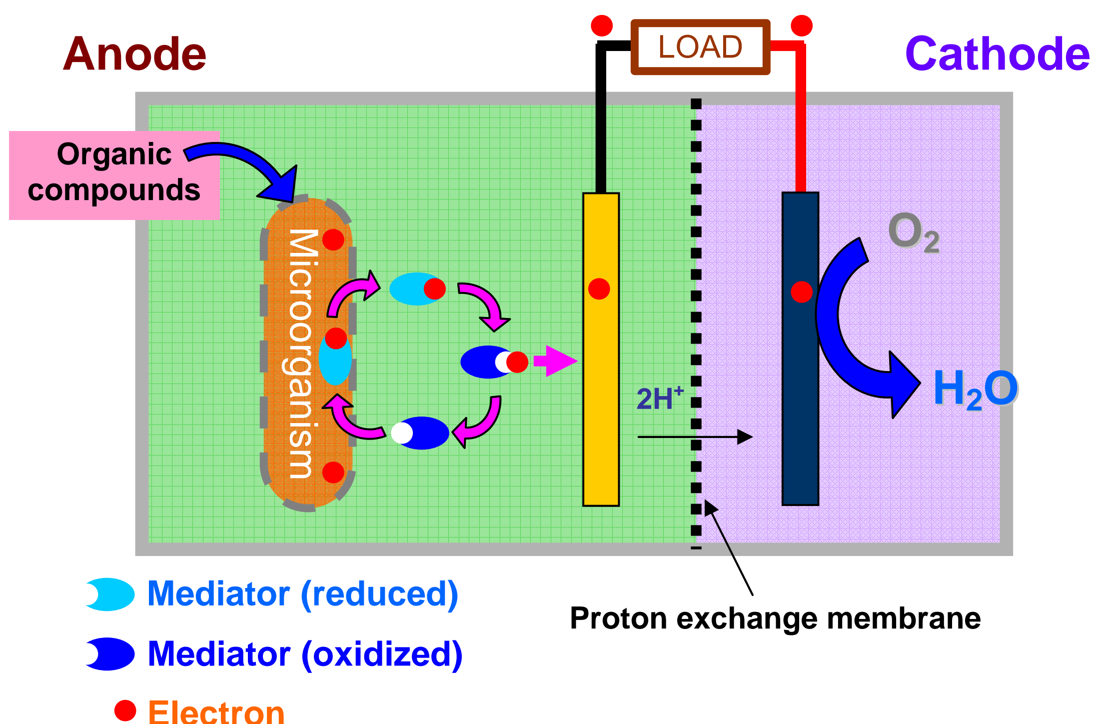

Figure 2.3 Schematic diagram of a MFC that use electron mediator in the system

The main factor that has to be more concerned is efficiency of electron transfer. Since if inefficiency of electron transfer to anode electrode occurred in MFC at anodic compartment including this part was not be operated to obtain enough an anoxic condition. As well as, oxygen function as greater oxidizer than electrode or mediator. Facultative bacteria in chamber could use oxygen as an electron acceptor easier than other electron acceptor.

2.3 Mediator-less microbial fuel cell

Mediator-less microbial fuel cell is the artificial mediator free system. It required electrochemically active microbes that can self-mediate electron transfer toward the anode electrode. This breakthrough in MFC technology was firstly proposed by Kim *et al*. (1999) [8]. His system was catalyzed by ferric reducing bacteria (FRB), *Shewanella putrefaciens* that can self-mediate electron transfer towards the anode electrode [7]. Many FRB, which have the ability to use soluble and insoluble ferric ion (Fe^{3+}) as an electron acceptor in anoxic environment, express electrochemical activity by using graphite electrode of mediator-less MFC as an electron acceptor [8-12]. Electrochemical activity of bacteria can be determined by cyclic voltammetry [8] which use to characterize oxidation and reduction of redox compounds including redox protein such as cytochromes on bacterial cell membrane. There are reports that membrane-bound cytochromes of *S. putrefaciens* that cultured in anoxic condition are represented on its outer membrane [25, 26]. It was believed that membrane-bound cytochromes involved in electron transfer of *S. putrefaciens* to electrode [7]. Phenomena of electron transferring via periplasmic c-type cytochromes of *S. oneidensis* MR-1 biofilm to closely attached anode simulated the reduction reaction of insoluble Fe(III) which served as an electron acceptor [27, 28].

In some cases, microorganisms such as *Pseudomonas aeruginosa* could produce their own mediators, phenazine pyocyanin, that can be used to facilitate the electron transfer to electrode [4]. Pyocyanin compounds such as phenazine cause pathogenic effect on human and inhibitory effect on other bacteria [29]. Not only, *Ps. aeruginosa* can use phenazine as electron mediator, but other bacteria such as *Lactobacillus amylovorus*, *Entrococcus faecium* [30] and *Brevibacillus* sp. [31] can also use phenazine for their electron transfer to electrode.

Moreover, *Geobacter* ***sulfurreducens*** used electrical pilli or nanowires to transfer electrons to electrode or insoluble ferric hydroxide as an electron acceptor for their respiration [32, 33]. In addition, *S. oneidensis* MR-1 also have bacterial nanowires for

transferring electrons to electrode [34], but the size of its nanowires is different from *G. sulfurreducens*.

In summary, there are three proposed mechanisms of self-mediate electrode transfer of bacteria that used in mediator-less MFC as shown in Fig. 2.4. First, electrons are transferred via membrane bound cytochromes to electrode that closely attached. Second, bacteria utilize self-produced electron mediator for use as electron shuttle between cell and electrode. Third, electrons are transferred through electrical pilli or “nanowires” that directly contact with electrode.

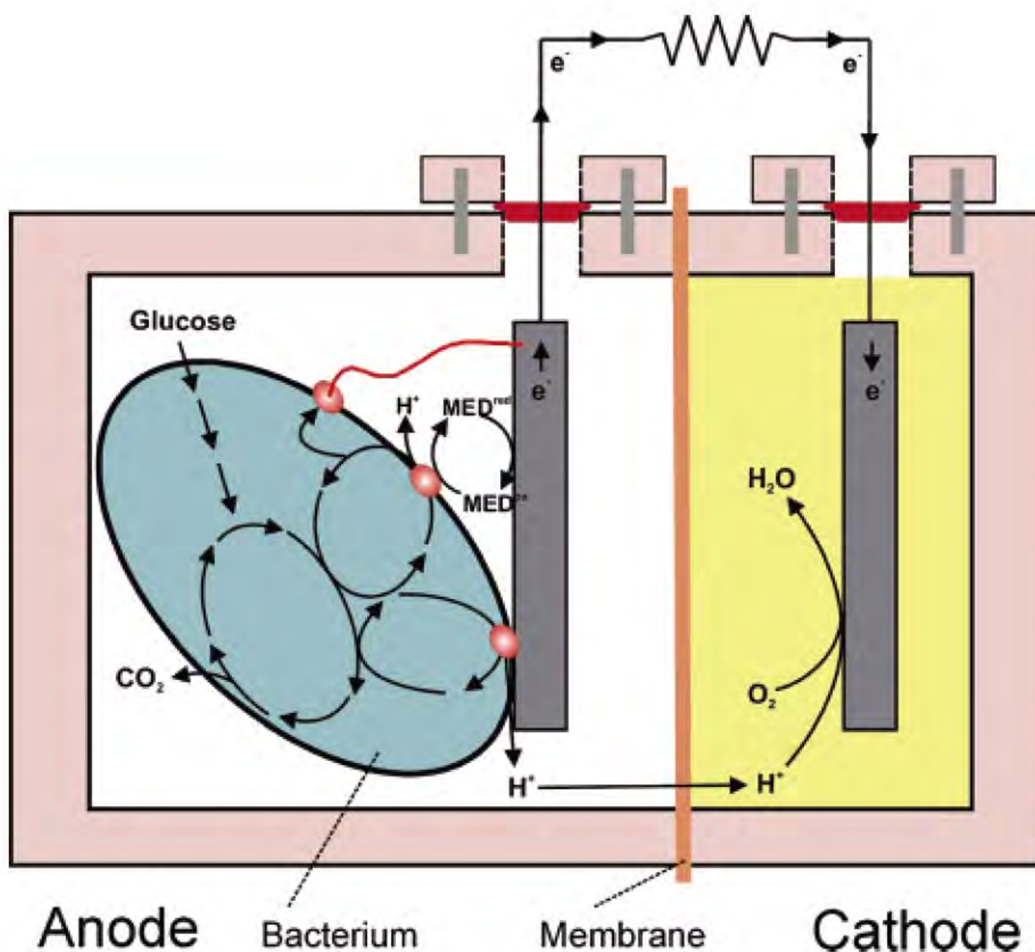

Figure 2.4 Mechanisms of electron transfer of bacteria in mediator-less MFC [35]

2.4 Ferric reducing bacteria as biocatalysts in mediator-less MFC

Ferric reducing bacteria (FRB) play a vital role in the iron nutrient cycling on the Earth's crust [36]. They can reduce ferric ion (Fe^{3+}) into ferrous ion (Fe^{2+}) by using ferric ion (Fe^{3+}) as terminal electron acceptor in anaerobic respiration. The ferric ion that is reduced by microorganisms may be in soluble form such as ferric citrate or insoluble form for example; limonite (FeOOH), goethite ($Fe_2O_3.H_2O$) and hematite (Fe_2O_3). Furthermore, many reports indicated that *Shewanella* sp. and other FRB can be operated effectively in mediator-less MFC. FRB hold great promise as microbes that use in mediator-less MFC because they have the potential for use graphite electrode as a final electron acceptor in their respiratory system. Culture of *S. putrefaciens* that grown in an anaerobic condition showed electrochemical activity greater than aerobically grown culture but both of anaerobically and aerobically -grown *E. coli* didn't show electrochemical activity [7]. Many researchers used pure culture of FRB such as *Shewanella putrefaciens* [7], *S. oneidensis* [34],*Clostridium butyricum* [9], *Geobacter sulferreducens* [10], *Aeromonas hydrophila* [11] and *Rhodoferrax ferrireducens* [12], as biocatalysts in mediator-less MFC. Not all ferric reducing bacteria can produce electricity current. FRB, *Pelobacter carbinolicus* could reduce ferric oxide but couldn't use electrode as an electron acceptor that resulted in none of electricity production [37].

2.5 The effect of poised potential on microbes in mediator-less MFC

In MFC system, Cho and Ellington (2007) [38] investigated the impact of poised potential on current generation of aerobically grown *S. oneidensis* inoculums. The effect of various potential from 0-500 mV that poised into anode chamber on lag period prior current generation and current output were observed. When poised potential increased lag period was decreased from 90 to 5 hour before current generation but not significantly different in maximum current productivity. They suggested that aerobically grown cells could be adapted for current generation in anaerobic condition by poised potential that supplied into anodic compartment of mediator-less MFC. The authors said that higher poised potential

above 750 mV inhibited the growth and current generation of *S. oneidensis* in mediator-less MFC (data not shown).

2.6 Application of Microbial fuel cell

MFCs have a wide range of applications as electrical generator for electronic device, electronics in space [39] and self-feeding robot [40]. Moreover, mediator-less MFC systems still have a potential for use and convert organic waste water into electrical energy [41-44]. In waste treatment system, mixed cultures of microbe or sludge were used as inoculums for treat various kind of organic compounds in waste water. Furthermore, mediator-less MFC concept was applied to construct sediment microbial fuel cell, also known as Benthic Unattended Generator (BUG) [45]. They consist of two plate graphite electrodes as anode and cathode, but only anode electrode was submerged into anoxic sediment. In this system, interfacial between sediment and water act as proton exchange membrane. BUGs generated electricity current by acetate oxidation of bacteria mainly in family *Geobateraceae* that can use graphite electrode as an electron acceptor as shown in Fig 2.5. They have a potential application to power electronic devices, such as monitoring equipment in remote locations [45].

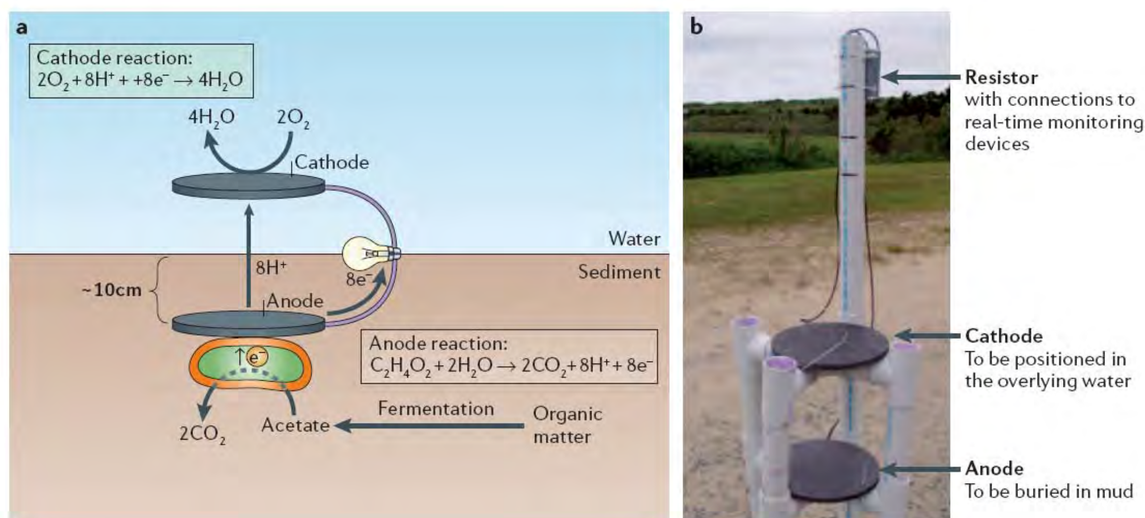

Figure 2.5 A sediment MFC [46]

(a) A schematic of sediment MFC

(b) An actual sediment MFC before deployment

CHAPTER III

MATERIALS AND METHODS

3.1 Materials and Chemicals

Materials and chemicals used in this research are listed as follows:

1. Bacto Agar, purchased from Difco Laboratories, U.S.A.
2. Crystal violet, purchased from Fluka, Sigma-Aldrich Co., Inc., Singapore.
3. Ethanol absolute, Analytical grade, ACS., purchased from Scharlau Chemie S.A., Spain.
4. Ethylene diamine tetra acetic Acid (EDTA), purchased from Sigma-Aldrich Co., Inc., Singapore.
5. Genome DNA Simax Kit, purchased from Beijing SBS Genetech Co., Ltd., China.
6. Glacial hydrochloric acid (HCl) (A.R. grade), purchased from Merck KGaA, Germany.
7. Glucose, purchased from Merck KGaA, Germany.
8. Hydrogen peroxide 30%, purchased from Merck KGaA, Germany.
9. Iodine, purchased from Merck KGaA, Germany.
10. Lysozyme, purchased from Sigma-Aldrich Co., Inc., Singapore.
11. Nutrient Agar (NA) purchased from Oxoid, UK
12. Peptone, purchased from Difco Laboratories, U.S.A.
13. Potassium dihydrogen phosphate (KH_2PO_4), purchased from Merck KGaA, Germany.

14. Potassium hexa cyanoferrate (III) ($K_3Fe(CN)_6$), purchased from May and Baker Co., Ltd., England.
15. Salfanin O, purchased from Merck KGaA, Germany.
16. Sodium chloride (NaCl) (A.R. grade), purchased from Merck KGaA, Germany.
17. Sodium dodexyl sulfate (SDS), purchased from Fluka, Sigma-Aldrich Co., Inc., Singapore.
18. Sodium hydroxide (NaOH), purchased from Merck KGaA, Germany.
19. Trizma base, minimum 99.9% titration, purchased from Sigma-Aldrich Co., Inc., Singapore.
20. Tryptic Soy Broth (TSB), purchased from Difco Laboratories, U.S.A.
21. Yeast Peptone Dextrose broth (YPDB), purchased from Difco Laboratories, U.S.A.

3.2 Instruments

1. Autoclave (Model Autoclave ES-315, Tomy Seiko Co., Ltd., Tokyo, Japan)
2. Cold room (Model Compakt 880(B)H, Foster Refrigerator (U.K) Ltd., U.K)
3. 4-Digit precision weighting balance (Model AG 204, Mettler Toledo, Switzerland)
4. DNA thermo cycler TP 600 (TaKaRa Bio Inc., Otsu, Shiga, Japan)
5. Electrophoresis chamber set (Mupid-ex, Bruker BioSpin Inc., Fällanden, Switzerland)
6. Gel Documentation system (Bio-Rad Laboratories Gel Doc ™ XR, California, U.S.A.)
7. High Speed Refrigerated Centrifuge (Beckman Coulter ™ Avanti J-30I, Palo Alto, California, U.S.A.)
8. Hot air oven (Model UC 30, Memmert GmbH and Co. KG., Western Germany)

9. Hot plate stirrer (Model C-MAG HS 10, Becthai Bangkok Equipment & Chemical Co., Ltd., Thailand)
10. Incubator (Model 800, Memmert GmbH and Co. KG., Western Germany)
11. Incubator shaker (Model SK-737, Amerex Instruments, Inc., USA)
12. Kubota Refrigerated Microcentrifuge 6500 (Kubota Corporation, Tokyo, Japan)
13. Laminar flow 'clean' (Model V6, Lab Service Ltd., Part)
14. Microscope (Model CH 30RF200, Olympus Optical Co., Ltd., Japan)
15. pH meter (Mettler-Toledo International Inc., New York, U.S.A.)
16. Spectrophotometer (Genesys 20 Model 4001/4, ThermoSpectronic, Rochester., New York, USA)
17. Vortex mixer (Model G-560E, Scientific Industries, Inc., Bohemia. N.Y., 11716, USA)
18. Water bath (Model WB14, Becthai Bangkok Equipment & Chemical Co., Ltd., Thailand)

3.3 Experimental Procedures

3.3.1 Screening and isolation of electric current enriched bacteria

3.3.1.1 Sample collecting

Few samples of soils from Phu Rua, Loei, Thailand, sub-sediments from Koh Larn, Chonburi, Thailand, and sediments from pond in front of Physic I building, Faculty of Science, Chulalongkorn University, were randomly collected. All samples were stored at 4 °C before use.

3.3.1.2 Enrichment by using electrical current

A sample of 5-gm was inoculated into 250 ml flask containing 150 ml of Nutrient broth (NB) and stainless steel electrode then covered with paraffin oil in order to

generate an anaerobic condition. Subsequently, the various electrical current, 6, 30, 60, 90 and 120 mill-amperes (mA) were supplied respectively, in each system by connecting with 50 Hz AC current generator (Bio-electronic Research Laboratory) to the stain-less steel electrode. After incubation for 5 days, electrodes were transferred into the new 250 ml flask contained 150 ml of NB then covered with paraffin oil, then supplied with electric current and incubated for another 5 days.

3.3.1.3 Isolation of enriched bacterial culture

After incubation in electrical environment for 5th electrode transferring (30 days), each of serial dilutions of electrode biofilm were spread on Nutrient agar (NA) (Oxoid) and NA with 20 mM of ferric citrate, and were incubated in anaerobic chamber, GENbox anaer (bioMérieux, France), at room temperature for 5-7 days. Mixed bacterial cultures were restreaked until pure culture was isolated.

3.3.2 Ferric reducing activity of enriched bacteria

Pure culture of electrode biofilm were streaked on Nutrient agar plate that supplemented with 20 mM ferric citrate and incubated in anaerobic chamber, GENbox anaer (bioMérieux, France), at room temperature for 5-7 days. The changing of NA with ferric citrate from the reddish-brown color into the light-green color was used to evaluate the ferric reduction activity.

3.3.3 Operation of mediator-less Microbial fuel cell (MFC)

3.3.3.1 Construction and configuration of mediator-less MFC

In this research, three models of MFC chamber were constructed and designed by using acrylic; Glass I and Glass II as architectural structure shown in Figs 3.1, 3.2 and 3.3, respectively. For the first one, acrylic is a clear acrylic-plated chamber designed and constructed by following the procedure of Ouitrakul (2007). In this system, all components except PEM were sterilized by ultraviolet (UV) radiation and 70% of ethanol as

disinfectant because acrylic couldn't maintain its structure when sterilization has been done by autoclaving. Secondly, Glass I was designed and constructed from 100 ml Glass bottle (Duran, Germany) that connected with glass socket for sampling port and the opposite side of sampling port was connected with cylindrical glass, 1.8 cm in diameter, for the connection anode to cathode compartment. Thirdly, Glass II was different from Glass I model at anode chamber that glass chamber was blew from cylindrical glass, 5 cm in diameter, and connected with three glass sockets. Those sockets were for injecting and sampling port, gas sampling port and electrical wire as shown in Fig.3.3. Three models were the same for electrode surface area, but different in working volume and PEM diameter or area as shown in Table 3.1. Neosepta® PEM (model CMS, ASTOM corporation, Japan) functioning as cation exchange membrane was installed between the anode and cathode compartments and sterilized by autoclaving at 110 °C for 15 minutes. A 3 cm X 3 cm carbon fiber cloth (ACELAN, Korea) — surface area (18 cm^2) — were used as electrodes in both of compartments. In acrylic model, electrode was sterilized by UV radiation, but in Glass I and II, electrode was installed inside the chamber and both were later sterilized by autoclaving at 121 °C for 30 minutes.

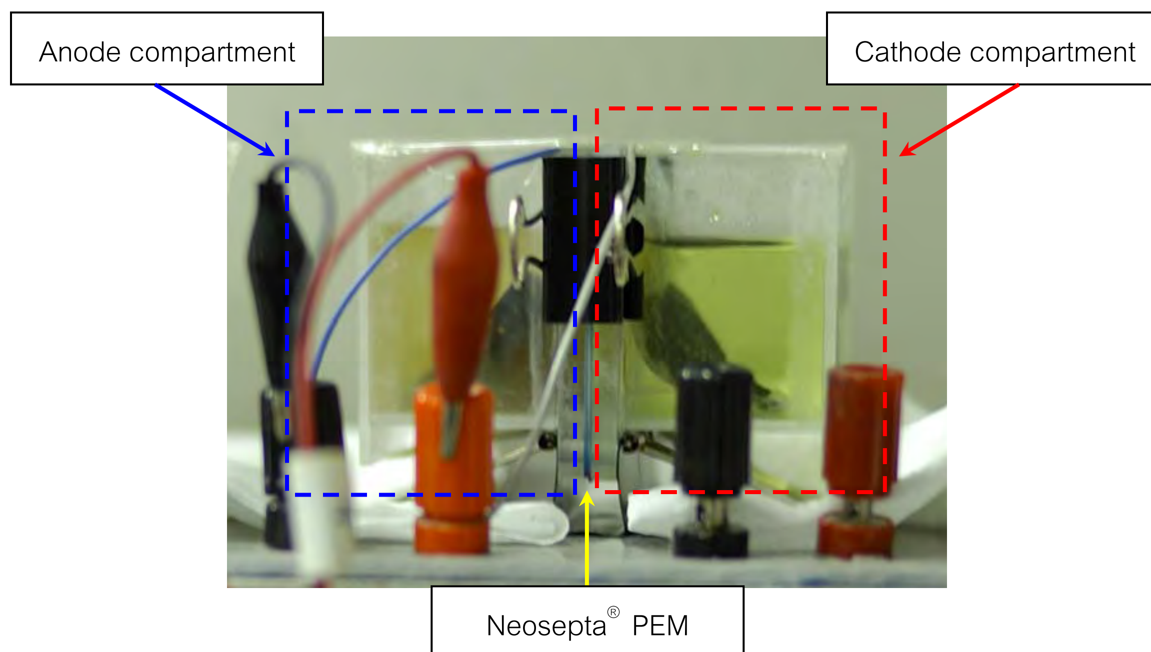

Fig 3.1 Configuration of Acrylic model of mediator-less MFC

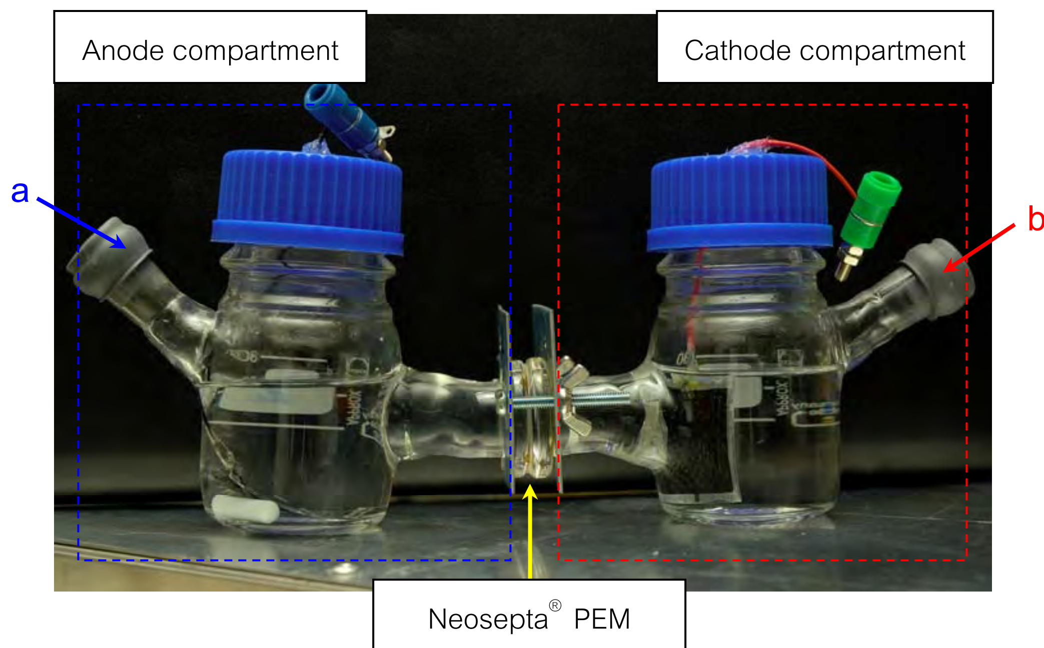

Fig 3.2 Configuration of Glass I model of mediator-less MFC

(a) and (b) injecting and sampling port

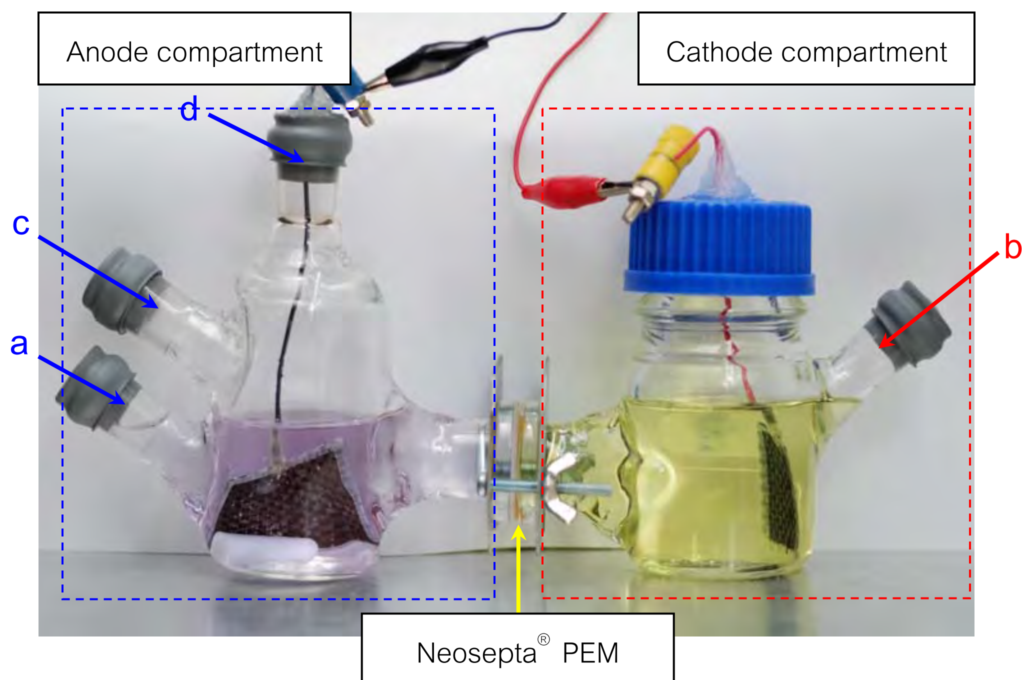

Fig 3.3 Configuration of Glass II model of mediator-less MFC

(a) and (b) injecting and sampling port

(c) gas sampling port (d) electrical wire

Table 3.1 Parameter of component that used in mediator-less MFC in different model

Model	PEM area (cm^2)	Parameters of both anode and cathode compartment of mediator-less MFC	
		Electrode surface area (cm^2)	Working volume (ml)
Acrylic	8.0	18	30
Glass I	3.0	18	90
Glass II	3.0	18	100

3.3.3.2 Electrolyte solution

3.3.3.2.1 Preparation of electrolyte solution and microorganism inoculum

Phosphate buffer pH 7.0 of KH_2PO_4 was prepared and added into anodic and cathodic chamber as electrically conductive medium. Glucose, as electron donor, and Potassium Ferric cyanide ($K_3Fe(CN)_6$), as electron acceptor were added into anodic and cathodic chamber, respectively. In Glass II model, L-cysteine HCl was used as oxygen scavenger for more anaerobic than Glass I model. Resazurin acted as oxygen indicator which indicated the presence of oxygen as shown in blue to red and the absence of oxygen was colorless. Prepared solution mediator-less MFC (Table 3.2) was sterilized by autoclaving at 121 ºC for 15 minutes.

3.3.3.2.2 Microorganism inoculum

Pure isolate of baker yeast (Fermipan), *Saccharomyces cerevisiae,* was cultured in Yeast Peptone Dextrose broth (YPDB) (Difco®) and incubated in rotary shaker at room temperature, 200 rpm for 24 hours. All selected isolates from sediments and sub-sediments were cultured in Trypticase soy broth (TSB) (Difco®) that added 20 mM of

ferric citrate and incubated in anaerobic condition at room temperature for 5 days. Yeast or bacterial cultures were collected by centrifugation at 10,000g, 25 ºC and washed with 100 mM of pH 7.0 KH_2PO_4 buffer for removal of the remaining medium broth. Cell pellet was resuspended with 5 ml of pH 7.0 KH_2PO_4 buffer for used as biological catalyst in the anode of mediator-less MFC. Information of 3.3.3.2.1 and 3.3.3.2.2 were combined as details shown in Table 3.2.

Table 3.2 Concentration of electrolyte solution and microorganism suspension that filled in anodic or cathodic chamber of the mediator-less MFC

Solution or suspension in mediator-less MFC	Final Concentration	
	Anode	Cathode
Phosphate buffer pH 7.0 (KH_2PO_4) (mM)	100	100
Potassium Ferric cyanide ($K_3Fe(CN)_6$) (mM)	-	1
microorganism concentration (CFU ml^{-1})	10^8-10^9	-
Glucose ($C_6H_{12}O_6$) (mM)	100	-
L-cysteine HCl (for model “Glass II” only) (w/v)	0.05 %	-
Resazurin (for model “Glass II” only) (w/v)	0.0001%	-

Note: - = no addition

3.3.4 Measurement

3.3.4.1 Electrical parameter measurement and calculation

Mediator-less MFC performances were evaluated in term of voltage, current density and power density that was supplied to external load or resistance. Mediator-less MFC system was connected to pico ADC-11 data acquisition unit (pico

technology, UK), and voltage was recorded every 15 seconds and transferred to the personal computer via parallel port. The voltage data output was displayed in pico recorder and pico player program (pico technology, UK), and the open circuit voltage (*V*oc) was obtained when the system was not connected to any load or resistance. For current density and power density, various external system resistances (*R*) as follows: 1, 2, 5.1, 10, 51 and 100 k**Ω,** were connected after a steady open circuit voltage obtained, and the current density (i) (mA/m^2) was deduced as shown in Equation 3.1 where V is the voltage (volts) and *a* is electrode surface area (m^2). The power density (P) (mW/m^2) was calculated as shown in Equation 3.2.

Current density: $i = (V / R) / a$ (3.1)

Power density: $P = (I \times V) / a$ (3.2)

The relationship between current used for enrichment and selection (I selection) and open circuit voltage (Voc), I selection and current density (i), and I selection and power density (*P*), were separately plotted as shown in graphs.

3.3.4.2 Monitoring parameters

In this experiment, three of parameters—pH, microorganism viable cell and glucose concentration—were determined before and after the operation of MFC for microbiological aspects. The pH of solution in anode was measured before and after the operation of MFC by using pH meter (Mettler-Toledo International Inc., New York, U.S.A.). Microorganism viable cell count (CFU ml^{-1}) was determined by total plate count technique. Culture suspension was serially diluted and each of dilution was spread on their appropriated medium plate in duplicate. Glucose concentration was determined by DNSA method when model glass II has been operated.

3.3.5 Identification of pure isolated bacterial culture

3.3.5.1 Morphological Examination and Biochemical tests

Bacterial identification based on morphology, Gram's straining, and ingredient utilization by using rapid identification kit API® 20E. Results from API kit were interpreted by using program API®WEB (bioMérieux, France). Coliforms were counted using selective agar, Eosin Methylene Blue agar (EMB).

3.3.5.2 Molecular technique based on 16s rDNA sequencing

Genomic DNA of overnight cultured was extracted using Simax Genome DNA Extraction Kit (Beijing SBS Genetech Co., Ltd., China). DNA extraction procedure followed the manufacturer's instructions manual. The PCR amplification of 16s rRNA gene were amplified by using two universal primer 16F27 (5'-AGA GTT TGA TCC TGG CTC AG-3') and 16R1522 (5'-AAG GAG GTG ATC CAG CCG CA-3') as described in Bayane *et al.* (2006) [47]. About 1,500 base-pairs of PCR amplicons were performed under the following conditions: denaturing 94 °C for 1 minute, annealing 55 °C for 1 minute and elongating 72 °C for 2 minutes by PCR thermal cycler TP600 (TaKaRa Bio Inc., Otsu, Shiga, Japan) for 35 cycles. PCR product was submitted for sequencing at Macrogen Inc. co. Ltd. (Seoul, Korea). Bacterial similarity was obtained after DNA sequence compared to the database of the National Center for Biotechnology Information (NCBI) using BLASTn (for nucleotide sequence) algorithm.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Screening, isolation and morphological characterization of electricity enriched isolates

4.1.1 Isolation of sediment from pond in front of Physic I building

For enrichment and isolation, 1 gram of sediment from pond in front of Physic I building, Faculty of Science, Chulalongkorn University were inoculated in four groups of experiment, and then stainless electrodes were connected with 50 Hz AC current generator for enrichment 3 and 4 as shown in Fig. 4.1. Enrichment 1 and 2 were performed as controls which were not supplied with electric current. The enrichment groups were divided into four groups as shown in Fig. 4.2.

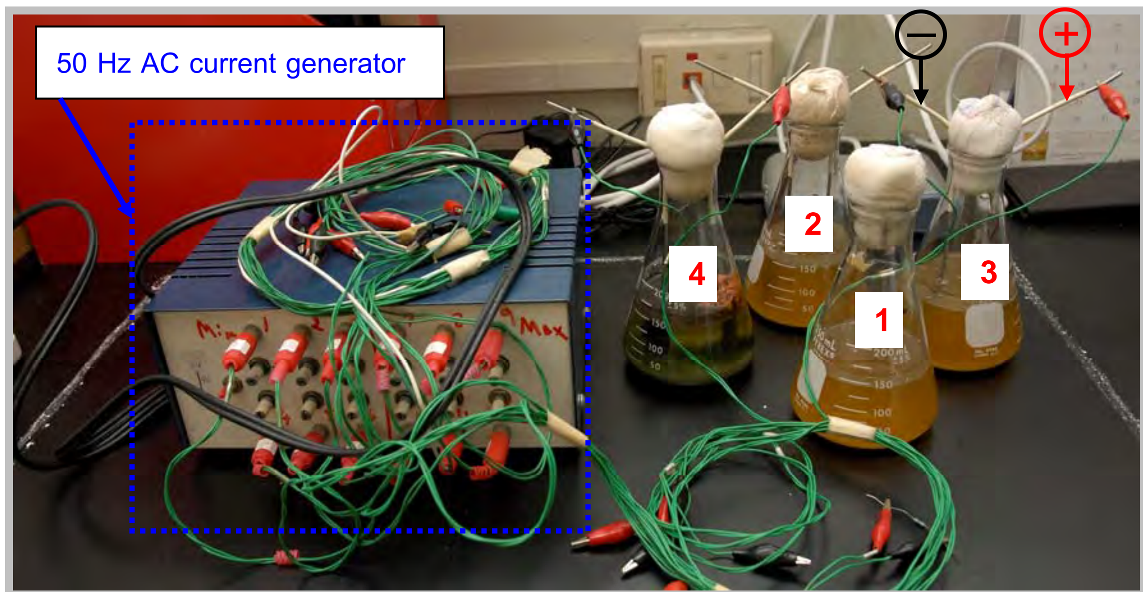

Figure 4.1 Electric current enrichment of sediment from pond in front of Physic I building

After incubation for 24 hours, total plate count of four groups was performed and results were shown in Table 4.1. From the results, total bacterial count of enrichment 1-3

were ~3-5 x 10^7 CFU ml $^{-1}$, but viable cell count of enrichment 4 supplied with high electric current (120 mA) was ~8.5 x10^5 CFU ml $^{-1}$. Obviously the reduction of ~10^2 CFU of total bacteria was found as compared to those of two control groups. In addition, Coliforms count of enrichment 1-3 was ~3-4 x 10^7 CFU ml $^{-1}$, but Coliforms count of enrichment 4 supplied with high electric current (120 mA) was 7.03 x 10^5 CFU ml $^{-1}$. The same reduction for ~10^2 CFU of Coliforms was determined between the values of two control groups and that from high electric current supply (120 mA). These results agreed with previous studies that electric current could reduce bacterial biofilm [48].

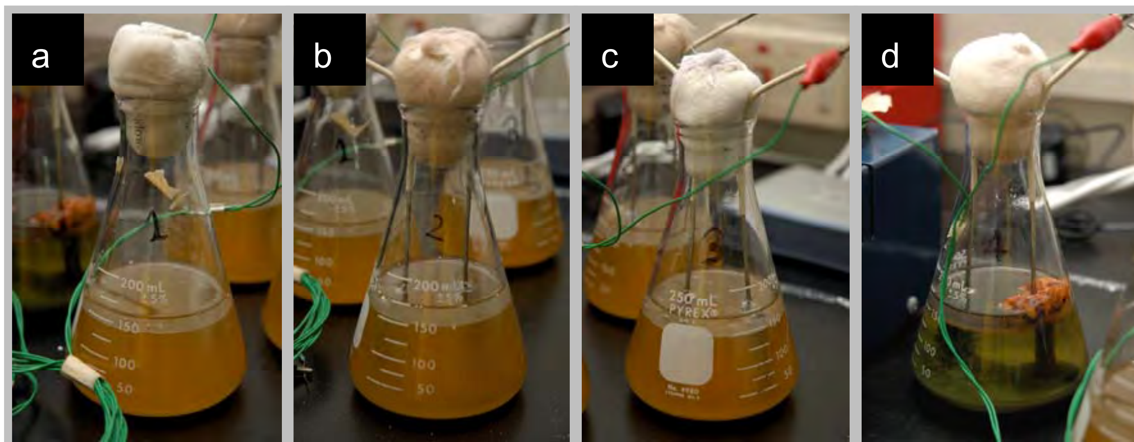

Figure 4.2 Four enrichments of sediment from pond after incubation for 5 days including:

(a) Enrichment 1: 150 ml NB broth

(b) Enrichment 2: 150 ml NB broth with stainless electrode

(c) Enrichment 3: 150 ml NB broth with stainless electrode and current 6 mA

(d) Enrichment 4: 150 ml NB broth with stainless electrode and current 120 mA

Table 4.1 Total plate count from four enrichments of sediment from pond

Media	Total plate count of four enrichment of sediment from pond (CFU ml $^{-1}$)			
	Enrichment 1	Enrichment 2	Enrichment 3	Enrichment 4
NA	3.23 x 10^7	3.86 x 10^7	4.73 x 10^7	8.50 x 10^5
EMB	3.00 x 10^7	3.40 x 10^7	4.00 x 10^7	7.03 x 10^5
Note: All values are from duplicated trial.				

After incubation of sediment in electrical environment broth for five days, bacterial biofilm was tested only at negative and positive electrode in 120 mA enrichment system as shown in Fig 4.3. Sub-sequential transferring of electrode to new broth were performed for 9 times to select for bacteria that can survive in electric current enriched condition. Finishing the 9th electrode transfer, biofilm that attached to stainless steel electrode were isolated. In enrichment 4 (120 mA), negative electrode has greenish and brownish-colored biofilm in bulky density and positive electrode also has biofilm but in darkish-colored that less amount than biofilm at negative electrode. Biofilm of enrichment 3 (6 mA) only was observed at negative electrode as white-colored biofilm as shown in Fig 4.4(a-c).

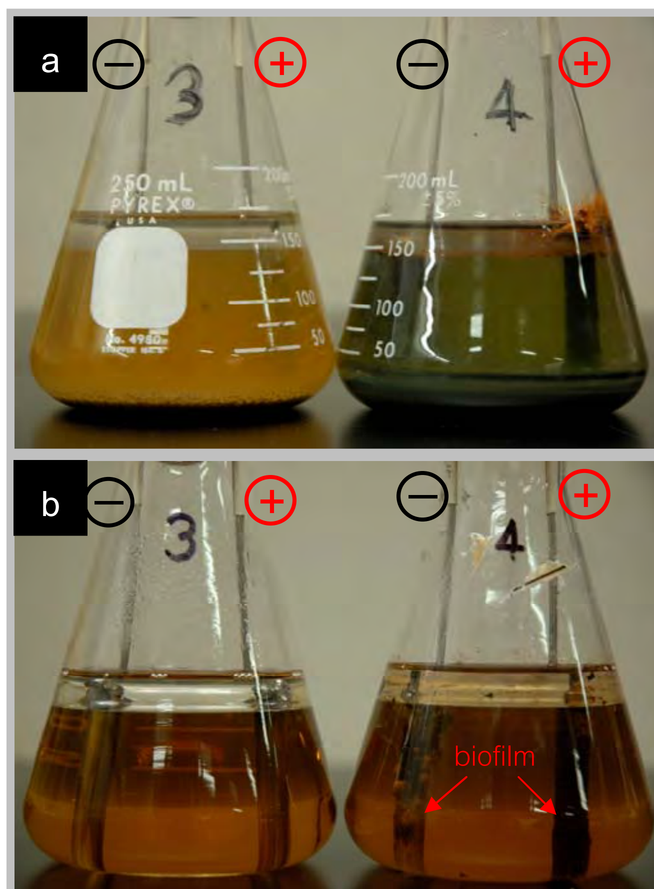

Figure 4.3 Enrichment 3 and 4 of sediment from pond

(a) Before transfer electrodes to new broth

(b) After transfer electrodes to new broth

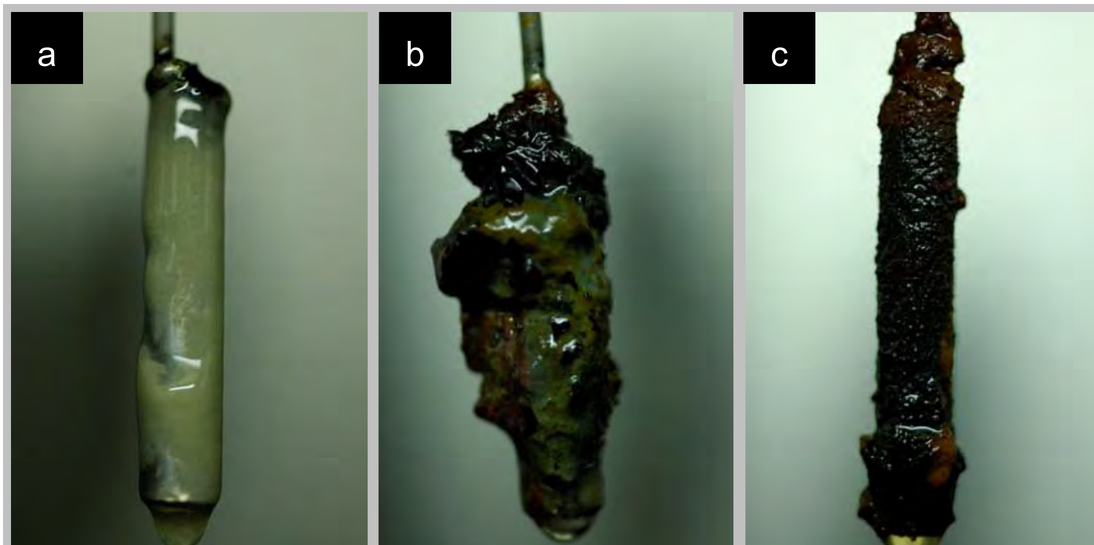

Figure 4.4 Biofilm formation at 10th transferred-electrode of enrichment 3 and 4

(a) Biofilm at negative electrode of enrichment 3 (6 mA)

(b) Biofilm at negative electrode of enrichment 4 (120 mA)

(c) Biofilm at positive electrode of enrichment 4 (120 mA)

The 28 pure cultures of electricity enriched biofilm from sediment from pond in front of Physic I building, Faculty of Science, Chulalongkorn University were isolated and designated as PH1-28. They were two Gram's positive cocci and 26 Gram's negative rod. All isolates were characterized based on Gram's staining, cell morphology under microscopic examination and ferric reduction activity as shown in Appendix B1.

4.1.2 Isolation of sub-sediments from Koh Larn

After sub-sediments from Koh Larn were enriched, microbe biofilm occurred in 60, 90 and 120 mA of enrichment system as shown in Fig 4.5. After incubation in electrical environment for 10th electrode transferring, biofilm that attached to stainless steel electrode were isolated. The pure culture of 40 isolates of electrode biofilm from sub-sediment from Koh Larn were isolated and designated as KL1-40. They were 16 Gram's positive bacilli and 24 Gram's negative rod. All isolates were characterized based on Gram's staining, cell

morphology under microscopic examination and ferric reduction activity as shown in Appendix B2.

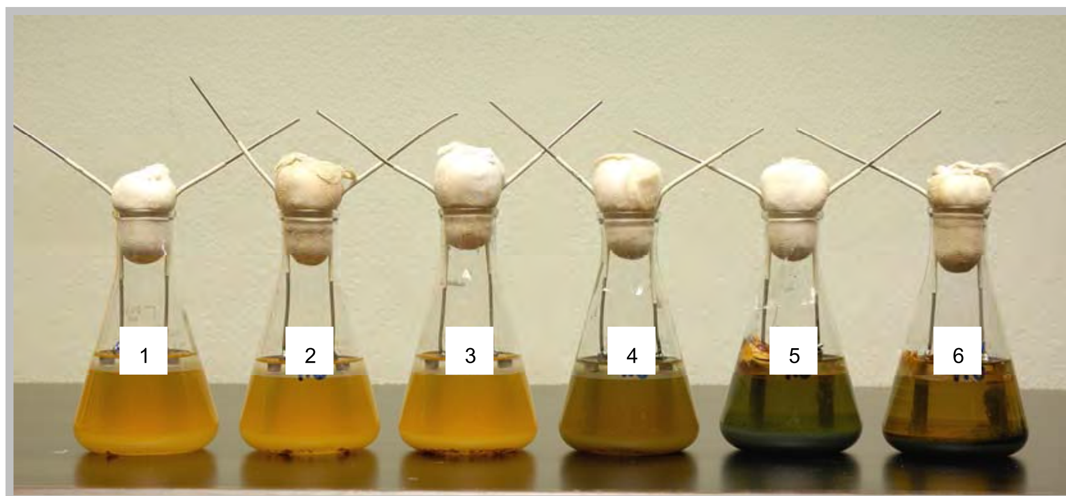

Figure 4.5 Enrichment of sub-sediment from Koh Larn after incubation for 5 days including:

Enrichment 1: 150 ml NB broth

Enrichment 2: 150 ml NB broth with stainless electrode and current 6 mA

Enrichment 3: 150 ml NB broth with stainless electrode and current 30 mA

Enrichment 4: 150 ml NB broth with stainless electrode and current 60 mA

Enrichment 5: 150 ml NB broth with stainless electrode and current 90 mA

Enrichment 6: 150 ml NB broth with stainless electrode and current 120 mA

Bacteria from anoxic environments such as sediment and sub-sediment were isolated for use as biocatalysts in mediator-less microbial fuel cell as mentioned earlier [7, 10, 12]. For example, facultative anaerobic bacteria such as *Shewanella putrefaciens* [7] and *Rhodoferrax ferrireducens* [12] isolated from sediment and sub-sediment were previously reported that they could be used in mediator-less MFC. They are also ferric reducing bacteria which have ability to use ferric ion and electrode as an electron acceptor. All of pure cultures that were isolated from electric current enrichment of sediment and sub-sediment in this study should also be classified as facultative anaerobic bacteria. Because of experimental procedure that used in this research was not controlled under strictly

anaerobic condition such as in anaerobic Glove box or Hungate's tube techniques. In order to fulfill our proposal in this study, ferric reduction activities of selected pure isolates were evaluated on reduction of soluble ferric ion such as ferric citrate [9], and be monitored for use in mediator-less MFC.

Four isolates of electricity enrichment of soil sample from Phu Rua were selected for ferric reducing bacteria (FRB) on NA plates containing ferric citrate as the electron acceptor. Restreak til pure cultures were obtained. Four isolates of FRB, which designated as B1-B4 were facultative anaerobic Gram-positive bacilli and spore forming bacteria. Due to B1-B4 isolated from iron-rich environment they may play important role in iron cycling as the same results from other *Bacillus* spp. as mentioned earlier [36]. Also, based on biochemical and physical examination and confirmed by 16S rDNAs sequence B1-B4 isolates were identified as *Bacillus* spp. Other isolates from electricity enrichment of soil from Phu Rua were mixed-culture that could not be isolated for pure culture either by using enriched media such as Blood agar and Brain heart infusion agar or using non-rich media such as nutrient agar. Therefore, only four isolates of *Bacillus* spp. were selected for preliminary use in mediator-less MFC. Since they grew very well and could be easily identified from contaminant.

4.2 Ferric reduction of electricity enriched isolates

Ferric reducing bacteria hold great promise as microbes that use in mediator-less MFC because they have the potential for use graphite electrode as a final electron acceptor in their respiratory system. Ferric reduction activity of all isolates was characterized under anaerobic condition by streaking pure cultures on NA plates containing ferric citrate. FRB could use ferric ions (Fe^{3+}) as the electron acceptor under the anaerobic condition, thus Fe^{3+} were reduced to be ferrous ions (Fe^{2+}) as shown in Fig 4.6. Pure isolate of FRB changed the reddish-brown color of NA with ferric citrate into the light green-colored. There are 8 isolates of sediment from pond and 31 isolates of sub-sediment from Koh Larn performed ferric reduction activity as shown in Appendix B1-2.

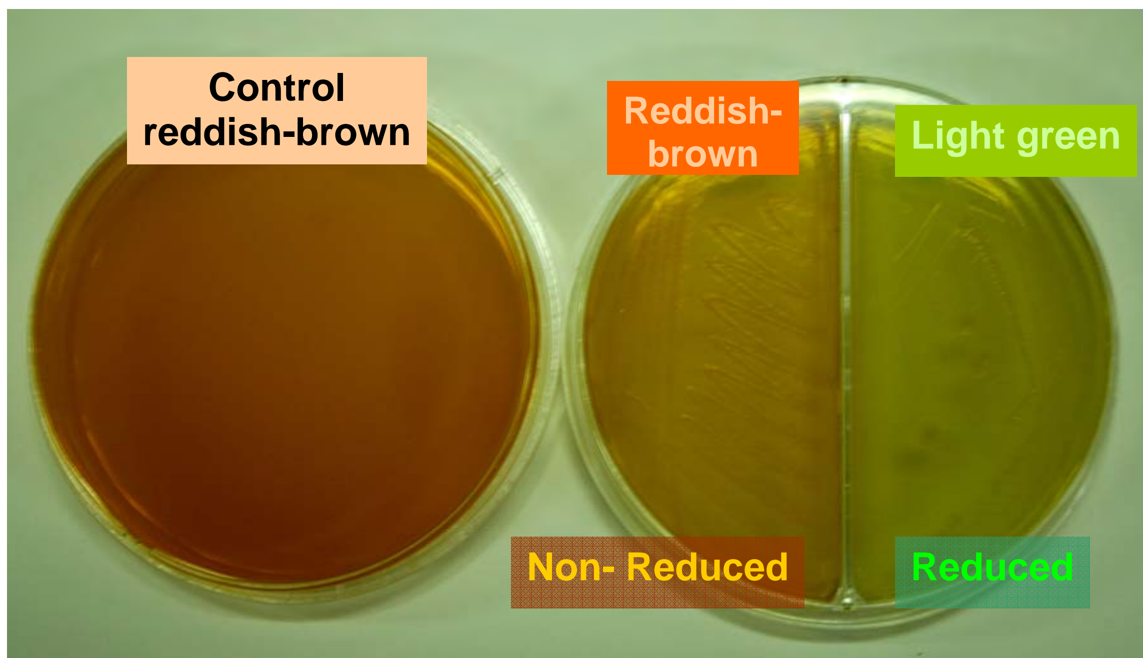

Figure 4.6 Ferric reduction activity on NA plate containing ferric citrate

4.3 Performance of pure isolates in mediator-less MFC

4.3.1 Potential development in Acrylic model of mediator-less MFC

Acrylic chambers of MFC were designed and constructed by following the procedure of Ouitrakul (2007) [14]. The potentials development of yeast, *Saccharomyces cerevisiae* were compared with four isolates of FRB from Phu Rua in mediator-less MFC, acrylic chamber as shown in Fig 4.7. The open circuit voltage—potentials that develop without load or resistance connection—of MFCs gradually increased after all components were sequentially added into the system. B1-isolate gives the highest maximum open circuit voltage of 0.511 volt and 0.387, 0.441, 0.481 and 0.274 volt for B2-B4 isolates and yeast, respectively (Fig. 4.7). After 15 hours, concentration of $K_3Fe(CN)_6$ in cathode chamber slightly decreased; consequently, the open circuit potentials of MFCs gradually decreased. But after 10 hours, potentials of B2 isolate suddenly decreased because decreasing in the concentration of $K_3Fe(CN)_6$ were used by microbial contamination. Contaminations that occurred in cathodic compartment were observed by changing of color from yellow to

colorless, and increasing of turbidity. As the results, contaminants reduce $K_3Fe(CN)_6$ and give the effect on potential development in mediator-less MFC. In mediator enhanced MFC, contamination cause less effect than mediator-less system. When methylene blue were added in the MFC system, potential reached steady value ~0.35 volt within half an hour. Total time for experimental procedure could be completed in 3-4 hours as the same period of time as mentioned earlier [14]. However, in mediator-less system, it took 10 hours to reach steady potentials (Fig. 4.7).

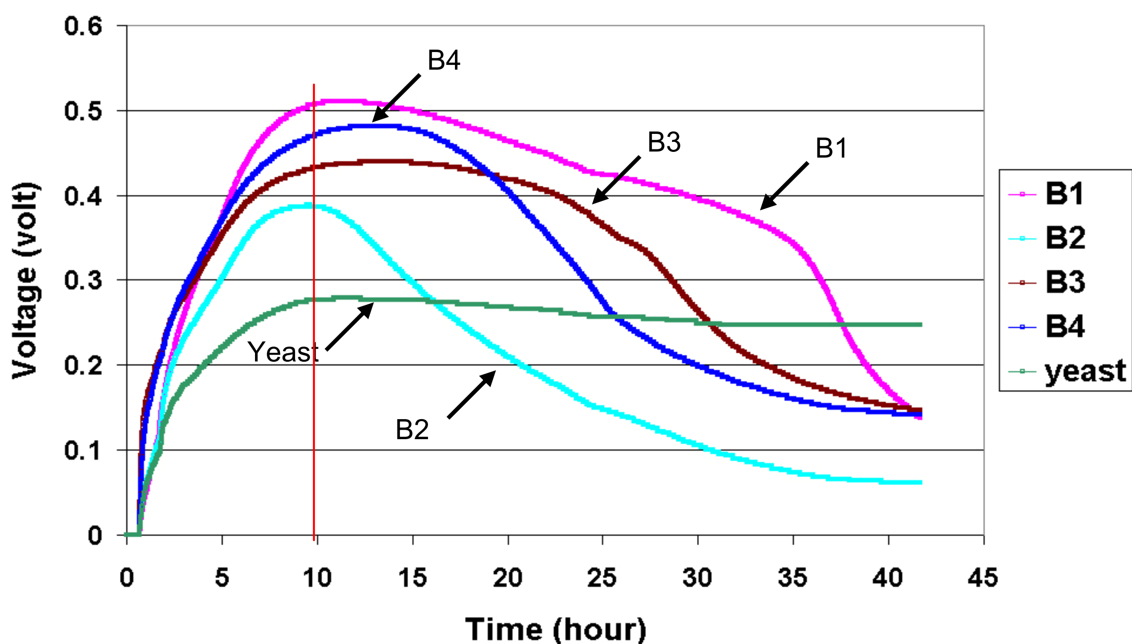

Figure 4.7 The open circuit voltage (volt) obtained from B1-B4 isolates and *Saccharomyces cerevisiae* in mediator-less MFC as a function of time (hour).

Therefore, mediator-less MFC chamber required sterile system for conducting the experiment that used pure culture as an inoculum, and for long period operation. Glass I and Glass II chamber were designed and constructed for sterile system that suitable for microbiological aseptic techniques.

In this study, only certain bacterial isolates were tested in different designed model of mediator-less MFC as shown in Table 4.2

Table 4.2 Number of bacterial isolates from different source and their identification of the best performance of the isolates

Source	Number of bacterial isolates	FRB	Non-FRB	Number of isolate tested in each model			Identified as
				Acrylic	Glass I	Glass II	
Phu Rua (PR) - soil	4	4	-	4	1 [c]	ND	*Bacillus* sp. [c]
Koh Larn (KL) - sub-sediment	40	31	9	ND	40	12 [e]	*Proteus vulgaris* [f]
Physic I CU (PH) - sediment	28	8	20	ND	8 [d]	ND	*Lactococcus garvieae* [g]
Control - baker yeast [a] - *E. coli* [b]	 - -	 - -	 - 1	 1 ND	 1 1	 ND ND	 ND ND

a – *Saccharomyces cerevisiae* (Fermipan®)

b – *E. coli* ATCC 25922

c – the highest open circuit voltage

d – only FRB

e – generated high current density in Glass I

f – generated the highest current density in Glass II

g – generated the highest current density

ND – not done

4.3.2 Potential, current density and power density development in Glass I model of mediator-less MFC

In the sterile system, Glass I model of mediator-less MFC were used to evaluate the performance of electricity enriched isolate on potential, current density and power density output. The current density and power density output were measured and calculated by connecting the various external resistances across the anode and cathode from 100 kΩ to1 kΩ.

First, potential development in Glass I model when using Yeast, *E. coli* and B1 isolate as biocatalysts were shown in Fig 4.8. After adding all components into both anode and cathodic compartment, MFC systems were operated for 12 hours, and then various load resistance were connected between anode and cathode, leading to actual voltage that system could supply to individual load resistance. The actual voltage from each load resistance was collected and used to calculate the current density and power density by using the averaged actual voltage for one hour before connecting to other load.

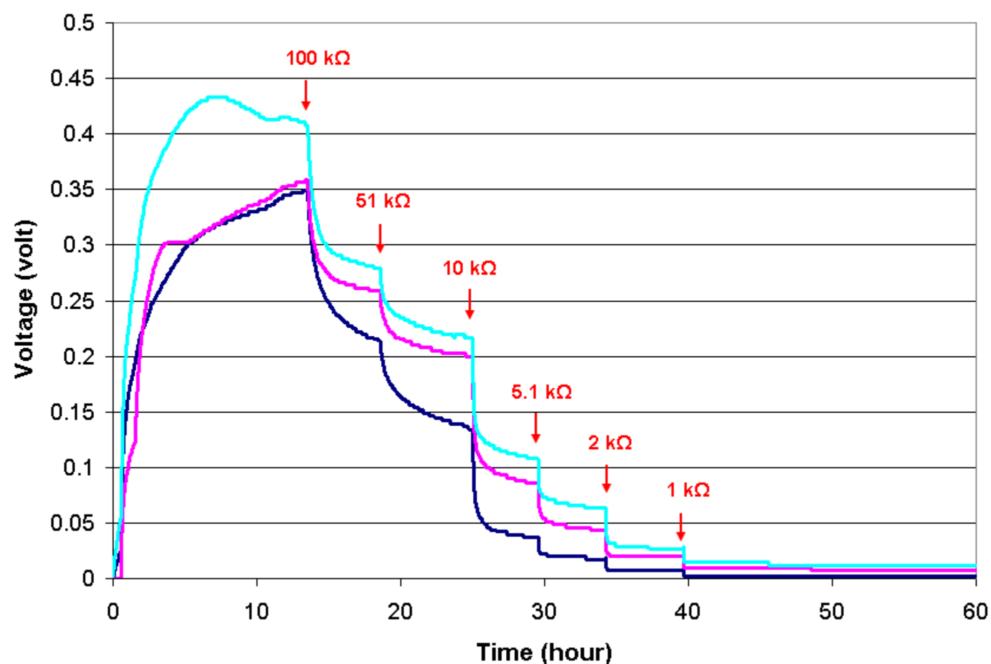

Figure 4.8 Voltage generations in Glass I model of mediator-less MFC when using yeast *S. cerevisiae*, *E. coli* and B1 isolate as biocatalysts

Relationship between actual voltage of each load resistance and current density was shown in Fig 4.9.

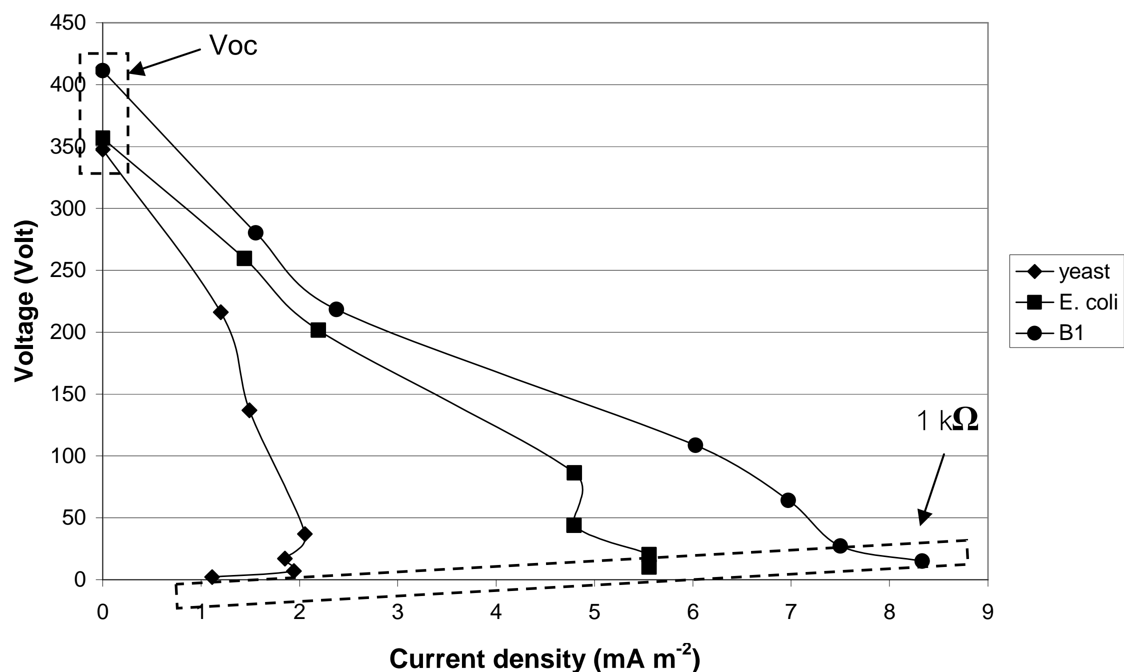

Figure 4.9 Voltage vs. current density of mediator-less MFC when using Yeast, *E. coli* and B1 isolate as biocatalysts

Figure 4.9 indicates the voltage – current density characteristic of the mediator-less MFC when using Yeast, *E. coli* and B1 isolate as biocatalysts. These results show that B1 isolate give the highest open circuit up to 411 mV and 356, 347 mV for *E. coli* and yeast, *S. cerevisiae,* respectively. For current density output of mediator-less MFC, it is found that B1 isolate give highest output of 8.33 mA m^{-2} at 1 kΩ of resistance and 5.55, 1.11 mA m^{-2} for *E. coli* and *S. cerevisiae,* respectively at the same load resistance.

As the results, B1 isolate generates the highest open circuit voltage and current density over the output from *E. coli* and *S. cerevisiae.* These results indicate that FRB-B1 isolate can transfer electron better than *E. coli* and *S. cerevisiae* in mediator-less MFC. To investigate the opportunity of electricity enriched bacteria for use in mediator-less MFC, pure culture of all isolates were tested for their ability to develop electricity in these Glass I mediator-less MFC.

Second, pure culture of 40 isolates of ferric reducing bacteria and non-ferric reducing bacteria from Koh Larn were determined the ability of their self-mediate electron transfer in mediator-less MFC. Current density and power density of 40 isolates that used to compare the performance of each isolate were calculated at 1 kΩ of load connection. The result are plotted between electric current (I) selection (electric current that used for selection) and Vmax (open circuit voltage), I selection and current density, and I selection and power density of 40 isolates were shown in Figs 4.10-15.

After 40 isolates from sub-sediment from Koh Larn were classified according to the ability of ferric reduction and Gram's staining. It was found that either ferric reducing or non-ferric reducing bacteria can produce Vmax (open circuit voltage) ~300-500 mV as shown in Appendix C1. Whereas, the highest Vmax of 500 mV was produced by Gram's positive bacteria as shown in Figs.4.10 and 4.11. Current density output from Gram's positive bacteria were less than 6 mA m^{-2} while Gram's negative bacteria gave ~4-14 mA m^{-2} as shown in Figs 4.12 and 4.13. Nevertheless, ferric reducing bacteria that were Gram's negative tentatively performed current density ~11-13 mA m^{-2}. Power density output of all isolate was concomitant of current density that Gram positive bacteria produced less than 0.06 mW m^{-2} but Gram's negative, ferric reducing bacteria gave the highest of 0.32 mW m^{-2} which was greater than Gram's positive for ~5 times.

As the results, Gram's negative bacteria were enriched by electric current less than 90 mA whereas Gram's positive bacteria were enriched when using electric current higher than 90 mA. Gram's positive bacteria isolated from Koh Larn were spore forming bacilli, facultative anaerobic bacteria and tentatively identified as *Bacillus* spp. which have a low electron transferring to electrode in anaerobic environment reflecting in low current density and power density output. As in previous studies (Pham, T.H., *et al.* 2008), facultative anaerobic, Gram's positive bacteria, *Brevibacillus* sp. PTH1 which was observed in mediator-lees MFC system also had poor generated electric current in anaerobic environment. However, its current generation was enhanced by phenazine-1-carboxamide, phenazine compound which produced by *Pseudomonas* sp. [31].

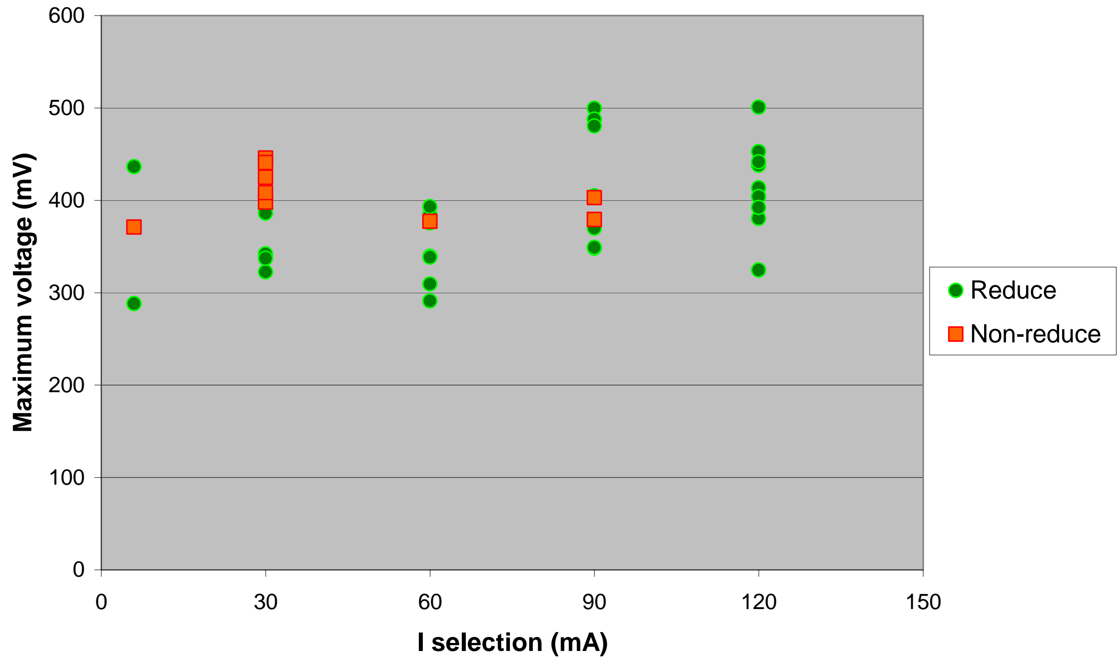

Figure 4.10 The electric current (I) selection vs Vmax on ferric reducing and non-ferric reducing bacteria among 40 isolates from Koh Larn

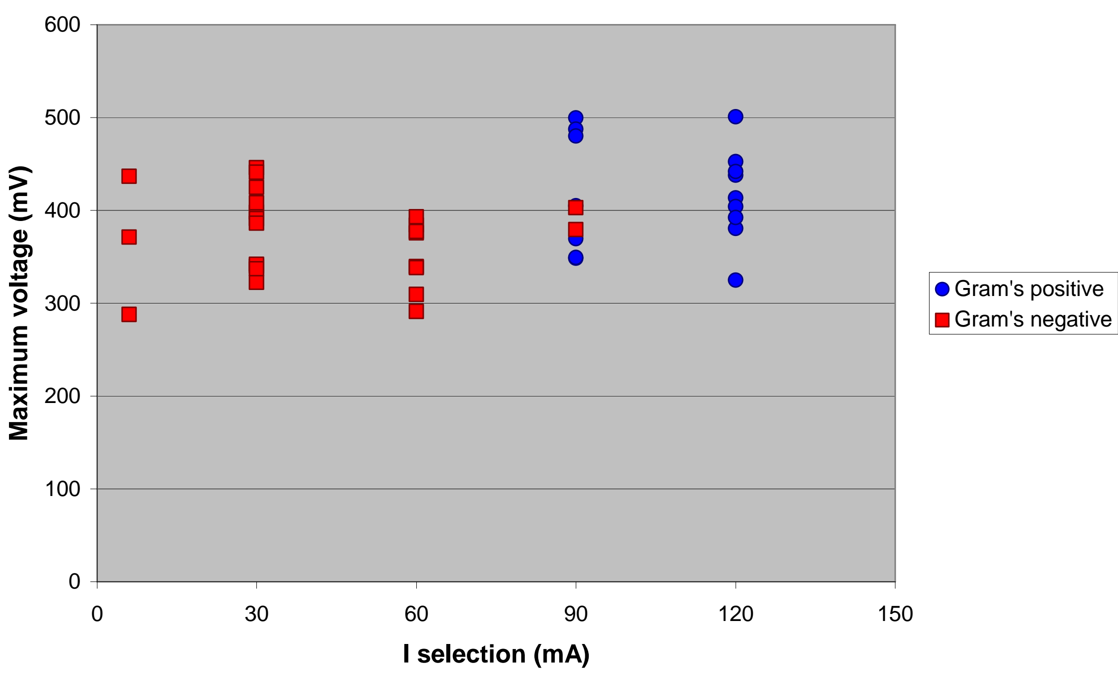

Figure 4.11 The electricity current (I) selection vs Vmax on ferric reducing and non-ferric reducing either Gram's positive or negative bacteria among 40 isolates from Koh Larn

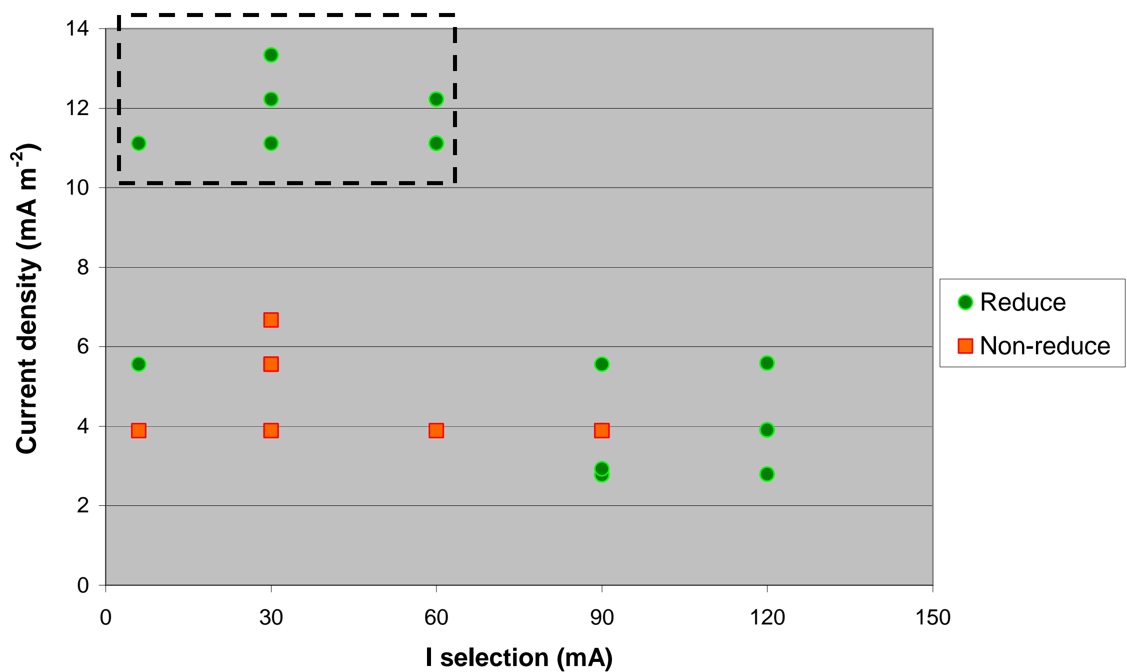

Figure 4.12 The electricity current (I) selection vs current density on ferric reducing and non-ferric reducing bacteria among 40 isolates from Koh Larn

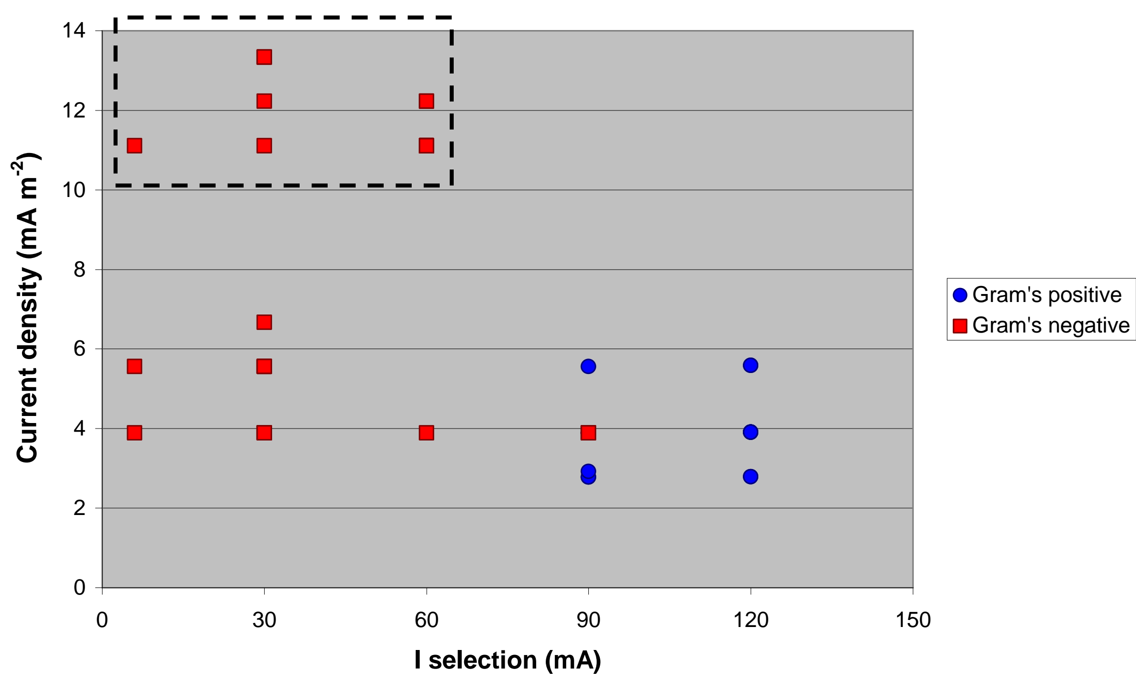

Figure 4.13 The electric current (I) selection vs current density on ferric reducing and non-ferric reducing either Gram's positive or negative bacteria among 40 isolates from Koh Larn

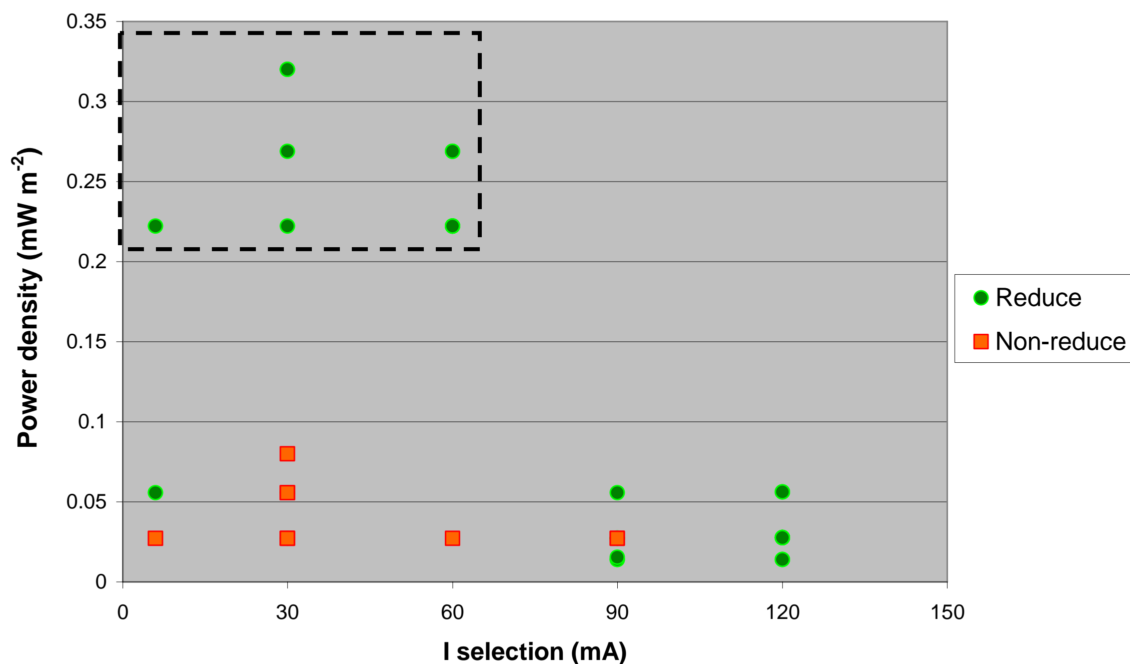

Figure 4.14 The electric current (I) selection vs power density on ferric reducing and non-ferric reducing bacteria among 40 isolates from Koh Larn

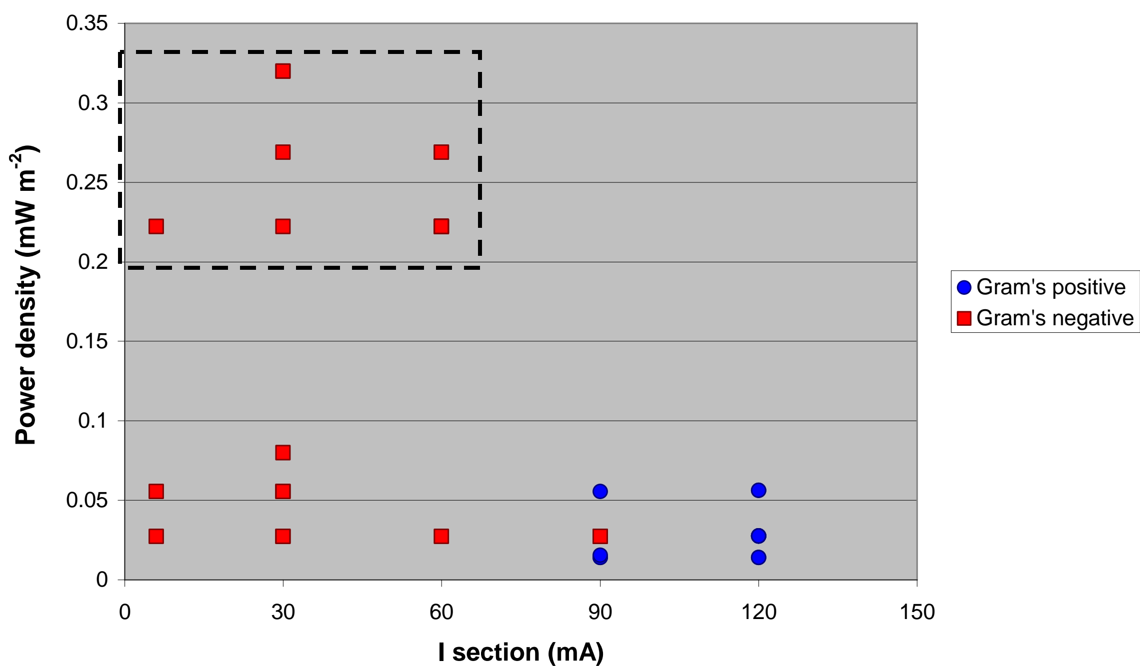

Figure 4.15 The electricity current (I) selection vs power density on ferric reducing and non-ferric reducing either Gram's positive or negative bacteria among 40 isolates from Koh Larn

Gram's negative, ferric reducing bacteria generated the highest current density output and either Vmax or current density output generated from ferric reducing bacteria was greater than non-ferric reducing bacteria. It can be concluded that ferric reduction activity has more impact than current that used for selection and enrichment on the electricity generation of isolates in mediator-less MFC. These results agree with previous research that ferric reducing bacteria have a potential to self-mediated electron transfer for use in mediator-less MFC [7]. Therefore, only ferric reducing bacteria that isolated from Physic I CU were tested in Glass I model, mediator-less MFC.

Pure culture of 8 isolates of ferric reducing bacteria from Physic I CU were determined the ability of their self-mediate electron transfer in Glass I, mediator-less MFC. Based on calculation at 1 kΩ of load connection, among 8 isolates, current density and power density performance obtained from each isolate were compared. Electricity output including, Vmax (open circuit voltage), Voltage, current density and power density are shown in Table 4.3.

Table 4.3 Bacterial Characterization and Electricity output of 8 isolates from Physic I CU

Bacterial isolate	Morphology	Gram's staining	Electricity output			
			Vmax (mV)	V at 1kΩ (mV)	i at 1kΩ (mA m^{-2})	P at 1kΩ (mW m^{-2})
PH2	Cocci	positive	374.53	5.00	2.78	0.014
PH5	**Cocci**	**positive**	**462.00**	**12.00**	**6.67**	**0.080**
PH7	Rod	negative	296.15	5.00	2.78	0.014
PH10	Rod	negative	305.00	7.00	3.89	0.027
PH11	Rod	negative	366.80	8.47	4.70	0.040
PH12	Rod	negative	338.11	2.00	1.11	0.002
PH13	Rod	negative	374.76	5.00	2.78	0.014
PH17	Rod	negative	379.96	5.00	2.78	0.014

Gram' positive cocci, PH5 isolate give the highest electricity output over other isolates (Table 4.3). PH5 generated Vmax of 462 mV and 12 mV, 6.67 mA m^{-2} and 0.08 mW m^{-2} for V (1kΩ), current density and power density, respectively. This results confirmed previous report (Rabaey, K., *et al*. 2004) that Gram's positive cocci such as *Enterococcus gallinarum* and *Lactococcus lactis* became a majority in mediator-less MFC that long operated with mix consortium of granular sludge from water treatment system [49]. PH5 was preliminary identified as *Lactococcus* sp. by biochemical tests and confirmed by 16s rDNA analysis.

4.3.3 Potential, current density and power density development in Glass II model of mediator-less MFC

Current generations of 12 isolates from Koh Larn that gave high current density and power density in Glass I model were tested in Glass II model which performed more anaerobic than Glass I by using L-cysteine HCl as oxygen scavenger. The performance of electricity output of 12 isolates in Glass II chamber compared with Glass I are shown in Table 4.4.

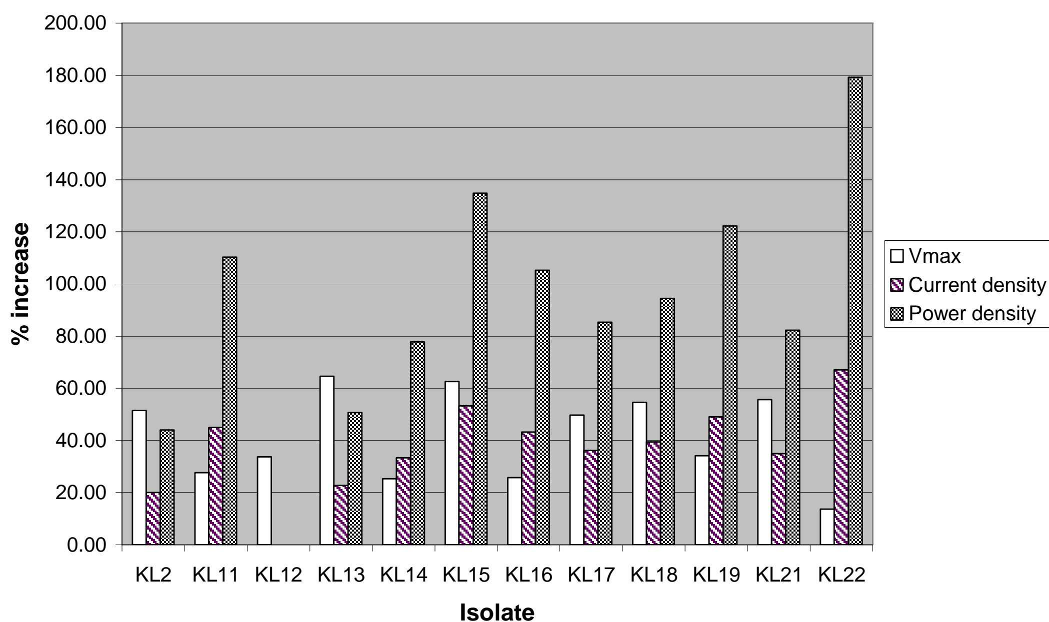

Figure 4.16 Percentage of increasing of electricity output from Glass II that is different from Glass I

Table 4.4 Summary of electricity output of 12 isolates from Koh Larn in mediator-less MFC, Glass I and Glass II model

Isolate	Glass I				Glass II				Differentiation (%)		
	Vmax (mV)	V at 1kΩ (mV)	i at 1kΩ (mA m^{-2})	P at 1kΩ (mW m^{-2})	Vmax (mV)	V at 1kΩ (mV)	i at 1kΩ (mA m^{-2})	P at 1kΩ (mW m^{-2})	Vmax	i	P
KL2	288.00	20.0	11.11	0.2222	436.41	24.00	13.33	0.3200	51.53	20.00	44.00
KL11	390.99	20.0	11.11	0.2222	498.96	29.00	16.11	0.4672	27.62	45.00	110.25
KL12	342.00	24.0	13.33	0.3200	457.59	24.00	13.33	0.3200	33.80	0.00	0.00
KL13	337.16	22.0	12.22	0.2689	555.26	27.00	15.00	0.4050	64.69	22.73	50.62
KL14	386.00	24.0	13.33	0.3200	484.00	32.00	17.78	0.5689	25.39	33.33	77.78
KL15	309.35	20.0	11.11	0.2222	502.98	30.65	17.03	0.5220	62.59	53.26	134.88
KL16	375.70	20.0	11.11	0.2222	472.23	28.66	15.92	0.4562	25.69	43.28	105.29
KL17	339.35	22.0	12.22	0.2689	507.96	29.95	16.64	0.4983	49.68	36.14	85.33
KL18	291.07	20.0	11.11	0.2222	450.00	27.89	15.49	0.4321	54.60	39.44	94.43
KL19	384.26	22.0	12.22	0.2689	515.44	32.79	18.22	0.5974	34.14	49.06	122.18
KL21	338.22	20.0	11.11	0.2222	526.45	27.00	15.00	0.4050	55.65	35.00	82.25
KL22	393.00	20.0	11.11	0.2222	447.00	33.42	18.57	0.6206	13.74	67.12	179.28

In Glass II model, KL22 gives the highest of 18.57 mA m^{-2} and 0.62 mW m^{-2} current density and power density, respectively. Fig 4.16 indicates the over all increasing of electricity output from 12 isolates except KL12 that did only Vmax increase. The highest percentages of increasing in current density of 67.12% and power density of 179.28 % is performed by isolate KL22.

From results as mentioned above, anaerobic condition in anodic compartment enhanced electron transfer to anode electrode resulted in the increasing of electricity output [50]. Viable cell of the inoculum before and after the experiment were determined by total plate count (TPC) and pH of electrolyte were also measured by pH meter as shown in Appendix D. After finishing the experiment (3 days), viable bacterial cell decreased because electrolyte in anodic compartment only contained glucose as carbon source and none of nitrogen source and other growth factor for cell proliferation existed. Decreasing of glucose in this MFC system confirmed that glucose was consumed and used as electron donor by all isolates for generating electricity as shown in Appendix E. All of 12 isolates were Gram's negative rod, swarming colony facultative anaerobic bacteria and tentatively identified as *Proteus* spp. KL14 and KL22 give high current density of 13.33 and 11.11 mAm^{-2}, respectively in Glass I chamber and also give high current density of 17.78 and 18.57 mA m^{-2}, respectively in Glass II. These two isolates, therefore, were subjected to be identified.

4.4 Identification of electricity enriched bacteria

Isolate B1 from Phu Rua, PH5 from Physic I CU, KL14 and KL22 from Koh Larn were identified by 16S rDNA analysis. Genomic DNA of the selected bacteria was extracted and used as template for 16S rRNA gene amplification using polymerase chain reaction (PCR). Then, PCR products were submitted for sequencing 16S rDNA at Macrogen Inc. co. Ltd. (Seoul, Korea). After that the obtained sequences of B1, PH5, KL14 and KL22 were 1,262, 1,109, 890 and 1,080 bp, respectively. All sequences were blasted and compared with the database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov).

According to the result, sequencing of the partial 16S rRNA gene of KL14 (Appendix F1-2) gave 99% of identity to the partial 16S rRNA gene of *Proteus* sp., accession number EF426446.1 and E-value 0.0. As regard to, sequencing of the partial 16S rRNA gene of KL22 (Appendix F3-4) gave 98% of identity to the partial 16S rRNA gene of *Proteus vulgaris*, accession number DQ499636.1 and E-value 0.0. Furthermore, sequencing of B1 (Appendix F5-6) gave 100% of identity to the partial 16S rRNA gene of *Bacillus* sp., accession number D84630 and E-value 0.0. And the last sequencing of PH5 (Appendix F7-8) gave 100% of identity to the partial 16S rRNA gene of *Lactococcus garvieae*, accession number AB300504.1 and E-value 0.0.

Identification of KL14 and KL22 were confirmed by using rapid identification for *Enterobacteracae*, API 20E identification kit (BioMérieux, France). The results showed in Appendix G. Results from API kit were interpreted by using program API®WEB (bioMérieux, France) that KL14 and KL22 were identified as *Proteus* sp. and *Proteus vulgaris*, respectively. The identity percentage (%ID) of KL14 and KL22 were 97.7% and 99.6%, they offered an excellent identification profile. From morphological characterization, 16S rRNA gene analysis and biochemical test using API 20E identification kit, it could imply that KL14 and KL22 were identified as *Proteus* sp. and *Proteus vulgaris*, respectively.

Table 4.5 Identification of B1, PH5, KL14 and KL22

Isolate	Morphological characterization	Identification procedure	
		16S rDNA analysis	API kit
B1	Gram's positive bacilli	*Bacillus* sp.	ND
PH5	Gram's positive cocci	*Lactococcus gravieae*	ND
KL14	Gram's negative rod	*Proteus* sp.	*Proteus* sp.
KL22	Gram's negative rod	*Proteus vulgaris*	*Proteus vulgaris*

ND – not done

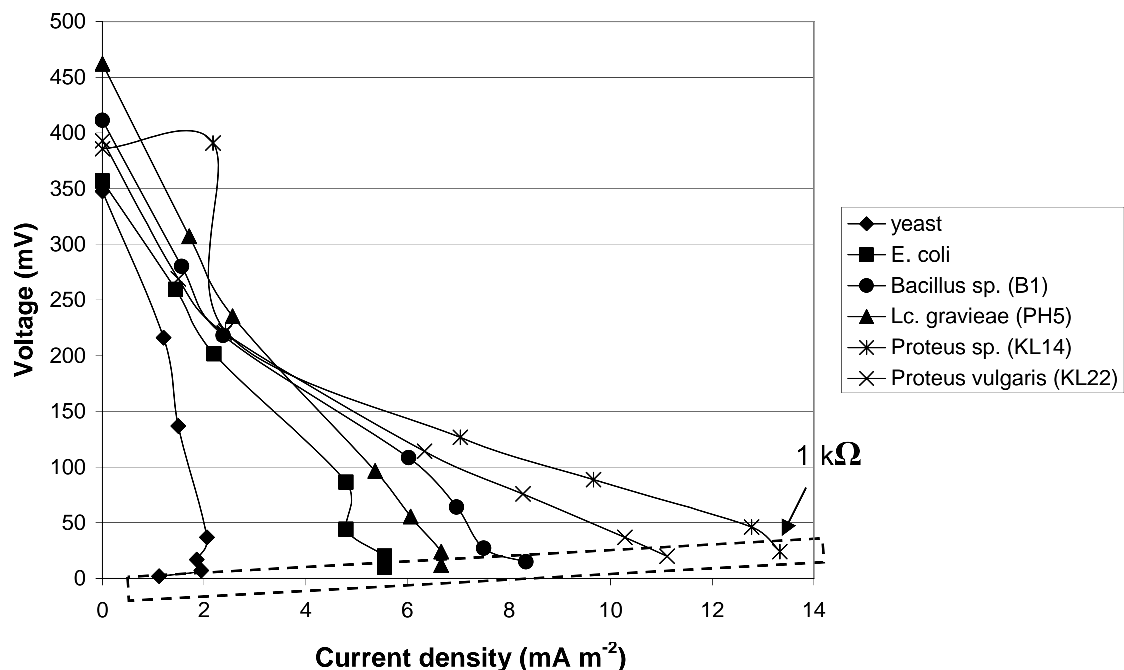

Figure 4.17 Voltage vs. current density of Glass I, mediator-less MFC when using Yeast, *E. coli*, B1, PH5, KL14 and KL22 isolate as biocatalysts

Figure 4.17 indicates the voltage – current density characteristic of the mediator-less MFC when using Yeast, *E. coli* and B1, PH5, KL14 and KL22 isolates as biocatalysts. For current density output of mediator-less MFC, it is found that KL14 isolate give highest output of 13.33 mA m^{-2} at 1 kΩ of resistance and 11.11, 8.33, 5.55, 1.11 mA m^{-2} for KL22, B1, PH5, ***E. coli*** and *S. cerevisiae,* respectively at the same load resistance.

As the results, Gram's negative, ferric reducing bacteria, KL14 and KL22 generated higher current density than those of Gram's positive, ferric reducing bacteria B1 and PH5. Ferric reducing bacteria generated current density greater than those of *E. coli* and yeast in mediator-less MFC. These results are consistent with previous research that yeast and *E. coli* did not directly transfer electron to electrode, and it required electron mediator for facilitating their electron transfer to the electrode. Moreover, many reports showed that Gram's negative, ferric reducing bacteria had three effective strategies to transfer electron to electrode in mediator-less system [43] but Gram's positive, ferric

reducing bacteria generated small amount of electric current [9, 49, 51] and also required electron mediator for improving their electron transfer [49, 51].

Geobacter sulferreducens [10] and *Rhodoferax ferrireducens* [12] generated current density of 65 and 31 mA m^{-2}, respectively whereas KL22 isolate, *Proteus vulgaris* generated lower current density of 18.57 mA m^{-2}. The reason may be because *Proteus vulgaris* may not directly attach to the electrode enough as do G. *sulferreducens* and *R. ferrireducens.* Further improvements by optimizing the physical and chemical parameters of microbial fuel cells for the sustainable energy in the future are required.

CHAPTER V

CONCLUSION

1. Sterile system of microbial fuel cell was designed constructed by using glass ware and designated as Glass I and Glass II that suitable for microbiological aseptic techniques.
2. Ferric reduction activity has more impact than electricity current that used for selection and enrichment on the electricity generation of isolates in mediator-less MFC.
3. Gram's negative, ferric reducing bacteria have potential for use in mediator–less MFC because they can self-mediate electron transfer to anode better than Gram's positive, ferric reducing bacteria.
4. KL22 isolated from sub- sediment from Koh Larn was identified as *Proteus vulgaris* which generated the highest current density (18.57 mA m^{-2}) and power density (0.62 mW m^{-2}) in Glass II model.
5. Anaerobic condition is required for the effective electron transfer to anode electrode led to the increasing of electricity output.
6. This study is firstly reported that *Proteus vulgaris* can act as biocatalyst in mediator-less MFC system.

Future Suggestion

1. Strictly anaerobic system and techniques are required for isolation of anaerobe or strictly anaerobic bacteria that have better cell-electrode interaction than facultative anaerobic bacteria.
2. Electron micrograph of anode electrode of mediator-less MFC that use KL22 as biocatalyst is required for observation of interaction between KL22 and electrode.
3. Optimizations of the physical and chemical parameters are required for reducing the overpotentials or internal resistance and improving electric current generation.

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Appendices

Appendix A

Media for microorganisms

A1. Nutrient Broth or Nutrient Agar (Oxoid)

Approximate Formula * Per Liter		
Lab-Lemco powder	1.0	g
Yeast extract	2.0	g
Peptone	5.0	g
Sodium Chloride	5.0	g
Agar (for Nutrient Agar)	15.0	g

pH 7.2 ± 0.2

Autoclave at 121 °C and pressure at 15 pounds/square inch for 15 minutes

A2. Tryptic Soy Agar (TSA) (Difco)

Approximate Formula * Per Liter		
Tryptone	17.0	g
Soytone	3.0	g
Dextrose	2.5	g
Sodium chloride (NaCl)	20.0	g
Di-Potassium hydrogenphosphate (K_2HPO_4)	2.5	g
Agar	15.0	g

pH 7.3 ± 0.2

Autoclave at 121 °C and pressure at 15 pounds/square inch for 15 minutes

A3. Eosin Methylene Blue Agar, Modified,Holt-Harris and Teague (EMB) (Difco)

Approximate Formula * Per Liter

Pancreatic Digest of Gelatin	10.0	g
Lactose	5.0	g
Sucrose	5.0	g
Dipotassium Phosphate	2.0	g
Eosin Y	0.4	g
Methylene Blue	65.0	mg
Agar	13.5	g

pH 7.2 ± 0.2

All of ingredients were dissolved in 1 L of distilled water. Mix it thoroughly and adjust pH to 7.2 ± 0.2. Heat in order to completely dissolve and autoclave at 121°C and pressure at 15 pounds/square inch for 15 minutes.

A4. Yeast Peptone Dextrose broth (YPDB) (Difco®)

Approximate Formula * Per Liter

Yeast extract	10.0	g
Peptone	20.0	g
Dextrose	20.0	g

pH 6.5 ± 0.2

Autoclave at 121 °C and pressure at 15 pounds/square inch for 15 minutes

Appendix B

Bacterial isolates

Table B1 Morphological characterization and ferric reduction activity of 28 isolates from pond in front of Physic I building, Chulalongkorn University

Isolate	Gram staining	Cell morphology	Ferric reduction
PH1	Negative	Rod	-
PH2	Positive	Cocci	+
PH3	Negative	Rod	-
PH4	Negative	Rod	-
PH5	Positive	Cocci	+
PH6	Negative	Rod	-
PH7	Negative	Rod	+
PH8	Negative	Rod	-
PH9	Negative	Rod	-
PH10	Negative	Rod	+
PH11	Negative	Rod	+
PH12	Negative	Rod	+
PH13	Negative	Rod	+
PH14	Negative	Rod	-
PH15	Negative	Rod	-
PH16	Negative	Rod	-
PH17	Negative	Rod	+
PH18	Negative	Rod	-

- non-ferric reduction activity

+ ferric reduction activity

Table B1 Morphological characterization and ferric reduction activity of 28 isolates from pond in front of Physic I building, Chulalongkorn University (continued)

Isolate	Gram staining	Cell morphology	Ferric reduction
PH19	Negative	Rod	-
PH20	Negative	Rod	-
PH21	Negative	Rod	-
PH22	Negative	Rod	-
PH23	Negative	Rod	-
PH24	Negative	Rod	-
PH25	Negative	Rod	-
PH26	Negative	Rod	-
PH27	Negative	Rod	-
PH28	Negative	Rod	-

- non-ferric reduction activity

+ ferric reduction activity

Table B2 Current selection, morphological characterization and ferric reduction activity of 40 isolates from Koh Larn, Chonburi

Isolate	Current selection (mA)	Gram's staining	Cell morphology	Ferric reduction
KL1	6	Negative	Rod	-
KL2	6	Negative	Rod	+
KL3	6	Negative	Rod	+
KL4	30	Negative	Rod	-
KL5	30	Negative	Rod	+
KL6	30	Negative	Rod	+
KL7	30	Negative	Rod	-
KL8	30	Negative	Rod	-
KL9	30	Negative	Rod	-
KL10	30	Negative	Rod	-
KL11	30	Negative	Rod	+
KL12	30	Negative	Rod	+
KL13	30	Negative	Rod	+
KL14	30	Negative	Rod	+
KL15	60	Negative	Rod	+
KL16	60	Negative	Rod	+
KL17	60	Negative	Rod	+
KL18	60	Negative	Rod	+
KL19	60	Negative	Rod	+
KL20	60	Negative	Rod	-

- non-ferric reduction activity

+ ferric reduction activity

Table B2 Current selection, morphological characterization and ferric reduction activity of 40 isolates from Koh Larn, Chonburi (continued)

Isolate	Current selection (mA)	Gram's staining	Cell morphology	Ferric reduction
KL21	60	Negative	Rod	+
KL22	60	Negative	Rod	+
KL23	90	positive	Rod	+
KL24	90	negative	Rod	-
KL25	90	positive	Rod	+
KL26	90	positive	Rod	+
KL27	90	positive	Rod	+
KL28	90	negative	Rod	-
KL29	90	positive	Rod	+
KL30	90	positive	Rod	+
KL31	90	positive	Rod	+
KL32	120	positive	Rod	+
KL33	120	positive	Rod	+
KL34	120	positive	Rod	+
KL35	120	positive	Rod	+
KL36	120	positive	Rod	+
KL37	120	positive	Rod	+
KL38	120	positive	Rod	+
KL39	120	positive	Rod	+
KL40	120	positive	Rod	+

- non-ferric reduction activity

+ ferric reduction activity

Appendix C

Electricity output

Table C1 Summary of electricity output of 40 isolates from Koh Larn

Electricity output		Classification			
		Ferric reduction		Gram's staining	
		Reduced (n=31)	Non-reduced (n=9)	Gram's negative (n=24)	Gram's positive (n=16)
Open circuit voltage (mV)	max	500.62	445.76	445.76	500.62
	min	288.00	370.96	288.00	324.63
	average	385.83	405.34	371.75	417.93
	SD	59.44	27.39	45.04	56.29
Current density (mA/m^2)	max	13.33	6.67	13.33	5.58
	min	2.78	3.89	3.89	2.78
	average	7.04	4.57	8.22	3.87
	SD	3.94	1.07	3.74	0.98
Power density (mW/m^2)	max	0.32	0.08	0.32	0.06
	min	0.0139	0.0272	0.03	0.01
	average	0.1161	0.0394	0.15	0.03
	SD	0.1115	0.0196	0.11	0.01

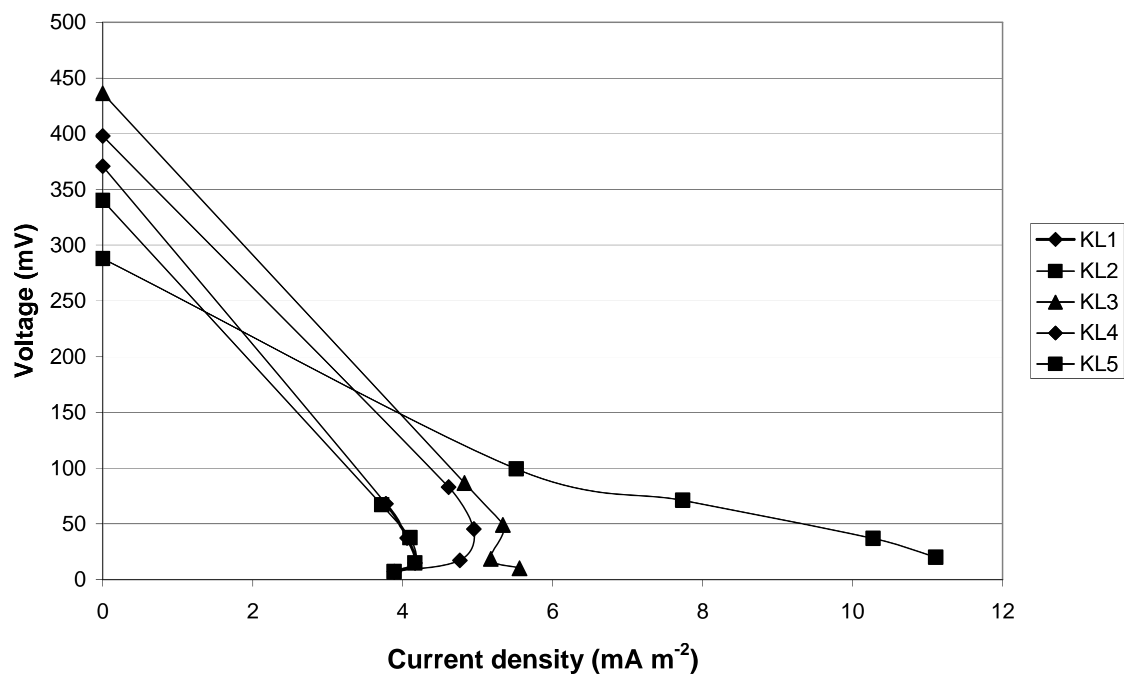

Figure C1 Voltage vs. current density of Glass I, mediator-less MFC when using K1-K5 as biocatalysts

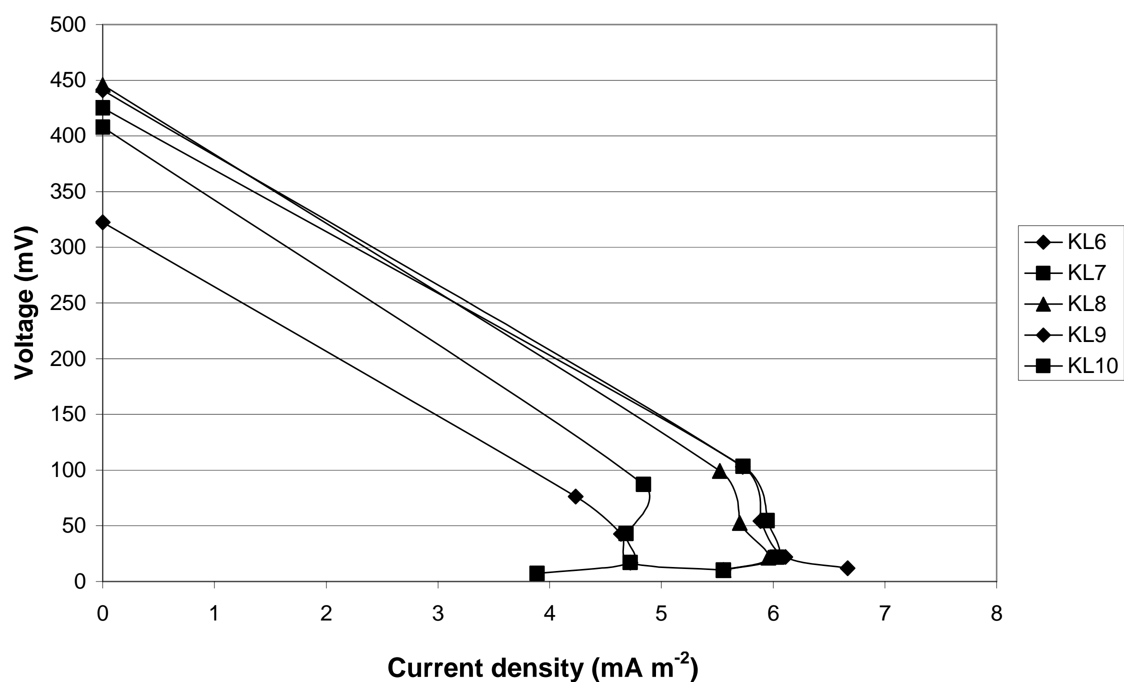

Figure C2 Voltage vs. current density of Glass I, mediator-less MFC when using K6-K10 as biocatalysts

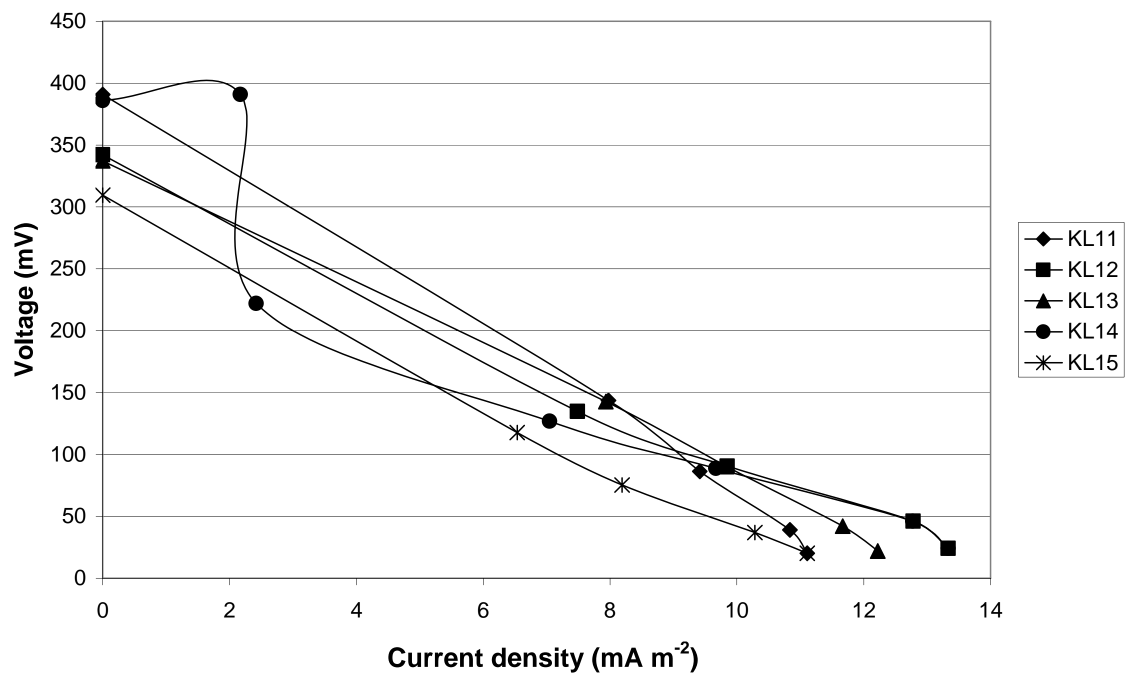

Figure C3 Voltage vs. current density of Glass I, mediator-less MFC when using K11-K15 as biocatalysts

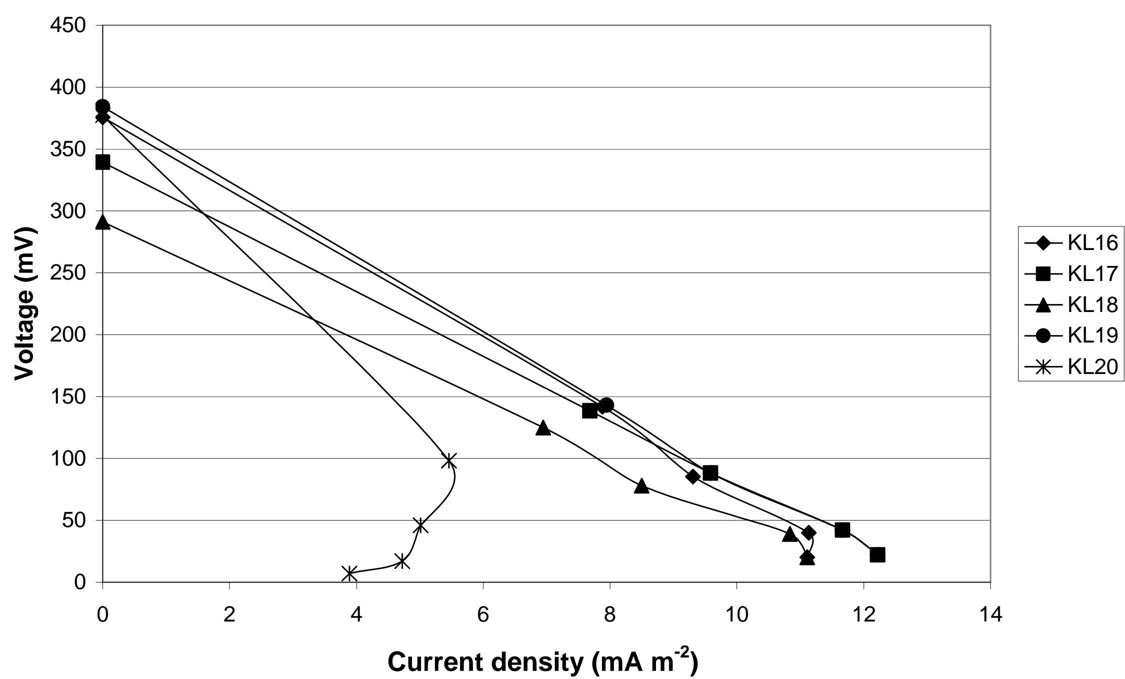

Figure C4 Voltage vs. current density of Glass I, mediator-less MFC when using K16-K20 as biocatalysts

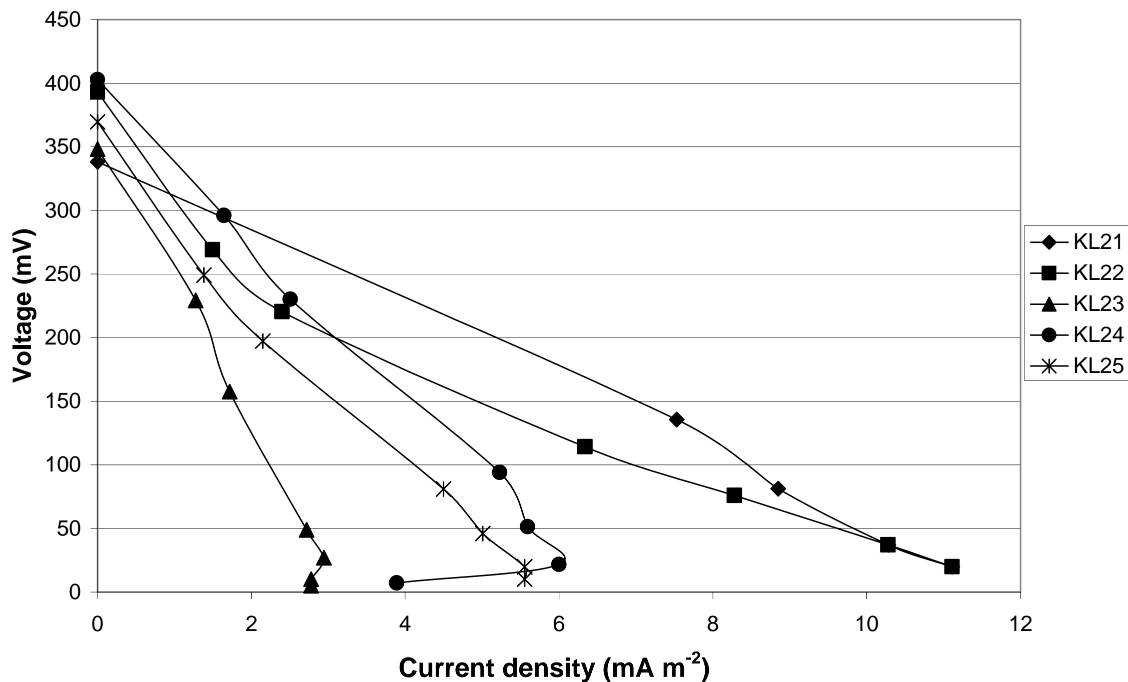

Figure C5 Voltage vs. current density of Glass I, mediator-less MFC when using K21-K25 as biocatalysts

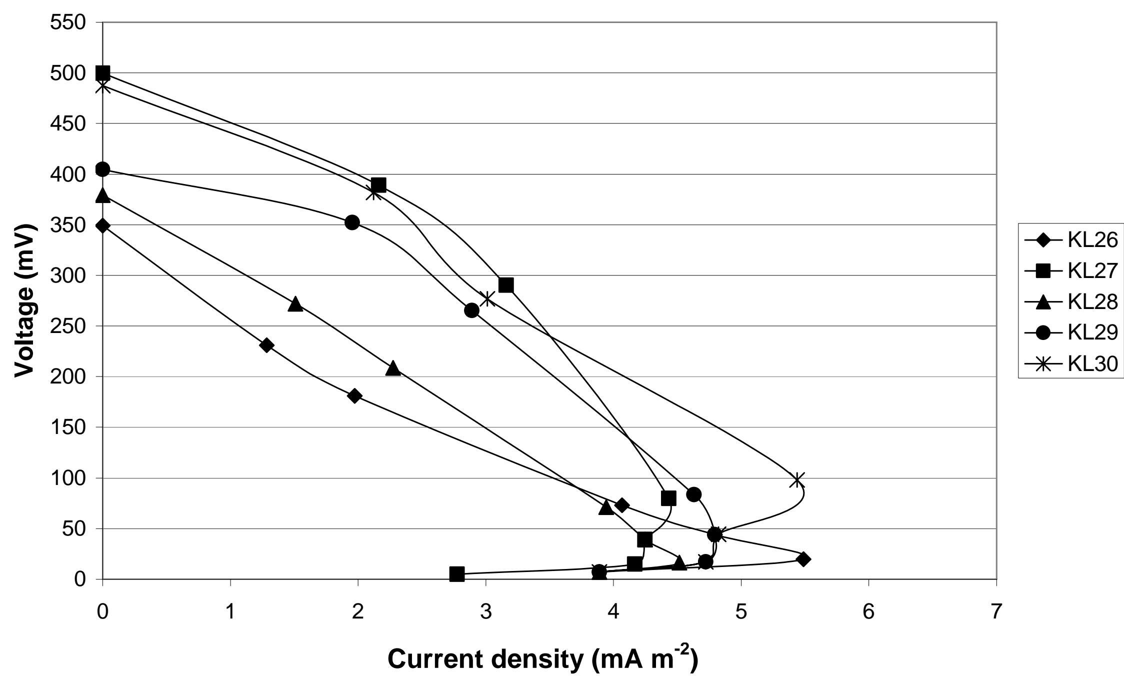

Figure C6 Voltage vs. current density of Glass I, mediator-less MFC when using K26-K30 as biocatalysts

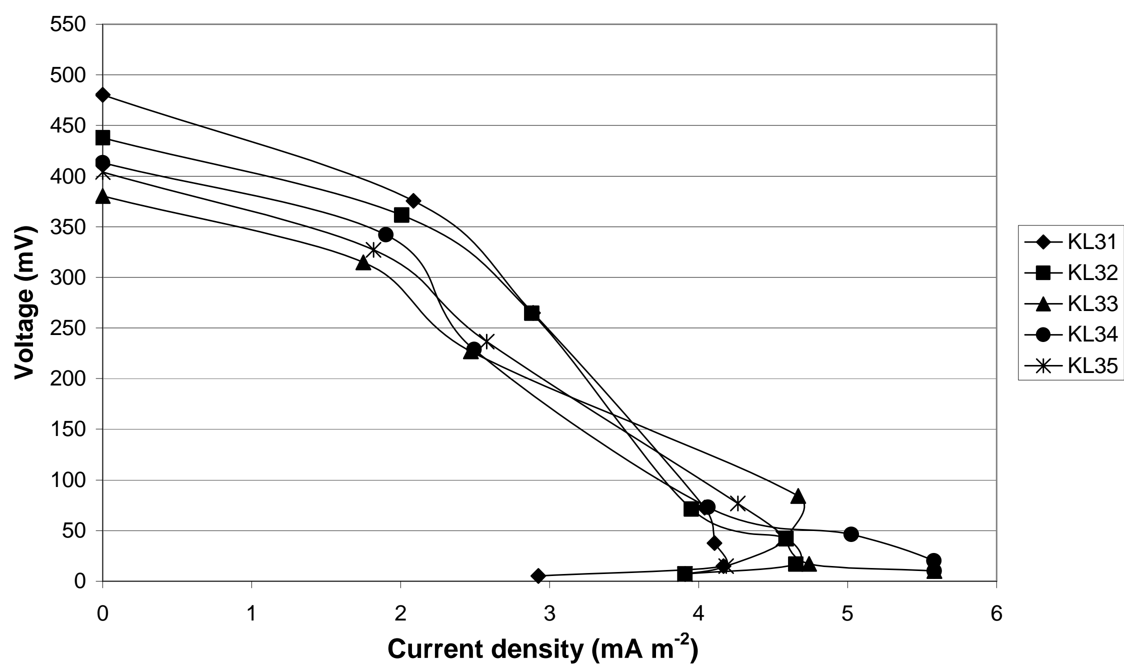

Figure C7 Voltage vs. current density of Glass I, mediator-less MFC when using K31-K35 as biocatalysts

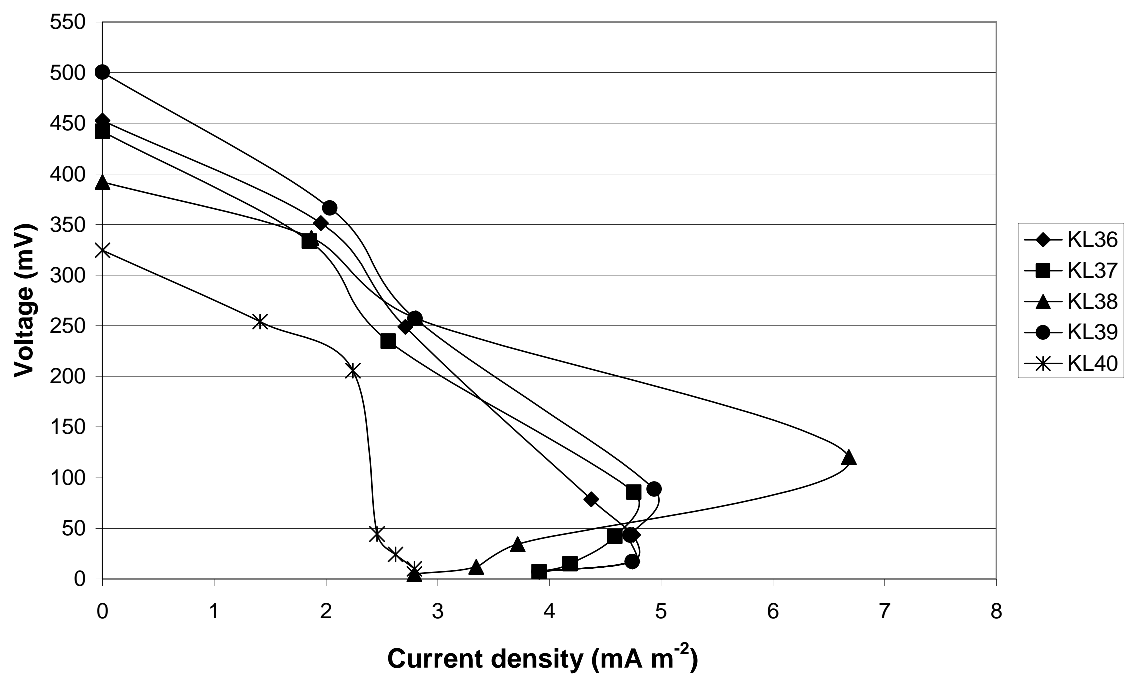

Figure C8 Voltage vs. current density of Glass I, mediator-less MFC when using K36-K40 as biocatalysts

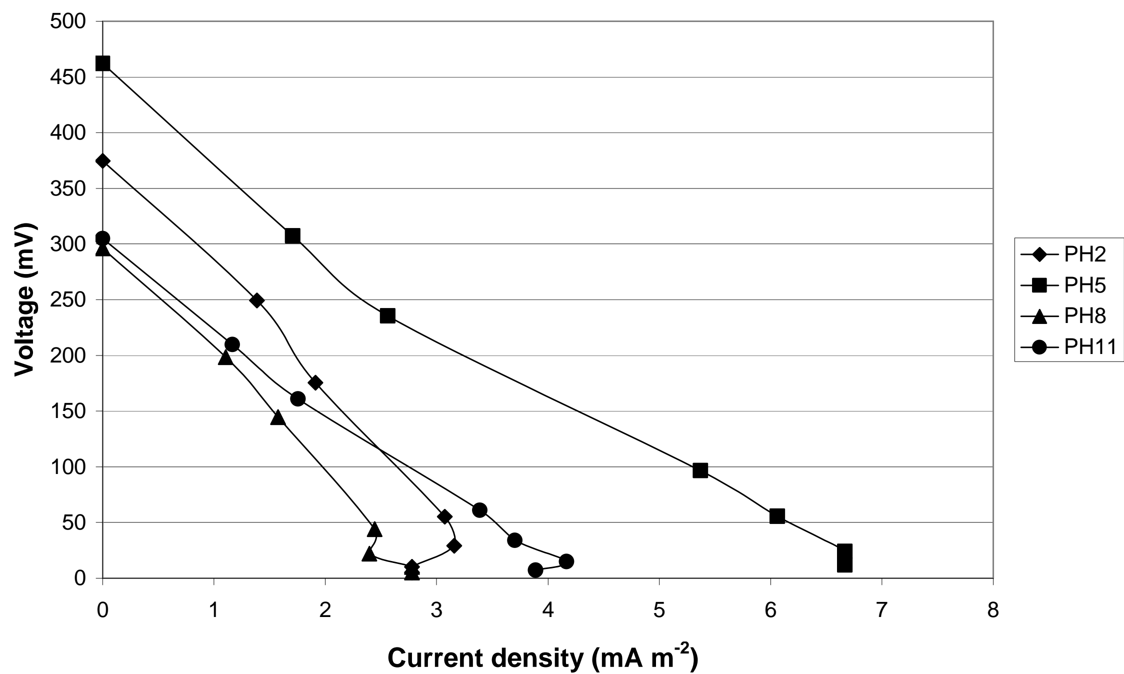

Figure C9 Voltage vs. current density of Glass I, mediator-less MFC when using PH2, PH5, PH8 and PH11 as biocatalysts

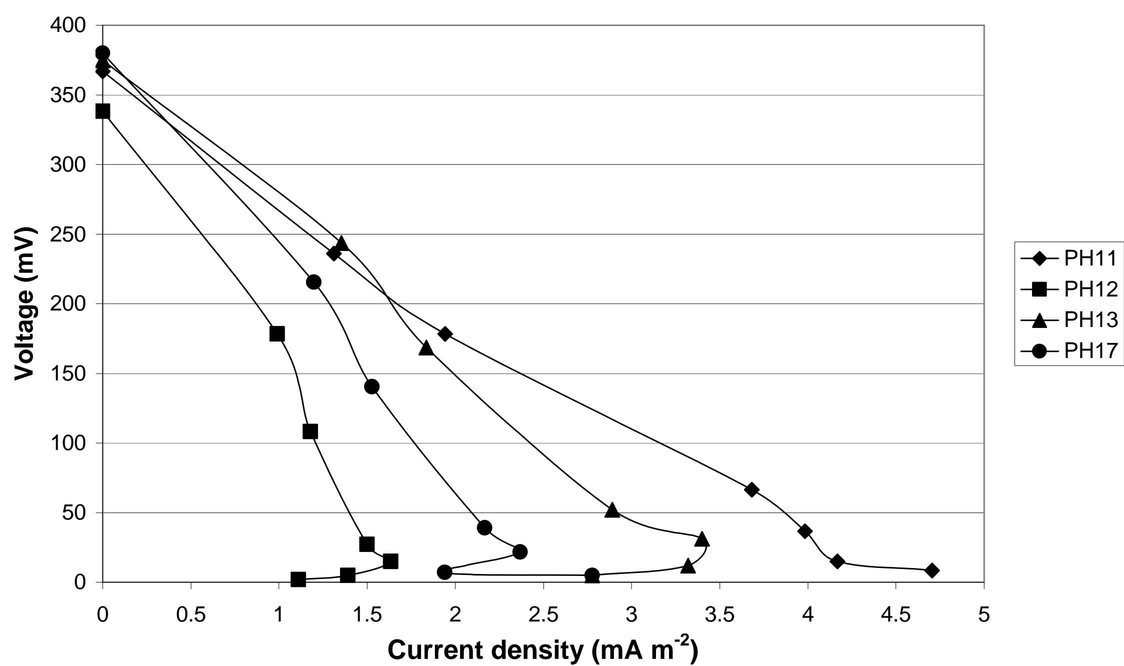

Figure C10 Voltage vs. current density of Glass I, mediator-less MFC when using PH11, PH12, PH13 and PH17 as biocatalysts

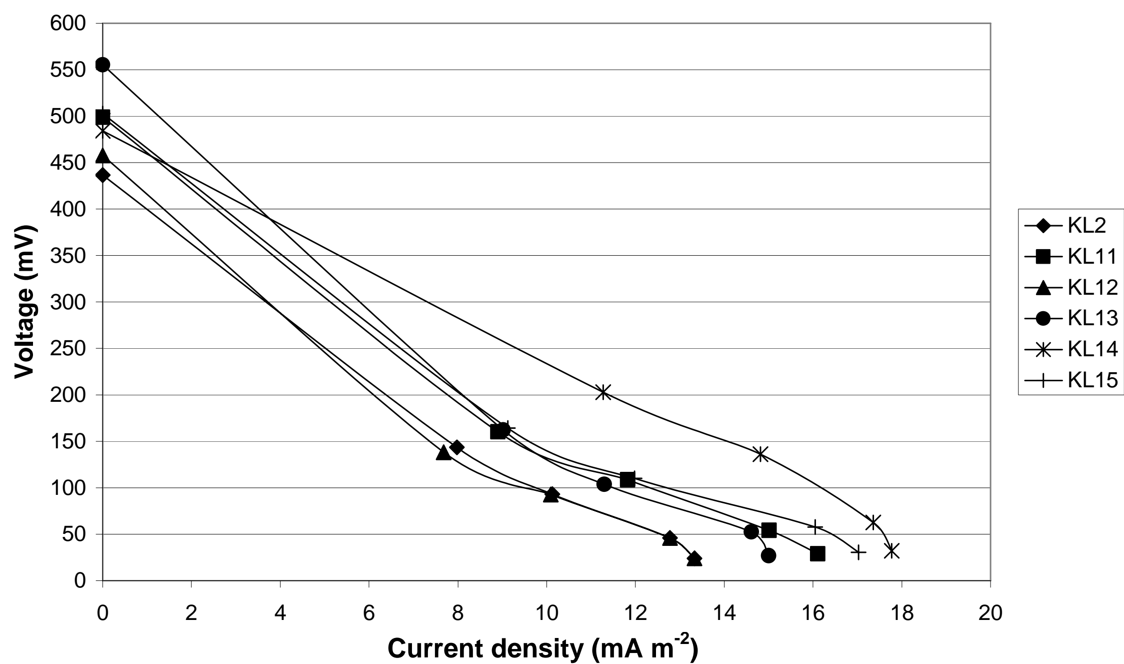

Figure C11 Voltage vs. current density of Glass II, mediator-less MFC when using KL2 and KL11-KL15 as biocatalysts

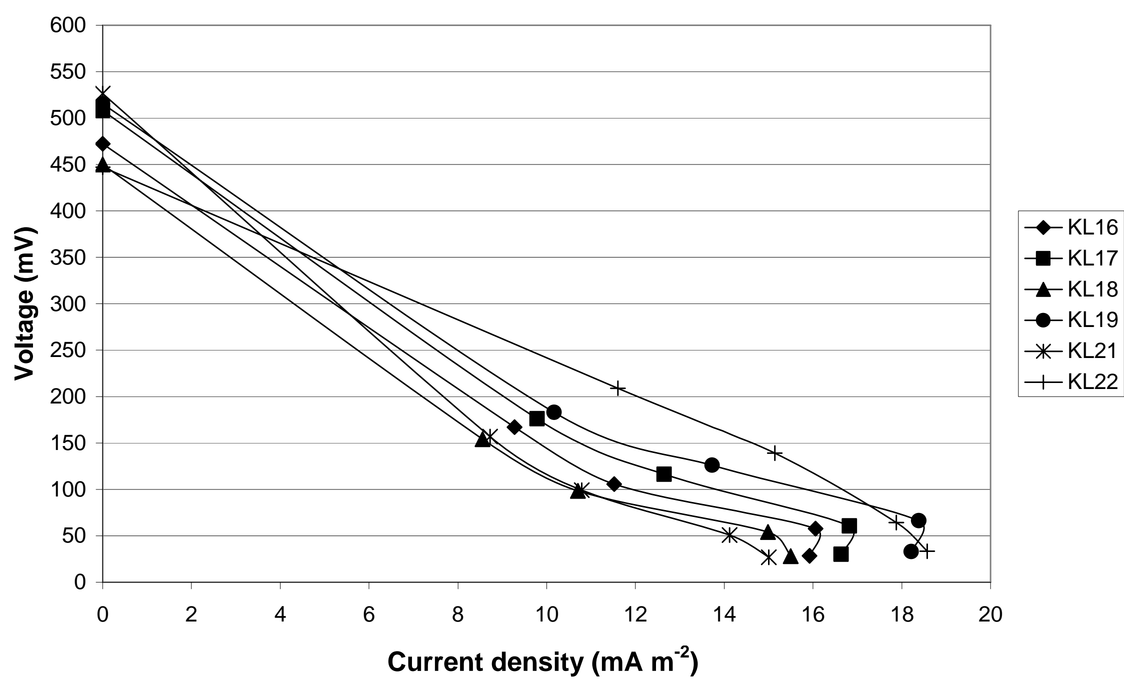

Figure C12 Voltage vs. current density of Glass I, mediator-less MFC when using KL16-KL19, KL21 and KL22 as biocatalysts

Appendix D

Table D Total plate counts (TPC) of 12 isolates from Koh Larn and pH of electrolyte in Glass II model before and after the experiment

Isolate	Total plate counts before starting the experiment (CFU ml^{-1})			Total plate counts after finishing the experiment (CFU ml^{-1})			% Decrease of TPC	pH measurement	
	1	2	average	1	2	average		initial	final
KL2	4.40×10^9	4.50×10^9	4.45×10^9	1.70×10^9	1.95×10^9	1.83×10^9	58.9	7.00	5.07
KL11	2.77×10^9	2.98×10^9	2.88×10^9	1.90×10^9	2.14×10^9	2.02×10^9	29.9	7.00	5.00
KL12	2.24×10^9	2.48×10^9	2.36×10^9	1.10×10^9	1.36×10^9	1.23×10^9	47.9	7.00	5.04
KL13	2.55×10^9	3.22×10^9	2.89×10^9	1.73×10^9	1.87×10^9	1.80×10^9	37.7	7.00	5.03
KL14	2.58×10^9	2.73×10^9	2.66×10^9	1.43×10^9	1.51×10^9	1.47×10^9	44.7	7.00	5.01
KL15	3.30×10^9	3.50×10^9	3.40×10^9	2.10×10^9	2.26×10^9	2.18×10^9	35.9	7.00	5.03
KL16	2.20×10^9	2.50×10^9	2.35×10^9	1.22×10^9	1.36×10^9	1.29×10^9	45.1	7.00	5.10
KL17	2.20×10^9	2.20×10^9	2.20×10^9	1.11×10^9	1.23×10^9	1.17×10^9	46.8	7.00	5.19
KL18	1.90×10^9	2.80×10^9	2.35×10^9	1.56×10^9	1.80×10^9	1.68×10^9	28.5	7.00	5.06
KL19	1.79×10^9	1.88×10^9	1.84×10^9	9.10×10^9	1.21×10^9	1.06×10^9	42.4	7.00	5.19
KL21	2.55×10^9	3.44×10^9	3.00×10^9	1.79×10^9	1.95×10^9	1.87×10^9	37.7	7.00	5.09
KL22	2.80×10^9	3.01×10^9	2.91×10^9	1.97×10^9	2.21×10^9	2.09×10^9	28.2	7.00	5.05

Appendix E

Table E1 O.D. at 540 nm of various concentration of Glucose by DNSA method

Tube	1 mg/ml glucose (ml)	DW (ml)	Final conc. of glucose (μg/ml)	O.D. at 540 nm			Average of O.D. at 540 nm
1	-	1.0	0	0	0	0	0.000
2	0.1	0.9	100	0.032	0.031	0.025	0.032
3	0.2	0.8	200	0.084	0.078	0.077	0.080
4	0.3	0.7	300	0.128	0.126	0.136	0.130
5	0.4	0.6	400	0.191	0.191	0.192	0.191
6	0.6	0.4	600	0.273	0.291	0.278	0.281
7	0.8	0.2	800	0.392	0.378	0.389	0.386
8	1.0	-	1000	0.501	0.495	0.486	0.494

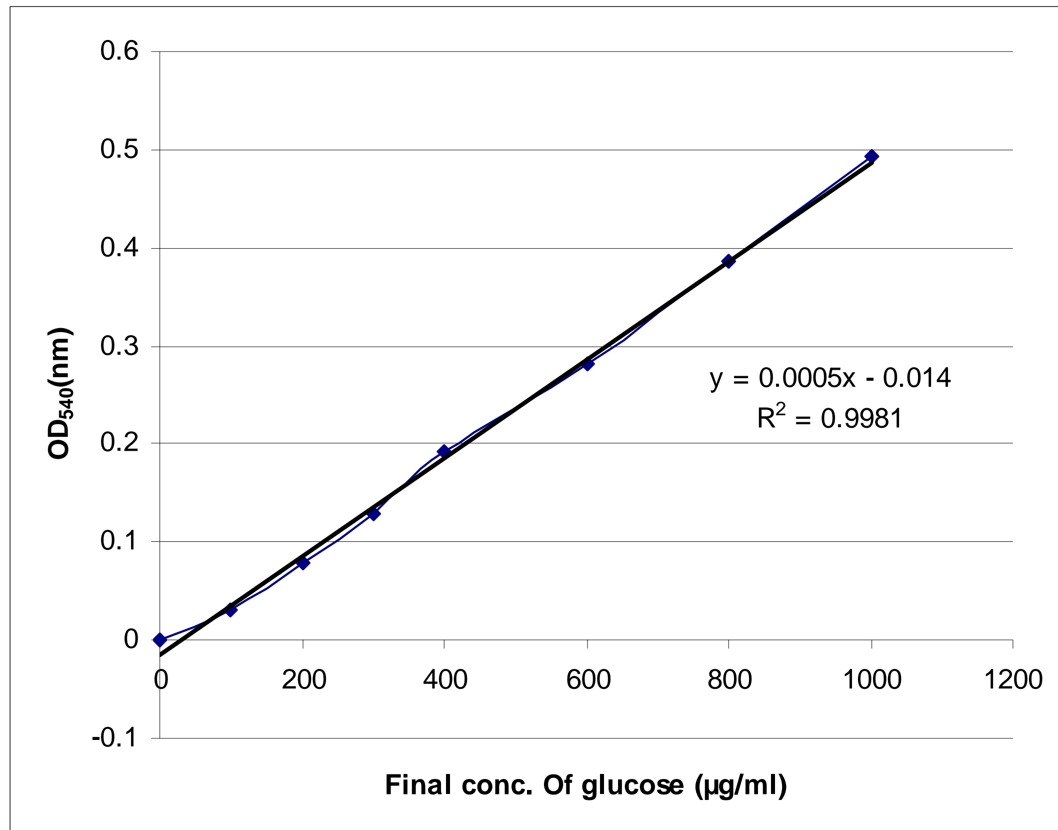

Figure E1 Standard curve of glucose by DNSA method

Determination of glucose concentration by DNSA method can be calculated as follow:

$$[\text{Glucose}]\ (\mu g/ml) = \frac{\text{O.D. x dilution factor}}{\text{Slope}}$$

Table E2 Glucose concentration in Glass II, mediator-less MFC before starting the experiment

Isolate	$O.D_{540}$			$O.D_{540}$ average	[Glucose] (mg/ml)
KL2	0.874	0.871	0.858	0.868	17.36
KL11	0.780	0.776	0.766	0.774	15.48
KL12	0.836	0.868	0.882	0.862	17.24
KL13	0.846	0.853	0.808	0.836	16.72
KL14	0.826	0.848	0.850	0.841	16.83
KL15	1.068	1.077	0.958	1.034	20.68
KL16	0.894	0.895	0.933	0.907	18.14
KL17	0.900	0.983	0.990	0.958	19.16
KL18	0.915	0.905	0.900	0.907	18.14
KL19	0.944	0.938	0.927	0.936	18.72
KL21	0.806	0.801	0.859	0.822	16.44
KL22	0.910	0.895	0.935	0.913	18.27

Table E3 Glucose concentration in Glass II, mediator-less MFC after finishing the experiment

Isolate	$O.D_{540}$			$O.D_{540}$ average	[Glucose] (mg/ml)
KL2	0.490	0.482	0.474	0.482	9.64
KL11	0.498	0.498	0.500	0.499	9.98
KL12	0.494	0.470	0.502	0.489	9.78
KL13	0.501	0.515	0.508	0.508	10.16
KL14	0.521	0.503	0.512	0.512	10.24
KL15	0.601	0.624	0.582	0.602	12.04
KL16	0.524	0.536	0.547	0.536	10.72
KL17	0.582	0.591	0.564	0.579	11.58
KL18	0.571	0.587	0.567	0.575	11.50
KL19	0.582	0.531	0.587	0.567	11.34
KL21	0.494	0.495	0.492	0.494	9.88
KL22	0.561	0.596	0.589	0.582	11.64

Appendix F

Nucleotide Sequences

F1. Nucleotide sequences of KL14 as followed:

5’-GCTTGCTTTCTTGCTGACGAGCGGCGGACGGGTGAGTAATGTATGGGGATCTGCC
CGATAGAGGGGGATAACTACTGGAAACGGTGGCTAATACCGCATGACGTCTACGGAC
CAAAGCAGGGGCTCTTCGGACCTTGCGCTATCGGATGAACCCATATGGGATTAGCTAG
TAGGTGGGGTAAAGGCTCACCTAGGCGACGATCTCTAGCTGGTCTGAGAGGATGATCA
GCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATA
TTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTAGG
GTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGTTAAGATTAATACTCTTAGCAATTGACG
TTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGG
GTGTAGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAATACCGGTGGC
GAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAA
CAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTAGAGGTTGTGGTCT
TGAACCGTGGCTTCTGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCA
AGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGCGAATCCTTTAGAGATA
GAGGAGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTT
GTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGCG
TGATGGCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGA
CGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCAGATACA
AAGAGAAGCGACCTCGCGAGAGCAAGCGGAACTCATAAAGTCTGTCGTAGTCCGGATT
GGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTA
CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTG
CAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCAC-3’

F2. Nucleotide of the partial 16S rRNA gene of *Proteus* sp., the database sequence for comparing with KL14

LOCUS: EF426446; 1505 bp DNA linear

DEFINITION: *Proteus* sp. L2 16S ribosomal RNA gene, partial sequence.

ACCESSION: EF426446

REFERENCE 1 (bases 1 to 1505)

AUTHORS: Lindh,J.M., Kannaste,A., Knols,B.G.J., Faye,I. and Borg-Karlson,A.-K

TITLE: Identification of volatiles and oviposition response of Anopheles gambiae s.s. mosquitoes (Diptera: Culicidae) to solutions containing bacteria previously isolated from An. gambiae s.l. midguts or oviposition sites

JOURNAL: Unpublished

REFERENCE 2 (bases 1 to 1505)

AUTHORS: Lindh,J.M., Borg-Karlson,A.-K. and Faye,I.

TITLE: Direct Submission

JOURNAL: Submitted (07-FEB-2007) Dept. of Genetics, Microbiology and Toxicology, Stockholm University, Svante Arrhenius v. 16E, Stockholm 10691, Sweden

ORIGIN

1 agagtttgat ggtggctcag attgaacgct ggcggcaggc ctaacacatg caagtcgagc

61 ggtaacagga ggaagcttgc tttcttgctg acgagcggcg gacgggtgag taatgtatgg

121 ggatctgccc gatagagggg gataactact ggaaacggtg gctaataccg catgacgtct

181 acggaccaaa gcaggggctc ttcggacctt gcgctatcgg atgaacccat atgggattag

241 ctagtaggtg aggtaatggc tcacctaggc gacgatctct agctggtctg agaggatgat

301 cagccacact gggactgaga cacggcccag actcctacgg gaggcagcag tggggaatat

361 tgcacaatgg gcgcaagcct gatgcagcca tgccgcgtgt atgaagaagg ccttagggtt

421 gtaaagtact ttcagcgggg aggaaggtga taaagttaat acctttatca attgacgtta

481 cccgcagaag aagcaccggc taactccgtg ccagcagccg cggtaatacg gagggtgcaa

541 gcgttaatcg gaattactgg gcgtaaagcg cacgcaggcg gtcaattaag tcagatgtga

601 aagccccgag cttaacttgg gaattgcatc tgaaactggt tggctagagt cttgtagagg

661 ggggtagaat tccacgtgta gcggtgaaat gcgtagagat gtggaggaat accggtggcg

721 aaggcggccc cctggacaaa gactgacgct caggtgcgaa agcgtgggga gcaaacagga

781 ttagataccc tggtagtcca cgctgtaaac gatgtcgatt tagaggttgt ggtcttgaac

841 cgtggcttct ggagctaacg cgttaaatcg accgcctggg gagtacggcc gcaaggttaa

901 aactcaaatg aattgacggg ggcccgcaca agcggtggag catgtggttt aattcgatgc

961 aacgcgaaga accttaccta ctcttgacat ccagcgaatc ctttagagat agaggagtgc

1021 cttcgggaac gctgagacag gtgctgcatg gctgtcgtca gctcgtgttg tgaaatgttg

1081 ggttaagtcc cgcaacgagc gcaaccctta tcctttgttg ccagcgcgtg atggcgggaa

1141 ctcaaaggag actgccggtg ataaaccgga ggaaggtggg gatgacgtca agtcatcatg

1201 gcccttacga gtagggctac acacgtgcta caatggcaga tacaaagaga agcgacctcg

1261 cgagagcaag cggaactcat aaagtctgtc gtagtccgga ttggagtctg caactcgact

1321 ccatgaagtc ggaatcgcta gtaatcgtag atcagaatgc tacggtgaat acgttcccgg

1381 gccttgtaca caccgcccgt cacaccatgg gagtgggttg caaaagaagt aggtagctta

1441 accttcggga gggcgcttac cactttgtga ttcatgactg gggtgaagtc gtaacaaggt

1501 acccg

F3. Nucleotide sequences of KL22 as followed:

5'-CTTGCTGACGAGCGGCTGACTGTCGGTATTGTTGGGGGCTGCCCGAGGAAAGGCGAT
AACTACTGCAACGGTGGCTAATACTCCATGACGTCTACAAACCAAATGATGGCTCTTCGG
ACCTTGCACTATCGGATGAACCCTTATGAGATTGTCTGGTAAAAGGCCTAACGGCTCACC
TAGGCCCATTCTTCGGCTGATCTGAGAGGATGATGGACCTGAAAGCGACTGACAAACGG
CCCTTACTCCTACGGGAGGCGAAAATGTTAATTAATTCAAAATGGTGACAGGCCCTTACTT
GTGTCAAATTGTCTACGAATTGCAAAGCTTTGTAATGTCATTACAAGGGGGAGGAGGGTA
ACACAAGAATACATTTACCCCTGACGTTACCCCCAGAAAATCCACGGGCTAACTCCGGG
CCAACTGCCAGCATACAGGATTAGATACCCTGGTAGTCCACGCTGTAACACGATGTCGAT
TTAGAGGTTGTGGTCTTGAACCGTGGCTTCTGGAGCTAACGCGTTAAATCGACCGCCTGG
GGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTG
GAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGCGA
ATCCTTTAGAGATAGAGGAGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTGTC
GTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTT
GTTTCCAGCGCGTGATGGCGGGAACTCAAAGGAGACTGCCGGTGATAATCCGGAGGAA
GGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAA
TGGCAGATACAAAGAGAAGCGACCTCTCGAGAGCAAGCGGAACTCATAAAGTCTGTCGT
AGTCCGGATTGGAGTCGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGAT
CAGAATGTTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCCCTCCATGGG
AGTGGGTTGCAAAAGAAGTAGGTAGCTTACCTTCGGTATATC-3'

F4. Nucleotide of the partial 16S rRNA gene of *Proteus vulgaris*, the database sequence for comparing with KL22

LOCUS: DQ499636; 1535 bp DNA linear

DEFINITION: *Proteus vulgaris* 16S ribosomal RNA gene, partial sequence.

ACCESSION: DQ499636

REFERENCE 1 (bases 1 to 1535)

AUTHORS: Cao,H. and Xu,H.

TITLE: Isolation, identification, phylogenetic analysis and related properties of a pathogen in Silurus meridionalis Chen.

JOURNAL: Acta Microbiol. Sin. (2006), In press

REFERENCE 2 (bases 1 to 1535)

AUTHORS: Cao,H. and Xu,H.

TITLE: Direct Submission

JOURNAL: Submitted (19-APR-2006) College of Life Science, Key Laboratory of Bio-Resources and Eco-Environment (Ministry of Education), Sichuan University, 24 South Section 1, Yihuan Road, Chengdu, Sichuan 610065, P.R. China

ORIGIN

1 agagtttgat cctggctcag attgaacgct ggcggcaggc ctaacacatg caagtcgggc

61 ggtaacagga gaaagcttgc tttcttgctg acgagcggcg gacgggtgag taatgtatgg

121 ggatctgccc gatagagggg gataactact ggaaacggtg gctaataccg catgacgtct

181 acggaccaaa gcaggggctc ttcggacctt gcgctatcgg atgaacccat atgggattag

241 ctagtaggtg gggtaaaggc tcacctaggc gacgatctct agctggtctg agaggatgat

301 cagccacact gggactgaga cacggcccag actcctacgg gaggcagcag tggggaatat

361 tgcacaatgg gcgcaagcct gatgcagcca tgccgcgtgt atgaagaagg ccttagggtt

421 gtaaagtact ttcagcgggg aggaaggtgt taagattaat actcttagca attgacgtta

481 cccgcagaag aagcaccggc taactccgtg ccagcagccg cggtaatacg gagggtgcaa

541 gcgttaatcg gaattactgg gcgtaaagcg cacgcaggcg gtcaattaag tcagatgtga

601 aagccccgag cttaacttgg gaattgcatc tgaaactggt tggctagagt cttgtagagg

661 ggggtagaat tccacgtgta gcggtgaaat gcgtagagat gtggaggaat accggtggcg

721 aaggcggccc cctggacaaa gactgacgct caggtgcgaa agcgtgggga gcaaacagga

781 ttagataccc tggtagtcca cgctgtaaac gatgtcgatt tagaggttgt ggtcttgaac

841 cgtggcttct ggagctaacg cgttaaatcg accgcctggg gagtacggcc gcaaggttaa

901 aactcaaatg aattgacggg ggcccgcaca agcggtggag catgtggttt aattcgatgc

961 aacgcgaaga accttaccta ctcttgacat ccagcgaatc ctttagagat agaggagtgc

1021 cttcgggaac gctgagacag gtgctgcatg gctgtcgtca gctcgtgttg tgaaatgttg

1081 ggttaagtcc cgcaacgagc gcaaccctta tcctttgttg ccagcgcgtg atggcgggaa

1141 ctcaaaggag actgccggtg ataaaccgga ggaaggtggg gatgacgtca agtcatcatg

1201 gcccttacga gtagggctac acacgtgcta caatggcaga tacaaagaga agcgacctcg

1261 cgagagcaag cggaactcat aaagtctgtc gtagtccgga ttggagtctg caactcgact

1321 ccatgaagtc ggaatcgcta gtaatcgtag atcagaatgc tacggtgaat acgttcccgg

1381 gccttgtaca caccgcccgt cacaccatgg gagtgggttg caaaagaagt aggtagctta

1441 accttcggga gggcgcttac cactttgtga ttcatgactg gggtgaagtc gtaacaaggt

1501 aaccgtaggg gaacctgcgg ctggatcacc tcctt

F5. Nucleotide sequence as followed:

5'-CATGCAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACG
GGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGG
CTAATACCGGATAATATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCA
CTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAA
CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCA
GACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAG
CAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTCTTGACATCCTC
TGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTT
GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGAT
CTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGA
AGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACA
ATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCT
CAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGG
ATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCAC
GAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTTATGGAGCCAGCCGCCTAAGGT
GGGACAGATGA-3'

F6. Nucleotide of the partial 16S rRNA gene of *Bacillus* sp., the database sequence for comparing with B1

LOCUS: D84630; 1512 bp DNA linear

DEFINITION: *Bacillus* sp. S23440 gene for 16S ribosomal RNA, partial sequence

ACCESSION: D84630

REFERENCE: 1

AUTHORS: Mitsui,H., Gorlach,K., Lee,H., Hattori,R. and Hattori,T.

TITLE: Incubation time and media requirements of culturable bacteria from different phylogenetic groups

JOURNAL: J. Microbiol. Methods 30, 103-110 (1997)

REFERENCE: 2 (bases 1 to 1512)

AUTHORS: Mitsui,H., Hattori,R., Watanabe,H., Tonosaki,A. and Hattori,T.

TITLE: Direct Submission

JOURNAL: Submitted (23-APR-1996) Contact:Hisayuki Mitsui Graduate School of Life Sciences, Tohoku University; 2-1-1, Katahira, Aoba-ku, Sendai, Miyagi 980-8577, Japan

ORIGIN

1 gagtttgatc ctggctcagg atgaacgctg gcggcgtgcc taatacatgc aagtcgagcg

61 aatggattga gagcttgctc tcaagaagtt agcggcggac gggtgagtaa cacgtgggta

121 acctgcccat aagactggga taactccggg aaaccggggc taataccgga taacattttg

181 aactgcatgg ttcgaaattg aaaggcggct tcggctgtca cttatggatg gacccgcgtc

241 gcattagcta gttggtgagg taacggctca ccaaggcaac gatgcgtagc cgacctgaga

301 gggtgatcgg ccacactggg actgagacac ggcccagact cctacgggag gcagcagtag

361 ggaatcttcc gcaatggacg aaagtctgac ggagcaacgc cgcgtgagtg atgaaggctt

421 tcgggtcgta aaactctgtt gttagggaag aacaagtgct agttgaataa gctggcacct

481 tgacggtacc taaccagaaa gccacggcta actacgtgcc agcagccgcg gtaatacgta

541 ggtggcaagc gttatccgga attattgggc gtaaagcgcg cgcaggtggt ttcttaagtc

601 tgatgtgaaa gcccacggct caaccgtgga gggtcattgg aaactgggag acttgagtgc

661 agaagaggaa agtggaattc catgtgtagc ggtgaaatgc gtagagatat ggaggaacac

721 cagtggcgaa ggcgactttc tggtctgtaa ctgacactga ggcgcgaaag cgtggggagc

781 aaacaggatt agataccctg gtagtccacg ccgtaaacga tgagtgctaa gtgttagagg

841 gtttccgccc tttagtgctg aagttaacgc attaagcact ccgcctgggg agtacggccg

901 caaggctgaa actcaaagga attgacgggg gcccgcacaa gcggtggagc atgtggttta

961 attcgaagca acgcgaagaa ccttaccagg tcttgacatc ctctgaaaac cctagagata

1021 gggcttctcc ttcgggagca gagtgacagg tggtgcatgg ttgtcgtcag ctcgtgtcgt

1081 gagatgttgg gttaagtccc gcaacgagcg caacccttga tcttagttgc catcattaag

1141 ttgggcactc taaggtgact gccggtgaca aaccggagga aggtggggat gacgtcaaat

1201 catcatgccc cttatgacct gggctacaca cgtgctacaa tggacggtac aaagagctgc

1261 aagaccgcga ggtggagcta atctcataaa accgttctca gttcggattg taggctgcaa

1321 ctcgcctaca tgaagctgga atcgctagta atcgcggatc agcatgccgc ggtgaatacg

1381 ttcccgggcc ttgtacacac cgcccgtcac accacgagag tttgtaacac ccgaagtcgg

1441 tggggtaacc tttatggagc cagccgccta aggtgggaca gatgattggg gtgaagtcgt

1501 aacaaggtag cc

F7. Nucleotide sequence as followed:

5'-CGGGTGAGTAACGCGTGGGAAATCTGCCGAGTAGCGGGGGACAACGTTTGGAAA
CGAACGCTAATACCGCATAACAATGAGAATCGCATGATTCTTATTTGAAAGAAGCAATTGC
TTCACTACTTGATGATCCCGCGTTGTATTAGCTAGTTGGTAGTGTAAAGGACTACCAAGGC
GATGATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC
AGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGGGCAACCCTGACCGA
GCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAA
CGTTAAGTAGAGTGGAAAATTACTTAAGTGACGGTATCTAACCAGAAAGGGACGGCTAAC
TACGTGCGATGAGTGCTAGCTGTAGGGAGCTATAAGTTCTCTGTAGCGCAGCTAACGCAT
TAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAG
GTCTTGACATACTCGTGCTATCCTTAGAGATAAGGAGTTCCTTCGGGACACGGGATACAG
GTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTTATTACTAGTTGCCATCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGA
TAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTAC
ACACGTGCTACAATGGATGGTACAACGAGTCGCCAACCCGCGAGGGTGCGCTAATCTCT
TAAAACCATTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATCGCT
AGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
CGTCACACCACGGAAGTTGGGAGTACCCAAAGTAGGTTGCCTAACCGCAAGGAGGGCG
CTTCCTAAGGTAAGACCGA-3'

F8. Nucleotide of the partial 16S rRNA gene of *Lactococcus garvieae*, the database sequence for comparing with PH5

LOCUS: AB300504; 1449 bp DNA linear

DEFINITION: *Lactococcus garvieae* gene for 16S rRNA, partial sequence, strain: 20-92.

ACCESSION: AB300504

REFERENCE: 1

AUTHORS: Yoshida,T., Nagamune,H., Uchiyama,S. and Ueno,T.

TITLE: Comparison of characters of strains classified into *Lactococcus garvieae*

JOURNAL: Unpublished

REFERENCE: 2 (bases 1 to 1449)

AUTHOR: Nagamune,H.

TITLE: Direct Submission

JOURNAL: Submitted (10-APR-2007) Contact:Hideaki Nagamune The University of Tokushima Graduate School, Institute of Technology and Science; #1, 2-chome, Minami-josanjima, Tokushima 770-8506, Japan

ORIGIN

1 agtcgagcga tgattaaaga tagcttgcta tttttatgaa gagcggcgaa cgggtgagta

61 acgcgtggga aatctgccga gtagcggggg acaacgtttg gaaacgaacg ctaataccgc

121 ataacaatga gaatcgcatg attcttattt aaaagaagca attgcttcac tacttgatga

181 tcccgcgttg tattagctag ttggtagtgt aaaggactac caaggcgatg atacatagcc

241 gacctgagag ggtgatcggc cacactggga ctgagacacg gcccagactc ctacgggagg

301 cagcagtagg gaatcttcgg caatggggc aaccctgacc gagcaacgcc gcgtgagtga

361 agaaggtttt cggatcgtaa aactctgttg ttagagaaga acgttaagta gagtggaaaa

421 ttacttaagt gacggtatct aaccagaaag ggacggctaa ctacgtgcca gcagccgcgg

481 taatacgtag gtcccaagcg ttgtccggat ttattgggcg taaagcgagc gcaggtggtt

541 tcttaagtct gatgtaaaag gcagtggctc aaccattgtg tgcattggaa actgggagac

601 ttgagtgcag gagaggagag tggaattcca tgtgtagcgg tgaaatgcgt agatatatgg

661 aggaacaccg gaggcgaaag cggctctctg gcctgtaact gacactgagg ctcgaaagcg

721 tggggagcaa acaggattag ataccctggt agtccacgcc gtaaacgatg agtgctagct

781 gtagggagct ataagttctc tgtagcgcag ctaacgcatt aagcactccg cctggggagt

841 acgaccgcaa ggttgaaact caaaggaatt gacgggggcc cgcacaagcg gtggagcatg

901 tggtttaatt cgaagcaacg cgaagaacct taccaggtct tgacatactc gtgctatcct

961 tagagataag gagttccttc gggacacggg atacaggtgg tgcatggttg tcgtcagctc

1021 gtgtcgtgag atgttgggtt aagtcccgca acgagcgcaa cccttattac tagttgccat

1081 cattaagttg ggcactctag tgagactgcc ggtgataaac cggaggaagg tggggatgac

1141 gtcaaatcat catgcccctt atgacctggg ctacacacgt gctacaatgg atggtacaac

1201 gagtcgccaa cccgcgaggg tgcgctaatc tcttaaaacc attctcagtt cggattgcag

1261 gctgcaactc gcctgcatga agtcggaatc gctagtaatc gcggatcagc acgccgcggt

1321 gaatacgttc ccgggccttg tacacaccgc ccgtcacacc acggaagttg ggagtaccca

1381 aagtaggttg cctaaccgca aggagggcgc ttcctaaggt aagaccgatg actggggtga

1441 agtcgtaac

Appendix G

Table G Ingredient utilization pattern of strains KL14 and KL22 using API 20E

Active ingredients	Isolate number	
	KL14	KL22
2-nitrophenyl-βD-galactopyranoside	-	-
L-arginine	-	-
L-lysine	-	-
L-ornithine	-	-
Trisodium citrate	-	-
Sodium thiosulfate	-	+
Urea	+	+
L-tryptophane	+	+
Sodium pyruvate	-	-
Gelatin (bovine origin)	+	+
D-glucose	+	+
D-mannitol	-	-
Inositol	-	-
D-sorbitol	-	-
L-rhamnose	-	-
D-sucrose	+	-
D-melibiose	-	-
Amygdalin	-	-
L-arabinose	-	-
Cytochrome-Oxidase	-	-
NO_2 production (Potassium nitrate)	-	+
Reduction to N_2 gas (Potassium nitrate)	-	-
Motility	+	+
MacConkey medium	+	+
Glucose (API OF Medium): OF-O	+	+
Glucose (API OF Medium): OF-F	+	+
Accession number	007602047	047600057

+ = positive; - = negative

VITAE

Mister Kamol Rodyou was born in February 21, 1983, Bangkok, Thailand. He graduated from Department of Microbiology, Faculty of Science, Chulalongkorn University in 2005 with the Bachelor degree of Science (Microbiology). Recently, he has pursued for Master degree of Program in Industrial Microbiology from the same institute and expected to finish by the academic year of 2008.

Scientific Presentation

Kamol Rodyou, Mana Sriyudthsak and Sirirat Rengpipat. Selection of ferric reducing bacteria for use in microbial fuel cell. Proceedings in the 20th Annual Meeting and International Conference of the Thai Society for Biotechnology (TSB), 14th-17th October 2008, Taksila Hotel, Mahasarakham, Thailand. (oral presentation and full text collected in CD- ROM)

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