



**Development of hydrogel bio-anode with
immobilized cells for improvement of
performance of microbial fuel cells**

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1 INTRODUCTION AND OUTLINE

1.1 Introduction

Due to scarcity and adverse effects of fossil fuels, community are looking for alternative energy sources which is renewable and ecofriendly. In that context, it is believed that microbial fuel cells (MFC) is a two-fold solution to resolve the dilemma of energy crunch and negative environmental impacts of fossil fuels. MFC is an environmentally benign system, where microorganisms convert organic materials into electricity. In MFC, electrons and protons are produced via anaerobic respiration of microorganism in anode chamber, and then while protons are traveled through the proton exchange membrane in the MFC to the aerobic cathode compartment, whereas electrons are transferred through the external circuit generating electricity. Despite it is very attractive technology, however, the application still faces numerous limitations such as low power density, low electrical potential, cost of catalyst in cathodic chamber etc. There are various factors affecting the performance of MFCs such as microorganisms, substrate, mediator, electrode material and construction of MFCs. Engineering of electrodes may good direction to enhance the efficiency of MFCs because of their electron production and transfer role. No doubt that the electrode biofilm and the electronic transport are important factors in the performance of MFCs.

Shewanella spp. is well-accepted to produce electricity in the MFC systems by several research groups worldwide. In the metabolic pathway, during the conversion of NAD^+ to NADH, CO_2 and organic acids, as well as H^+ and electrons are generated. Furthermore, several strains of *Shewanella* produce mediators, such as riboflavin, flavin etc. to facilitate electron transfer to the anode (terminal electron acceptor). Interestingly, it was reported that bacterial cells can utilize both exogenous (externally added into the medium) or self-produced (endogenous) shuttle compounds as extracellular electron transporter. In recent years, electron mediators or electron acceptors such as methylene blue, methyl red, humic acid, ferricyanide, riboflavin were used in most biological fuel cells to boost the electricity generation. The electron transfer rate could be enhanced by shuttling the electron from donor microorganism to acceptor electrode. In recent studies, riboflavin was used as an electron shuttle to transport electron to the anode. The efficiency of application of mediator, however, strongly depends on its quality and actual quantity that can improved by new technique known as immobilization of mediators onto anode electrode. The advantage of this method was able to reuse the anode electrode with mediators and minimize their washing out in continuous operation.

Carbon paper, cloth, foams or graphite rods, felt, foams, plates are commonly used as anode material in MFC. For example, conductive polymer polyaniline (PANI) is one of materials

that use to fabricate anode electrode because it played a great role in the energy storage (polyaniline based electrode materials for energy storage and conversion). Polyaniline is well known as a low cost, mechanical flexibility and stability material. In the previous study, our group used alginate/polyaniline/titanium-dioxide/graphite for the immobilization microorganism and make hydrogel bio-anode and the increase in electrical power of MFC was obtained. Bacterial cellulose (BC) is well known a non-toxic, low cost polymer, as well as it has some outstanding characteristics compared to plan cellulose such as renewability, ultrafine network structure, higher purity, water retention capability, porosity, biological interaction, mechanical strength. Conducting polymers-cellulose composites including BC coated with conducting polymers is a new promising polymer and received interest in recent years because of their largely potential applications such as batteries, sensors and electrical devices. BC/PANI is a typical example for combination of BC and conducting polymer with the integration of several properties such as tensile strangle, biocompatibility, high surface areas and electrical conductivity. Müller *et al.* (2012); Wang *et al.* (2012) have been successful in fabrication of BC/PANI material with high electrical conductivity. In many researches, the PANI coated anode was successfully used in microbial fuel cells to enhance power density. Furthermore, the modified PANI polymers with the presence of titanium dioxide have generated enhanced current densities.

One of the main parts in MFCs is the anode, where exo-electrons are generated by the biocatalysts (bacteria) and transferred to the electrode. The performance of MFC is strongly affected by the quality and activity of microorganisms as well as quality and construction of the anode. Additionally, its performance also depends on some factors affected the efficiency of electron transfer such as the distance between microorganism cells and electrode, the internal resistance, mediators etc.

1.2 Outline of dissertation

In the last decade, due to intensive development of conductive composites materials and application of mediators, the engineering of anode in the MFC has turned onto a new stage. New type of bio-anode can be formed applying conductive composite materials, mediators and it may lead to enhance efficiency of electron-transfer between bacterial cells and electrode, thus improvement of performance of MFC. Connecting to this field, my PhD research focused on development of hydrogel electrode with immobilized bacterial cells (bio-anode). Detailed tasks are following:

- Growth of *Shewanella xiamenensis* species bacteria
- Investigation of riboflavin production by *Shewanella xiamenensis*
- Fabrication and characterization of conductive hydrogel composites anode electrode
- Incorporation and immobilization of bacterial cells into hydrogel electrode
- Construction of MFC using hydrogel bio-anode with immobilized cells
- Stability and operational performance of MFC

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

Riboflavin, yeast extract, marine agar, sodium chloride and ammonium persulfate were purchased from Reanal (Budapest, Hungary). Sodium hydroxide, hydrochloric acid, iron citrate and aniline were purchased from Merck (Darmstadt, Germany); tryptone was obtained from Oxoid Limited (Basingstoke, United Kingdom); Na-alginate was from Cargill (Hungary); titanium dioxide was from VWR (Hungary); graphite powder was from Pannoncolor (Hungary); cellulase enzyme was from Sigma-Aldrich (Hungary) and Bio-Rad Protein Assay Kit was from Bio-Rad (USA).

2.2 Microorganisms

Shewanella xiamenensis DSMZ 22215 was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany.

Acetobacter xylinum (ATCC 23768) was from Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh city, Vietnam.

2.3 Effect of exogenous riboflavin and pH on growth of *S. xiamenensis* and riboflavin production

2.3.1 Effect of exogenous riboflavin

Batch mode experiments were performed with modified LB medium supplemented with different concentrations of riboflavin, ranging from 0 – 20 nmol/mL to understand the system dynamics. Initial pH of the medium was 7.0. For each experiment, initial cells number was maintained to 10^5 CFU/mL. Programmed incubation temperature 30 °C under anaerobic condition and shaking speed 200 rpm for 120 hours was considered for this purpose. At every 24h intervals, 10 mL of samples from each Erlenmeyer flasks were collected in aseptic way and cells concentration was measured immediately. Subsequently, collected microbial broth was

centrifuged with 12,000 rpm for 10 minutes at temperature 20 °C. Supernatant was collected, and concentrations of extracellular riboflavin, pH in microbial broth were measured.

2.3.2 Kinetics of growth and riboflavin production of *S. xiamenensis*

The kinetic parameters were determined by Monode model (Eq. 2.1) and the relationship of cell growth to riboflavin formation was considered by Luedeking and Piret (Eq. 2.2).

$$\mu = \frac{\mu_{\max} \cdot S}{K_S + S} \quad (\text{Eq. 2.1})$$

where:

μ is the specific growth rate (1/h)

μ_{\max} is the maximum specific growth rate (1/h)

K_S is the saturation constant (g/L)

S is substrate concentration (g/L)

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (\text{Eq. 2.2})$$

where:

α is growth-associated product formation coefficient

β is non-growth-associated product formation coefficient

P is product concentration (g/L)

X is biomass (g/L).

The α and β were calculated using regression analysis of experimental data. Both correlation coefficients (R^2) and t-probe of each estimated constants were checked for statistical significances.

2.3.3 Effect of pH on growth of *S. xiamenensis* and riboflavin production

S. xiamenensis was growth in modified LB medium with different initial pH without exogenous riboflavin. For each experiment, initial cells number was maintained to 10^5 CFU/mL and initial pH of the medium was ranged from 6 – 10. Cells numbers, riboflavin concentration, pH was measure after 24 hours intervals.

2.3.4 Optimization of riboflavin production

Central Composite Design (CCD) was used to investigate the effects of two independent variables. Riboflavin was determined at 72nd hour of operation. The concentration of exogenous

riboflavin ranged from 10 to 20 nmol/mL and initial pH was in pH 7.0 – pH 9.0. The second-order polynomial model used in the response surface analysis was as following:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_{11}X_1^2 + a_{22}X_2^2 + a_{12}X_1X_2 \quad (\text{Eq. 2.3})$$

where Y (riboflavin concentration) is the response variable; X_1 (exogenous riboflavin) and X_2 (pH) are the independent variables; a_0 is the interruption coefficient; a_1 and a_2 are the coefficients of the linear effects; a_{11} and a_{22} are the coefficients of the quadratic effects; and a_{12} is the coefficient of the interaction effect.

The central composite design consisted of 11 experimental points was desined and applied. The fit of the models was evaluated by the determination coefficients (R^2) and adjusted R^2 (R^2 -adj).

2.4 Fabrication of bio-anode

2.4.1 Fabrication of hydrogel bio-anode with the riboflavin and bacterial cells

A hydrogel composites containing gel-entrapped bacteria in alginate/polyaniline/TiO₂/graphite composites was prepared as previously described by Szöllösi *et al.* (2017) with some modification. At the end of the process, before dropping down the mixed solution into calcium chloride, bacteria cells (10^7 CFU/mL *S. xiamenensis*) was added with graphite powder and different concentration of riboflavin from 5 to 20 nmol/mL. Composites coated hydrogel particles were formed with 0.3 cm in diameter.

The hydrogel gel composites with the immobilization of *S. xiamenensis* cells and riboflavin were used to create hydrogel bio-anode. The composites gel was placed in a bag made by graphite cloth. The size of the bag was $2.5 \times 3.5 \times 0.7$ cm. The joints of bag were sewn with copper wire. The bag was filled approximately 50% with hydrogel composite. The upper part of the bag was also connected to the conductive copper wire.

2.4.2 Fabrication of bacterial cellulose based bio-anode composites

Bacterial cellulose sheet produced by *Acetobacter xylinum* ATCC 23768 strain was cut into 2×3 cm pieces and sterilized in an autoclave at 121 °C for 15 min before use.

BC/PANI composites were prepared *in situ* aniline oxidative polymerization by using ammonium persulfate (APS) or iron (III) chloride hexahydrate (FeCl₃.6H₂O) as oxidant according to description by Müller *et al.* (2012); Wang *et al.* (2012) with some modifications. Briefly, BC sheet was immersed in distilled water (1:10 w/v) and then aniline was added. Ultrasonication (Clifton MU-8 sonicator, 40 kHz, 30 W) technique was used for 2 hours. In the next

step, the oxidant (ammonium persulfate or iron (III) chloride hexahydrate) was mixed. The BC was synthesized in the 2nd cycle of ultra-sonication (40 kHz, 30 W) for 2 hours at in ice. In the case of fabrication of electrically conducting BC/PANI/TiO₂ composites, TiO₂ was mixed simultaneously with oxidants.

In the immobilization procedure, bare BC, BC/PANI and BC/PANI/TiO₂ composite membranes were incubated in *S. xiamenensis* suspension (10⁹ CFU/mL in isotonic phosphate buffered saline) at 30 °C and 200 rpm for 72 – 96 hours. After that, the composite membranes were washed 3 times with PBS to remove free cells.

2.5 Construction batch and semi-continuous batch of MFC

Dual-chamber MFC system with similar volume of anode and cathode chambers (24 mL) was used. The chambers were separated by the Nafion 117 proton-exchange membrane. The cathode chamber was filled with 0.1 M of potassium-hexaferrocyanate and 0.5 M of Sorensen phosphate buffer (pH = 7). The aeration was provided by external pump connected directly to cathode chamber. Anode chamber was filled with modified LB medium (1g/L glucose, 5 g/L yeast extract, 10 g/L NaCl and 1 g/L tryptone). Graphite sheets with surface area 8 cm² as electrode were placed in the chambers. Then electrical circuit were closed with an external resistance (500 Ω) parallely with a digital multimeter wired between two electrodes. Voltage output value was measure and power density was calculated during operation of systems. Batch and semi-continuous operation modes used the same MFC system.

The effect of iron ferric on the performance of MFC was also studied. Anode chamber with BC/PANI/TiO₂/APS anode was filled with modified LB medium supplemented with different Fe(III) concentration (Fe(III)-citrate) ranging from 3 mM to 12 mM.

2.6 Analytical methods

The cells numbers of microbes in the growth medium was determined by plate count technique and optical density (OD_{600nm}) using UV-visible spectrophotometer at 600 nm wavelength.

The riboflavin concentration, reducing sugars and organic acids in solution were determined by the high-performance liquid chromatography (HPLC) method.

Polarization curve of MFC was performed once the voltage stabilized after the MFC was operated. Various external resistances (from 0 Ω to 1 MΩ) were connected between two electrodes of the MFC. Current density and power density were calculated and the relationship between voltage and current density, power density will be plotted.

Cyclic voltammetry (CV) was conducted using the open source potentiostat (IO Rodeo, USA) with saturated calomel electrode (SCE) and platin wire as the reference electrode and counter electrode, respectively.

The conductivity of BC/PANI, BC/PANI/TiO₂ was measured with a conventional four-point probe technique.

The infrared spectra of bare BC, BC/PANI and BC/PANI/TiO₂ composite membranes were obtained on a JASCO-4700 infrared spectrometer using KBr pellets. The wavelength range is 4000 to 400 cm⁻¹ with a resolution of 4 cm⁻¹ at room temperature.

Scanning electron microscope (SEM, JSM-6480LV-JED 2300, Jeol, Japan) was used to analyze the morphology and structure of surface of bare BC, BC/PANI and BC/PANI/TiO₂.

The cell number of immobilized *S. xiamenensis* in bare BC, BC/PANI and BC/PANI/TiO₂ were determined by plate count method on Marine agar.

Iron(III)-reduction was determined by the ammonium-thiocyanate (NH₄SCN) method.

The voltage was continuously monitored by Multimeter connected to personal computer. Current was calculated according to the Ohm's law

$$I = \frac{V}{R} \quad (\text{Eq. 2.4})$$

where,

V is the voltage (V)

R is the external resistance (Ω)

The power is calculated based on the electric current $P = I.V$. Power density is obtained according to

$$P_d = \frac{I.V}{d} \quad (\text{Eq. 2.5})$$

where d is the volume of the hydrogel bio-anode composite

Coulombic efficiency (CE) is calculated as

$$CE = \frac{8 \int_0^{tb} Idt}{FV_{MFC} \Delta COD} \quad (\text{Eq. 2.6})$$

where,

I is current

F = Faraday's constant

V_{MFC} is the volume of the anode chamber

COD is the chemical oxygen demand

3 RESULTS AND DISCUSSION

3.1 Effect of exogenous riboflavin and initial pH on growth of *S. xiamenensis* and riboflavin production

The growth medium was supplemented with different amounts of exogenous riboflavin (from 0 nmol/mL to 20 nmol/mL). Cell count and riboflavin concentration were monitored. The cells number of bacteria in sample with initial exogenous riboflavin 20 nmol/mL grew rapidly after 24 hours (from 10^5 CFU/mL to 3.3×10^8 CFU/mL), increased slowly after 72 hours and then stabilized. At 72nd hours of operation, the cells number of bacteria in the samples with exogenous riboflavin concentration 15; 20 nmol/mL were around 1.3-fold higher than in the sample with exogenous riboflavin concentration 5 nmol/mL and significantly higher than in the samples with 0; 5 nmol/mL.

The kinetics of riboflavin production by *S. xiamenensis* in the sample without exogenous riboflavin was studied. Maximum specific cell growth rate (μ_{\max}) of model was determined as 0.079 1/h with Monod constant (K_S) was 0.15 g/L. The ratio of the amount of biomass produced to the amount of glucose consume (Y_{XS} biomass yield) was 0.001 g biomass/g glucose. Additionally, the ratio of the amount of riboflavin produced to the amount of glucose consume (Y_{PS} riboflavin yield) was 0.003 g riboflavin/g glucose. The growth-associated (α) was determined as 3.3277 and the biomass-associated (β) was 0.002.

Riboflavin production by *S. xiamenensis* at different initial pHs of medium was also studied. Overall, riboflavin production at all pH levels increased after 24 hours of operation, then reached peaks after 72 hours. At 72nd hours, there were significant differences in riboflavin production among pH 6, pH 7, pH 10 and pH 8, pH 9. The riboflavin production of pH 6, pH 7 and pH 10 was lower than that of other pHs. The riboflavin production at pH 9 was the highest (4.89 ± 0.51 nmol/mL), followed by pH 8 (4.85 ± 0.72 nmol/mL) and pH 7 (4.54 ± 0.54 nmol/mL). In addition, the maximum of cell concentration (5.78×10^8 CFU/mL) peaked in the sample with initial pH 9 meaning 1.3-fold and 1.6-fold higher than in the samples with initial pH 6 (4.43×10^8 CFU/mL) and pH 10 (3.58×10^8 CFU/mL), respectively.

Effects of exogenous riboflavin (X_1) and initial pH (X_2) on riboflavin production (Y) of *S. xiamenensis* was also investigated using Response Surface Methods (RSM). According to results of regression analysis, the following second-order polynomial model (Eq. 3.1) was suggested to describe riboflavin production. The optimal exogenous riboflavin and pH were determined to be 18 nmol/mL and pH 8.2, respectively.

$$Y = 6.295 + 0.092X_1 + 0.12X_2 - 0.233X_2^2 \quad (\text{Eq. 3.1})$$

where Y is riboflavin production (nmol/mL), X_1 is exogenous riboflavin concentration (nmol/mL) and X_2 is initial pH value.

3.2 Engineering of hydrogel composites based bio-anode

MFC1, MFC2, MFC3, MFC4 and MFC5 were set-up with hydrogel composite bio-anode included 0, 5, 10, 15 and 20 nmol/mL riboflavin, respectively. The power density speedily increased in MFCs with higher riboflavin concentration (MFC4 and MFC5) in compared with control system MFC1. In the case of MFC5, the maximum power density was $6.06 \pm 0.15 \text{ W/m}^3$ at 36th hours, and it was 2.7-fold higher than one ($2.23 \pm 0.31 \text{ W/m}^3$) in the case of MFC1. In the end of period (from around 50th hours), the power density of MFC4, MFC5 was decreased rapidly.

The polarization curves of MFCs with different riboflavin concentration were also calculated. The open-circuit voltage of MFC5 (0.33 V) was 1.94-fold higher than MFC1 (0.17 V). The MFC1 produced a maximum power density of 2.4 W/m^3 , while MFC5 got a peak at 5.82 W/m^3 .

In the case of cyclic voltammetry, the redox peaks were found for the systems of bio-anode with riboflavin from 5 to 20 nmol/mL. The oxidation and reduction peaks of all systems with bio-anode composite with riboflavin were -0.4 V and -0.7 V, respectively. The area of the CV curves of bio-anode composite with riboflavin was larger than the system with lower riboflavin.

Semi-continuous MFCs were set-up after the power density of MFCs decreased to the low value (around $0.11 - 0.19 \text{ W/m}^3$ at 85th hours of every stage) to consider the effectiveness of hydrogel bio-anode with the immobilization of riboflavin and *S. xiamenensis*. The anode chambers of MFCs were fed fresh substrate. The maximum values of MFCs in the second and third cycle were lower than in the first cycle. The maximum power density in the third cycle peaked at $5.64 \pm 0.09 \text{ W/m}^3$ in MFC4.

3.3 Engineering of bacterial cellulose based bio-anode

3.3.1 Fabrication of bacterial cellulose based composites and bio-anode

The bacterial cellulose/polyaniline (BC/PANI) electrical conductivity composites were strongly dependent upon reaction conditions. The effect of preparation conditions (aniline, iron (III) chloride hexahydrate, ammonium persulfate concentration) on BC/PANI composites conductivity was investigated using the Central Composite Design (CCD). The maximum

BC/PANI composites conductivity could be attained when prepared as follows: concentration of aniline 0.2 mol/L; molar ratio of ammonium persulfate to aniline 1.2:1; molar ratio of iron (III) chloride hexahydrate to aniline 1.5:1; reaction temperature 0-5 °C; polymerization reaction time 14 hours. The conductivity of BC/PANI/APS composites obtained 2.67 ± 0.15 S/m and 2.29 ± 0.11 S/m with BC/PANI/FeCl₃.6H₂O composites. In addition, BC/PANI composite was coated with TiO₂ to enhance conductivity. The maximum of electrical conductivities of BC/PANI/APS prepared with 0.3 mol/L TiO₂ concentration and of BC/PANI/FeCl₃.6H₂O with 0.2 mol/L TiO₂ were 3.71 ± 0.2 S/m and 2.9 S/m, respectively.

S. xiamenensis cells were immobilized in BC, BC/PANI and BC/PANI/TiO₂ composites. Maximum cell numbers reached after 48 hours of immobilization of microorganism on BC/PANI and BC/PANI/TiO₂ electrodes and were stable for further 24 hours. The maximum cells number of BC/PANI/TiO₂ using ammonium persulphate and FeCl₃.6H₂O as oxidants were 1.2×10^6 CFU/g and 1.1×10^6 CFU/g, respectively.

3.3.2 Performance of bacterial cellulose based bio-anode

Five types of bioanodes namely bare BC, BC/PANI/FeCl₃.6H₂O, BC/PANI/APS, BC/PANI/TiO₂/FeCl₃.6H₂O and BC/PANI/TiO₂/APS with the immobilization *S. xiamenensis* were used in different MFC systems MFC6, MFC7, MFC8, MFC9, MFC10, respectively. The power density rapidly increased in MFCs with BC/PANI/TiO₂ anode (MFC9 and MFC10) compared with the control system bare BC (MFC6). The power density of MFC10 was 15-fold higher than MFC6 with bare BC anode (2.57 W/m³). In the case of BC/PANI/TiO₂ (MFC9) using FeCl₃.6H₂O as an oxidant – the power density value peaked around 23.95 - 29.30 W/m³, was significantly lower than MFC10.

The polarization curve of all MFC systems was calculated. The maximum power density of MFC10 system was 40.66 W/m³ with a current density of 116.72 A/m³.

To consider the effectiveness of BC/PANI/TiO₂/APS with immobilization of *S. xiamenensis*, semi-continuous batch was set-up after voltage output of MFC10 decreased to near nil. The maximum power density of each cycle peaked around 35.81 W/m³ after 30 hours meaning the MFCs are able to produce electricity when the fuel was fed. The cycle time of MFCs took about 70-72 hours from the feeding to exhaust of glucose.

The CV response of MFC10 with BC/PANI/TiO₂/APS composite anode was determined. The redox peaks were found for the systems of BC/PANI/TiO₂/APS anode.

3.3.3 Effect of exogenous iron(III)

The MFCs with BC/PANI/TiO₂/APS with immobilization of *S. xiamenensis* cells was used in this study. At the initiation, Fe(III) was added into anode chambers with different concentration from 3 mM to 12 mM. The maximum of power density ranged from 49.05 ± 1.24 to 51.544 ± 1.29 W/m³ between 20th hours and 40th hours in the MFC system with 12 mM Fe(III) concentration. These values were 1.3-fold and 1.4-fold higher than MFC systems with 3 mM and without Fe(III) concentration, respectively.

The maximum coulombic efficiencies (CE) reached $56.92 \pm 1.21\%$ of MFC with 12 mM Fe(III) concentration, compared to $49.71 \pm 0.98\%$ of the MFC without exogenous Fe(III) in anode chamber. Moreover, the maximum of Fe(III) reduction rate (0.033 ± 0.004 mM/h) was detected in MFC at 12 mM of Fe(III) comparing with 0.021 ± 0.004 mM/h and 0.024 ± 0.003 mM/h in MFC at 3 mM and 6 mM of Fe(III), respectively.

4 NOVEL CONTRIBUTION

1. The effect of exogenous riboflavin and pH on growth of *S. xiamenensis* and riboflavin production was studied. Exogenous riboflavin, initial pH and bacterial cell number were optimized for production of riboflavin by *S. xiamenensis*. The optimal conditions of exogenous riboflavin concentration, initial pH and inoculated cell numbers were initial 18 nmol/mL pH 8.2 and 10⁵ CFU/mL, respectively in the LB fermented broth. The fermentation should be carried out for 72 hours.
2. Hydrogel bio-anode of alginate/polyaniline/titanium-dioxide/graphite composites with the immobilization of riboflavin mediator and *S. xiamenensis* cells was successfully fabricated and applied in MFC. The presence of riboflavin increased in the transportation of electrons from the cells to the electrode. New bio-anode improved the efficiency and stability of MFCs. The maximum power density (6.06 ± 0.15 W/m³) was obtained in the case of MFC using hydrogel bio-anode with the immobilization of riboflavin concentration as 15 nmol/mL and 20 nmol/mL.
3. Electrical conducting composites based on bacterial cellulose were successfully fabricated. The effect of aniline concentration and ammonium persulfate or iron (III) chloride hexahydrate as an oxidant was determined. The highest electrical conductivity (2.62 ± 0.15 S/m) of BC/PANI composites using ammonium persulfate as an oxidant could be obtained when use of 0.2 mol/L aniline and molar ratio of ammonium persulfate to aniline was 1.2:1. In the case of use of iron (III) chloride hexahydrate as an oxidant,

the electrical conductivity of BC/PANI composites was 2.21 ± 0.11 S/m at 0.2 mol/L aniline, and the molar ratio of iron(III) chloride hexahydrate to aniline was 1.5:1. The electrical conductivity of BC/PANI can be improved by coating with TiO₂. The electrical conductivity of TiO₂ coated BC/PANI/APS was 1.4-fold higher than sample without TiO₂.

4. New bio-anode of BC/PANI/APS/TiO₂ composites immobilized *S. xiamenensis* was fabricated and applied in the MFC. This bio-anode significantly improved the power density of MFC from 2.57 W/m³ (bare BC) to around 38.89 W/m³ meaning 15-fold higher.
5. The performance of MFC was improved by supplement of Fe(III). With 9 mM initial Fe (III), the coulombic efficiency of MFC was $56.71 \pm 1.13\%$, the maximum of power density was around 49.28 ± 2.34 to 51.11 ± 2.29 W/m³ between 20th hour and 40th hour. The maximum Fe (III) reduction rate was 0.031 ± 0.004 mM/h.

5 PUBLICATIONS

Journal articles with IF

- Duy H. Truong**, Mai S. Dam, Erika Bujna, Judit Rezessy-Szabo, Csilla Farkas, Vu Ngoc Ha Vi, Olivia Csernus, Vuong D. Nguyen, Nicholas Gathergood, László Friedrich, Mohamed Hafidi, Vijai Kumar Gupta and Quang D. Nguyen (2021): *In situ* fabrication of electrically conducting bacterial cellulose-polyaniline-titanium-dioxide composites with the immobilization of *Shewanella xiamenensis* and its application as bioanode in microbial fuel cell. *Fuel* 285: 119259. DOI: 10.1016/j.fuel.2020.119259. **IF₂₀₁₉: 5.578.**
- Duy H. Truong**, Mai S. Dam, Erika Bujna, Csilla Farkas, Judit M. Rezessy-Szabo, Vu Ngoc Ha Vi, Vuong D. Nguyen, Quang D. Nguyen. Kinetic of riboflavin production by *Shewanella xiamenenses* and the effect of exogenous riboflavin on the performance of microbial fuel cell. *Acta Alimentaria* (Accepted, in press).
- Csilla Farkas, Judit M. Rezessy-Szabó, Vijai Kumar Gupta, **Duy H. Truong**, László Friedrich, József Felföldi, Quang D. Nguyen (2019): Microbial saccharification of wheat bran for bioethanol fermentation. *Journal of Cleaner Production* 240: 118289. DOI: 10.1016/j.jclepro.2019.118269. **IF: 6.395.**

International conferences

- Truong Hoang Duy**, Edina Nagy, Levente Horvanth, Dam Sao Mai, Erika Bujna, Quang D. Nguyen, *Effect of exogenous riboflavin and initial pH on growth Shewanella xiamenesis, and conductivity of ferment broth*, EuroFoodChem XIX Conference, 2017, Budapest - Hungary
- Duy H. Truong**, Edina Nagy, Mai S. Dam, Erika Bujna, Quang D. Nguyen. The effect of ferric ion on electricity generation by *Shewanella xiamenensis* with cell immobilization technique in Microbial fuel cell. 3rd FoodConf (Conference on Food Science and Technology), 2018, Budapest, Hungary.

National conferences

- Truong Hoang Duy**, Edina Nagy, Levente Horvanth, Dam Sao Mai, Erika Bujna, Quang D. Nguyen, *Effect of exogenous riboflavin on growth and production of some metabolites by Shewanella xiamenensis and Shewanella japonica*. Chemical Engineering Conference, 2017, Veszprem – Hungary.
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