Biodegradation of Penicillin and Electricity Generation from Swine Wastewater using Ceramic Microbial Fuel Cell

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***Abstract*-** In order to improve the penicillin removal from the swine wastewater, the main challenge is to developed practical system for penicillin removal with low-operating cost. In this study, the low-cost ceramic microbial fuel cell (CMFC) was integrated with the penicillin degrading consortium. The electrochemical properties such as open circuit voltage (OCV), current density (CD), and power density (PD) were measured. In term of wastewater treatment, the laccase activity, penicillin and chemical oxygen demand (COD) removal efficiencies were studied. The results indicated that the maximal OCV, CD, and PD of the CMFC of 972.88±2.03 mV, 2.00±0.15 A/m^3 and 0.92±0.02 W/m^3 were achieved at the stationary phase. The extracellular laccase of 2.54±0.08 U/mL was released. The maximal penicillin and COD removal of 95.10±1.30% and 96.15±2.25%. This work provided the new knowledge in using the low-cost ceramic separator microbial fuel cell for penicillin removal and electricity generation from the swine wastewater.

***Index Terms*-** Bioelectricity generation, Microbial fuel cell, Penicillin, Swine wastewater

I. INTRODUCTION

The rapidly growing human population led to the economic development of enhanced feedstock-based food demand. Among meat demand, pork and poultry have broadly consumed worldwide [1]. Swine wastewater is a high organic and nutrient content as well as hormones, heavy metal and antibiotic drugs resulting from feces, feed and washing water [2-3]. It has become a critical polluting situation for antibiotic drugs. The previous report has shown that only 10-30% of consumed antibiotics are metabolized by animals. The non-consumed drug has been discharged into the swine wastewater in original or metabolite forms [4]. The results of Hoekstra and Chapagain reported the production of 1 kg generated approximately 4.8 m^3 of swine wastewater [5]. This wastewater should be improved before discharging municipal wastewater treatment plants or groundwater. Various processes have been developed for swine wastewater treatment such as electrochemical oxidation, ozonation, UV/hydrogen peroxide, photocatalysis, Fenton process and biological treatment [6-8]. The study of Domingues et al. has indicated that antibiotics can inhibit total effluent biological treatment [9]. The major classes of antibiotics used for feedstock growing are tetracyclines, penicillin, macrolides and sulfonamides [10].

Penicillin is a broadly applied antibiotic in feedstock growing especially swine and cattle [11]. It can be discharged to the wastewater and caused the high toxic impact for environment. The penicillin could bind to the specific protein called, penicillin-binding protein (PBP) of bacteria led to the inhibition of cell wall synthesis [12].

Laccase is the well-known biocatalyst owing to its broadly substrate specification led to wide range of application [13]. Nowadays, bacterial laccase has more interested than other microbes owing to its ability to tolerate high alkaline and temperature [14]. The bacterial laccase can be applied for various application such as antibiotic degradation [15], decolorization [16], bisphenol A (BPA) degradation [17], and biosensor [18].

Microbial fuel cell (MFC) is considered as green biotechnology for electricity generation and simultaneously wastewater bioremediation [19]. It has been used for different wastewater treatment such as pharmaceutical wastewater [20], municipal wastewater [21], sanitary wastewater [22], urine wastewater [23], rice mill wastewater and swine wastewater [25]. In Ren et al., the constructed wetland-microbial fuel cell has successfully used for swine wastewater treatment and electricity generation. The results showed that the maximal chemical oxygen demand (COD) of 72% and power output of 33.3 mW/m^3 were obtained. Whereas the antibiotic removal efficiency was not gained [26].

Ceramic microbial fuel cell (CMFC) has been shown to be the economically model of wastewater treatment and electricity generation [27]. Moreover, the CMFC can reduce approximately 60% of the material cost led to the suitable model for large-scale application [28]. The study of Gajda et al. has reported the CMFC can stability generation of electrical power up to 19 months [29].

Therefore, the CMFC was designed and conducted in this study for simultaneously removing penicillin from the synthetic swine wastewater. The removal efficiency of COD and penicillin were examined. The laccase activity and electrochemical properties were investigated.

MATERIAL AND METHOD

CMFC model

The stack CMFC used in this experiment was constructed from the modified model by Gajda et al. [29]. The terracotta cylinder (5.0 cm of diameter, 10.0 cm of height, and 0.3 cm of thickness) was used as a ceramic separator. The MFC chamber was made from the plastic tube with 500 mL of working volume. The activated carbon granule and stainless-steel mesh were used as electrodes. The electrodes were linked by copper wire (Fig. 1).

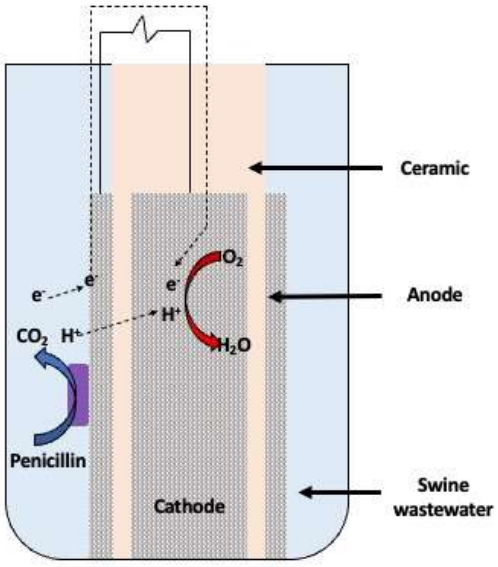

Figure 1: Diagram of the CMFC used in this experiment.

Microbial consortium

The penicillin degrading bacterial consortium was achieved from the Microbial Fuel Cell & Bioremediation Laboratory, Faculty of Science, Thaksin University, Thailand. It was mainly comprising of *Morganella morganii*, *Pseudomonas multiresinivorans*, and *Clostridium senegalense*. The consortium was maintained in the nutrient broth (3.0 g/L of beef extract and 5.0 g/L of peptone). The consortium was prepared at the concentration of 1.0×10^8 cell/mL using the spectrophotometry (OD 600 nm) and the standard curve before it was used.

Swine wastewater

The synthetic swine wastewater was prepared according to Cheng et al. [30], the wastewater contains 3.00 g/L of glucose ($C_6H_{12}O_6$), 0.45 g/L of ammonium chloride (NH_4Cl), 0.13 g/L of potassium dihydrogen phosphate (KH_2PO_4), 0.05 g/L of magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$), and 0.01 g/L of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$). The synthetic swine wastewater was sterilized at 121 °C for 15 mins to avoid the microbial contamination. The 100 µg/mL penicillin was added and kept at 4 °C before it was used in the next section.

CMFC operation

The 50 mL of 48-hr old consortium seed was inoculated into the CMFC chamber and the 450 mL of swine wastewater was added. The seed and wastewater were fermented for 48-hr under room temperature to immobilizing the penicillin degrading consortium on the anodic electrode. The 500 mL of fermented solution was fed out. Then, the 500 mL of fresh swine wastewater was fed in to determine the electrochemical properties of system. The open circuit voltage (OCV) was monitored every 60 mins for 1,440 mins. The close circuit voltage (CCV) was collected at the stationary phase of the electrical cycle. The polarization curve was measured at 300 – 1,000 Ω and plotted for study in the maximal power output generated from the CMFC system. The electrochemical properties such as current (A), power (W), current density (A/m^3) and power density (W/m^3) were calculated follows:

$$I = V/R \quad (1)$$
$$P = IV \quad (2)$$
$$CD = I/A \quad (3)$$
$$PD = P/A \quad (4)$$

where I is the current (A), V is the CCV at the external resistance of 1,000 Ω (V), R is the external resistance (Ω), P is the power (W), CD is the current density per working volume (A/m^3), A is the working volume (m^3) and PD is the power density per working volume (W/m^3).

Enzyme monitoring

The laccase activity was monitored using the UV-Vis spectrophotometry at 420 nm according to Chaijak et al. [31]. The swine wastewater was centrifuged at 12,000 rpm for 10 mins under 4 °C to preserve the crude enzyme activity. The 5 µL of supernatant was mixed with the 95 µL of ABTS solution. The colorimetric assay was determined. The laccase activity was measured every 360 mins for 1,440 mins.

Wastewater treatment

The swine wastewater was centrifuged at 12,000 rpm for 10 mins, the supernatant was collected. The penicillin removal was studied according to Yang et al. [32], the absorbance was determined at 325 nm using UV-Vis spectrophotometry. The penicillin removal was monitored every 360 mins for 1,440 mins.

The COD removal was measured using the High Range Plus COD kit (Hach, United States). The 0.25 mL of supernatant was added into the COD vial and heated in the DRB200 reactor for 2 hr. Then, the vial was cooled down at room temperature and measured the COD data using colorimetric procedure.

II. RESULTS AND DISCUSSION

The 48-hr old active consortium was inoculated onto the surface of anodic electrode in the CMFC. The swine wastewater with 100 μg/mL penicillin was fed in to prove the wastewater treatment and electricity generation efficiencies. The log phase was covered during time-0 min to thime-720 min. The stationary phase was covered during time-720 to time-1,440 min (Fig. 2). The maximal OCV of 972.88±2.03 mV was obtained at the stationary phase.

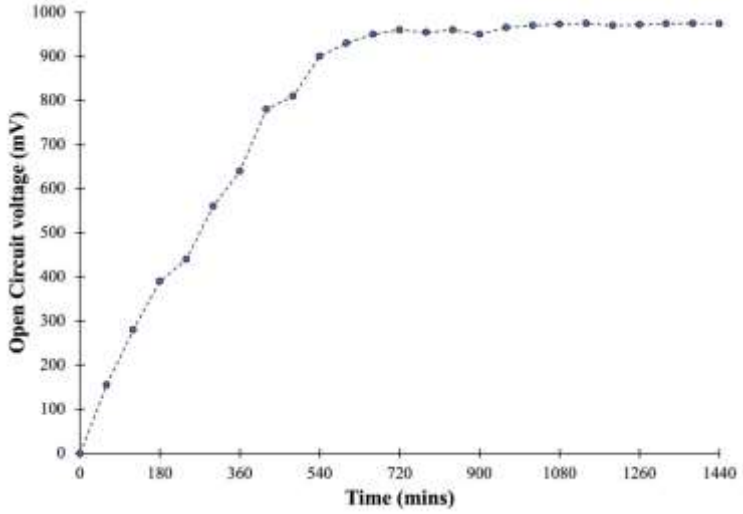

Figure 2: The open circuit voltage of the CMFC with the penicillin degrading consortium.

The polarization curve was calculated when the external resistances of 300-1,000 Ω were applied with the CMFC at the stationary phase. The Ohm's law was used for the calculation of the electrochemical properties. The maximal current density and power density of 2.00±0.15 A/m^3 and 0.92±0.02 W/m^3 were gained without the expensive metal catalyst. The polarization curve was showed in Fig. 3.

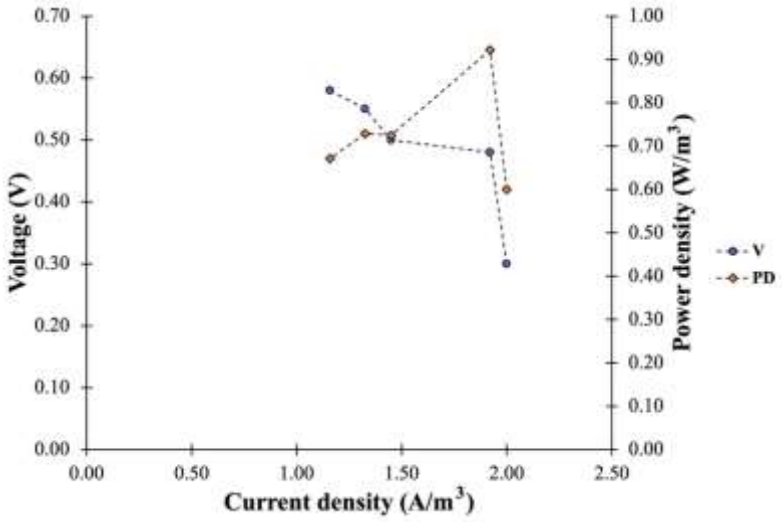

Figure 3: The polarization curve of the CMFC with the penicillin degrading consortium.

The extracellular laccase activity of the penicillin degrading consortium immobilized on the anodic surface was determined using the colorimetric method. The supernatant collected from the wastewater was used. The laccase activity was monitored every 360 mins or 6 hr. The laccase activity was found when the 48-hr old active consortium was contacted with the swine wastewater for 6 hr. The maximal laccase activity of 2.54±0.08 U/mL was achieved at time-1,080 min (Fig. 4).

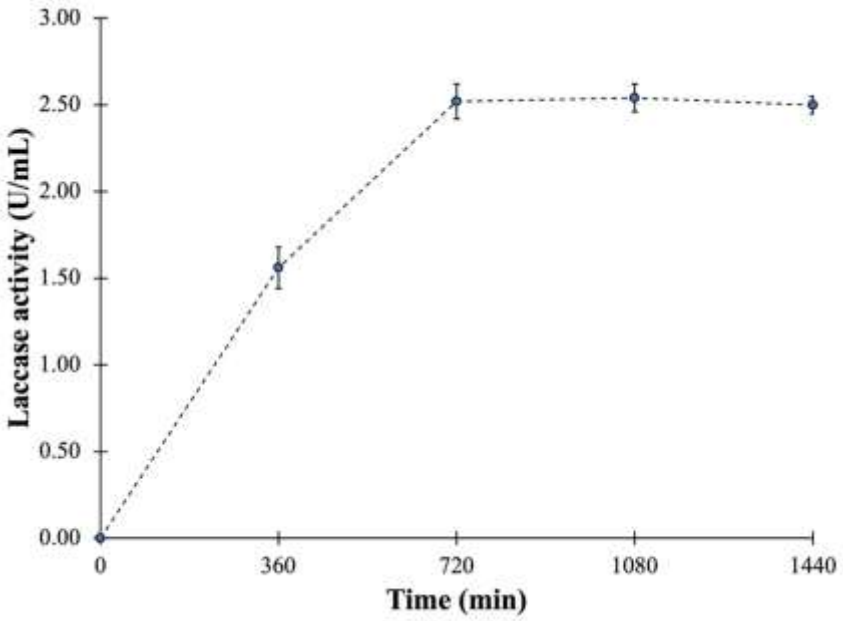

Figure 4: The laccase activity during the CMFC operation.

The penicillin removal efficiency from the swine wastewater using the CMFC was measured every 360 mins where the initial penicillin concentration of 100 μg/mL. The maximal penicillin removal of 95.10±1.30% was achieved (Fig. 5).

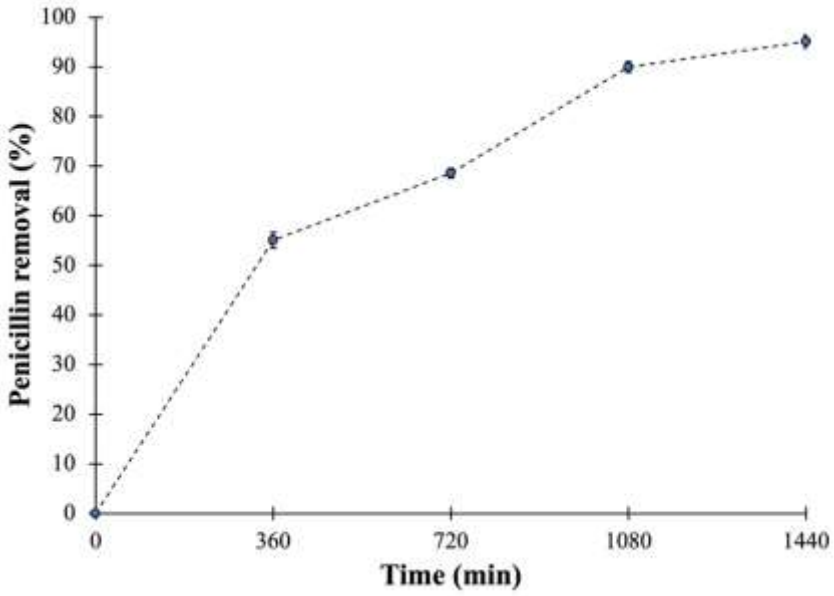

Figure 5: The penicillin removal (%) of the CMFC in this experiment.

The COD removal efficiency of the CMFC system was measured to study of the wastewater treatment potential of this system. The maximal COD removal of 96.15±2.25% was obtained. The COD removal of the CMFC with the penicillin degrading consortium was shown in Fig. 6.

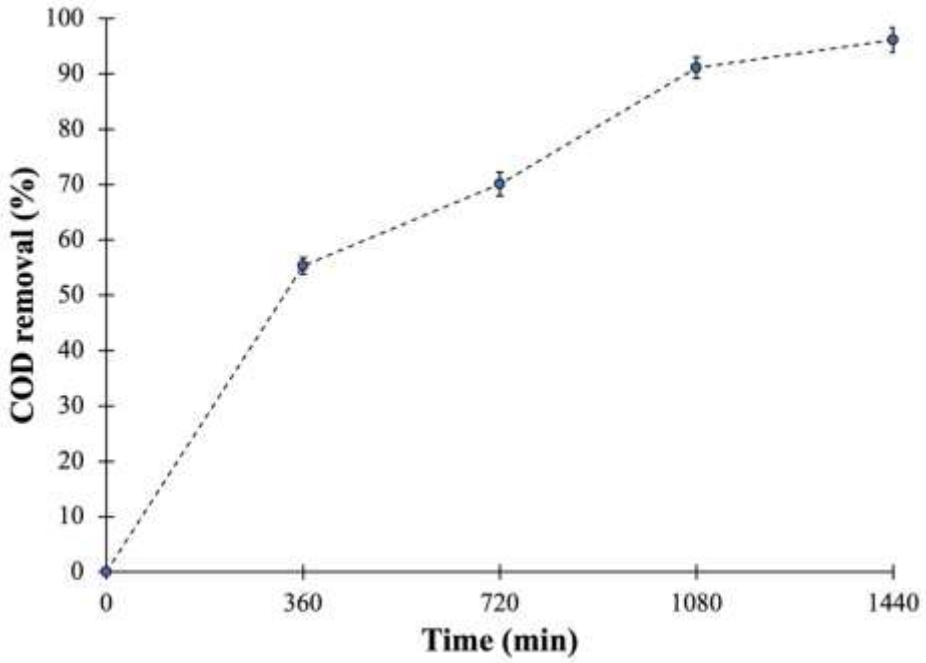

Figure 6: The COD removal (%) of the CMFC in this experiment.

On the other hand, the swine wastewater was treated using the constructed wetland-microbial fuel cell with the macrophyte Ipomoea aquatica as a biocatalyst. The maximal power output of 0.50 W/m^3 was gained where the *Comamonas* sp. was immobilized on the anodic surface for wastewater treatment [33]. In the study of Zhang et al., the photosynthetic algae (*Chlorella vulgaris*) MFC was used for power generation in the swine wastewater. The maximal voltage and power output of 747 mV and 3.72 W/m^3 were gained. However, the antibiotic removal efficiency was not achieved [34].

Moreover, the single chamber MFC with air-cathode of platinum-coated carbon cloth has been used for the swine wastewater treatment and simultaneously electricity generation. The maximal power output of 0.6 to 2.2 W/m^3 were generated along with the COD removal of 52% [35]. Li et al. indicated the airlift photosynthetic MFC using the swine wastewater as substrate was developed. It has performed in mixotrophic cultivation and enriched electrogene. The maximal power density of 1.92 KW/m^3 was gained [36]. As the result of Ni et al., the dual-chamber MFC was used for the swine wastewater treatment. The maximal voltage output of 634 mV was generated with the maximal COD removal efficiency of 80.10% [37].

Various processes have been developed in term of antibiotic removal from the agricultural and municipal wastewater such as photocatalysis, adsorption, membrane technology, hybrid technology, anaerobic treatment, aerobic treatment, electrochemical oxidation, wet oxidation and ozonation [38]. The comparison of antibiotic removal technologies potential of this study and other studies has presented in Table 1.

Table 1: The antibiotic removal efficiency of this experiment and other work.

Technology	Antibiotic type	Removal (%)	Chemical/Power input	By-product	Reference
CMFC	Penicillin	95.10±1.30	-	Electrical power	This work
Anaerobic treatment	Sulfonamide β-lactam	95.00	Electrical energy	None	[39]
Adsorption	Tetracycline	100.00	Electrical energy Biochar	None	[30]
Biodegradation	Sulfonamide	90.00	Electrical energy Chemical supplement	None	[40]
Modified constructed wetland	Tetracycline	98.00	Electrical energy	None	[41]

CONCLUSION

In conclusion, the ceramic microbial fuel cell (CMFC) has been developed for penicillin removal from the swine wastewater and simultaneously electricity generation. The maximal penicillin removal of 95.10±1.30% was reached when the extracellular laccase has releasing from the anodic surface. The maximal power output of 0.92±0.02 W/m^3 was produced. This work gained the new knowledge in using the low-cost ceramic separator microbial fuel cell for penicillin removal and electricity generation from the swine wastewater.

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