บทความวิจัย

การเพิ่มประสิทธิภาพการผลิตกระแสไฟฟ้าชีวภาพด้วยเซลล์เชื้อเพลิงจุลินทรีย์เซรามิกรูปแบบใหม่ โดยใช้ขั้วแคโทดที่มีเอนไซม์แลคเคสเป็นคะตะลิสต์

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บทคัดย่อ

เยื่อแลกเปลี่ยนโปรตอนเป็นองค์ประกอบสำคัญที่มีอิทธิพลต่อต้นทุนในการเดินระบบ การบำบัดสารปนเปื้อนในน้ำเสียและการผลิตกระแสไฟฟ้าในเทคโนโลยีเซลล์เชื้อเพลิงจุลินทรีย์ จากการศึกษาที่ผ่านมาวัสดุที่มีรูพรุนหลากหลายชนิดถูกนำมาประยุกต์ใช้ รวมถึงแผ่นเซรามิก ในงานวิจัยนี้เซลล์เชื้อเพลิงจุลินทรีย์เซรามิกรูปแบบใหม่ถูกพัฒนาขึ้น โดยใช้ขั้วแคโทดที่ผิวมีจุลินทรีย์แบบใช้อากาศเคลือบอยู่ซึ่งที่ประกอบขึ้นจากการเพาะเลี้ยงเชื้อยีสต์ *Galactomyces reessii* ที่ผลิตแลคเคสบนวัสดุใยมะพร้าวและวางบนแผ่นตาข่ายสเตนเลส ใช้ผ้าคาร์บอนเป็นขั้วแอโนด และใช้แผ่นเซรามิกที่มีความหนา 0.2 เซนติเมตร เป็นแผ่นกั้นประจุ ใช้น้ำเสียยางพาราที่มีความเข้มข้นซัลเฟตเริ่มต้นที่ 500 มิลลิกรัมต่อลิตร และค่าซีโอดีเริ่มต้นที่ 1,000 มิลลิกรัมต่อลิตร จากการศึกษาพบว่าระบบเซลล์เชื้อเพลิงจุลินทรีย์เซรามิกรูปแบบใหม่ที่พัฒนาขึ้นซึ่งทำการเดินระบบแบบกะ สามารถผลิตความหนาแน่นกำลังไฟฟ้าสูงสุดที่ 310.78 ± 1.94 มิลลิวัตต์ต่อลูกบาศก์เมตร ความหนาแน่นกระแสไฟฟ้าสูงสุดที่ $3,392.09 \pm 16.07$ มิลลิแอมแปร์ต่อลูกบาศก์เมตร และมีค่าความต้านทานภายในระบบเท่ากับ 330 โอห์ม ระบบนี้สามารถบำบัดซีโอดีได้ที่ร้อยละ 78.70 ± 0.56 และบำบัดสารซัลเฟตที่ร้อยละ 88.85 ± 0.50 ดังนั้นเซลล์เชื้อเพลิงจุลินทรีย์เซรามิกรูปแบบใหม่มีศักยภาพในการพัฒนาเพื่อใช้ในการบำบัดน้ำเสียในระดับอุตสาหกรรม

คำสำคัญ: แผ่นกั้นเซรามิก ใยมะพร้าว พลังงานไฟฟ้า แลคเคส เซลล์เชื้อเพลิงจุลินทรีย์ สแตนเลส ซัลเฟต

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Enhancing Bio-Electricity Generation Using Novel Model of Ceramic-Separator Microbial Fuel cell with a Laccase-Based Cathode

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ABSTRACT

In microbial fuel cell (MFC), a proton exchange membrane (PEM) is an important part that affects the cost of the operation and the performance in contaminants elimination and electricity production. Various types of porous materials have been studied as alternatives to ion separators including ceramic. In this work, a novel model of ceramic-separator microbial fuel cell (CMFC) consisted of air-cathode based on coconut coir with laccase producing yeast *Galactomyces reessii* on stainless steel net, the carbon cloth anode and ceramic plate with 0.2 cm of thickness. The rubber wastewater (initial 500 mg/L sulfate concentration and 1,000 mg/L COD concentration) was used as an anolyte. The maximum power density of 310.78±1.94 mW/m^3, the maximum current density of 3,392.09±16.07 mA/m^3 and the internal resistance of 330 Ω were obtained for CMFC by laccase-based cathode under batch processing. Moreover, the COD removal of 78.70±0.56% and the sulfate removal of 88.85±0.50% were achieved. Hence, the ceramic-separator microbial fuel cell with a laccase-based cathode are potential candidate for the development of industrial-scale wastewater treatment plants.

Keywords: Ceramic-separator, Coconut coir, Electrical energy, Laccase, Microbial fuel cell, Stainless steel, Sulfate

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Introduction

Microbial fuel cell (MFC) is a technology that uses microbes as a biocatalyst to degrade both organic and inorganic materials, and then generate electrical power [1]. To treat wastewater by MFC, microbes are used to convert the chemical energy of contaminants in wastewater into electrical energy [2]. In MFC, a proton exchange membrane (PEM) is an essential part that affects the cost of the operation and the performance in contaminants elimination and electricity production. Various types of PEM such as Nafion have often been used. However, Nafion is extremely expensive, reaching 3,000 USD per m^2. Accordingly, a low-cost PEM alternative is required [3]. Various types of porous materials have been studied as alternatives to ion separators including ceramic [4]. Previous report showed that porous material can perform better than PEM as the separator in MFC with lower internal resistance and reduction in power overshoot [5].

Another main problem of the MFC system is the slow kinetic rate on the cathode surface during the reduction of oxygen [6]. Therefore, the catalysts need to be used to overcome reaction overpotential and accelerate the kinetic reaction. Previous studies indicated that the applications of different types of biocatalyst such as ascorbate oxidase [7], bilirubin oxidase [8] and laccase [9] could reduce the molecular oxygen (O_2) to molecular water (H_2O), and remove specific contaminants such as nitrate and sulfate in wastewater [10].

Laccase (or benzenediol: oxygen oxidoreductase) is an essential multi-copper containing polyphenol oxidase which catalyzes on various substrates. It is widely distributed in diverse organisms including higher plants, insects, bacteria, and fungi [11]. Several reports are available for filamentous fungal laccases such as *Lenzites elegan* [12], *Phanerochaete chrysosporium* [13], and *Penicillium simplicissimum* [14]. Moreover, the laccase has been found in some yeast strains such as *Galactomyces reessii* [15] and *Galactomyces geotrichum* [16].

Natural rubber of *Hevea brasiliensis* is used for the production of an extensive range of elements such as tires, adhesives, and gloves [17]. The rubber wastewater released from a rubber industry is generally treated by the stabilization pond processes, following a residual latex particle recovery by adding the sulfuric acid [18]. However, this system is limited by a vast area requirement and emits the highly toxic gas, hydrogen sulfide (H2S) [19]. For these reasons, the alternative ceramic separator and laccase producing yeast *G. reessii* were used to develop the novel model cost-effective MFC and then evaluated regarding performance and application area in rubber wastewater treatment.

Materials and Methods

Microbes

The laccase producing yeast *G. reessii* [15] was achieved by the Department of Biotechnology, Faculty of Science, Thaksin University, Thailand. This yeast was maintained on the potato dextrose agar (PDA) at 4°C until used. The sludge of the rubber wastewater was collected from the natural rubber industry in Phatthalung province, Thailand. The microbial community of sludge was described in our previous study [20]. The 10 g of sludge was inoculated into 100 mL of nutrient broth (NB) and then it was incubated at 30°C for 24 hour without shaking. The 24-hour-old mixed culture was used as an MFC anode starter.

Laccase fermentation

One plug (1×1 cm) of 7 days-old *G. reessii* was inoculated into 100 mL of potato dextrose broth (PDB) and incubated at 25°C for 7 days with shaking. The 7-days-incubated broth was used as a consortium for laccase production under solid state fermentation. The 0.5 cm size coconut coir was washed with tap water and deionized water (DI water). It was autoclaved at 121°C for 15 mins to remove the contaminant cultures and dried at 60°C until constant weight was achieved.

The 4.9 mL of 7-days-incubated broth was transferred into 7 g of weighing invariably sterilized coconut coir (to adjust the initial moisture of 70%). It was fermented at 25°C for 7 days. To confirm the laccase activity, the 1 g of 7-days-fermented coconut coir was soaked in the sodium acetate buffer. The crude enzyme in the supernatant was determined by laccase activity [15].

MFC design

The diagram of the novel model ceramic-separator MFC (CMFC) with the laccase-based stainless steel cathode is presented in Figure 1. The anode chamber was made of a 200-mL glass beaker. The cathode chamber was made from 2.0 mm thick ceramic cylinder (6.0 cm height, 6.4 cm outer diameter, and 6.0 cm inner diameter) that was prepared according to the previous study [21]. It was soaked in DI water overnight before used. The cathode chamber was dipped in 1.0 cm below the anolyte surface (interface area between the anode and the cathode was 52.3 cm^2), and it was opened to the atmosphere to obtain necessary oxygen. The working volume of the anode chamber was 150 mL.

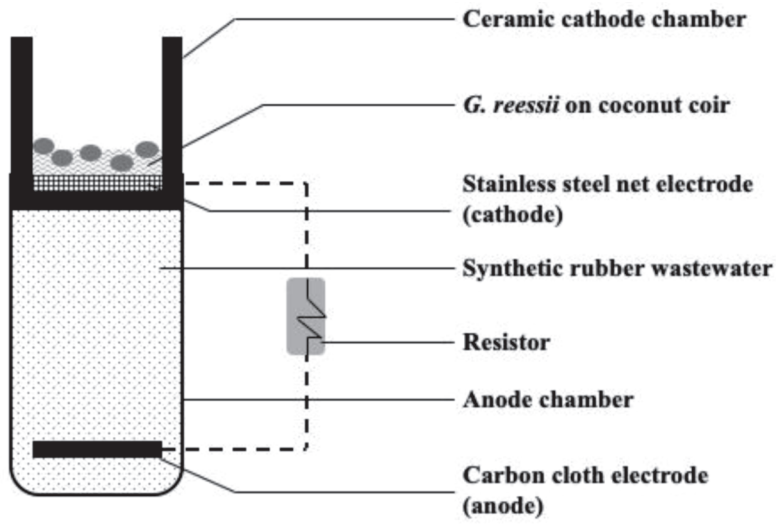

Figure 1 The diagram of the novel model CMFC with the whole cell bio-catalyst on a cathode.

The plain carbon cloth (5×2 cm) was used for the anode. The stainless steel net (5×2 cm) was used for cathode with the laccase-based cathode (Lac-cathode) and the negative control with sterile coconut coir (Co-cathode). The 5×2 cm of platinum (Pt) coated carbon cloth (0.3 mgPt/cm^2) was used for positive control (Pt-cathode). The 1.0 mm diameter stainless steel wire was used for a link between electrodes.

Operation

The rubber wastewater containing sulfate (500 mg/L) and initial COD concentration (1,000 mg/L) was prepared and sterilized at 121°C for 15 mins according to modified method [20], and stored at 4°C until used as anolyte.

To operate the CMFC, the 15-mL of starter was mixed in 135-mL anolyte and transferred to the anode chamber. The 6 g of 7-days-fermented coconut coir was filled on the stainless steel net in the ceramic cathode chamber (Lac-cathode). The 6 g of sterile coconut coir was used for negative control (Co-cathode).

The open circuit voltage (OCV) was collected every 10 mins for 24 h using LabView software (version 2015, National Instruments, United States). Five different resistors (150, 270, 330, 390 and 1,002 Ω) were used for the closed circuit voltage (CCV).

Analysis and calculation

The sulfate removal was determined by the turbidimetric method [22]. The sulfate removal (%) was calculated by:

$$\text{Sulfate removal} = [(A-B)/A] \times 100 \quad (1)$$

where A and B are the initial and final absorbance, respectively.

The COD concentration was monitored by the COD test kit (Hach, United States). The COD removal (%) was calculated by:

$$\text{COD removal} = [(COD_{before} - COD_{after})/COD_{before}] \times 100 \quad (2)$$

where COD_{before} and COD_{after} are the COD (mg/L) before and after treatment, respectively.

The electrochemical properties such as current density (CD) and power density (PD) were calculated according to the previously studied [23]. The polarization curves were plotted according to Wu and co-workers [23]. The internal resistance was determined based on the maximum power transfer theorem [24].

Results

Laccase activity

To produce the extracellular laccase under solid-state fermentation, the 7-days-old yeast *G. reessii* consortium was added to sterile coconut coir (70% initial moisture) and then incubated for 7 days. The results revealed that the yeast *G. reessii* could produce the 154.04±2.40 U/mL (N=3) without adding exogenous media and chemical inducer.

Electrochemical properties

To study the application of the biocatalytic cell to enhance electricity generation in CMFC, the laccase producing yeast was grown on a stainless steel net electrode to catalyze an electron accepting of atmospheric oxygen. Figure 2 shows the Lac-cathode CMFC that generates the OCV of 960.82±1.30 mV (N=3) at 540 mins. While the Pt-cathode CMFC and Co-cathode CMFC generate the OCV of 965.17±2.81 mV (N=3) at 430 mins, and 446.35±12.29 mV (N=3) at 100 mins respectively. The results indicated that the Lac-cathode generated the higher OCV than the Co-cathode (negative control) by 53.54%. However, it produced the minor lower OCV than the Pt-cathode (positive control) by 0.45%.

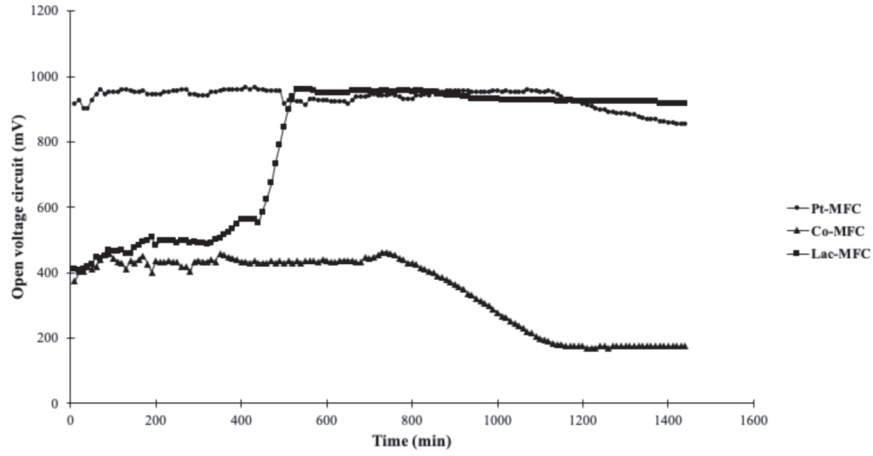

Figure 2 The open circuit voltage of the novel model CMFC with the difference cathodes.

The five different resistors (150, 270, 330, 390 and 1,002 Ω) were connected to study CMFC performance in CCV with 150 mL of anode working volume. The electrochemical properties of three different types of air-cathode CMFCs was shown in Table 1. The Lac-cathode achieved the maximal CD and PD about 3,392.09±16.07 mA/m^3 (N=3), and 310.78±1.94 mW/m^3 (N=3) respectively. While the Pt-cathode reached the maximal CD and PD of 3,472.76 ±8.46 mA/m^3 (N=3), and 471.68±3.30 mW/m^3 (N=3), respectively. The Co-cathode achieved the maximal CD and PD of 128.59±0.44 mA/m^3 (N=3) and 0.59±0.00 mW/m^3 (N=3), respectively.

Table 1 The electrical properties of CMFC (N=3).

Resistance (Ω)	Pt-cathode		Lac-cathode		Co-cathode	
	CD (mA/m^3)	PD (mW/m^3)	CD (mA/m^3)	PD (mW/m^3)	CD (mA/m^3)	PD (mW/m^3)
150	3,472.76±8.46	271.35±1.32	3,392.09±16.077	258.89±2.45	128.59±0.444	0.37±0.00
270	3,234.81±20.34	423.80±5.34	2,691.19±56.09	293.41±12.18	100.27±1.37	0.41±0.01
330	3,086.87±10.81	471.68±3.30	2,505.66±7.82	310.78±1.94	108.79±0.37	0.59±0.00
390	2,708.60±8.77	29.19±2.78	2,218.63±6.45	287.96±1.67	90.66±4.40	0.48±0.05
1,002	1,570.41±30.27	370.76±14.33	1,271.92±13.52	243.17±5.19	41.14±0.20	0.25±0.00

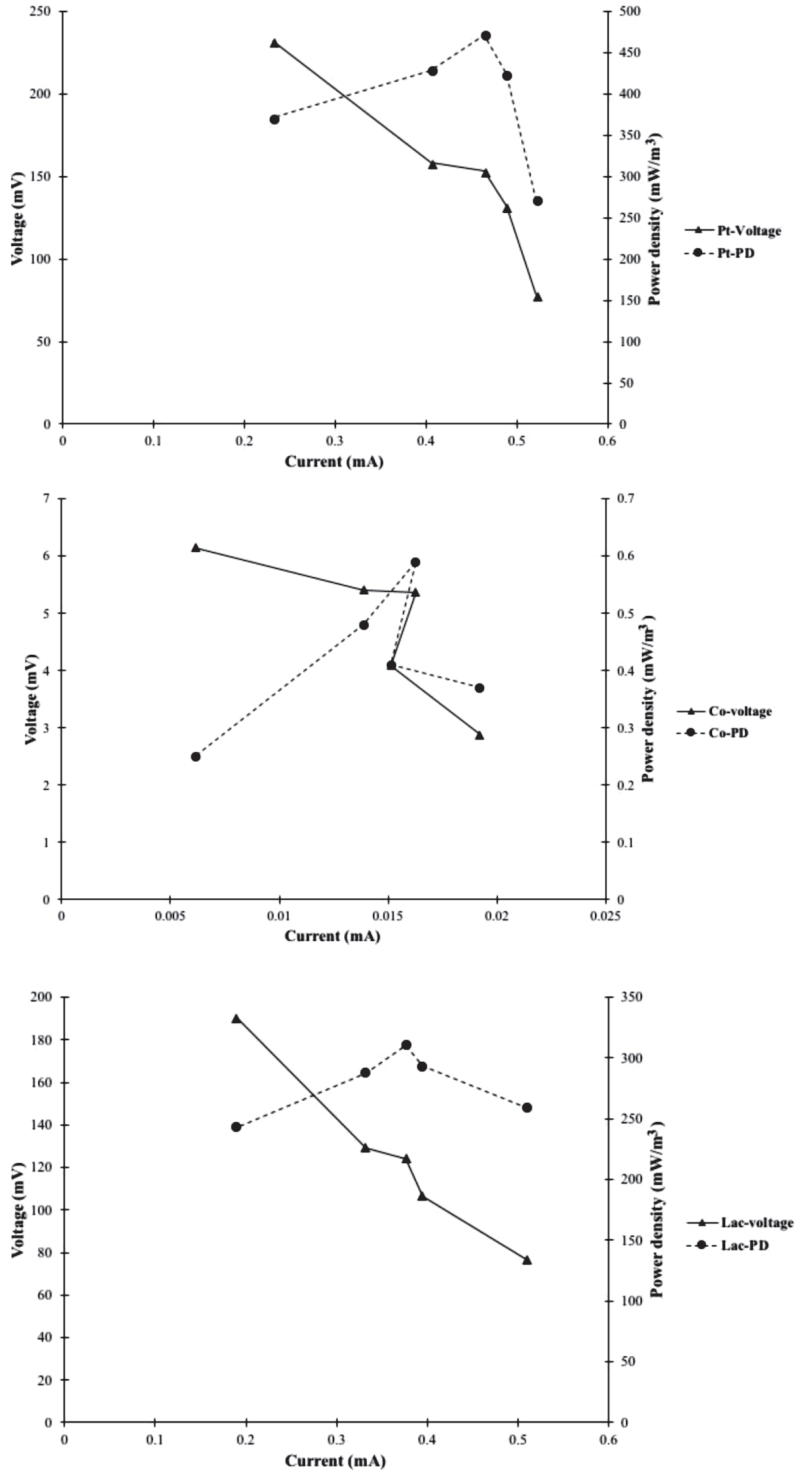

Figure 3 The power density cure of the novel model CMFC with the difference cathodes.

The power density curve of the novel CMFC models (Figure 3) show the internal resistance of 330 Ω. This study indicated that the Lac-cathode provided the lower PD than the positive control (Pt-cathode) by 34.11% but it generated higher PD than the negative control (Co-cathode) by 99.81%. Thus, the Lac-cathode CMFC is an interesting alternative low-cost system to generate electrical power.

Sulfate and COD removal efficiencies

After 24 h of operation in rubber wastewater treatment (initial 500 mg/L sulfate and 1,000 mg/L COD), the sulfate removal efficiencies by the CMFCs were monitored. The Lac-cathode, Pt-cathode, and Co-cathode achieved the sulfate removal of 88.85±0.50% (N=3), 88.95±0.40% (N=3) and 88.84±0.64% (N=3) respectively. Moreover, the COD removal efficiencies by CMFCs showed that the Lac-cathode, Pt-cathode, and Co-cathode achieved the COD removal of 78.70±0.56% (N=3), 78.33±0.61% (N=3), and 78.40±0.36% (N=3), respectively.

Discussion and Conclusion

Comparison of laccase activity using solid-state fermentation of the G. reessii and other studies showed in Table 2. The result shows that the *G. reessii* produce higher laccase activity than other works about 15.36 to 96.60% [12, 16, 25-27].

Table 2 Comparison of laccase activity of the *G. reessii* and other studies.

Microbe	Substrate/ Carbon source	Inducer/ Supplement	Laccase activity (U/mL)	Reference
Galactomycess reessii	coconut coir	–	154.04±2.40	This study
Brevundimonas sp.	glucose	copper sulfate guaiacol	5.24	[25]
Marasmiellus palmivorus	glucose	casein	3,420.00	[26]
Lenzites elegans	glucose	veratryl alcohol	156.79	[12]
Galactomyces geotrichum	rice bran	–	130.38	[16]
Spirulina platensis	glucose	guaiacol	582.92	[27]

Comparison of laccase-based CMFC and other MFCs were shown in Table 3. The results indicate that the CMFC showed the higher PD than other MFCs by 27.61% to 99.03% [28-33].

Table 3 Comparison of laccase-based CMFC and other MFCs.

MFCs type	Anode consortia	Cathode catalyst	Wastewater	PD (mW/m^3)	Reference
CMFC	rubber wastewater sludge	laccase	rubber wastewater	310.78±1.94	this study
dual chamber MFC	winery wastewater sludge	laccase	molasses wastewater	66.67	[30]
dual chamber MFC	wastewater treatment plant sludge	laccase	dye-wastewater	224.98	[28]
single chamber MFC	oil-cracking wastewater sludge	laccase	dye-wastewater	51.15	[31]
dual chamber MFC	*Shewanella oneidensis MR-1*	laccase	dye-wastewater	160.00	[32]
single chamber MFC	wastewater treatment plant sludge	laccase	sulfate-nitrate wastewater	64.00	[33]
upflow bio-filter circuit	rubber wastewater sludge	–	rubber wastewater	3.00	[29]

Comparison of the COD and sulfate removal efficiencies of laccase-based CMFC and other MFCs were shown in Table 4. The laccase-based CMFC with the stainless steel electrode provided the higher efficiencies (COD and sulfate removal) Therefore, it can be used as a model to develop wastewater treatment plant.

Table 4 Comparison of COD and sulfate removal efficiencies of laccase-based CMFC and other MFCs.

MFC type	COD removal (%)	Sulfate removal (%)	PD (mW/m^3)	Reference
CMFC	78.70	88.85	310.78±1.94	This study
Upflow Bio-filter circuit	80.00	70.00	3.00	[29]
Single chamber MFC	90.00	17.60	64.00	[33]
Single chamber MFC	72.00	67.10	32.00	[34]

The economic CMFC model is an example of clean technology that uses whole cell biocatalyst to generate electricity and treat the wastewater without addition the chemical and exogenous media. It can remove sulfate-rich wastewater and produce electrical energy under short time batch operation. The maximum PD of 310.78 mW/m^3, the COD removal of 78.70% and the sulfate removal of 88.85% were obtained. The work provided a promising method for electricity generation and high pollutant remove from wastewater with low-cost operation.

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