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Electricity Generation in Cellulose-Fed Microbial Fuel Cell Using Thermophilic Bacterium, *Bacillus* sp. WK21

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The cellulose-fed microbial fuel cell (MFC) is a biotechnological process that directly converts lignocellulosic materials to electricity without combustion. In this study, the cellulose-fed, MFC-integrated thermophilic bacterium, *Bacillus* sp. WK21, with endoglucanase and exoglucanase activities of 1.25 ± 0.08 U/ml and 0.95 ± 0.02 U/ml, respectively, was used to generate electricity at high temperatures. Maximal current densities of 485, 420, and 472 mA/m^2 were achieved when carboxymethyl cellulose, avicel cellulose, and cellulose powder, respectively, were used as substrates. Their respective maximal power was 94.09, 70.56, and 89.30 mW/m^3. This study demonstrates the value of the novel use of a cellulase-producing thermophilic bacterium as a biocatalyst for electricity generation in a cellulose-fed MFC.

Keywords: **Thermotolerant bacteria, cellulase, cellulose, microbial fuel cell, electricity generation**

Owing to the rapidly rising human population, the requirement for energy is increased while fossil fuel is depleted. Hence, the researcher is focusing on the investigation of alternative energy resources such as bioenergy from energy crops and agricultural wastes [1, 2]. For the second generation, biofuel is obtained from the conversion of waste materials. Among waste residue, the lignocellulose biomass is one of the most interesting substrates due to its potential for converting to bioenergy [3]. Almost 50% of lignocellulose is composed of biodegradable cellulose derivatives, it is a non-branched polysaccharide that comprises approximately 14,000 D-glucose linked by β-glycosidic linkage [4, 5]. To release the glucose from the lignocellulose substrate, microbial cellulolytic enzymes such as exoglucanase (EC 3.2.1.91), endoglucanase (EC 3.2.1.4), and cellobiase (EC 3.2.1.21) have been used [6]. However, the high-temperature reaction is used for removing microbial contamination and achieving faster chemical reactions during bioenergy production. so, the thermophilic enzyme has been investigated owing to its flexibility and high stability at high temperatures [7].

Moderate thermophile is the microbe that has optimal growth temperature between 50–60℃ [8]. In Shiratori *et al.*, the moderate cellulolytic bacteria *Clostridium clariflavum* and *Clostridium caenicola* with optimal growing at 60℃ have been isolated from anaerobic sludge of cellulose-degrading methanogenic bioreactor [9].

Microbial fuel cell (MFC) has recently gained more interest due to its ability to generate electricity from various waste materials such as sugar, synthetic substrate, carbon source substrate, nitrogen substrate, organic acid, livestock wastewater, swine wastewater, food-based wastewater, dairy wastewater, agro-processing wastewater, refinery wastewater, and distillery wastewater [10, 11]. Cellulose-fed MFC is the alternative electricity generation process that uses cellulolytic

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bacteria to decompose cellulose and gain electrical power [12]. In Tockzylowska-Maminska *et al*., the maximal power density of 44 mW/m^2 was achieved from the single-chamber MFC with air-cathode. the microbial community was dominated by *Parabacteroides*, *Proteiniphilum*, *Catonella,* and *Clostridium* [13]. This work aims to investigate the electricity generation from cellulose-fed MFC with moderate-thermophilic bacterium *Bacillus* sp. WK21 under high temperature at 50℃.

The moderate thermophilic bacteria *Bacillus* sp. WK21 was isolated from the hot spring sediment in Nakhon Si Thammarat, Southern Thailand (8º 26' 11" N and 99º 57' 47"). It was maintained in the nutrient broth (1.0 g/l beef extract, 2.0 g/l yeast extract, 5.0 g/l peptone, and 5.0 g/l NaCl) at 50℃. The endoglucanase and exoglucanase activities were determined by DNS (3,5-dinitrosalicylic acid) assay at 540 nm. One unit of the enzyme was defined as the amount of enzyme that hydrolyzes carboxymethyl cellulose (CMC) and microcrystalline cellulose-avicel then releases 1 μmol of glucose per min [14, 15].

The dual-chamber MFC was constructed, the 50 ml cell-culture bottles were used as cathode and anode chambers. The 1 M KCl salt bridge gel (10% w/v agarose) was used to transfer a proton from the anode chamber to the cathode chamber [16]. The 4 cm^2 of microwave-expanded graphite plates were used as electrodes. The 200 mg/l $KMnO_4$ in distilled water was used as a catholyte [17]. The 1% (w/v) of substrates (CMC, avicel, and cellulose powder) in 50 mM phosphate buffer saline (PBS) was used as an anolyte. The 10% (v/v) of liquid culture was mixed with an anolyte and filled into an anode chamber. The electrodes were linked by copper wire.

The MFC was operated at 50℃, the opened circuit voltage (OCV) was monitored every 6 h for 72 h. The closed-circuit voltage (CCV) was measured at fixed external resistance (1000 Ω). Current (I) and power (P) were calculated according to Ohm's law. The current density (CD) and power density (PD) was calculated based on electrode area and working volume.

Endoglucanase can randomly hydrolyze internal sites of cellulose, whereas exoglucanase can act at the reducing and non-reducing ends of cellulose [14]. In this study, the endoglucanase and exoglucanase activities of 1.25 ± 0.08 U/ml and 0.95 ± 0.02 U/ml were shown when the *Bacillus* sp. WK21 was grown at 50℃. Previous studies on endoglucanase and exoglucanase activities of thermophilic bacteria isolated from natural sources were generally low (Table 1). In Zhao *et al.*, the cellulolytic bacterial consortium contains *Geobacillus* sp., *Thermus* sp., *Bacillus* sp., and *Anoxybacillus* sp. was gained from the hot spring sediment in China. However, the endoglucanase and exoglucanase activities were not analyzed [23].

The maximal OCV of the dual-chamber MFC with *Bacillus* sp. WK21 as an anodic catalyst of 890 ± 5 mV, 650 ± 10 mV, and 860 ± 10 mV was gained when the 1% (w/v) CMC, avicel, and cellulose powder were used as a substrate (Fig. 1A). Currently, there have been few studies that have developed the cellulose-fed MFC for second-generation clean energy production (compared in Table 2). In this study, the new knowledge of using dual-chamber MFC was integrated with thermophilic cellulolytic bacterium *Bacillus* sp. WK21 for operation under high temperature at 50℃ was gained.

Table 1. Review of endoglucanase and exoglucanase activities of thermophilic bacteria isolated from a natural source.

Microbe	Source	Optimal temperature (℃)	Endoglucanase (U/ml)	Exoglucanase (U/ml)	Reference
Bacillus sp. WK21	Hot spring sediment	50	1.250 ± 0.080	0.95 ± 0.02	This study
Bacillus licheniformis 2D55	Compost	50	0.330	NA	[18]
Bacillus sp. C1CA5507	Sugarcane bagasse	70	0.370	NA	[19]
Bacillus subtilis BY-3	Tibetan pig feces	60	4.323	NA	[20]
Geobacillus sp.	Hot spring sediment	60	0.096	0.156	[21]
Bacillus aerius CMCPS1	Hot spring sediment	50	2.980	1.76	[22]

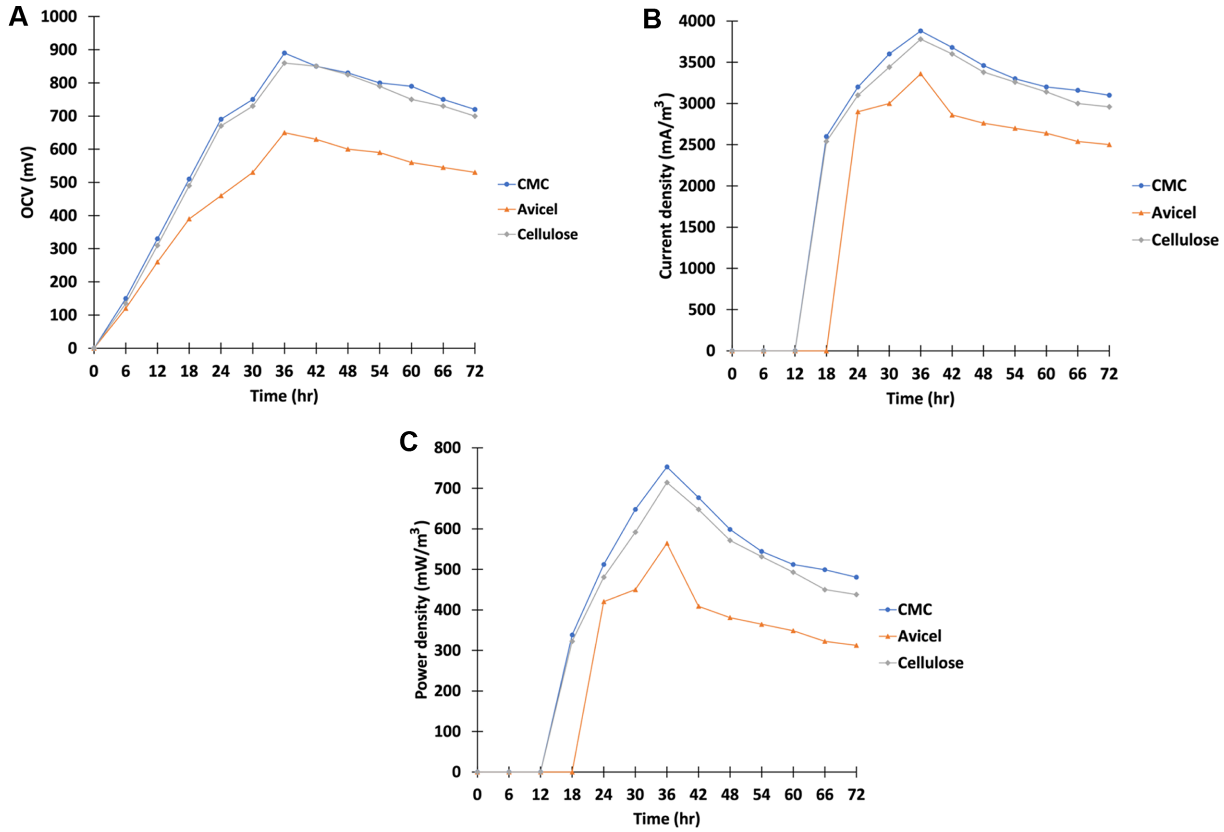

Fig. 1. The electrochemical properties of dual-chamber MFC in this experiment. (A) The OCV (mV) of dual-chamber MFC. (B) The generated current density based on the working volume (mA/m^3) of dual-chamber MFC. (C) The generated power density based on the working volume (mW/m^3) of dual-chamber MFC.

Table 2. Comparison of cellulose-fed MFCs.

Microbe	MFC Type	Specification	Cellulose type	PD (mW/m^2)	Reference
Bacillus sp. WK21	Dual-chamber	Graphite electrodes and potassium permanganate catholyte	CMC	94.09 ± 0.00	This study
			Avicel	70.56 ± 0.01	
			Cellulose powder	89.30 ± 0.01	
Enterobacter cloacae	Dual-chamber	Carbon cloth anode, carbon fiber cathode, and ferricyanide catholyte	Cellulose powder	5.4	[24]
Clostridium sp. and *Comamonas* sp.	Dual-chamber	Graphite electrodes and ferricyanide catholyte	Cellulose powder	55	[25]
Rhizobiales sp., *Clostridiales* sp., *Chloflexi* sp., and *Methanobacterium* sp.	Dual-chamber	Graphite electrodes and ferricyanide catholyte	Avicel	10	[26]
Clostridium sp. and *Comamonas* sp.	Single-chamber	-	Cellulose powder	44	[13]
Rhodopseudomonas sp., and *Clostridium* sp.	Single-chamber	-	Corn stover	10	[27]

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Agarwal AK. 2007. Biofuels (alcohols and biodiesel) applications as fuels for internal combustion energies. *Prog. Energy. Combust. Sci.* **33**: 233-271.
2. Weng JK, Li X, Bonawitz ND, Chapple C. 2008. Emerging strategies of lignin engineering and degradation for cellulosic biofuel production. *Curr. Opin. Biotechnol*. **19**: 166-172.
3. Robak K, Balcerek M. 2018. Review of second generation bioethanol production from residual biomass. *Food Technol. Biotechnol*. **56**: 174-187.
4. Bayer EA, Chanzy H, Lamed R, Shoham Y. 1998. Cellulose, cellulase, and cellulosomes. *Curr. Opin. Struct. Biol*. **8**: 548-557.
5. Dey N, Vickram S, Thanigaivel S, Subbaiya R, Kim W, Karmegam N, *et al.* 2022. Nanomaterials for transforming barrier properties of lignocellulosic biomass towards potential applications – A review. *Fuel* **316**: 123444.
6. Hobdey SE, Donohoe BS, Brunecky R, Himmel ME, Bomble YJ. 2015. Direct microbial conversion of biomass to advanced biofuels, pp. 111-127. 1st Ed. Elsevier B.V., Golden, Colorado.
7. Taylor MP, Eley KL, Martin S, Tuffin MI, Burton SG, Cowan DA. 2009. Thermophilic ethanologenesis: Future prospects for second-generation bioethanol production. *Trends Biotechnol*. **27**: 398-405.
8. Gupta GN, Srivastava S, Khare SK, Prakash V. 2014. Extremophiles: An overview of microorganism from extreme environment. *Int. J. Agric. Environ. Biotechnol*. **7**: 371-380.
9. Shiratori H, Sasaya K, Ohiwa H, Ikeno H, Ayame S, Kataoka N, *et al.* 2009. *Clostridium clariflavum* sp. nov. and *Clostricium caenicola* sp. nov., moderately thermophilic, cellulose-/cellobiose-digesting bacteria isolated from methanogenic sludge. *Int. J. Syst. Evol. Microbiol.* **59**: 1764-1770.
10. Almatouq A, Babatunde AO, Khajah M, Webster G, Alfodari M. 2020. Microbial community structure of anode electrodes in microbial fuel cells and microbial electrolysis cells. *J. Water Process Eng*. **34**: 101140.
11. Thulasinathan B, Jayabalan T, Arumugam N, Kulanthaisamy MR, Kim P, Govarthanan M, *et al.* 2022. Wastewater sunstrates in microbial fuel cell system for carbon-neutral bioelectricity generation: An overview. *Fuel* **317**: 123369.
12. Toczylowska-Maminski R, Szymona K, Madaj H, Wong WZ, Bala A, Brutkowski W, *et al.* 2015. Cellulolytic and electrogenic activity of *Enterobacter cloacae* in mediatorless microbial fuel cell. *Appl. Energy* **160**: 88-93.
13. Toczylowska-Maminski R, Szymona K, Krol P, Gliniewicz K, Pielech-Przybylska K, Kloch M, *et al*. 2018. Evolving microbial communities in cellulose-fed microbial fuel cell. *Energies* **11**: 124.
14. Islam F, Roy N. 2018. Screening, purification and characterization of cellulase from cellulase producing bacteria in molasses. *BMC Res. Notes* **11**: 445.
15. Junior FLS, Dias ACF, Fasanella CC, Taketani RG, Lima AOS, Melo IS, *et al*. 2013. Endo- and exoglucanase activities in bacteria from mangrove sediment. *Braz. J. Microbiol*. **44**: 969-976.
16. Parkash A, Aziz S, Soomro SA. 2015. Impact of salt concentration on electricity generation using hostel sludge based dual chambered microbial fuel cell. *J. Bioprocess. Biotech*. **5**: 1000252.
17. You S, Zhao Q, Zhang J, Jiang J, Zhao S. 2006. A microbial fuel cell using permanganate as the cathodic electron acceptor. *J. Power Sourc.* **162**: 1409-1415.
18. Kazeem MO, Shah UKM, Baharuddin AS, Rahman AA. 2017. Prospecting agro-waste cocktail: supplementation for cellulase production by a newly isolated thermophilic *Bacillus licheniformis* 2D55. *Appl. Biochem. Biotechnol*. **182**: 1318-1340.
19. Padiha IQM, Carvalho LCT, Dias PVS, Grisi TCSL, Honorato da Silva FL, Santos SFM, *et al.* 2015. Production and characterization of thermophilic carboxymethyl cellulase synthesized by *Bacillus* sp. growing on sugarcane bagasse in submerged fermentation. *Braz. J. Chem. Eng.* **32**: 35-42.
20. Meng F, Ma L, Ji S, Yang W, Cao B. 2014. Isolation and characterization of Bacillus subtilis strain BY-3, a thermophilic and efficient cellulase-producing bacterium on untreated plant biomass. *Lett. Appl. Microbiol*. **59**: 306-312.
21. Hajiabadi S, Mashreghi M, Bahrami AR, Ghazvini K, Matin MM. 2020. Isolation and molecular identification of cellulolytic bacteria from Dig Rostam hot spring and study of their cellulase activity. *Biocell* **44**: 63-71.
22. Ganesan M, Mathivani Vinayakamoorthy R, Thankappan S, Muniraj I, Uthandi S. 2020. Thermotolerant glycosyl hydrolases-producing *Bacillus aerius* CMCPS1 and its saccharification efficiency on HCR-laccase (LccH)-pretreated corncob biomass. *Biotechnol. Biofuels* **13**: 124.
23. Zhao C, Chu Y, Li Y, Yang C, Chen Y, Wang X, *et al.* 2017. High-throughput pyrosequencing used for the discovery of a novel cellulase from a thermophilic cellulose-degrading microbial consortium. *Biotechnol. Lett.* **39**: 123-131.
24. Rezaei F, Xing D, Wagner R, Regan JM, Richard TL, Logan BE. 2009. Simultaneous cellulose degradation and electricity production by *Enterobacter cloacae* in a microbial fuel cell. *Appl. Environ. Microbiol*. **75**: 3673-3678.
25. Rismani-Yazdi H, Christy AD, Dehority BA, Morrison M, Yu Z, Tuovinen OH. 2007. Electricity generation from cellulose by rumen microorganisms in microbial fuel cells. *Biotechnol. Bioeng.* **97**: 1398-1407.
26. Ishii S, Shimoyama T, Hotta Y, Watanabe K. 2008. Characterization of filamentous biofilm community established in a cellulose-fed microbial fuel cell. *BMC Microbiol.* **8**: 6.
27. Wang X, Feng Y, Wang H, Qu Y, Yu Y, Ren N, *et al.* 2009. Bioaugmentation for electricity from corn stover biomass using microbial fuel cells. *Environ. Sci. Technol.* **43**: 6088-6093.