Journal of Sustainable Bioenergy Systems, 2019, 9, 169-178
https://www.scirp.org/journal/jsbs
ISSN Online: 2165-4018
ISSN Print: 2165-400X

Enhancement of Power Generation in Microbial Fuel Cells through Supplementation of *Platycodon grandiflorum* in Doraji Roots

Jung-In Park[1,2], Hyunjin Kim[1], Dae Kyung Go[3], Peter S. Park[4], Sang Heon Lee[1,5], Min Jung Koh[2], Jonathan Jo[2], Donghoon Lee[1], Seungshin Lee[4], Paul S. Chung[1,2]

[1]Fuzbien Technology Institute, Rockville, MD, USA
[2]Youth with Talents, Fairfax, VA, USA
[3]St. Johnsbuury Academy, Jeju, Korea
[4]Department of Biology, School of Arts and Sciences, Cornell University, Ithaca, NY, USA
[5]School of Arts and Sciences, Johns Hopkins, Baltimore, MD, USA
Email: paulschung08@gmail.com

How to cite this paper: Park, J.-I., Kim, H.-J., Go, D.K., Park, P.S., Lee, S.H., Koh, M.J., Jo, J., Lee, D.-H., Lee, S.S. and Chung, P.S. (2019) Enhancement of Power Generation in Microbial Fuel Cells through Supplementation of *Platycodon grandiflorum* in Doraji Roots. *Journal of Sustainable Bioenergy Systems*, **9**, 169-178.
https://doi.org/10.4236/jsbs.2019.94011

Received: September 19, 2019
Accepted: December 20, 2019
Published: December 23, 2019

Abstract

The current study reports the evidence of enhancement in power generation from cellulosic biomass in microbial fuel cell (MFC) systems by supplementing dried Doraji (*Platycodon grandiflorum*) roots powder. Mediator-less two chamber H-type MFCs were prepared using rumen fluid as anode inocula to convert finely ground pine tree (Avicel) at 2% (w/v) to electricity. Dried Doraji roots were ground to pass 1 mm sieve and added to the anode of MFC at 0.1% w/v dosage for treatment. MFC power and current across an external resistor were measured daily for 10 d. At the end of incubation on d10, collected gases were measured for total gas volume and analyzed for gas composition on gas chromatography. Supplementation of Doraji roots powder to MFC anode chamber increased power generation and CO_2 production. Over the 10d experimental period, power density normalized to anode surface area were between 17.0 and 37.7 with average of 32.5 mW/m^2 in Doraji MFCs, and between 16.8 and 19.8 with average of 18.2 mW/m^2 in control group. CO_2 production increased and methane to CO_2 ratio decreased in Doraji root treatment comparing to control group. These observations imply that Doraji root components would inhibit methanogenesis and alter microbial fermentation of cellulose compounds favorable to produce bioenergy efficiently in MFC.

Keywords

MFC, *Platycodon grandiflorum*

1. Introduction

Fossil fuels have served as the main energy resources for industrialization and economic growth for the past century [1], and represented 80% of the global primary energy use [2], but the use of these also contributed 94% to 96% of greenhouse emission in the USA [3] including CO_2, methane, CO and nitrous oxide (N_2O), which cause global warming and pollution [4]. Thus, greater efforts are currently being undertaken to develop technologies generating clean and sustainable energy sources that would replace and/or displace fossil fuels [5].

Microbial fuel cell (MFC) is one of such technologies that directly convert biomass including organic waste to electricity [6]. MFC has shown tremendous electron donor versatility including simple substrates such as glucose, acetate, and lactate [7] [8] [9] complex substrates such as municipal and industrial wastewaters [10] [11] and cellulose [1] [12] [13] [14].

Cellulosic biomass is particularly attractive renewable resources because of its relatively low cost, plentiful supply [15] [16], and neutral carbon balance [17] and furthermore cellulose is a significant component in the annual production of 250 million tons municipal solid wastes and 40 billion cubic meters waste water [18]. To utilize cellulosic biomass in MFC, the anodic process requires cellulose degradation, but often the microorganisms that are electrochemically active did not show cellulolytic activity, thus, it requires products of cellulose fermentation as electron donors to generate electricity [19] [20]. Therefore, rumen fluid from cow [14] [21] or goat [22] had been studied for electricity generation from cellulose or cellulosic biomass because rumen microorganisms include both strict and facultative anaerobes, which effectively hydrolyze cellulose, and conserve energy via anaerobic respiration or fermentation [23]. However, one of major products from cellulose fermentation is acetic acid, and the acetate concentration and anaerobic condition influence the growth of methanogens which contribute significantly to limiting power generation in MFC. Methanogenesis diverts electron from the anode and methanogens act as substrate competitors to the exoelectrogens; acetoclastic methanogens compete for electron donors and hydrogenotrophic methanogens utilize the hydrogen produced in the reactor [24].

Supplementation of saponin or saponin rich plant materials has shown methanogenesis inhibitions [25] [26] [27] or proliferation of fiber degrading bacteria [28] in cultures of rumen microorganisms. *Platycodon grandiflorum* root (Korean name, "Doraji", Japanese name, "Kikyo", and Chinese name, "Jiegeng") has been reported to contain saponins [29] [30]. However, either Doraji root effects on methanogenesis in culture of microorganisms or saponin effects on anolyte fermentation characteristics has not been reported.

We hypothesized that supplementation of Doraji root in anolyte would inhibit methanogenesis and (or) proliferate cellulolytic microorganisms in anode chamber and subsequently ferment cellulosic biomass more efficiently and (or) enhance power generation in MFC. In the current study, MFCs were constructed

with stained rumen fluid as anolyte and cellulose as electron donor, and effects of Doraji root supplementation on power generation and fermentation gas production were investigated.

2. Materials & Methods

2.1. Microorganisms and Culture Media

For MFC anode compartment inoculum (anolyte), rumen fluid was collected from a dry dairy cow and while flushing of CO_2 gas, filtered through 4 layers of cheesecloth to remove feed debris and transferred to an Erlenmeyer flask, then bubbled with CO_2 gas for 10 min and closed with cotton ball and stored in an incubator at 39˚C until inoculated to MFCs.

Culture medium containing 0.048% KH_2PO_4, 0.048% K_2HPO_4, 0.048% $(NH_4)_2SO_4$, 0.096% NaCl, 0.5% Trypticase peptone, 5% yeast extract, 0.05% cysteine-HCl, 0.013% $CaCl_2{\cdot}2H_2O$, 0.02% $MgSO_4{\cdot}7H_2O$, 0.4% Na_2CO_3, 0.1% sodium fumarate, and 1 ppm of resazurin, was prepared anaerobically and autoclaved at 121˚C for 30 min and stored at room temperature. Phosphate buffered saline pH7.4 (PBS) consisted of 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4 and was autoclaved at 121˚C for 30 min and stored at room temperature.

2.2. Microbial Fuel Cells

H-type MFCs consisted two 125 mL-volume glass bottles joined at branched tube. Cation exchange membrane (CMI-7000S, Membranes International Inc., NJ) was placed and clamped between branched tube of anode and cathode compartments. Two gram of cellulose (Avicel PH-101, Sigma-Aldrich, MO) was weighed into anode chamber and 80 mL of culture medium, 20 mL strained rumen fluid were transferred, then suspended using a magnetic bar on agitator. Graphite plates (12 cm^2) were used as electrodes for both anode and cathode. Electrode connected with copper wire fixed to butyl rubber stopper was placed in anode chamber. 100 mL of PBS was transferred to cathode chamber and electrode connected with copper wire was placed in the middle of cathode. Rubber stopper was capped on cathode but left open to air through tubing. Anode and cathode were connected externally through a copper wire with a resistor (300 ohm). MFCs were placed in a water bath at 39˚C for operation.

After 9 d of MFC pretrial operation, 100 mg of dried Doraji root (C&M Food, Seoul, Korea) ground to pass through 1 mm screen was added into anode chamber of treatment group. Two L-volume Mylar balloons were connected to each anode to collect biogas produced during experiment.

2.3. Measurements and Calculation

Using a digital multimeter, voltage across an external resistor (closed circuit voltage), open circuit voltage, and current were recorded daily for 10d. The power density normalized to electrode surface area was calculated using following equations.

$$P = \frac{I\ V}{A} \quad \text{and} \quad I = \frac{V}{R}$$

where, $I(A)$ is the current, $V(V)$ is voltage, R(ohm) is the external resistance, and $A(m^2)$ is the projected area of the anode.

The volume of biogases produced in anode collected in Mylar balloons were measured using a 250 mL glass syringe, and gas components were analyzed using an Agilent 6890 series gas chromatograph equipped with a thermal conductivity detector and a stainless steel packed column prepared with 60/80 Carboxen 1000 (12390-U Supelco, Sigma-Aldrich, MO).

2.4. Statistical Analyses

Effects of Doraji root addition to anode chamber of MFC on electricity generation, fermentation gas production and gas composition were analyzed using the one way ANOVA procedure of JPM 14.1.0 (SAS Institute Inc., NC). When the effect was significant ($P < 0.05$), means between treatments were separated using Student's t-test ($P < 0.05$). Means for operation time (d) were separated using Tukey HDS where the operation time effects exist ($P < 0.05$).

3. Results and Discussion

3.1. Power Generation

MFCs were stabilized and operational prior to treatment addition. Voltage across resistor, current density and power density were 96.4 ± 4.87 mV, 186.8 ± 9.43 mA/m^2 and 18.0 ± 0.64 mW/m^2, respectively.

In control group, voltage across resistor (Table 1) were steady ($P = 0.9766$) with operation time and average was 96.8 ± 5.22 mV ranged from 93 to 101 mV, and end point potential (Table 2) also did not change ($P = 0.9865$) with operation time and average was 392 ± 33.6 mV ranged from 374 to 423 mV during 10d operation.

Voltage across resistor in MFCs received Doraji root powder increased ($P < 0.05$) at d 2 and maintained through d10 (Table 1), but greater ($P < 0.05$) end point potentials than d 0 were observed only at d 2, 5, 7 and 8 (Table 2). In comparison to control group, MFCs received Doraji root powder had greater ($P < 0.05$) voltage across resistor (Table 1) at d 2 and thereafter through 10 d experimental period, and end point potential (Table 2) at d 5, 7, 8 and 10.

Power density (Table 3) in control group did not change ($P = 0.9766$) through operation time which reflects the steady cellulolysis and electricity generation during 10d operation. Within Doraji received group, power density increased ($P < 0.05$) at d 2 from 17.0 to 35.6 mW/m^2 and these higher power density than d0 and d1 was maintained until the end of operation. Between treatments, from d 2 to d 10, power density in Doraji group were greater ($P < 0.05$) than in control group.

The enhanced power generation ($P = 0.0043$) must be attributed to the action

Table 1. Closed circuit voltage across 300 ohms resistor measured from microbial fuel cells established with strained rumen fluid and 2% of cellulose with or without Doraji (*Platycodon grandiflorum*) roots powder addition.

Day	Voltage across resistor (300 ohms), mV			
	Control	Doraji	SEM[1]	P[2]
0	97	94[b]	4.0	0.6513
1	95	94[b]	4.0	0.8769
2	97	136[a]	3.6	0.0166
3	93	139[a]	3.6	0.0121
4	100	137[a]	3.9	0.0208
5	95	135[a]	0.7	0.0006
6	95	136[a]	5.4	0.0328
7	98	135[a]	4.5	0.0280
8	101	133[a]	0.8	0.0013
9	96	140[a]	4.4	0.0196
10	100	135[a]	2.2	0.0073
SEM[1]	4.4	2.7		
P[3]	0.9766	<0.0001		

[ab]Means within a treatment with different superscripts differ, $P < 0.05$. [1]Standard error of means. [2]P-value: probabilities that treatments effect is not significant within the day. [3]*P*-value: probabilities that day effect is not significant within the treatment.

Table 2. Terminal (open circuit) voltage measured from microbial fuel cells established with strained rumen fluid and 2% of cellulose with or without Doraji (*Platycodon grandiflorum*) roots powder addition.

Day	Open circuit voltage, mV			
	Control	Doraji	SEM[1]	P[2]
0	385	356[b]	29.4	0.5517
1	406	412[ab]	26.2	0.8861
2	378	526[a]	35.8	0.0997
3	383	502[ab]	22.8	0.0665
4	381	459[ab]	38.4	0.2895
5	401	530[a]	15.2	0.0267
6	394	502[ab]	33.8	0.1533
7	395	522[a]	20.2	0.0465
8	400	509[a]	15.8	0.0390
9	374	464[ab]	36.9	0.2271
10	423	466[ab]	6.8	0.0466
SEM[1]	29.9	24.7		
P[3]	0.9865	0.0141		

[ab]Means within a treatment with different superscripts differ, $P < 0.05$. [1]Standard error of means. [2]P-value: probabilities that treatments effect is not significant within the day. [3]P-value: probabilities that day effect is not significant within the treatment.

Table 3. Power density measured from microbial fuel cells established with strained rumen fluid and 2% of cellulose with or without Doraji (*Platycodon grandiflorum*) roots powder addition.

Day	Open circuit voltage, mV			
	Control	Doraji	SEM[1]	P[2]
0	18.1	17.0[b]	1.46	0.6624
1	17.3	17.0[b]	1.71	0.8719
2	18.3	35.6[a]	1.34	0.0118
3	16.8	37.4[a]	1.33	0.0082
4	19.2	36.4[a]	1.50	0.0150
5	17.5	35.3[a]	0.37	0.0009
6	17.6	35.9[a]	2.14	0.0263
7	18.7	35.3[a]	1.77	0.0220
8	19.8	34.0[a]	0.33	0.0011
9	17.9	37.7[a]	1.68	0.0141
10	19.2	35.3[a]	1.12	0.0095
SEM[1]	1.66	1.13		
P[3]	0.9766	<0.0001		

[ab]Means within a treatment with different superscripts differ, $P < 0.05$. [1]Standard error of means. [2]P-value: probabilities that treatments effect is not significant within the day. [3]P-value: probabilities that day effect is not significant within the treatment.

of Doraji root powder supplementation that most likely causes favorable anode respiration by exoelectrogens by methanogenesis inhibitions [25] [26] [27] and (or) by cellulolysis extension with proliferation of fiber degrading bacteria [28] in MFCs constructed with rumen fluid as anolyte and cellulose as electron donor.

3.2. Biogases Production

Total gas productions in anode for 10d MFC operation were similar ($P = 0.7072$) and the volumes were 328 and 335 mL in control and Doraji root treatment, respectively (**Figure 1**). Biomethane production were also similar ($P = 0.0248$) and amounts were 220 and 197 mL in control and Doraji root treatment, respectively. However CO_2 production was greater ($P = 0.0305$) in Doraji root treatment (138 mL) than in control group (108 mL). The increase in CO_2 and numerical difference in biomethane were clearly reflected in biomethane to CO_2 ratio, and the ratio was less ($P = 0.0461$) in Doraji root treatment (1.44) than in control group (2.03).

Biogas is produced from biomass fermentation, and it was from cellulose in the current study. Because of the identical carbon balance between methane and CO_2 and no difference in total gas production between treatment, the extend of cellulolysis might not influenced by treatment in the current study. Therefore, the enhanced power generation in Doraji rood added MFCs should be attributed to the methanogenesis reduction. Methanogenesis does not only divert electron from the anode but also reflects the competition of methanogens for substrates

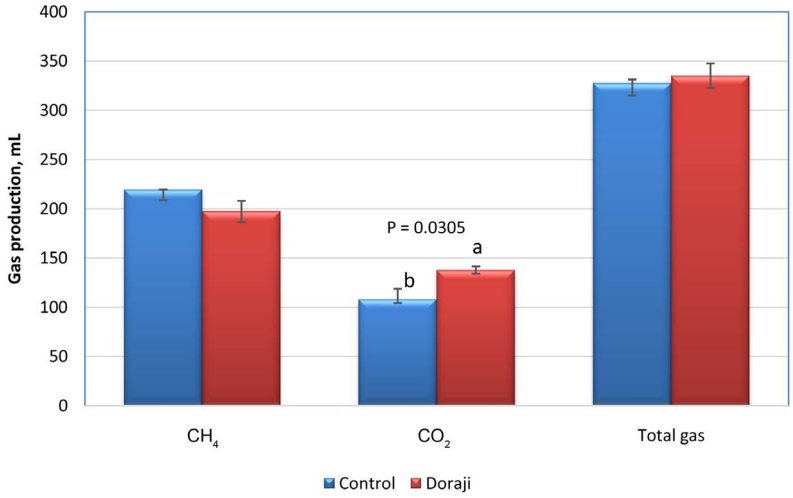

Figure 1. Accumulated gas production in the anode chamber of microbial fuel cells (MFCs). MFCs were built with rumen microorganisms and 2% of cellulose (Avicel®) with or without Doraji (*Platycodon grandiflorum*) roots powder addition Accumulated volume of gases were measured and analyzed for gas components on d10.

to exoelectrogens which transfer electrons to anode [24]. Aacetoclastic methanogens compete for electron donors ($CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$) and hydrogenotrophic methanogens utilize the hydrogen produced in the reactor ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$), and methanogenesis consumes exogenous energy (Kaur *et al.*, 2014), and it is certainly not favorable for exoelectrogens establishment on anode which is critical in electricity generation in MFC.

4. Conclusion

Microbial Fuel Cell is one of the technologies that generate clean sustainable bioenergy from cellulosic biomass; however, methanogenesis remains as a major factor limiting MFC performance. In the current study, rumen microorganisms were employed as anolyte and cellulose served as electron donors, and Doraji (*Platycodon grandiflorum*) roots powder was tested at 0.1% of anolyte as methanogenesis inhibitor and cellulolytic microorganisms growth promoter. Cellulolysis did not change, however enhanced power generation and decreased methane to CO_2 ratio were observed with Doraji roots powder addition to anode in MFC which were hypothesized on the basis of saponine effects on rumen microbial fermentation. Results from the current study imply that Doraji root addition would inhibit methanogenesis and enhance MFC efficiency in cellulosic electricity generation. Optimal dose and preparation methods of Doraji roots may be elucidated by further studies to maximize the methanogenesis inhibition and power generation in MFCs from cellulosic biomass.

Acknowledgements

The authors acknowledge the STEM Research Institute and Youth with Talents for sponsoring this research project (SRI-2018-5).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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