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Improvement of Renewable Bioenergy Production in Microbial Fuel Cells with Saponin Supplementation

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Abstract

Microbial fuel cell (MFC) is one of renewable biofuel production technology that directly converts biomass to electricity. Cellulosic biomass is particularly attractive renewable resources for its low cost and abundance and neutral carbon balance. However, methanogenesis remains as a major factor limiting MFC performance. The current study reports that saponin addition at 0.05% w/v dose to anolyte in MFCs inhibited methanogenesis and improves power generation and cellulose fermentation. Mediator-less two chamber H-type MFCs were prepared using rumen fluid as anode inocula at 20% v/v of anolyte to convert finely ground pine tree (Avicel) at 2%, w/v to electricity. Saponin was added to the anode of MFC at 0.005% or 0.05% v/v dosage for treatment. MFC power and current across an external resistor were measured daily for 10d. On d10, collected gases from anode compartment were measured for total gas volume and analyzed for gas composition on gas chromatography. Supplementation of saponin to MFC at 0.005% did not have any effects on electricity generation or biogas production and composition. Saponin at 0.05% dose reduced 10% of methane production and increased 40% of CO_2 production and 6.4% of total gas production for 10d MFC operation. Voltage across resistor prior to treatment addition (d0) was 164.75 ± 9.07

mV. In control group, voltage across resistor did not change (P = 0.9153) with time course and mean was 167.8 ± 8.20 mV ranged from 157 to 174.5 mV during 10d operation. In 0.05% Saponin group, voltage across resistor increased (P < 0.0001) after d2 and mean was 187.3 ± 4.30 mV ranged between 161.5 and 204.0 mV and the 10d mean of voltage across resistor in 0.05% Saponin was greater (P < 0.0001) than in control group. 0.05% Saponin also had greater voltage across resistor at d5 (P = 0.0030) and d6 (P = 0.0246) than control. End point potential increased (P < 0.0001) in 0.05% Saponin after d2. 0.05% Saponin had greater (P < 0.05) end point potentials than control at d1, d4, d7, d10, and also 10d mean was greater (731.9 vs 606.5 mV; P < 0.0001) in 0.05% Saponin. Power density increased (P < 0.0001) after d2 in 0.05% Saponin. 0.05% Saponin MFCs had greater (P < 0.05) power density than control at d5 and d6, and also a greater (P < 0.0001) overall mean of 10d operation. The current study provides strong background for potential use of saponin and saponin containing natural resources for methanogenesis inhibitor and cellulolysis enhancer in MFC and also cellulolysis reactors.

Keywords

Microbial Fuel Cells, Saponin, Bioenergy

1. Introduction

Fossil fuels represented about 80% of the global energy use [1], and fossil fuel combustion and natural gas and petroleum systems for energy contributed 95.3% of greenhouse emission in the USA [2] which cause global warming and pollutions [3]. Therefore, development of technologies generating clean and sustainable energy to reduce fossil fuels usage has been undertaken [4]. Microbial fuel cell (MFC) is one of renewable biofuel production technology that directly converts biomass to electricity [5] MFC has shown tremendous electron donor versatility including simple substrates like glucose and organic acids [6] [7], complex substrates such as municipal and industrial wastewaters [8] [9]; and cellulosic biomass [10] [11].

Cellulosic biomass is particularly attractive renewable resources for its low cost and abundance [12] [13] and neutral carbon balance [14]. To utilize cellulosic biomass in MFC, the anodic process requires cellulose degradation, but electrochemically active microorganisms did not possess cellulolytic activity, thus cellulose fermentation by cellulolytic microorganisms is required as electron donors to generate electricity [15] [16]. Therefore, rumen fluid had been studied for MFCs because microorganisms in the rumen effectively hydrolyze cellulose and conserve energy via anaerobic respiration or fermentation [17]. However, methanogens in anaerobic microbial community contribute significantly to limiting cellulosic 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 hy-

drogenotrophic methanogens utilize the hydrogen produced in MFCs [18].

Supplementation of tea (*Camellia sinensis*) seed saponin [19], tea saponin extract [20] and saponin rich yucca schidigera extract [21] have shown methanogenesis inhibitions and also addition of saponin rich fractions from different plant materials [22] induced proliferation of fiber degrading bacteria on in vitro cultures of rumen microorganisms. Furthermore, improvement of power generation and methanogenesis reduction in MFC was reported with supplementation of bellflower (*Platycodon grandiflorum*) root which is known to contain saponins [23]. However, direct effects of saponin on power generation and methane production in MFC had not been studied. Understanding of saponin effects on MFC efficacy would expand the potential use of saponin rich natural resources as MFC supplements.

Therefore, we hypothesized that addition of saponin would decrease methanogenesis and enhance the power generation from cellulose in MFCs. The objectives of the current study were 1) to investigate the direct effect of saponin on methanogenesis and power generation in MFC; and 2) consequently to provide rationale to expand the use of saponin rich natural resources in biofuel production.

2. Materials & Methods

2.1. Microbial Fuel Cell Construction

H-type MFCs consisted two 125 mL-volume glass bottles, anode and cathode. Two compartments joined at branched tube and cation exchange membrane (CMI-7000S, Membranes International Inc., NJ) was placed and clamped between branched tube. Two gram of finely ground pine tree (Avicel PH-101, Sigma-Aldrich) was weighed, 80 mL of culture medium and 20 mL strained rumen fluid were transferred into anode chamber, then well suspended using agitator. 100 mL of phosphate buffered saline pH 7.4 (PBS) was transferred to cathode chamber. Electrodes (Graphite plates, 12 cm^2) connected with copper wire was placed in the middle of anode and identical anode electrode of which copper wire was fixed to butyl rubber stopper was placed in anode chamber. Rubber stopper was placed 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 9d of MFC stabilization operation, two MFCs were randomly assigned to Saponin or Control groups and 5 mg of Saponin (MFCD00081981, VWR) for Experiment 1 or 50 mg of Saponin for Experiment 2 was added into anode chamber of treatment group. Control MFCs did not receive anything for Experiment 1 or 2 either. Two L-volume Mylar balloons were connected to each anode to collect biogas produced during experiment.

2.2. 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 [24] and was autoclaved at 121˚C for 30 min and stored at room temperature.

2.3. Measurements and Calculation

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

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

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.

On d10, the volume of biogases produced in anode collected in Mylar balloons were measured using a 250 mL glass syringe. Gas components were analyzed using an Agilent 6890 series gas chromatograph equipped with a thermal conductivity detector and a packed column prepared with 60/80 Carboxen 1000 (12390-U Supelco, Sigma-Aldrich). Argon gas flow rate was 20 ml/min and the injector and detector temperatures were 250˚C for both. Oven temperature ramped between 50˚C and 150˚C.

2.4. Statistical Analyses

The treatments included 2 doses of saponin, 0.005% and 0.05% (w/v). Experiment 1 (Exp 1) was conducted with duplication of 0.005% MFCs and control MFCs. Experiment 2 (Exp 2) was done in the same conditions but with 0.05% dose and control MFCs after Exp 1 was completed. Effects of each dose of saponin was compared to only controls in the same experiment.

Electricity generation, fermentation gas production and gas composition were analyzed using the one way ANOVA procedure of JPM 14.1.0 (SAS Institute Inc.). When the effect was significant ($P < 0.05$), means between treatments were separated using Student's t-test ($P < 0.05$).

3. Results

3.1. Biogases Production

Experiment 1. Methane and carbon dioxide productions during 10d MFC operation were not different between control and 0.005% Saponin (**Figure 1**: Exp1). Total gas volume and methane to carbon dioxide ratio were also similar between treatments.

Experiment 2. Supplementation of Saponin at 0.05% increased ($P < 0.05$) carbon dioxide and total gas production and decreased ($P < 0.05$) methane production and methane to carbon dioxide ratio during 10d MFC operation (Figure 1: Exp2). Methane reduction was 20 mL which is 9.7% of production in control and increased carbon dioxide was 40.3 mL which is 37.2% of production in control by 0.05% Saponin.

3.2. Power Generation

Experiment 1. Voltage across resistor prior to treatment addition (d0) was 172.8 ± 7.14 mV. Voltage across resistor (Table 1) were steady with time course for both control ($P = 0.3412$) and 0.005% Saponin ($P = 0.3803$). Average of voltage across resistor were similar ($P = 0.6561$) between treatments and values were 172.0 ± 4.51 and 172.9 ± 4.02 mV for control and 0.005% Saponin, respectively, although 0.005% Saponin had lower ($P = 0.0377$) voltage at d9.

End point potential (Table 2; open circuit voltage) increased with time in both control ($P = 0.0007$) and 0.005% Saponin ($P = 0.0037$). Between treatments, 0.005% Saponin had the greater (614 vs 602 mV; $P = 0.0330$) end point potential than control at d4, however overall means were not different ($P = 0.7304$) between treatments.

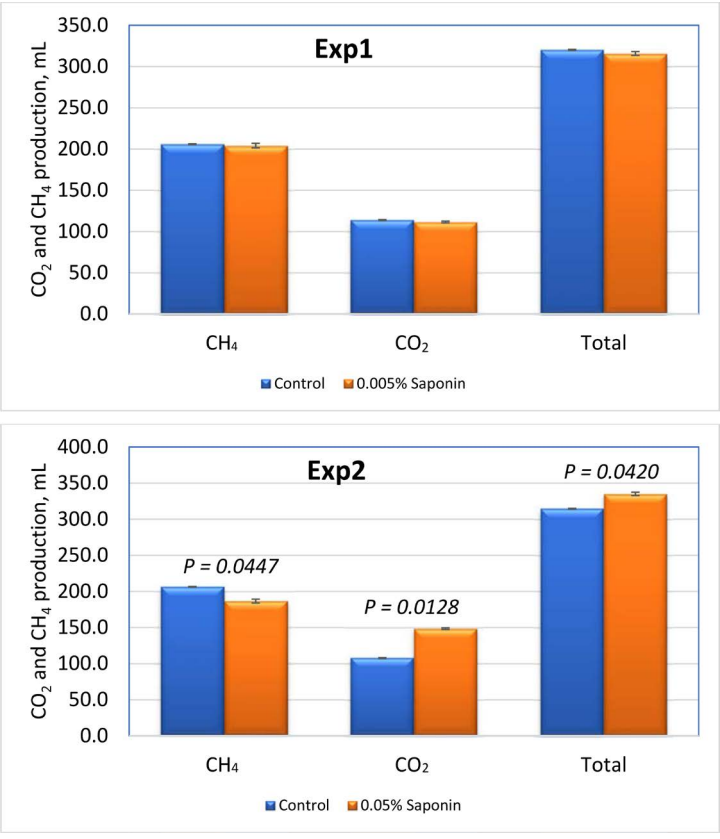

Figure 1. Accumulated gas production on d10 of operation in the anode compartment of MFCs. MFCs were built with 20% rumen fluid as inocula and 2% of cellulose (Avicel®) with or without saponin at 0.001% (Exp1) or 0.01% (Exp2).

Table 1. Closed circuit voltage across 300 ohms resistor measured from microbial fuel cells built with 20% rumen fluid as inocula and 2% of cellulose (Avicel®) with or without saponin at 0.005% (Exp1).

Day	Control	0.005% Saponin	SEM[1]	P[2]
0	160.5	167.0	2.06	0.0876
1	174.5	173.0	2.25	0.8075
2	177.5	181.0	1.44	0.2965
3	166.5	175.0	2.59	0.0541
4	177.5	173.0	1.65	0.2137
5	170.0	177.0	4.79	0.5799
6	174.5	167.0	2.29	0.0532
7	174.5	175.5	1.87	0.8457
8	169.5	174.0	4.99	0.7396
9	175.0	167.5	2.25	0.0377
10	172.5	172.0	3.47	0.9584
SEM[1]	4.5054	4.0170		
P[3]	0.3412	0.3803		

[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. End point potential measured from microbial fuel cells built with 20% rumen fluid as inocula and 2% of cellulose (Avicel®) with or without saponin at 0.005% (Exp1).

Day	Control	0.005% Saponin	SEM[1]	P[2]
0	595.0	576.5	5.75	0.0712
1	571.5	571.0	8.86	0.9837
2	561.5	565.0	4.70	0.7849
3	555.5	564.0	3.09	0.2065
4	602.0	614.0	3.58	0.0330
5	609.5	596.5	8.95	0.5809
6	624.0	618.0	2.71	0.3604
7	599.5	604.5	10.45	0.8619
8	627.5	623.5	7.64	0.8489
9	631.0	624.0	5.44	0.6285
10	643.0	630.0	5.39	0.3041
SEM[1]	9.9109	10.5356		
P[3]	0.0007	0.0037		

[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.

Power density (**Figure 2**: Exp 1) in either control group (P = 0.3548) and 0.005% Saponin (P = 0.3869) did not go up or down through time course during

10d operation. 0.005% Saponin had smaller (P = 0.0363) power density than control at d9, but 10d means (P = 0.6661) were 57.5 and 58.0 mW/m^2 for control and 0.005% Saponin, respectively.

Experiment 2. Voltage across resistor prior to treatment addition (d0) was 164.75 ± 9.07 mV. In control group, voltage across resistor (Table 3) did not change (P = 0.9153) with time course and mean was 167.8 ± 8.20 mV ranged from 157 to 174.5 mV during 10d operation. In 0.05% Saponin group, voltage across resistor increased (P < 0.0001) after d2 and mean was 187.3 ± 4.30 mV ranged between 161.5 and 204.0 mV. This 10d mean of voltage across resistor in 0.05% Saponin was greater (P < 0.0001) than in control group. 0.05% Saponin also had greater voltage across resistor at d5 (P = 0.0030) and d6 (P = 0.0246) than control.

End point potential (Table 4) in control did not change (P = 0.7094) with

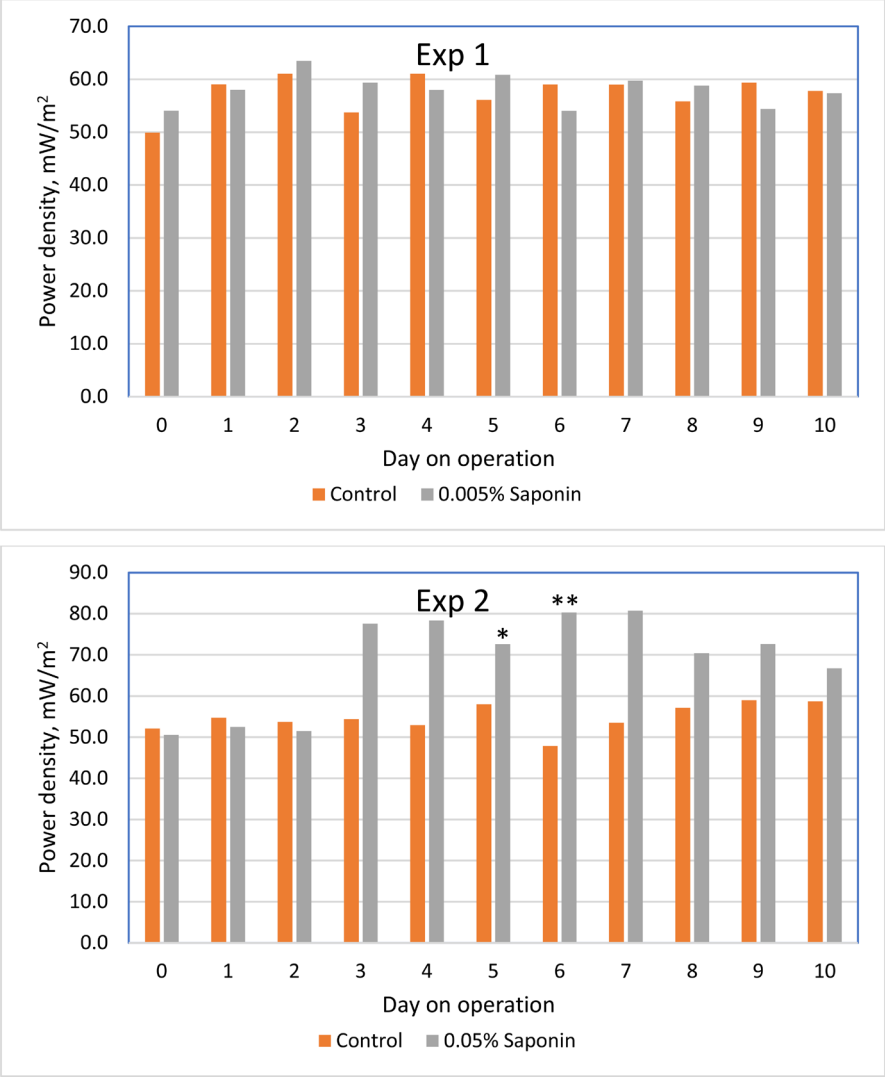

Figure 2. Power density measured from MFCs built with 20% rumen fluid as inocula and 2% of cellulose (Avicel®) with or without saponin at 0.005% (Exp1) or 0.05% (Exp2). * P < 0.05; ** P < 0.01.

Table 3. Closed circuit voltage across 300 ohms resistor measured from microbial fuel cells built with 20% rumen fluid as inocula and 2% of cellulose (Avicel®) with or without saponin at 0.05% (Exp2).

Day	Control	0.05% Saponin	SEM[1]	P[2]
0	163.5	161.5	5.14	0.8877
1	168.0	164.5	2.69	0.6242
2	166.0	163.0	4.99	0.8265
3	167.0	200.0	10.84	0.1209
4	165.0	201.0	11.29	0.0796
5	173.0	193.5	5.94	0.0030
6	157.0	203.5	13.76	0.0246
7	166.0	204.0	11.80	0.0701
8	171.5	190.5	6.82	0.1957
9	174.5	193.5	5.82	0.0570
10	174.0	185.5	4.13	0.1963
SEM[1]	8.1951	4.3038		
P[3]	0.9153	< 0.0001		

[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 4. End point potential measured from microbial fuel cells built with 20% rumen fluid as inocula and 2% of cellulose (Avicel®) with or without saponin at 0.05% (Exp2).

Day	Control	0.05% Saponin	SEM[1]	P[2]
0	575.5	565.0	10.26	0.7045
1	606.0	688.0	24.04	0.0154
2	529.5	613.5	25.69	0.0562
3	615.0	812.0	63.69	0.1071
4	619.0	771.0	45.01	0.0250
5	612.5	715.5	36.35	0.1820
6	646.5	754.0	40.23	0.2286
7	616.0	784.0	49.65	0.0233
8	593.0	775.5	58.84	0.1046
9	622.0	751.5	39.45	0.0523
10	636.5	821.0	54.04	0.0144
SEM[1]	38.1245	17.75		
P[3]	0.7094	< 0.0001		

[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.

time course, however it increased ($P < 0.0001$) in 0.05% Saponin after d2. Between treatments, 0.05% Saponin had greater ($P < 0.05$) end point potentials

than control at d1, d4, d7, d10, and also 10d mean was greater (731.9 vs 606.5 mV; $P < 0.0001$) in 0.05% Saponin.

Power density (**Figure 2**: Exp 2) had similar trends for each group. It did not change ($P = 0.9204$) with time course in control group, but increased ($P < 0.0001$) after d2 in 0.05% Saponin. 0.05% Saponin MFCs had greater ($P < 0.05$) power density than control at d5 and d6, and also a greater ($P < 0.0001$) overall mean of 10d operation.

4. Discussion and Conclusion

Methane inhibition with supplementation of saponin extracted from tea (*Camellia sinensis*) seed to in vitro rumen culture was reported [19] and in their fermentation system, methane production decreased linearly with dose of saponin up to 0.8% of substrate, which is equivalent to 0.005% (w/v) of in vitro culture. Another study [20], where inhibited methanogenesis and increased cellulolytic bacteria *Fibrobacter succinogenes*, included 0.4% of tea saponin in rumen fluid or pure culture. In the current study, anolyte for MFCs were 20% rumen fluid and substrates (electron donor) were 2% cellulose (finely ground pine tree), and saponin was added at the dose of 0.005% and 0.05% (w/v). Addition of 0.005% saponin did not modify the biogas production or methanogenesis while 0.05% of saponin decreased methanogenesis and increased carbon dioxide production. Therefore, the strong evidence of saponin effects in MFCs was observed from this study and the minimal effective dose of saponin can be deduced between 0.005% and 0.05%. While 20 mL of methane decreased, 40 mL of carbon dioxide increased with 0.05% saponin addition. As a result of carbohydrate fermentation, even in anaerobic digestion ending with short chain fatty acids such as acetate, propionate and butyrate, the increase in carbon dioxide reflects the increase in substrates fermentation because of the identical carbon balance between methane and carbon dioxide. Increase in cellulolysis with 0.05% saponin can be inferred because substrate was cellulose, and it may coincide with the proliferation of cellulolytic bacteria with saponin addition [19]. Methanogenesis does not only divert electron from the anode but also cause the competition of methanogens for substrates to exoelectrogens which transfer electrons to anode [18]. Aacetoclastic methanogens compete for electron donors ($CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$) and hydrogenotrophic methanogens utilize the hydrogen produced in the anode of MFC ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$), and methanogenesis consumes exogenous energy, thus it is certainly not favorable for exoelectrogens establishment on anode which is critical in electricity generation in MFC. Therefore, improved power generation in MFC is expected with methane inhibition.

In experiment 1, the power generation was constant during 10d operation which reflected steady fermentation of cellulose in both control and 0.005% saponin added MFC, and no difference in energy production was observed with 0.005% saponin supplementation. At this dose, saponin did not show any effect on microorganism involved cellulolysis, methanogenesis or electron transferring

to anode in MFC although this dose or less was reported effective in animal studies. In experiment 2, saponin concentration was increased to 0.05% to observe the effects on MFC productivity. The end point potential increased with 0.05% saponin comparing to control as early as d1. Improvement in power generation was observed not only at MFC operation time points but also through 10d means with 0.05% saponin addition. The difference of 10d mean of power density is 13.8 mW/m^2 (54.7 vs 68.5 mW/m^2) which is 25.2% of power density in control, however the accumulated differences measured daily for 10d is 151.7 mW/m^2 which is equivalent to 277.2% of average of power density in control. Electricity generation is continuous in MFC while experimental observation was once a day, therefore the improvement in electricity generation presented might be underestimated. Various natural resources consist saponin and some of its by-products are also known to contain saponin such as ginseng marc. The current study provides strong background for potential use of saponin and saponin containing natural resources for methanogenesis inhibitor and cellulolysis enhancer in MFC and also cellulolysis reactors.

Microbial Fuel Cell is a technology that generates clean sustainable bioenergy from cellulosic biomass including municipal wastewater and industrial organic waste. 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 in MFC, and 0.05% (w/v) saponin addition improved the cellulose fermentation and the electricity generation, and inhibited the methanogenesis. Results from the current study elucidate the effects of saponin rich resource on cellulose fermentation and imply that application of saponin and saponin containing natural resource would be beneficial to maximize the methanogenesis inhibition and power generation from cellulosic biomass in MFCs.

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Conflicts of Interest

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

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