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Microbial Fuel Cell: New Assay for Nutritive Value Determination of Whole Cottonseed Used in Ruminants Nutrition

Research Article

M. Besharati[1*] and A. Taghizadeh[2]

[1] Department of Animal Science, Ahar Faculty of Agriculture and Natural Resources, University of Tabriz, Tabriz, Iran
[2] Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

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*Correspondence E-mail: mbesharati@tabrizu.ac.ir

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ABSTRACT

The aim of this study was to determine the relationship between gas produced through *in vitro* gas production test and microbial fuel cell (MFC) technique and introduce new assay for evaluation of ruminants feedstuffs. The treatments were 1) control diet (without whole cottonseed, monensin and vitamin E; CD), 2) diet containing 20.00% whole cottonseed (CSD), 3) diet including 20.00% whole cottonseed plus vitamin E (12000 IU per day; CSDE) and 4) diet containing 20.00% whole cottonseed plus monensin (24 ppm kg^{-1} dry matter/day; CSDM). For MFC trial, the homemade MFC configuration consisted of two plexiglass cylinders (dual-chambered) was used. The results showed that treatment containing vitamin E had higher gas production compared to the other treatments. Gas production in the monensin treatment was lower than the other treatments. The vitamin E treatment had higher voltage, current and power values in comparison with other treatments. The obtained data showed that addition of monensin in comparison with CSD treatment improved the MFC performance. The results indicated that there were high positive correlations between obtained gas production values and achieved MFC performance in experimental treatments.

KEY WORDS gas production, microbial fuel cell, monensin, rumen microorganisms, vitamin E.

INTRODUCTION

The *in vitro* gas production (GP) method has been widely used for evaluation of the feedstuffs nutritive value. Useful data about digestion kinetics of feedstuffs come from GP method. Some researchers have used several gas measuring techniques and *in vitro* gas techniques. Profits and imperfections of these methods have been reviewed by Getachew *et al.* (1998). The GP method concentrates on the appearances of fermentation products. While a substrate with buffered rumen fluid incubated *in vitro*, the carbohydrates (CHO) are fermented to produce gases (mainly methane and carbon dioxide), microbial cells and short-chain fatty acids (SCFAs). Mainly produced gas is the results of CHO fermentation to acetate, propionate and butyrate. The GP from protein content of feedstuffs fermentation is relatively smaller than from CHO fermentation. The GP from feedstuff's fat content is also slight. For example, obtained data from several studies have showed that GP from cellulose and casein fermentation was higher than GP from different fat sources fermentation (for example palm kernel oil, coconut oil or soybean oil) (Getachew *et al.* 1998; Makkar *et al.* 1995).

Produced gas is the direct produced gas as a fermentation result and the indirect produced gas from the buffering of volatile fatty acids (VFAs). The amount of CO_2 produced from buffering of VFAs (at very high molar propionate concentration) represents about 60 percent of whole GP.

Every mmol of VFAs generated from fermentation acquits 0.80 to 1 mmol of CO_2 from the buffered rumen fluid

solution, relying on the amount of phosphate buffer existence (saliva). The researchers have observed a highly significant correlation between GP and VFAs (Makkar *et al.* 1995; Getachew *et al.* 1998). With increasing amount of VFAs, GP increases too.

When the feedstuffs are fermented to butyrate and acetate, the gas is produced. With production of propionate, lower gas is produced. Rapidly fermentable CHO produce relatively higher propionate compared to acetate. The shift in the proportion of SCFA will be reflected by changes in GP. The GP technique has a disadvantage, when VFAs production goes into propionic acid production; the gas volume comes down and it is a problem in feedstuffs evaluation (Makkar *et al.* 1995; Getachew *et al.* 1998).

Microbial fuel cells (MFCs) are a tool that can facilitate the study of microorganism physiological roles in complex ecosystems (Aelterman *et al.* 2008). The MFCs are bio-electrochemical reactors converting biochemical energy stored in organic compounds into electrical energy through the catalytic reaction of microorganisms (Kim *et al.* 2002; Rabaey and Verstraete, 2005). Organic compounds and anode act as electron donors and electron receptors for microorganisms in MFCs, respectively. The MFC is composed of anode and cathode components separated by cation-exchange membranes. The bacteria in anode oxidize the substrate as an electron donor and produce electrons and protons (Bond and Lovley, 2003). The electrons are transmitted through connective wire current to the cathode and protons are transmitted through the membranes. Electrons and protons are used in the cathode and restore oxygen to water (Benetto, 1990; Madden and Schollar, 2001). Today, MFCs are less commonly used in ruminal production and health studies (Rismani-Yazdi *et al.* 2007).

The MFCs are an advanced technology used to study the microbial physiology in the form of electron and / or proton transport (Schroder, 2007). The MFCs provide a medium for studying complex microbial systems such as the rumen as well as new advanced tools for changing the dynamics between these systems. A comprehensive understanding of the diversity of ruminal microbial communities will have a great impact in the nutritional digestibility, production efficiency, metabolic diseases and environmental effects perception.

Given the inherent advantage of a precise oxidation-reduction control, MFCs can play a new and important role in the cultivation of rumen microorganisms. Studies of MFCs have shown that the microbial ecosystems of the systems have changed according to the conditions of the substrate composition and the electrochemical performance (Jung and Regan, 2007; Ishii *et al.* 2008).

But, many studies are needed to investigate these effects on the microbial population. Ruminal liquid is used as an inoculum in MFCs to identify biocatalysts capable of producing electricity from cellulose (Rismani-Yazdi *et al.* 2007). Production of methane in the rumen is associated with production efficiency. The consumption of carbon in the form of methane is a waste of energy for the livestock. Methanogenic bacteria often use hydrogen and carbon dioxide to produce methane. Hydrogen removal is an essential step for maintaining fermentation activity by other groups and there is a balance between the VFAs production and methane (Van Soest, 1982). Hydrogen can be used as an electron donor for exoelectrogenic microbial populations and thus facilitates the conditions that are very suitable for propionate and acetate production. This process can be evaluated by MFCs.

The aim of this study was to determine the relationship between *in vitro* GP and MFC and introduce a new method for feedstuffs evaluation.

MATERIALS AND METHODS

In vitro gas production

Ruminal fluid was collected two hr after morning feeding from two rumen-fistulated sheep. The animals were fed 400 g alfalfa hay, 200 g barley and 200 g soybean meal. Ruminal fluid was immediately squeezed through four layers of cheesecloth and transported to the laboratory in a sealed thermos.

The resulting ruminal fluid was purged with deoxygenated CO_2 before use as an inoculum. The GP was measured by Fedorak and Hrudy (1983) method. Approximately 300 mg of dried and ground (2 mm) dietary treatments (Table 1) samples were weighted and placed into serum bottles. The treatments were included 1) control diet, 2) diet containing 20.00% whole cottonseed (CSD), 3) diet containing 20.00% whole cottonseed plus 12000 IU of vitamin E (Lutavit®, Bern, Swiss; CSD+E) and 4) diet containing 20.00% whole cottonseed plus 24 ppm monensin (*Razak* Co., Tehran, Iran; CSD+M).

Buffered rumen fluid with McDougall, (1984) buffer (20 mL; Merck,) was pipetted into each serum bottle. The GP was recorded after 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 hr of incubation. Total gas values were corrected for the blank incubation and reported gas values are expressed in mL per 1 g of dry matter (DM).

The MFC trial

Figure 1 shows the MFC setup (there were three setups) employed in this research.

Table 1 Ingredients and chemical composition of the experimental diets

Ingredient (g kg^{-1} DM)	Dietary treatment			
	Control	CSD	CSD + E	CSD + M
Alfalfa hay	190	190	190	190
Corn silage	270	270	270	270
Barley grain	190	100	100	100
Corn grain	150	90	90	90
Cottonseed meal	90	40	40	40
Soybean meal	80	80	80	80
Wheat bran	20	20	20	20
Whole cottonseed	-	200	200	200
Salt	2	2	2	2
Ca carbonate	2	2	2	2
Mineral premix[1]	4	4	4	4
Vitamin premix[2]	4	4	4	4
Monensin (ppm cow^{-1} per day)	-	-	-	24
Vitamin E (IU cow^{-1} per day)	-	-	12000	-

[1] Each kg (DM basis) of mineral premix contained: Ca: 200 g; Mg: 90 g; Mn: 13.50 g; Fe: 17.50 g; Zn: 14.30 g; Cu: 3.50 g; I: 210 mg; Co: 35 mg and Se: 90 mg.
[2] Each kg (DM basis) of vitamin premix contained: vitamin A: 1500000 IU; vitamin D_3: 400000 IU; vitamin E: 6000 mg and Antioxidant: 400 mg.
Control: diet without whole cottonseed or vitamin E or monensin supplementation; CSD: control diet plus whole cottonseed 200 g kg^{-1} dry matter (DM); CSD + E: CSD diet plus 12000 IU of vitamin E per day and CSD + M: CSD diet plus 24 ppm of monensin kg^{-1} DM per day.

This homemade configuration consists of two plexiglass cylinders each of about 125 cm^3 volume with a 3 cm wide O-ring fitted aperture between them to set a 3.10 cm^2 Flemion (Asahi Glass Co., Headquarters: Tokyo, Japan) cation membrane. Anode in the shape of a rectangle with a total geometrical area of 24.30 cm^2 was made of commercial electrical grade graphite plate. Distance between cathode and anode was 8.50 cm. Catholyte solution composition was as follows: 60 mM biological phosphate buffer solution with pH= 7.40, $MgSO_4$ (0.90 g L^{-1}; Merck) and NH_4Cl (1.00 g L^{-1}; Merck, Germany). Ruminal fluid was collected as described above. Buffered rumen fluid along with McDougall's buffer (Merck; 100 mL) with 1 g of each treatment (CSD, CSD+E and CSD+M) was pipetted into the anodic chamber.

After inoculation of the bacterial culture into the anodic section, anaerobic condition was observed in that compartment by sealing of all apertures around the anode using water-proof silicone sealant. Whole of the cell was kept at 39 ˚C in a homemade thermostatic chamber. This temperature is necessary for mixed culture bacteria to grow and maintain activity.

Self-organized, vertically oriented and uniformly distributed TiO_2 nanotube arrays on a pure titanium substrate (TiO_2 nanotube/Ti electrode) were prepared by anodizing of pure titanium sheet in a non-aqueous fluoride-containing electrolyte. Titanium cathode in the shape of a rectangle having an area of 15 cm^2 was cut from a graphite sheet (purity of 99.99% and 1.00 mm thickness) and degreased by acetone and ethanol followed by rinsing with distilled water.

Anodic TiO_2 films were grown from titanium by potentiostatic anodizing in an ethylene glycol electrolyte containing 25 × 10^{-4} W/V ammonium fluorideat at a constant voltage of 40 V using a platinum sheet as a counter electrode. After anodizing of titanium, the cathode was ultrasonically cleaned in distilled water for 5 to 10 min to remove surface contaminants.

Palladium nanoparticles were deposited on the nanotubes by electroless method (Hosseini *et al.* 2007).

After electroless plating, the cathode was rinsed, dried and subjected to the characterization. Morphology, alignment and composition of the TiO_2 nanotube array and palladium coating on TiO_2 nanotubes matrix were characterized with a scanning electron microscope (model XL30; Carl Zeiss, Jena, Germany). The MFC performance was registered after 24, 48, 72, 96, 120 and 144 hr incubation.

Statistical analysis

Data obtained from *in vitro* GP study were subjected to ANOVA as a completely randomized design with three replicates by the generalized linear model (GLM) procedure of SAS version 9.2 (SAS, 2004). Correlation between *in vitro* GP and MFC was estimated using Microsoft Office Excel software (version 15.0; Microsoft Corporation, Redmond, USA).

RESULTS AND DISCUSSION

Total GP volumes (mL g^{-1} DM) in incubation times are shown in Table 2. At the early incubation times (2 and 4 hr), there was no significant difference among treatments.

Table 2 Total gas production volume (mL g^{-1} dry matter (DM)) in incubation times

Treatments	Incubation times (hr)										
	2	4	6	8	12	16	24	36	48	72	96
Control	29.27	52.74	83.75^{a}	116.93^{a}	163.93^{a}	198.69^{a}	229.12^{a}	252.23^{a}	277.27^{ab}	296.98^{ab}	307.91^{ab}
CSD	29.83	51.07	72.09^{b}	96.73^{c}	140.02^{b}	173.83^{b}	208.48^{b}	237.91^{b}	267.61^{b}	291.99^{b}	304.58^{b}
CSD + E	28.16	52.74	84.19^{a}	112.49^{ab}	156.22^{a}	191.25^{a}	227.24^{a}	255.67^{a}	284.71^{a}	307.08^{a}	317.67^{a}
CSD + M	29.05	51.18	77.75^{ab}	103.39^{bc}	137.58^{b}	167.83^{b}	198.93^{b}	220.37^{c}	237.20^{c}	253.58^{c}	262.67^{c}
SEM	0.832	1.16	2.47	2.83	3.19	3.22	3.37	3.54	3.51	3.48	3.77

Control: diet without whole cottonseed or vitamin E or monensin supplementation; CSD: control diet plus whole cottonseed 200 g kg^{-1} dry matter (DM); CSD + E: CSD diet plus 12000 IU of vitamin E per day and CSD + M: CSD diet plus 24 ppm of monensin kg^{-1} DM per day.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 The effect of treatments on the performance of microbial fuel cells

Treatments	Times (hr)					
	24	48	72	96	120	144
Open circuit-potential (milli-volt)						
CSD	126	60	28	22	33	4
CSD + E	205	106	73	23	68	76
CSD + M	165	74	45	18	22	10
Short circuit current (micro-ampere)						
CSD	160	28	55	22	22	0
CSD + E	390	150	163	44	146	46
CSD + M	230	175	104	36	44	11
Power (milli-watt)						
CSD	20.16	1.68	1.54	0.48	0.73	0.00
CSD + E	79.95	15.90	11.90	1.01	9.93	3.50
CSD + M	37.95	12.95	4.68	0.65	0.97	0.11

Control: diet without whole cottonseed or vitamin E or monensin supplementation; CSD: control diet plus whole cottonseed 200 g kg^{-1} dry matter (DM); CSD + E: CSD diet plus 12000 IU of vitamin E per day and CSD + M: CSD diet plus 24 ppm of monensin kg^{-1} DM per day.

At the most incubation times, control treatment had the highest GP among treatments (P<0.05). Adding whole cottonseed to diet decreased (P<0.05) GP values. With incubation time progression, the treatments with monensin or vitamin E had the lower (P<0.05) and higher (P<0.05) GP compared to other treatments, respectively.

The GP in the monensin treatment was lower (P<0.05) than other treatments. The effects of treatments on the performance of MFCs are shown in Table 3. Treatment containing vitamin E showed higher voltage, current and power values compared to other treatments and indicated that the addition of vitamin E improves the function of bacteria.

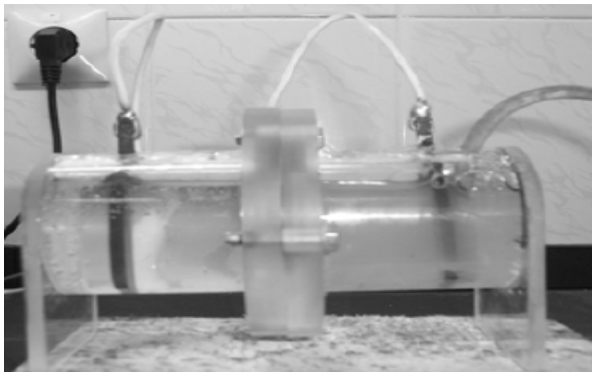

Figure 1 Dual-chambered microbial fuel cell setup

Power density was achieved as high as 79.95 mW per m^2 with vitamin E addition. The curves of power, current and voltage produced by MFC are given in Figures 2, 3 and 4. Figures 5, 6, 7, 8, 9 and 10 show correlations between the obtained data using the gas production technique and MFCs. There was strong correlation between gas production values and MFC performance of treatments.

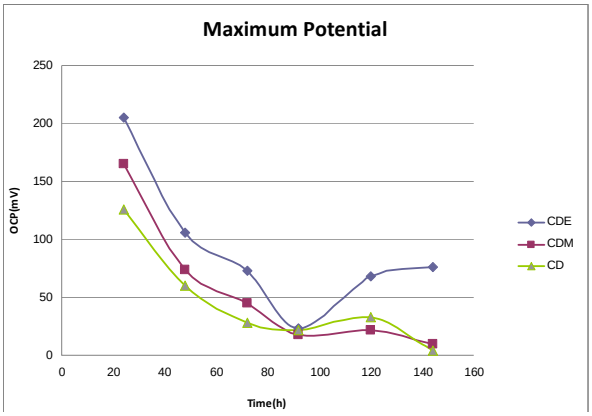

Figure 2 Cell open circuit potential (OCP) variation versus time

The results showed that treatment with vitamin E produced more gas than other treatments. High unsaturated fats in rumen are toxic for rumen bacteria and cause low bacteria activity in the rumen.

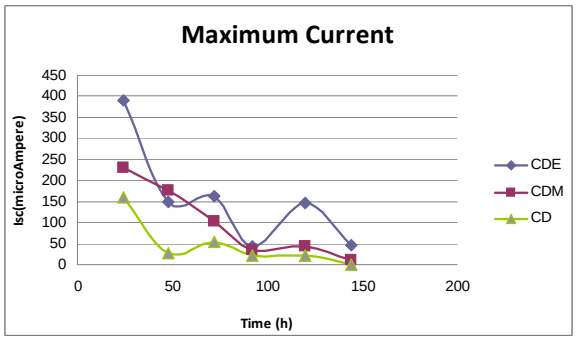

Figure 3 Short circuit current (ISC) variation versus time

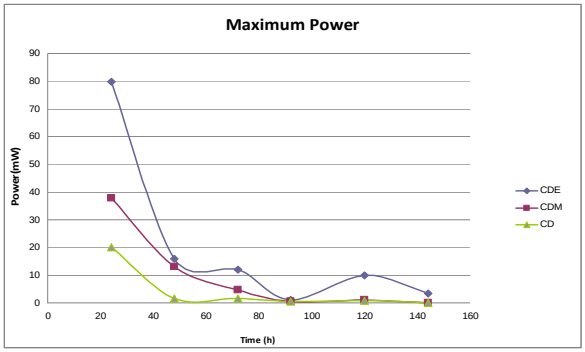

Figure 4 Maximum power variation versus time

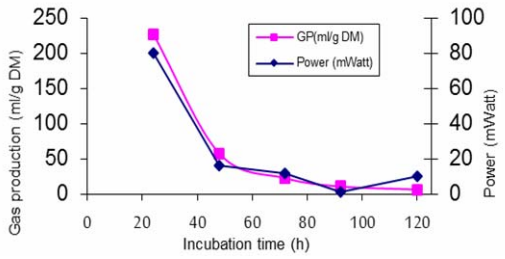

Figure 5 Gas production and MFC power production in CDE treatment

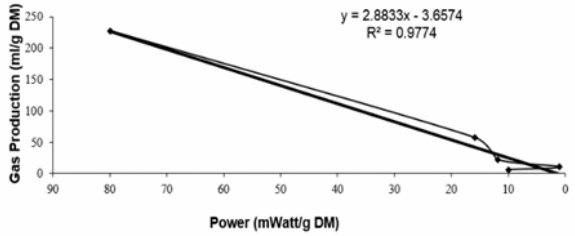

Figure 6 Correlation between gas production and MFC power versus time in treatment CDE

This study showed that the addition of vitamin E in diets high in monounsaturated fats improves the activity and function of rumen bacteria and reduces the toxic effects of unsaturated fatty acids. Increased GP in CSM + E treatment can be related to the activity of bacteria.

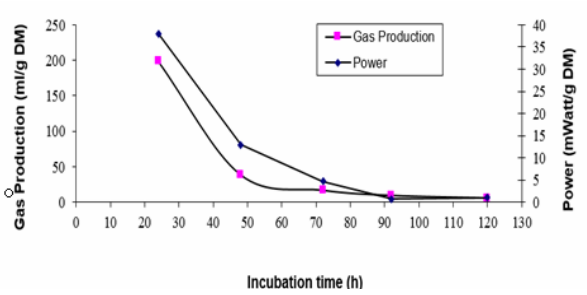

Figure 7 Gas production and MFC power production in treatment CDM

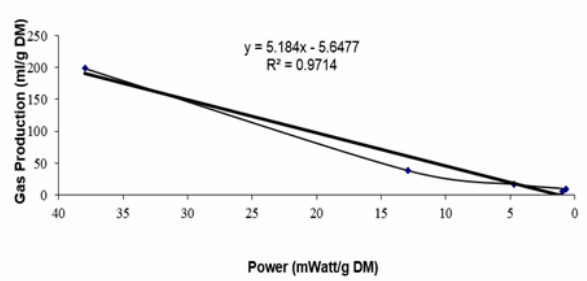

Figure 8 Correlation between gas production and MFC power versus time in treatment CDM

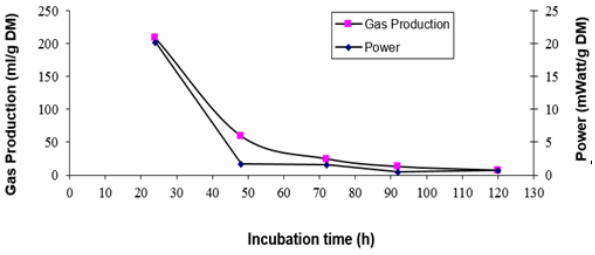

Figure 9 Gas production and MFC power production in treatment CD

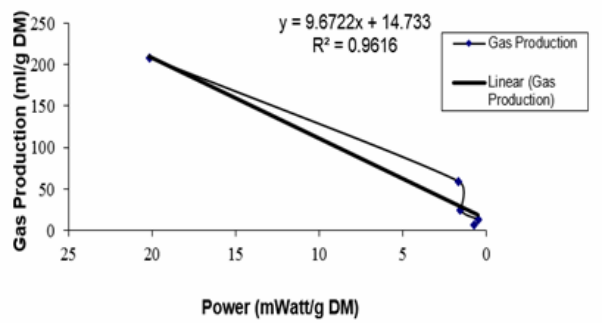

Figure 10 Correlation between gas production and MFC power versus time in treatment CD

Treatment containing vitamin E showed the higher voltage, current and power values compared to the other treatments and indicated that the addition of vitamin E improved the function of bacteria that was in agreement

with the reported results of the other experiments (Rismani-Yazdi *et al.* 2007). Electron flow commenced immediately upon inoculation of the diets-containing medium in the anodic chamber. The current declined gradually as feedstuffs were depleted. There was a remarkable current production in the presence of rumen microorganisms with or without using monensin or vitamin E in the medium and it was higher in treatment containing vitamin E than the other treatments (Table 3).

Power density was achieved as high as 79.95 mW per m^2 with vitamin E addition. This power output was higher than that achieved in the study of Rismani-Yazdi *et al.* (2007) using cellulose as a substrate. The MFCs demonstrated here exhibited constant power generation without the need for exogenous electron transfer mediators.

The obtained data showed that the addition of monensin in comparison with CSD treatment improved the MFC performance, whereas GP of treatment with monensin was lower than CSD treatment. Studies have showed that monensin improves feedstuffs digestion in the rumen and increases propionic acid production causing GP volume reduction (Taghizadeh *et al.* 2015). The GP technique could not show monensin effect through measuring GP volume, but MFC demonstrated its effect very clearly.

CONCLUSION

In conclusion, regarding to high correlations between the obtained results of MFC and GP techniques, MFC can be a substituted GP technique especially for energetic concentrates to predict degradabilities parameters. The *in vitro* GP method indirectly evaluates DM degradation by gas yield determination, whereas the MFC technique determines power during incubation in the anode through microbial degradation. As several advantages are generally pointed out to the MFC, vast possible research area can explore the application of this technique to directly estimate the fermentable metabolizable energy using recording of feedstuffs electron releasing potential. Some of these experiments are now being conducted by this research team, but for receiving sufficient information, further researches can be conducted in future.

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