Vol. 18, No. 3 (2019) 865-874
Revista Mexicana de Ingeniería Química

ASSESSMENT OF MICROBIAL ELECTROLYSIS CELLS FED HYDROLYSATE FROM AGAVE BASSASE TO DETERMINE THE FEASABILITY OF BIOELECTROHYDROGEN PRODUCTION

EVALUACIÓN DE CELDAS DE ELECTRÓLISIS MICROBIANAS ALIMENTADAS CON HIDROLIZADO DE BAGAZO DE AGAVE PARA DETERMINAR LA FACTIBILIDAD DE PRODUCCIÓN DE BIOELECTROHIDRÓGENO

R. García-Amador[1], S. Hernández[1], I. Ortiz[1], B. Cercado[2]*

[1] *Universidad Autónoma Metropolitana Unity Cuajimalpa. Av. Vasco of Quiroga 4871, Santa Fe Cuajimalpa, 05348 Mexico City, Mexico.*

[2] *Centro de Investigación y Desarrollo Tecnológico en Electroquímica S.C. Parque Tecnológico Querétaro, Sanfandila, Pedro Escobedo, 76603 Qro. Mexico.*

Received: September 6, 2018; Accepted: November 10, 2018

Abstract

The use of biomass for alternative energy production is of great interest because of the low cost of raw material and the high added value of the product as far as hydrogen is concerned. Steam explosion is used as pretreatment of lignocellulosic biomass to increase the accessibility of sugars. An approach based on sustainability and development of biorefineries allows proposing the integrated use of agave bagasse pretreatment fractions to produce bio-hydrogen. In this work, hydrolysates from steam-explosion pretreated agave bagasse were used as substrate in microbial electrolysis cells (MEC). Hydrolysates were evaluated at concentrations of 20 %, 40 % and 100 % (v/v). The use of hydrolysates was compared by analyzing two inoculum sources, compost leachate and granular sludge. The highest bioelectrohydrogen production was 8.5 mL L^{-1} d^{-1}, which was achieved using 20 % hydrolysate, 10 % compost leachate at the 0.8 V/Ag/AgCl as anodic potential in a two-chamber microbial electrolysis cell.

Keywords: agave bagasse, bioelectrohydrogen, microbial electrolysis cells, inoculum source, bioenergy.

Resumen

El uso de biomasa para la producción de energía alternativa ha ganado un gran interés debido al bajo costo de la materia prima y el alto valor de los productos generados tal como el hidrógeno. La explosión de vapor es utilizada como pretratamiento de biomasa lignocelulósica para aumentar la disponibilidad de azúcares. Una propuesta basada en la sustentabilidad del proceso y el desarrollo de biorefinerías es el uso de fracciones del pretratamiento del bagazo de agave para producir biohidrógeno. En este trabajo, los hidrolizados del pretratamiento por explosión de vapor del bagazo de agave se utilizaron como substrato en celdas de electrólisis microbianas (CEM). Los hidrolizados fueron evaluados a concentraciones de 20 %, 40 % y 100 % (v/v). Adicionalmente, dos fuentes de inóculo fueron comparadas, lixiviado de composta y lodo granular. La producción más alta de bioelectrohidrógeno fue 8.5 mL L^{-1} d^{-1}, la cual se obtuvo utilizando 20 % de hidrolizados, 10 % de lixiviado de composta y un potencial anódico de 0.8 V/Ag/AgCl en una celda de dos cámaras.

Palabras clave: bagazo de agave, bioelectrohidrógeno, celdas de electrólisis microbianas, fuente de inóculo, bioenergía.

1 Introduction

Residual biomass from food-processing industry is a diverse, available and low-cost source of alternative energy to replace oil. In Mexico, the production of agave bagasse was reported to have been 4,709,000 tons in 2016 (Gallardo, 2017), so this residue has a vast potential as a source for bioenergy production.

Cellulosic biomass is composed of cellulose, hemicellulose and lignin; the ratios of these polymers depend on the origin of biomass. Corn residues are composed of 70 % cellulose and hemicellulose, and just 15 % to 20 % of lignin (Zuo *et al*. 2006), whereas wheat residues are composed of 30 % to 40 % cellulose, 20 % to 30 % hemicellulose and 20 % lignin

* *Corresponding author. E-mail*: bcercado@cideteq.mx
https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Garcia
issn-e: 2395-8472

(Thygensen *et al*. 2011a). Agave bagasse composition is estimated to be 43 % cellulose, 19 % hemicellulose, and 15 % lignin. Sugars that make up cellulose and hemicellulose must be first released and only then used in other energy production processes.

Biomass pretreatments basically consist in breaking the fiber and releasing polymers. Different pretreatments include enzymatic pretreatments, microbial degradation (Wang *et al*. 2006), acid or alkaline hydrolysis, and hydrothermal pretreatments, such as steam explosion. These treatments convert hemicellulose into soluble sugars, mainly pentoses like xilose, whereas lignin is just partially degraded. Degradation products from cellulose also include phenolic compounds, 2-furfural and carboxylic acids (Carvalheiro *et al*. 2008). Phenolic compounds can have inhibitory effect on microorganisms (Catal *et al*. 2008).

Output currents from cellulosic biomass treatments are used in the production of liquid biofuels such as bioethanol, gaseous biofuels such as hydrogen (Carvalheiro *et al*. 2008), and recently, hydrolysates have been evaluated for the production of electricity in microbial fuel cells (MFC) (Thygesen *et al*. 2011a) or hydrogen production in microbial electrolysis cells (Li *et al*. 2015).

Microbial electrochemical cells are devices that combine characteristics of a fixed biomass biological reactor and an electrochemical cell. These devices have been grouped in fuel cells that spontaneously produce electric current through the difference of potential between their electrodes, and the electrolysis cells that require energy supply to change the potential of electrodes and allow for the occurrence of reactions that otherwise would not take place.

Microbial electrochemical cells are fed with organic matter and inoculated with microorganisms, mainly in the anode chamber; these conditions give rise to biological oxidation processes causing a flow of electrons from the anode towards the cathode (Kadier *et al*., 2016). This biological characteristic is what distinguishes bioelectrochemical cells from the electrochemical ones, and consequently microbial bioelectrochemical process depend on biofilm properties as well as on the flux of compounds in the biofilm matrix (Peraza-Baeza *et al*., 2016).

The cathode chamber, however, is similar to abiotic cells. In the case of fuel cells, the cathode chamber is where oxygen reduction reactions take place, giving rise to water formation, while in the electrolysis cells, it is the place where water reduction occur to produce gaseous hydrogen. There is an increasing interest in microbial bioelectrochemical cells because of the possibility of using waste material to reduce the degree of contamination through its biological oxidation and simultaneously produce energy. Liquid waste materials, such as effluents of agricultural, food, paper and other industries can be used for that purpose (Pant *et al*., 2016; Kadier *et al*., 2016).

Cellulose hydrolysates have been used as substrate in microbial fuel cells. Corn residues have produced up to 63 A m^{-3} (Zuo *et al*., 2006). However, the use of hydrolysates in microbial electrolysis cells is less frequent.

As has been previously described and is shown in Table 1, cellulosic biomass hydrolysates can be used in both fuel and electrolysis cells. Concept tests have been focused on corn and wheat wastes, and our work proposes the use of hydrolysates from steam explosion pretreatment of agave fibers for a possible regional application of microbial electrolysis cells (MEC).

Here, agave bagasse hydrolysates were first evaluated in MEC to determine the feasibility of their simultaneous use as substrate and inoculum. Then, dilutions made of raw hydrolysates were added external inoculum source, and finally experiments were carried out for an extended period of time to increase their performance in the generation of electric current and electrical charge, which in turn have the potential to produce hydrogen.

2 Materials and methods

2.1 Inoculum

Two inoculum sources, compost leachate and granular sludge, were evaluated in order to enrich native population of hydrolysates. Compost leachate was prepared as a mixture of yard compost (Rancho el Molino, Mexico) and distilled water in a 2:3 ratio, then the mixture was supplemented with 10 mM sodium acetate as carbon source and 50 mM KCl as base electrolyte. Leachates were obtained by filtering the compost mixture utilizing a felt cloth. Granular sludge was obtained from a hydrogen-producing reactor operating in the Instituto de Ingeniería at UNAM, Juriquilla campus.

Table 1. Investigations on the use of biomass in microbial electrochemical cells.

Biomass	Treatment	Hydrolysate composition	System[a]	System performance	Contribution	Ref.
Wheat straw	Steam explosion neutral and acid-1.2 % sulfuric acid and 190 °C or 220 °C	Content (gL^{-1}) neutral; acid: Glucose 2.3; 9.8 Xylose 20.8; 32.9	MFC	933 mW m^{-2}, neutral. 971 mW m^{-2}, acid	Power improvement due to the use of air-cathode and the increase in conductivity to 20 $mScm^{-1}$ solution	Zuo *et al*. (2006)
Corncob	Steam explosion	Solid residues from a pretreatment. Cellulose 68% Hemicellulose 17 % Lignin 15 %	MFC	331 mWm^{-2}	Improvement of productivity due to addition of microorganisms acclimatized to the substrate	Wang *et al*. (2009)
Wheat straw	Liquefaction	37.9 g COD L^{-1} 32.05 g acids L^{-1} Furfurals Phenolics Xylose Glucose	MFC	123 mWm^{-2}	Microbial community characterization: *Bacteroidetes*, *Alphaproteobacteria*, *Bacillus* and *Deltaproteobacteria*.	Zhang *et al*. (2009)
Wheat straw	Hydrothermal at 180°C for 15 min and 190°C for 3 min	Content (g L^{-1}): Xylane 17 Acetate 4.8 Phenolics 4.5	MFC	148 mWm^{-2}	Treatment of residual water in mixture with straw hydrolysates	Thygesen *et al*. (2011a)
Wheat straw	Hydrothermal	Cellulose 4% Hemicellulose 50%	MEC, 0.7 V	0.61 $m^3m^{-3}d^{-1}$ 0.4 kg H_2-COD $m^{-3}d^{-1}$ Maximum 0.8 H_2-COD $m^{-3}d^{-1}$ 100-200 Am^{-3}	Production of hydrogen and coproduction of xylane and polyphenols to reach 53 % w/v	Thygesen *et al*. (2011b)
Corncob	Diluted sulfuric acid and 121 °C	Content (g L^{-1}): Glucose 1.7 Xylose 10 Arabinose 2 Acetic ac 1.2	MFC/MEC 100 kΩ 0.8 V	Produced voltage 745 mV 23.3 H_2-mmol acetate-mol^{-1}	Sequential production of hydrogen and electricity in the same reactor.	Yan *et al*. (2015)
Corncob	Subcritical H_2O with diluted HCl and 230 °C	Reducing sugars 469.7 mg total solids-g^{-1}	MEC, 0.8 V	1060 mL H_2 COD-g^{-1} 3.4 $m^3m^{-3}d^{-1}$	Hydrogen production in two sequential stages: fermentation and MEC.	Li *et al*. (2015)
Corncob	2% (v/v) sulfuric acid at a ratio of 1:5 (w/v). Detoxification by $Ca(OH)_2$, NaOH, activated carbon.	External addition of individual sugars at 15 g L^{-1}	MEC -0.6 V to 2.6 V	Not produced	Production of succinic acid. Detoxification of the hydrolysate.	Zhao *et al*. (2016)
Molasses	Polyacrylamide pretreatment	Sugars 15-75 g L^{-1}	MEC -1.0 V	Not produced	Production of succinic acid.	Zhen *et al*. (2018)

a. MFC Microbial fuel cell; MEC Microbial electrolysis cells.

2.2 Substrate

The bagasse of blue agave tequilana from Amatitlán, Jalisco, was used as raw material (supplied by the Instituto Potosino de Investigación Científica y Tecnológica, IPICYT). The composition of agave bagasse was 56 %, 11 % and 15 % of cellulose, hemicellulose and lignin, respectively. The agave bagasse hydrolysates (ABH) are the liquid fraction obtained by submitting the fibers to a pretreatment by steam explosion. Here, the fibers were submitted to 154 ºC (6 kgf cm^{-2}) and after 20 min, sudden expansion was performed. The characteristics and composition of hydrolysates were pH 4.0 ± 0.1, 9560 ± 10 mg COD L^{-1}, 6.73 g VS L^{-1}, and 9.63 ± 0.005 mS cm^{-1}.

2.3 Electrochemical cell design and operation

Two-chamber glass electrochemical cells, each chamber having 130 mL operation volume and a total volume of 200 mL, were used. The chambers were separated by a cation exchange membrane of 5 cm diameter (CMI-7000 Ultrex Membranes International, USA). The distance between the electrodes was 12.5 cm and the total cell length was 17 cm. The electrodes used as anode were made of carbon felt (2 cm x 2 cm x 0.5 cm, Carbon Rooe, Mexico) and electrodes used as cathode were prepared with stainless steel mesh (2 cm x 2 cm x 0.1 cm, Sommer, México). The cell was fed in batch and kept at 25°C in a water bath. The anodic potential was maintained at 0.8 V/Ag/AgCl for 5 or 21 days. The gas produced at the cathodic chamber was quantified using the water displacement method.

2.4 Physicochemical analyses

Microbial biomass was quantified by the volatile solids (VS) method (Eaton *et al*. 2005). In brief, the method consists in eliminating the water from one sample of 25 mL by evaporation and oven desiccation; the sample is then calcined at 550 °C for 1 hour to obtain the weight of ashes, and finally, the difference of weights determines the amount of volatile matter, which is an approximation of microbial biomass.

The substrate or feed to the cells was quantified by chemical oxygen demand (COD) using the potassium dichromate method in digestion vials (Method 8000, DRB 200 Hach, USA). For that, 2 mL of sample were incubated with a potassium dichromate solution in sulfuric acid during 2 h at 550°C; after digestion, chrome ion was quantified in a spectrophotometer at 600 nm (DR 1900, Hach USA).

The pH and conductivity of solutions were determined using a multiparameter meter (Oakton PCD 650, USA).

2.5 Electroanalytical techniques

The analysis of electrochemical parameters was made using a potentiostat-galvanostat (BioLogic VSP, EC-Lab ver. 10.44). The open circuit potential was measured for 2 h, and subsequently cyclic voltammetry was performed in the potential interval of +1.0 V a -1.0 V/Ag/AgCl at a scanning rate of 5 mV s^{-1}. Chronoamperometry at a fixed potential of 0.8 V/Ag/AgCl was used to analyze the electroactive biofilm growth and the production of cathodic hydrogen for 5 days with raw hydrolysates, and for 20 days with diluted hydrolysates.

3 Results and discussion

3.1 Evaluation of untreated and diluted ABH

The ABH was evaluated as raw substrate (100%) and substrate diluted at 20% with phosphate buffer solution. The generation of electric current was followed under an anodic potential of 0.8 V/Ag/AgCl during 5 days (Fig. 1).

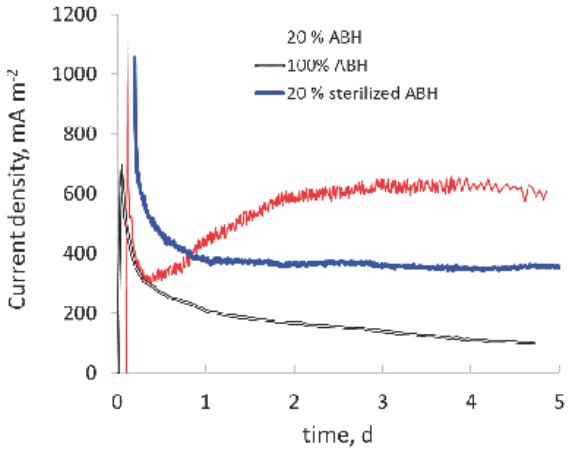

Fig. 1. Chronoamperograms of carbon felt in untreated ABH and diluted at 20% with buffer solution. Anodic potential 0.8 V/Ag/AgCl.

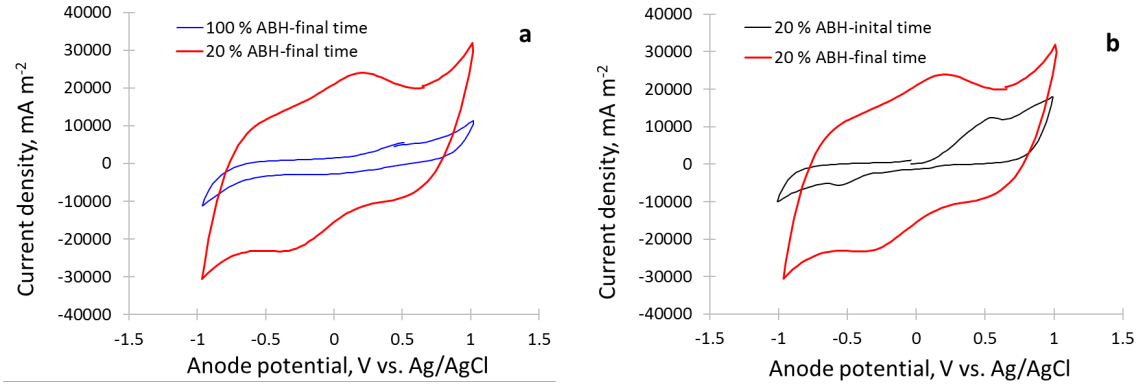

Fig. 2 Voltammograms of carbon felt immersed in ABH. a) Comparison of untreated and diluted ABH, b) Comparison of ABH diluted at 20% at initial and final experimental time.

Current density showed a decreasing profile from 695 mA m^{-2} to 100 mA m^{-2} when using untreated ABH, whereas the diluted ABH resulted in an increase in the current from 298 mA m^{-2} to 658 mA m^{-2}, which was stable for 3 days.

The behavior of both chronoamperograms suggests that electroactive microorganisms are present in ABH as native community, and that its dilution with buffer solution allowed the reduction of organic matter content simultaneously with the adjustment of pH of the medium. The pH of raw ABH was 4.0 ± 0.1 but once ABH was diluted, the pH increased to 6.4, which favored the bacterial growth as it was close to neutrality. It is widely recognized that the universe of microbial species is majority around pH 7 (Mardigan *et al*. 2009).

In addition to the change of pH due to dilution, the raw ABH was found to exhibit conductivity of the order of 9.6 mS cm^{-1}, which is low as compared to diluted ABH that exhibited values from 56 mS cm^{-1} to 87 mS cm^{-1}. The increase in conductivity upon diluting ABH was due to the salts in the buffer solution. Although the use of buffer solution provided a benefit, an inexpensive pH adjustment of ABH should be foreseen in order to maintain process sustainability.

Another factor that favored the development of electroactivity was the dilution of organic matter. Raw ABH exhibited a COD of 9570 mg L^{-1}, and after dilution COD decreased to 2320 mg L^{-1}. High concentrations of substrate have been previously reported to cause a decrease in electroactivity by diverting the electron flow from the electrode towards the chemical reduction of other organic compounds (Pant *et al*. 2016).

In order to verify whether the increase in current with the use of diluted ABH was due to the microbial electroactivity, the medium was sterilized and the test was repeated. The obtained chronoamperogram was similar to that observed with the raw ABH, that is to say, the current decreased and stabilized at 370 mA m^{-2}, even though the current density was greater when using sterilized ABH (Fig. 1). This result was probably caused by the formation of oxidizable organic compounds during sterilization process, which takes place at high pressure and temperature.

Oligomers of pentose and hexose units might have been oxidized at the anode operating potential (0.8 V/Ag/AgCl). Electro-oxidation of organic compounds is commonly used for removal of pollutants, therefore the current observed when using diluted and sterile ABH is possibly due to the oxidation of sugars at the electrode (Parpot *et al*. 2004).

An additional test to verify the microbial electroactivity was carried out using cyclic voltammetry. Voltammograms of the electrodes were analyzed at the final experiment time, i.e., when the presence of electroactive biofilm in the cell installed with the diluted ABH was expected. Under this condition, the oxidation current peak barely reached 5231 mA m^{-2} at a potential of 0.47 V/Ag/Ag/Cl with raw ABH, but increased until a maximum of 23927 mA m^{-2} at a potential of 0.22 V/Ag/AgCl with diluted ABH (Fig. 2a).

The presence of electroactive biofilm was confirmed upon comparing the voltammograms of the electrode immersed in the diluted medium at the initial and final experiment time. The voltammogram at the initial time showed a lower oxidation current peak than at the final time (Fig. 2b).

The voltammogram at the final experiment time appeared to have less defined oxidation and reduction peaks due to a capacitive current, which is attributed to the biofilm formed on the electrode. Current density

observed at the final time is the sum of the capacitive and faradaic currents. The capacitive current has been attributed to the structural constituents of the biofilm, such as exopolymers of sugars, proteins, and lipids in addition to the presence of other bacterial cells which storage electrical charge. While the faradaic current is attributed to the charge transfer from the membrane of bacteria towards the electrode, said charge has its origin in the oxidation of organic matter by electroactive microorganisms (Fricke *et al*. 2008). Thus, it can be assumed that the increase in current observed in voltamperograms at the final time was due to the formation of the electroactive biofilm.

These results altogether show that the use of untreated ABH as a source of inoculum and substrate in microbial bioelectrochemical systems is not feasible due to the high charge of organic matter, and low pH and conductivity. However, an adjustment of pH close to neutrality and an increase in conductivity allowed the formation of the electroactive biofilm from native microorganisms, which produced up to 650 mA m^{-2} under a fixed potential of 0.8 V/Ag/AgCl and up to 23927 mA m^{-2} at of 0.22 V/Ag/AgCl during the potential scan.

Even though electroactivity supported by the components typical of ABH was found, a strategy to increase the current density is to use an external inoculum in a bioaugmentation-like process. The process of bioaugmentation requires a previous pre-selection of divers inocula that are known by their electroactive microorganisms content.

3.2 *Selection of external inoculum*

Leachates of compost and granular sludge were compared as sources of external inoculum in combination with ABH diluted at 20% as a substrate.

The chronoamperograms showed a slightly higher production of electrical current when using compost leachates compared to those of granular sludge. The experimental charge was 6.11 C and 4.37 C for compost leachates and granular sludge, respectively (specific charge 1.21 C/g SV and 1.26 C/g SV).

The amount of volatile solids in compost leachates was 5% and its pH was 6.7, while in sludge this amount was 3.5% and pH 6.4. Based on the differences in their chemical characteristics, the compost leachate was selected as inoculum, because of the higher concentration of volatile solids and a pH slightly closer to neutrality.

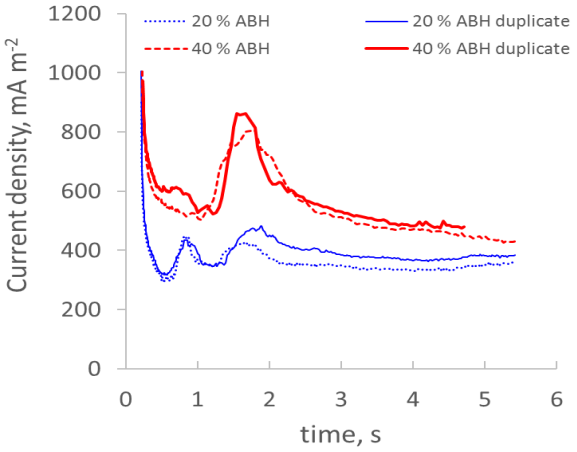

Fig. 3. Chronoamperograms of carbon felt in ABH diluted at 20% as substrate and compost leachates as inoculum at 10%. Anodic potential 0.8 V/Ag/AgCl.

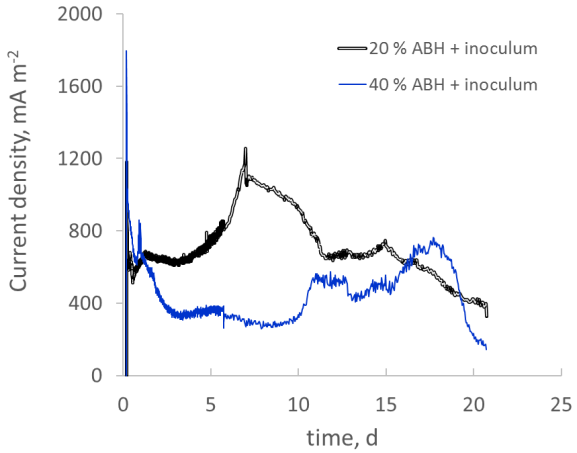

Fig. 4. Chronoamperograms of carbon felt in ABH diluted at 20% and 4 % supplemented with 10% of inoculum. Anodic potential 0.8 V/Ag/AgCl.

3.3 *Inoculum-substrate system*

Once the improved generation of current with diluted ABH was established and compost leachate selected as source of external inoculum, 2 dilutions were compared for production of current using ABH at 20%, 40%; these solutions were inoculated at 10% (v/v). It was hypothesized that an increase in current density would be observed due to an increased content of organic matter in the medium.

The chronoamperograms showed a higher current density for the 40% dilution, however, this improvement was only present within the first days of operation. Afterwards, the difference in current density between the two dilutions was minor, and

current density was in the range from 383 mA m^{-2} to 448 mA m^{-2} on day 5 (Fig. 3).

The lack of a major difference between chronoamperograms, obtained with 20% and 40% of ABH, after 5 days of operation can be attributed either to a dilution interval, insufficient to give a visibly differentiated response, or to the fact that the mixture of external inoculum and the native microbial community required a longer period to establish an inter-species equilibrium and start a synergistic process of charge transfer.

In order to prove the hypothesis of the need for a prolonged time of adaptation between the native community and the external inoculum, the experiment time was extended to 20 days. The results showed that the current density was higher for ABH diluted at 40% only during the very first day of the test. From then on, current density was higher in the cell containing 20% of ABH than in the cell with 40% of ABH (Fig. 4).

The current density peak in the cell with 20% ABH was 1,255 mA m^{-2} and was achieved after 7 days approximately, whereas the peak in the cell containing 40% ABH was 762 mA m^{-2} and was achieved after 17.7 days.

The reason for a lower current obtained at the higher ABH concentration (40%) may be attributed to the presence of phenol- and furfural-type inhibitors in ABH (Parpot *et al*. 2004), thus the more diluted medium presented a lower inhibitory effect.

At higher ABH concentration (40%), the current density peak was lower and appeared quite late, these results suggest the existence of an adaptation process of the microbial community towards the aforementioned inhibitors, however, further investigation aimed at identifying and quantifying the inhibitors in ABH is required.

Although current density peaks can be higher in one cell with regard to another cell, the experimental charge may present an opposite order, since the charge refers to the area under the curve of current in a period of time, whereas the current peak is punctual in time. Therefore, the experimental charge was calculated for the period of 20 days, being 116 C and 82 C for 20% and 40% of ABH, respectively (specific charge of 133 C/g VS and 101 C/g VS).

Experimental charge can be associated with the formation of products on the cathode of an electrochemical cell according to the Faraday Law (Brockris *et al*. 1998). One of the most extended uses of the electrical charge in MEC is the production of gaseous hydrogen as energy vector.

Based on the experimental charge data and the cathodic reaction of Eq. (1)

$$3H_2O + 2e^- \rightarrow H_{2(g)} + 2OH^- + H_2O \qquad (1)$$

an estimation of the theoretical production of hydrogen in the MEC was carried out by means of Eq. (2).

$$n_{H2,I} = \frac{\int Idt}{2F}[=]\frac{A \cdot s}{\frac{2e^- mol}{H_2 mol} \cdot 96485 \frac{C}{e^- mol}}[=]H_2 mol \qquad (2)$$

where I is the experimentally observed current in a period (*dt*), F is the Faraday's constant, the number 2 corresponds to the electrons required to form gaseous hydrogen and one Coulomb is defined by one Ampere by second. The bioelectrohydrogen that could theoretically be produced from the current generated with 20% and 40 % of ABH was 52 mL L^{-1} d^{-1} and 32 mL L^{-1} d^{-1} respectively. However, experimentally only 4.7 mL of hydrogen were harvested from the cell with 20% of ABH which is equivalent to 9×10^{-4} m^3 hydrogen m^{-3} reactor d^{-1}.

The experimental and even the maximum theoretical hydrogen production rate is far of that reported for MECs fed biomass hydrolysates. For instance, Thygesen *et al*. (2011b) reached a productivity of 0.61 m^3 m^{-3} d^{-1} from wheat straw hydrolysates, whereas Li *et al*. (2015) reported a productivity 5.6-fold superior using corncob. The strategy followed by Li *et al*. (2005) was to link two bioprocesses in addition to the thermal-chemical pretreatment, thus obtaining an effluent rich in organic compounds. The strategy enabled to increase hydrogen productivity in the MEC, but time spent and materials were also increased.

The broad difference between the theoretical and experimental production of hydrogen is explained by various factors. One of the main challenges of electrolysis cells is to avoid the leakage of gaseous products, so it is very likely that there were gas leaks in the cathode chamber of the electrochemical cell used in the present work. Moreover, the experimental charge could have been consumed in parasite reactions, that is, in the reactions that generate no products of interest. This phenomenon is also frequent during electrochemical processes (Larminie and Dicks, 2003).

The real production of hydrogen can be associated with changes of pH in the electrolyte. Changes of pH of both anodic and cathodic electrolytes due to the evolution hydrogen reaction have been previously reported (Nam and Logan, 2012).

Table 2. pH values in anolyte and catholyte of MEC fed with ABH at 20% and 40% and amended with 10% of inoculum.

	pH		Conductivity (mS cm^{-1})	
	Anolyte	Catholyte	Anolyte	Catholyte
20% ABH				
Initial	6.58	6.73	83.73	112
Final	5.65	10.32	85.78	121.9
Difference	-0.93	3.59	2.05	9.9
40% ABH				
Initial	6.32	6.73	67.23	112
Final	5.48	7.53	96.61	116.1
Difference	-0.84	0.8	29.38	4.1

Anolyte pH is usually acidified due to the process of acidogenesis occurring in anaerobic media as well as to the production of protons during oxidation of highly reduced carbonaceous compounds, such as reducing sugars. On the other hand, catholyte pH usually turns to alkaline values when hydrogen production process starts at a neutral pH. As specified in Eq. (1), the product of reaction is a hydroxyl ion that alkalinizes the medium.

The above-described phenomenon was confirmed by the changes of pH found in electrolytes of the cells after 20 days (Table 2).

Anolyte acidification and catholyte alkalization were greater in the cell with 20% of ABH, which confirms that the hydrogen evolution reaction indeed occurred more effectively in the cell with a more diluted ABH.

Clearly, the MEC system used in the present work, i.e. inoculum, substrate concentration, and electrochemical cell design is optimizable. From an engineering point of view, the electrochemical cell is of major importance and it is expected to be improved in subsequent investigation.

It can be concluded that ABH is feasible for use as inoculum and substrate in MEC due to the presence of native electroactive microorganisms. The use of external inoculum as strategy to increase the current density with 20 % diluted ABH was successful, however, it requires a period of microbial adaptation. The degree of dilution is a factor that requires optimization to simultaneously provide sufficient carbonaceous compounds and native microorganisms. At the same time, the inhibition due to a complex substrate as well as the presence of furfural-type compounds need to be investigated.

Conclusions

The use of the effluents generated by residual biomass-based processes, such as pretreatment by steam explosion of agave bagasse, sustains the development of biorefineries. The exploitation of the liquid fraction resulting from the bagasse pretreatment as feed to microbial electrolysis cells allows extracting additional energy in form of electrical current and gaseous biofuels. In this work, the best conditions for electrical current production were found for a fixed anodic potential of 0.8 V vs. Ag/AgCl, inoculation of 10 % of compost leachates, and agave bagasse leachates diluted at 20 %. Electrical current corresponded to the observed maximum electrical charge (133 C/g VS) which enabled a production of 8.5 mL H2 L^{-1} d^{-1} from the system inoculum-substrate-cell design used in the present research. These findings will undoubtedly be a guide for coupling thermal and bioelectrochemical processes for biomass valorization.

Acknowledgements

R. García-Amador is grateful for the support from Consejo Nacional of Ciencia and Tecnología (CONACYT) for his graduate studies fellowship (CVU 799528). The project was financed by the CONACYT-SENER-FSE project 247006.

Abbreviations

MEC & microbial electrolysis cells
COD &chemical oxygen demand
ABH & agave bagasse hydrolysate

References

Bockris, O., Reddy, A., & Gamboa-Aldeco, M. (1998). *Modern Electrochemistry 2A, Fundamentals of Electrodics*. New York: Kluwer Academic Plenum.

Gallardo J. (2018). Industria del tequila y generación de residuos. Ciencia y Desarrollo CONACYT. http://www.cienciaydesarrollo.mx/?p=articulo &id=287. Consultado el 01 of October of 2018

Larminie, J., & Dicks, A. (2003). *Fuel Cell Systems Explained*. West Sussex, England: Wiley.

Carvalheiro, F., Duarte, L. C., & Girio, F. M. (2008). Hemicellulose biorefineries: a review on biomass pretreatments. *Journal of Scientific & Industry Research 67*, 849-864.

Catal, T., Fan, Y. Z., Li, K. C., Bermek, H., & Liu, H. (2008). Effects of furan derivatives and phenolic compounds on electricity generation in microbial fuel cells. *Journal of Power Sources 180*, 162-166. doi:10.1016/j.jpowsour.2008.02.052

Fricke, K., Harnisch, F., & Schroder, U. (2008). On the use of cyclic voltammetry for the study of anodic electron transfer in microbial fuel cells. *Energy & Environmental Science 1*, 144-147. doi:10.1039/b802363h

Kadier, A., Simayi, Y., Kalil, M. S., Abdeshahian, P., & Hamid, A. A. (2014). A review of the substrates used in microbial electrolysis cells (MECs) for producing sustainable and clean hydrogen gas. *Renewable Energy 71*, 466-472. doi:10.1016/j.renene.2014.05.052

Li, Y. H., Bai, Y. X., Pan, C. M., Li, W. W., Zheng, H. Q., Zhang, J. N., . . . Hou, H. W. (2015). Effective conversion of maize straw wastes into bio-hydrogen by two-stage process integrating H-2 fermentation and MECs. *Environmental Science and Pollution Research 22*, 18394-18403. doi:10.1007/s11356-015-5016-3

Mardigan, M., & Martinko, J. (2009). *Brock: Biology of Microorganisms*. Madrid: Pearson.

Nam, J. Y., & Logan, B. E. (2012). Optimization of catholyte concentration and anolyte pHs in two chamber microbial electrolysis cells. *International Journal of Hydrogen Energy 37*, 18622-18628. doi:10.1016/j.ijhydene.2012.09.140

Pant, D., Van Bogaert, G., Alvarez-Gallego, Y., Diels, L., & Vanbroekhoven, K. (2016). Evaluation of bioelectrogenic potential of four industrial effluents as substrate for low cost microbial fuel cells operation. *Environmental Engineering and Management Journal 15*, 1897-1904.

Parpot, P., Bettencourt, A. P., Chamoulaud, G., Kokoh, K. B., & Beigsir, E. M. (2004). Electrochemical investigations of the oxidation-reduction of furfural in aqueous medium - Application to electrosynthesis. *Electrochimica Acta 49*, 397-403. doi:10.1016/j.electacta.2003.08.021

Peraza-Baeza, I., Perez-Hernandez, A., Blanco-Cocom, L., Dominguez-Maldonado, J., & Alzate-Gaviria, L. (2016). A mathematical model of the oscillations of pH for the anodic biofilm formation in a microbial fuel cell. *Revista Mexicana de Ingeniería Química 15*, 763-771.

Thygesen, A., Poulsen, F. W., Angelidaki, I., Min, B., & Bjerre, A. B. (2011a). Electricity generation by microbial fuel cells fuelled with wheat straw hydrolysate. *Biomass & Bioenergy 35*, 4732-4739. doi:10.1016/j.biombioe.2011.09.026

Thygesen, A., Marzorati, M., Boon, N., Thomsen, A. B., & Verstraete, W. (2011b). Upgrading of straw hydrolysate for production of hydrogen and phenols in a microbial electrolysis cell (MEC). *Applied Microbiology and Biotechnology 89*, 855-865. doi:10.1007/s00253-010-3068-3

Wang, X., Feng, Y. J., Wang, H. M., Qu, Y. P., Yu, Y. L., Ren, N. Q., . . . Logan, B. E. (2009). Bioaugmentation for electricity generation from corn stover biomass using microbial fuel cells. *Environmental Science & Technology 43*, 6088-6093. doi:10.1021/es900391b

Wang, Z., Li, H., Feng, J., Zhang, A., Ying, H., He, X., Jiang, M., & Chen, K. (2018). Enhanced succinic acid production from polyacrylamide pretreated cane molasses in microbial electrolysis cells. *Journal of Chemical Technology and Biotechnology 83*, 855.860. doi.org/10.1002/jctb.5440

Yan, D., Yang, X. W., & Yuan, W. Q. (2015). Electricity and H-2 generation from hemicellulose by sequential fermentation and microbial fuel/electrolysis cell. *Journal of Power Sources 289*, 26-33. doi:10.1016/j.jpowsour.2015.04.164

Zeng, X. F., Collins, M. A., Borole, A. P., & Pavlostathis, S. G. (2017). The extent of fermentative transformation of phenolic compounds in the bioanode controls exoelectrogenic activity in a microbial electrolysis cell. *Water Research 109*, 299-309. doi:10.1016/j.watres.2016.11.057

Zhang, Y. F., Min, B. K., Huang, L. P., & Angelidaki, I. (2009). Generation of electricity and analysis of microbial communities in wheat straw biomass-powered microbial fuel cells. *Applied and Environmental Microbiology 75*, 3389-3395. doi:10.1128/aem.02240-08

Zhao, Y., Cao, W. J., Wang, Z., Zhang, B. W., Chen, K. Q., & Ouyang, P. K. (2016). Enhanced succinic acid production from corncob hydrolysate by microbial electrolysis cells. *Bioresource Technology 202*, 152-157. doi:10.1016/j.biortech.2015.12.002

Zuo, Y., Maness, P. C., & Logan, B. E. (2006). Electricity production from steam-exploded corn stover biomass. *Energy & Fuels 20*, 1716-1721. doi:10.1021/ef0600331