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Diana Ramírez Sáenz
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ELECTRICITY GENERATION USING BIOHYDROGEN: EVALUATION OF SOME ENVIROMENTAL PARAMETERS AND SYSTEM INTEGRATION

D. Ramírez-Sáenz[a], L.G. Arriaga-Hurtado[b] and E.I. García-Peña[a,*].

[a] Bioprocesses Department. Unidad Profesional Interdisciplinaria de Biotecnología-IPN, Avenida Acueducto s/n., DF, Mexico, Po Box 07340

[b] Hydrogen and Fuel Cells Department. Centro de Investigación y Desarrollo Tecnológico en Electroquímica-CONACyT. Parque Tecnológico Querétaro, **S**anfandila, Pedro Escobedo, Queretaro, Mexico. Po Box 76703

*corresponding author Email: inesppu3@yahoo.com.mx; Ph.: (+52) 5557 296000 ext 56386; Fax: (+52) 5557 296000 ext 56305

ABSTRACT

Heat and acid pretreatments of the sludge obtained from an anaerobic digester have been used to inhibit the activity of the H_2 consumers and to harvest high yield of H_2 at two incubation temperatures (35 and 55ºC). The best results was found with the heat pretreated microbial community (HPMC) incubated at 35ºC, 99% removal of 10 $g_{glucose}$/L in 24 h and a yield of 2.8 molH_2/mol $_{glucose}$. Also, batch tests were conducted at increasing initial glucose concentrations (3 to 30 g/L) using HPMC. The maximum H_2 production (461 mL) and yield (3.6 molH_2/mol$_{glucose}$) were accomplished with 15 and 10 $g_{glucose}$ /L, respectively. Acetic acid was the major metabolic product present in the systems with higher yields. Using a concentration of 10 $g_{glucose}$/L and the HPMC, a combination of anaerobic reactor and PEMFC could generate electricity with an output power, voltage and current of 0.1W, 1.03 V and 0.068 A, respectively.

Keywords: Biohydrogen production, anaerobic fermentation, pretreatments, substrate concentration, acetic acid, fuel cell, electricity generation.

NOMENCLATURE

APMC: Acid Pretreated Microbial Community.

AR: Anaerobic Reactor.

HPMC: Heat Pretreated Microbial Community.

IGC: Initial Glucose Concentration.

MAD: Methanogenic Anaerobic Digester.

MEA: Membrane Electrode Assembly

OSW: Organic Solid Waste.

PEMFC: Proton Exchange Membrane Fuel Cell.

SS: Seed Sludge.

1 INTRODUCTION

Biological H_2 production using dark fermentation is an environmentally friendly and less energy intensive process [1,2]. Furthermore, organic solid wastes (OSW) can be used as substrates for biological H_2 production facilitating both waste elimination and energy recovery. When methanogenic anaerobic digester (MAD) sludge is used as seed for H_2 production, a pretreatment to inhibit H_2 consumers (especially methanogens) is necessary. Previous studies have demonstrated that heat and acid pretreatments are able to suppress the growth of H_2 consumers and accordingly to enhance H_2 production. [3-7]. Heat pretreatment seems to activate spore forming H_2 producing microorganisms (such as *Clostridium*) and kill vegetative cells of non-spore forming microorganisms some of which consume H_2 (such as methanogens) but it could also attack H_2 producing microorganisms (such as *Enterobacter sp.*) which are unable to sporulate [8]. Valdez-Vazquez et al.

[9] found a strong interaction between induction pretreatment and incubation temperature. According with the authors exist a strong negative interaction between heat pretreatment and subsequent incubation at 55°C.

On the other hand, hydrogen production is directly related with the fermentation substrates. Each substrate has its own fermentation pathways and end products, which leads to different hydrogen yields [10-12]. Several monosaccharides, including hexoses and pentoses, have been often used as model substrates [13-15]. The effect of substrate concentration is a very important parameter, determining the economic and technical feasibility of fermentative hydrogen production [2,16]. Furthermore, the amount of substrate is important to activate germination and to prevent re-sporulation in cultivated spore forming bacteria [14].

Nath et al. [17] evaluated initial glucose concentrations (IGC's) between 2 - 14 g/L and found the highest H_2 production (956.8 mL) and the highest yield (3.31mol H_2/mol $_{glucose}$) at an IGC of 10 g/L. Jianzheng et al. [15] reported an H_2 yield of 82.4 mL H_2 / g $_{glucose}$ with an IGC of 10.1 g/L using sewage sludge as inocula. Pan et al. [18] found the optimal IGC for the maximal yield (4165.9 mL H_2/L_{media}), using *Clostridium sp. Fanp2*, at 23.75 g/L. Other study, testing IGC's between 5 and 25 g/L, found the highest H_2 production (425.8 mL H_2/g $_{dry\ cell}$ h) at an IGC of 21 g/L [19]. It has been reported that substrate inhibition gets predominant at higher glucose concentration because this modifies the metabolic pathways [5].

H_2 produced biologically can be use to electricity production via proton exchange membrane fuel cell (PEMFC), nevertheless, few reports have describe the integration of biological hydrogen production and fuel cell systems for electricity generation [2,20,21]. Nakada et al. [20] reported that photosynthetic bacterial hydrogen production could generate electricity of 1W with a fuel cell system. According to these authors, the advantage of this system is that the biogas produced by

photosynthetic bacterial contained high H_2 purity (88.1%), and thus can be used by a fuel cell to generate power directly. However, this system require of intensive light energy supply.

Most articles have discussed PEMFC applications on mobile and household power generator [22-27]. According to Levin et al. [2], the choice of a PEMFC is based on the idea that biohydrogen systems might best be used as a means of delivering small, distributed power systems to communities; if a biohydrogen system can deliver enough H2 to power a PEMFC for 24 h, on a continuous basis, and the fuel cell system can produce enough electricity to supply the electrical demand year round, then the biohydrogen system could have a truly useful, and potentially commercial, application.

A fuel cell is an electrochemical device (a galvanic cell) which converts free energy of a chemical reaction into electrical energy; by products are heat and water/steam if hydrogen and air are the reactants [28]. Characteristic of fuel cell systems is generally high efficiency since it is not limited by Carnot efficiency. Efficiency can be very high (up to 55-65%) for the fuel cells with a combined cycle and/or cogeneration compared to the system efficiency of current power generation of up to about 40-45%. Fuel cell power can reduce costly transmission lines and transmission losses for a distributed system. No moving parts in fuel cells and a very few moving parts in the fuel cell system so that it has higher reliability compared to an internal combustion or gas turbine power plant [28].

Previous studies developed in an MAD used for treating sorted OSW of the Central of supplies showed that under the conditions of the OSW, low pH and easily degraded organic matter [29], the H_2 production could be favored instead of the CH_4 production.

In this research, the effect of two pretreatments methods, incubation temperature and substrate concentration (glucose) was investigated; furthermore, the biohydrogen production was integrated with a PEMFC for electricity generation.

2 METHODOLOGY.

2.1 Effect of pretreatment and incubation temperature.

The seed sludge (SS) was obtained from an OSW methanogenic digester with 30 L of total volume. To allow H_2 production, heat and acid pretreatments were evalauted. The heat pretreatment was carried out in a boiling water bath at 80 °C for a period of 30 min just before the inoculation. For acid pretreatment, a sample of methanogenic sludge was introduced in a sealed bottle of 125 mL, and pH was reduced and maintained around 4 by adding an HCl solution (10% v/v). The system was incubated at 35 °C and 150 rpm for 24 h before the inoculation.

50 mL of mineral media (containing: KH_2PO_4, 0.6 g/L; $(NH_4)_2SO_4$, 3 g/L; K_2HPO_4, 2.4 g/L; $MgSO_4 \cdot 7H_2O$, 1.5 g/L; $CaSO_4$, 0.15 g/L; $FeSO_4$, 0.03 g/L and glucose, 10 g/L) was introduced in serological bottles of 125 mL of total volume, sealed and flushed with N_2 (for air evacuation and gas replacement). The serological bottles were then inoculated with 5 mL of the heat pretreated microbial community (HPMC) or the acid pretreated microbial community (APMC), respectively. A system was inoculated with the anaerobic sludge without a pretreatment, as a control. The systems were incubated at 35 and 55 °C, and 150 rpm. The experimental design used is shown in table 1. All the experiments were made with replicates. To avoid the methanogenic inhibition for pH effect, it was controlled at 7 ± 0.5 with a NaOH solution (0.8 N).

2.2 Effect of substrate concentration

The tests were carried out in similar conditions of the ones described previously with increasing IGC's of 3, 5, 7, 10, 15, 20 and 30 g/L. The systems were inoculated with 5 mL of the HPMC and incubated at 35°C and 150 rpm. All the experiments were made with replicates. The pH was maintained at a value of 5.5 ± 0.5 with the addition of a NaOH solution (0.8 N).

2.3 Electricity generation

A schematic description of the biohydrogen-fuel cell electricity generation system is shown in figure 1a. The anaerobic reactor (AR) consists in a bottle of 2L of total volume with 1.44 L of mineral media and 160 mL of HPMC were used as inocula. The IGC was 10 g/L, the pH value was controlled at 5.5± 0.5 with the addition of a NaOH solution (0.8 N) and the system was incubated at 35°C and 150 rpm. The bioreactor was operated on a semi-continuous mode. To avoid the HPMC inhibition for metabolic products effect, 800 mL of exhaust media were replaced with fresh media, periodically (around 15 days). 10 g/L of glucose were feeding once between 48 and 72 h.

The biogas was feed in a 2L bottle containing 500 mL of a NaOH solution (2 N), the CO_2 was trapped via a quick reaction with NaOH and CO_2 to form $NaHCO_3/Na_2CO_3$ precipitates. The effluent stream was fed to the fuel cell device (al a flow of 0.6 L/h) to generate electricity that turned on a motor. At the same time air was pumped into a bottle contained 500 mL of distillated water, the humidify air was fed to the fuel cell device. The biogas volume produced was measurement with a displacement system.

A PEMFC was used for electricity generation in this experiment (figure 1b). The membrane electrode assembly (MEA) consists of a Nafion 117 membrane (Dupont) with catalyst layers of platinum (BASF, 30% Pt/Vulcan) loading of 1 mg/cm^2 at anode and at cathode. The MEA was made by hot pressing process under optimum conditions. While the gas separator plates were made of the carbon material (Technoquip). The gas separator plate is 5 cm^2 and 1mm. Two steel plates of 6 cm x 6 cm x 1 mm and two acrylic plates of 6 cm x 6 cm x 1 cm were used to complete the fuel cell. The fuel cell was operated at 25 °C.

2.4 Analytical methods.

Samples of the headspace were periodically used to evaluate H_2, CH_4 and metabolic products. H_2 and CH_4 production were measured using a gas chromatograph (Gow Mac 550 series) equipped with a thermal conductivity detector and 18'x1/8'x0.085'' silica-gel 60/80 column (Alltech). The column temperature was 30ºC, the injector and detector temperatures were 75 and 120 ºC, respectively. Nitrogen was used as carrier at a flow of 65 mL/min. Metabolic products (acetic acid, butyric acid, methanol and ethanol) concentrations were measured in a gas chromatograph Buck Scientific, equipped with a flame ionization detector. A 4 - 6.9 feet packed column (Hayesep R 80/100 Mesh, Chromatography Research Supplies, Inc, Louisville, USA) was used for the analysis. Temperatures in the column and injector/detector were 190 and 200ºC, respectively. Nitrogen was used as carrier at a flow of 30 mL/min. Glucose consumption was determined in liquid samples and quantified by using DNS technique [30]. The pH was measured with a potentiometer (Hanna Instrumets HI-8424).

A digital multimeter (microcontroller AVR, ATMEGA8-16PU, and LCD) was used to measurement voltage and current generated by the AR–PEMFC system.

3 RESULTS AND DISCUSSION.

3.1 Effect of pretreatment and incubation temperature.

The seed sludge (SS) was obtained from a methanogenic anaerobic digester feed with OSW for 3 years. In order to enrich the inoculums with H_2 producing bacteria, it was necessary to conduct a pretreatment of the anaerobic sludge for eliminating the methanogens, which use the H_2 to produce CH_4. During the study, CH_4 production was not found in the acid and heat pretreatment systems. Methanogenic activity was observed only in control systems (N35 and N55, figure 2). The incubation temperature showed a significant effect over the CH_4 production in the systems without pretreatment. Higher CH_4 production was observed in systems incubated at 55°C. These results are in agreement with data reported by Mata-Alvarez et al. [31]. According with this authors, the biogas and CH_4

production yields seem more favorable at thermophilic temperature, optimal conditions depend on the type of substrate (biodegradability) and type of system (one/two-phase) used.

Both pretreatments applied on the SS showed positive influence on the H_2 productions and molar yields compared to ones obtained in control systems without a pretreatment (N35, N55).

H_2 production obtained under the different conditions is presented in figure 3. A lag phase was observed for both, APMC and HPMC, this fact is due to the need of the microorganisms of an adaptation period to a new environment when they are transferred from an extremely unfavorable condition (due to the pretreatment) to a favorable one, before consumption of glucose to generate H_2 [32]. The lag phase for the APMC (12 h) was longer than the one obtained for HPMC (4 h), this fact suggests that the H_2 producing microorganisms might recover its biological activity quickly after a heat pretreatment than after an acid pretreatment. Similar effect was found for Valdez-Vazquez et al. [33]; according to these authors after an acid pretreatment an acclimatization time of the inoculum is required.

The enhanced H_2 production obtained with the HPMC could be related to the fact that heat allows the survival of spore-forming microorganisms [8]. It is well known, that in a heat pretreatment a higher amount of spores forming H_2 producing fermentative microorganisms, like *Clostridium,* are recovered in the consortium. The spores are resistant to heat and cannot be inactivated easily even by harsh conditions [7]. Microorganism from *Clostridium* genera showed higher H_2 molar yields [18,34-37] compared to other H_2 producers as *Enterobacter* or *Bacillus* strains [38,39] depending on the culture conditions.

With respect to incubation temperature, the mesophilic temperature at 35 ºC with both, HPMC (H35) and APMC (A35), reached higher H_2 volume productions (289 and 221 mL, respectively) and H_2 molar yields (2.85 and 2.24 mol H_2/mol $_{glucose}$, respectively) compared to the systems incubated at 55 ºC, H55 and A55 (table 2).

Incubation temperature also had significant influence on glucose consumption (figure 4). In systems incubated at 35 °C the total glucose consumption was observed between 24 and 32 h of culture. Meanwhile, in the systems incubated at 55 °C, 99% glucose consumption was attained in 32 and 58 h of cultivation, respectively.

In table 2 the final H_2 volume produced, CH_4 production, H_2 molar yields and H_2 production rate obtained under the different conditions are presented. The HPMC incubated at 35 ºC (H35) showed the highest H_2 volume production (289 mL), production rate (0.014 L/h) and yield (2.85 mol H_2 / mol $_{glucose}$). The results obtained in the present work are in agreement with the data reported by other authors. Iyer et al. [40], Van Ginkel et al. [41] and Mu et al. [7] found H_2 molar yields for an HPMC of 1.8, 2.5 and 2 mol H_2 / mol $_{glucose}$, respectively. Fang and Liu [4], Lin et al. [42] and Mu et al. [7] reported H_2 molar yields for an APMC of 2.1, 3.5 and 1.3 mol H_2 / mol $_{glucose}$, respectively.

3.2 Effect of substrate concentration.

The IGC is an important factor that determines the economic and technical feasibility of the fermentative hydrogen production [2,16]. Glucose concentration plays an important role on the yield and production rate of hydrogen [43]; a low IGC results in the low rate of the fermentation steps, and the fermentation time increases as the initial substrate concentration increases [19].

H_2 production obtained under the different glucose concentrations is presented in figure 5. Once again, a lag phase was observed, this fact confirm the need of those microorganisms to be adapted after the pretreatment, before consumption of glucose to generate H_2 [32]. The maximum H_2 volume (461 mL) was accomplished using 15 g/L of IGC (figure 5); this corresponds to a H_2 molar yield of 3.1 mol H_2/mol $_{glucose}$. Nevertheless, the highest molar yield (3.6 mol H_2/mol $_{glucose}$) was obtained with an IGC of 10 g/L, at this concentration an H_2 volume of 365 mL was produced (figure 5).

With an IGC of 20 and 30 g/L the H_2 production was inhibited and the molar yield decrease to 1.4 and 1.3 mol H_2 / mol $_{glucose}$ (table 3). The low conversion efficiency suggests that the glucose concentration was in excess and the excess may have been converted to alcohols and butyric acid rather than hydrogen [13]. Nevertheless, Pan et al. [18] found the highest yield (4.16 L H_2/ L $_{media}$) with an IGC of 22 g/L using sewage sludge as inoculum.

In table 3 the final H_2 volume produced, H_2 molar yields and H_2 production rates obtained under the different conditions are presented; at a relatively low IGC, 3 and 5 g/L, the H_2 production rates were low (0.008 and .015 L/h, respectively); a similar effect was observed at high glucose concentrations, at 20 and 30 g/L the H_2 production rates were 0.005 and 0.006 g/L, respectively; at high glucose concentrations, the low H_2 production rate indicates that the carbon flux goes to the production of reduced by products such as ethanol and organic acids [44].With IGC's of 7, 10 and 15 g/L the H_2 production rates were very similar, 0.021, 0.023 and 0.024 L/h, respectively. Wu and Lin [45] observed a similar trend between the substrate concentration and the H_2 production rate, in this study the highest production rate was found at 4% (m/v) of molasses concentration using a mixed culture.

The results obtained in the present work are in agreement with the data reported by other authors. Nath et al. [17] found an H_2 molar yield of 3.31 mol H_2 / mol $_{glucose}$ with an IGC of 10 g/L using *Enterobacter cloacae DM11*. Jianzheng et al. [15] reported an H_2 yield of 82.4 mL H_2 / g $_{glucose}$ with an IGC of 10.1 g/L using sewage sludge as inoculum.

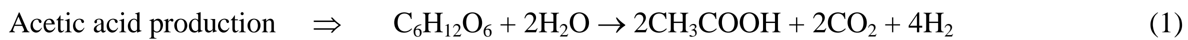
Figure 6 show the glucose consumption at different initial concentrations; a complete consumption (99%) was determined for IGC's of 3, 5, 7, 10 and 15 g/L; glucose concentrations of 3 and 5 g/L were consumed before 18 h of culture; higher glucose concentrations of 7, 10 and 15 g/L were assimilated around 24 h of culture; whereas, 20 and 30 g/L of glucose concentrations were slowly and incompletely consumed by the HPMC, after 58 h of culture 94 and 79% of the initial glucose was

transformed, respectively. This data suggest that an excessive glucose concentration cause a microbial activity inhibition.

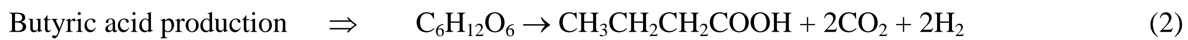
It has been reported that substrate inhibition gets predominant at higher glucose concentration because this modifies the metabolic pathways [5].

3.3 Metabolic Pathways.

Pretreatment, incubation temperature and IGC also have an effect over the maximum specific growth, substrate utilization rate and the metabolic pathway of the microorganisms in the system, resulting in a shift of by product compositions [13,46]. There are mainly four fermentation types in the anaerobic H_2 production using glucose as substrate, namely acetic acid fermentation, propionic acid type fermentation, butyric acid type fermentation and ethanol type fermentation [15,47]. In general, production of acetic and butyric acid favors the production of H_2, according to the next equations [47]:

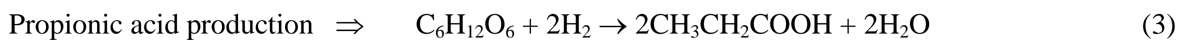


Acetic acid production $\Rightarrow$ $$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

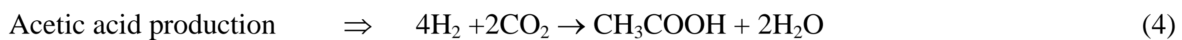


Butyric acid production $\Rightarrow$ $$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2)$$

Nevertheless, acetic acid and propionic acid can be produced by H_2 consumers [47] such as bacteria of the *Acetobacterium* and *Clostridium* genera [48,49]:



Propionic acid production $\Rightarrow$ $$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (3)$$



Acetic acid production $\Rightarrow$ $$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad (4)$$

In the case of ethanol production the H_2 balance is zero, no H_2 is consumed or produced [47]:

Ethanol production $\Rightarrow$ $$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (5)$$

Metabolic products generation was followed throughout all the experiments in the gas phase. The concentrations of the main metabolic products measured at each experiment are presented in figures 7 and 9. Acetic acid and butyric acid were determined during the process; ethanol and methanol were also detected. The distributions of volatile fatty acids and alcohols were different in all cases, resulting in various fermentative types (figure 10).

In pretreatment testing, methanol production was almost at the same levels for acid and heat pretreatments (0.42 – 0.49 mg/L), whereas in the control systems this compound was consumed by methanogenic microorganisms to CH_4 generation after 32 h of culture (figure 7c), according to the next equation [48,49]:

Methane production $\Rightarrow$ $3CH_3OH + 6H^+ \rightarrow 3CH_4 + 3H_2O$ (6)

The concentrations of acetic acid were similar (around 1 mg/L) for the acid pretreated system at both incubation temperatures (figure 7a). The maximum butyric acid concentration (0.53 mg/L) was observed in A35 system (figure 7b), suggesting butyric fermentation (figure 10, eq.2). Meanwhile, ethanol production at 55°C shown a lag phase around 12 h (figure 7d) and the highest production was 1.87 mg/L, suggesting alcoholic fermentation (figure 10, eq.5).

In the systems inoculated with HPMC, the highest acetic acid production (1.56 mg/L) was observed in the system incubated at 35°C (figure 7a); this suggests that the favored pathway is acetate fermentation (figure 10, eq.1). The concentrations of butyric acid were similar (around 0.4 mg/L) in both incubation temperatures (figure 7b). The alcoholic fermentation was favored at 55 °C obtaining 1.22 mg/L (figure 7d). in the case of methanol a lag phase of around 12 h was observed in the system H35 (figure 7c).

In control system incubated at 55°C metabolic products were lower than the obtained in the other systems since these were consumed for methanogens to CH_4 generation (figure 7).

The differences in all the systems might be attributed to the diversity of microbial population in the inoculum after enrichment. Iyer et al. [40] identified *Clostridium acetobutylicum* as the major bacterial population in heat treated anaerobic sludge. Furthermore, Fang et al. [50] found mixed type fermentation with enriched mixed cultures by acid pretreatment, closely related to species in the genera *Citrobacter*, *Clostridium*, or *Klebsiella.*

Figure 8 show the effect of incubation temperature and pretreatment on metabolic products generation. The acid pretreatment favors the alcoholic fermentation at both incubation temperatures. While, heat pretreatment favors the acetic acid fermentation at an incubation temperature of 35°C.

With respect to the effect of IGC, at 10 g/L the acetic acid concentration (1.84 g/L) was more than 5 times higher than other metabolites in the same system (figure 9), this suggest that the favored pathway is acetate fermentation (figure 10, eq.1) and also explain the high molar yield obtained in the system (3.6 mol $H_2/mol_{glucose}$).

On the other hand, with an IGC of 20 and 30 g/L the butyric acid (0.81 and 0.94 g/L, respectively), methanol (0.87 and 1.07 g/L, respectively) and ethanol (0.83 and 1.09 g/L, respectively) concentrations were higher compared with the other glucose concentrations evaluated (figure 9). This suggest that the possibility of produce some acid, as butyric acid (figure 10, eq.2), and alcohols (figure 10, eq.5) increase with high IGC [44,51]. Data reported by Chin et al. [52] are in agreement with the results obtained in the present study; the authors showed that an increase in butyrate concentration corresponds with a significant and steady decrease in specific hydrogen production rate.

Higher substrate concentration may quickly become inhibitory through pH depletion, acid production, or increased hydrogen partial pressure [17]; similar effects were reported by other authors [13,14].

3.4 Electricity generation.

Anaerobic fermentation is known to be the most promising way of H_2 production, thereby feasible for continuous electricity generation [21]. The use of biohydrogen for electricity generation can be possible via PEMFC or microbial fuel cell. The advantage of microbial fuel cell is that a natural biological material could be used in a special fuel cell without having to first convert it to H_2, but there are still many problems to overcome, related to the poor ability of the biological substances to conduct, i.e., to transfer electrons to the external electrodes [53]. The advantages of PEMFC are many [53,54]: they are also smaller in volume and lighter in weight, which make them perfect for automotive and portable applications; they are modular and can be installed at nearly the same price per kW at any size; they can work with H_2 generated by any process; they operate at relatively low temperatures, which makes them easier to contain and reduces thermal losses.

Levin et al. [2] designed a continuous stirred anaerobic bioreactor with an H_2 production capacity of 3 L/h to generate 5W of electricity via the PEMFC (using a stack) by calculating the minimum amount of H_2 required powering the PEMFC. According with these authors if a biohydrogen system can deliver enough H_2 to power a PEMFC for 24 h for at least one year, then the biohydrogen system could have a truly useful and potentially commercial application.

In order to know if the H_2 production rate was enough to electricity production experiments at laboratory level were carried out with an AR of 1.6 L of operation volume. Since a stable and constant supply of H_2 is extremely important for a successful biohydrogen-fuel cell electricity generation system, the persistence and stability of an AR for long term operation was assessed operated on a semi-continuous mode at 35°C and 150 rpm, inoculated with an HPMC, media replaced periodically (around 15 days) and an IGC of 10 g/L feeding once between 48 and 72 h. Similar results were reported by Lin et al. [21]; in order to match the required H_2 producing performance to generate electricity, this authors carried out tests with a continuous stirred anaerobic

bioreactor conducted at an hydraulic retention time of 6 h with an influent sucrose concentration of 30 g COD/l, obtaining a molar yield of 3.71 ± 0.18 mol H_2/mol $_{sucrose}$ and an H_2 content in biogas of 40.6 ± 1.4 %.

After start-up, a lag fase was observed for around 5 days (figure 11). The AR began to produce biogas with an H_2 content of over 17 %; nevertheless, the highest H_2 content observed was 45 %. CH_4 was not found. Glucose conversion was around 99%, indicating an efficient utilization of the carbon substrate, giving the highest molar H_2 yield of 3.4 mol H_2/mol $_{glucose}$. The highest H_2 production rate was 0.71 L/h. The main metabolic product was acetic acid. As in previously testes presented in this paper, the predominant acetic acid production suggests that AR system carried out metabolic pathways in favor of H_2 production. After 70 days of operation, the H_2 content was maintained stable, 43 ± 2 %, at a pH of 5.5 ± 0.5 (controlled during all the process).

The H_2 produced in the AR was used to generate electricity whit a PEMFC. Since the H_2 content in the gas effluent was around 43%, H_2 purity needs to be increased before introducing it to the PEMFC. The biogas was introduced into a CO_2 absorber for H_2 purification and then was entering to the fuel cell at a flow rate of 0.6 L/h.

Theoretically, the maximum cell voltage (E_{max}) is the voltage that would result if all the fuel's energy were transformed into electrical energy:

$$E_{max} = -\frac{\Delta\overline{h}_f}{zF} \qquad (7)$$

Where, F is the Faraday constant, 96.485 Coulombs (C); for H_2 fuel, $\Delta\overline{h}_f = -285.85$ kJ/mol and z=2 [2]. Therefore, E_{max} = 1.48 V. According to Larminie and Dicks [55] and Levin et al. [2] the voltage of a fuel cell is around 0.7 V. In the circuit the output voltage, power and current were 1.03 V, 0.1W (at 25 ◦C) and 68 mA, respectively. This data indicates that the lab scale AR–PEMFC system was very stable and has the potential for commercial application in a small power system. The electricity

generated was used to turn on a small motor, which requires 1 V to work, it can works for a long term.

Water generation from the electrochemical reaction in the fuel cell might decrease power density of the fuel cell [21]. However, no serious problems of water generation were observed, this is due to the scale of the PEMFC was very small.

As the AR system operated for more than 90 days giving a stable H_2 production, the AR–PEMFC system shows potential for semi-continuous and electricity generation (table 5), nevertheless, the results found in this research are lower than other reported in AR-PEMFC systems [2,21], this is due to the fact of the experiments in this papers were carried out with racks and not with single fuel cell. Nevertheless, the cell efficiency was around 70%, it's higher than the reported, around 50% [2,21]. For practical applications the H_2 purification method and operating conditions of the fuel cells may need to be improved to attain better PEMFC system efficiency.

4 CONCLUSIONS

The results obtained in this study demonstrate that heat and acid pretreatments were effective for eliminating methanogenic microorganisms and enriching H_2 producing inoculums from anaerobic sludge. Nevertheless, the highest H_2 yield, 2.85 mol H_2/mol $_{glucose}$, was achieved with the HPMC at an incubation temperature of 35°C. The acid pretreatment favors alcoholic and butyric fermentation, whereas heat pretreatment favors acetic acid production during the H_2 production process. On the other hand, a thermophilic temperature of incubation (55ºC) promotes the alcoholic fermentation process.

The highest H_2 molar yield of 3.6 mol H_2/mol glucose was achieved with an IGC of 10 g/L, while the lowest yield of 1.3 mol H_2/mol glucose was obtained with an IGC of 30 g/L. High IGC's favor

alcoholic and butyric fermentation, whereas 10 g/L of IGC favors acetic acid production as intermediate during the H_2 production process which allows a higher H_2 production.

The results suggest that predominant genera were *Clostridium,* nevertheless more experiments and molecular studies, are necessary to elucidate this point.

A combination of AR-PEMFC systems could generate electricity with an output power of 0.1 W and a voltage and current of 1.03 V and 0.068 A, respectively. This work clearly shows that with simple H_2 purification step, hydrogen produced from a dark fermentation process can be used to power a PEMFC system for on-line electricity generation.

However, to reduce the cost of H_2 production, a cheaper and more sustainable substrate (OSW) should be used as the feedstock for biohydrogen production.

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FIGURE CAPTIONS.

FIGURE 1 a) A schematic description of anaerobic bioreactor and its integration with PEMFC. 1, AR; 2, temperature control; 3, mixed control; 4, NaOH solution; 5, air pump; 6, distillate water; 7, PEMFC; 8, motor; 9, valve; 10, flow meter. b) PEMFC used.

FIGURE 2 CH_4 production obtained in microcosms systems using two different pretreatments (heat, H, and acid, A) for inocula obtention and two incubation temperatures (35 and 55ºC). H35 (◆) and H55 (■) inoculated with the HPMC and incubated at 35 and 55ºC, respectively. A35 (▲) and A55 (×) inoculated with the APMC and incubated at 35 and 55ºC, respectively. Systems without a pretreatment N35 (✳) and N55 (●) incubated at 35 and 55ºC, respectively, used as controls.

FIGURE 3 H_2 production obtained in microcosms systems using two different pretreatments (heat, H, and acid, A) for inocula obtention and two incubation temperatures (35 and 55ºC). H35 (◆) and H55 (■) inoculated with the HPMC and incubated at 35 and 55ºC, respectively. A35 (▲) and A55 (×) inoculated with the APMC and incubated at 35 and 55ºC, respectively. Systems without a pretreatment N35 (✳) and N55 (●) incubated at 35 and 55ºC, respectively, used as controls.

FIGURE 4 Glucose consumption obtained in microcosms systems using two different pretreatment (heat, T, and acid, A) for inocula production and two temperatures of incubation (35 and 55ºC). H35 (◆) and H55 (■) HPMC incubated at 35 and 55ºC, respectively. A35 (▲) and A55 (×) APMC incubated at 35 and 55ºC, respectively. Systems without a pretreatment N35 (✳) and N55 (●) incubated at 35 and 55ºC, respectively, used as controls.

FIGURE 5 H_2 production obtained in microcosms systems at increasing IGC. 3 g/L (◆), 5 g/L (×), 7 g/L (▲), 10 g/L (✳), 15 g/L (●), 20 g/L (■) and 30 g/L (▬).

FIGURE 6 Glucose consumption obtained in microcosms systems at increasing IGC. 3 g/L (◆), 5 g/L (×), 7 g/L (▲), 10 g/L (✳), 15 g/L (●), 20 g/L (■) and 30 g/L (▬)

FIGURE 7 The main metabolic products obtained in microcosms systems using two different pretreatments (heat, H, and acid, A) for inocula obtention and two incubation temperatures (35 and 55ºC). H35 and H55 inoculated with the HPMC and incubated at 35 and 55ºC, respectively. A35 and A55 inoculated with the APMC and incubated at 35 and 55ºC, respectively. Systems without a pretreatment N35and N55 incubated at 35 and 55ºC, respectively, used as controls. a) Acetic acid; b) Butyric acid; c) Methanol; d) Ethanol.

FIGURE 8 Effect of incubation temperature and pretreatment on metabolic products generation. AP, acid pretreatment; HP, heat pretreatment; NP, no pretreatment (controls); AA, acetic acid; BA, butyric acid; ME, methanol; ET, ethanol.

FIGURE 9 The main metabolic products obtained at different IGC: a) Acetic acid; b) Butyric acid; c) Methanol; d) Ethanol.

FIGURE 10 Flow chart showing the different metabolic pathways of glucose (Antonopoulou, et al., 2008).

FIGURE 11 H_2 production in anaerobic reactor using an IGC of 10 g/L, inoculated with HPMC, incubated at 35°C and pH controlled at 5.5 ± 0.5.

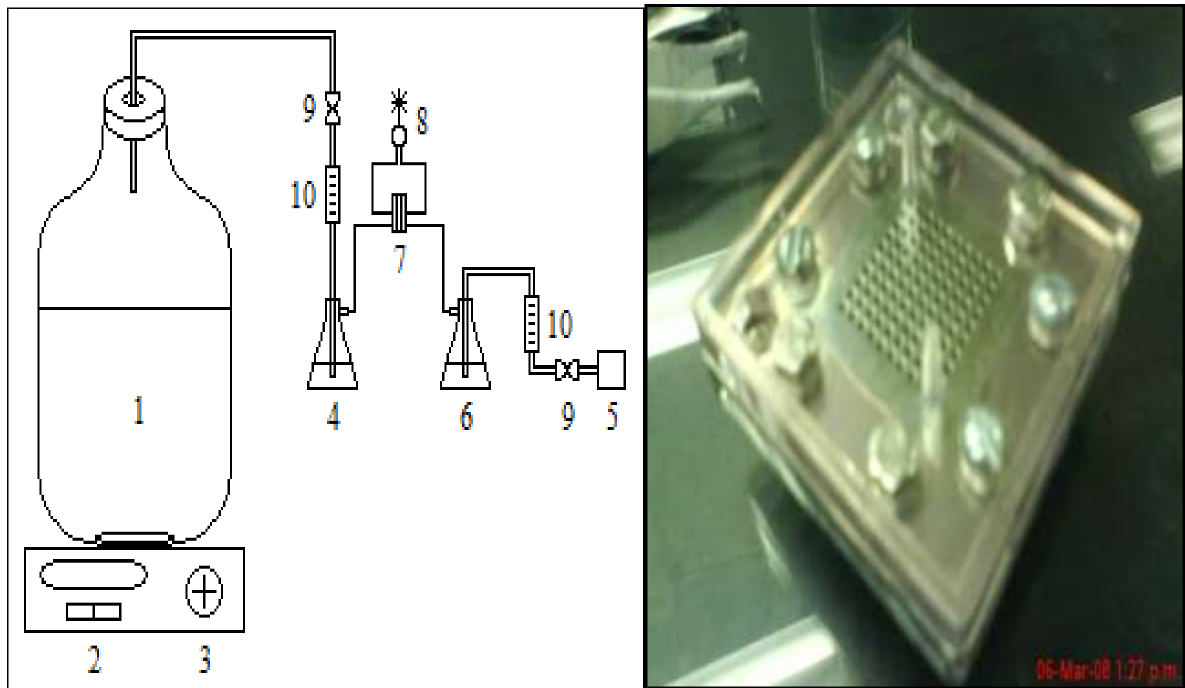
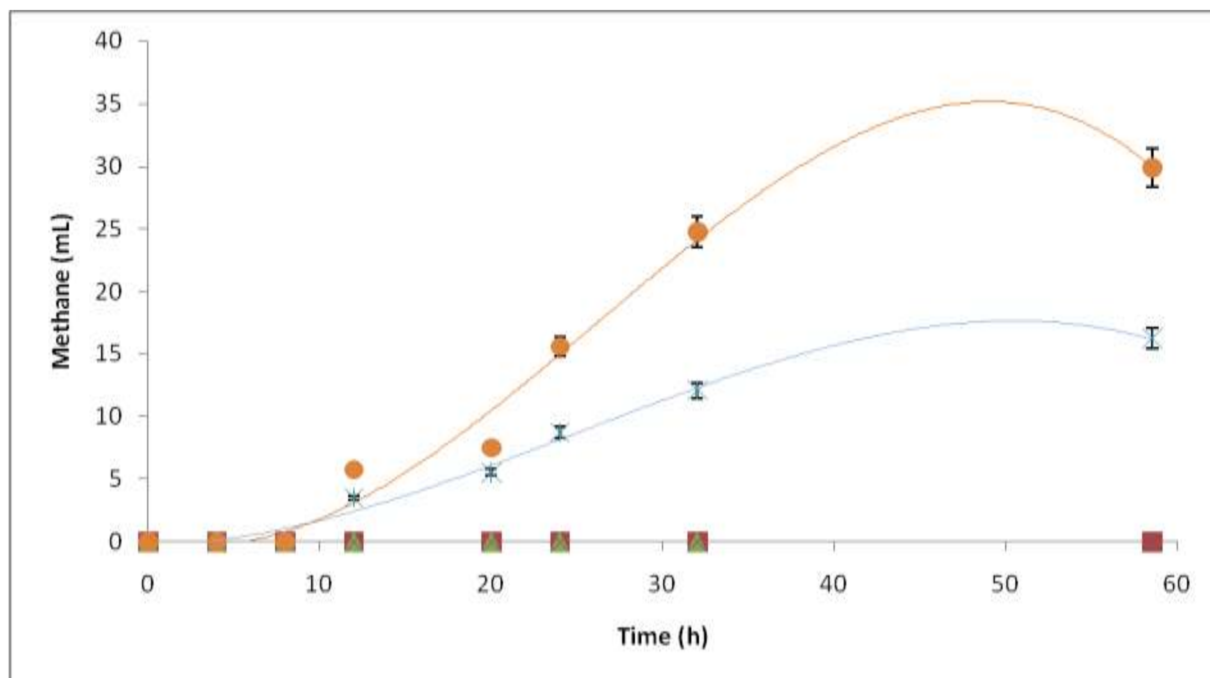


FIGURE 1.



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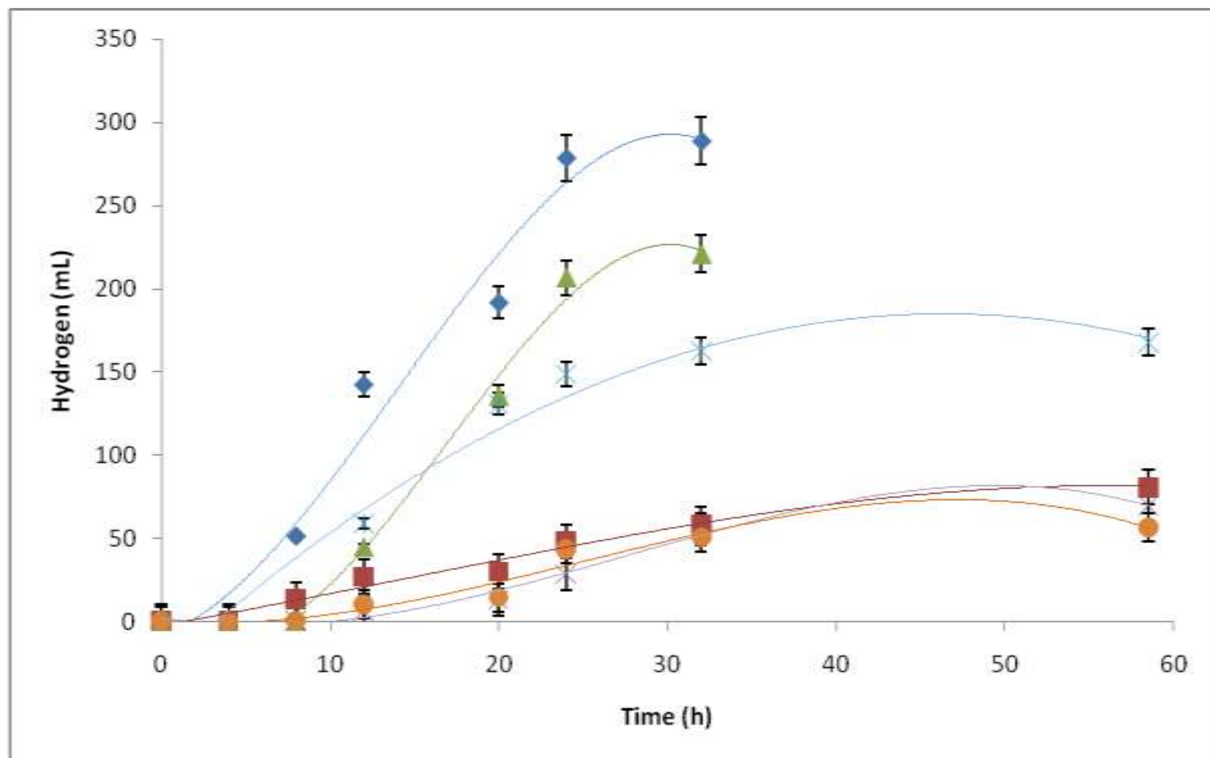


FIGURE 3.

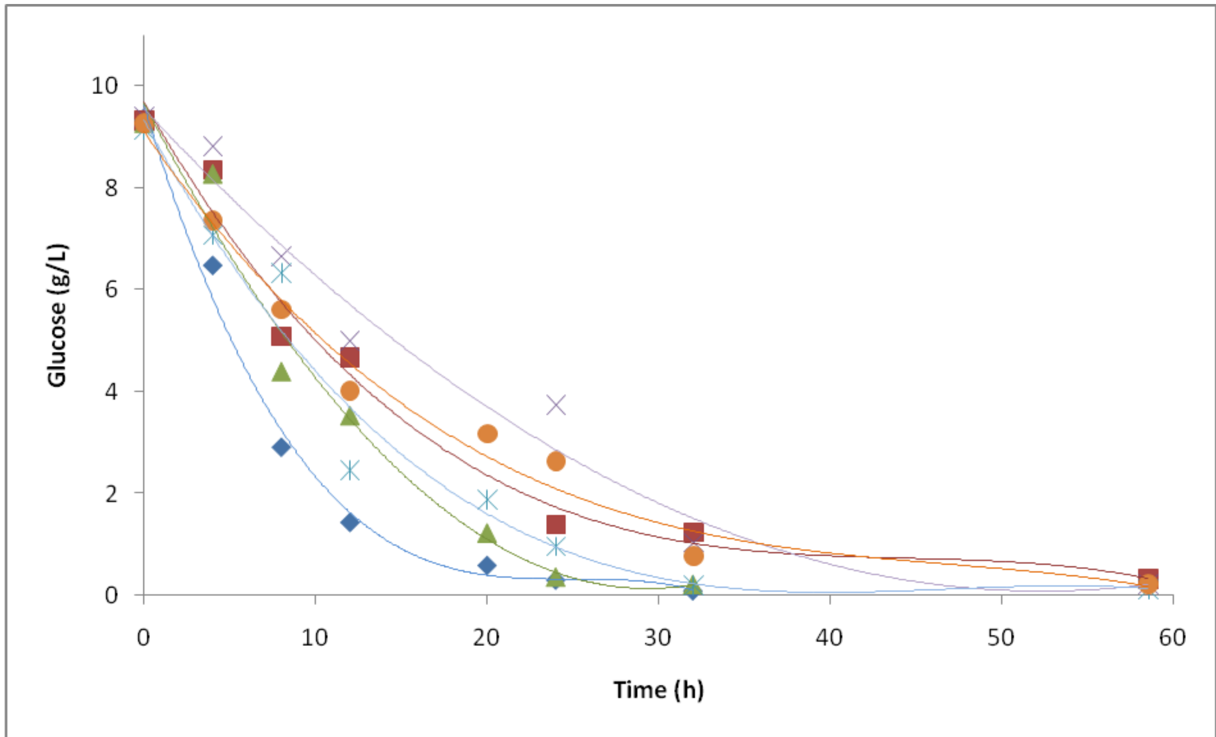


FIGURE 4.

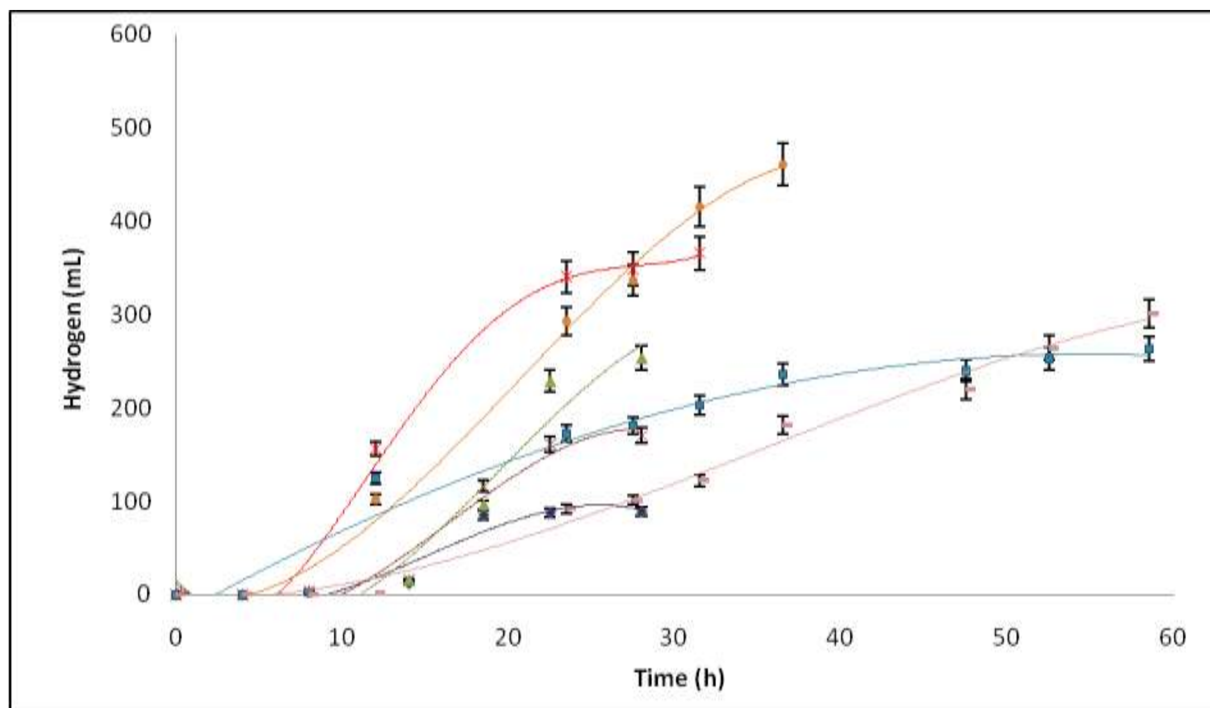
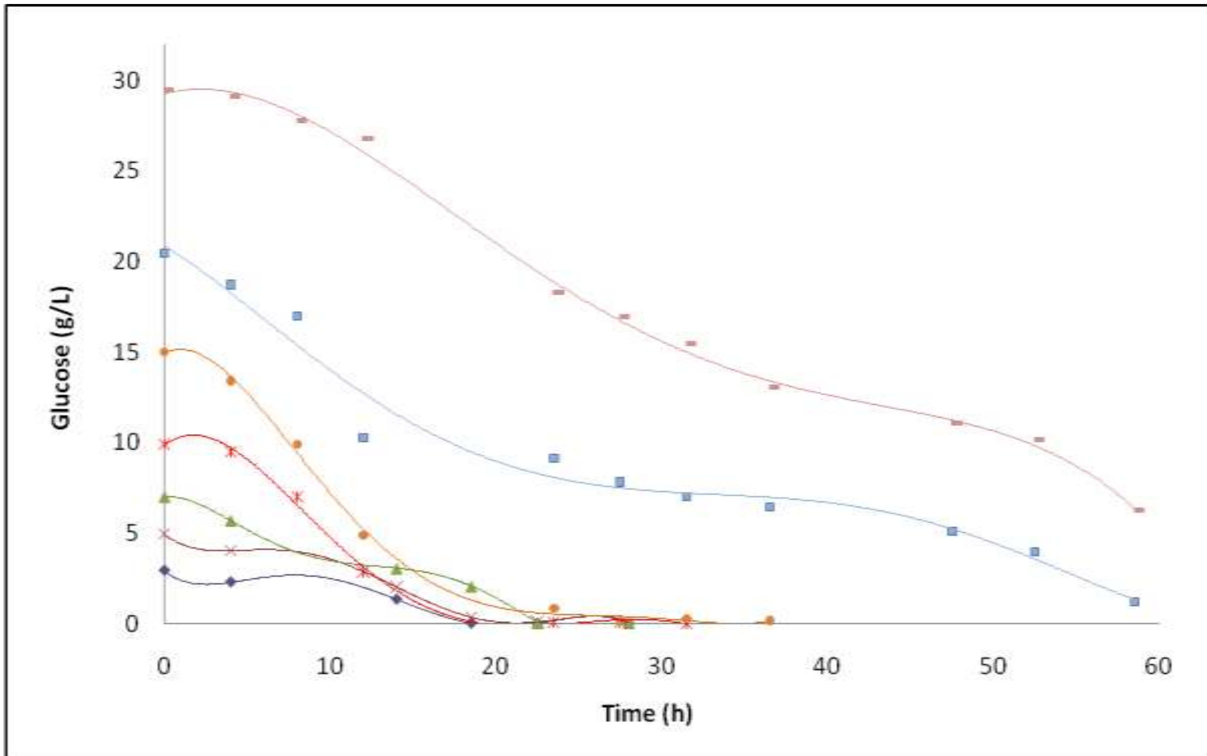


FIGURE 5.



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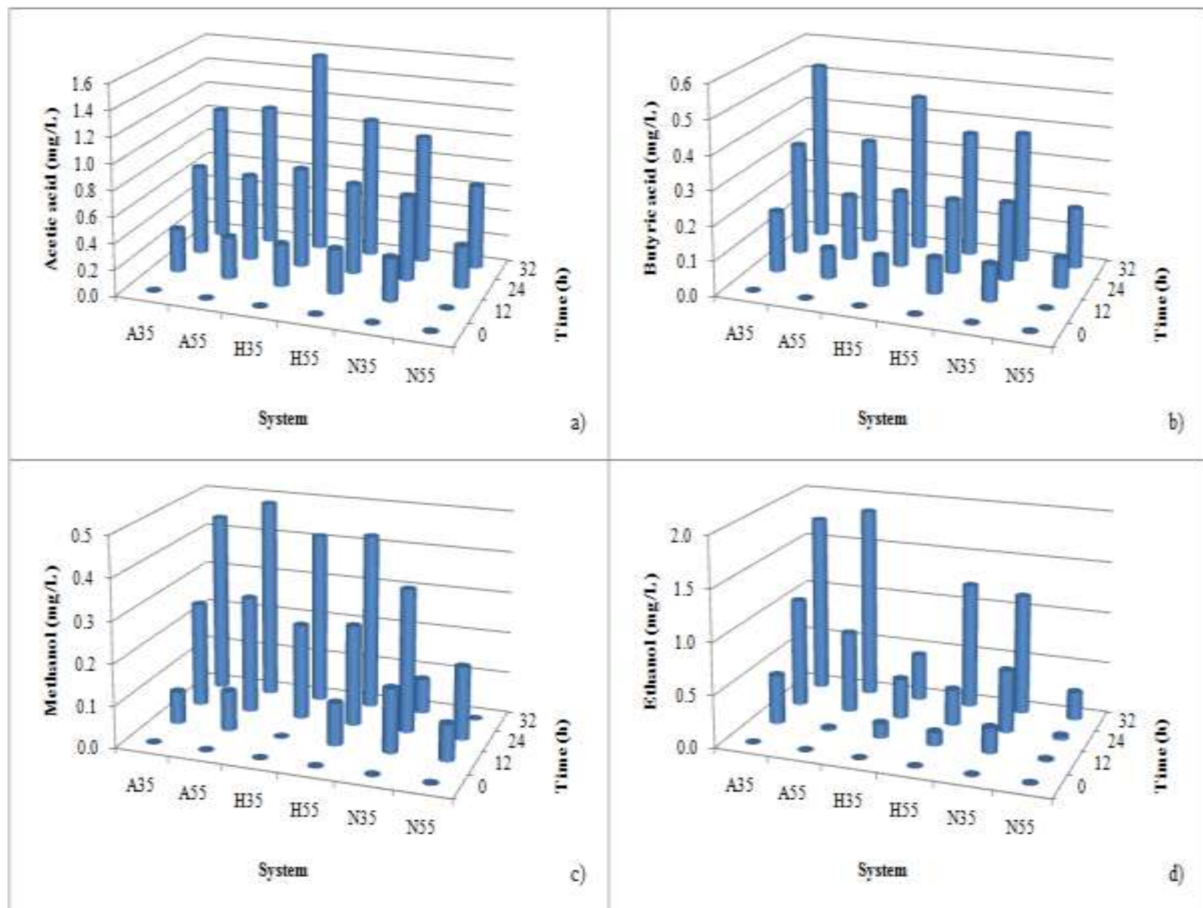

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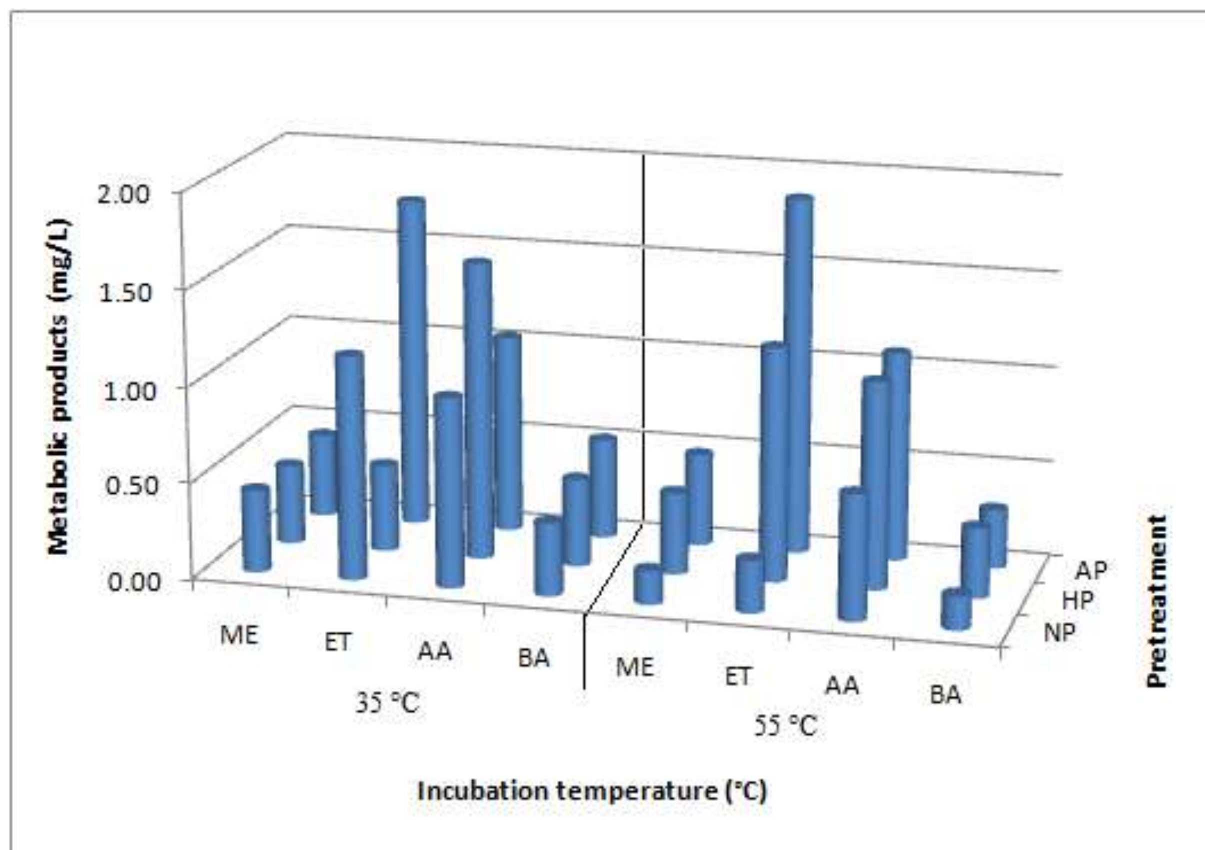

FIGURE 8.

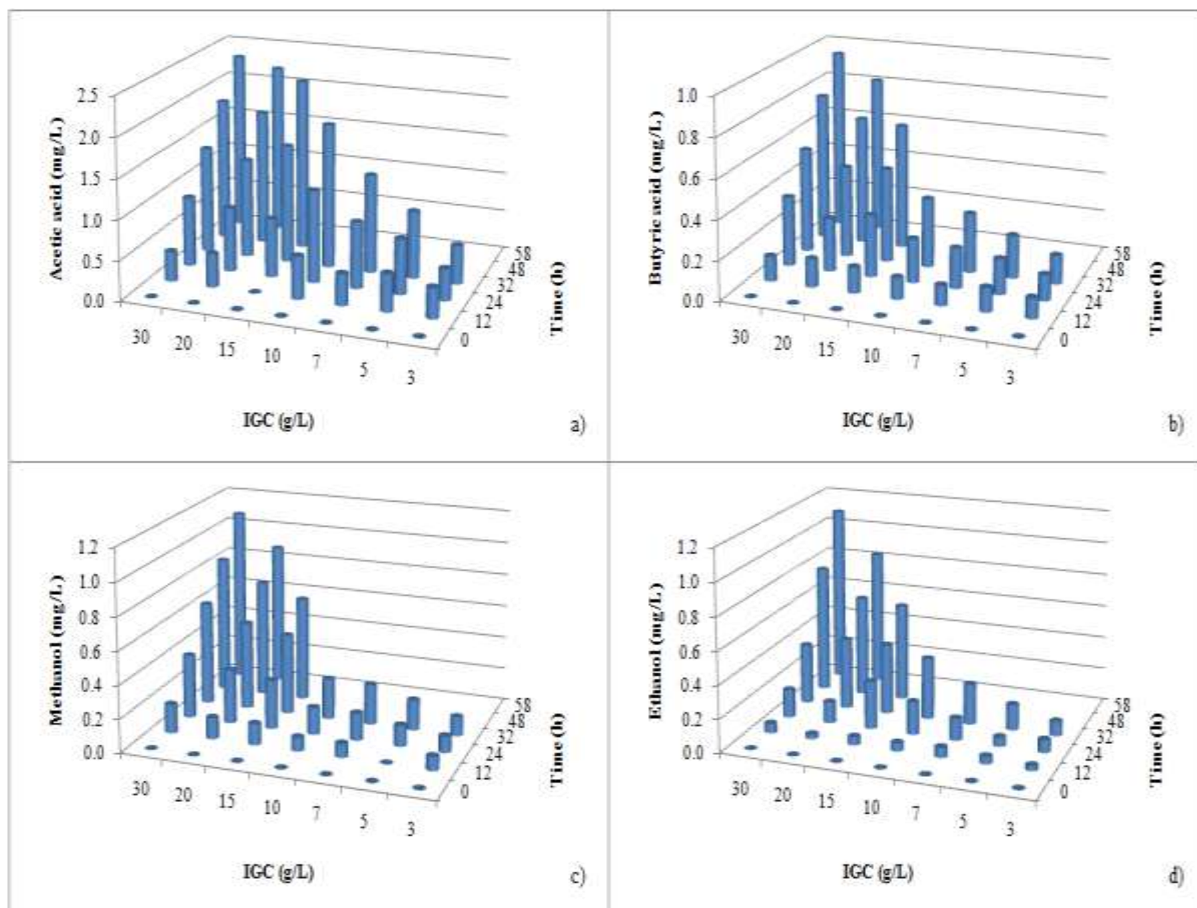

FIGURE 9.

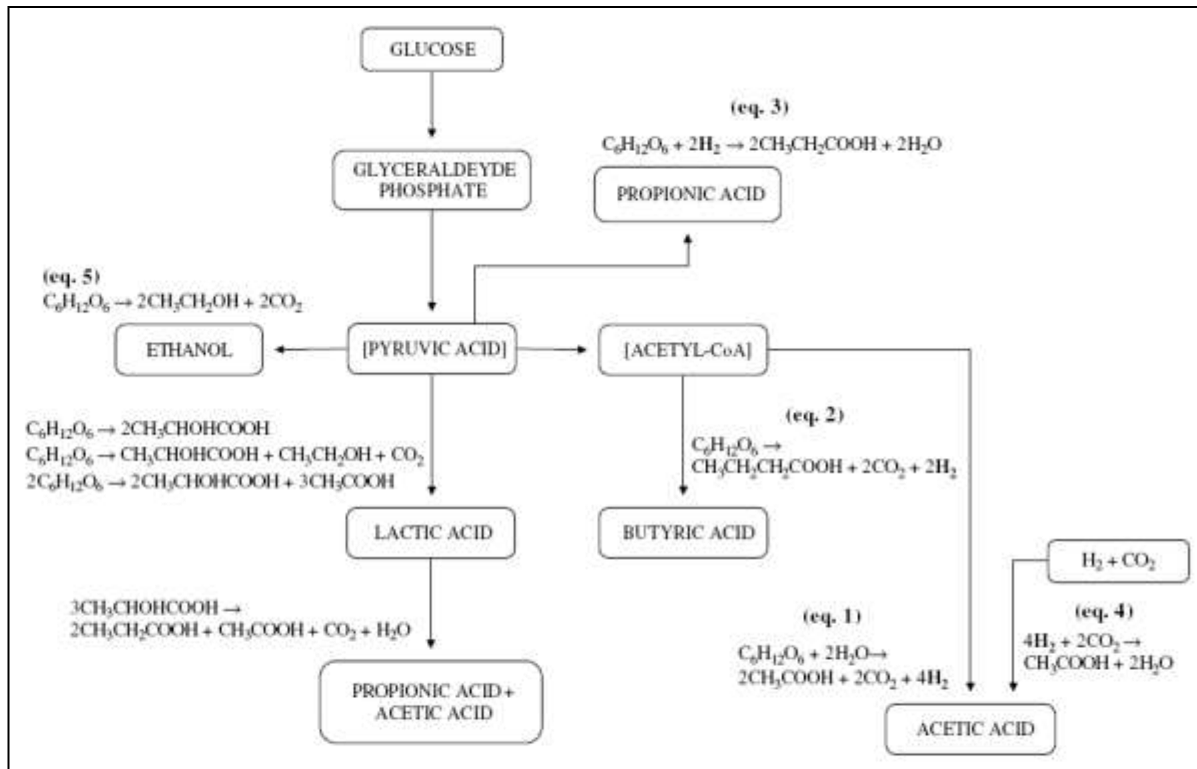

FIGURE 10.

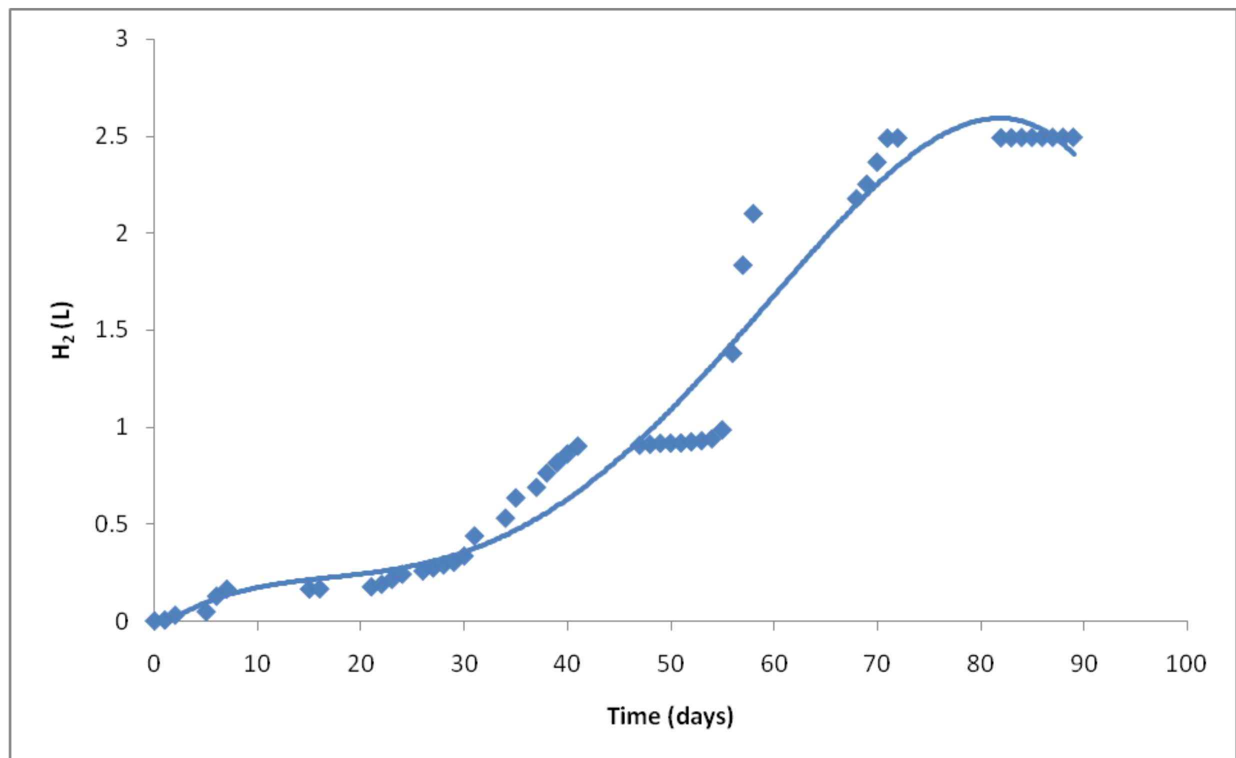

FIGURE 11.

TABLE CAPTIONS.

TABLE 1 Experimental design used to evaluate the effect of pretreatment and incubation temperature.

TABLE 2 H_2 volume produced, CH_4 production, H_2 molar yields and H_2 production rate obtained under the different conditions evaluated.

TABLE 3 H_2 volume produced, H_2 molar yields and H_2 production rate obtained under the different conditions evaluated.

TABLE 4 Performance of anaerobic fermentation (glucose concentration = 10 g/L).

TABLE 5 Results obtained in the integration of AR and PEMFC for electricity generation.

TABLE 1.

Condition	Incubation temperature (°C)	System
Heat Pretreatment	35	H35
	55	H55
Acid Pretreatment	35	A35
	55	A55
No pretreatment	35	N35
	55	N55

TABLE 2.

Pretreatment	**H_2 (mL)**	**CH_4 (mL)**	**H_2 Yield [a] (mol/mol$_{glucose}$)**	**H_2 Production Rate (L/h)**
H35	289	0	2.85	0.014
H55	81	0	0.82	0.001
A35	221	0	2.24	0.013
A55	70	0	0.70	0.001
N35	168	16	1.71	0.007
N55	56	30	0.57	0.001

[a] H_2 yield = the amount of H_2 formed (mol) ÷ the amount of glucose consumed (mol).

TABLE 3.

Glucose concentration (g/L)	**H_2 (mL)**	**H_2 yield [a] (mol/mol$_{glucose}$)**	**H_2 production rate (L/h)**
3	89	3.0	0.008
5	174	3.4	0.015
7	245	3.5	0.021
10	365	3.6	0.023
15	461	3.1	0.024
20	263	1.4	0.005
30	301	1.3	0.006

[a] H_2 yield = (the amount (mol) of H_2 formed) ÷ (the amount (mol) of glucose consumed).

TABLE 4.

pH	**H_2 content (%)**	**H_2 Yield [a] (mol/mol$_{glucose}$)**	**H_2 Production Rate (L/h)**	**Glucose conversion [b] (%)**
5.5 ± 0.5	43 ± 2	3.2 ± 0.2	0.69 ± 0.02	98 ± 1

[a] H_2 yield = the amount of H_2 formed (mol) ÷ the amount of glucose consumed (mol).

[b] Glucose conversion = the amount of glucose utilized (g) ÷ the amount of glucose fed (g).

TABLE 5.

Parameter	Values	
	This research	**Lin et al., 2007**
H_2 feeding rate (L/h)	0.6	1.72 ± 0.07
Output power (W)	0.1	0.87
Output voltage (V)	1.03	2.28
Output current (A)	0.068	0.38
Efficiency (%)	70	50