BIOHYDROGEN PRODUCTION FROM DIARY PROCESSING WASTEWATER BY ANAEROBIC BIOFILM REACTORS.

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Abstract

This article describes biological hydrogen production from diary wastewater via anaerobic fermentation using pretreated heat shock (100°C, 30 min.) and acid (pH 3.0, 24 h) treatment procedures to selectively enrich the hydrogen producing mixed consortia prior to inoculation to batch reactors. Bioreactor used for immobilization consortia was operated at mesophilic (room) temperature (20±3°C), under acidophilic conditions (pH 4.0-4.5), HRT (2h), and a natural support for generate hydrogen producing mixed consortia biofilm: *Opuntia imbricata*. Reactor was initially operated with sorbitol (5g/L) for 60 days of operation. Batch tests were conducted using 20±0.02g of natural support with biofilm. Batch experiments were conducted to investigate the effect of COD (2.9-21.1 g-COD/L), at initial pH of 7.0, 32±1°C. Maximum hydrogen yield was obtained at 21.1 g-COD/L. Experiments of pH effect were conducted using the optimal substrate concentration (21.2 g-COD/L), at pH 4 to 7 and 11.32 (pH diary wastewater) ,and 32±1°C. Experiments results indicate the optimum initial cultivation was pH 4.0, but we can consider also a stable hydrogen production at pH 11.32 (pH diary wastewater), so we can avoid to fit the pH, and use diary wastewater as it left the process of cheese manufacture. The operational pH of 4.0 is 1.5 units below that of previously reported hydrogen producing organisms. The influence of the effect of temperature were conducted using the optimal substrate concentration (21.2 g-COD/L), two pH levels: 4.0 and 11.32, and four different temperatures: 16±3°C (room temperature), 32±1°C, 45±1°C y 55±1°C.Optimal temperature for hydrogen production from diary wastewater at pH 4.0 was 55±1°C, and for pH 11.32 was 16±3°C.Therefore, the results suggests biofilm reactors in a natural support like *Opuntia imbricata* have good potential to be employed for hydrogen production.

Key Words: Biohydrogen, diary wastewater, Opuntia imbricata, biofilm.

1.- Introduction.

At the present, 90% of the world´s energy requirements are fulfilled by fossil fuels, which are regarded as an endless and cheap source(1)Their indiscriminate use has caused global problems, such as the greenhouse effect, ozone layer depletion , acid rain and pollution (2).Cleaner fuels and more effective energy conversion technologies are in greater demand due to local and global environment protection measures(3), there are many candidates , such as synthetic gasoline, synthetic natural gas (methane), methanol, ethanol, and hydrogen(2).Approximately 95% of the hydrogen produced today comes from carbonaceous raw material, primarily fossil in origin. Only a fraction of this hydrogen is used for energy purposes; the bulk serves as a chemical feedstock for petrochemical, food, electronics, and metallurgical processing industries. (4) Recent reviews on hydrogen indicated that the worldwide need on it is increasing with a growth rate nearly 10% per year for the time being (5) and contribution of hydrogen to total energy market will be 8-10% by 2025 (6).

Much recent interest has been expressed in the biological production of hydrogen using fermentative bacteria. Fermentative hydrogen production has been reported from numerous waste and wastewater sources including paper mill (2), swine manure (7), municipal solid waste (8), rice winery (9), and dairy waste (10). Dairy wastewater is a potential source (11) of many environmental problems, due to there are many of manufacturers produced using traditional methods, so improperly managed diary wastewater can result in severe consequences to the environment, such as malodor, attraction of rodents, insects, other pests and groundwater contamination, so the main affected natural resource is water due to for each liter of milk produced the process consumes two liters of water.

Hydrogen production by sewage sludge is influenced by many factors (12-15) including the type of inoculums, pretreatment, pH, temperature, and wastewater specificity. Several types of pretreatment procedures (heat treatment, chemical treatment, pH treatment, etc) have been reported in literature for a variety of inocula (7, 11).

In this paper, we report experimental data pertaining to batch studies performed on mixed consortia immobilized in a natural support, *Opuntia imbricata* which is an abundant shrub in the northeast region of Mexico and is therefore easily available. Biofilm reactors are viable alternatives for the development of stable microbial populations with great activity and for efficient biodegradation of wastewater when compared to other conventional treatment processes (16, 17). The biofilm behavior depended on the type of support used (1, 17).

2.- Materials and Methods.

2.1 Seed Inoculum.

A granular sludge coming from a mesophilic full-scale UASB reactor treating wastewater from a beer industry was used like inoculum after beat the granular condition, using a manual mixer.

2.1.1 Acid and Heat pretreatments.

Acidic pretreatment involved decreasing the pH of the sludge to 3.0 using 0.1N HCl solution for 24h and readjustment of pH back to 7.0 by 0.1N NaOH solution. After the acidic pretreatment, the inoculum was treated by heat-shocked by heating the sludge at 100°C for 30 minutes, then cool, it was ready for inoculation.

2.2 Support treatment.

Opuntia imbricata trunk was cut into pieces of approximate 1.5 cm×0.5cm×0.5cm dimensions. Five hundred grams of *Opuntia imbricata* pieces were watered with 1L of water, and then the support was washed and dried at 60°C for 24 h. Then the support was watered again for a week and was weighted for the formation of biofilm.

Table 1. Characteristics of media

Parameter	Specifications
Type	*Opuntia imbricata*, fixed type
Producer	Natural support
Dimensions	0.230 g
Density	0.838 g /cm^3
Specific surface are	0.599 m^2/g (from BET)

2.3 H_2 producing mixed consortia in a biofilm.

After inoculation, mixed consortia was subjected to acid environment (pH 4-4.5), using sorbitol as substrate at 5 g/l to restrain the growth of methanogenic bacteria at the same time to selectively enrich the H_2 producing acidogenic bacteria, operating conditions were: 20±3°C, HRT=2h. Immobilized consortia were formed in 60 days and then used to startup the batch reactors.

2.4 Substrate.

Diary wastewater was used as substrate, it was collected from a moderate size commercial milk and cheese factory located in Saltillo, Coahuila, Mexico, was stored at 4^0 C. Characteristics of dairy wastewater used are given in Table 2.

Table 2. Characteristics of dairy wastewater

Parameter	Dairy wastewater
pH	11.32
COD (mg/l)	21140
Conductivity (m/S)	2640
Density	0.838 g /cm^3
TSS (mg/l)	2190

2.5 Influence of initial substrate concentration on H_2 production

Experiments were conducted in 120 ml serum bottles to investigate the effect of concentration of diary wastewater on hydrogen production. Experiments were conducted using wastewater concentration from the range of 2.91 to 21.14 g COD/l. 40 ml of diary wastewater was added to each reactor and 20±0.020 g of support (containing 0.550 ± 0,10 g biomass). Reactors were first purged with helium for 15 min and capped tightly with silicon rubber and aluminum caps to avoid gas leakage form the bottles. Reactors were then placed in an incubator at 32±1^{0}C.

2.6 Influence of initial ph on H_2 production.

Experiments were carried out under the same conditions that point 2.5, using the optimal substrate concentration (21.14) but unless the value of pH was established from 4.0 to 7.0 with increments of 0.5, and 11.32

2.7 Influence of temperature on H_2 production.

Experiments were carried out under the same conditions that point 2.5, using the optimal substrate concentration (21.14), to pH 4.0 and 11.32, and 4 different temperatures: 16±3°C, 35±1°C, 45±1°C, 55±1°C.

2.8 Analytical methods.

Hydrogen and methane content in biogas was measured by a gas chromatography Varian 3400 using a thermal conductivity detector and a AT-Mole Sieve PLOT GC column packed with molecular sieve 5ª using helium as a carrier gas, and a glass syringe of 1-100 µl .Temperatures of injector, detector and column were kept at 50 C, 200 C, 200 C, respectively. At the same time, samples removed from the liquid phase everyday were centrifuged at 10,000 rpm to remove solids from the liquid media and were analyzed for Chemical oxygen demand (COD) using a quick COD microwave sealed digestion measuring system according to Standard Methods (APHA, 1998) and the pH, Conductivity and TSS values were determinate by Standard Methods.

3.-Results and discussion.

Reactor operation with biofilm on *Opuntia imbricata*.

Biofilm configured systems are reported to be well suited for the treatment of wastewater containing poorly degradable compounds (18). In this study two pretreatment techniques were adopted to limit methanogenic group in the mixed cultures. Heat-shock (100°C; 30 min.) and acid (pH-3; 24 h) were applied on anaerobic mixed culture for hydrogen generation. Both techniques had good results in removing non-spore forming methanogenic groups from inoculums. Besides another strategy utilized in this study to obtain a predominant biohydrogen producing microbial population from an anaerobic consortium, consists of cultivate the consortium at mesophilic temperatures, using generally simple substrates (glucose, sucrose, etc), pH in the range of 4-7 (for our study was at 4-4.5) and maintaining short hydraulic retention times (HRT =2 h) (8).

Effect of Initial Concentration on H_2 production.

Experimental results shown in the fig. 1, the production of bio-hydrogen obtained utilizing different concentrations of the diary wastewater at pH of 7, temperature at 32 ± 1°C at different initial COD concentrations: 2.91, 4.91, 8.676, 9.81, 12.176, 13.443, 15.276 y 21.143 g/l, a production accumulated of 433 ml of hydrogen was obtained for COD= 12.176 g/l , thus was enlarging for the greater concentrations to this being produced a maximum value of production accumulated of 2181 ml of hydrogen at COD=21.143 g/l. As well as it can be observed for a better understanding the efficiency of the production of the hydrogen with the analysis of the reduction of the COD among the start and end of the experiments (Table 5).

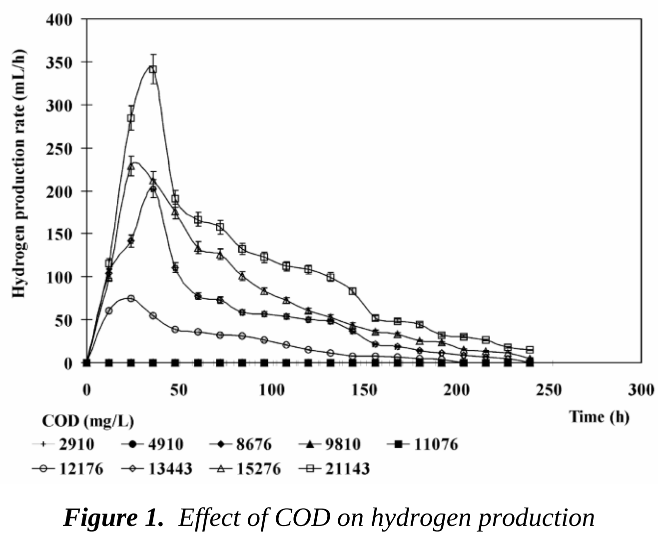

Figure 1. *Effect of COD on hydrogen production*

Methane concentration below detectable limit was observed during the operation, whereas the hydrogen concentration showed a gradual build-up with time. Hence it can be concluded that low activity of methanogenic population in the anaerobic inoculums was due to the persistent acidophilic microenvironment maintained during the reactor operation. Moreover, using mixed microbial cultures is considered to be practical, cost-effective and promising approach to achieve hydrogen production in large scale. The behavior of pH for the influence of the concentration of substrate is shown in the Table 1, it was observed that concentrations of substrate that produced hydrogen, correspond to ph´s reported in the literature; as well as the concentrations of substrate that didn´t produce hydrogen, they were established in a rank of ph of 6-8, being obtained at the same time low concentrations of methane, so due to the optimum pH for methanogenic bacteria is reported between 6.0 and 7.5 (19).

It is evident from the experimental data that, diary wastewater showed relatively high and rapid hydrogen yield (Fig. 2). This might be attributed to sufficient adaptation time for the biofilm with the dairy wastewater and higher concentration of substrate, which obviously showed higher availability of substrate, resulting in active substrate metabolism leading to an early and higher yield.

Table 3.
Hydrogen production at various substrate concentrations during 240 h experiments.

Substrate concentration	pH		COD reduction effficiency (%)	H_2 yield (mM/g COD)
mg COD/L	Initial	Final		
2910	6.935	6	46.98	0.000
4910	6.935	6	51.61	0.000
8676	6.857	6	65.31	0.000
9810	6.908	6	62.18	0.000
10676	6.936	6	56.62	0.000
11076	6.946	6	58.08	0.000
12176	6.959	5	63.24	2.304
13443	6.999	5	67.45	4.962
15276	7.009	5	69.61	5.976
21143	7.015	5	73.63	5.735

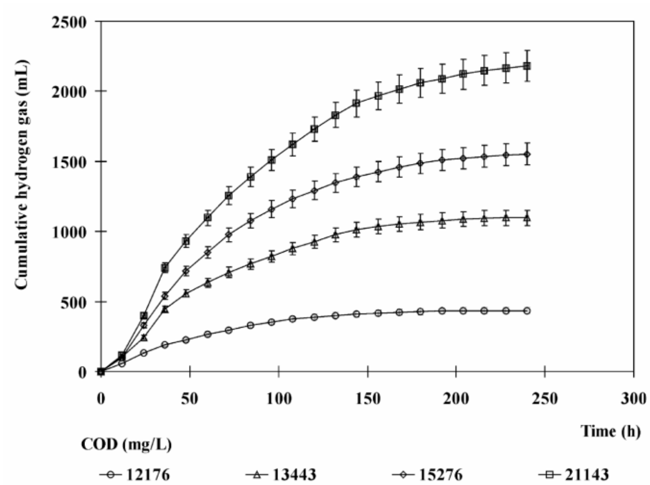

Figure 2. *Effect of COD on cumulative hydrogen production.*

Effect of initial pH on Hydrogen production.

Optimum concentration of COD=21.1 g/l was utilized to carry out the study on the influence of the initial pH, at temperature of 32±1°C, being taken as minimum value a pH of 4.0, being increased in intervals of 0.5, to a pH maximum of 7.0.Results obtained in the fig. 3 of production of hydrogen (ml) shows us that the greater accumulation of hydrogen produced was to a pH of 5.0, but this production decays rapidly and to determine the pH optimum we can observe on fig 4, pH of 4.0 produced greater accumulated hydrogen production.

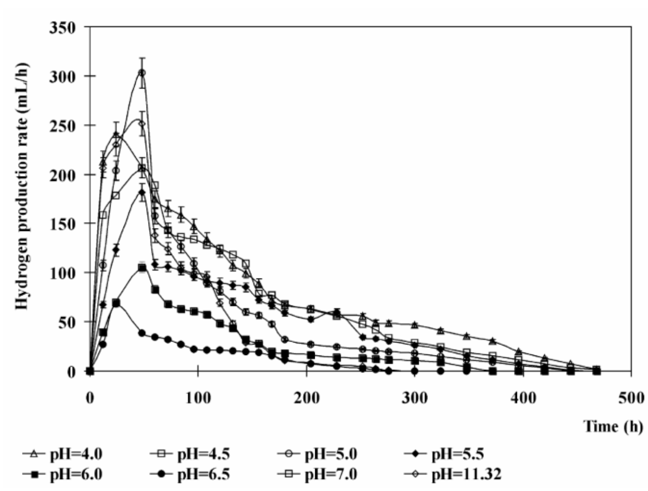

Figure 3. *Effect of pH on hydrogen production*.

In the fig. 4 can also be observed for the pH in which diary wastewater was collected from cheese factory (11,32) ,can be obtained a stable production accumulated of hydrogen, taking into account for feasibility this diary wastewater generated a stable production of hydrogen. During the first 12 hours pH drop was considered to be favorable microenvironment for effective h2 yield by inhibiting the methanogenic group of bacteria. pH drop was considered to be the congenial pH range for the functioning of acidogenic bacteria and at the same time for inhibiting the activity of metanogenic consortia. Higher pH drop resulted in rapid h2 production (Table 4),

Table 4
Hydrogen production of microbial community at various initial cultivation pH values during 468 h fermentation

pH		COD (mg/L)		COD reduction effficiency (%)	H_2 yield (mM/g COD)
Initial	Final	Initial	Final		
pH=11.32	5.19	22560	10827	52.01	3.616
pH=4.0	4.37	22560	8443	62.58	5.833
pH=4.5	4.48	22560	8735	61.28	5.526
pH=5.0	4.51	22560	9043	59.92	4.403
pH=5.5	4.52	22560	8835	60.84	4.305
pH=6.0	4.54	22560	9377	58.44	2.235
pH=6.5	4.53	22560	9360	58.51	0.933
pH=7.0	4.53	22560	9454	58.09	0.407

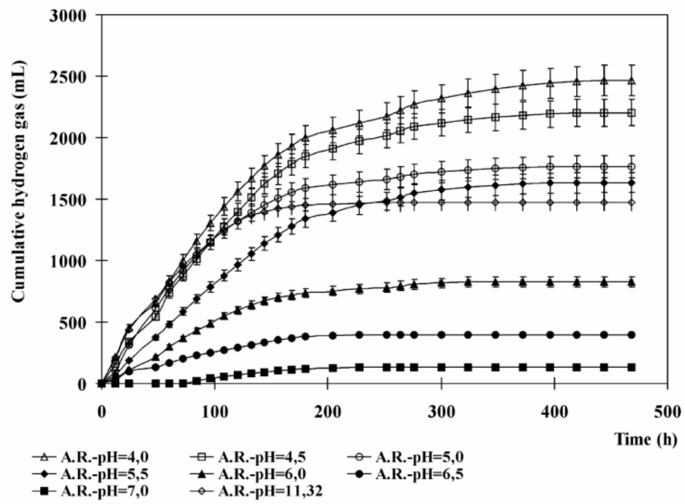

Figure 4. *Effect of pH on cumulative hydrogen production.*

Effect of temperature on H_2 production.

As presented in figs. 5, 6 we studied the effect of temperature on biohydrogen production at pH l1.32 and 4.0, so we can observe a considerable effect on the cumulative hydrogen production and content. An examination of fig. 5 for the pH level of 11.32 indicates that a 24-h lag of hydrogen production occurred at 35±1°C, and a 72-h lag of hydrogen production occurred at 16±3°C, 45±1°C, 55±1°C, these differences in the lag phase suggested that the microorganisms need a longer time to adapted; also in fig. 7, 8 it was observed the cumulative hydrogen production.

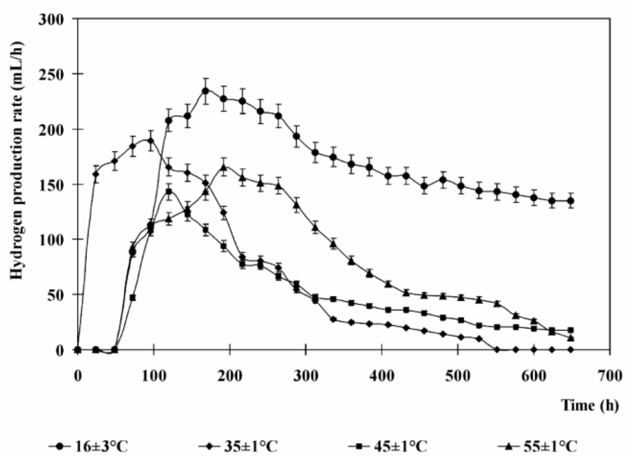

Figure 5. *Effect of temperature on hydrogen production using diary wastewater pH 11.32*

As shown in fig. 6 for the ph level of 4.0 indicates that a 96-h lag of hydrogen production occurred at 35±1°C and 55±1°C, and a 168-h lag of hydrogen production occurred at 45±1°C; and there was no hydrogen production at temperature of 16±3°C, in comparison to the other temperatures at ph of 4.0 were the most cumulative hydrogen production (Table 5); it could be due because the diary wastewater wasn´t homogeneous because it had segregated particles when the pH was adjusted. Optimal temperature for the best hydrogen production was found 55±1°C at pH of 4.0 (Fig. 6), an optimal temperature for the best hydrogen production at pH of 11.32 was found 16±3°C (Fig. 5).

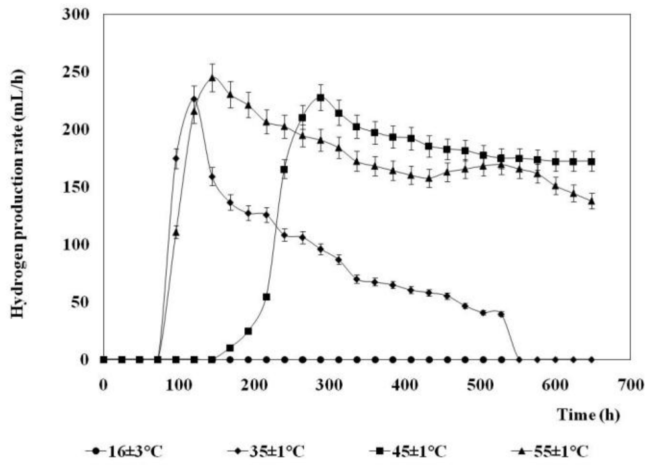

***Figure 6**. Effect of temperature on hydrogen production using diary wastewater pH 4.0*

Higher pH drop resulted in rapid h2 production, as noted pH drop from 11.32 to 5.48, 5.10 and 5.49 for 16±3°C, 45±1°C and 55±1°C respectively was in 72 h, therefore hydrogen production began; for 35±1°C takes 48 h to produce hydrogen and the pH was 5.07; after hydrogen production began the variation in pH was observed in a narrow range indicating a stable system.

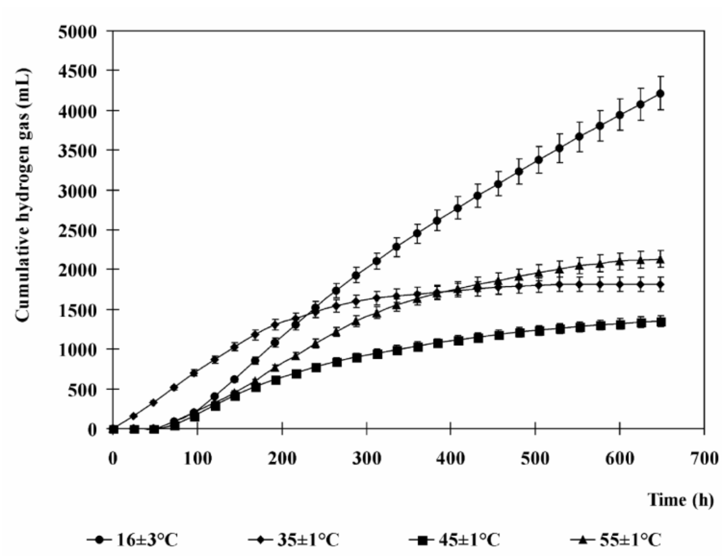

***Figure 7**. Effect of temperature on cumulative hydrogen production using wastewater pH 11.32*

Also pH variation from 4.0 to 4.189, 4.385 for 35±1°C, and 55±1°C respectively was in 96 h, therefore hydrogen production began; but for 45±1°C takes 168 h to produce hydrogen and the pH was 4.142; after hydrogen production began the variation in pH was observed in a narrow range indicating a stable system. the influence of the temperature in the accumulated production of hydrogen is observed in figures 7 and 8.

Table 5

Hydrogen production of microbial community at various temperature during 648 h fermentation

Temperature °C	pH		COD (mg/L)		COD reduction effficiency (%)	H_2 yield (mM/g COD)
	Initial	Final	Initial	Final		
16±3°C	4.0	4.029	22.9	13.04	43.06	0
16±3°C	11.3	4.977	22.9	11.57	49.48	12.731
35±1°C	4.0	4.267	22.9	8.18	64.28	5.138
35±1°C	11.3	4.993	22.9	12.04	47.42	5.583
45±1°C	4.0	4.047	22.9	9.24	59.65	7.767
45±1°C	11.3	5.033	22.9	12.21	46.68	4.822
55±1°C	4.0	3.940	22.9	9.98	56.42	11.047
55±1°C	11.3	5.350	22.9	12.51	45.37	7.894

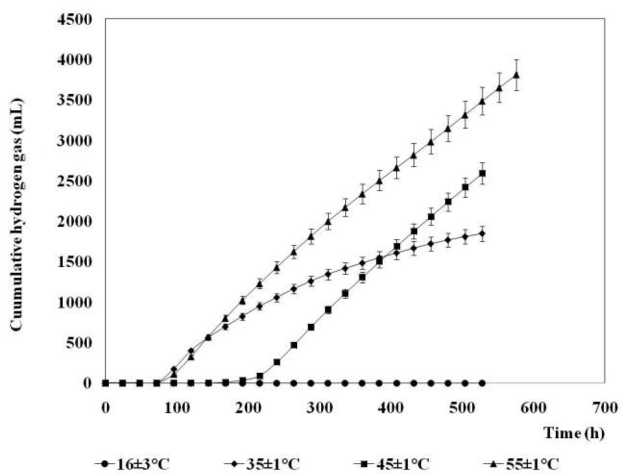

Figure 8. *Effect of temperature on cumulative hydrogen production using wastewater pH 4.0.*

4.-Conclusions

A good composition of environmental factors is extremely useful in starting-up anaerobic hydrogen-producing process. In this study, initial concentration of substrate, pH and temperature levels were selected as target factors for enriching anaerobic hydrogen-producing microorganisms from heat-shocked sludge and acidhopilic pretreatments using diary wastewater as sustrate. Batch experiments, in duplicate, were performed to assess the influence and optimize factors on the biofilm generating hydrogen.

The results indicated that:

1.-The production of biohydrogen showed no methanogenesis (or lower level) throughout the study indicates that the acidic and heat-shocked pretreatments, also the biofilm growth in a high hydraulic retention times were effective to produce hydrogen-producing microorganisms.

2.-The optimal initial concentration of diary wastewater was determinate in 21.1 g COD/L, and pH of 4.0 was optimal for hydrogen production, also for practical effects we studied the hydrogen production at pH 11.32. The influence of the temperature studies were made in the two levels of initial pH: 4.0 and 11.32, at the following temperatures: 35±1°C, 45±1°C, 55±1°C; the optimal temperature for pH 4.0 was 55±1°C, this operational pH is 1.5 units below that of previously reported hydrogen producing organisms; so for 16±3°C, the optimal was pH 11.32 .

3.-The results obtained confirmed that at higher hydrogen conversion was resulted from a higher COD reduction.

4.- The results obtained for hydrogen production by means of biofilms developed in *Opuntia imbricata* using diary wastewater were effective, efficient and economical way to obtain hydrogen. The use of *Opuntia imbricata* as support was favorable for hydrogen production, besides is an abundant natural support.

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