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On the optimization of fermentative hydrogen production from waste glycerol: effect of pH, substrate concentration and concentration of different cations in the medium on yields and distribution of metabolites

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Abstract:*Hydrogen is a clean, effective and renewable fuel which can be produced by different methods including biological ones, namely fermentation and biophotolysis. To improve fermentative hydrogen production the strategies, implicating use of by-products, utilization of carbon containing organic wastes and optimization of biotechnology process conditions, are developed. Glycerol, a biodiesel by-product, can serve as a cheap carbon containing source to produce hydrogen. Recent data on metabolic pathways, responsible hydrogenases and dependence of hydrogen production on external factors during glycerol fermentation are summarized. The strains are constructed to enhance hydrogen yield. In this study, hydrogen production from waste glycerol via dark fermentation was conducted by using mixed culture in both batch and continuous reactor. Furthermore, experiments have shown the best parameters and conditions about both the pH value, the effect buffer solution (Na or K) and the substrate concentration for the highest hydrogen production and metabolic products.*

Keywords:*hydrogen production, dark fermentation, anaerobic sludge, waste glycerol, pH, substrate concentration, optimal conditions.*

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I. INTRODUCTION

Hydrogen is known to be a good alternative renewable fuel in replacing the fossil fuels in future for the reason that it can offer a clean and sustainable environment; its combustion only produce water as by-product (Abdeshahian et al., 2014). The advantage of using hydrogen as fuel depends on the type of primary energy source used for its production (Salemme et al. 2014). Currently, most hydrogen is produced from non-renewable sources, such as oil, natural gas and coal. Hydrogen can also be produced from renewable sources, such as biomass, which makes these processes a promising avenue for the hydrogen production as an environmentally friendly fuel (Chaubey et al. 2013).

Biologically, hydrogen can be produced using biophotolysis, photo-fermentation, dark fermentation, or combination of these ways (Abo-hashesh et al., 2011). Although both chemical and biological processes have got their own advantages and disadvantages, biological approach is more economical and clean as chemical conversion releases greenhouse gases (Abdeshahian et al., 2014). The reason is why there is a forming CO_x, NO_x, SO_x, C_xH_y compounds, ash, and other organic compounds which have adverse effect to environment (Azwar et al., 2014 ; Sargsyan et al. 2016). Dark fermentation is reported as the best method because the production can occur continuously without the need for the light and is considered as the simplest process (Azwar et al., 2014 ; Wongthanate et al. 2015). The dark fermentation process begins with bacterial hydrolysis of the fed organic materials in order to break down insoluble polymers, making them available for microorganism consumption. Acidogenic bacteria then convert the products of hydrolysis into carbon dioxide, hydrogen, ammonia, alcohols and organic acids.

Furthermore, this method offers a high rate production of hydrogen by a variety of available cheap feedstocks and wastes (e.g: crude glycerol, biomass, animal waste, etc) (Azwar et al., 2014 ; Trchounian et al., 2015), a variety of fermentative anaerobic bacteria that have high growth rates (Abo-hashesh et al., 2011), low energy requirement, and produce valuable by-product such as volatile fatty acids and alcohols (Lee et al., 2014).Industrial crude glycerol was once mentioned as waste due to its great abundance which leads to its descending price and high purification cost However, crude glycerol can offer many industrial beneficial products such as hydrogen, 1.3-propanediol etc and thus it can also be used as a cheap substrate to substitute glucose in the fermentation processes (Dounavis, 2015a ; Dounavis et al., 2015b ; Dounavis et al., 2016).

Specifically, Dounavis et al. 2015b has mentioned that the yield in hydrogen production from crude glycerol (101 ± 5.0 mg CODprod./g COD of consumed glycerol) was very satisfactory in relation to other studies in the literature.

The aim of this study is to compare the hydrogen production via dark fermentation using crude glycerol in both batch and continuous reactors from the same mixed culture of microorganisms which has developed in the UFCB (Dounavis et al., 2015b). What is more, experiments have been studied in order to optimize the basical parameters in the hydrogen production of the reactor such as the pH value, the effect buffer solution and the substrate concentration in the feed.

II. EXPERIMENTAL PROCEDUREON BATCH REACTORS

2.1 Effect on substrate concentration (Experiment A)

The arrangement of the batch reactors was simple with a volume of 1.23 L and active volume 0.55 L with high resistance to high temperature and high pressure (Picture 1 (1)). During the experiment, the reactor was mounted on a magnetic stirrer so that the growing anareobic conditions were ensured in an incubator which was operating steadily at 35º C. In addition, the reactor provided its capability liquid and gaseous sampling.

Specifically, during the experiments, 50 mL from the reactor H_2-UFCB (Dounavis et al., 2015b) operating at a hydraulic retention time of 24, 36 and 48 h at steady state, were mixed with the substrate concentration (waste glycerol) of 10, 15, 20 and 25 g/L and buffer solyution consisting of K_2HPO_4 and KH_2PO_4, so that the pH was within the desired levels (6, 6.5 and 7 respectively). In addition, a trace element solution, yeast extract and $FeSO_4$ $7H_2O$ solution were extracted .

2.2 Determination of optimal conditions: effect of the buffer solution K and Na (Experiment B)

In addition, experiments were performed on batch reactors with volume 160 mL and active volume 50 mL (Picture 1 (2)) and the effect of sodium and potassium buffer was studied as a function of initial concentration of the substrate in the production of metabolic products, where five different initial glycerol concentrations were used: 10, 15, 20, 25 and 30 g/L.The start-up of the batch reactor was carried out with 5 mL from the reactor H_2-UFCB (Dounavis et al., 2015b) and a trace element solution, yeast extract and $FeSO_4$ $7H_2O$ solution were extracted .

Two different phosphate buffers solutions were tested: a) $(Na_2HPO_4)2H_2O$ and $(NaH_2PO_4)2H_2O$ with 41.6 and 72.6 g/L respectively and b) K_2HPO_4 και KH_2PO_4 with 37.4 and 86.8 g/L respectively. The pH value was 6 in both cases. During the experiment, the reactor was mounted on a magnetic stirrer so that the growing anareobic conditions were ensured in an incubator which was operating steadily at 35º C. In addition, the reactor provided its capability liquid and gaseous sampling.

2.3 Determination of optimal conditions: effect of the initial substrate concentration (10 and 20 g/L), on dark fermentation process, at different initial pH conditions (Experiment C)

What is more, experiments were performed on batch reactors with volume 160 mL and active volume 50 mL (Picture 1 (2)) and the effect of the initial substrate concentration (10 and 20 g/L), on dark fermentation process, at different initial pH conditions and in each case the pH value was 6, 6.5 and 7 respectively. The start-up of the batch reactor was carried out with 5 mL from the reactor H_2-UFCB (Dounavis et al., 2015b) and a trace element solution, yeast extract and $FeSO_4$ $7H_2O$ solution were extracted . During the experiment, the reactor was mounted on a magnetic stirrer so that the growing anareobic conditions were ensured in an incubator which was operating steadily at 35º C. In addition, the reactor provided its capability liquid and gaseous sampling.

Picture 1. Batch reactors: (1) for the 2.1, (2) for the 2.2 and 2.3 respectively

III. RESULTS AND DISCUSSIONS

3.1. Comparison yields for experiments in batch reactor for each time period (Experiment A)

Table 1 presents the Chemical Oxygen Demand (COD) about the main metabolic product (1.3-propanediol, ethanol, hydrogen and volatile fatty acids produced (vfas)) compared with the COD the consumed glycerol in all batch experiments were carried out. The stoichiometries production of metabolites is expressed in $gCOD_{product}/gCOD_{consumed\ glycerol}$, and the sum is compared with the unit price.

As seen from the values in Table A, it is apparent that the sum of the measured COD in the reactor product was approximately equal to or less than the COD of the consumed glycerol in all experiments, which indicates that identified all possible products produced by the degradation of glycerol. The hydrogen production was ranged from 0.035-0.068 gCOD in all experiments and was quite low.

Table 1. Yields of metabolic products

Experiments	Yields of metabolic products, $gCOD_{product}/gCOD_{consumed\ glycerol}$					
	H_2	SCFAs	Ethanol	1.3-propanediol	Biomass	sum
Batch from Steady State A	0.035	0.060	0.195	0.365	0.078	0.733
Batch from Steady State B	0.040	0.059	0.262	0.715	0.063	1.139
Batch from Steady State C	0.055	0.062	0.245	0.472	0.066	0.900
Batch from Steady State D	0.039	0.076	0.295	0.326	0.044	0.780
Batch from Steady State E	0.068	0.053	0.524	0.424	0.080	1.149
Batch from Steady State F	0.039	0.125	0.263	0.736	0.077	1.240
Batch from Steady State G	0.051	0.069	0.291	0.287	0.048	0.746
Batch from Steady State H	0.042	0.092	0.150	0.630	0.036	0.950

3.2. Experiments batch to determine the optimal conditions

The experiments in batch reactors were designed to draw conclusions as to the optimum operating conditions, in terms of substrate concentration. Specifically, the buffer function of the initial charge of substrate and the initial substrate concentration in the fermentation procedure were studied, at various initial pH conditions and the production of high value products. The results were used as a reference for the operating conditions in the experiments of the fermentative hydrogen production.

3.3. Effect buffer solution (Na and K) in the production of metabolic products (Experiment B)

The results of experiments were carried out in batch reactors (flasks - serum bottles), volume 160 mL and active volume 50 mL and five different initial concentrations of glycerol were used: 10, 15, 20, 25 and 30 g/L. In these experiments were observed production of hydrogen, volatile fatty acids, 1.3-propanediol and ethanol. The results are shown in the following figures.

Figure 1 shows the hydrogen production per Liter of culture, for the two cases with the different buffers. As shown, the productivity of hydrogen systems, for both phosphate solutions, was approximately 1000 mL / culture L. Notably, no significant differences in the production of hydrogen for the different initial organic loadings.

However, observing the performance of reactors in hydrogen yield(Figure 2), reactors with an initial organic loading 10 g/L showed higher yield for both buffers solutions. Specifically, the reactor was operated with a buffer solution of Na presented yield 0.53 mol H_2/mol consumed glycerol and the corresponding buffer

solution of K was yield 0.52 mol H_2/mol consumed glycerol, for an initial concentration of glycerol 10 g/L. On the other original organic loadings, the yield rates were presented significantly reduced, with rates ranging from 0.20 up to 0.35 mol H_2/mol glycerol. Finally, the solution of Na showed slightly higher yields of hydrogen, particularly in systems with starting loadings of organic 25 and 30 g/L. Nevertheless, the differences in yields between the two buffers solutions are not particularly significant.

Figure 2. Hydrogen production (mL/L culture) in phosphate Na buffer solution and in phosphate K buffer solution

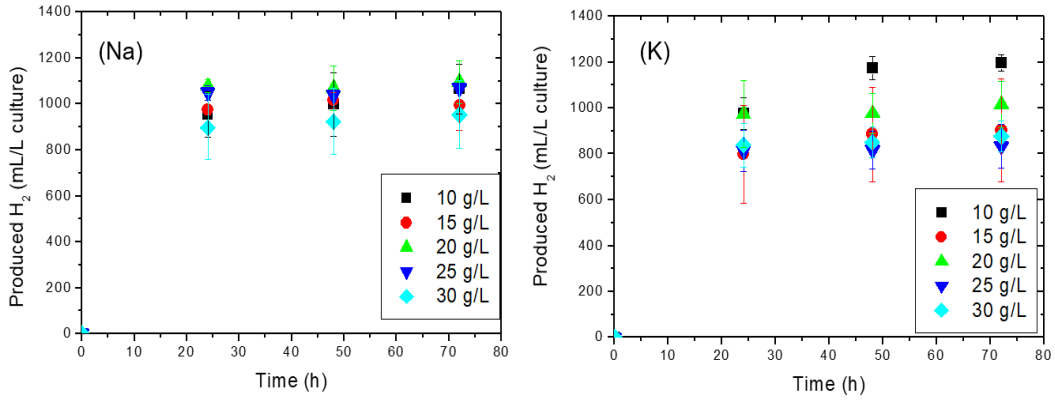
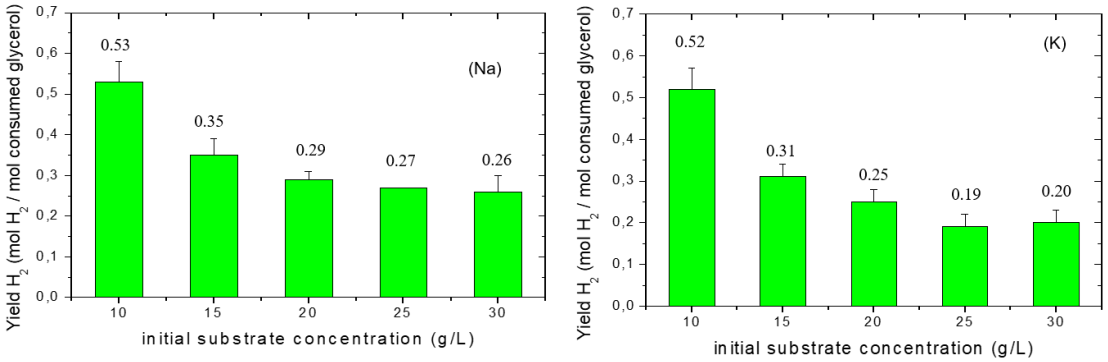


Figure 2. Hydrogen yield mol H_2/mol consumed glycerol in phosphate Na buffer solution and in phosphate K buffer solution



In both experiments with two buffer solutions, mainly acetic acid production and, in some cases detected small amounts of butyric acid was observed. Moreover, as it can be seen, there were no significant differences in productivity acids, between both different buffers solutions. However, in experiments with the phosphate Na buffer solution, there were variations in productivity to acids between different initial substrate concentrations, unlike the phosphate K buffer solution.

With regard to products, 1.3-propanediol (PDO) and ethanol production were observed. Typically, the productivity of PDO with the phosphate Na buffer solution was ranged to values between 3.5-4.5 g/L, while the relationship between the initial organic load and productivity to PDO was analogous. The behaviour of the reactor with the phosphate K buffer solution was different, as the initial organic load did not affect the productivity (3.5 g/L). Very significant difference was noted for the ethanol production, since the reactors with the phosphate Na buffer solution showed multiple productivity in this particular product, compared with the reactors, where was used the phosphate K buffer solution.

For experiments with the phosphate Na buffer solution was observed mean the disolved COD consumption of 30% for all of the initial substrate concentrations of glycerol in the medium, except the initial concentration of 25 g/L, which was removed 23.57% of the disolved COD. Similar results were presented both experiments which was used the phosphateK buffer solution. Moreover, in these experiments the interesting is shown for the initial substrate concentration of 15 g/L, since the consumption was 37% of the disolved COD.For the experiments with higher initial substrate concentration, greater quantity of glycerol consumption was

observed by the microorganisms. Therefore, the use of different buffers solutions did not affect the glycerol consumption.

The glycerol reduction was greater for reactors with smaller initial organic loadings of glycerol in the medium. More glycerol consumption rates were observed in the reactors with an initial concentration of 10/L (93.5 and 94.5% of the phosphate Na and K buffer solution, respectively) and then to those of the original 20 g/L concentration, in an amount of 87-87.5% in both pH-adjusting solutions. For larger initial substrate concentrations, the percentage consumption showed a downward tendency.

Biomass production presented differences both in the initial glycerol concentration in the feed systems and the use of different buffers solutions.All reactors of the pH value decreased by one unit and then remained stable near the value 5. The reduction observed is due to acid production by glycerol fermentation.

Table 2. Experimental results for the batch reactors with the phosphate Na buffer solution

Buffer Solution Na	**Initial substrate concentration (g/L)**				
	10	**15**	**20**	**25**	**30**
pH (0h)	6.2± 0.0	6.2± 0.0	6.2± 0.0	6.2± 0.0	6.2± 0.0
pH (72h)	5.2± 0.0	5.0± 0.1	4.9± 0.1	5.0± 0.0	4.8± 0.0
Glycerol (0h) (g/L)	9.7± 0.1	15.1± 0.5	26.4± 0.8	22.0± 0.5	31.1± 0.4
Glycerol (72h) (g/L)	0.6± 0.2	1.9± 1.1	6.3± 1.4	3.2± 2.3	11.6± 1.0
Consumed Glycerol (g/L)	9.1±0.2	13.2± 0.5	20.1±0.9	18.8±0.5	19.5±0.5
H_2 (L/Lculture)	1.1± 0.1	1.0± 0.1	1.1± 0.2	1.1± 0.1	1.0± 0.2
1.3-propanediol (g/L)	3.7± 0.3	3.6± 0.4	4.2±0.4	3.8± 0.3	4.6± 0.3
Ethanol (g/L)	1.5± 0.1	1.6± 0.2	2.8± 0.2	1.7± 0.1	2.9± 0.4
Acetic acid (g/L)	0.1± 0.0	0.2± 0.0	0.5± 0.0	0.4± 0.0	0.4± 0.0
Butyric acid (g/L)	>0.07	>0.07	>0.07	>0.07	>0.07
VSS (g/L)	1.3± 0.1	1.5± 0.1	1.3± 0.0	1.3± 0.0	1.4± 0.0

Table 3. Experimental results for the batch reactors with the phosphate K buffer solution

Buffer Solution K	**Initial substrate concentration (g/L)**				
	10	**15**	**20**	**25**	**30**
pH (0h)	6.1± 0.0	6.1± 0.0	6.1± 0.0	6.1± 0.0	6.1± 0.0
pH (72h)	5.1± 0.0	4.9± 0.1	4.1± 0.1	4.9± 0.0	4.9± 0.0
Glycerol (0h) (g/L)	11.2± 0.6	15.2± 0.6	21.7± 0.8	26.2± 0.9	31.9± 0.1
Glycerol (72h) (g/L)	0.6± 0.0	2.0± 1.3	4.9± 0.3	8.2± 0.3	14.7± 1.7
Consumed Glycerol (g/L)	10.6±0.5	13.3±0.4	16.8±0.8	18.0±0.7	17.2±0.5
H_2 (L/Lculture)	1.2± 0.0	0.9±0.2	1.0± 0.1	0.8± 0.1	0.9± 0.1
1.3-propanediol (g/L)	3.4± 0.2	3.5± 0.4	3.5± 0.3	3.4± 0.2	3.5± 0.4
Ethanol (g/L)	0.6± 0.0	0.4± 0.0	0.6± 0.0	0.5± 0.0	0.5± 0.4
Acetic acid (g/L)	0.5± 0.0	0.5± 0.0	0.5± 0.0	0.5± 0.0	0.5± 0.0
Butyric acid (g/L)	>0.07	>0.07	>0.07	>0.07	>0.07
VSS (g/L)	1.1± 0.0	1.2± 0.0	1.2± 0.0	1.1± 0.0	1.1± 0.0

Table 4. Yields of metabolic products (mole product/mole consumed glycerol)with the phosphate Na buffer solution

Buffer solution Na	Initial substrate concentration (g/L)				
	10	**15**	**20**	**25**	**30**
H_2	0.48	0.31	0.24	0.22	0.20
1.3-propanediol	0.43	0.29	0.22	0.23	0.26
Ethanol	0.29	0.21	0.17	0.26	0.28
Acetic acid	0.01	0.01	0.03	0.03	0.03
VSS	0.08	0.06	0.03	0.03	0.04

Table 5. Yields of metabolic products (mole product/mole consumed glycerol)with the phosphate K buffer solution

Buffer solution K	Initial substrate concentration (g/L)				
	10	**15**	**20**	**25**	**30**
H_2	0.48	0.28	0.25	0.19	0.22
1.3-propanediol	0.35	0.27	0.21	0.19	0.21
Ethanol	0.11	0.04	0.05	0.05	0.05
Acetic acid	0.06	0.06	0.04	0.04	0.04
VSS	0.05	0.05	0.03	0.03	0.02

3.4. Effect of initial substrate concentration in the production of metabolic products (Experiment C)

Initially, the hydrogen productivity and yield is shown in Figures 3 and 4. As shown in Figure 3, the reactors which started up with an initial loading of glycerol 20 g/L, were showed greater hydrogen production at different pH conditions.

The above results are confirmed by Figure 4, where yields in hydrogen are presented relative to the substrate consumption. For those experiments that took place in the pH value feed of 6, yields resulted equal namely 0.46 mol H_2/mol of glycerol. For the experiments, however, they started up with pH value feed of 6.5 and 7, yield in hydrogen was greater in reactors which used initial glycerol concentration of 20 g/L. Specifically, for pH value of 6.5 and starting up the substrate loading of 10 g/L, the yield was 0.42 mol H_2/mol of consumed glycerol, and starting up the substrate loading of 20 g/L, the yield was 0.63 mol H_2/mol of consumed glycerol. Even greater was the difference in pH value of 7, where for initial glycerol concentrations of 10 and 20 g/L, respectively, yields were 0.44 and 0.74 mol H_2/mol of consumed glycerol respectively.

Figure 3. Hydrogen production (mL/L culture) with initial substrate concentration 10 and 20 g/L

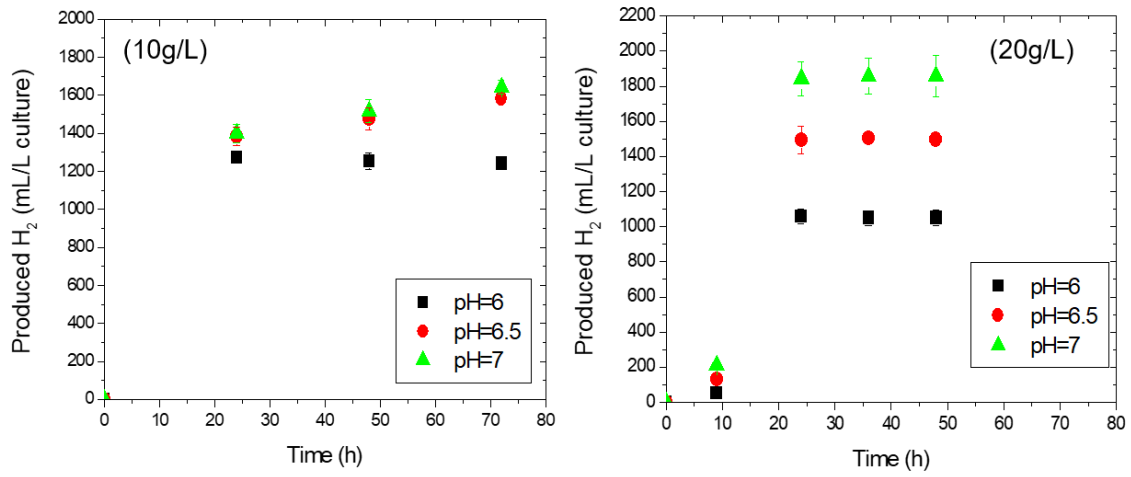
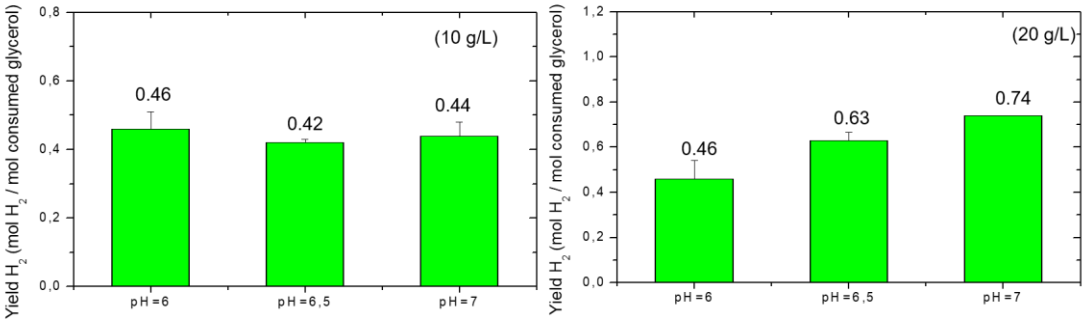


Figure 4. Hydrogen production (mL/L culture) with initial substrate concentration 10 and 20 g/L



The main acid produced was acetic, while smaller amounts detected butyric acid. Larger acid production was showed in reactors with initial glycerol concentration of 20 g/L in the feed. Noteworthy is also the fact that the major amount of acid produced in 48 h for reactors with starting up substrate loading of 10 g/L, while in 24 h for corresponding to 20 g/L. Concerning the pH value, lower volatile fatty acids production was observed for the value of 6 in both cases. Better results were obtained for pH value of 6.5 at 10 g/L, while initial substrate loading of 20 g/L for the pH value 7 was resulted in higher production of acids.

The productivity in 1.3-propanediol (PDO) was approximately doubled for the reactors with an initial substrate concentration of 20 g/L, whilst best results were showed for the pH value of 6.5. In contrast, the reactors showed reduced ethanol production up to 60%. As for the differences being due to the different initial pH value, there was not any certain conclusion.

There are no differences in the disolved COD consumption with respect to different pH values. Additionally, it was indicated that neither between different initial concentrations in the medium there are no significant differences in the disolved COD consumption by microorganisms.

Glycerol contained in the medium (10 g/L) was consumed entirely in pH value conditions of 6.5 and 7, whilst a major proportion of a pH value equal to 6. Also, in all experiments, the greatest substrate percentage was consumed in the first 24 hours.Indeed, initial substrate concentration of 10 g/L, the glycerol consumption was performed at 100% at a pH value of 6.5 and 7, and at a pH value of 6 was performedat 96.25% of the initial substrate concentration was removed. As for the initial substrate concentration of 20 g/L, the glycerol consumption rates were lower, while an increase in pH value resulted in greater glycerol consumption.

Table 6. Experimental results with initial substrate concentration 10 g/L

Initial substrate concentration 10 g/L	**Initial pH value**		
	6	**6.5**	**7**
pH (0h)	6.1± 0.0	6.5± 0.0	7.0± 0.0
pH (72h)	5.1± 0.0	5.3± 0.0	5.7± 0.0
Glycerol (0h) (g/L)	9.8± 0.2	9.8± 0.8	10.3± 0.1
Glycerol (72h) (g/L)	0.4± 0.0	0.0	0.0
Consumed Glycerol (g/L)	9.4± 0.7	9.8± 0.5	10.3± 0.9
H_2 (L/Lculture)	1.25± 0.02	1.58± 0.00	1.64± 0.04
1.3-propanediol (g/L)	2.8± 0.3	2.7± 0.2	2.4± 0.1
Ethanol (g/L)	0.9±0.0	0.8± 0.0	0.7± 0.0
Acetic Acid (g/L)	0.5± 0.0	0.2± 0.0	0.3± 0.0
Butyric Acid (g/L)	> 0.1	0.8± 0.0	0.5± 0.0
VSS (g/L)	1.1± 0.1	1.3± 0.1	1.3± 0.0

Table 7. Yields of metabolic products (mole product/mole consumed glycerol) with initial substrate concentration 10 g/L

Initial substrate concentration 10 g/L	InitialpH value		
	6	6.5	7
H_2	0.54	0.66	0.65
1.3-propanediol	0.27	0.28	0.21
Ethanol	0.17	0.14	0.12
Acetic acid	0.06	0.02	0.04
VSS	0.06	0.07	0.06

Table 8. Experimental results with initial substrate concentration 20 g/L

Initial substrate concentration 20 g/L	Initial pH value		
	6	6.5	7
pH (0h)	6.1± 0.0	6.6± 0.0	6.9± 0.0
pH (72h)	4.6± 0.0	4.9± 0.1	5.6± 0.1
Glycerol (0h) (g/L)	19.9± 0.6	21.0± 0.7	21.5± 0.5
Glycerol (72h) (g/L)	5.7± 0.2	3.8± 0.5	1.3± 0.3
Consumed Glycerol (g/L)	8.1± 0.8	8.9± 0.6	11.2± 0.9
H_2 (L/Lculture)	1.05± 0.05	1.50± 0.03	1.86± 0.12
1.3-propanediol (g/L)	5.3± 0.3	5.2± 0.4	4.8± 0.5
Ethanol (g/L)	0.5± 0.0	0.3± 0.0	0.5± 0.0
Acetic Acid (g/L)	0.9± 0.0	1.1± 0.0	1.2± 0.0
Butyric Acid (g/L)	> 0.1	> 0.1	> 0.1
VSS (g/L)	1.0± 0.0	1.2± 0.0	1.2± 0.0

Table 9. Yields of metabolic products (mole product/mole consumed glycerol) with initial substrate concentration 20 g/L

Initial substrate concentration 10 g/L	InitialpH value		
	6	6.5	7
H_2	0.53	0.69	0.68
1.3-propanediol	0.52	0.65	0.44
Ethanol	0.07	0.04	0.07
Acetic acid	0.15	0.17	0.15
VSS	0.06	0.07	0.06

IV. CONCLUSION

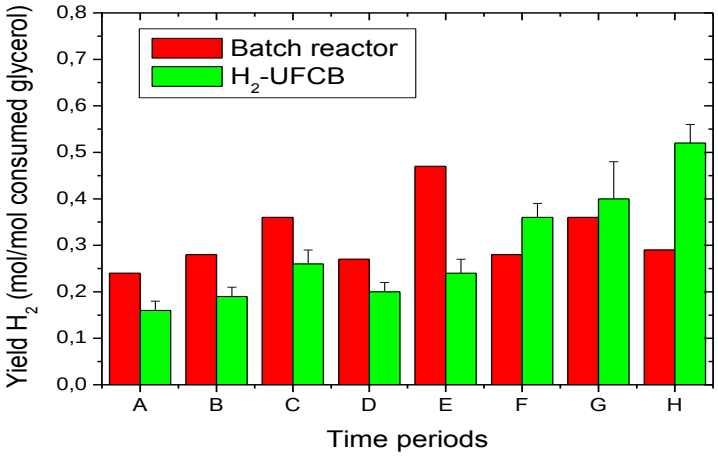
4.1 Comparison yields for experiments both batch reactors and the reactor H_2-UFCB for each time period(Experiment A)

In Figure 5, a comparison in stoichiometric production of hydrogen from glycerolis shownfor all time periods, as calculated for the batch-type experiments work and continuous reactor hydrogenogenic (H_2-UFCB). It is obvious that in the first five periods, yields in hydrogen production were greater in batch-type experiments in contrast to H_2-UFCB, although there is a large deviation, except to the period E. Then, the yield decreased to batch experiments and it was risen to the H_2-UFCB experiments. This result means that the increase of the pH value in the substrate plays a key role in the efficiency of hydrogen production in the two kinds of experiments(Dounavis et al., 2015b).

Concerning the metabolic products, in batch experiments it was observed that acetic acid was the major acid, which was produced, whilst butyric and hexanoic acid were detected at very low concentration (except to the experiment period G). Ethanol was showed in very high concentrations (1.25-3 g/L). In the experiments of the reactor H_2-UFCB, butyric, acetic and hexanoic acid were identified at higher concentrations compared to the batch experiments, while ethanol was ranged ethanol at concentrations between 0.2-0.5 g/L. The 1.3-propanediol was the principal soluble metabolite, which was identified in all experiments(Dounavis et al., 2015b).The difference in the distribution of metabolic products is due to the fact that in one case the

experiments were conducted in a reactor upflow H_2-UFCB bed and in the other case in batch mixed reactors. In addition, a remarkable role played by the fact that the reactor H_2-UFCB used with ceramic rings (poro-ring) for the immobilization of microbial culture. These elements are one possible explanation for the distribution and range of the metabolic products.

Figure 5. Hydrogen yields both batch reactors and H_2-UFCB for all the periods and comparison



4.2 Effect buffer solution (Na and K) in the production of metabolic products (Experiment B)

No significant differences were observed with respect to the experimental results, which led to the conclusion that these buffers solutions do not affect significantly the yields of the metabolic products. Additionally, a very significant observation regarding the above experiments has to do with the fact that the reactors with initial glycerol concentration of 10 and 15 g/L, mass balances were satisfied to a major level, in contrast to the corresponding higher preloads systems. The possible causes are the discrepancies encountered in the experimental procedures and the possibility of a by-product, which has not been identified.

4.3 Effect of initial substrate concentration in the production of metabolic products (Experiment C)

In these experiments, the differences were examined which can result in the production of desired products, the initial substrate concentration in the feed solution, for three different pH values. They compared two initial concentrations, 10 and 20 g/L. The choice of these values is a continuation of the previous cycle experiments (Effect of buffer (Na or K) in the production of metabolic products) as for these initial glycerol concentrations the reactors responded better in relation to the rest studied.

The conclusion drawn is that the initial substrate concentration of 20 g/L resulted in significantly higher yields, mainly 1.3-propanediol and acetic acid, while for the yield in hydrogen the results were similar. The ethanol was an exception because the reactors with the initial substrate concentration of 10 g/L showed higher yields.

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