Evaluation of Pre-Treatments on the First Stage of an Anaerobic Digester for Enhancing Bio-H_2 Production

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ABSTRACT

Wastewater Treatment (WWT) is an essential public service that simultaneously consumes a large amount of energy and produces a significant amount of by-products (e.g. sludge). There is, however, excellent conservation potential through the production of biogas in Anaerobic Digestion (AD), and the use of it as a renewable source of energy in-situ. It is well know that each cubic meter (m^3) of biogas contains the equivalent of 5-7.5 kWh of calorific value, if the composition of CH_4 lies between 50-75% of the total biogas composition [1]. Nevertheless, to increase the conservation potential in a two-stage digester, a pre-treatment to the feedstock and seed should be applied. This pre-treatment increases the yield of hydrogen (H_2) by 10% in the first stage of the AD [2]. Additionally, the pre-treatment should selectively inhibit methanogenesis and increase the production of acetic acid and acetate, thus achieve the highest possible H_2 yield [3]. Furthermore, several techniques have been appointed as potential pre-treatments due to their simplicity, contribution to hydrolysis of organic material presented in the biomass, and inactivation of methanogenic bacteria [4]. Moreover, H_2 has the highest energy content per unit weight of any known fuel (120.21 MJ/kg) [3]. This is particularly interesting, as there are additional socio-economic benefits for using bio-H_2 as a source of energy. These include the reduction of green-house gas (GHG) emission, by reducing the final amount of CH_4 produced through AD, and the creation of a viable renewable energy source.

This study focused exclusively on 4 pre-treatments: temperature shock, pH control, chemical addition and a combination of the above mentioned. Therefore, the aims of this research were:

1) To study the influence of different pre-treatments on the first stage of a two-stage anaerobic digester, using glucose as substrate.
2) To select the most suitable pre-treatment for enhancing the bio-H_2 production for scaling-up.

Keywords: Anaerobic digestion; Bio-hydrogen production; Climate Change; Pre-treatment;

1. Introduction

One of the biggest challenges to overcome in Wastewater Treatment Plants (WWTPs) in developing countries is the reduction of energy consumption, and optimization of the different processes and services at the facility. From a technical point of view, in these countries, the use of Anaerobic Digestion (AD) of sewage sludge reduces the transportation costs of dry sludge to landfills, and partially eliminates the need for filter presses or any other drying systems. Therefore, one of the first resulting indirect benefits is the reduction of the amount of sludge sent to landfills, reducing the Green House Gas (GHG) emissions (as methane) at the landfill. Some other environmental benefits from AD include odor reduction, pathogen control, minimization of sludge production, and conservation of nutrients [5]. In addition, WWTPs are large energy users with excellent conservation potential because of biogas production, which has become one of the main sources of renewable energy [1]. Literature references report that 0.29 to 0.33 Nm^3 of CH_4 can be produced for each kilogram of Chemical Oxygen Demand (COD) digested at 35°C [6, 7]. Furthermore, hydrogen (H_2) represents one of the most promising steps toward a sustainable energy system, due to its high energy content per unit weight 120.21 MJ/kg (while CH_4 is only 50.2 MJ/kg), and its potential as a renewable energy source [3]. H_2 is a clean green fuel only if it is produced from renewable sources (e.g. wind, biomass) or through AD; making it easy to transport and store [5]. Recent works suggest that the theoretically yield of hydrogen is 4 mol H_2/mol substrate [8, 9, 10]; however, practically 1.5 to 2.5 mol H_2/mol glucose can be produced [3, 9]. Additionally, some challenges to overcome in the following years for the commercialization of bio-H_2 production are: a) the use of efficient microbial strains which can use different organic materials as feedstock, b) the low rate of H_2 production after the complete process, c) the comprehension of the metabolic pathway that drives the production of H_2, d) the cost and mass production of certain pre-treatments, and e) the improvement of the H_2 yields of the processes using cheaper raw material as substrates [5].

In biogas production through a single-stage AD process, the CH_4 formation takes away a significant portion of the reactants, acetate and H_2, which are produced by "H_2-producing bacteria" and simultaneously consumed by "H_2-consuming bacteria". In contrast, a two-stage AD produces H_2 and carbon dioxide (CO_2) in the first stage, whereas the second stage produces CH_4 and CO_2. One of the main characteristics of the pre-treatments is the selective inhibition of methanogenesis, increasing the production of acetic acid and acetate, and thus achieving the highest possible H_2 yield [4]. Several pre-treatments have been appointed for enhancing the production of H_2 in a two-stage digester, such as a low pH control [11], temperature shock of the inoculums for removing hydrogen consuming non-spore forming bacteria [8], and chemical addition by means of specific methanogenic inhibitors [4]. Special attention has been given to pre-treatments with Microwave (MW), due to its uniformity on heating and the precise control of the process temperature that is applied to the sludge. Significant concentration of soluble COD (sCOD), phosphate and ammonia are released; reduction of capillary suction and improvement of the sludge dewaterability and high water content in the sewage sludge can absorb MW energy efficiently [12, 13]. Furthermore, MW irradiation seems to be a potential method because of its synergetic effect on pathogenic destruction and thermal heating for anaerobic digestion at 35°C. In addition, MW energy has a strong ability to penetrate dielectric material to produce thermal and non-thermal effects on microbes, increasing potential food for methane producing bacteria, and lowering the hydraulic retention time (HRT) [13]. Further, sludge is a multiphase medium that can be effectively absorbed by the MW energy, but the degree of degradation depends on the intensity of the MW irradiation [12].

Based on an extensive literature review, three main pre-treatment have been identified as the most cost-effective and adequate techniques. These includes: (i) heat-shock as microwaves (MW) and water bath and WB; (ii) combination of heat shock with chemical addition (specific methanogen inhibition); and (iii) addition of specific methanogen inhibitors, or chemical additions of bromoethanosulfonate (BES), iron (Fe III) chlorhidric acid (HCl) or chloroform ($CHCL_3$). In addition, the objective of this paper was to identify the specific condition and concentration for applying the pre-treatment to the feedstock and seed in a real-case WWTP in Mexico.

2. Experimental

Two reactors (R1 and R2), made of borosilicate glass, clear, with round bottom, were used for the experiments. The reactors have a total volume of 12 L, with a working volume of 10 L (sludge) and 2 L headspace volume (biogas). Two point nine liters of inoculum (sludge) for the reactors (R1 and R2) were taken from the anaerobic digester of the Wastewater Treatment Plant for Research and Education (LFKW) at the University of Stuttgart (Germany). The anaerobic digested sludge (ADS) or inoculum was diluted to 7.2% Total Solids (TS) concentration and strained through a 10 mm sieve to eliminate coarse material that could interfere with further analysis. The ADS was placed inside R1 and R2 under continuous stirred conditions for the guarantee of well-suspended biomass in the mix liquor, and to represent the composition of a real effluent. In addition, the R1 and R2 were installed in a controlled temperature room (37°C), while the temperature of the sludge was 35ºC. The pH was regulated by means of a pH glass electrode and a pH programmable controller, which controlled 2 solenoid dosing pumps for automatic addition of a sodium hydroxide (NaOH) solution 25% or a hydrochloric acid (HCl) solution 25% to maintain the operation pH level at 5.6. This value has been reported to be the optimum for batch bio-H_2 production [3, 9, 10]. It is important to clarify that the initial pH of the ADS was 7.8 and was gradually reduced until reach the operation pH value of 5.6. Figure 1 shows the setup of experiments that were used for these batch experiments.

According to previous work at our laboratory [2] two important conditions were considered: glucose or substrate was used as feedstock to represent a real effluent from municipal wastewater with an Organic Loading Rate (OLR) of 10 g COD/L and a specific solution of nutrients was added to ensure healthy bacteria growth. The produced biogas quantity was measured with a drum-type gas meter twice per day. This gas was collected for each experiment in gas bags, and then analyzed with a gas analyzer equipped with an infrared detector for CH_4 and CO_2 and a thermal conductivity detector for H_2. The biogas amount was registered into a log book. The analyses of concern were determined according to the German DIN-Norm and performed twice weekly: one for the influent and again for the effluent. The analyses included: Total Solids (TS), Volatile Solids (VS), Chemical Oxygen Demand (COD), nitrogen (N) and phosphate (PO4); these last three parameters were analyzed as total and soluble form.

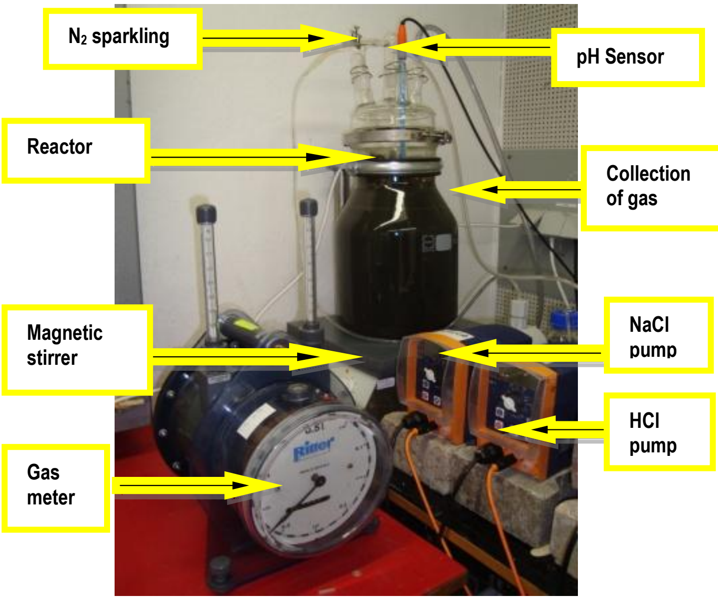

Figure 1 Set up of Experiment

Glucose and sucrose were determined spectrophotometrically after enzymatic digestion by a test kit according to the manufacturer's instructions. Gas Chromatography was also used to analyze organic acids (Volatile Fatty Acids- VFA). VFAs were analyzed in a GC Perkin Elmer equipped with a capillary injector, a FI detector, and a Varian column at a detection / injector temperature of 280°C with a programmable temperature as: 3 min. 70°C, in 6 min. 100°C, 20 min. 240°C. Each set of experiment was operated in batch during 120 h and pH 5.6.

Previous experiences have shown that this operation time gives a better control for short operation time experiment under a batch mode [2]. Table 1 outlines the 21 different pre-treatments under study, including the order in which the mix of nutrients and glucose (feedstock) were added.

Table 1 Pre-treatments

Pre-treatment	Label	Description	Conditions	Label	Pre-treatment	Description	Conditions
i Heat shock	WB 90°C/20 min	WB + G + n	@90°C for 20min	pH3	iii Chemical addition	G + n + HCl	addition of HCL 25% for pH3 during 24hr
	MW 2.5min @900W	MW + G + n	2.5 min under 900W	BES 5mM		G +n + BES	5 mM
	MW 5 min @900W		5 min under 900W	BES 7mM			7 mM
	MW 7 min @900W		7 min under 800W	BES 8 mM			8 mM
	MW 10min @900W		10 min under 800W	BES 10 mM			10 mM
ii Combination: heat shock and chemical addition	WB 90°C + pH4	WB + HCl + G + n	@ 90°C for 20min + pH4 during 17hr	CHCL3 0.05%		G + n + CHCl3	0.05 %V/V
				CHCL3 0.75%			0.075%V/V
	MW 5min @800W + pH4	MW + HCl + G + n	5 min @ 800W + addition of HCL 25% for pH4 during 17hr	CHCL3 0.10%			0.10%V/V
	MW 3min@800W + pH4	MW + HCl + G + n	3 min @ 800W + addition of HCL 25% for pH4 during 17hr	CHCL3 0.15%			0.15%V/V
	CHCL3 0.075 + pH4	G + n + CHCl3	0.075 %V/V + addition of HCL 25% for pH4 during 17hr	Fe III (5mM)		G + n + Fe III	5 mM
	Fe III (7mM) + pH4	G + n + Fe III	7mM +pH4 (17hr)	Blanc glucose	CONTROL SAMPLES	Blanc Glucose	G+n+ no pre-treatment

	Fe III (7mM) + pH6		7mM+pH6 (17hr)	Blanc no glucose		Blanc no Glucose	No G+ no pre-treatment

G: Glucose; n: nutrients; WB: Water Bath; MW: Microwaves; $CHCL_3$: Chloroform; Fe III: Iron 3 Chloride; HCl: Hydrochloric acid; BES: Bromo-ethane-sulfonate;

3. Results and discussion

The biogas measurement for each pre-treatment (Figure 2) included CH_4, H_2 and CO_2, registered in L over 120 h. No CH_4 was detected at any of the pre-treatments. The most representative performances in terms of cumulative biogas production were: *WB 90°C +pH4* by means of 61.7 L of biogas; *MW 7min@800W; BES 8mM* and *MW 5 min @ 800W+pH4* by means of 44.5; 41.6 and 33.10 L of biogas respectively; *$CHCl_3$ 0.10%* and *BES 7mM*, by means of 24.8 and 25 L of biogas, respectively. The production of H_2 and CO_2 was in the following order (H_2; CO_2, in L): *MW 5min @ 800W +pH4 (15; 17) > BES 8mM (13.9; 26.8)> WB 90°C +pH4 (9.7; 4.8) > BES 7mM (8.11; 10) > CHCl30.10% (5.28; 17.6 L) > MW 7min@800W (1; 9).* The rest of the pre-treatments produced, in average, less than 1.5 L of H_2, and less than 9 L of CO_2 .

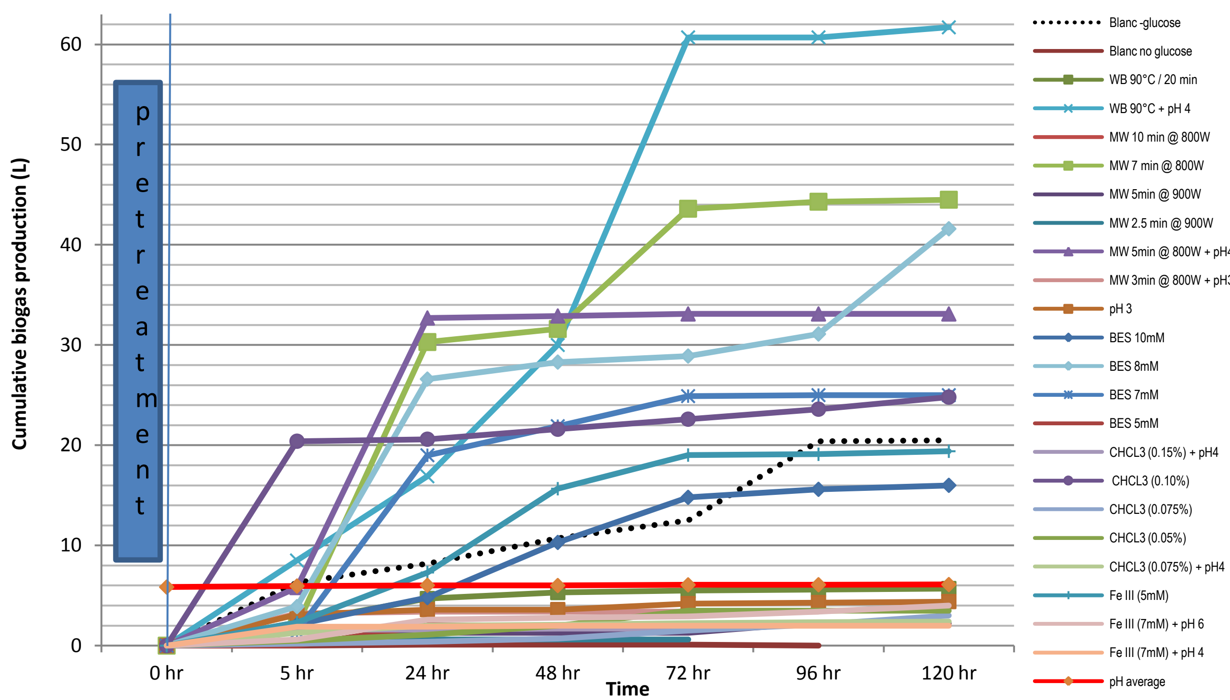

Figure 2 Gas productions after different pre-treatments (under pH 5.6, over 120 hr)

According to previous experiment in our laboratory, the maximum H_2 yield was 0.59 to 0.66 mol H_2 / mol glucose in a 120 h batch process, if applying a heat shock pre-treatment and a specific mix of nutrients [2]. Figure 3 shows the yields of H_2 for each pre-treated sample. The best performances were as follow: *MW5min@ 800W+pH4* and *BES 8mM* by means of 0.96 and 0.88 mol H_2/mol glucose respectively; *WB 90°C +pH4* and *BES 7mM* by means of pH of

0.62 and 0.52 mol H_2/mol glucose respectively; *$CHCl_3$ (0.10%)* be means of 0.34mol H_2/mol glucose. The rest of the pre-treatments showed a low performance, in comparison to *Blanc 1 and Blanc 2*.

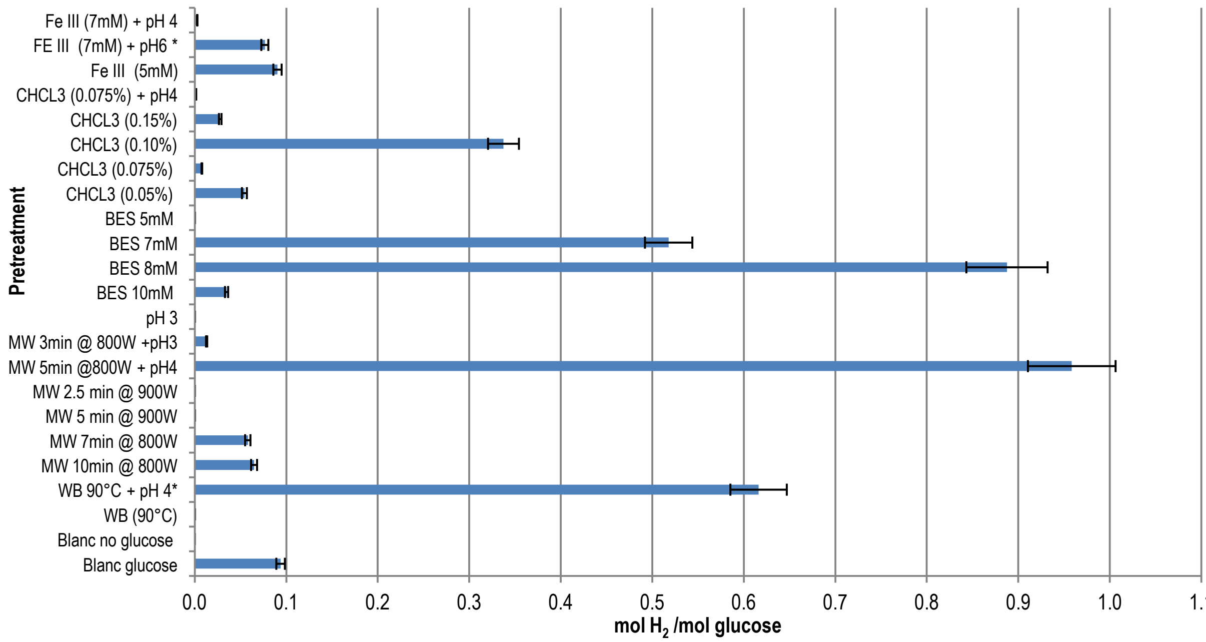

Figure 3 Hydrogen yield production from digested sludge

Conversely, the relationships soluble (s) and total (c) Chemical Oygen Demand (sCOD/cCOD) were calculated to give an idea of the solubilization of organic matter and its indirect relation to volatile fatty acids (VFA) production. Additionally, this relationship will be used as a design parameter for scaling up an anaerobic reactor in a WWTP in Mexico. The experimental results showed that the initial value for sCOD/cCOD for the *Blanc no glucose* (no pre-treatment) was 0.05, reducing to 0.02 after 5 days; while the same relation for the *Blanc glucose* (no pre-treatment) was 0.42, reducing to 0.36 after the same time. This reduction was observed in the 21 experiments, with an initial average sCOD/cCOD relationship of 0.46 and a final average sCOD/tCOD relationship of 0.09. In other words, the average reduction of sCOD was 19.4%. Nonetheless, two pre-treatments pointed out a different behavior, where the sCOD/tCOD ratio doubled. The situation corresponded to the pre-treatments: *BES 10 mM and $CHCL_3$ (0.05%)* by means of 0.83 and 0.89 respectively. Finally, the highest solubilization of organic materia was observed in: *MW 5 min@900W* by means of 33.6%, while the lowest solubilization was observed in *$CHCl_3$ (0.015%)* by means of 7.86%. While the increase of sCOD in comparison to the *Blancs*, was observed mainly in nine pre-treatment (in mg/L): *MW 5 min@900W* (12500) > *FeIII (5mM)* (11500) > *pH 3* (10600) > *MW5min @ 800W+pH4* (10500) > *WB*

90°C +pH4 (10400) > *Fe III (7mM)+pH6* (10100) > *BES (5mM)* (9860) > *BES (8mM)* (9800) > *BES (7mM)* (9490) > *Blanc 1*(9370).

One final parameter to consider is the "food to microorganism" relationship (F/M), which is a controlling parameter suggesting that microorganisms must satisfy their maintenance energy requirements prior to synthesizing new biomass. Decreasing the supply of substrate per unit biomass resulted in gradual decrease in the biomass yields, but at the same time it resulted in gradual increase in the bacteria mediated inert COD as a side effect. According to previous studies in our department [2], for anaerobic digestion, the F:M lowest limit is 1.5, while the upper limit is 3.5. The optimal value for this research was set between and 2-2.5. This condition followed by almost all pre-treated samples. The F:M ratio after pre-treatment for 9 specific parameters is listed as follow: *MW 5min @ 900W + pH 4* as 2.44; *BES 5mM* as 2.24; *WB 90°C+pH 4*as 2.62; *MW 5 min @ 900W* as 2.58; *pH 3* 2.25; *BES 7mM* as 2.18; *Fe III (5mM)* as 2.06; *BES 8mM* as1.71; and *Fe III (7mM)+pH6* as 1.26.

In order to achieve the highest possible H_2 yield, glucose has to be fermented to acetate. In addition, it has been reported [9, 14] that H_2 is not produced in propionate fermentation, rather in butyrate and acetate-ethanol fermentation, especially after a pre-treatment, which enhance the formate production. Therefore, butyrate-acetate fermentation has been appointed by several researchers as the main pathways followed by the bacteria for bio-H_2 production, due to its potential to change to butanol production (where H_2 is directly consumed or it production is inhibited). Additionally, under a pH controlled environment, the most stable pathway is the ethanol-acetate fermentation, because only acetic acid is produced as the main acid in this pathway. One more point that must be considered before analyzing the results is: that the hydrolization and fermentation of carbohydrates, proteins and lipids to VFA are pH dependent, thus the higher the initial pH is, the lower the total H_2 production potential is [11].

This section shows the results of nine selected pre-treatments and the their VFA production. The VFA of interests were: acetic acid (HOAc), propianic acid (HOPr), butyric acid (HOBu), valeric acid (HOVa) iso-valeric acid (iso-HOVa) and caproic acid (HOCa). Especially attention was given to HOAc, due to its relationship with the acetate-ethanol fermentation and a possible high yield of H_2. Additionally, the yield of H_2 can be very low when propionate or any other reduced products such as alcohol or lactic acid are formed [14]. The working pH remained under 5.6. Mainly 3 VFA were produced: HOAc, HOPr and HOBu; while iso-HOVa, HOVa and HOCa were detected in very low concentrations. Figure 4 presents the amount of VFA for the initial (in) and final (out) condition for 9 selected pre-treatments. The HOAc was produced in the following order (mg/L): *MW 5min@800W+pH4* (3828) > *BES 5mM* (3091) > WB *90°C + pH 4* (3308) > *MW 5min@900W* (2240) > *pH 3* (2036) > *BES 7mM* (2128) > *BES 8mM* (1996) > *Fe III (5mM)* (2016) > *Fe III (7mM) +pH6* (1888). While for the same pre-treatment, the HOBu was as follows: (mg/L): *pH 3* (238) < MW *5min@800W+pH4* (681) < *BES 5mM* (693) < *BES 7mM* (990) < *Fe III (5mM)* (1344) < WB *90°C + pH 4* (1360) < *Fe III (7mM) +pH6* (1369) < *BES 8mM* (1852) < *MW 5min@900W* (2690). The results suggest that the metabolic pathway, followed by the majority of pre-treatments during this research, was the acetate fermentation (acetogenesis), with CO_2, H_2 and acetate as main products from the acetogenesis. These products will be converted to methane in a second-stage through methanogenic bacteria. In addition, the tendency to produce Butyrate or Acetate was described by the relationship HOAc:HOBu (out). Literature reports for 1 mol H_2/mol hexose a ratio HBu:HAc of 0.75-1.25 with butyrate as main product and HOAc:HOBu ratio between 3-4, with acetate as main product, under a pH range 5.5 to 5.7 [11]. For the 9 experiments, the average HOAc:HOBu ratio was 4.6.

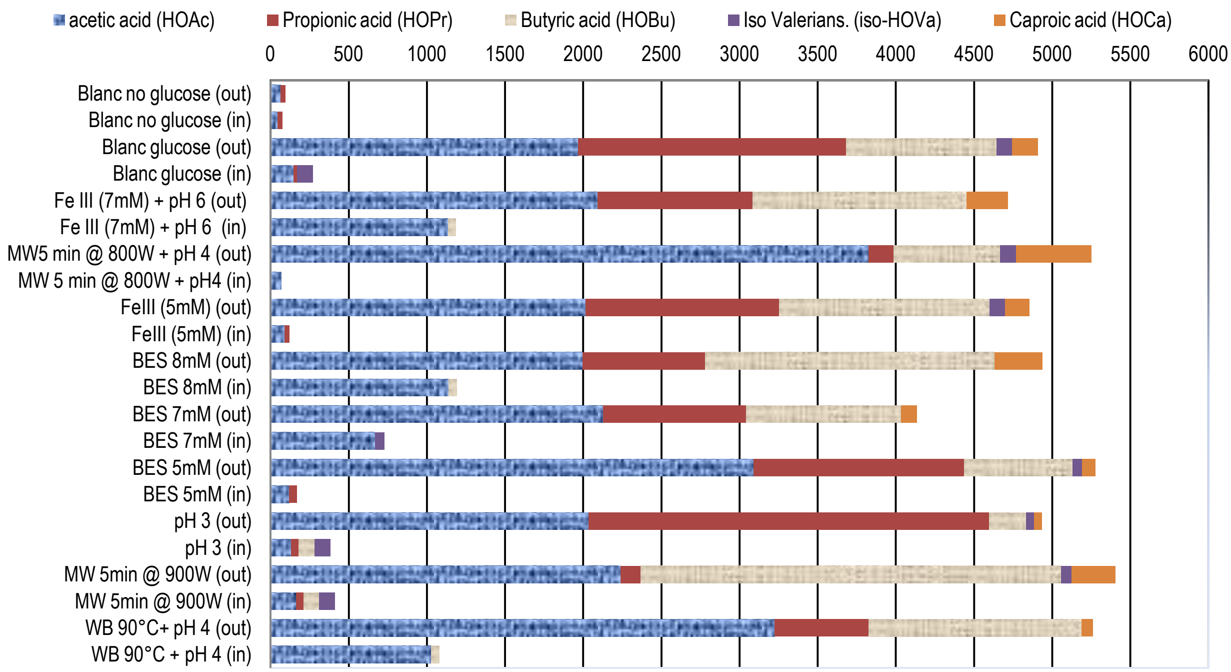

Figure 4 Volatile Fatty Acids production (9 experiments -input and output in mg/L)

4. Conclusions

After a deep literature review, the operation parameters for the first stage of a two-stage anaerobic digester were selected. Additionally, 4 pre-treatments were evaluated in 21 set of experiments. Previous work at our laboratory suggested the use of a specific nutrient of mix for guaranteeing a healthy F:M relationship. These conditions were the key factor for a good performance of the 21 experiment. It was confirmed that the combination of heat shock and chemical treatment with HCl (for working under a controlled pH level) enhance the H_2 production in the first stage of a two-stage anaerobic digester. Especial attention was given to the use of MW as the most suitable heat shock pre-treatment. These set of experiment helped to select design parameters for the scale-up of an anaerobic digester in a WWTP located in Mexico city. At the moment the 3 most representative pre-treatment for the full design are: a) *MW 5min@800W+pH4* with a H_2 yield of 0.96molH2/mol glucose, HOAc and HOBu production by means of 3828mg/L and 681 mg/L respectively; b) *WB 90°C + pH4* with a H_2 yield of 0.62mol H_2/mol glucose, HOAc and HOBu production by means of 3224mg/L and 1360 mg/L respectively; c) *BES 7mM* produced a H_2 yield of 0.52 mol

H_2/mol glucose, HOAc and HOBu production by means of 2128mg/L and 990 mg/L respectively. For the final implementation of a pre-treatment, a cost-benefit analysis and energy balance should be performed. Additionally, it was found that the use of selective inhibitor of methanogenesis (e.g. BES) has to include an environmental impact assessment, since there are not enough studies focused on the environmental effects of its by-products.

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