Comparative effect of acid and heat inoculum pretreatment on dark fermentative biohydrogen production

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Introduction

Dark fermentation can be an attractive option for hydrogen production due to its low environmental footprint and potential for large-scale implementation, replacing or operating in parallel with conventional anaerobic digesters that produce methane (Camacho et al., 2022). However, as it is a microbiological process, all metabolic pathways competing with the production of hydrogen (hydrogenotrophic, homoacetogenic, sulfate-reducing, lactic-acid) or the presence of microorganisms that consume this gas must be dynamically controlled inside the digesters to prevent hydrogen production yield from being negatively affected (Westerholm et al., 2019). That is why it is imperative to design mixed fermentative cultures through appropriate strategies of selective inoculum enrichment to make biological hydrogen production technologically and economically viable, compared to other more conventional methods.

There are many methods investigated for the pretreatment of the inoculum before its introduction into the reactor in order to favour the production of hydrogen, with very different results depending on the substrate used and the operating conditions (Jain et al., 2022). Among them, heat shock and acid treatment are perhaps the most interesting with a view to a future industrial scaling of the process, but it is necessary to carry out a detailed study under optimal conditions of inoculum pre-treatment and process development to find the dominant microorganisms and metabolic profiles to lay the foundations for the design of a larger-scale process. That is why this work proposes to carry out a systematic study to evaluate and compare the effect of both pretreatments, under mesophilic conditions, on the dynamics of the microbial community, on the metabolic profiles, and on the yield of biohydrogen.

Materials and Methods

Digestate from an anaerobic reactor operating in a municipal wastewater treatment plant and glucose were used as inoculum and carbon source, respectively. Biogas production was measured automatically using pressure transmitters (Desin Instruments, TPR-14/N, ranging 0-1 bar) connected in the headspace of each reactor. Biogas composition was analyzed on samples collected in Tedlar bags using a Varian CP-4900 Micro-GC chromatograph with a thermal conductivity detector. Volatile fatty acids (VFA) profiles (i.e. acetic, propionic butyric, isobutyric, valeric, isovaleric, caproic and isocaproic) were determined using a gas chromatograph (GC2014, Shimadzu, Japan) equipped with a flame ionisation detector (GC-FID) while a high performance liquid chromatography equipment (HPLC1100, Agilent) with refractive index detector was use to analyse glucose and ethanol in the dark fermentation digestate.

Glass serum bottles of 1000 mL with working volume of 500 mL were used as batch reactors. 10 g/L of glucose and inoculum were taken to achieve a substrate to inoculum (S/X) ratio of 2 in the reactors. A solution of macro and micronutriens (1 mL/L) were also added (NH_4Cl 170 g/L, KH_2PO_4 38 g/L, $CaCl_2.2H_2O$ 8 g/L, $MgSO_4.4H_2O$ 9 g/L, $FeCl_3.4H_2O$ 2000 mg/L, $CoCl_2.6H_2O$ 2000 mg/L, $MnCl_2.4H_2O$ 500 mg/L, $CuCl_2.2H_2O$ 30 mg/L, $ZnCl_2$ 50 mg/L, H_3BO_3 50 mg/L, $(NH_4)_6Mo_7O_{24}$ 90 mg/L, $Na_2SeO_3.5H_2O$ 100 mg/L, $NiCl_2.6H_2O$ 50 mg/L, EDTA 1000 mg/L, resorcine 500 mg/L). After taking the substrate and inoculum, the reactors were flushed with nitrogen gas to purge the air remaining in the headspaces for ensuring anaerobic conditions. The reactors were hermetically sealed and placed on a shaking table in a thermostatic room at 34±1°C until exhaustion of biogas production. Each experiment was carried out in triplicate and also blank test (without substrate) were prepared to calculate the remaining endogenous biogas production of the inoculum and proceed with the corresponding results correction.

In reference to the pretreatment of the inoculum, the methods and operational conditions selected for this study are collected in Table 1, considering their potential to quickly select the fermentative bacteria of interest and inhibit or suppress methanogens. The treated inocula were stored in a thermostated chamber at 34 ºC for acclimation, 24 hours before being used for the experiments.

Table 1. Inoculum pretreatment methods and operational conditions for the different tiers and trials.

Tier 1: Heat shock					
Time/Temperature	60 ºC		80 ºC	100 ºC	
15 min	Trial 1		Trial 4	Trial 7	
30 min	Trial 2		Trial 5	Trial 8	
60 min	Trial 3		Trial 6	Trial 9	
Tier 2: Acid (HCl) pretreatment vs Heat shock					
	Trial 10	Trial 11	Trial 12	Trial 13	Trial 14
pH	5.5	-	5.5	-	5.5
Tª (ºC)	-	80	80	100	100
Time (min)	-	30	30	30	30
Tier 3: Acid (HCl) pretreatment vs Acid (H_2SO_4) pretreatment					
	Trial 15	Trial 16			
pH	5.5	5.5			
Acid	HCl	H_2SO_4			

Results and main conclusions

Tier 1

In a first experimental run, three temperature levels (60º, 80º, and 100º) and three time levels (15, 30 and 60 minutes) were studied in order to verify the most effective combination in the pretreatment to maximize production of hydrogen. After introducing the mixture of substrate and inoculum into the reactor, the pH was adjusted to 5.5 to create an acidic environment. In general, a slight positive effect of temperature on biogas production (CO_2+H_2) was observed, but nevertheless, the heating time factor did not seem to have a clear effect on the result. Figure 1 shows, as an example, the curves obtained in the case of the trials at 60 ºC and 100 ºC (trials 1-3 and 7-9, according Table 1). The analysis of the composition of the generated biogas reveals, in all cases, a percentage of hydrogen in the mixture of around 43% and total absence of methane.

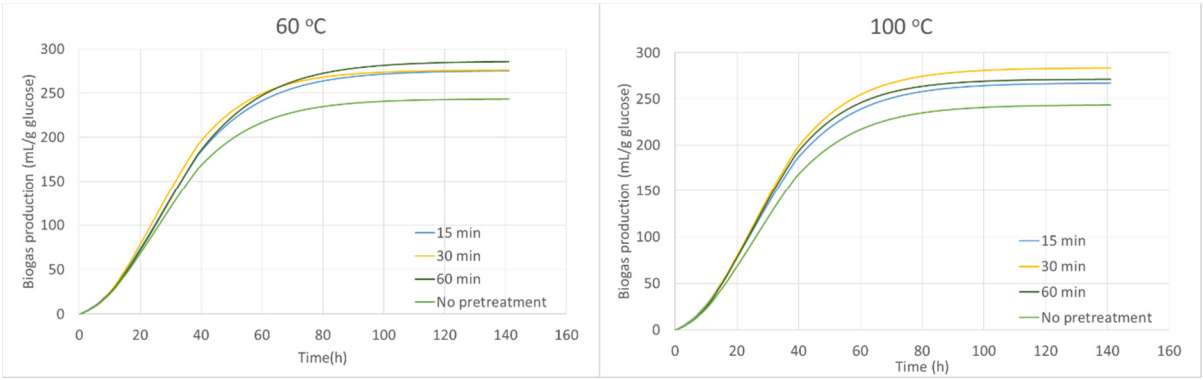

Figure 1. Biogas (H_2+CO_2) production with inoculum thermally pretreated.

Tier 2

Since the different conditions tested for the thermal pretreatments in Tier 1 did not seem to show differences among themselves in the production of hydrogen, the following step was to check if the adjustment of pH to acidic conditions that was carried out at the beginning of the test, was being dominant over the thermal treatment for the inhibition of methanogens. In the Tier 2, the thermal pretreatment was compared with the acid pretreatment. For this, three different operating conditions were established: pH adjustment only, thermal shock only (for two temperature levels), pH adjustment + thermal shock.

As observed in Figure 2, the biogas production is lower for the samples in which the acid pretreatment was not applied. These had an initial lag phase and their maximum production was lower than in the samples with the acid pretreatment. However, between the samples with acid pretreatment and those that received both pretreatments, there is no clear difference in biogas production. This indicates that the pH adjustment is being critical in the production of biogas, therefore in Tier 1 the results were very similar to each other, despite having applied different thermal pretreatment conditions. The acidification of the medium seems to have more influence on the production of hydrogen, making the influence of the thermal pretreatment not appreciable if the sample is also acidified. The analysis of the composition of the generated biogas reveals a percentage of hydrogen in the mixture around 43% when the inoculum has been acidified, but this percentage falls to 40% when exclusively thermal pretreatment is applied, and this result is common for both temperatures tested.

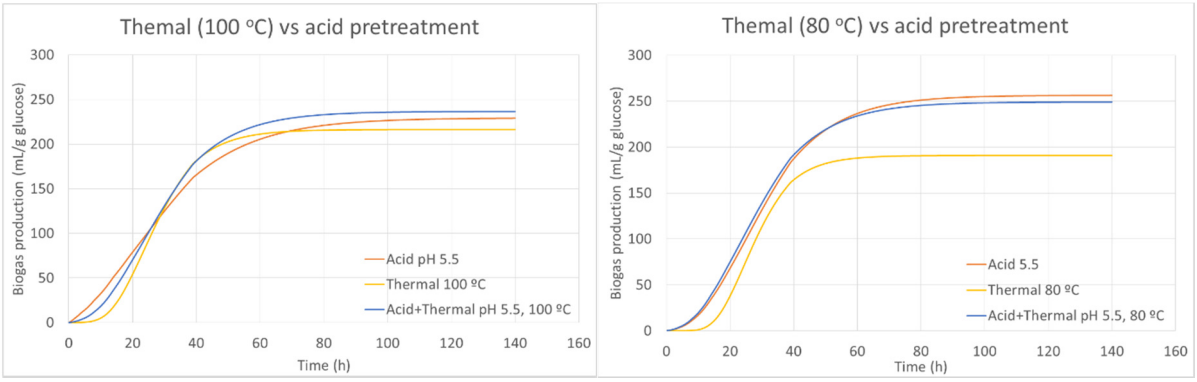

Figure 2. Biogas (H_2+CO_2) production with inoculum pretreated thermally vs acid.

Tier 3

In Tier 3, the objective was to study whether the performance of the process was affected depending on the type of acid used in the pretreatment of the inoculum. For this, two tests were replicated with the only difference that in one of them hydrochloric acid was used in acidification and in the other sulfuric acid was used. Figure 3 shows the result of this comparison. The evolution of the tests is practically identical in both cases, as is the percentage of hydrogen in the generated biogas that reaches 43% for the two tests, replicating the results obtained in the previous cases.

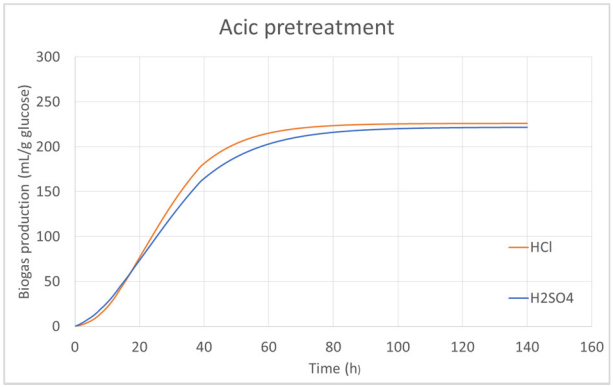

Figure 3. Biogas (H_2+CO_2) production with inoculum pretreated with HCl vs H_2SO_4.

It is concluded that the acid pretreatment of the inoculum in dark fermentation is more convenient than the thermal one, due to the higher biohydrogen production yields achieved in the process, the ease of application of this pretreatment and the lower associated energy cost.

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