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

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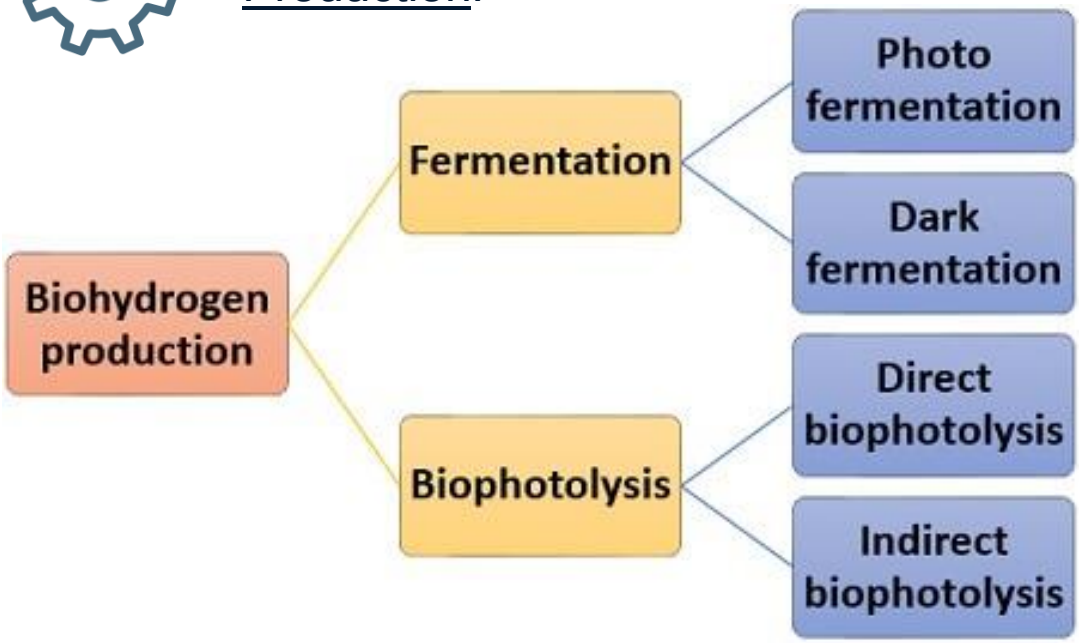
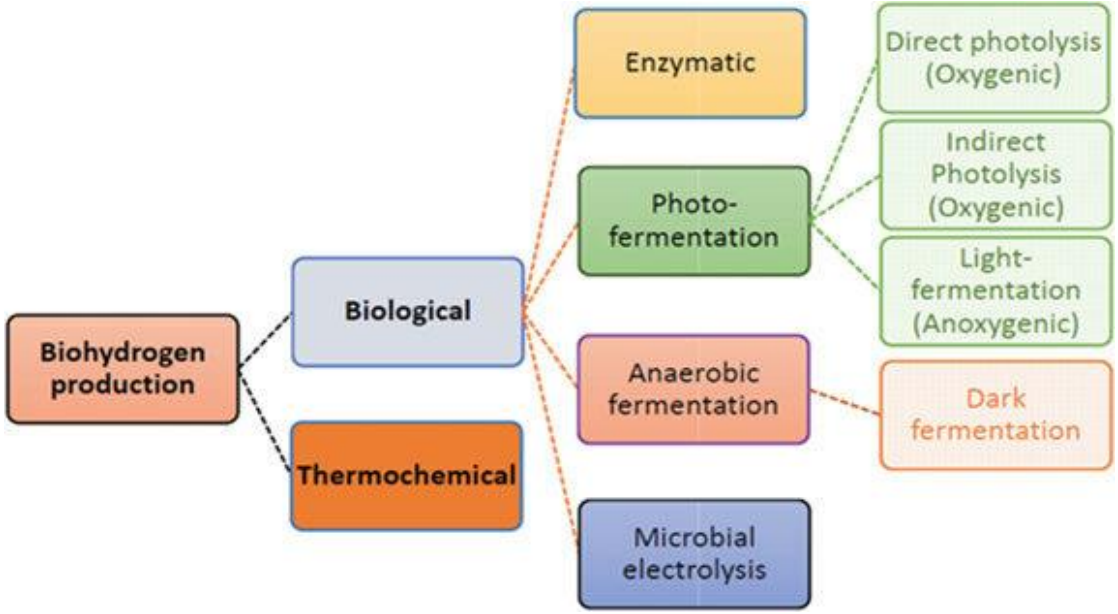
# Introduction


**Biohydrogen** is defined as:

- The biofuel or the source of energy that uses living microorganisms to produce hydrogen via biological processes.
- The hydrogen produced from bio-feedstocks.

Production:



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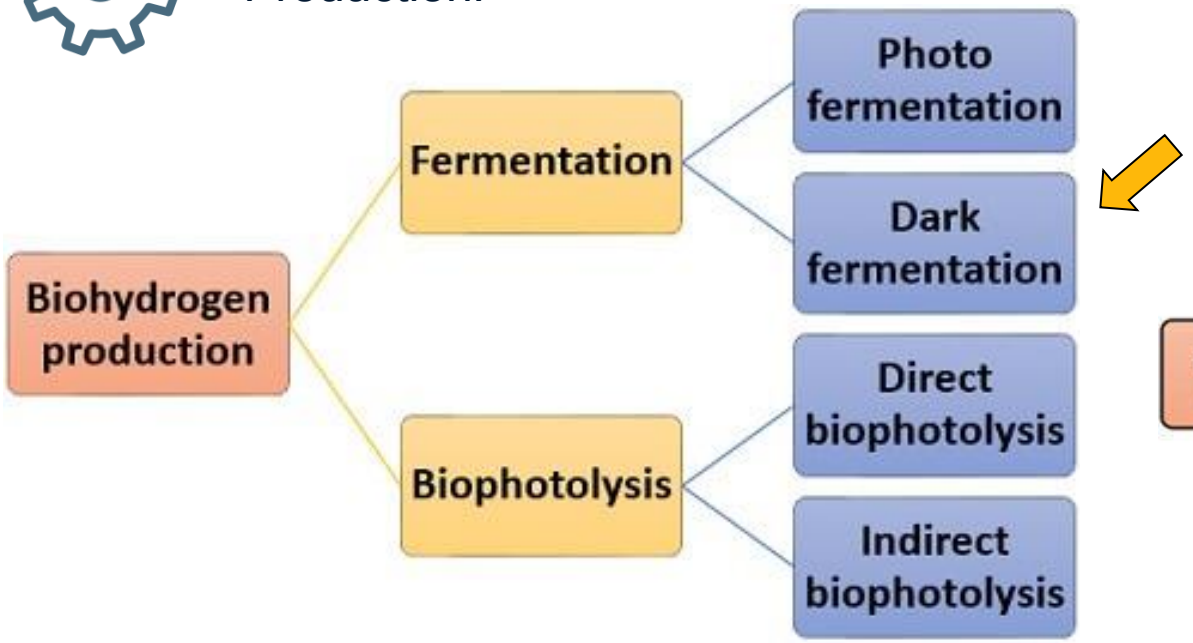


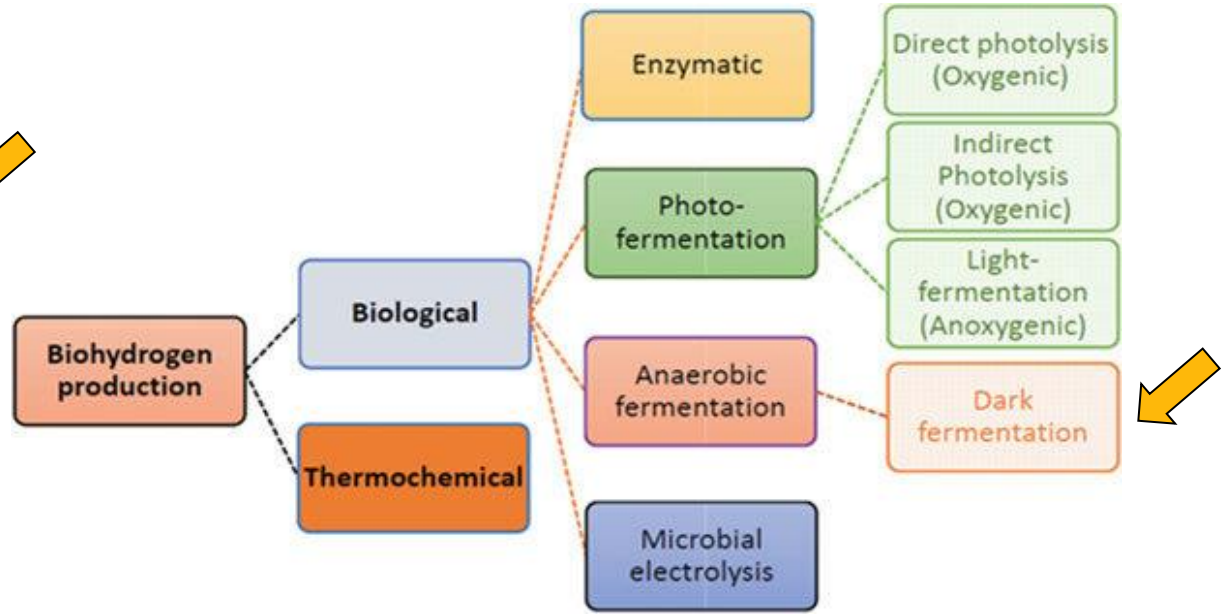

# Introduction

**Dark fermentation** is a part of the acidogenic step of anaerobic digestion.

Involves conversion of organic substrates to hydrogen, carbon dioxide, and non-gaseous products including volatile fatty acids.

# Introduction

**Dark fermentation** can be an attractive option for hydrogen production due to:

- low environmental footprint, and
- potential for large-scale implementation, replacing or (better) operating together with conventional anaerobic digesters that produce methane.

However, as it is a microbiological process:

- all metabolic pathways compete with the production of hydrogen (hydrogenotrophic, homoacetogenic, sulfate-reducing, lactic-acid), and
- the presence of microorganisms that consume this gas must be dynamically controlled inside the digesters to prevent hydrogen production yield from being negatively affected.

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**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.**

# Introduction

There are many ways to pretreat the inoculum before its introduction into the reactor in order to favour the production of hydrogen.

Among them, **heat shock and acid treatment** are perhaps the most interesting with a view to a future industrial scaling of the process.

However, 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.

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**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

## Inoculum and substrates

- **Digestate** from an anaerobic reactor operating in a municipal wastewater treatment plant, was used as inoculum.
- **Glucose** was used as substrate.

## Inoculum pretreatment process

- The methods and operational conditions were selected 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.

# Materials and methods

## Inoculum pretreatment methods and operational conditions

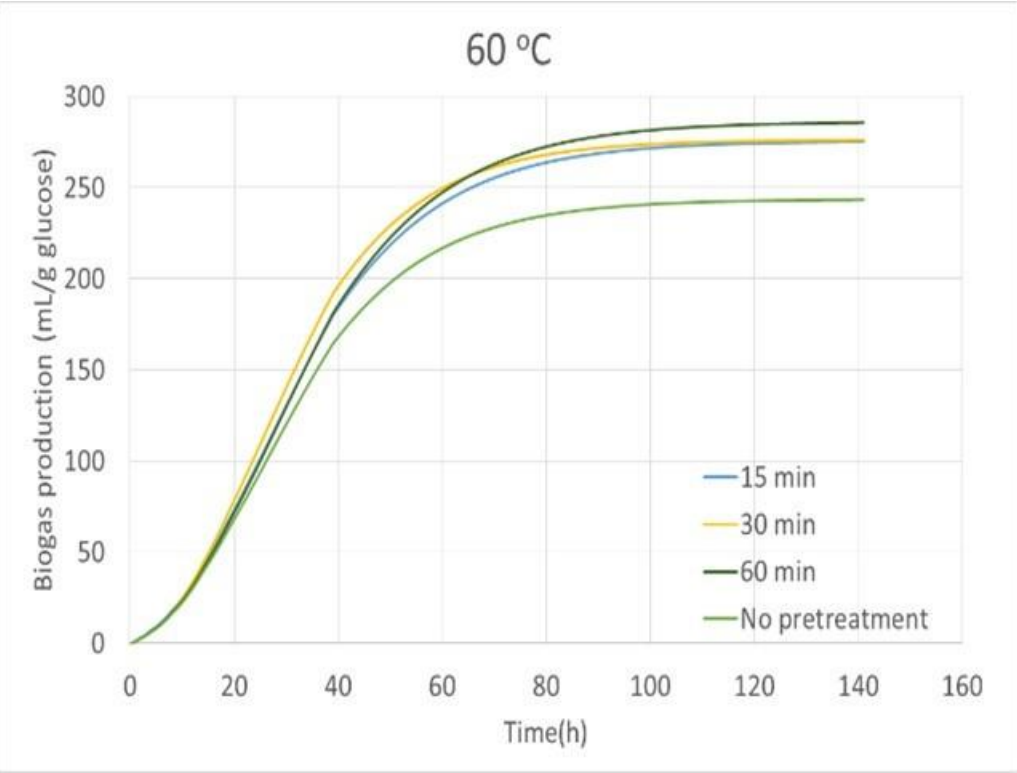
| **Tier 1: Heat shock** | | | | | |
|---|---|---|---|---|---|
| **Time/Temperature** | 60 ºC | | 80 ºC | 100 ºC | |
| **15 min** | Trial 1 | | Trial 4 | Trial 7 | |
| **30 min** | Trial 2 | | *Trial 5* | *Trial 8* | |
| **60 min** | Trial 3 | | Trial 6 | Trial 9 | |
| **Tier 2: Acid(HCl) pretreatment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

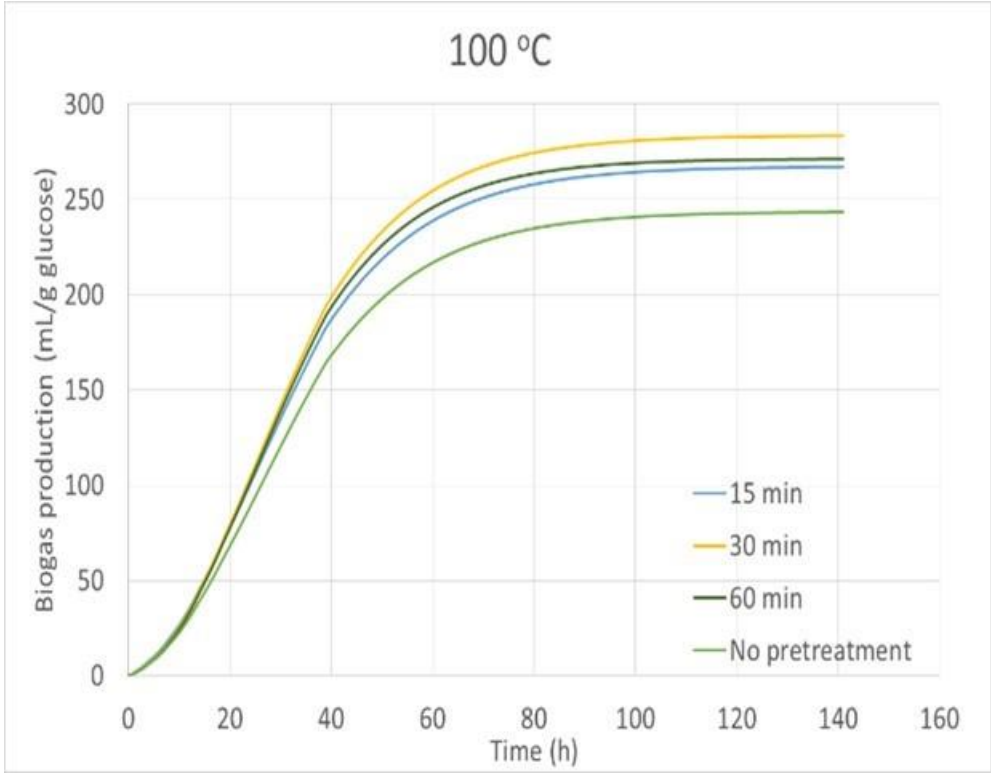
# Materials and methods

## Experimental set up

# Results: Tier 1

- three temperature levels (60º, 80º, and 100º) and three time levels (15, 30 and 60 minutes)




- 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 (CO2+H2) was observed.
- The heating time factor did not seem to have a clear effect on the result.
- The analysis of the composition of the generated biogas reveals, in all cases, a percentage of hydrogen in the mixture of around 47-53% and total absence of methane.

# Results: Tier 2

- After introducing the mixture of substrate and inoculum into the reactor, the pH was adjusted to 5.5 to create an acidic environment.

- 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.

Tier 2: pH adjustment only, thermal shock only (for two temperature levels), pH adjustment + thermal shock

# Results: Tier 2

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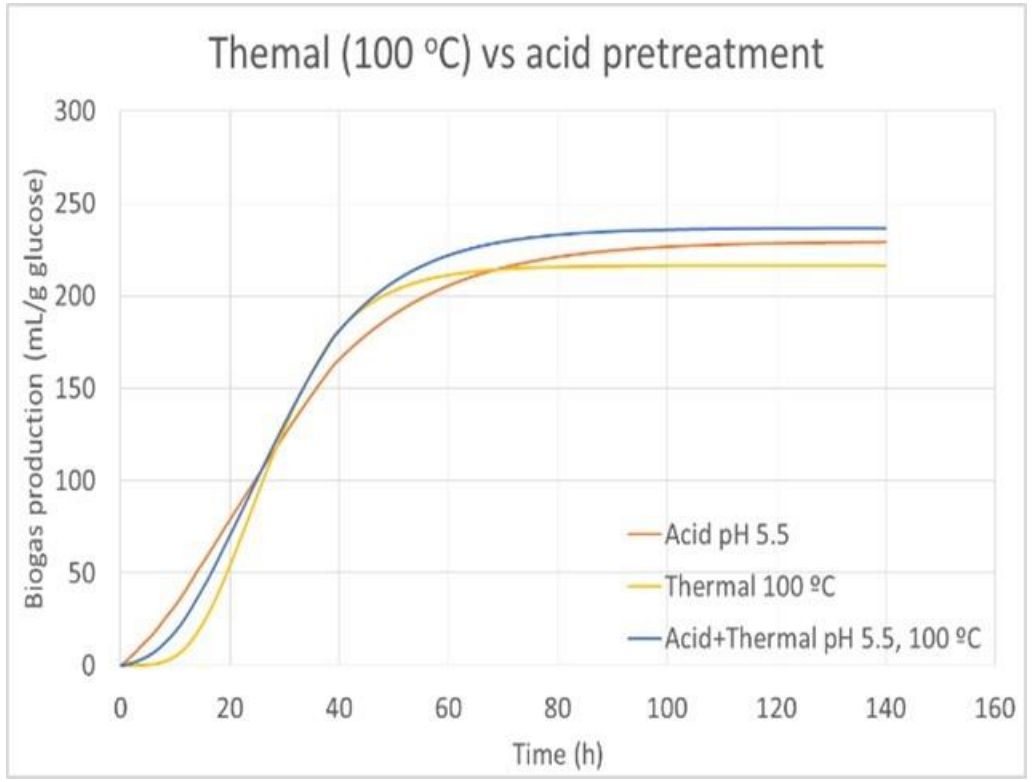
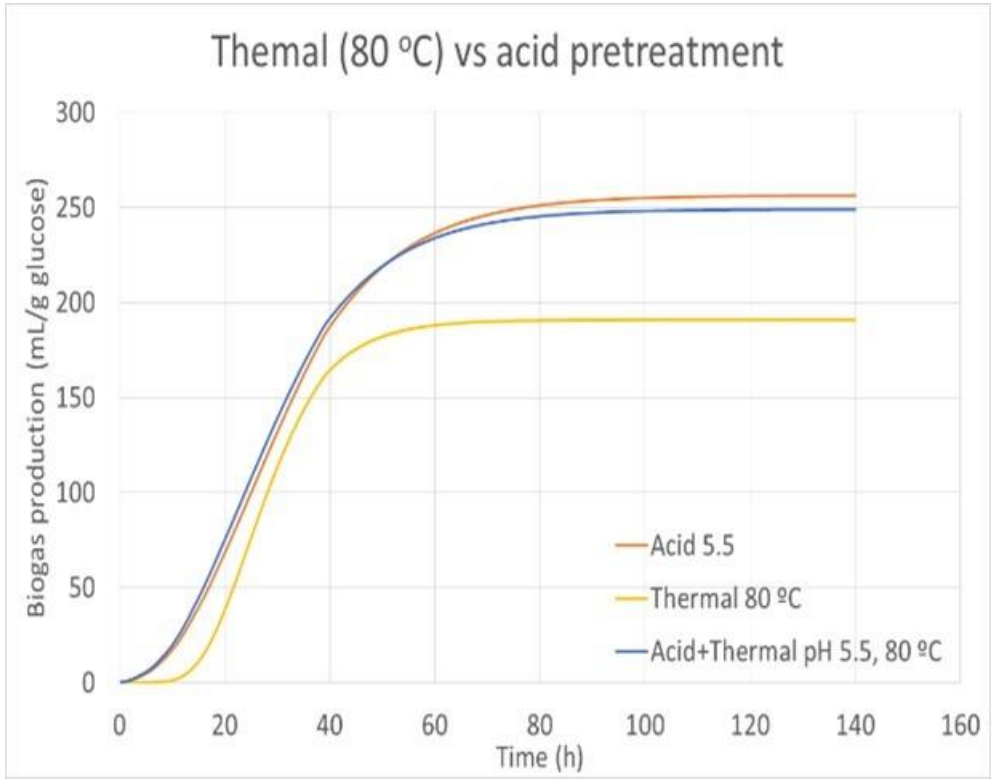


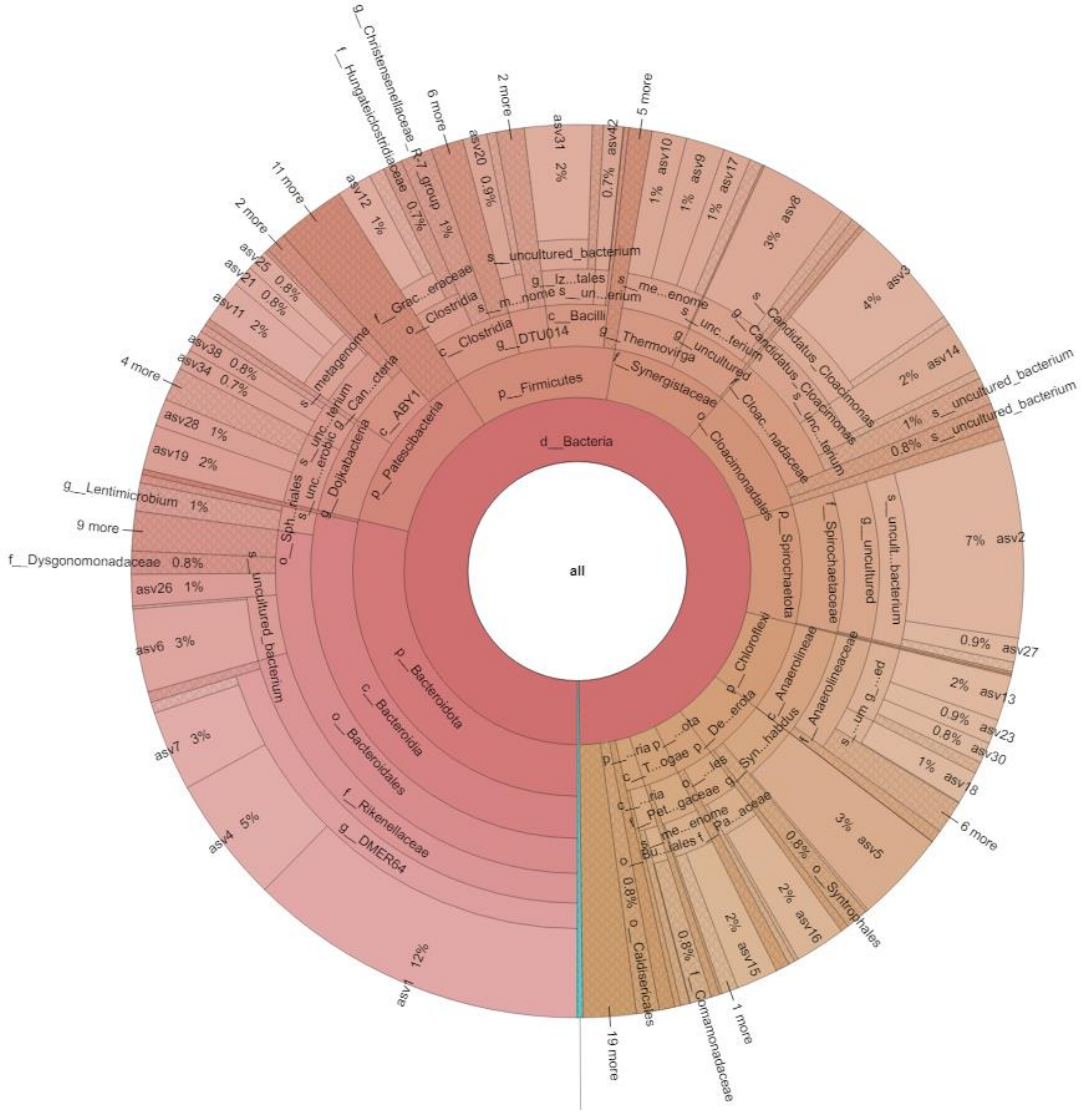

- The biogas production is lower for the samples in which the acid pretreatment was not applied. These had an initial lag phase and their maximum production was lower than in the samples with the acid pretreatment.
- However, between the samples with acid pretreatment and those that received both pretreatments, there is no clear difference in biogas production. This indicates that the pH adjustment is being critical in the production of biogas (H2).

# Results: Tier 2

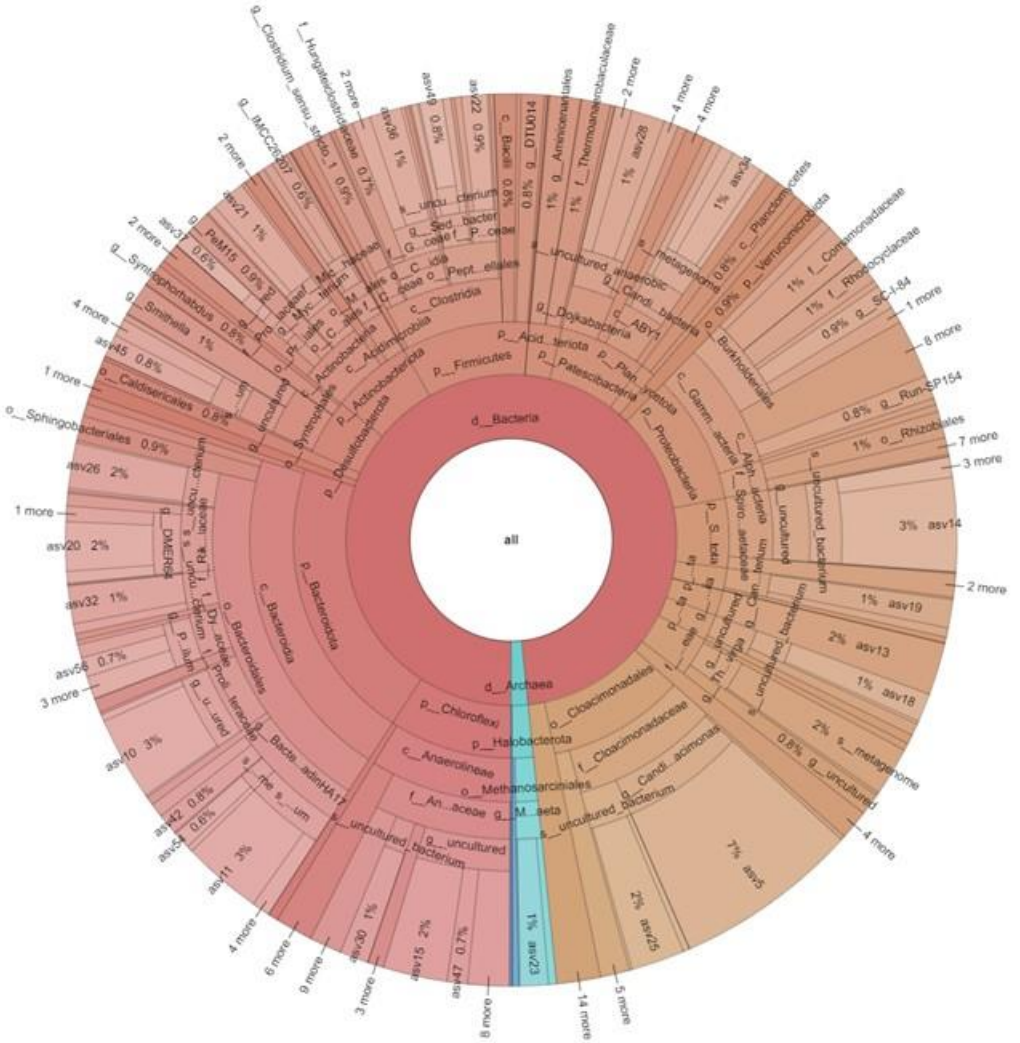
- The acidification of the medium seems to have more influence on the production of hydrogen, making the influence of the thermal pretreatment not appreciable if the sample is also acidified.

- The analysis of the composition of the generated biogas reveals a percentage of hydrogen in the mixture around 55% when the inoculum has been acidified, but this percentage falls to 53% when exclusively thermal pretreatment is applied, and this result is common for both temperatures tested.

# Microbiota after pretreatment




After thermal pretreatment



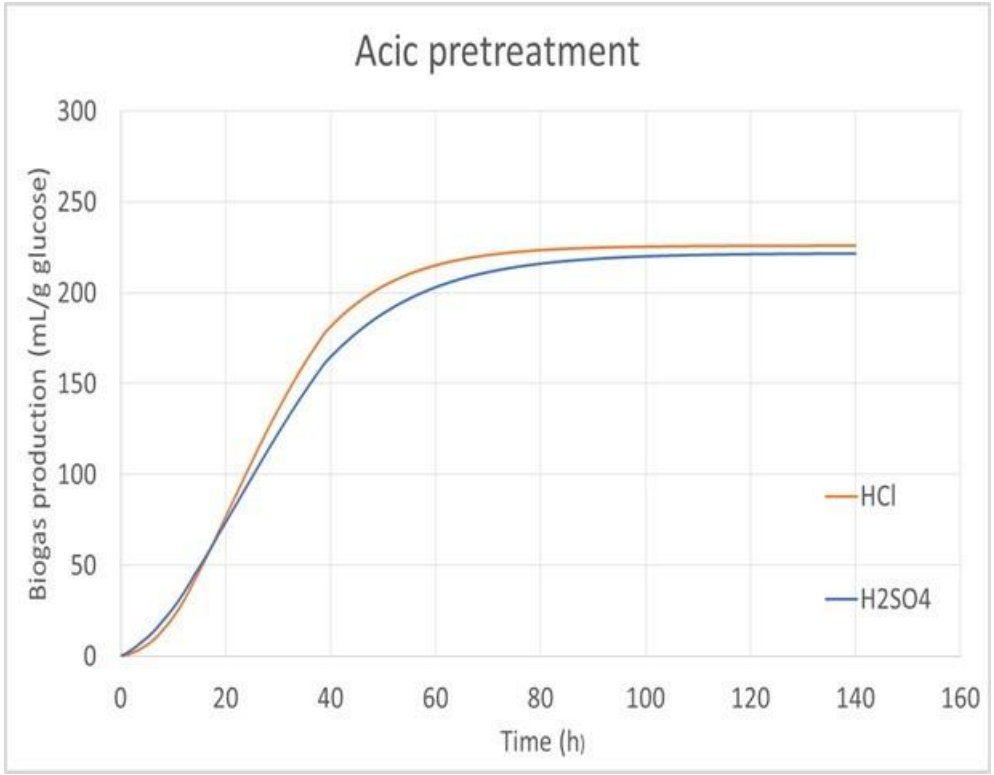

After acid pretreatment

# Results: Tier 3

- pH adjustment only with HCl vs H2SO4

- 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.




- The evolution of the tests is practically identical in both cases, as is the percentage of hydrogen in the generated biogas that reaches 53% for the two tests, replicating the results obtained in the previous cases.

# Summary and Conclusions

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.

# Next steps

What about industrial wastewater streams as carbon source?

# Results: Tier 4

- Influence of the type of inoculum and the type of carbon source:
  - Carbon source: 1) glucose (G) and 2) sugar beet factory wastewater (SBWW).
  - Inoculum: 1) municipal WWTP digestate (MD) and 2) sugar beet factory WWTP digestate (SBD).

| | |
|---|---|
| G + MD | SBWW + MD |
| G + SBD | SBWW + SBD |

- The pH was adjusted in the same way as in previous assays (with HCl).
- Since the amount of organic matter and solids varies in each substrate and digestate, an S/X ratio of 2 g COD/g VS and 2 g COD fed per reactor was set.
- This quantity was determined considering that the concentrations of VS are very different between the digestates used, with a value of 4.7 g/L for the SBD and 12.21 g/L for the MD.

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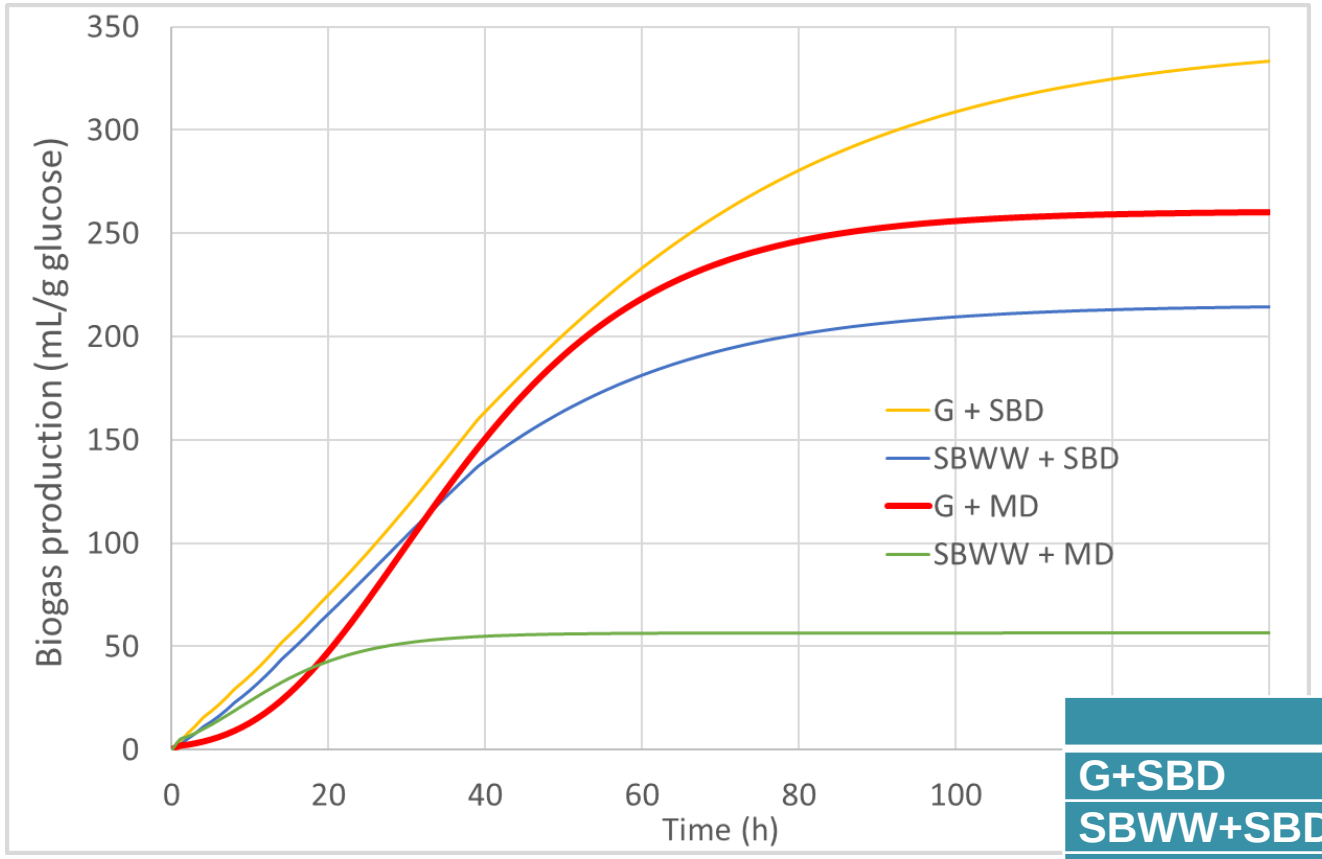


| | %H2 | %CH4 | pH 0 | pH final |
|---|---|---|---|---|
| G+SBD | from 12 to <1 | from 18 to 60 | 5,5 | 6,7 |
| SBWW+SBD | < 1 | 65 | 5,5 | 6,9 |
| G+MD | 50 | 0 | 5,5 | 3,6 |
| SBWW+MD | < 1 | 19 | 5,5 | 6,4 |

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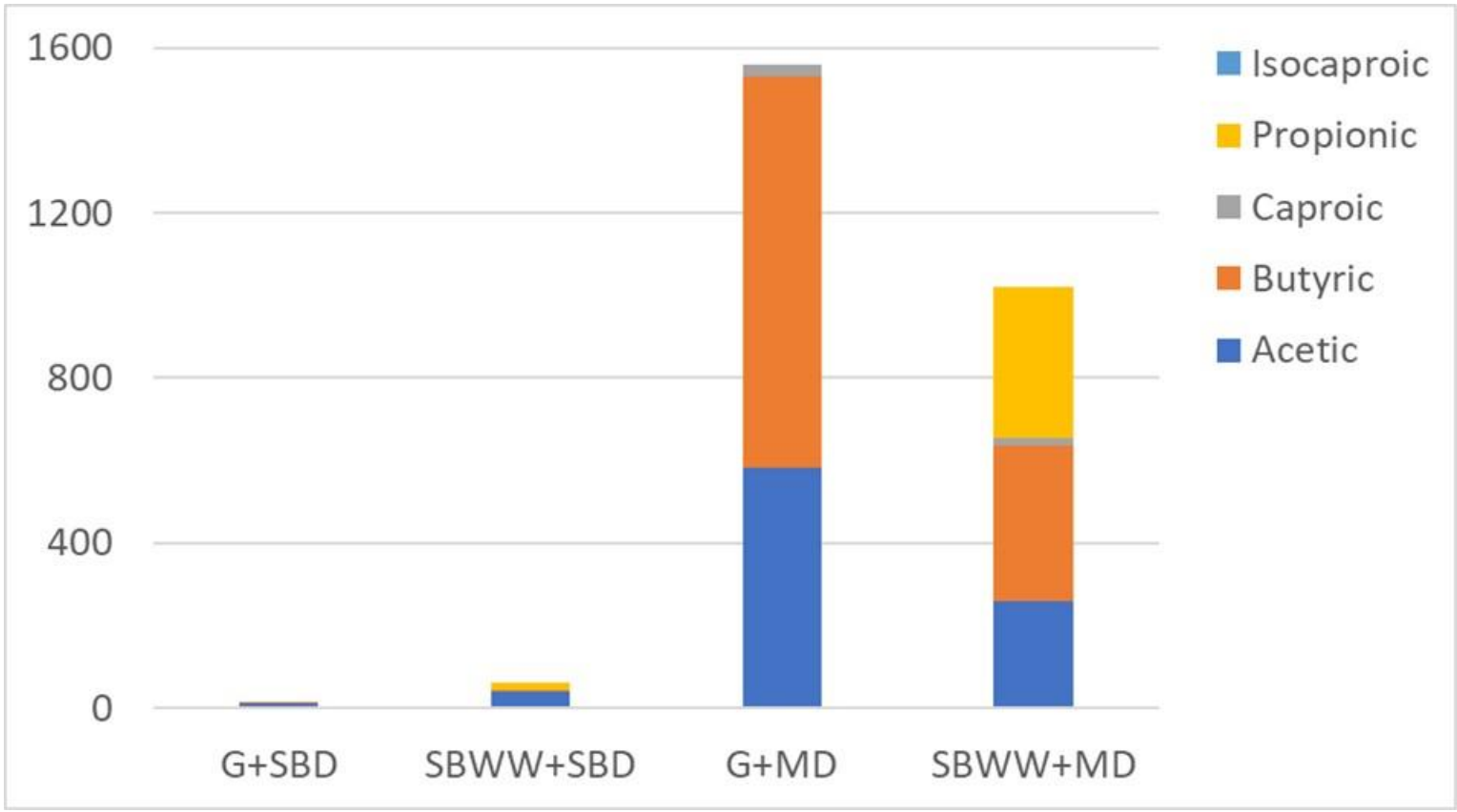


NEW
KEEP
CALM
BECAUSE
RESULTS ARE
COMING SOON

# Thank you for your attention

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