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**Enhanced biohydrogen production by dark
fermentation using additives**

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Introduction

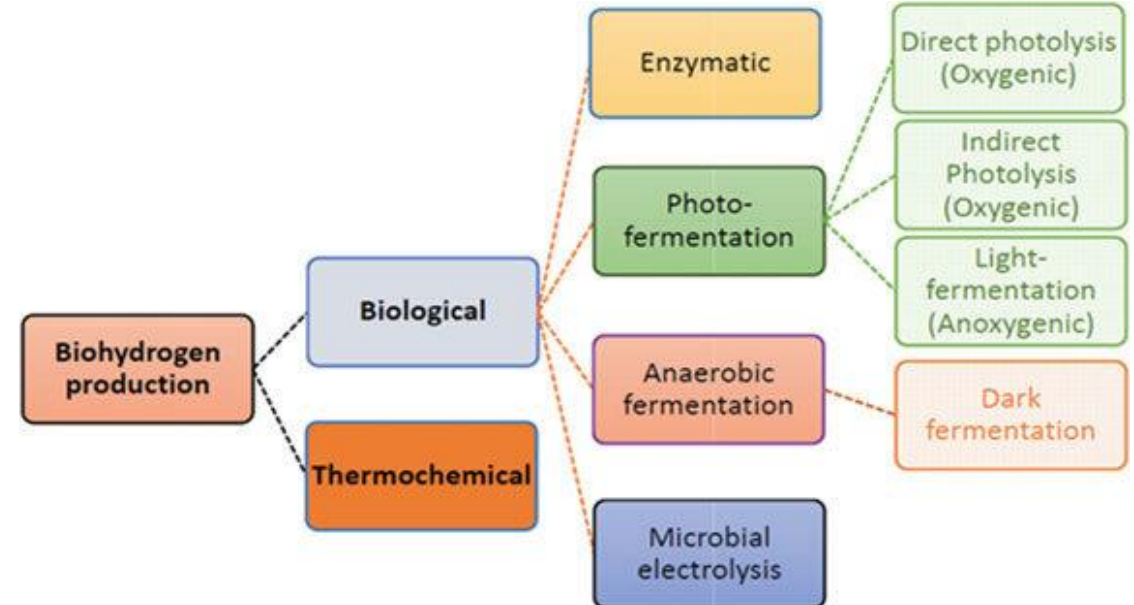
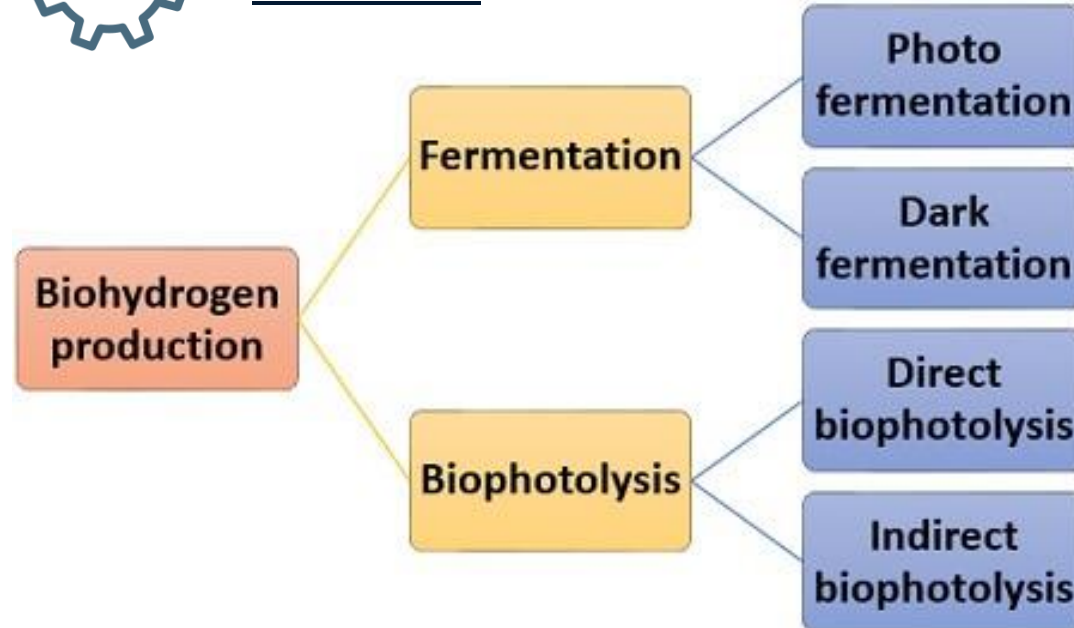


Biohydrogen is defined as:

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



Production:



Introduction

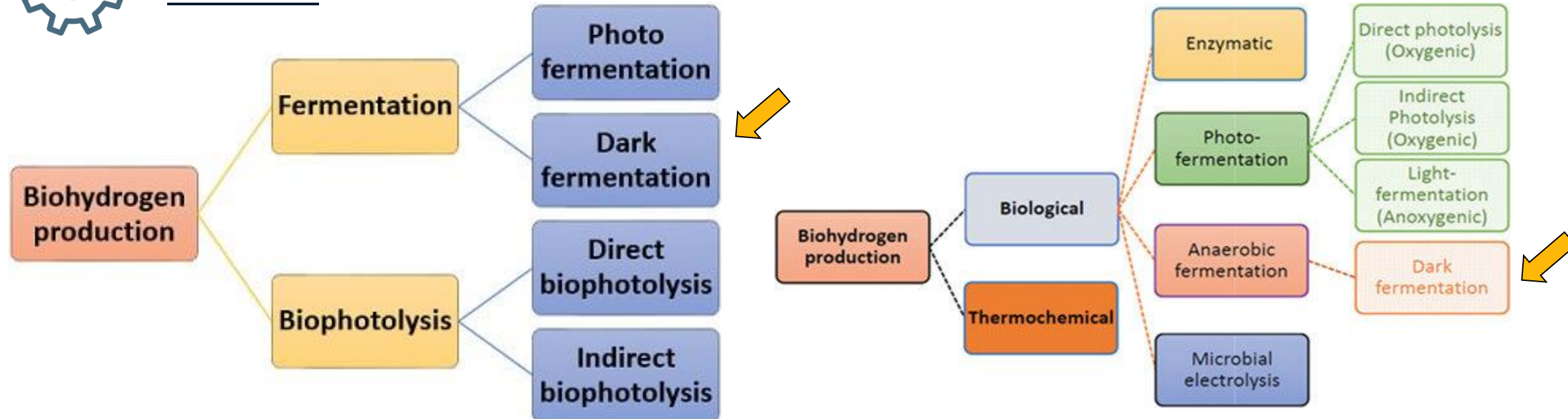


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Production:



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.





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:

- dark fermentation is commonly associated with a low yield of hydrogen production compared to traditional thermochemical processes.



It is imperative to design appropriate strategies to make biological hydrogen production technologically and economically viable when compared to other more conventional methods.

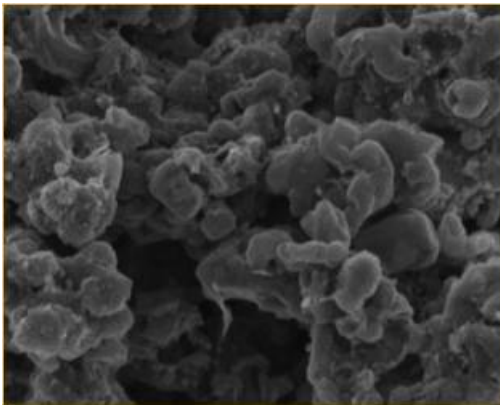
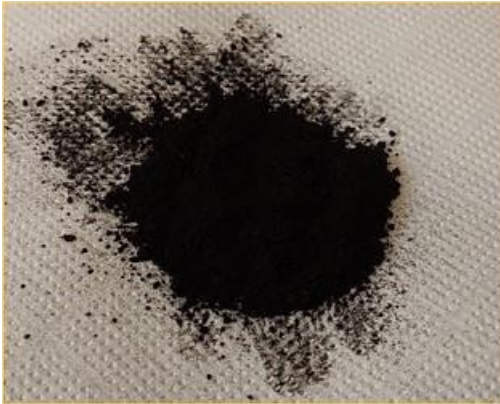
Supplementation of additives to the fermenter with the aim of intensifying the process is an interesting option.

Additives can facilitate by different ways **the microbial growth and enzymatic activity** in dark fermentation, thereby leading to the enhancement of process performance.

The effects of these additives can vary depending on factors such as their concentration, interaction with the microbial community, and the specific conditions of the dark fermentation process. Therefore, their effectiveness and impact on fermentation outcomes may differ in different systems and applications.

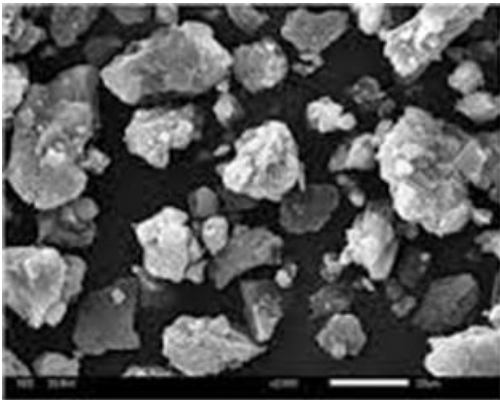
This work aims to shed some light on this process through the comparative study of three types of additives analyzing their effect on the dark fermentation process and hydrogen generation.

Additive 1: Zero-valent iron nanoparticles (Fe^0 NP)



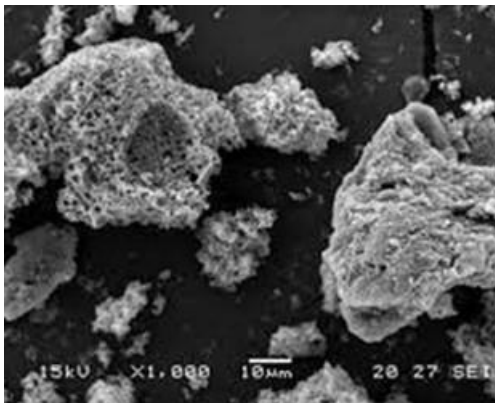
- Fe^0 NP have unique properties that make them useful in various applications, including dark fermentation.
- They can act as catalysts for certain microbial reactions, such as the conversion of organic compounds into hydrogen or other desired fermentation products.
- Fe^0 NP can enhance the efficiency of dark fermentation by providing a surface for microbial adhesion and facilitating electron transfer processes, thus promoting the production of target products.
- They can also have antimicrobial effects, which can help maintain a favorable microbial community during fermentation.

Additive 2: **Active carbon**



- Activated carbon is a highly porous material that can adsorb organic compounds.
- In dark fermentation, activated carbon can be used as an additive to remove inhibitory substances or byproducts from the fermentation medium.
- It can help in detoxifying the substrate by adsorbing compounds that may inhibit microbial activity, thus improving the fermentation efficiency.
- Additionally, activated carbon can serve as a support material for bacterial attachment, providing a surface for microbial colonization and promoting biofilm formation.

Additive 3: **Hydrochar**



- Hydrochar is a carbonaceous material produced through the hydrothermal carbonization of biomass.
- It has a high carbon content and can be rich in nutrients and beneficial compounds.
- When used as an additive in dark fermentation, hydrochar can serve as a carbon source for microbial growth.
- It provides additional organic matter that can be readily utilized by fermentative bacteria, leading to increased microbial activity and improved fermentation performance.
- Hydrochar can also contribute to the stability of the fermentation medium by acting as a buffer, helping to maintain optimal pH conditions.

Materials and methods

- 1 L stainless steel reactors, with 500 mL of working volume were used.
- Digestate from an anaerobic reactor operating in a municipal wastewater treatment plant and glucose were used as inoculum and carbon source, respectively in the first tiers (**Tier 1, Tier 2, Tier 3**).
- Digestate from an anaerobic reactor operating in a sugar beet factory and residual effluent from this factory were used in the second group of test (**Tier 4**).
- **Thermal preatreatments** were carried out ranging between 60 and 100 °C in periods of 15, 30 and 60 minutes.
- **HCl** was used to perform the acidification of the inoculum, bringing all tests to pH 5.5.



Materials and methods

- Anaerobic conditions were established before sealing.
- The reactors were placed on a shaking table in a thermostatic room at $34\pm 1^{\circ}\text{C}$ until exhaustion of biogas production.
- Pressure was monitored by sensors connected to each reactor.
- Biogas production curves were fitted to the Gompertz equation.
- Gas samples were extracted and analysed periodically using gas chromatography.
- VFAs (Volatile fatty acids) and pH were measured before and after the experiment.

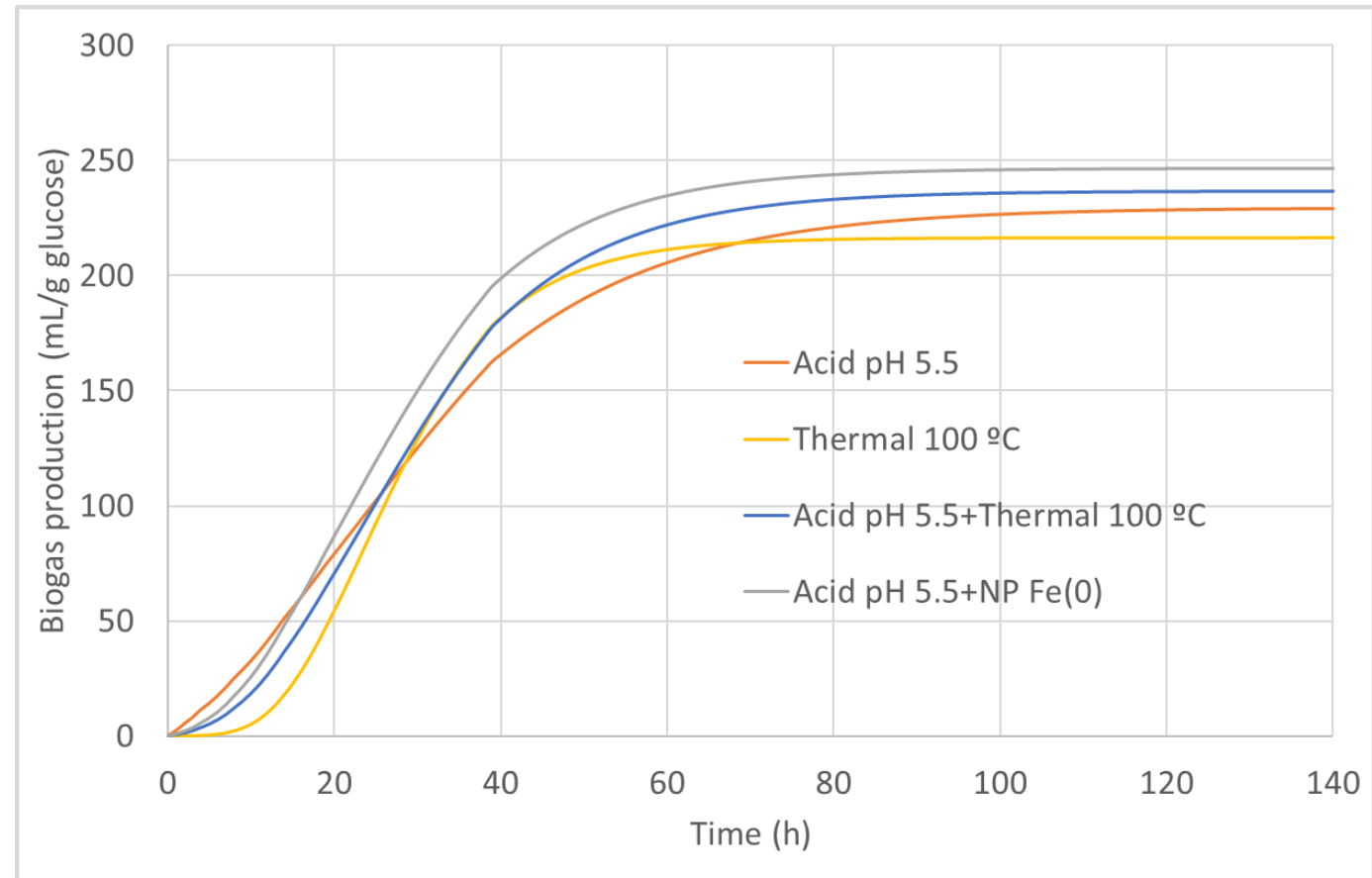


Results: Tier 1

- Influence of inoculum pretreatment and NP addition:
pH adjustment only, thermal shock only (100°); pH adjustment + thermal shock; pH adjustment + 200 mg/L NP Fe(0)
- Glucose as carbon source and digestate from municipal WWTP as inoculum.

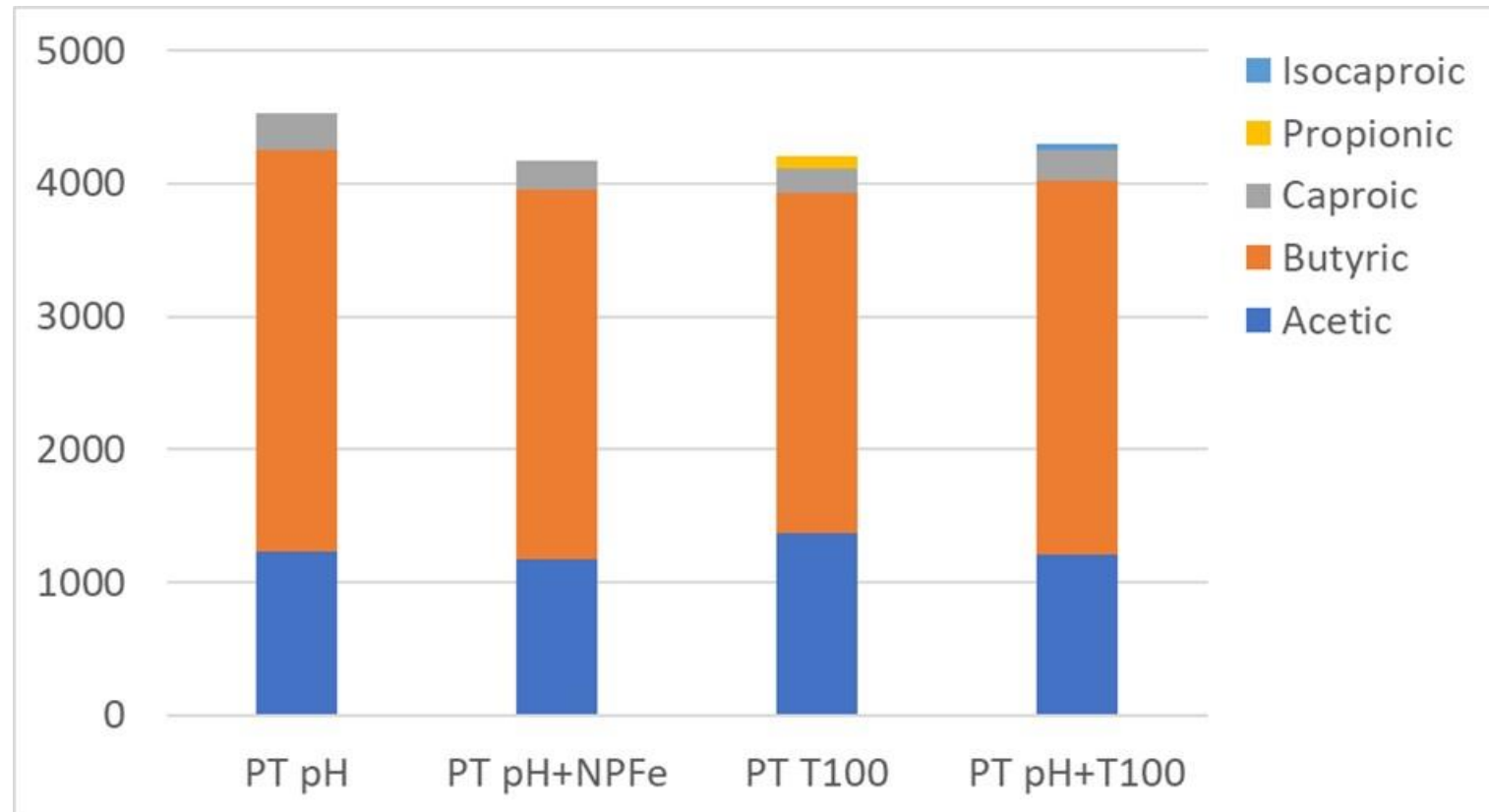
- The biogas production was similar for all samples, with the **NP-added samples being the most productive** and the exclusively thermally pre-treated samples being the least productive.

- The generated biogas contained around **50% H₂** in all cases except for the one with exclusively thermally pre-treated inoculum, where this percentage decreased to 47%.



Results: Tier 1

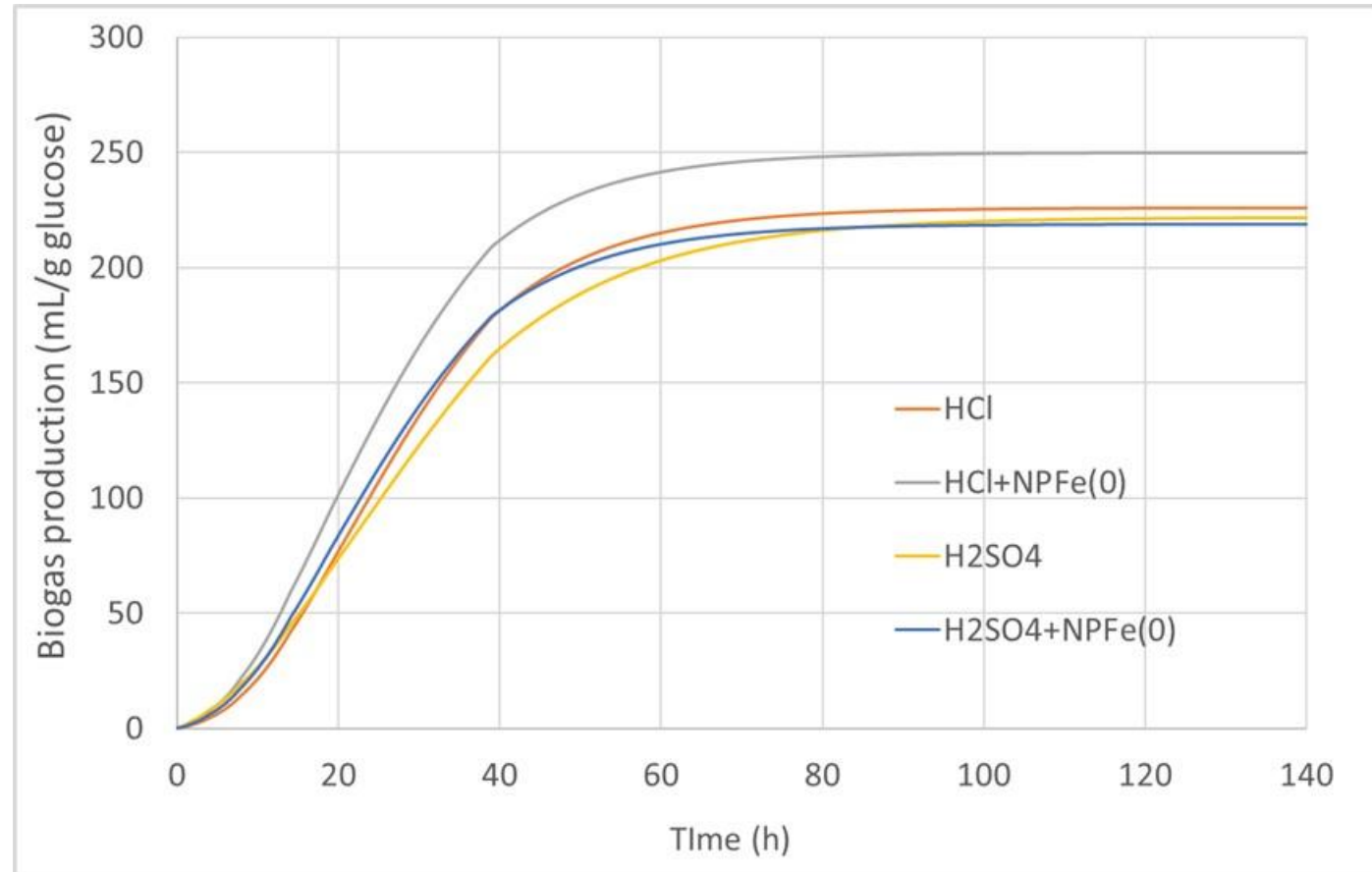
- Influence of inoculum pretreatment and NP addition:
pH adjustment only, thermal shock only (100°); pH adjustment + thermal shock; pH adjustment + 200 mg/L NP Fe(0)
- Glucose as carbon source and digestate from municipal WWTP as inoculum.



Results: Tier 2

- Influence of acid compound used during pretreatment: HCl vs H_2SO_4 , when NP Fe(0) is added.
- Glucose as carbon source and digestate from municipal WWTP as inoculum.

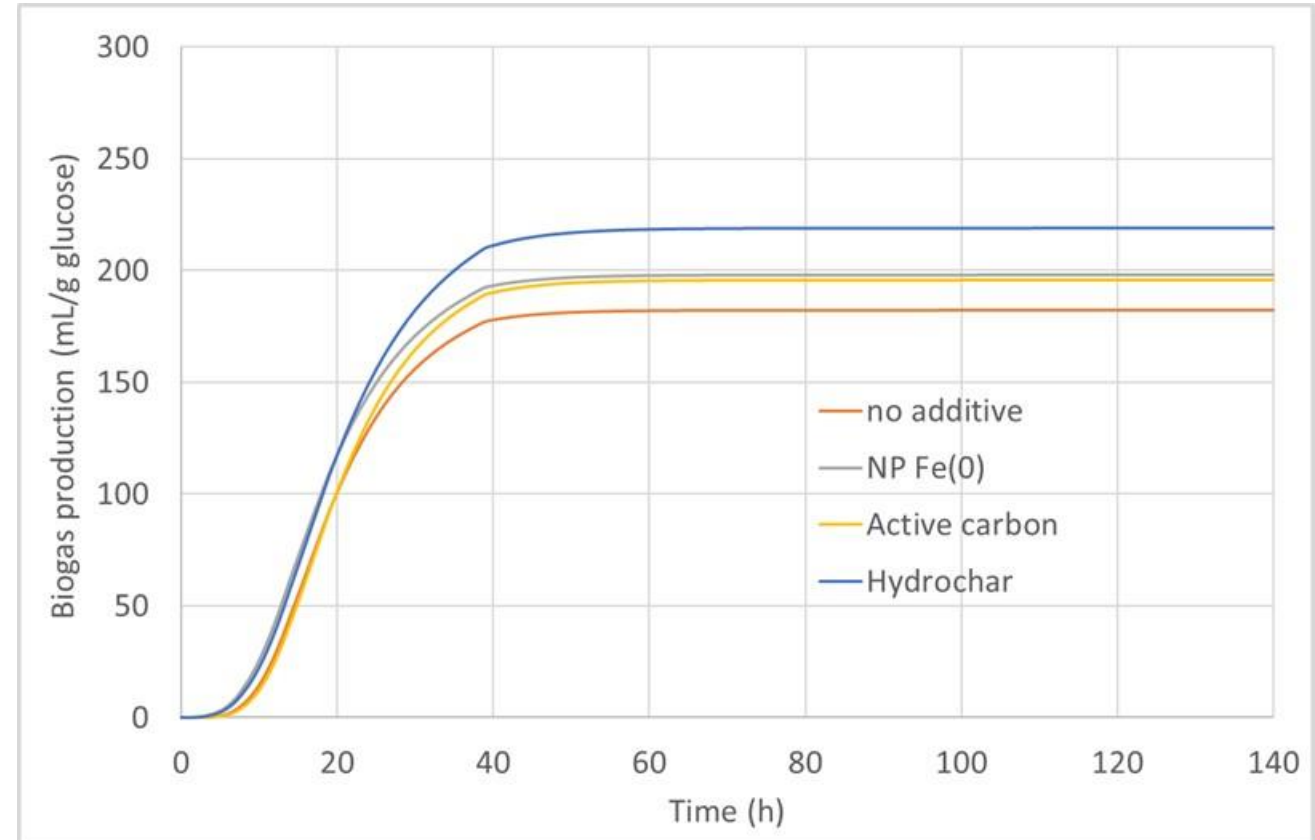
- The sample with NP Fe(0) addition and inoculum pre-treatment with HCl stood out in biogas production.
- The generated biogas contained around 50% hydrogen in all cases.



Results: Tier 3

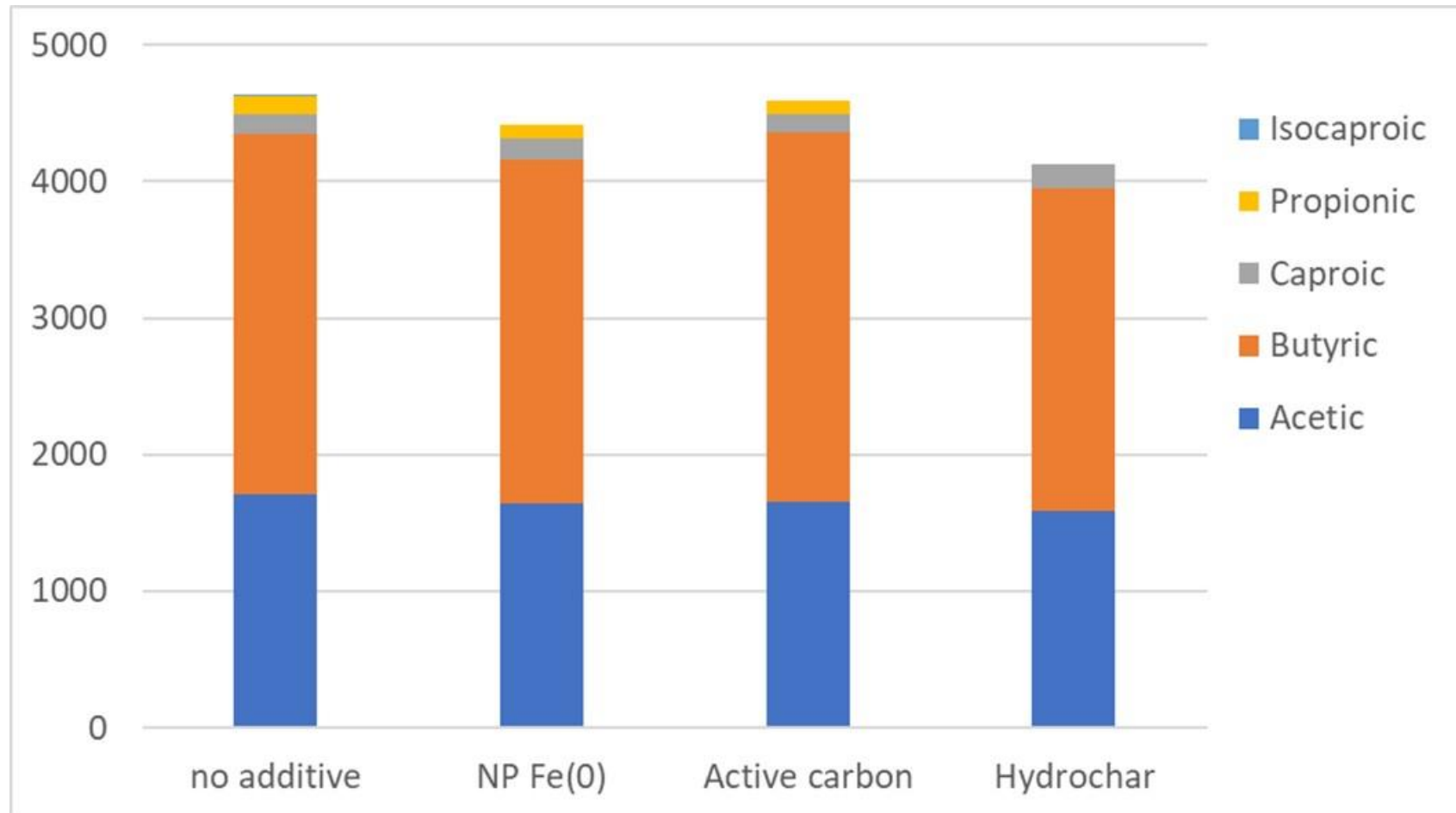
- Influence of different additives: NP Fe(0), activated carbon and hydrochar (200 mg/L).
- Glucose as carbon source and digestate from municipal WWTP as inoculum.

- The use of additives revealed to affect positively the biogas yield.
- Maximum biogas production is obtained by using hydrochar as an additive.
- The curves corresponding to the test with activated carbon and iron nanoparticles.
- Clearly, the test without the use of additive was the one that generated less biogas.
- The generated biogas contained around 50% hydrogen in all cases.



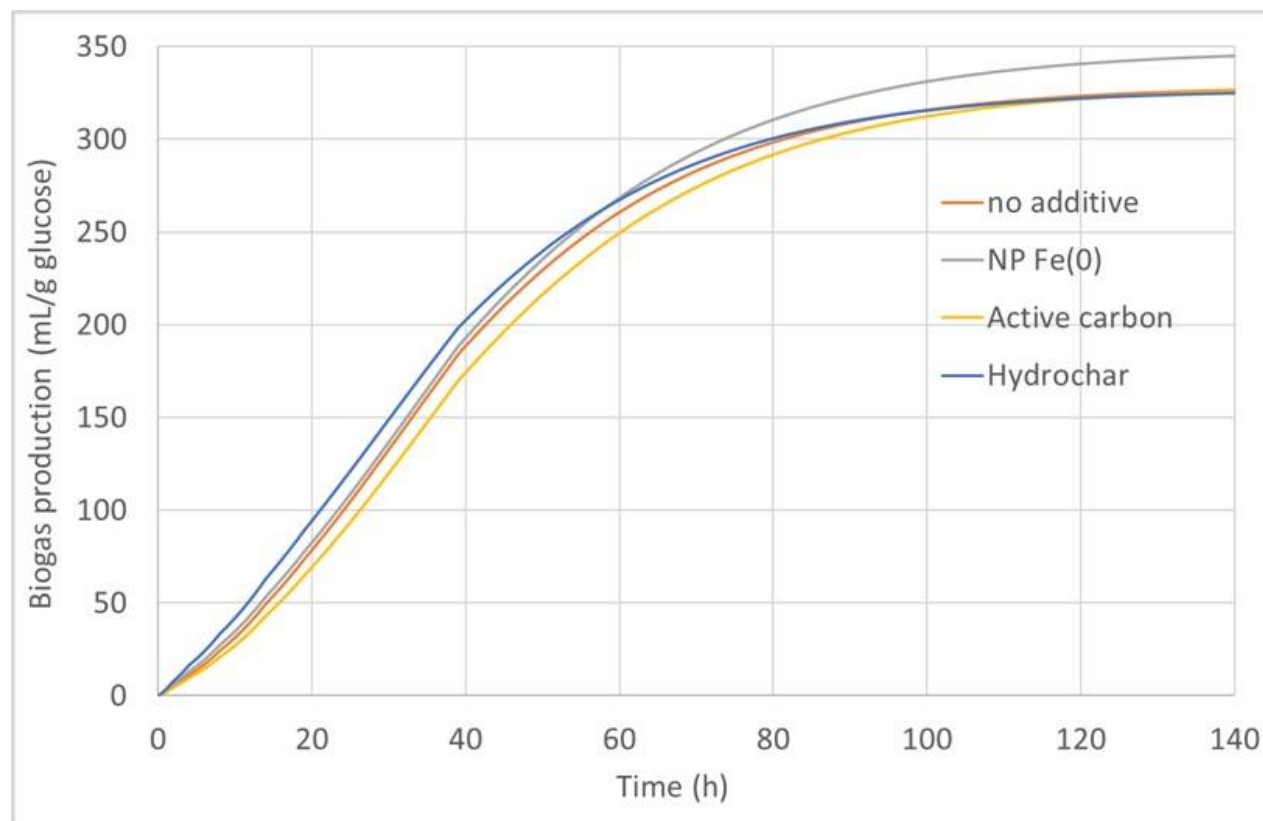
Results: Tier 3

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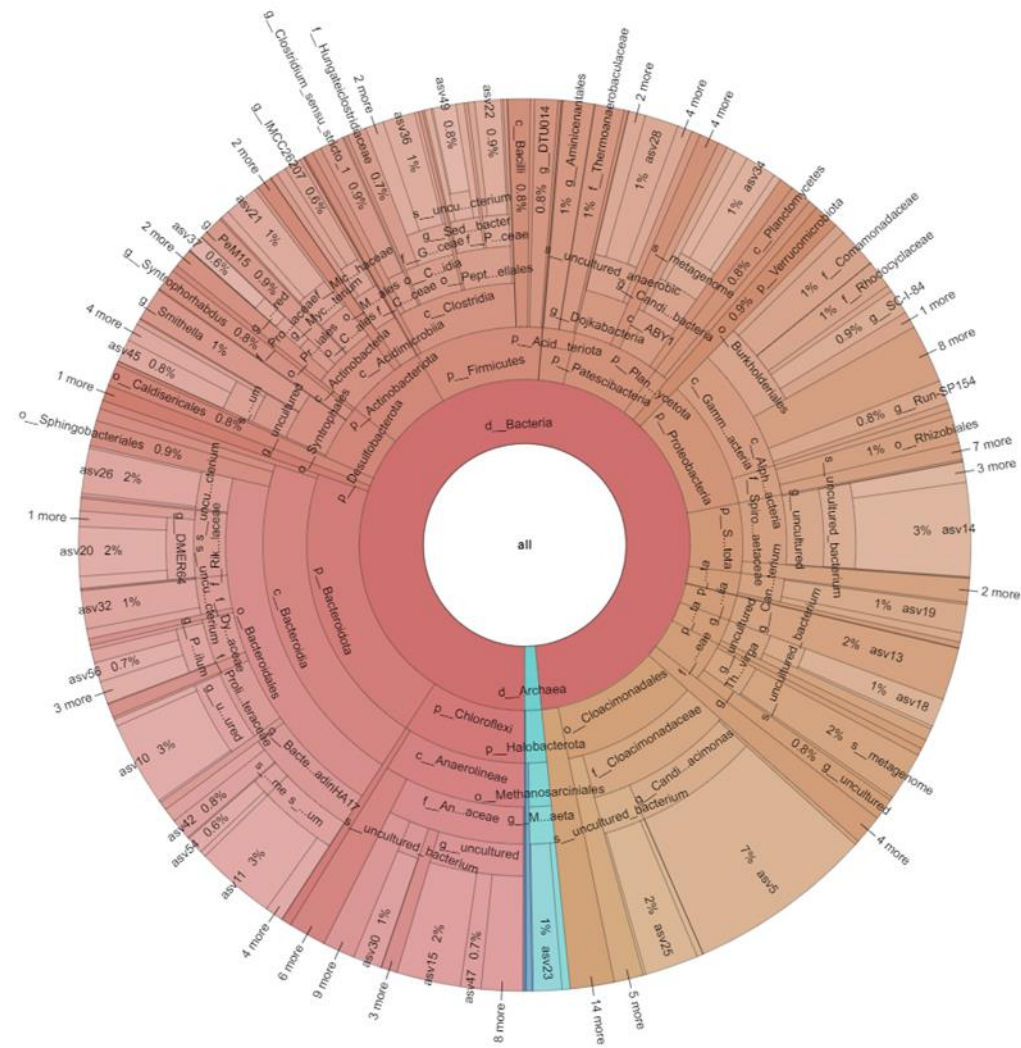


Results: Tier 4

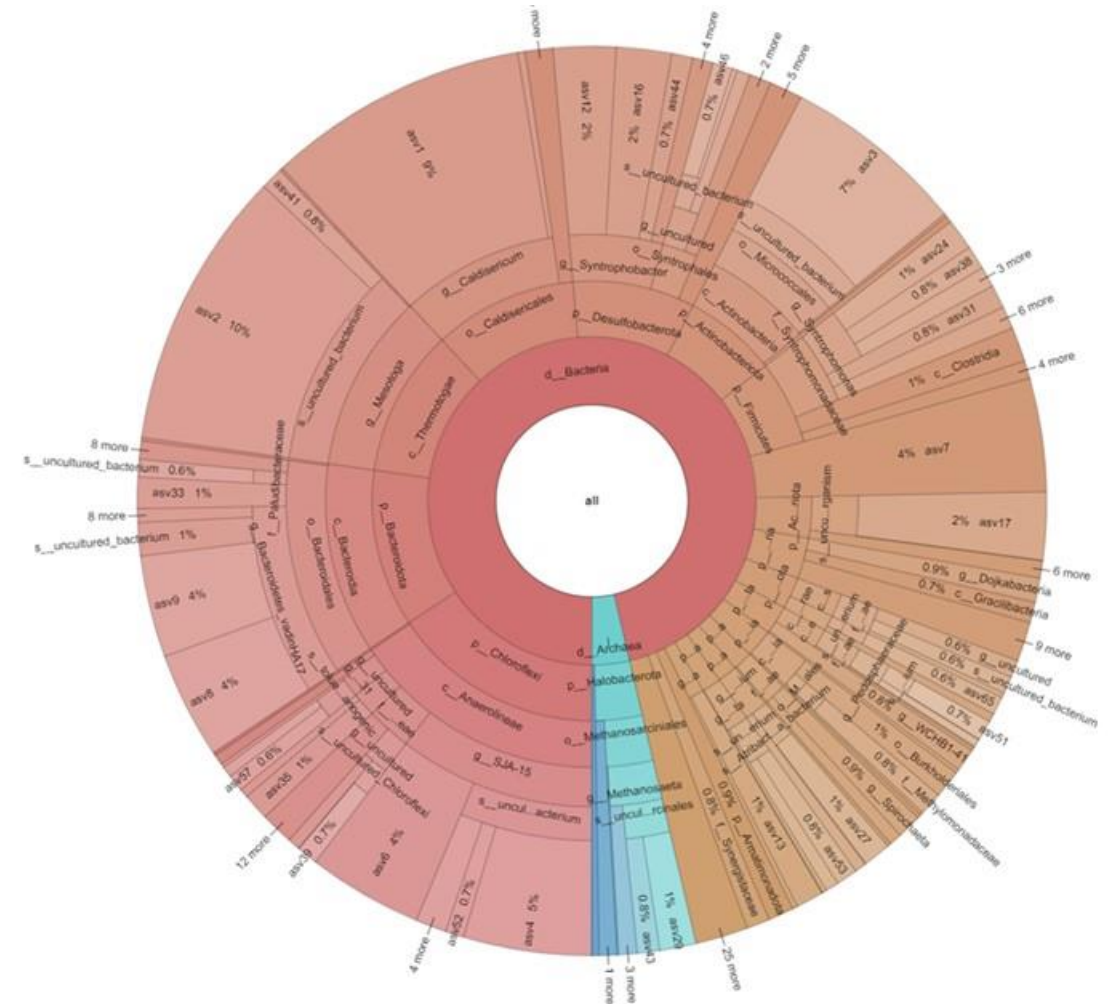
- Performance of additives with real wastewater:
 - Carbon source: sugar beet factory wastewater (SBWW); Inoculum: sugar beet factory WWTP digestate (SBD).
- The reactors were adjusted to pH 5.5 at the beginning of the assay using HCl.
- After the reaction, a **pH close to 7** was reached in all tests.
- Biogas analysis revealed a majority composition **of CH₄ (>60%)** and the absence of H₂ except in the initial moments.
- The **high alkalinity** of the SBWW sample (> 500 mg/L) is considered to be the cause of pH recovery and the emergence of conditions that favor methanogenesis.



Microbiota after acid pretreatment



Digestate from municipal WWTP



Digestate from sugar beet WWTP

Tested additives - **NPFe(0)**, **active carbon** and **hydrochar** - effectively enhanced the fermentation process:

- Under acidic conditions (pH 4-5.5) **H₂** production is clearly stimulated, hydrochar being the additive showing better results.
- At pH>6, **CH₄** production is favored, with NP Fe(0) as the most efficient additive.
- **Alkalinity** of the influent is key in the performance of the fermentation.
- High alkalinity levels hinder fermentation evolution to H₂ production.

Thank you for your attention

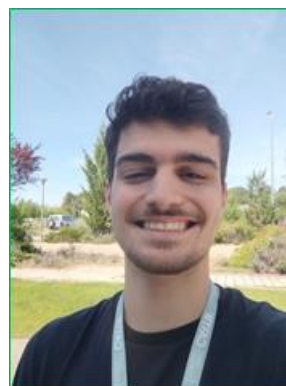


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