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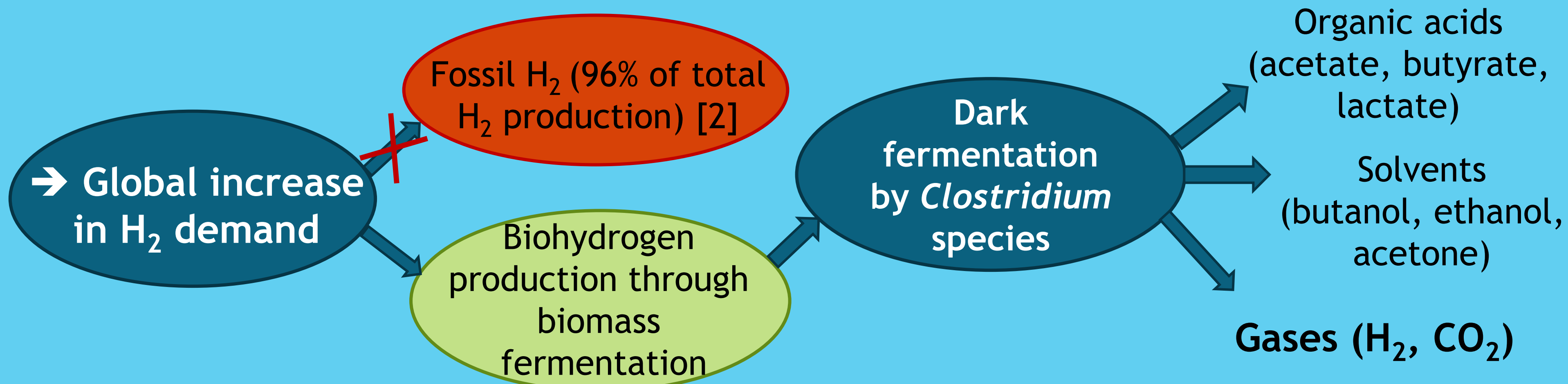
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Comparative analysis and improvement of biological hydrogen production in *Clostridium* genus

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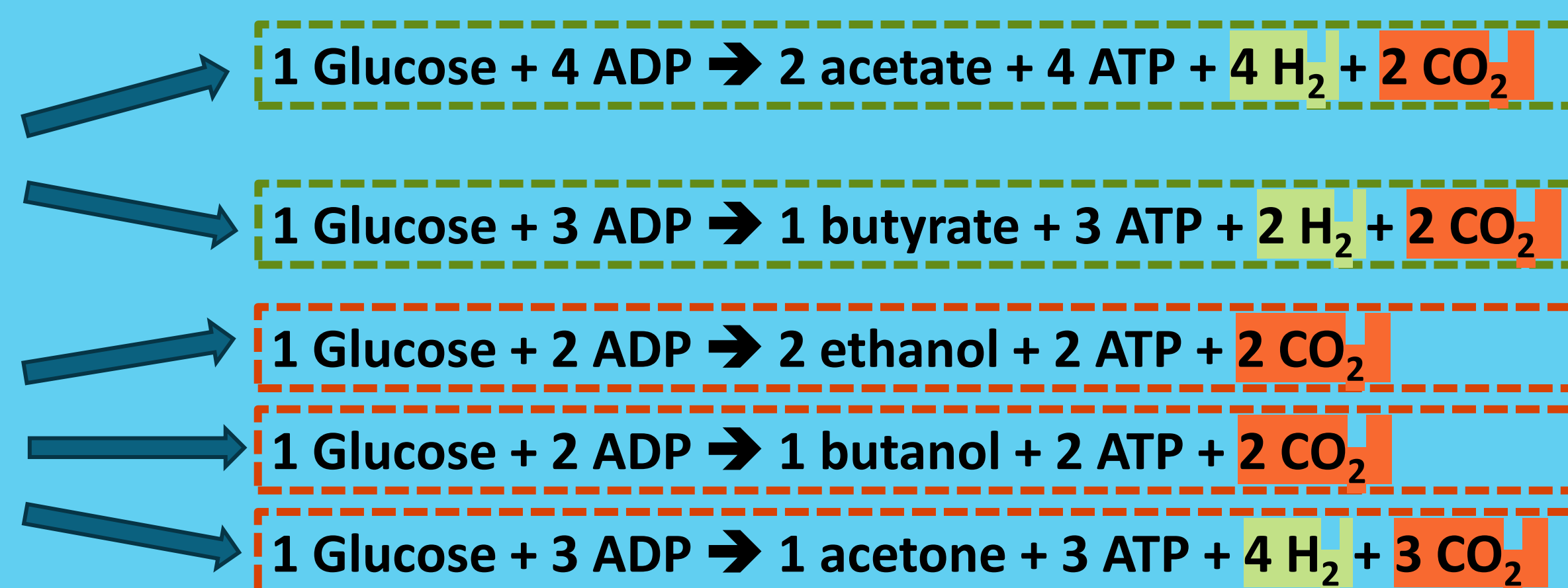
Scientific context and objectives

Many industries already use gaseous hydrogen (H₂) for their activities (ammonia and methanol production refining of oil products). Now, H₂ is seen as a potential energy vector for other industries, resulting in the need to produce more of this gas [1].



→ Need to identify the most sustainable strain and operating conditions to enhance H₂ production.

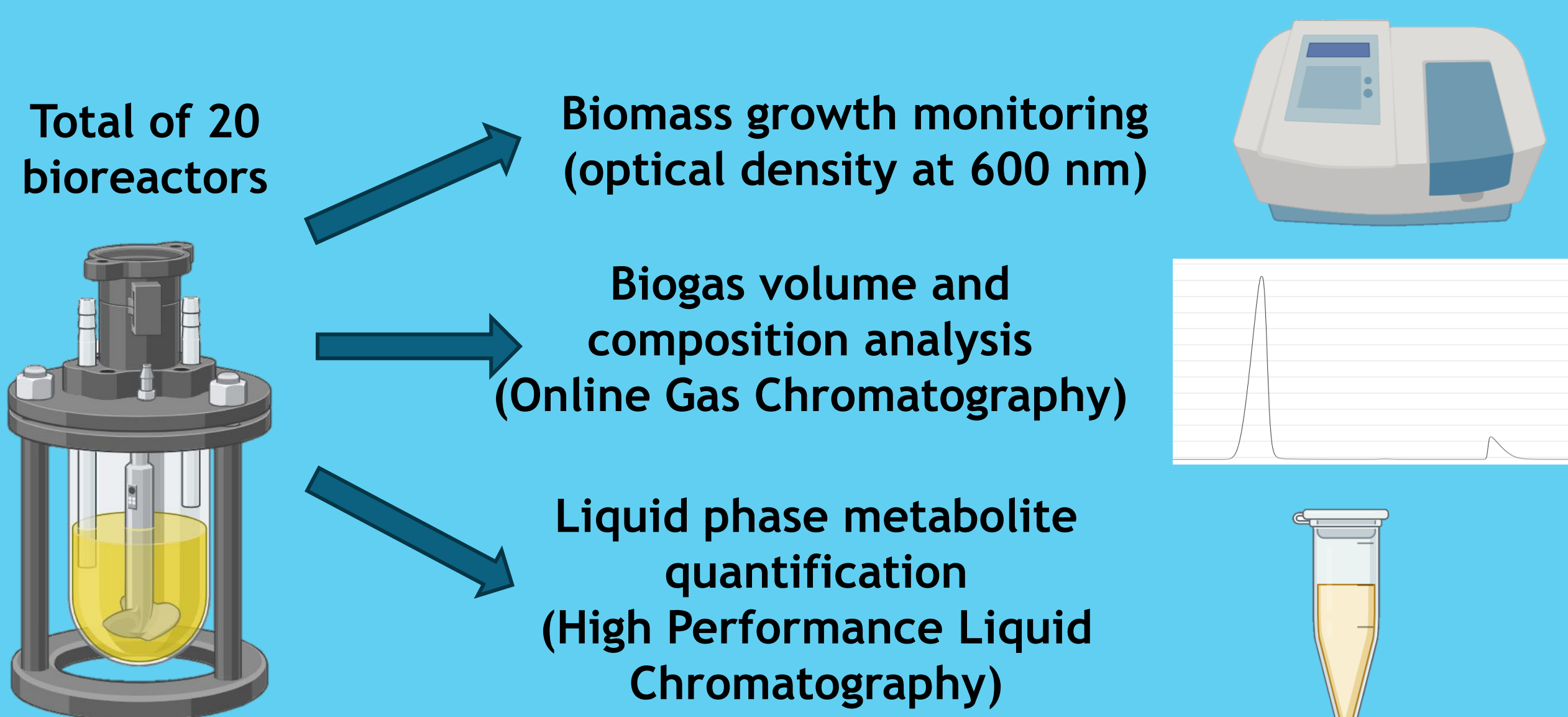
In the genus *Clostridium*, the anaerobic dark fermentation takes place in two phases :
 -An **acidogenic phase** (pH between 5 and 7) where organic acids are produced as the main metabolites.
 -A **solventogenic phase** (pH between 4 and 5) where organic acids are consumed back, and solvents are produced.



→ To obtain a maximum H₂ production rate, it is necessary to maintain the acidogenic phase, and especially to favor the acetate pathway thanks to a pH regulation.

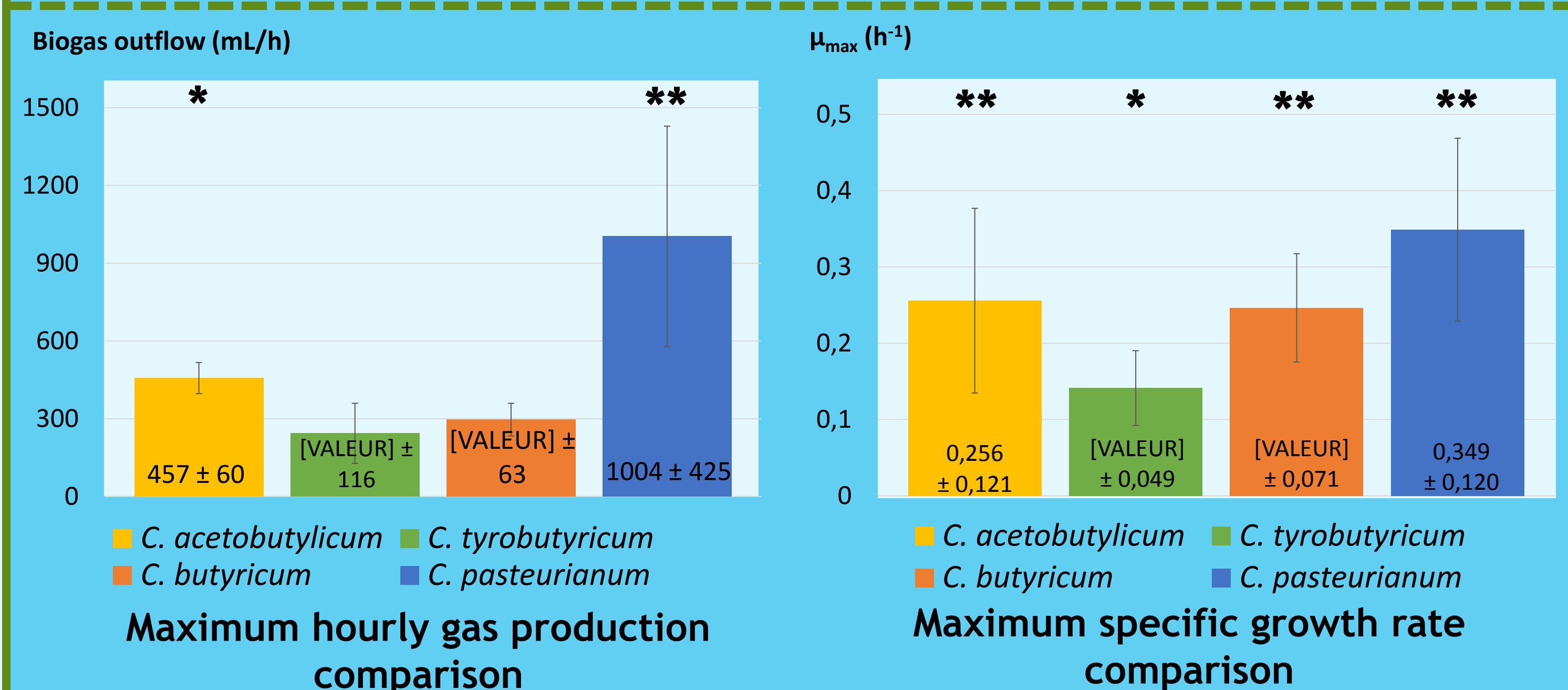
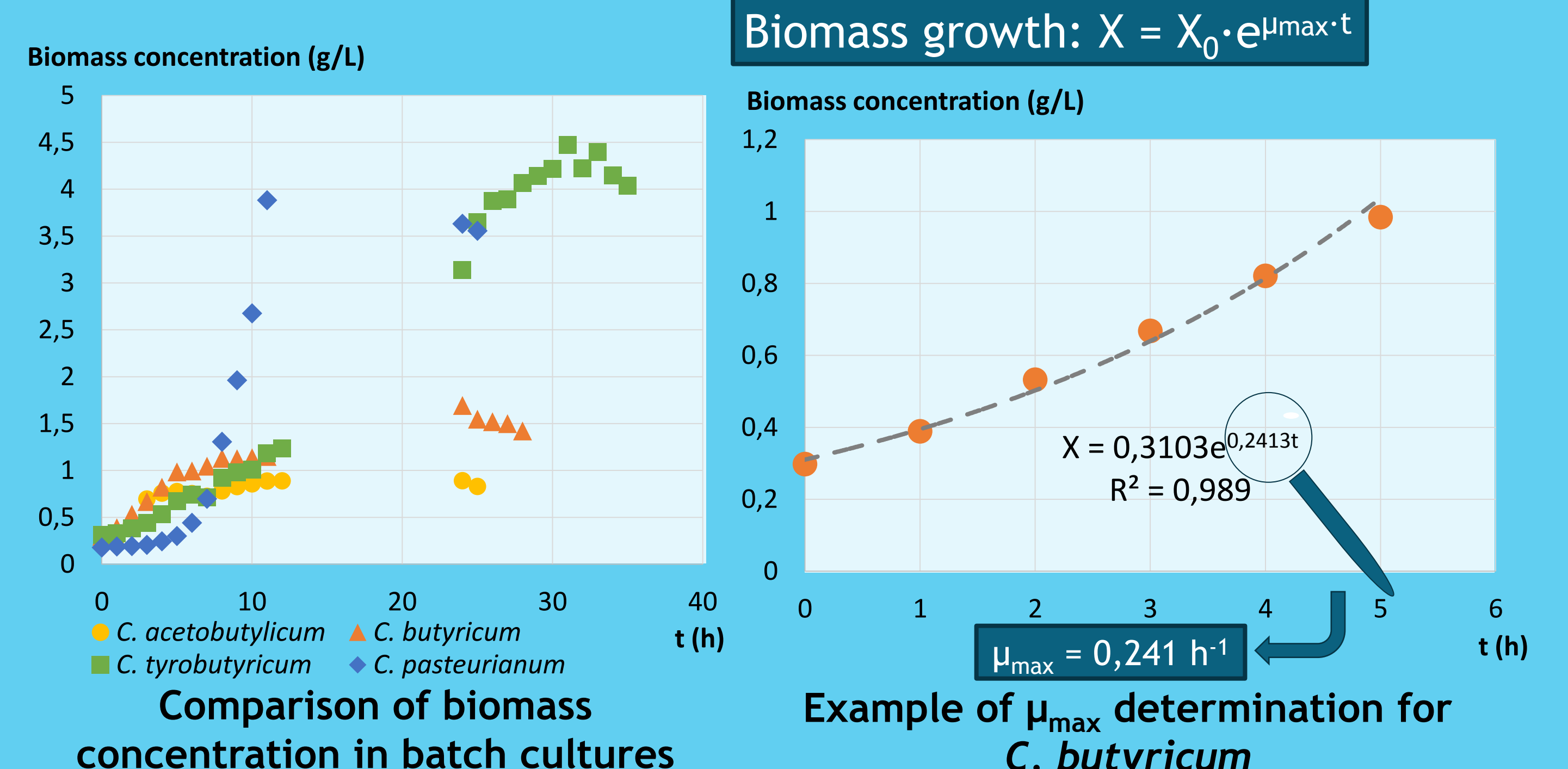
Experimental methodology

- Among all the strains of *Clostridium*, there are several ones that are known as high hydrogen producer and are often cited in bibliographic studies. [3]
 In our study, four strains belonging to collection strains were considered :
- *Clostridium acetobutylicum* ATCC 824
 - *Clostridium pasteurianum* ATCC 6013
 - *Clostridium tyrobutyricum* CNRZ 510
 - *Clostridium butyricum* DSM 5431
- Batch cultures (5 repetitions for each strain)
 Operating conditions :
- Bioreactor volume = 1.6 L
 - pH = 6 (regulated with NaOH 1M)
 - Temperature = 37 °C
 - Initial glucose concentration = 20 g/L
 - Agitation with a 4-blade Rushton turbine at 150 rpm
 - Culture monitoring until substrate depletion and biomass decrease



→ In terms of performances for biohydrogen production, the evaluation criteria considered are the maximum specific growth rate (μ_{max}), the biogas production rate and the H₂/CO₂ ratio (correlated with the metabolite production rate). In this work, the highest responses are targeted.

Main results



H₂/CO₂ ratio between for each culture of *Clostridium*

	<i>C. acetobutylicum</i>	<i>C. tyrobutyricum</i>	<i>C. butyricum</i>	<i>C. pasteurianum</i>
H ₂ /CO ₂ ratio	50,4% / 49,6% (±1,4%)	51,1% / 48,9% (±1,4%)	52,9% / 47,1% (±0,3%)	52,8% / 47,2% (±0,4%)

→ After performing statistical tests (Student t-test), *C. pasteurianum* shows the best results concerning both biohydrogen production and a fast and strong growth.

Perspectives

→ Now that an effective strain has been selected, further work can be done with *C. pasteurianum* in continuous cultures. The stable conditions of the chemostat could enable the constant emission of biogas in terms of productivity and H₂/CO₂ ratio. Other parameters such as dilution rate, pH or concentration of feed substrate concentration can be considered to optimize the process and achieve a maximal steady H₂ outflow. Other substrates could be used to study biowaste valorization.

→ H₂ in addition to CO₂ are part of the biomethanation reaction, performed by many hydrogenotrophic archaea species during anaerobic digestion. The biogas could be transferred to enhance methane (CH₄) production. Not only the biogas, but also the liquid phase from the fermenter, with a high acetate concentration, could be transferred to a digester, where acetotrophic archaea would be able to produce additional methane.