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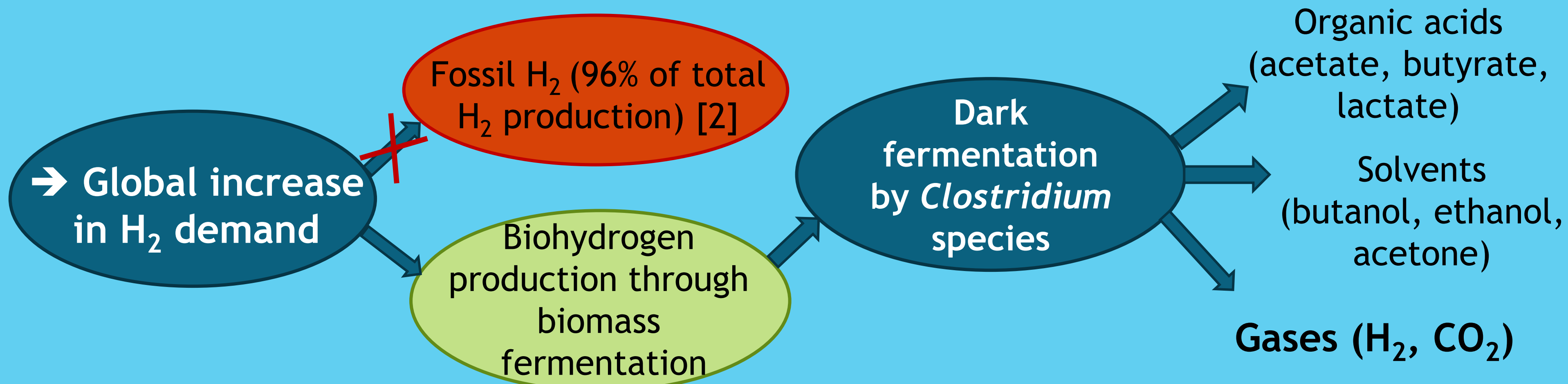
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# Comparative analysis and improvement of biological hydrogen production in *Clostridium* genus

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## Scientific context and objectives

Many industries already use gaseous hydrogen (H<sub>2</sub>) for their activities (ammonia and methanol production refining of oil products). Now, H<sub>2</sub> is seen as a potential energy vector for other industries, resulting in the need to produce more of this gas [1].



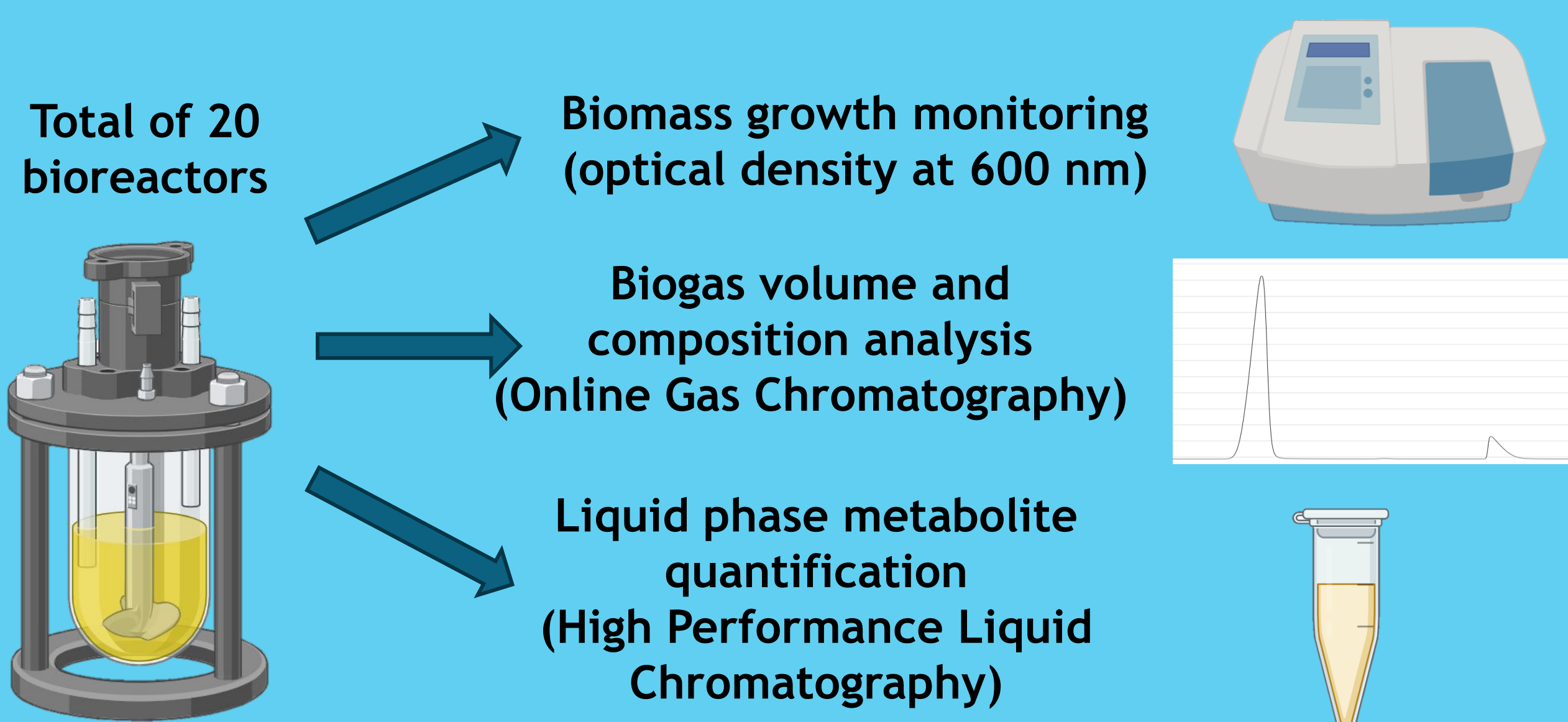
In the genus *Clostridium*, the anaerobic dark fermentation takes place in two phases :  
 -An **acidogenic phase** (pH between 5 and 7) where organic acids are produced as the main metabolites.  
 -A **solventogenic phase** (pH between 4 and 5) where organic acids are consumed back, and solvents are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



→ To obtain a maximum H<sub>2</sub> production rate, it is necessary to maintain the acidogenic phase, and especially to favor the acetate pathway thanks to a pH regulation.

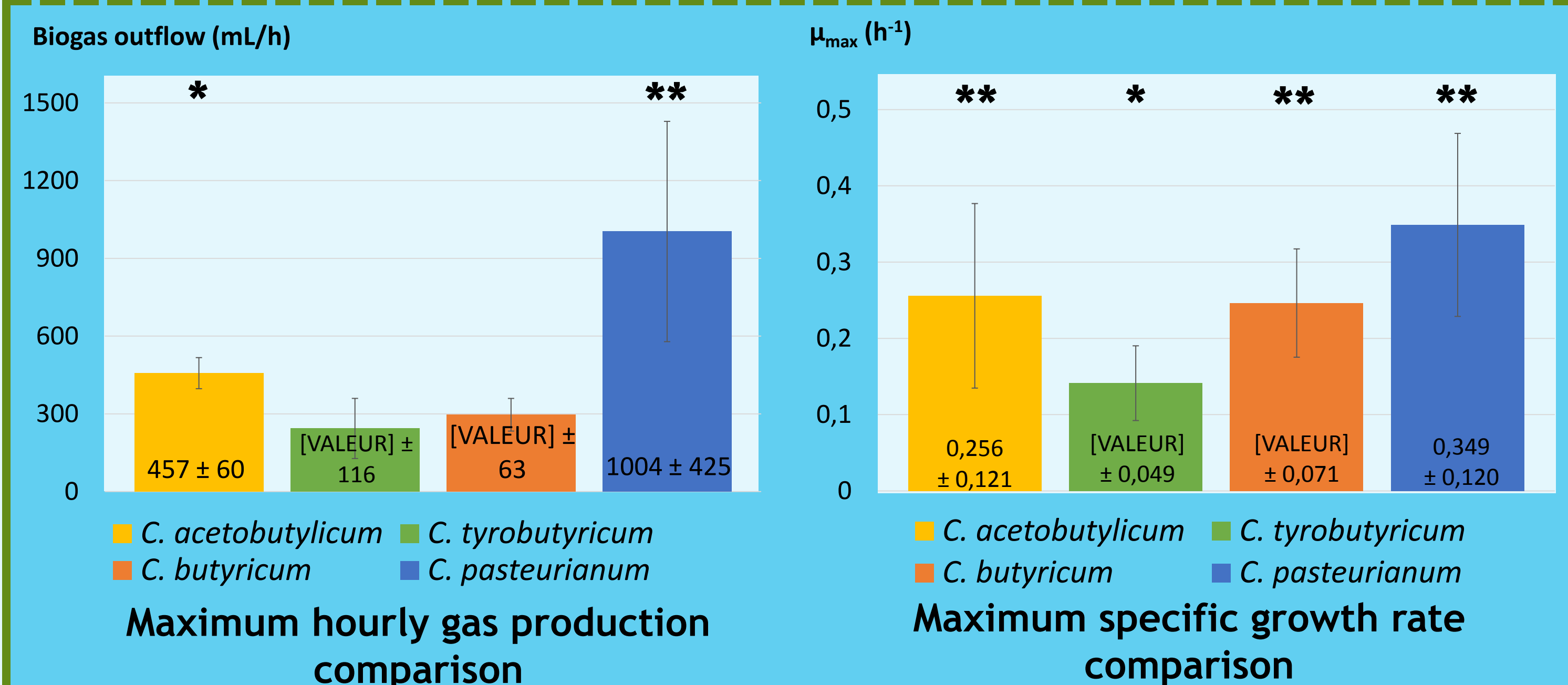
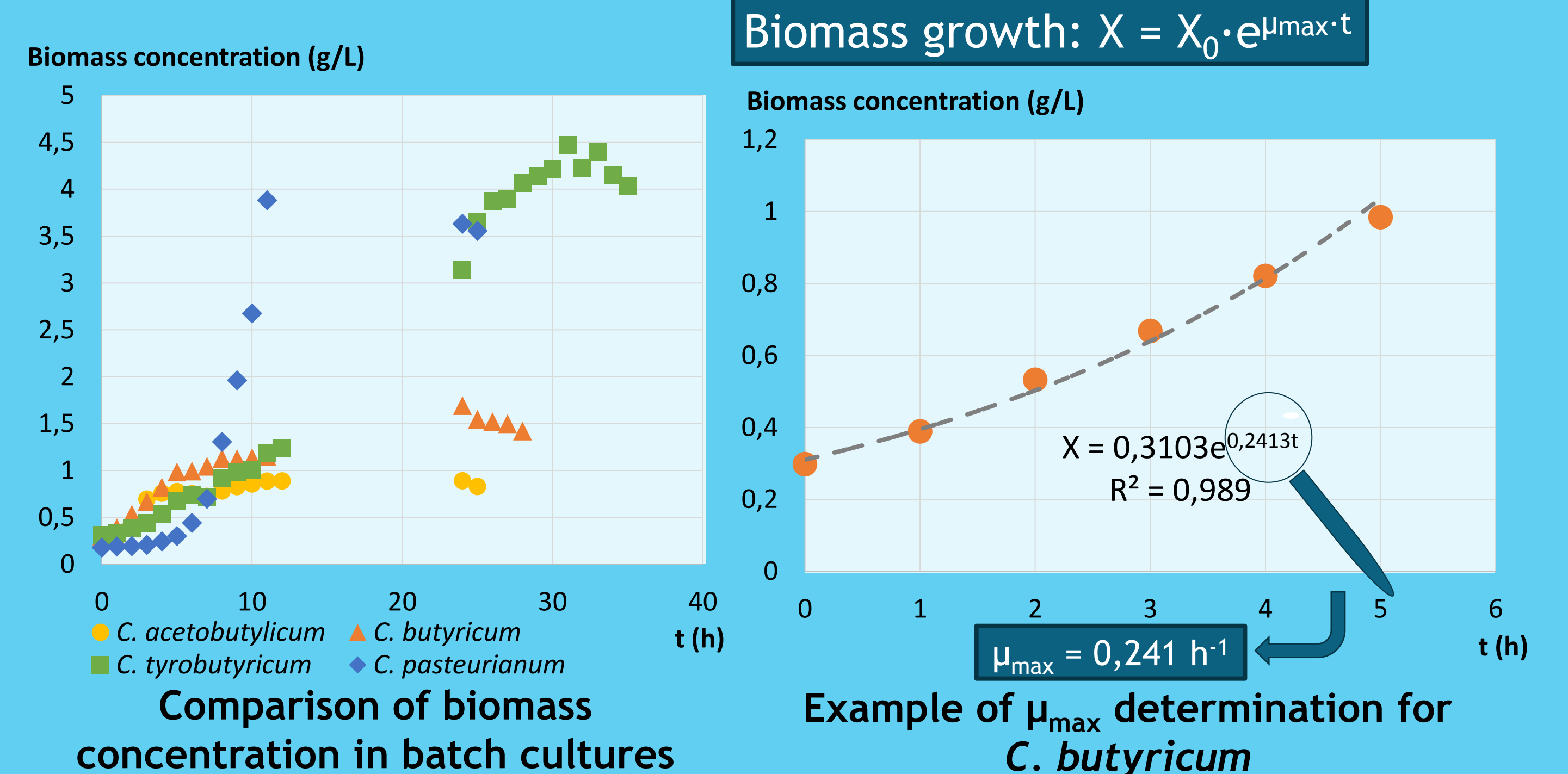
## Experimental methodology

- 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** ( $\mu_{max}$ ), the **biogas production rate** and the **H<sub>2</sub>/CO<sub>2</sub> ratio** (correlated with the metabolite production rate). In this work, the highest responses are targeted.

## Main results



H<sub>2</sub>/CO<sub>2</sub> ratio between for each culture of *Clostridium*

	<i>C. acetobutylicum</i>	<i>C. tyrobutyricum</i>	<i>C. butyricum</i>	<i>C. pasteurianum</i>
H <sub>2</sub> /CO <sub>2</sub> 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 H<sub>2</sub>/CO<sub>2</sub> 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 H<sub>2</sub> outflow**. Other substrates could be used to study biowaste valorization.

→ H<sub>2</sub> in addition to CO<sub>2</sub> are part of the **biomethanation reaction**, performed by many hydrogenotrophic archaea species during **anaerobic digestion**. The **biogas** could be transferred to **enhance methane (CH<sub>4</sub>) 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**.