



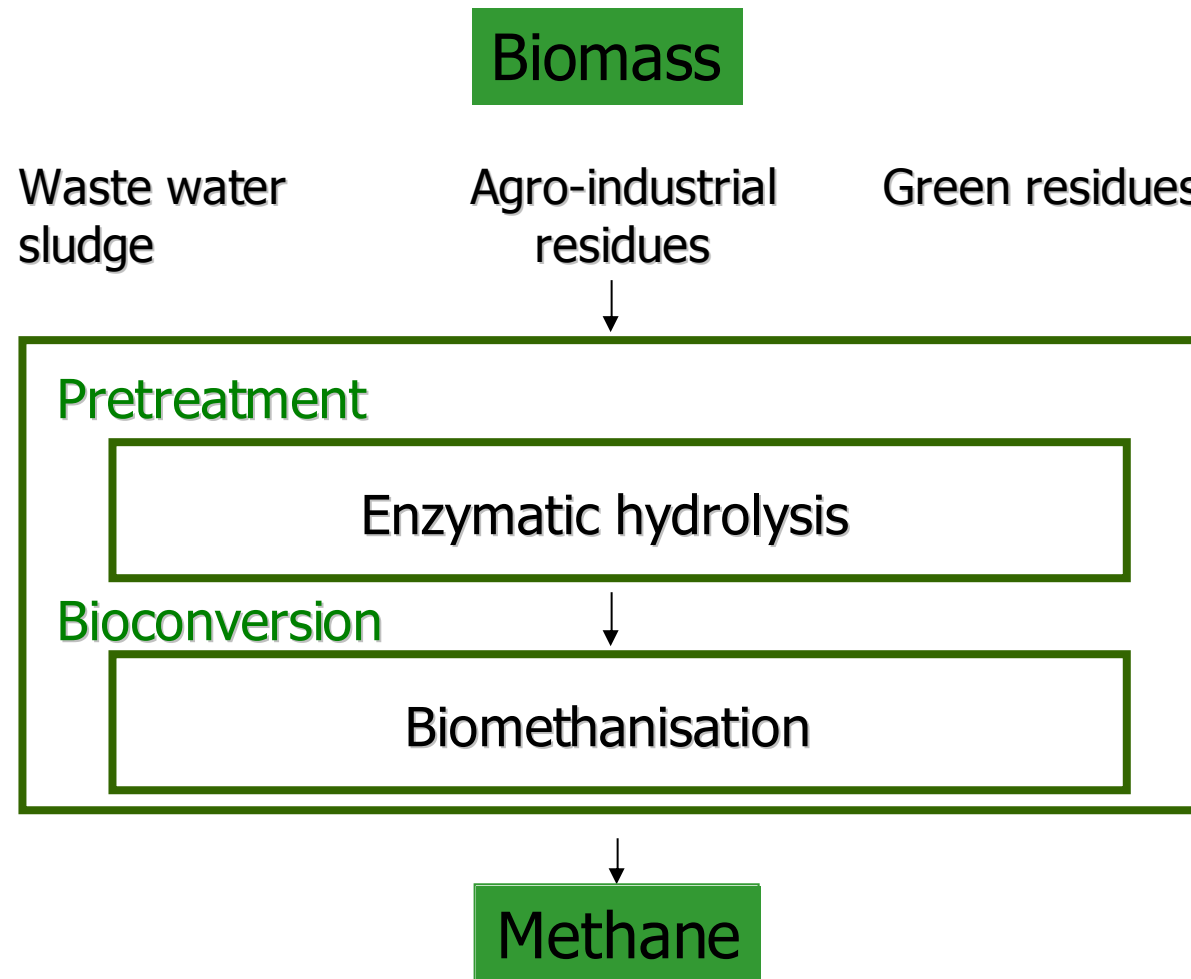
Investigating the kinetics of biogas production: *managing the experimental interferences*

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Context: what is the influence of enzymatic hydrolysis pretreatment on biomass anaerobic conversion ?





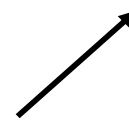
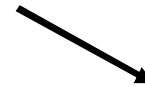
How to measure the enzyme action ?

Biomass biodigestibility?

What are the limiting factors?

Biodigestibility gain?

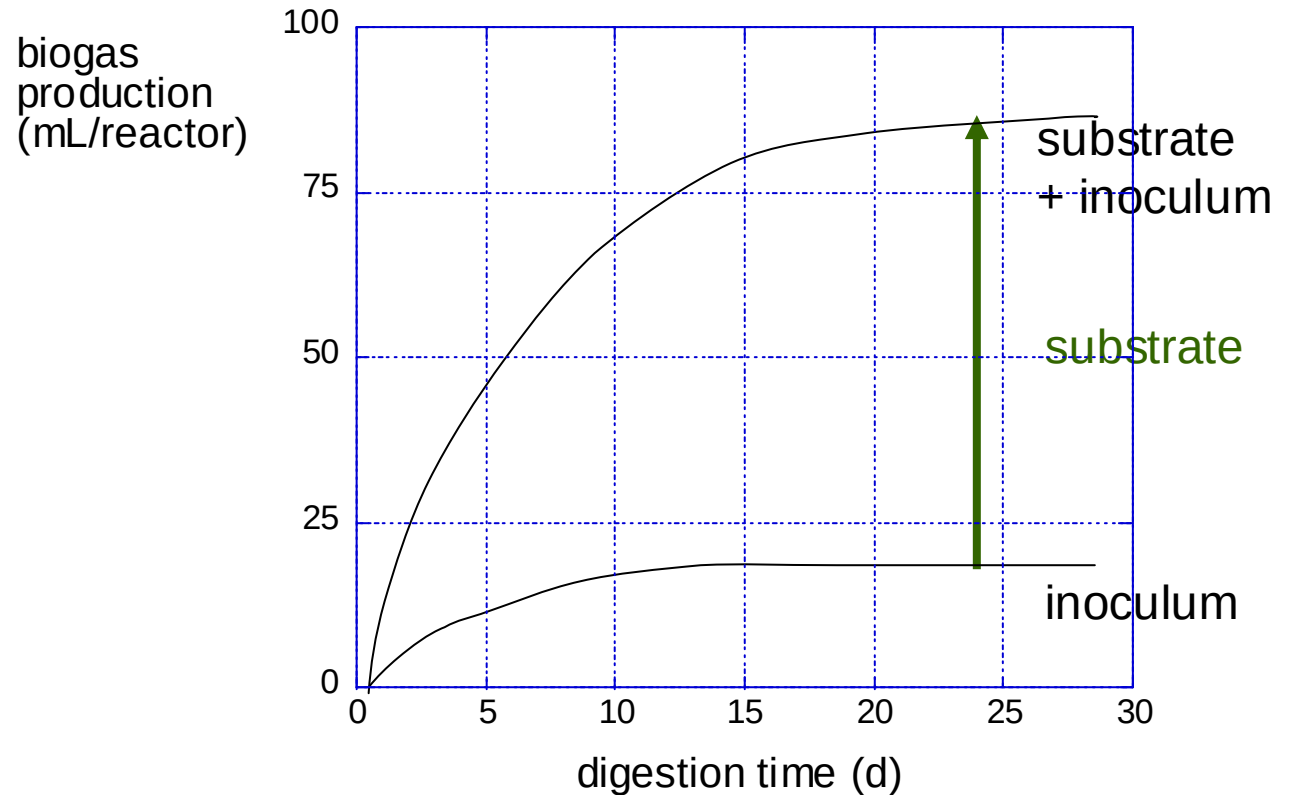
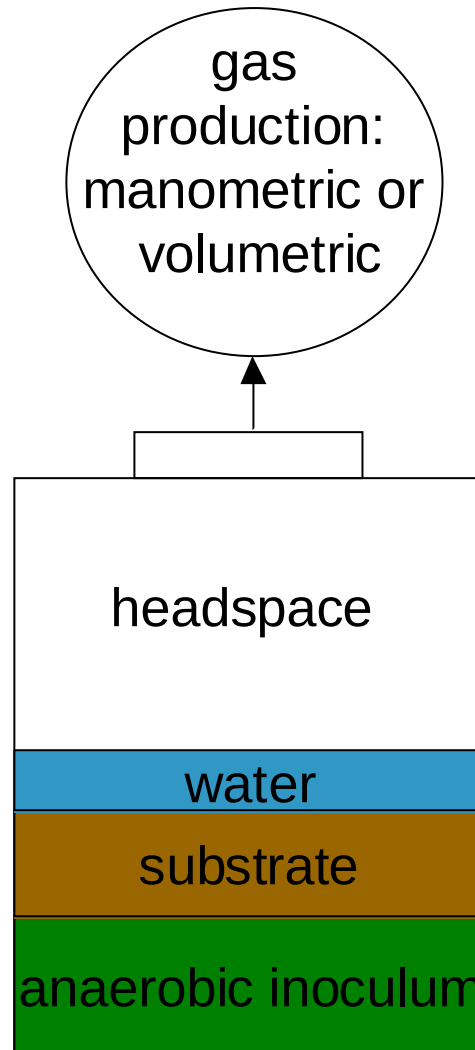
What is the enzyme efficiency?



**Biodigestibility
assays**

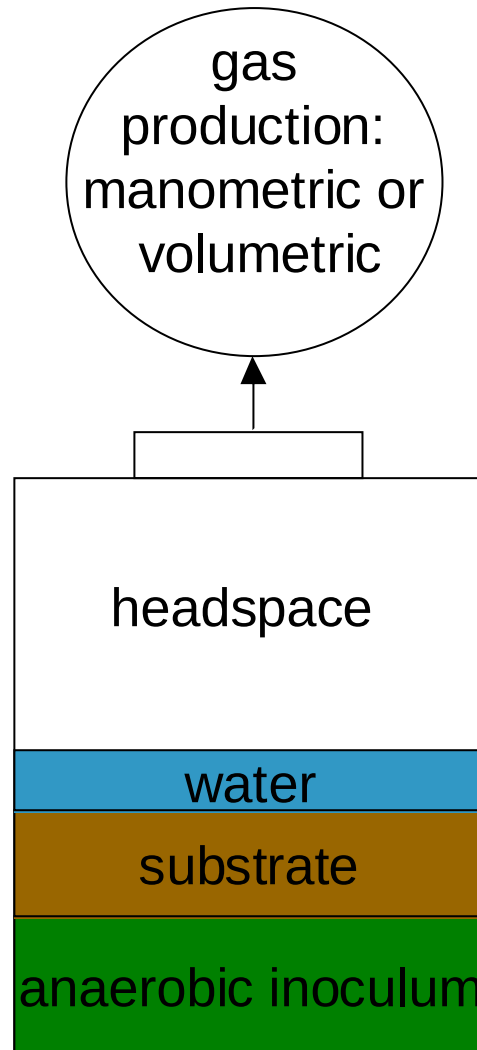


Biodigestibility assays are conducted under anaerobic batch conditions

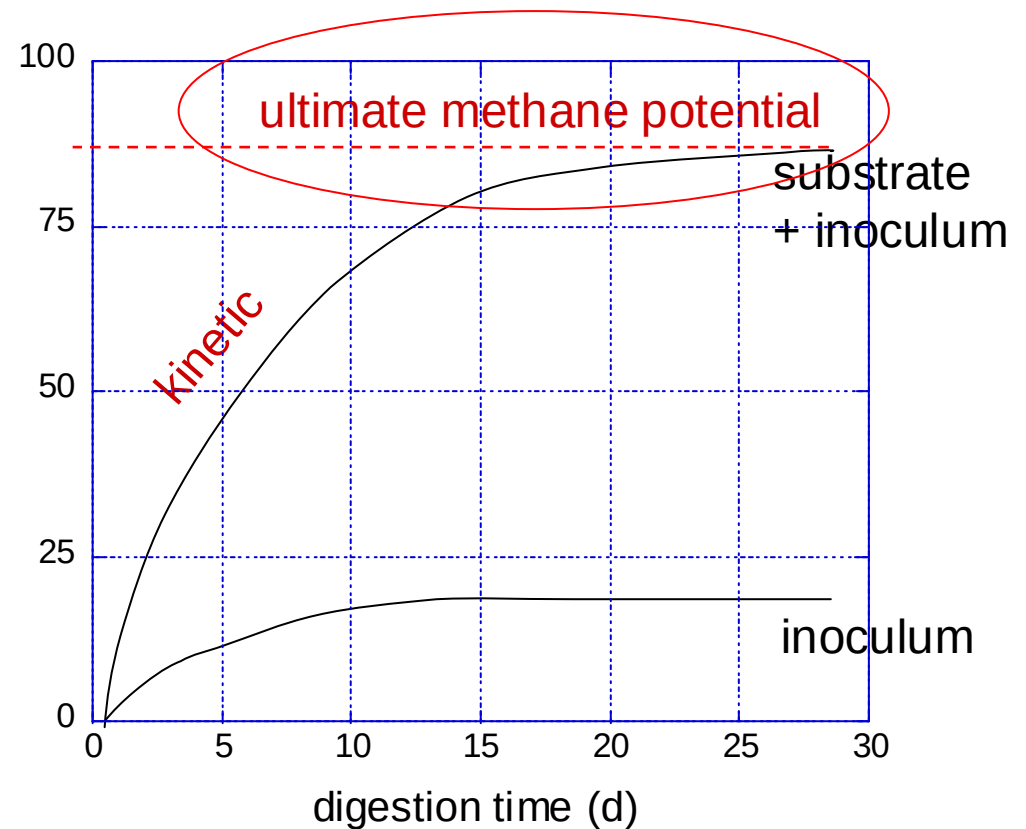




Anaerobic biodegradability is typically assessed by the ultimate methane potential

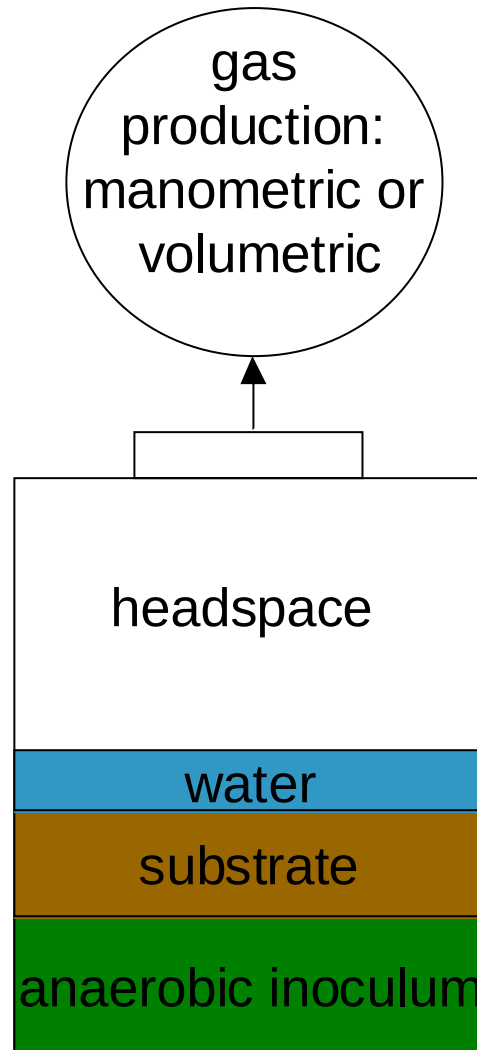


biogas
production
(mL/reactor)

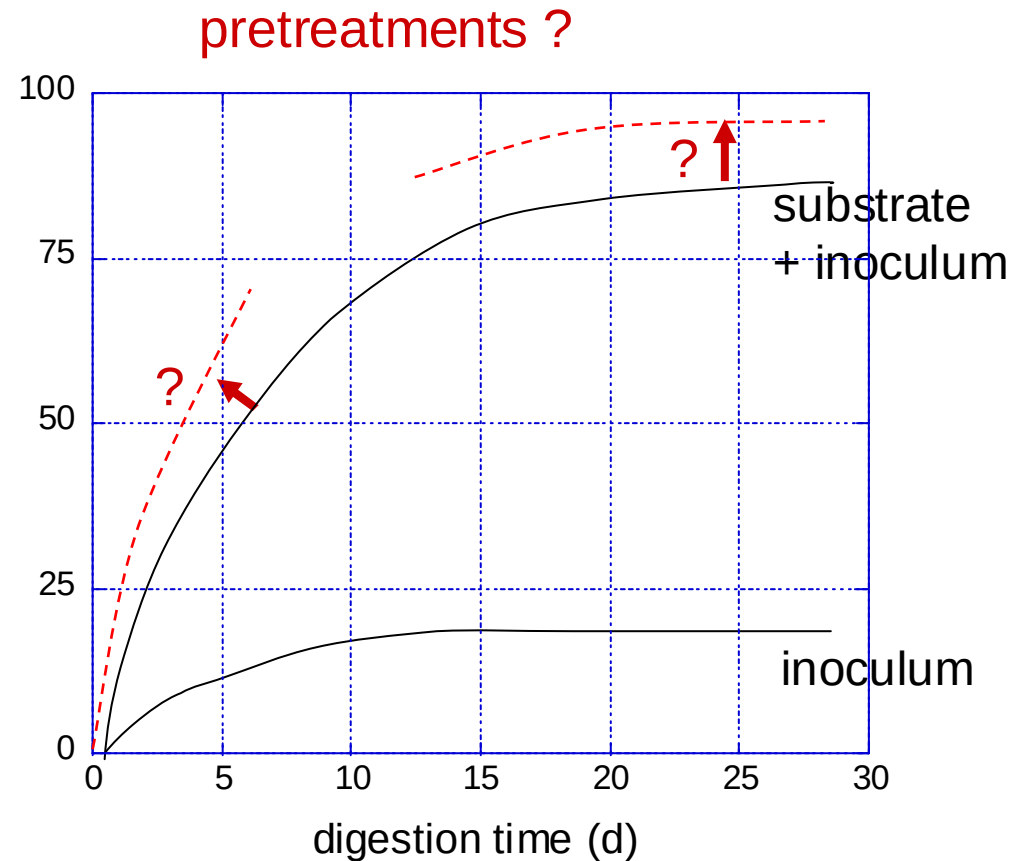




Some pretreatments lead to kinetic improvement which is not always correlated to ultimate methane production gain

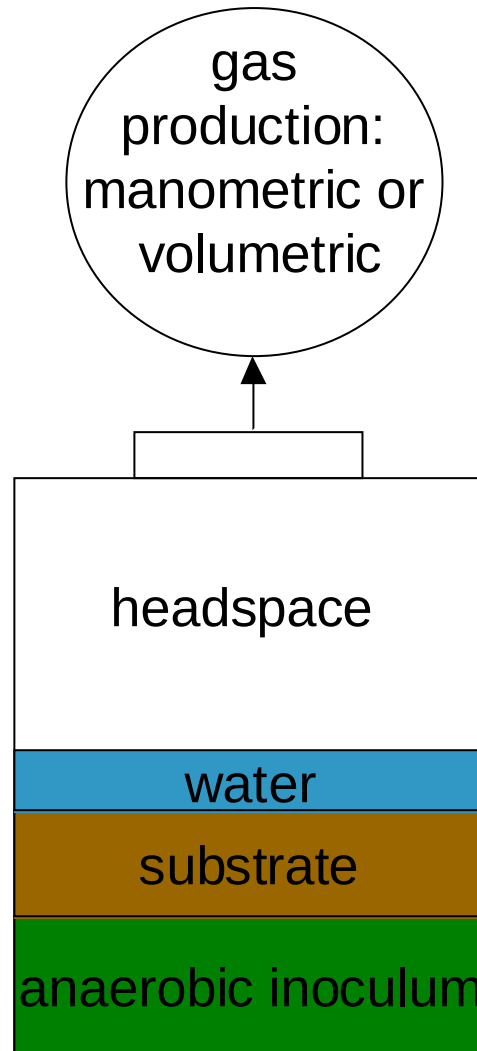


biogas production (ml/reactor)

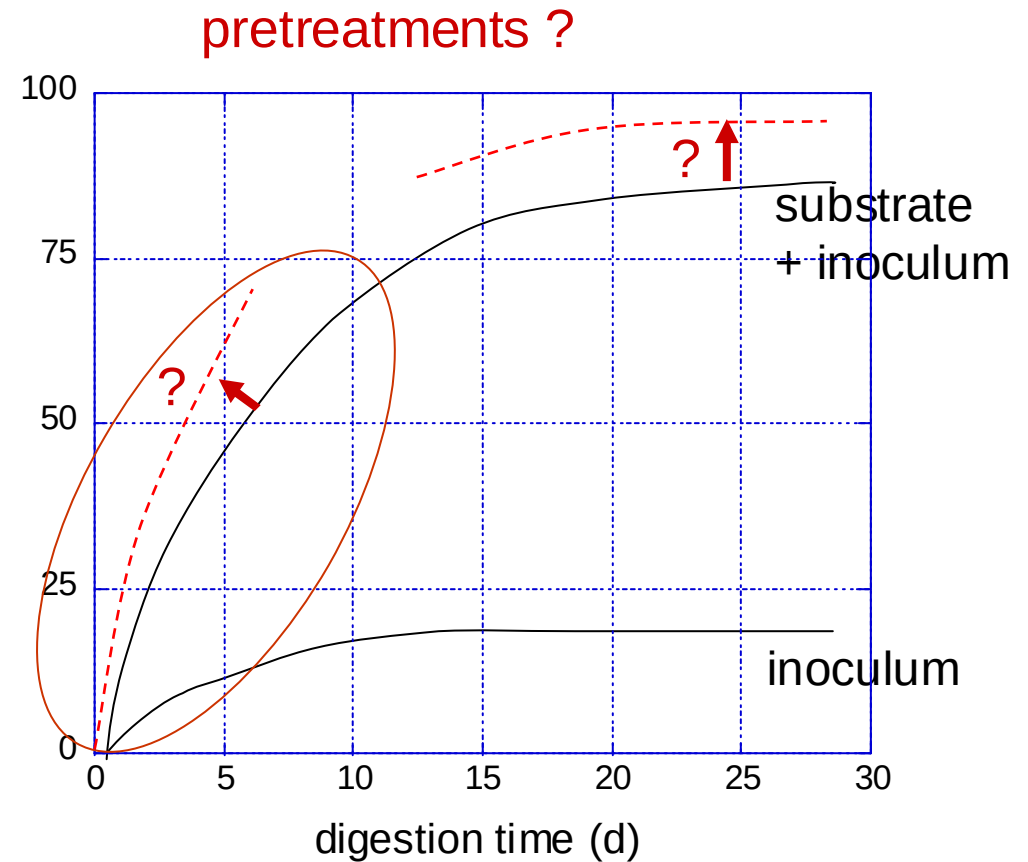




We want to investigate the influence of pretreatment on the kinetics of biogas production



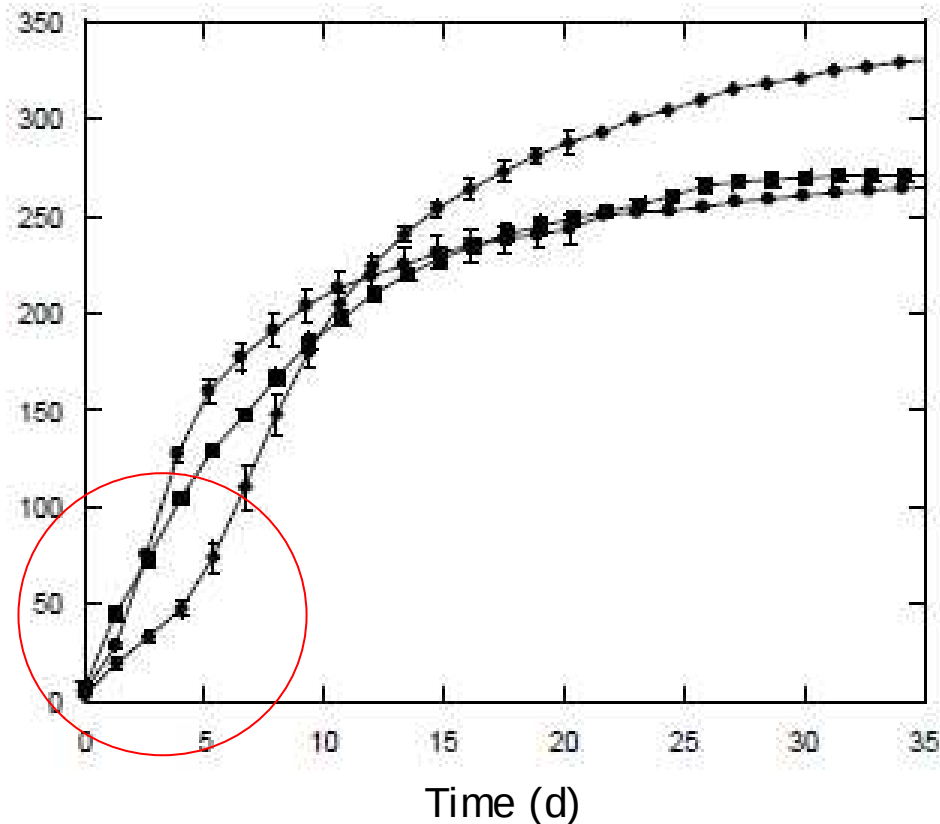
biogas
production
(ml/reactor)





Example: anaerobic digestion of white rot fungi (WRF) pretreated maize digestate

biogas production
(mL/g_{COD})



substrate
(maize digestate)

WRF 1 pretreated substrate

WRF 2 pretreated substrate



Aim: how to optimise the experimental procedure to reliably measure the kinetics of biogas production ?

Factors to investigate:

Inoculum

- inoculum bioactivity
- substrate/inoculum ratio

Gas / liquid equilibria

- O_2
- CO_2 & pH
- flushing gas temperature
- flushing gas humidity

Instrument performances

The influence of these factors is not taken into account in most published data



Inoculum

- inoculum bioactivity
- substrate/inoculum ratio

Gas / liquid equilibria

- O_2
- CO_2 & pH
- gas temperature
- gas humidity

Instrument performances



Inoculum characteristics ?

Goals

1.
Measurement sensitive
to small variations of rapidly
digestible substrates



Inoculum characteristics

Low inoculum gas production
compared to substrate

2.
Kinetics controlled by the easily
digestible substrate and
not by the inoculum bioactivity

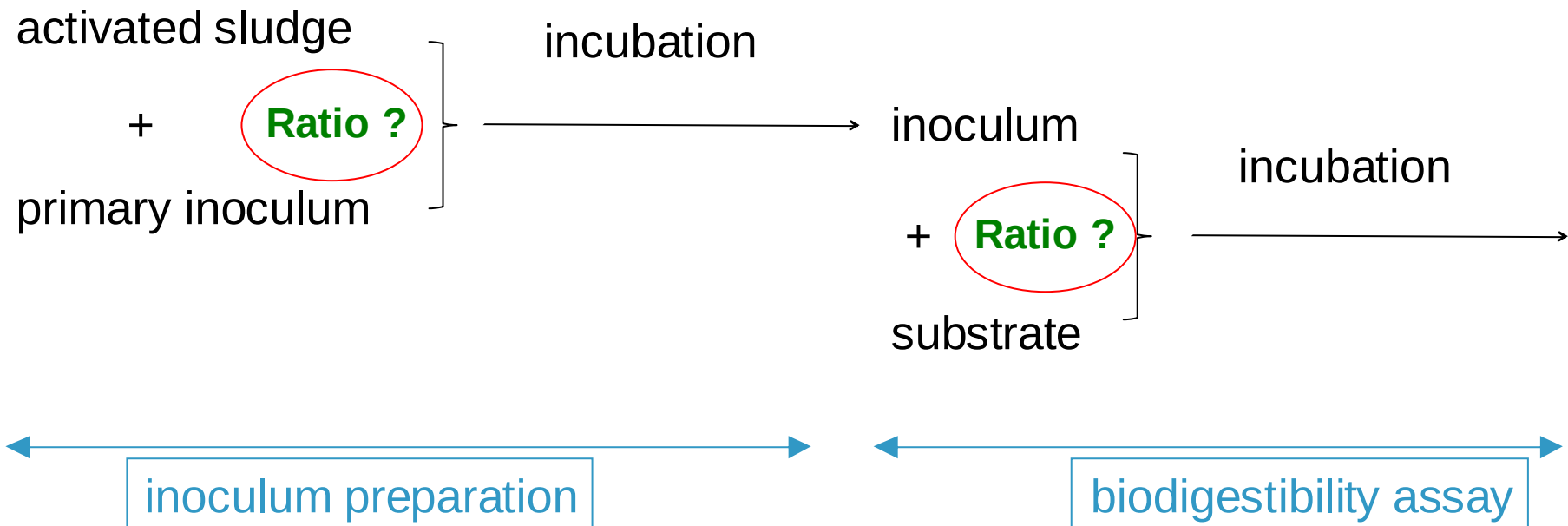


High activity of inoculum
microorganisms



Anaerobic inoculum = incubation (primary inoculum + activated sludge)

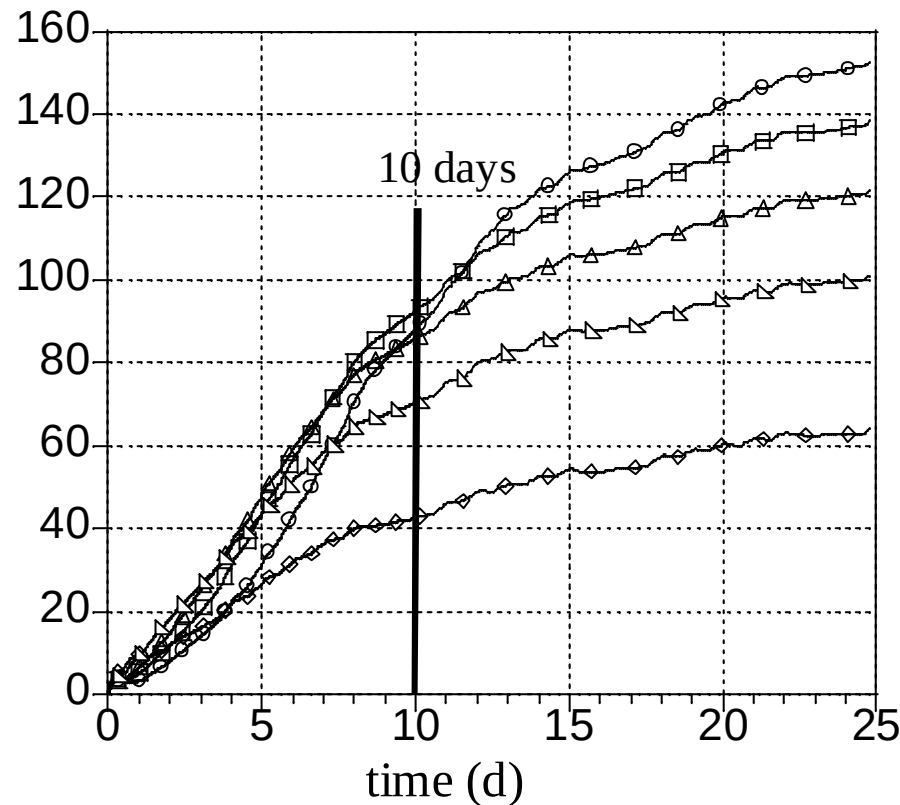
= complex substrate to activate
inoculum digestion capacity
+ source of additional micro-
organisms





Inoculum preparation: reasonable activated sludge / primary inoculum ratio?

biogas production
 $\text{mL}_{\text{biogas}} / \text{g}_{\text{COD}}$



$\frac{\text{activated sludge quantity}}{\text{primary inoculum quantity}} =$

9 g_{COD}/g_{COD}

4 g_{COD}/g_{COD}

2.33 g_{COD}/g_{COD}

1 g_{COD}/g_{COD}

0.33 g_{COD}/g_{COD}



Biodigestibility assay: Reasonable substrate / inoculum ratio?

Goals

Kinetics controlled by the availability of the easily digestible fraction of the substrate

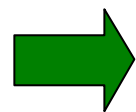


Biogas production rate proportional to the concentration of easily digestible substrate

Kinetics controlled by the substrate fraction made more digestible by the hydrolytic pretreatment



Biogas production rate proportional to the concentration of easily digestible substrate (glucose) added to the main substrate



Maize digestate : $1 \text{ g}_{\text{MS-substrate}}/\text{g}_{\text{COD-inoculum}}$

Waste water sludge: $0.2 \text{ g}_{\text{COD-substrate}}/\text{g}_{\text{COD-inoculum}}$



Inoculum

- inoculum bioactivity
- substrate/inoculum ratio

Gas / liquid equilibria

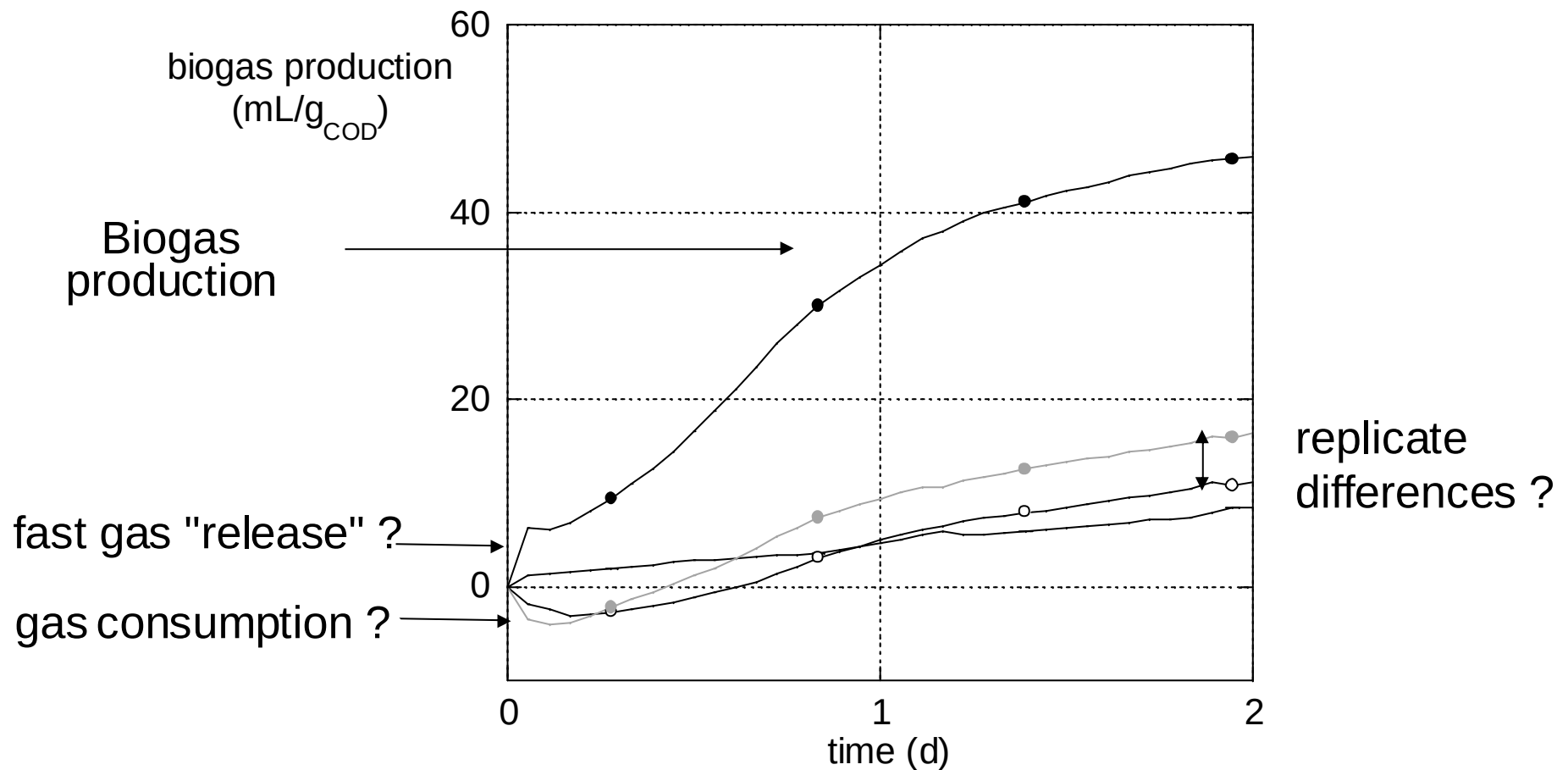
- O_2
- CO_2 & pH
- gas temperature
- gas humidity

Instrument performances



Observation: several interfering phenomena affect the measured biogas production

Manometric monitoring in the range 0-100 mbar_{relative_pressure}



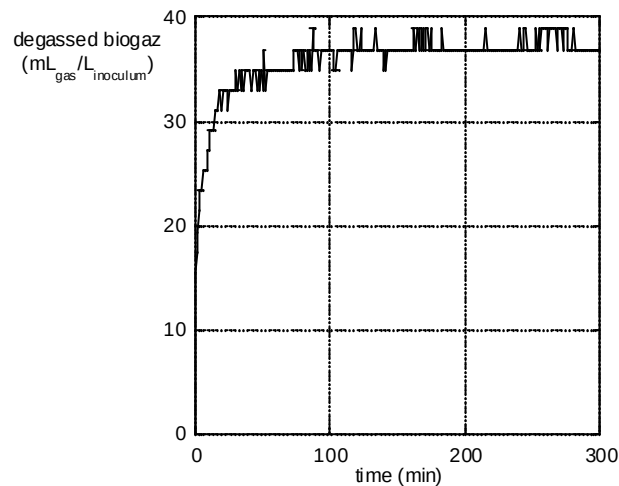


Pressure changes are not exclusively due to biogas production but affect the apparent final biogas production

1. Insufficient flushing → remaining O_2 absorbed in liquid → pressure drop

Solution 1: extensive flushing

2. Inoculum passing from 50% CO_2 at high pressure to 0% CO_2 headspaces → CO_2 degassing → pressure increase



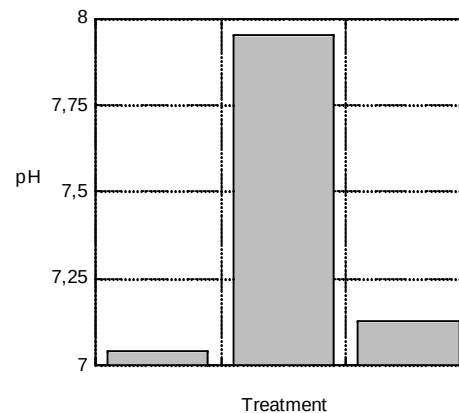
Solution 2: depressurise inoculum 2 hours before use



Pressure changes are not exclusively due to biogas production but affect the apparent final biogas production

3. Carbonate solubilised in inoculum or substrate → CO₂ degassing during pretreatments → pH changes → CO₂ absorption or degassing → pressure drop or increase

Solution 3: adjust pH

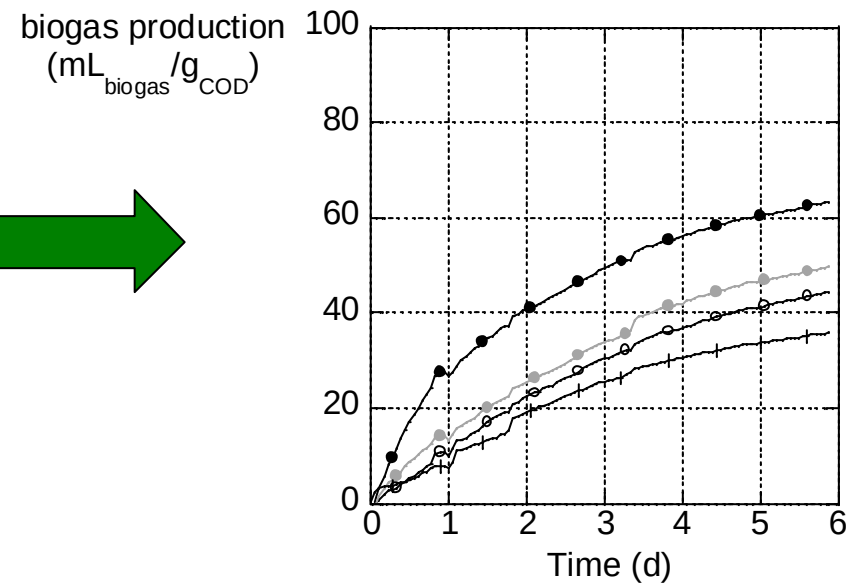
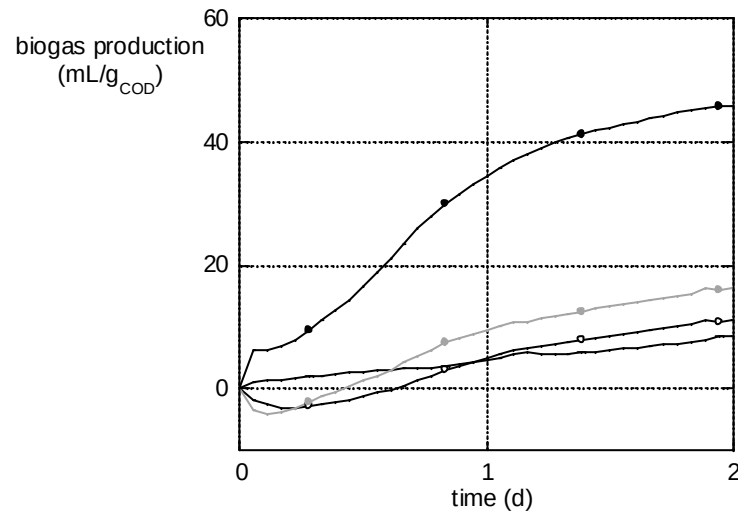


4. Flushing with dry N₂ → equilibrates with water vapour pressure → pressure increase (56 mbar_(35°C))
5. Flushing with N₂ at 20°C → temperature increase to 35°C during incubation → pressure increase (52 mbar)

Solution 4 & 5: prior bubbling the flushing N₂ in a 35°C water column



The proposed solutions improved reproducibility and accuracy of the results





Inoculum

- inoculum bioactivity
- substrate/inoculum ratio

Gas / liquid equilibria

- O₂
- CO₂ & pH
- gas temperature
- gas humidity

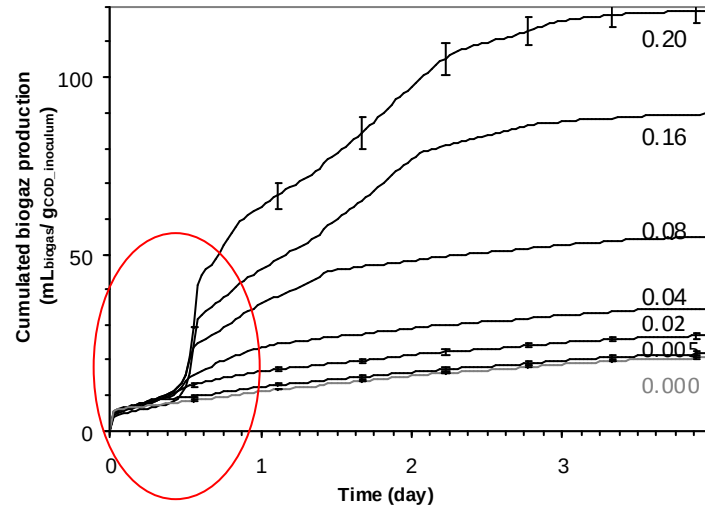
Instrument performances



Measuring the production kinetics of gaseous metabolites remains a challenge

1. CO₂ diffusion through most "practical" storage materials → biased biogas composition

2. Gas metabolites (CO₂, CH₄, H₂) production rate evolving at the hour scale



3. Many bioreactors to be monitored in parallel

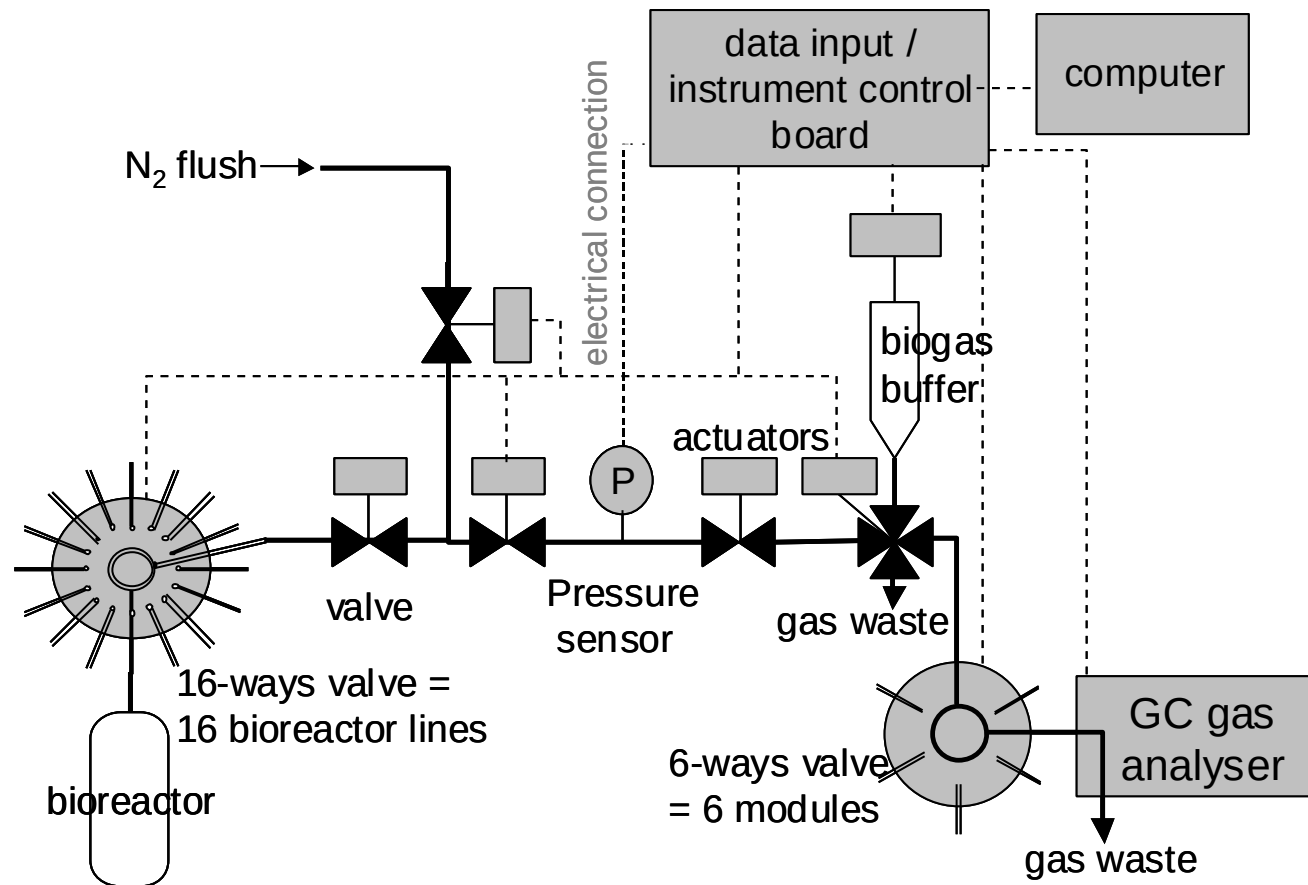
triplicates * $\left[\begin{array}{l} \text{inoculum} \\ n \text{ negative pretreatment control} \\ n' \text{ pretreated substrates} \\ n'' \text{ calibration substrates} \end{array} \right] = \text{typically 30-60 bioreactors}$

4. Commercial instruments not reliable / do not meet all requirements

=> Research strategy: development of automatic instrument:
fast monitoring of gas pressure and composition



Automatic equipment is on development and test to measure pressure and composition on line



analysis of small gas volumes at high frequency

collaboration with UCL / Thermodynamics and fluid mechanics (TFL)



Conclusions

We were able to master phenomena interfering with biogas production kinetics and not taken into account in most published data
=> more accurate and reproducible biogas production rates

Monitoring the gas metabolites at the hour scale is required to investigate :

- the bioconversion of easily digestible substrates
- the influence of substrate hydrolysis pretreatments

Perspectives

Instrument under development will enable to monitor the gas metabolites produced by various bioprocesses

- biomethanation : CH_4 , CO_2 , (H_2)
- acidogenic fermentation: H_2 , CO_2 , (CH_4)
- H_2 fermentation: H_2 , CO_2