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Characterization of bacterial population adapted to methane production from partially hydrolyzed *Scenedesmus sp.* biomass in a high-rate anaerobic digester

ABSTRACT

Using anaerobic digestion (AD) to convert microalgae *Scenedesmus sp*. AMDD into methane represents a promising biomass-to-biofuel scenario. However, there is still a need for optimization. In this study, two pre-treatment strategies have been elaborated to obtain higher and/or faster methane yield.

Microalgal biomass was hydrolyzed by a combination of enzymatic (pectate-lyase and cellulase) and thermal (121-150°C), or caustic (NaOH) and thermal (121°C) pretreatments. Pretreated microalgal biomass was fed to two Upflow Anaerobic Sludge Blanket digesters, to assess methane production at different organic loading rates (OLR) and a fixed hydraulic retention time (six days). Bacterial community was monitored using next generation sequencing technology.

Both pretreatments successfully hydrolyzed up to 75% of microalgal biomass. Increasing the OLR applied to each digester yielded progressively higher methane production, as expected. The highest volumetric methane production was obtained after the caustic-thermal pretreatment, but the conversion ratio of hydrolyzed algal biomass was only marginally higher than those obtained in previous studies. A shift in the bacterial population was highlighted for both digesters, with *Bacteroidetes* becoming dominant in both adapted populations. Acetate-, H_2/CO_2- and formate-producer *Syntrophus* was the only emerging genus in the bacterial population after caustic-thermal pretreatment, while various VFA and H_2/CO_2-producers, including *Syntrophus*, emerged in the bacterial population after enzymatic-thermal pretreatment.

The much shorter time to produce methane and the reduced footprint of the AD process in an algal biorefinery are very promising results. Identification of bacterial species particularly adapted to the bioconversion of microalgal biomass into methane will be helpful for future process optimization.

INTRODUCTION

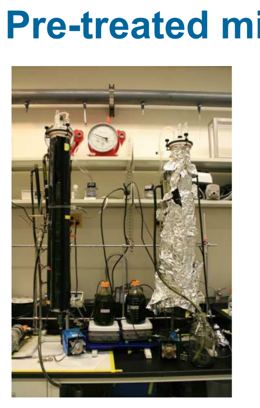
- **Growing concern about global warming and greenhouse gases (GHG) emissions**
- **Unsustainability and fluctuating price of fossil-fuels**
  - → Increased interest in the production of renewable energies
  - → Special attention on biomass-based energies.
- **Algal biorefineries: algal biomass converted into energies**
  - → Conversion of CO_2 emissions into algal biomass, then converted into biofuels & other value-added products
  - → Maximize GHG emissions reduction from the net CO_2 abatement associated to the algal culture
- **Anaerobic digestion (AD) of microalgal biomass: bioconversion into CH_4**
  - → Best energetic balance among the biomass-to-biofuel scenario for microalgae containing less than 40% lipids
- **Microalgae *Scenedesmus sp.* Strain AMDD**
  - → Potential for CH_4 production with a conversion ratio of 50% over 15-28 days of incubation
  - → Need for an optimized conditioning of the biomass prior to its digestion in a high-rate digester

OBJECTIVES

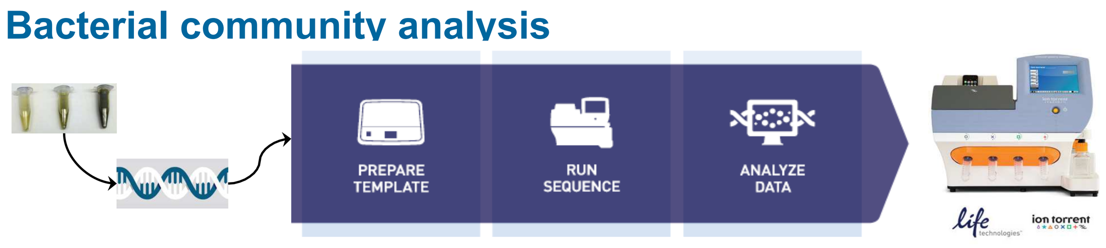
- **Evaluation of different pre-treatment strategies**
  - → Evaluation of the best strategies for microalgal biomass pre-treatment
  - → Achievement of higher and/or faster CH_4 yields
- **Characterization of the adapted bacterial population**
  - → Identification of bacterial species particularly adapted to the bioconversion of microalgal biomass into CH_4

MATERIALS & METHODS / EXPERIMENTAL DESIGN

- **Strategies of pre-treatments selected**
  - → 1^{st} = combination of enzymatic and thermal pre-treatments
    - 24h with pectate-lyase [1340 U/gTVS] & cellulase [970 U/gTVS]
    - 30 min at 121°C (weeks 1 to 24), 30 min at 150°C (weeks 25 to 34)
    - 34 weeks of operation
  - → 2^{nd} = combination of caustic and thermal pre-treatments
    - 24h with 0.2N NaOH
    - 30 min at 121°C
    - 17 weeks of operation
- **Pre-treated microalgal biomass fed to anaerobic digesters**



  - → UASB (Upflow Anaerobic Sludge Blanket) digesters
    - working volume of 3.5L
  - → Different organic loading rates (OLR)
    - from 1.3 to 4.7 gTVS/L_{rx}.d in the 1^{st} digester
    - 2.2 - 3.9 & 1.1 gTVS/L_{rx}.d in the 2^{nd} digester
  - → Fixed hydraulic retention time (HRT) of six days
  - → Mesophilic conditions (35°C)
- **Bacterial community analysis**



- → Samples taken at the beginning, regularly along the process & at the end
- → Genomic DNA extraction (MoBio soil kit) and purification (Qiagen)
- → Next generation sequencing technology (Ion PGM™ sequencer)
- → 16S rDNA gene targeted
- → RDP Pyrosequensing pipeline (http://pyro.cme.msu.edu/) and RDP classifier (http://rdp.cme.msu.edu/classifier)

RESULTS

Broad screening approach for selection of the best pre-treatment strategy

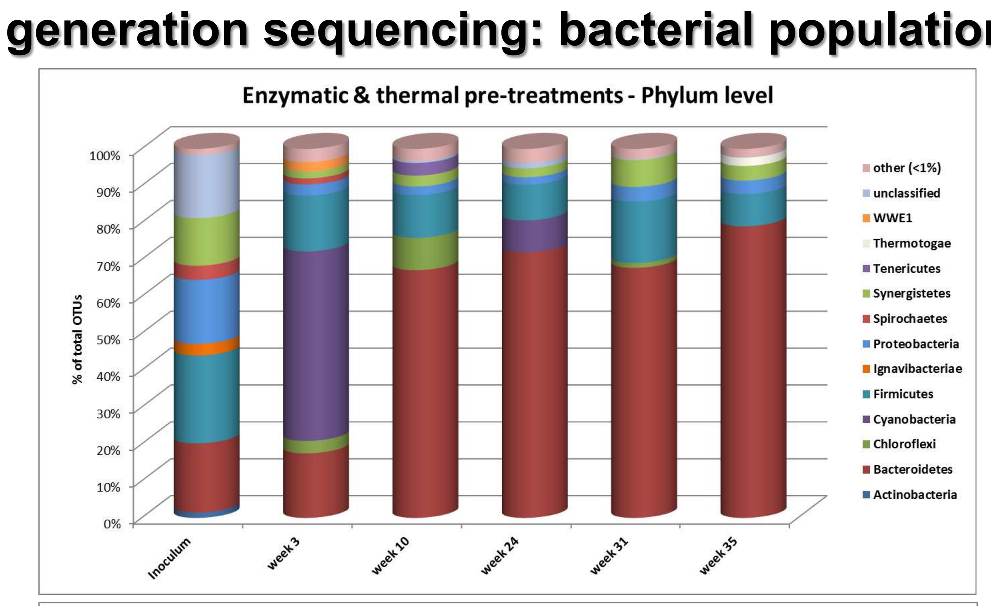
- **Best strategies = both combination of [enzymatic & thermal] and [caustic & thermal] pre-treatments**

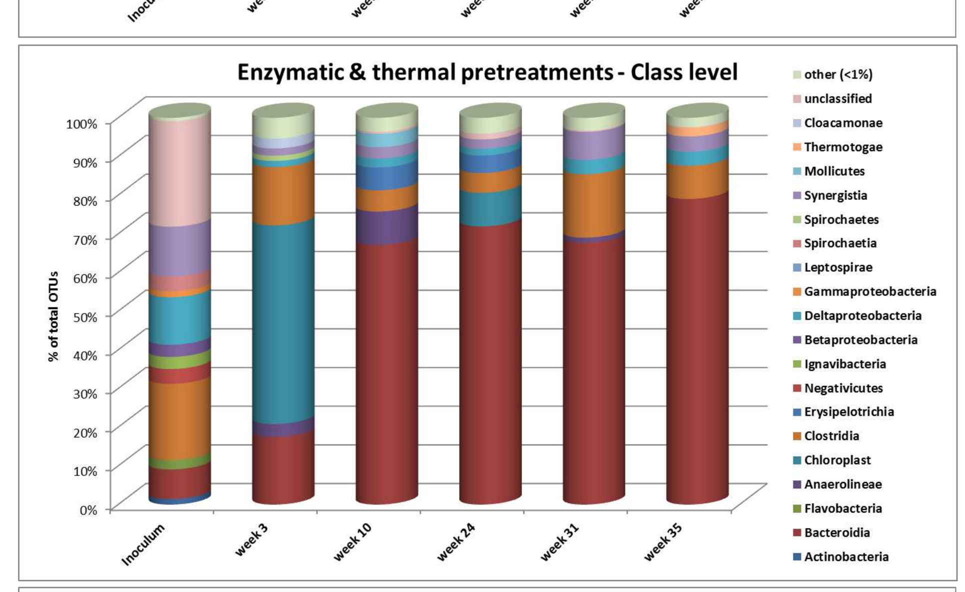
Effect of the selected pre-treatments on digesters' performance

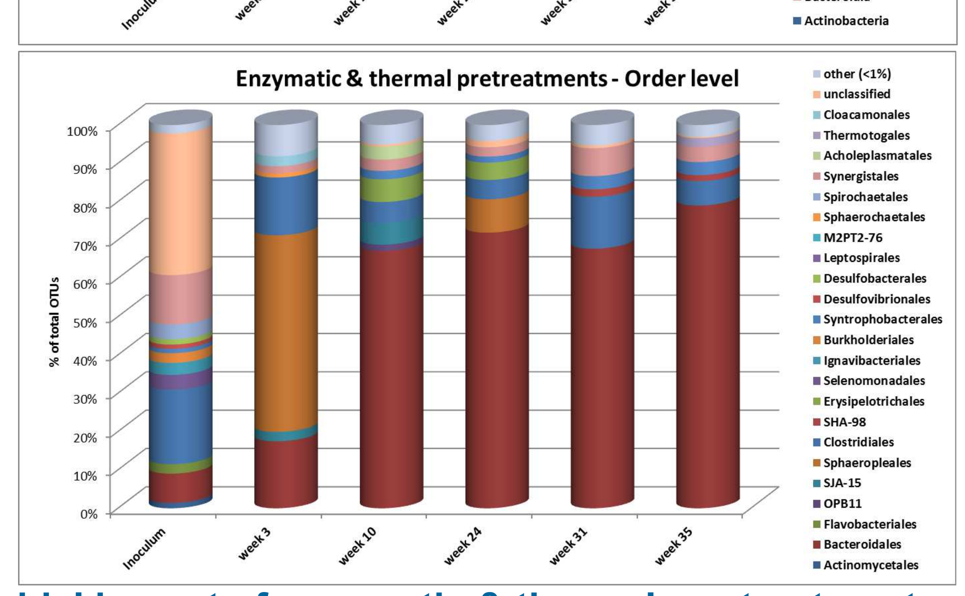
- **Only limited impact of both pre-treatment strategies in UASB tests**
  - → Best results obtained at mid OLR (~ 2g TVS/L_{RXR}.d) after the combination of caustic & thermal pre-treatments
  - → No substantial improvement of degradation efficiency
  - → Only limited improvement in methane production

Parameters	Best performances				
	Control UASB	UASB after pre-treatments			
Pre-treatment	No	Enzymatic & thermal (121-150°C)		Caustic & thermal (150°C)	
Operational time (weeks)		1 to 24	25 to 34	1 to 13	14 to 17
Solubilization ($[sCOD-sCOD_{T0}]/[totCOD_{T0}-sCOD_{T0}]$)	n/a	up to 25%		up to 66%	
HRT (d)	4	6		6	
OLR (gTVS/L_{RXR}.d)	2.95	1.3 to 4.7	2.2 to 4.2	2.2 to 3.3	1.1
Degradation (%, 1-TVS_{eff}/TVS_{in})	52	up to 52	up to 59	up to 66	up to 50
sCOD (g/L)	0.1 - 0.2	0.4 to 2.4	2.6 to 4.9	4.1 to 10.4	12.9
Y CH_4 (L/TVS_{in})	0.18	up to 0.23	up to 0.27	up to 0.36	0.35

Next generation sequencing: bacterial population







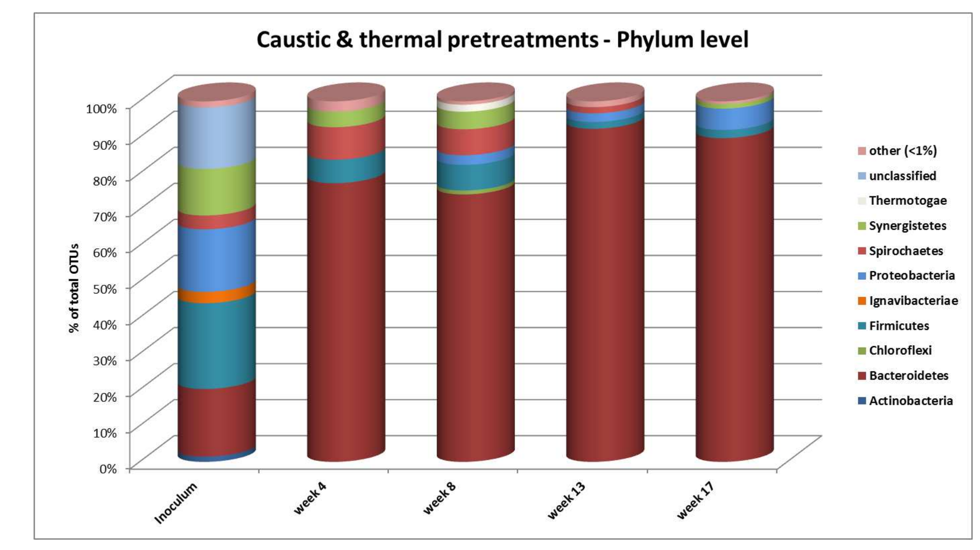
- **Microbial impact of enzymatic & thermal pre-treatments**

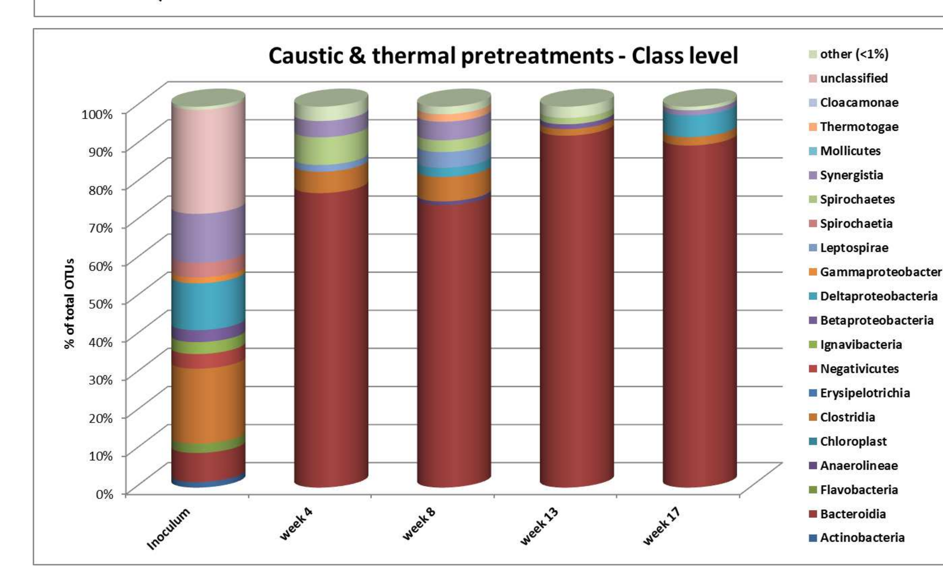
Important impact on bacterial population

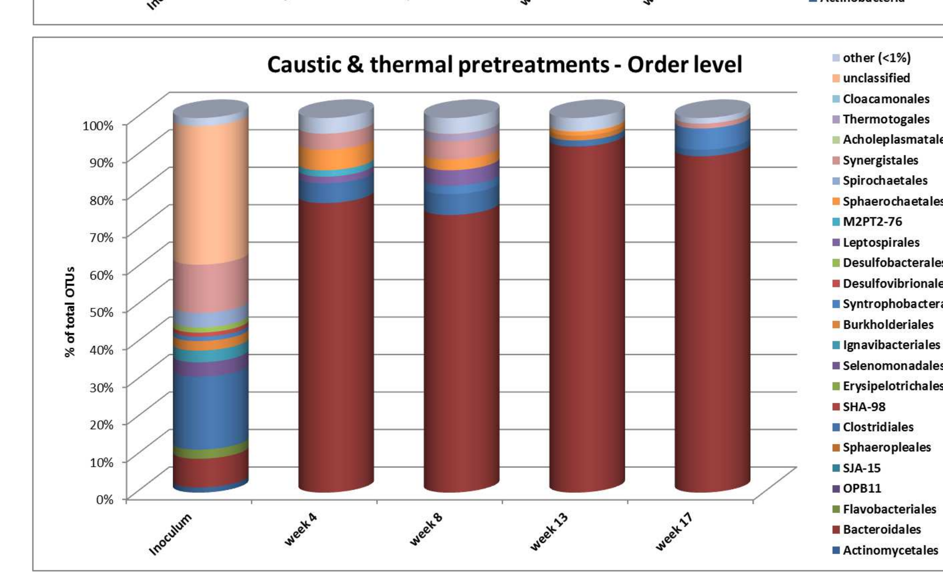
- → Huge increase of *Bacteroidetes*, especially of class *Bacteroidia* / order *Bacteroidales* (~80% of the population)
- → Huge decrease of *Proteobacteria*, *Firmicutes* and *Synergistetes*
- → Importance of 4 genera: *Paludibacter* (*Bacteroidetes*), *Syntrophus*, *Kosmotoga* and *Lutispora*
- → Variations at the genus level between 121°C and 150°C and when ORL varies

Microalgae not completely solubilyzed at 121°C

- → Microalgae (*Scenedesmus*) dominant after 3 weeks (51.3% of the population) and still detected after 24 weeks of operation
- → No microalgae detected at 150°C







- **Microbial impact of caustic & thermal pre-treatments**

Important impact on bacterial population

- → Huge increase of *Bacteroidetes*, especially of class *Bacteroidia* / order *Bacteroidales* (~80% of the population)
- → Huge decrease of *Proteobacteria*, *Firmicutes* and *Synergistetes*
- → Importance of one genus: *Syntrophus*
- → Variations at the genus level when ORL varies

Microalgae completely solubilyzed

- → Microalgae (*Scenedesmus*) not detected

CONCLUSIONS & PERSPECTIVES

- **Best stragegy for microalgal biomass pre-treatment**
  - → Best results obtained after the combination of caustic & thermal pre-treatments
  - → Best performance at mid OLR (~ 2g TVS/L_{RXR}.d)
- **Benefits from operating a high-rate digester on pre-treated microalgal biomass**
  - → Much shorter time to produce methane (HRT of 6 days vs 15-28)
  - → Reduction of the footprint of the AD process in an algal biorefinery
  - → Slight improvement in methane production
- **Characterization of the bacterial population adapted to methane production from microalgal biomass**
  - → Important shift in the bacterial population for the two digesters, with a particular importance of *Bacteroidetes* in the adapted populations
  - → Importance of the syntrophic bacterium *Syntrophus* (acetate-, H_2/CO_2- and formate-producer) in the best adapted population
  - → *Paludibacter* (VFA-producer), *Kosmotoga* (acetate and H_2/CO_2-producer) and *Lutispora* (VFA-producer) are genera of interest
- **Characterization of the adapted archeal population to be done**

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