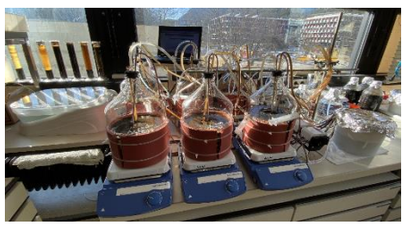

BioApp
Biointeractions - from basics to application

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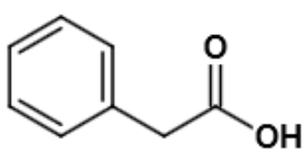
Microbial and phenyl acid dynamics during the start-up phase of anaerobic straw degradation in meso- and thermophilic batch reactors



PAA

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Prem et al. (2019)

Introduction

Organic waste materials coming from municipalities or agricultural facilities are valuable substrates for biogas production. One obstacle of using (pre-treated) organic wastes is the increased entry of aromatic compounds like lignocellulose or secondary plant metabolites. Aromatic compounds can cause poor biogas production due to intracellular malfunctions and biochemical restrictions[1]. However, the effects on AD communities are numerous and depend on several - not fully understood - microbiological and biochemical factors. Previous studies concluded that phenylacetate (PAA) and phenylpropionate (PPA) can be important and early detectable intermediates during AD of aromatic compounds. The objectives of this study were to i) initiate phenyl acid formation during the start-up phase of anaerobic straw degradation, ii) monitor microbial communities during high concentrations of straw from grain and iii) detect biomarkers for high phenyl acid concentrations.

Material and Methods

Meso- and thermophilic batch reactors were set up with different loadings (low: LCL, medium: MCL, high: HCL) of straw from grain as described in Prem et al.[2]. Gas as well as phenyl acid concentrations were measured chromatographically and the microbial community structure was analysed via high throughput amplicon sequencing of the 16S rRNA.

Results

On day 28, mesophilic MCL and thermophilic HCL samples produced significantly more methane than the control and LCL samples, respectively (Fig. 1). A cascade-like pattern – from PAA to PPA to phenylbutyrate (PBA) – was observed for meso- and especially for thermophilic samples. **Mesophilic** reactors: The highest phenyl acid concentrations were detected in MCL and HCL samples with 123 ± 3 mg L^{1} PAA in HCL samples on day 2, and 73 ± 3 mg L^{1} PPA on day 14 and 307 ± 24 mg L^{1} PBA in MCL samples on day 28. **Thermophilic** reactors: The highest phenyl acid concentrations were observed in HCL straw samples with 119 ± 9 mg L^{1} PAA on day 7, 202 ± 8 mg L^{1} PPA on day 14, and 1593 ± 80 mg L^{1} PBA on day 21. *Methanosarcina* – despite its previously described susceptibility to aromatic compounds – was the prevailing methanogenic genus in all mesophilic samples and in thermophilic HCL samples. *Methanoculleus* spp. was present in all thermophilic samples and was dominating in control, LCL and MCL reactors; the genus might have cooperated with syntrophic acetate oxidisers like *Syntrophaceticus* spp., *Tepidanaerobacter* spp. or Clostridia DTU014 genus. Genera like *Petrimonas* (mesophilic, MCL) and *Hydrogenispora* (thermophilic, HCL) – representative species are fermentative and produce acetate and hydrogen[3,4] - were significant biomarkers when methane and phenyl acid production were high.

	C-load (Class)	*LEfSe* Biomarkers (LDA Score ≥ 4.0)
Mesophilic	control	***Anaerolineaceae* uncult. genus** ***Clostridia* DTU014 genus** ***Bacteroidetes* vadinHA17 genus**
	MCL	***Bacteroides*** ***Petrimonas***
	HCL	***Caproiciproducens*** ***Ruminococcaceae* genus** ***Lachnospira*** ***Clostridium* sensu stricto 1** ***Methanobacterium*** ***Clostridia* D8A-2 genus**
Thermophilic	control	***Defluviitoga*** ***Syntrophaceticus*** ***Clostridia* MBA03 genus** ***Lactobacillus***
	HCL	***Hydrogenispora*** ***Methanosarcina*** ***Ruminococcaceae* UCG-010** ***Caproiciproducens*** ***Proteiniphilum***

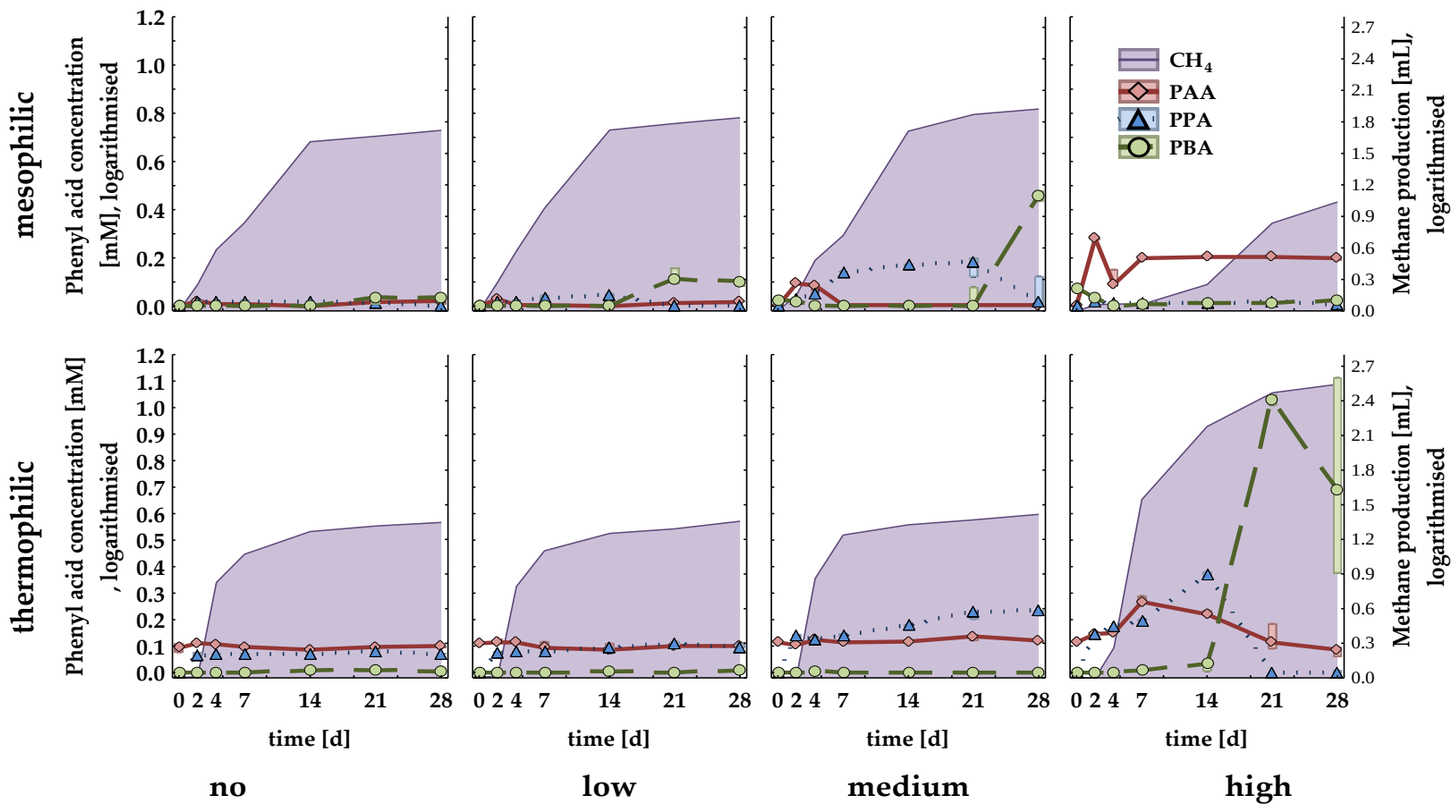
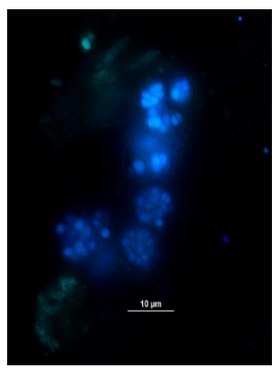


Figure 1: Cumulative methane production (violet area) and phenyl acid concentrations of no (controls), low, medium, and high C-load samples of the mesophilic (top) and thermophilic (bottom) biogas reactors, respectively, from day (d) 0 to day 28. Marker points and boxes show means and percentiles (25–75%), respectively.

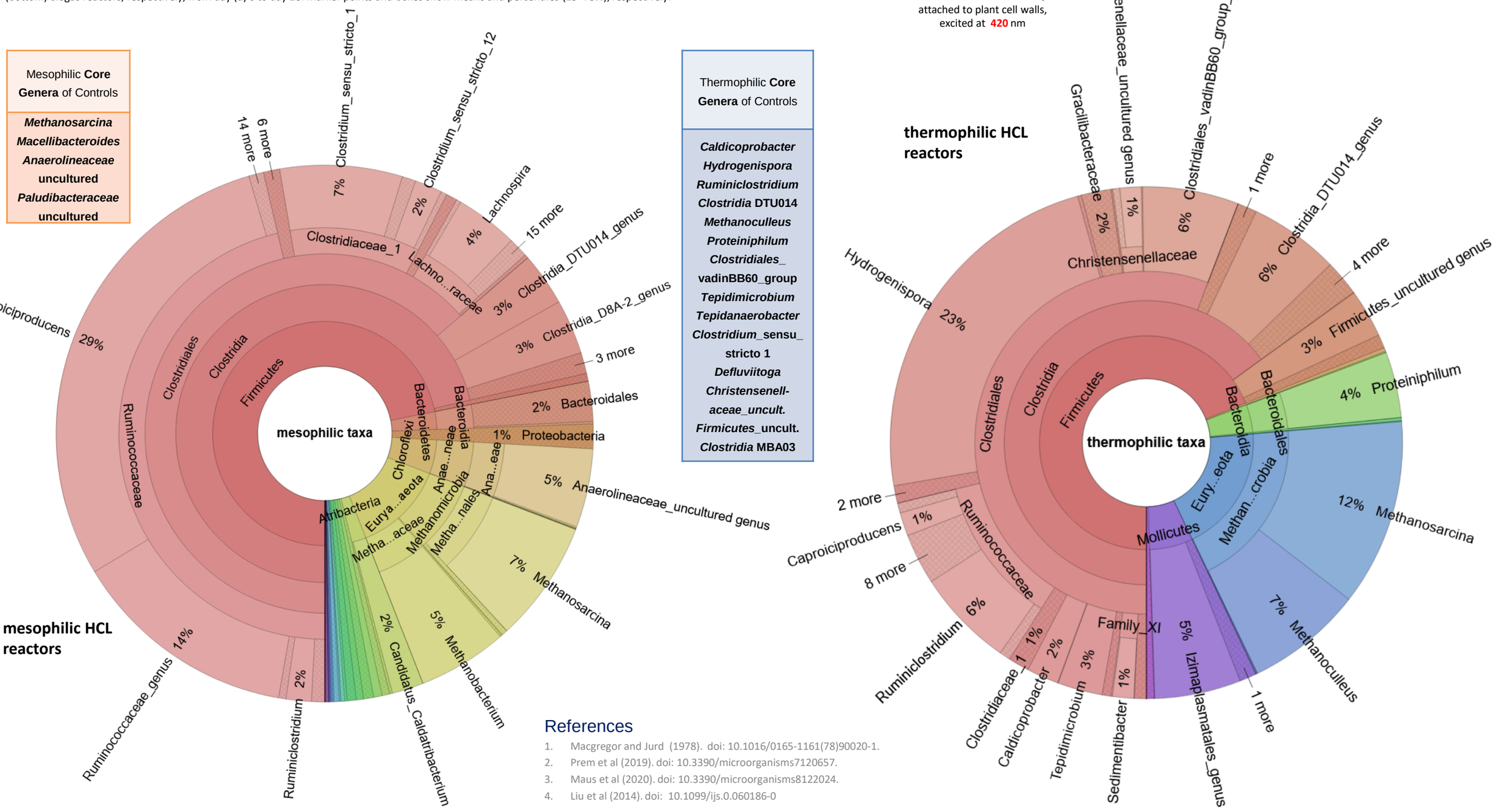
Batch reactor with straw (high load, HCL)



Thermophilic HCL reactors: *sarcina*-like cell structures, attached to plant cell walls, excited at **420** nm

Conclusions and Outlook

- While PAA was an early indicator for AD of straw material, an increase in PBA probably indicated the end of the start-up phase and the beginning of the degradation of more complex lignocellulosic materials.
- Results showed that aromatic compounds do not inevitably lead to process failures and that *Methanosarcina* spp. can thrive at high concentrations of lignocellulosic material under certain conditions which are not fully understood yet.
- Nonetheless, these preliminary results on phenyl acid dynamics might be interesting for biogas plant operators using lignocellulosic substrates.



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