Use of Microbial Consortia for Conversion of Biomass Pyrolysis Liquids into Value-Added Products
Outline

- Pyrolysis
- Bio-oil
- AD

\[
\{ \text{Py-AD} \}
\]

- Illumina Sequencing
- Mass Spectrometry
- Relatedness
Biomass burned at high temperatures (300°C - 600°C) in the absence of oxygen
Thermal depolymerisation of lignocellulosic biomass
Products are char, bio-oil & syngas
Bio-oil

- Product of pyrolysis of biomass
- Dark brown organic liquid
- High water content (~25 wt %)
- Extremely high oxygen content
- + 1000s other compounds
- Ages instantly
- Composition dependant on feedstock
- Low pH & biocatalyst inhibitors
Anaerobic Digestion

Hydrolysis
• High molecular weight organic polymers split into smaller more bioavailable monomers.
  • Proteins > Amino acids | Carbohydrates > Monosaccharides | Fats > Fatty acids | + H₂

Acidogenesis
• Acidogenic fermentation of hydrolysed products to short chain...
  • volatile acids (propionic, butyric, acetic, formic, lactic)
  • alcohols (ethanol, methanol)
• H₂ + CO₂ + NH₃ + H₂S

Acetogenesis
• Further digestion of acids by acetogens to H₂, CO₂ and acetic acid.

Methanogenesis
• Methanogenic archaea convert H₂ and acetic acid to CH₄, CO₂.
Vast range of microorganisms capable of bioconversion across a spectrum no single species could accomplish.

An ideal platform for the detoxification of complex organic mixtures such as bio-oil.

Enables relevant primary energy savings of non-renewable sources without worsening abiotic resources depletion + a strong reduction of GHGs emissions.

(Fabbri & Torri, 2016)
Hohenheim Biogas Yield Test

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- 12 × 100 ml glass syringes
- 30 ml Seafield water treatment plant anaerobic digestate
- Supplemented with 10 g/l COD bio-oil, dried anaerobic digestate (AD), wood pellets (WP) or seaweed (SW)
- Mesophilic (~37°C) for 102 days

(adapted Mittweg et al., 2012)

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![Graph showing biogas generation over time for different samples: DIGESTATE, AD BO, WP BO, SW BO.](image)
Illumina sequencing

Target gene 16S rRNA V4 hypervariable region

Conserved region

5’ Illumina adapter (adapted)
Primer pad
Primer linker
Forward primer

Reverse primer
Primer linker
Primer pad
Golay barcode
3’ Illumina adapter

Read 1

Read 2

Index

Adapted V4 forward primer

Coverage (%)

Archaea
Bacteria

V3 V4 aV4

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<thead>
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**Illumina sequencing**

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**Archaenal top 10**

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![Graph showing relative abundance of different archaeal phyla and classes](image-url)
Illumina sequencing

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Phylum | Class | Order | Family | Genus
--- | --- | --- | --- | ---
Thermotogae | Thermotogae | Petrotogales | Petrotogaceae | Defluviitoga
Cloacimonetes | W5 | uncultured | uncultured | uncultured
Bacteroidetes | Sphingobacteriia | Sphingobacteriales | Lentimicrobiaceae | uncultured
Bacteroidetes | Bacteroidia | Bacteroidales | Porphyromonadaceae | Proteiniphilum
Firmicutes | Clostridia | D8A-2 | uncultured | uncultured
Firmicutes | Clostridia | Thermoanaerobacteriales | Thermoanaerobacteraceae | Gelria
Bacteroidetes | Bacteroidia | Bacteroidales | Porphyromonadaceae | uncultured
Proteobacteria | Gammaproteobacteria | Pseudomonadales | Pseudomonadaceae | Pseudomonas
Firmicutes | Clostridia | Clostridiales | Caldicoprobacteraceae | Caldicoprobacter
Firmicutes | BSA1B-03 | uncultured | uncultured | uncultured

Relative Abundance (%)
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-1.5 -1 0 0.5 1 1.5
-2 -1 0 1 2
NMDS 1

-1.5 -1 -0.5 0 0.5 1.5
NMDS 2

d102 DIG1 d102 DIG2 d102 DIG3
d102 WP2 d102 WP3
d102 WP1 d102 AD1 d102 AD2 d102 AD3
d102 SW1 d102 SW2 d102 SW3

-2 -1 0 1 2
NMDS 1

-1.5 -1 -0.5 0 0.5 1.5
NMDS 2
Relatedness

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Relatedness

Defluviitoga Abundance (24.4% of total variation)

Cloacimonetes Abundance (38.2% of total variation)
Analysis:
- Distance-based linear model (DistLM).
- Multivariate chemical data matrix using the abundance profiles of Candidatus Cloacimonetes and the Defluviitoga as the predictor variables.
- The predictor variables are additionally plotted as vectors (annotated arrows).
- The abundance profiles of these two microorganisms are cumulatively able to explain 62.61% of the chemical variation observed.

- Candidatus Cloacimonetes phylum abundance correlates with the chemical pattern separating reactor conditions – propionate degradation?

- Defluviitoga suggests that increases in its abundance are related to the chemistry observed for digestate-only control reactors – specific inhibition by bio-oil?
Importance

• Patterns in reactor chemistry can be correlated to fluxes in microbial community

• Understanding the microbial players involved at each stage of AD

• Longitudinal studies: continuous sampling of both the chemical and biological species involved to identify process bottlenecks

• Strategies to overcome inhibition
Biochar supplementation aids AD by the adsorption of inhibitory compounds and via the adherence of microbial cells in biofilms.

- High surface area, biofilm formation
- Biofilms show increased resistance to environmental stresses
- Partially conductive to the flow of electrons, capable of supporting direct interspecies electron transfer (DIET)

Thank you!

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