ERA CoBioTech

BioTech Research and Innovation Hack 2021

Mid-term seminar of the projects from 2. call of ERA CoBioTech

SYNBIOGAS: Synthetic landfill microbiomes for enhanced anaerobic digestion to biogas

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SYNBIOGAS

@synbiogas

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant 722361

27.09.2021

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Project partners

SYNBIOGAS

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Natural Resources Wales

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- Plus several AD / landfill operators
- Total project budget: €1.2m
- Start: Jan 2020 - End: Dec 2022

@synbiogas

Introduction

- By 2025, global cities will generate approximately 2.2 billion tonnes of solid waste biomass per year (The World Bank, 2017).
- Lignocellulosic plant biomass is the major waste product.
- Microorganisms convert waste biomass to methane-rich biogas.
- Anaerobic digestion (AD) plants and landfills are engineered environments where microorganisms are harnessed for waste decomposition and biogas production.

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Introduction

SYNBIOGAS

- There are over 17,000 AD plants and 500,000 landfill sites in the EU alone.
- Enhancing microbial biomass conversion to biogas in AD plants and landfill provides a sustainable and renewable green-energy source.
- Typically, microorganisms from animal faeces / slurry used as inoculum for AD.

Güllert et al. Biotechnol Biofuels (2016) 9:121
DOI 10.1186/s13068-016-0534-x
Biotechnology for Biofuels

Conclusions: Our data indicate that a relatively lower abundance of bacteria affiliated with the phylum of *Bacteroidetes* and, to some extent, *Fibrobacteres* is associated with a decreased richness of predicted lignocellulolytic enzymes in biogas fermenters. This difference can be attributed to a partial lack of genes coding for cellulolytic GH enzymes derived from bacteria which are affiliated with the *Fibrobacteres* and, especially, the *Bacteroidetes*. The partial deficiency of these genes implies a potentially important limitation in the biogas fermenter with regard to the initial hydrolysis of biomass. Based on these findings, we speculate that increasing the members of *Bacteroidetes* and *Fibrobacteres* in biogas fermenters will most likely result in an increased hydrolytic performance.

AMERICAN SOCIETY FOR MICROBIOLOGY

RESEARCH ARTICLE
Applied and Environmental Science
July/August 2017 Volume 2 Issue 4 e00300-17
https://doi.org/10.1128/mSphere.00300-17

Lignocellulose-Degrading Microbial Communities in Landfill Sites Represent a Repository of Unexplored Biomass-Degrading Diversity

Emma Ransom-Jones[a], Alan J. McCarthy[b], Sam Haldenby[c], James Doonan[a], James E. McDonald [a]

	Bacteroidetes	Fibrobacteres	Firmicutes	Proteobacteria	Spirochaetes
Auxiliary Activities	51	0	78	4	35
Carbohydrate Binding Modules	831	9	638	21	90
Carbohydrate Esterases	538	0	370	13	82
Cohesin	11	0	37	0	0
Dockerin	5	0	129	0	0
Glycoside Hydrolases	1665	13	938	46	286
Glycosyl Transferases	1006	2	584	44	105
Polysaccharide Lyases	115	2	28	3	6
S-layer Homology Domain	1	0	583	2	0

Ransom-Jones et al., 2017

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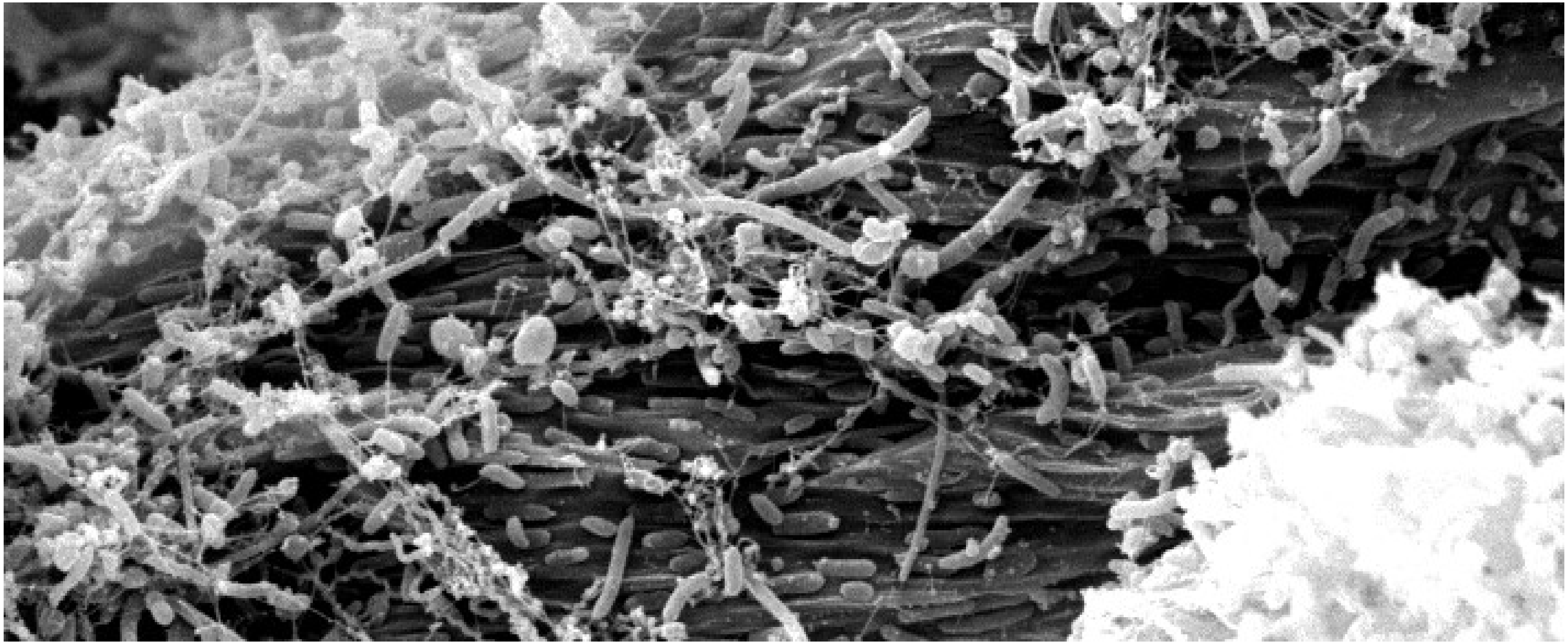
Research questions

SYNBIOGAS

Could microbiota from landfill sites enhance biomass conversion in AD plants?

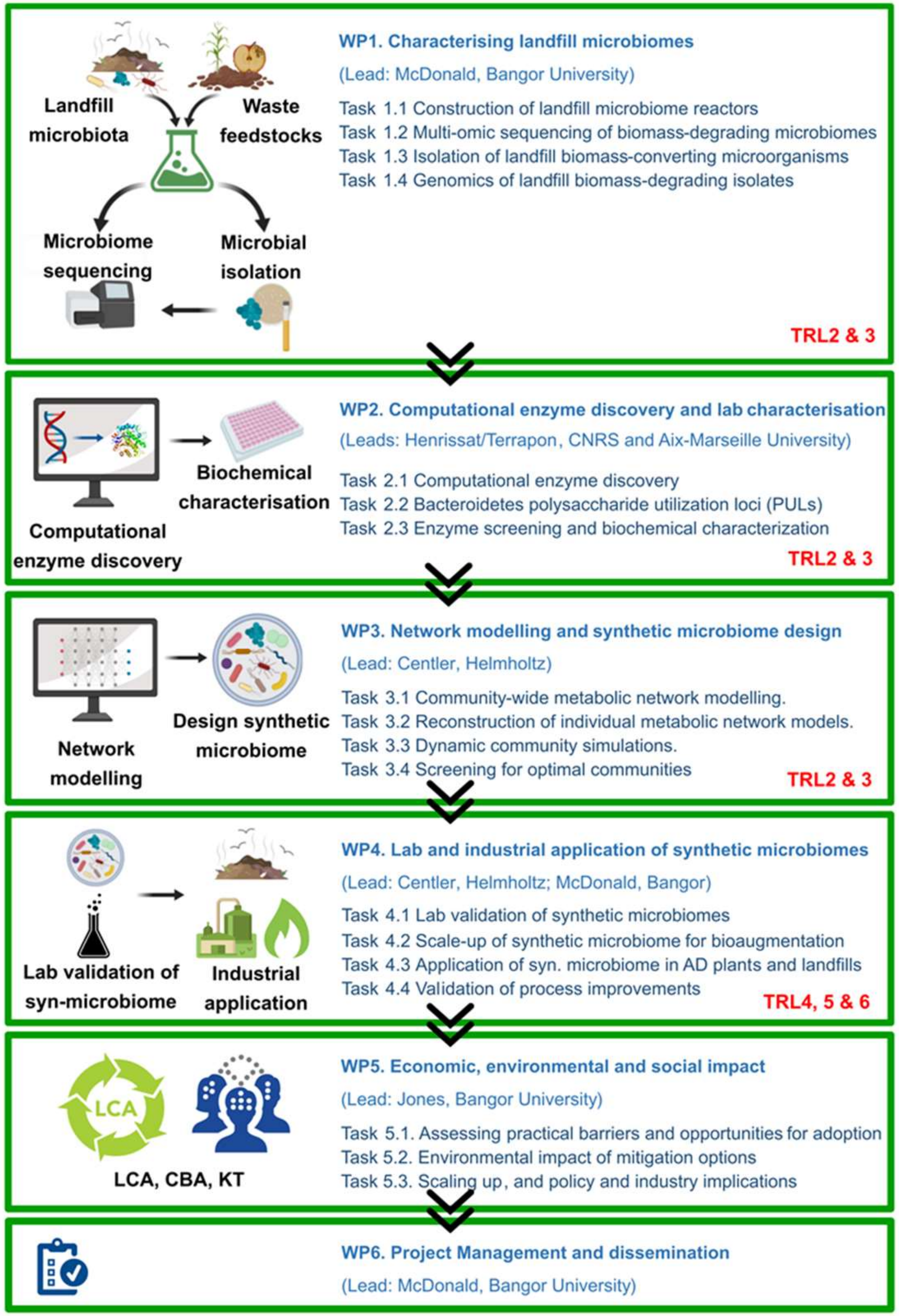
Can synthetic landfill microbiomes be used for bioaugmentation of AD and landfill processes?



Project plan

Aims of the SYNBIOGAS project:

- Characterise anaerobic landfill microbiomes associated with waste biomass conversion.
- Design optimal synthetic microbiomes for anaerobic digestion.
- Application and validation of synthetic microbiomes for bioaugmentation of landfill sites and AD plants.



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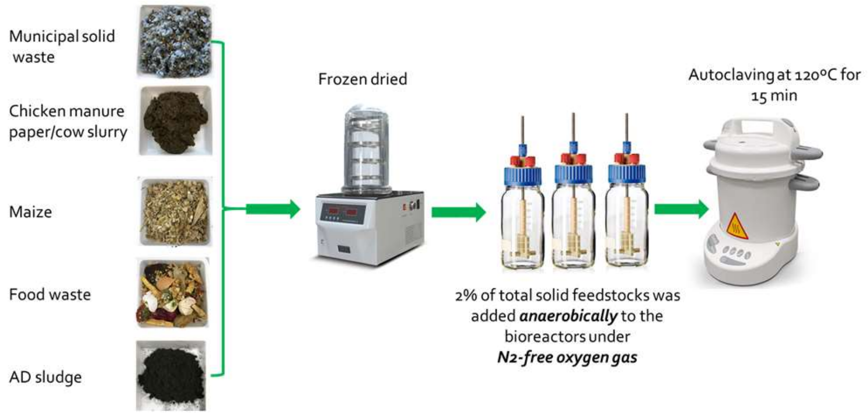
Technical overview
WP1: Chracterising landfill microbiomes

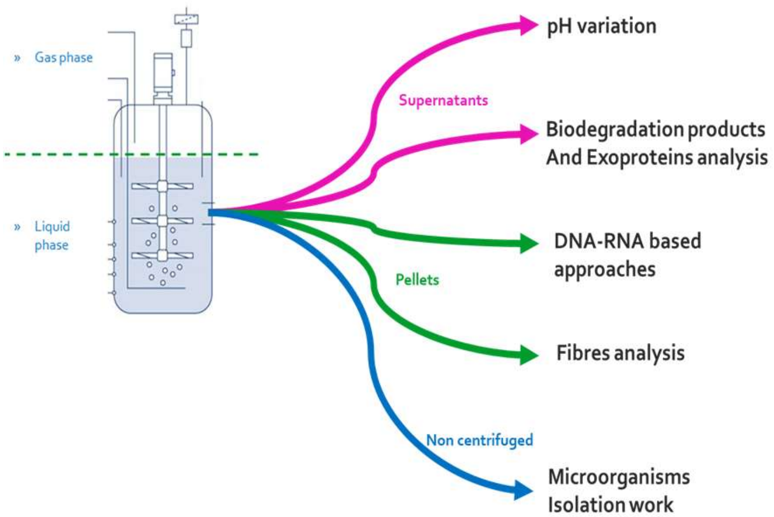
SYNBIOGAS

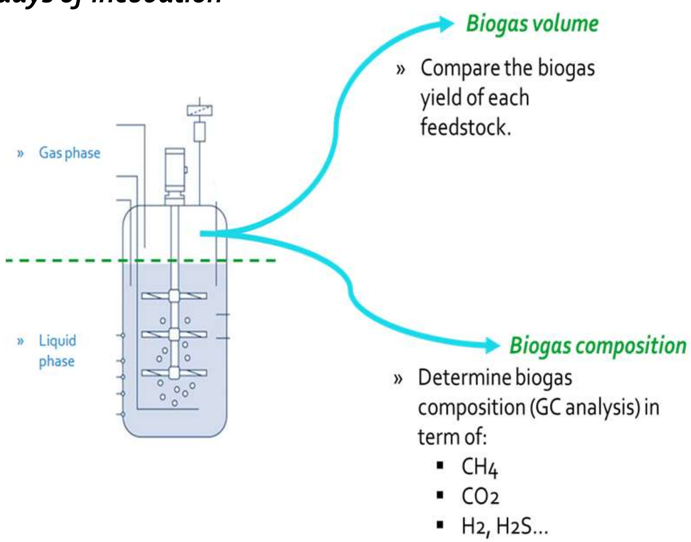
Landfill bioreactors set up



Landfill Microbial communities

Monitoring of the bioreactors during 28 days of incubation



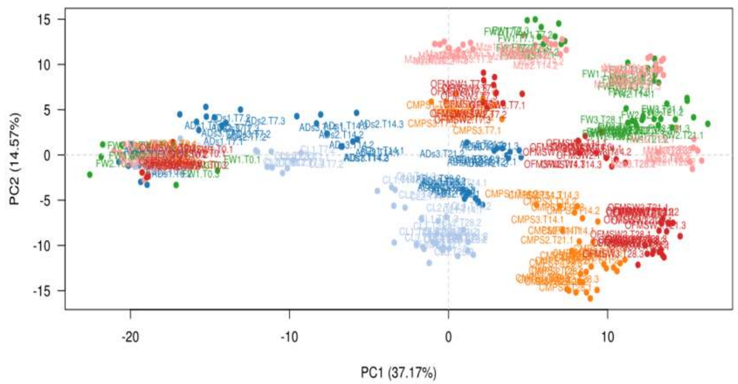


Task 1: Construction of landfill microbiome reactors

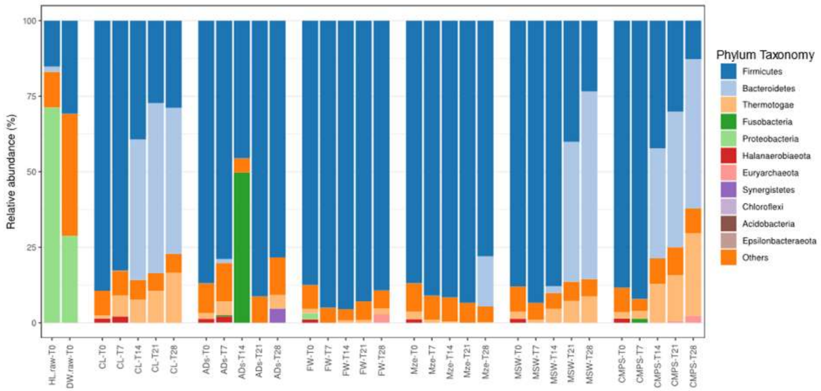
Technical overview
WP1: Chracterising landfill microbiomes

- ***Single gene community profiling:*** sequencing of the V4 region of the 16S rRNA gene: 276 samples in total were analysed.

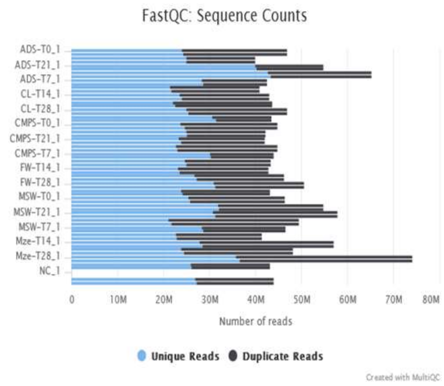
PCA Analysis: Clear separation by sampling time/feedstock

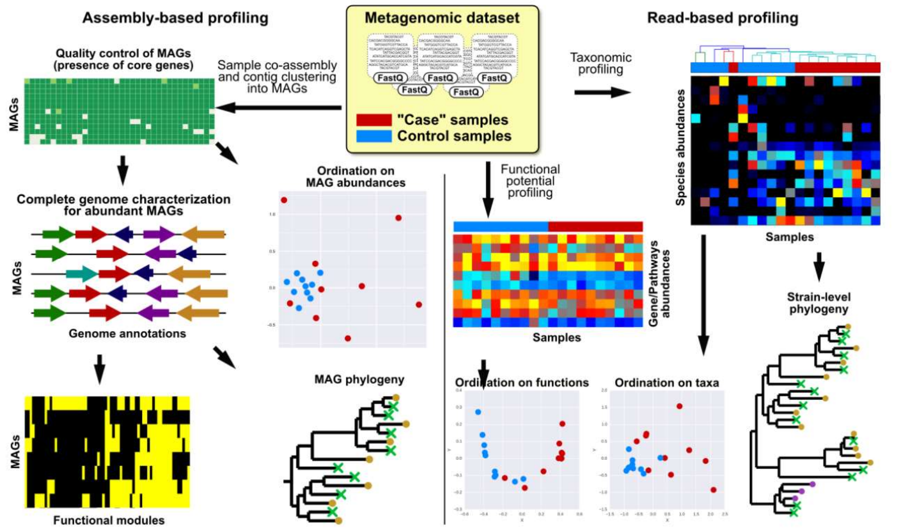


Community profiling: Total of 513 ASVs were found in all the samples



- ***Shotgun metagenomes sequencing:*** 32 DNA Libraries in total were sequenced, assembled, and analysed.



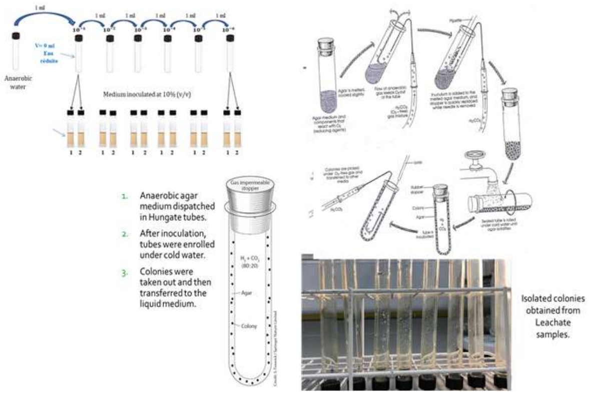


Task 2: Multi-omic sequencing of biomass degrading microbiome

Technical overview
WP1: Chracterising landfill microbiomes

- ***Isolation work:*** more than 600 strains were isolated belonging to 61 different species.

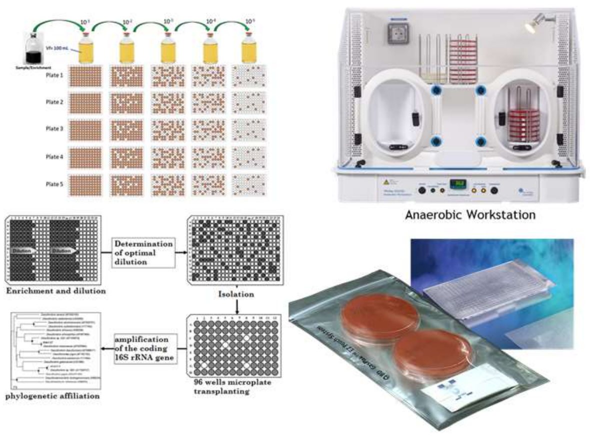
Hungate techniques



Phylum Taxonomy

- Firmicutes
- Bacteroidetes
- Thermotogae
- Fusobacteria
- Proteobacteria
- Halanaerobiaeota
- Euryarchaeota
- Synergistetes
- Chloroflexi
- Acidobacteria
- Epsilonbacteraeota
- Others

High-throughput isolation in microplates



Genus Taxonomy

Caldicoprobacter	Enterococcus
Caproiciproducens	Keratinibaculum
Proteiniphilum	Tissierella
Clostridium	Fastidiosipila
Garciella	Halocella
Tepidanaerobacter	Hydrogenispora
Anaerosalibacter	Desulfotomaculum
Defluviitoga	Acetomicrobium
uncultured	Pseudomonas
Sporanaerobacter	Anaerosporobacter
Fusobacterium	Vibrio
Tepidimicrobium	Salmonella
Bacillus	Methanoculleus
Thiopseudomonas	Fermentimonas
Anaerococcus	Thermotoga
Lactobacillus	Staphylococcus

- ***Enrichment and characterisation of of targeted groups:*** *e.g., cellulose degrading, and methanogens communities.*

Enrichment on Avicel of *Fibrobacters.*

Enrichment of *Spirochaete.*

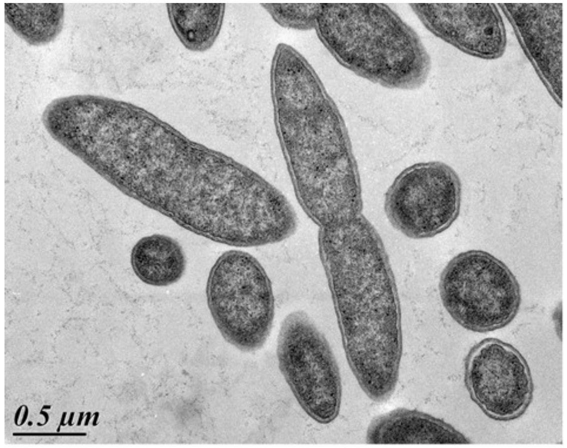
Enrichment of methanogens, microorganisms responsible of methane production in the bioreactors.

Task 3&4: Isolation and genomics of biomass degrading microbiome

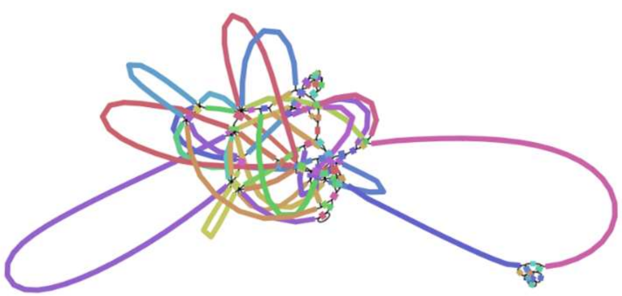
Technical overview

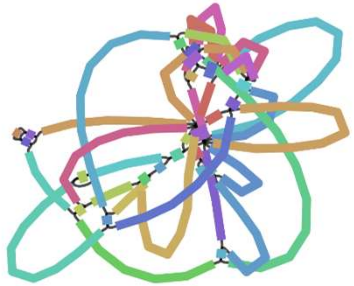
WP1: Chracterising landfill microbiomes

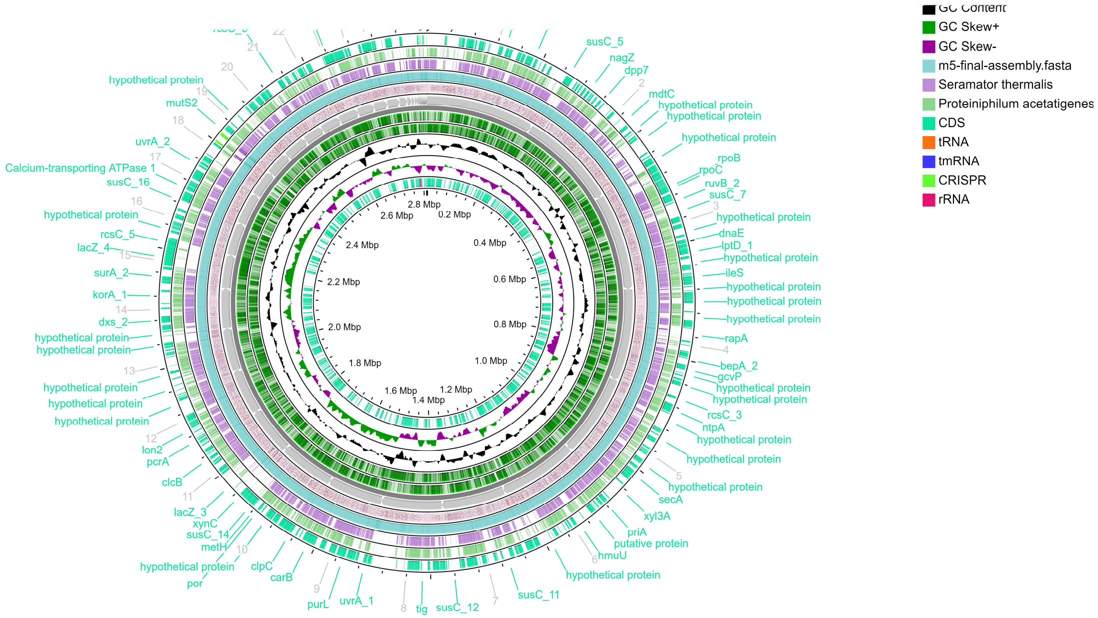
- ***Description and characterisation of novel species:*** 15 novel species were isolated representing a novel species, genera or even novel families.



- ***Genomics of isolates of interest:*** Whole genome sequencing and genome annotation of the novel species isolates.







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Task 3&4: Genomics of biomass degrading microbiome

Technical overview
WP2: Computational enzyme recovery

SYNBIOGAS

- *Context*
  - ***CAZy*** → *specialist database for carbohydrate assembly/breakdown*
  - ***Annotation*** → *identification of protein domain families*
  - ***Functional interpretation*** → *families with high substrate specificity*
  - ***Subfamilies*** → *improve functional annotation in large/diverse families*

- *Results*
  - ***Annotation** of the whole gene catalog*

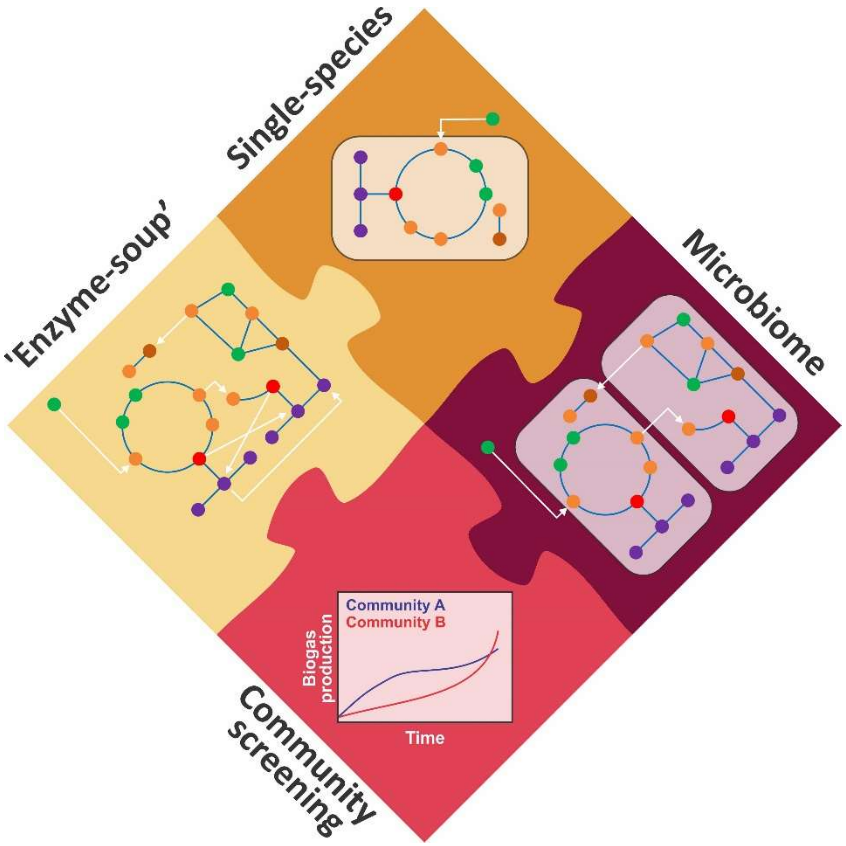
  → *map to samples for **interpretation***

  → *select candidates for **biochemistry***
  - ***Large subfamilies delineation** facilitated by a **Graph Theory criterion***

  → *Proof-of-concept manuscript (in prep) for 3 CAZy families, more to come!*

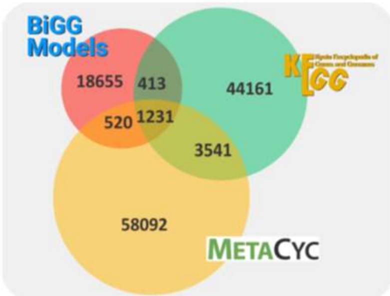
Technical overview
WP3: network modelling

- **Task 1:** Reconstruction of single-species metabolic network models
- **Task 2:** Community-wide metabolic network modeling ('enzyme-soup')
- **Task 3:** Microbiome simulations
- **Task 4:** Screening for optimal communities



WP3: Project outcomes so far

- Pipeline for metabolic reconstruction of multiple metabolic network models:

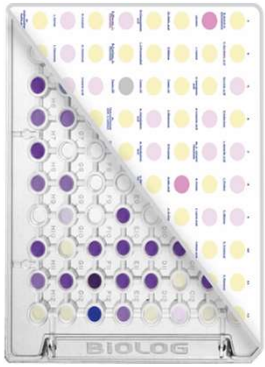
CarveMe + 

+ BioCyc KEGG Organisms + MinPath

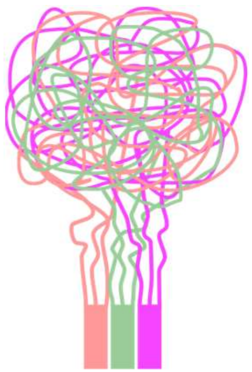
Software to reconstruct metabolic models	Reaction database: combination of KEGG, BiGG, and MetaCyc	Identification of pathways likely present in organisms	Identification of pathways present in models

WP3: Project outcomes so far

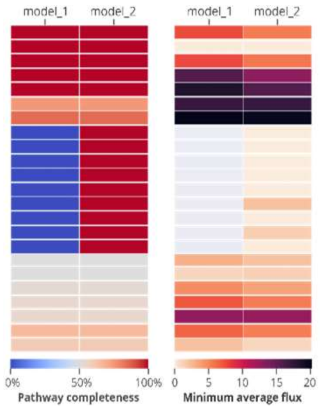
- Web application to analyze, compare and curate metabolic network models. It is available at *sbmlcomp.bioinf.uni-leipzig.de*



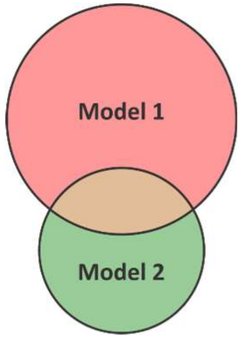
In silico
growth phenotype



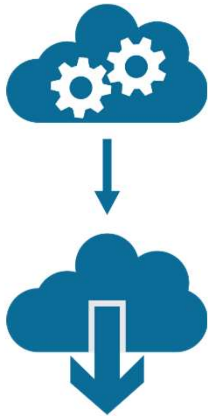
Reactions mapping
to KEGG and BioCyc pathways



Calculate pathway activity
and identify
essential reactions



Identify reactions
present
in multiple models



Export model after
deleting or including
reactions

Project outcomes and next steps

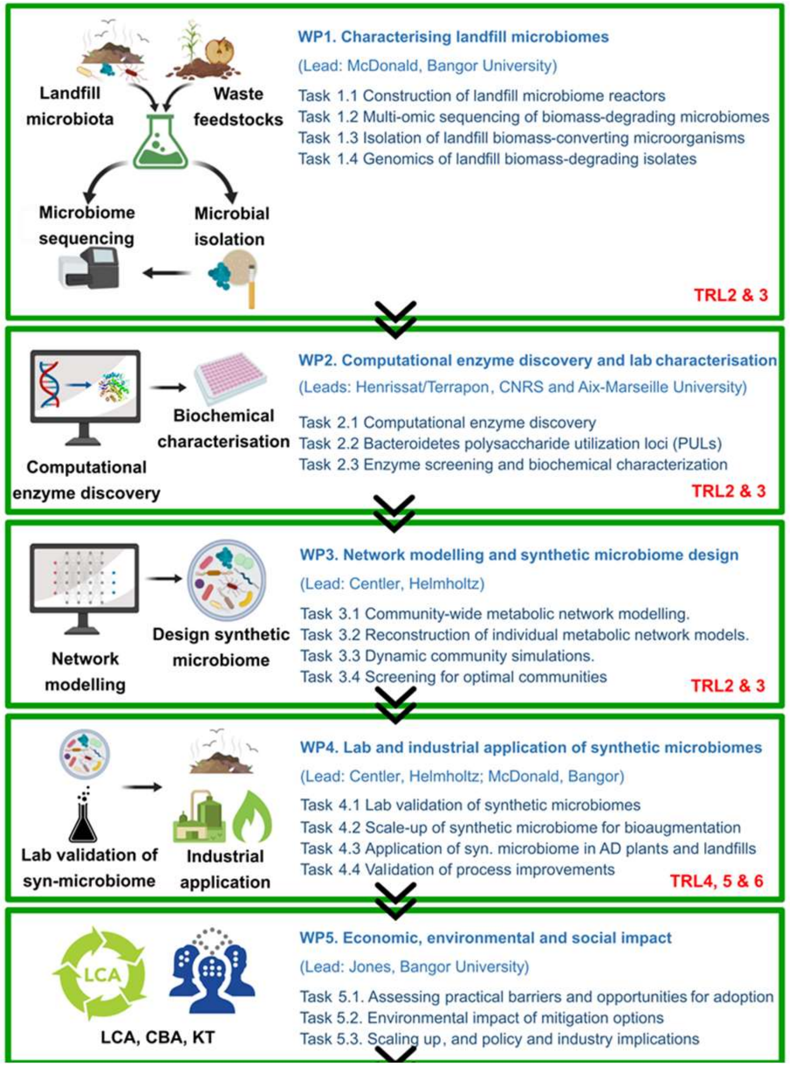
Outcomes:

Bioreactors:
16S rRNA gene & shotgun metagenomes. >500 isolates, 61 species, 15 new species.

Enzyme discovery:
CAZYme detection. Subfamilies for 3 GH families.

Network modelling:
Pipeline for metabolic model reconstruction. Web application.

5 manuscripts in prep



Next steps:

Genome sequencing of isolates

Enzyme characterisation

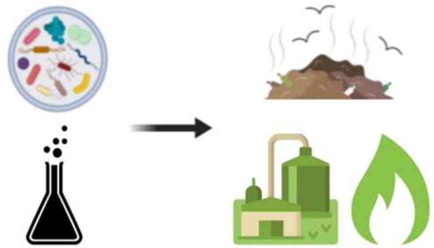
Network modelling

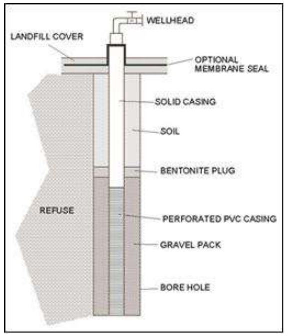
Testing and application of synthetic microbiomes

Problems: Covid-19, inability to meet in person (as a team, and with stakeholders)

Expected outcomes

- Social impacts:
  - sustainable options for waste management
  - reduce environmental impact of waste biomass
  - development of key enabling technologies for sustainable green-energy generation
- Scientific outcomes:
  - Collection of microbial isolates from landfill sites
  - CAZYmes with enhanced catalytic activity and substrate specificity
  - high resolution datasets on SLM activity
  - metabolic process models of AD processes
  - validated SLMs for AD bioaugmentation
  - life cycle assessment and roadmap for tech implementation





Next steps

- *What is planned*
  - *In person team meeting*
  - *Stakeholder-focused dissemination & discussion events*
  - *WP4 research co-design with stakeholders*
  - *Outreach activities – animation, science festivals, press releases.*
  - *Stakeholder handbook*

- *What should be achieved*
  - *Several manuscripts in preparation.*
  - *Bioaugmentation tests in landfill sites and AD plants.*

Policy recomendations

- *Recommendations for political measures to overcome current and potential obstacles for biotech research, market implementation of biobased products, processes and technologies*

Follow-on funding to support the development and commercialisation of technologies emerging form the projects.

Contact details

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