Flow cytometry fingerprinting for the design of a cellulolytic synthetic microbial community

R. Kinet [1*#], Ph. Dzaomuho [1], S. Hiligsmann [1], C. Tarayre[1], B.Taminiau[2], G. Daube[2], F. Delvigne[1]

[1] ULg – Gembloux Agro-Bio Tech, Microbial Processes and Interactions. Passage des Déportés, 2, Gembloux, B-5030, Belgium. *corresponding author: r.kinet@doct.ulg.ac.be [#] founded by FNRS-FRIA

[2] Fundamental and Applied Research for Animal & Health (FARAH), Food Science Department, Faculty of Veterinary Medicine, University of Liège, Sart-Tilman, B43b Liège, B-4000 Belgium.

Context and objectives

ANAEROBIC DIGESTION

Lignocellulosic biomass

Complex microbial population

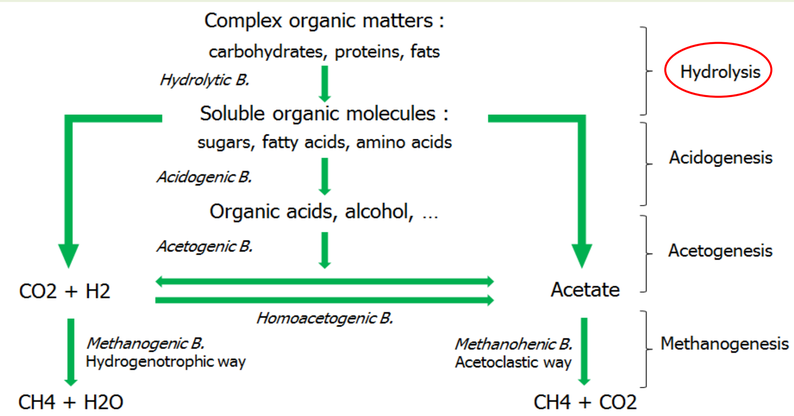
Microbiome's behavior = blackbox

Biogas : CH4, CO2

Digestate

1. **Hydrolysis of lignocellulosic biomass = Limiting step**
2. **Microbiome's behavior = Blackbox**

1. **Design of a cellulolytic synthetic microbial community**
2. **Monitoring of microbiome**

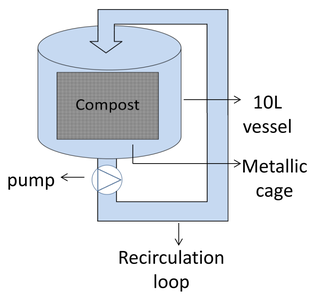


Method

Step 1 : Microbial cellulolytic community isoaltion

➢ **Experimental conditions**

- Compost as microbial source
- Solid/liquid (water) extraction
- Anaerobia
- Thermophilia



Step 2 : Assessment of Cellulose anaerobic digestion improvement

➢ **Experimental conditions:**

- Cellulolytic community, leachate or mix of consortium and leachate in 1:1 proportion as inoculum
- Anaerobia, 55°C, static
- Cellulose as substrate 1% (w/v)

Step 3 : Microbiome monitoring during anaerobic digestion

A. Metagenomics analysis

3 days

Raw sample → DNA extraction, amplification and sequencing → Data processing → OTU's distribution <-> Metagenomics fingerprint

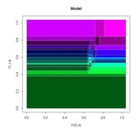
B. Flow cytometry analysis

1 h

Raw sample → DNA Staining → Flow Cytomety analysis → Data processing → Cytometric fingerprint

Flow cytometric data processing – Flow FP package :

- Cytometric space modelization (geometrical grid composed of defined number of bins)
- Application of grid model to samples' cytometric pattern
- Extraction of cell number per bin for each sample



Results

➢ Improving cellulose anaerobic digestion thanks to cellulolytic community

○ ***Cellulose Hydrolysis rate***

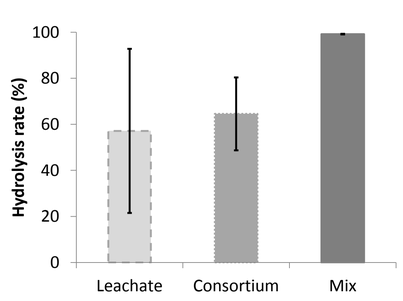


Figure 1 : Final hydrolysis rates (%) obtained after anaerobic and thermoophilic (55°C) digestion of cellulose (10 g/l) by (1) leachate microflora (10% v/v), (2) isolated consortium (10% v/v) and (3) mix 1:1 of leachate and isolated consortium (10% v/v).

○ ***Biogas production***

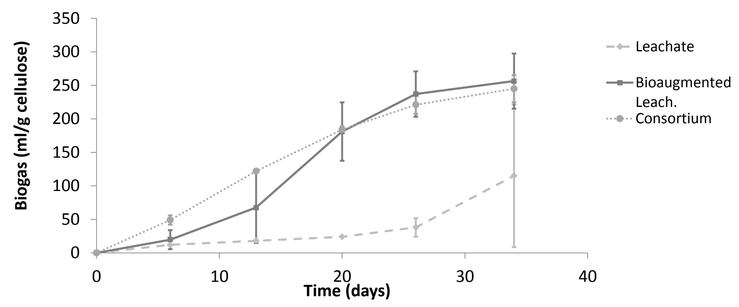


Figure 2 : Evolution of biogas production (ml/g cellulose) during anaerobic and thermophilic (55°C) digestion of cellulose (10 g/l filter paper) by (1) leachate microflora (10% v/v), (2) isolated consortium (10% v/v) and (3) mix 1:1 of leachate and isolated consortium (10% v/v).

➢ **Addition of cellulolytic community induces improvement of leachate cellulolytic potential and biogas production**

➢ **Maximal hydrolysis rate and biogas production obtained when populations are mixed**

➢ Microbiome monitoring during anaerobic digestion

○ ***Metagenomics analysis***

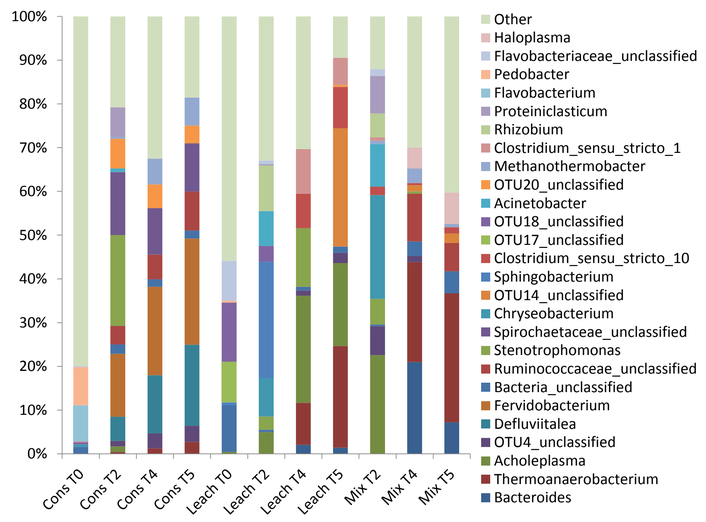


Figure 3 Evolution of microbial populations (metagenomics analysis) during anaerobic and thermophilic (55°C) digestion of cellulose (10 g/l) by (1) isolated consortium (10% v/v), (2) leachate microflora (10% v/v) and (3) mix 1:1 of leachate and isolated consortium (10% v/v). Only genus with relative abundance superior to 5% in one of the sampels are presented individually, others are regrouped in "other" group.

○ ***Flow cytometry analysis***

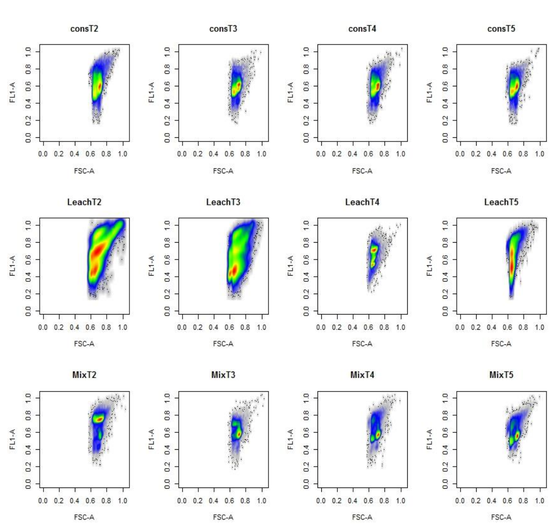


Figure 4 Evolution of microbial populations (cytometry analysis) during anaerobic and thermophilic (55°C) digestion of cellulose (10 g/l) by (1) isolated consortium (10% v/v), (2) leachate microflora (10% v/v) and (3) mix 1:1 of leachate and isolated consortium (10% v/v). High cell density is represented by red color while blue represents low cell density.

○ ***Calculation of similarity between samples***

Flow cytometry data

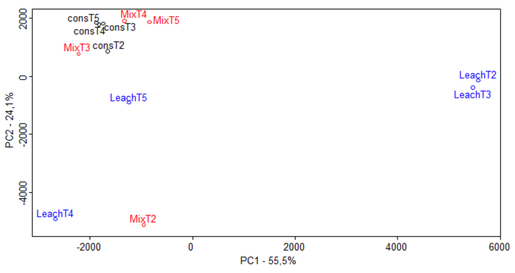


Figure 5 Distribution of samples' flow cytometric patterns in 2 dimensional space. In a first time, samples' flow cytometric patterns are processed thanks to Flow FP package to obtain fingerprints of each sample. Next, PCA is applied to all fingerprints to calculate distances between samples. Here, samples are represented according to two first principal components.

	consT2	consT3	consT4	consT5	LeachT2	LeachT3	LeachT4	LeachT5	MixT2	MixT3	MixT4	MixT5
consT2	0											
consT3	1546	0										
consT4	1263	377	0									
consT5	1417	261	170	0								
LeachT2	7676	7979	8008	8090	0							
LeachT3	7673	7948	8007	8075	4849	0						
LeachT4	6098	6817	6804	6845	9836	9758	0					
LeachT5	3635	5140	4837	4994	8297	7885	6419	0				
MixT2	6224	7500	7356	7468	8899	8496	3648	4517	0			
MixT3	1601	1186	1263	1233	8245	8226	5714	5016	6748	0		
MixT4	1240	767	578	695	7571	7609	7045	4666	7359	1712	0	
MixT5	1356	1723	1483	1628	7034	7347	7258	4162	7158	2436	981	0

Table 1 Euclidian distance between different microbial populations (flow cytometric pattern) according to their coordinates in principal components space.

Metagenomics data

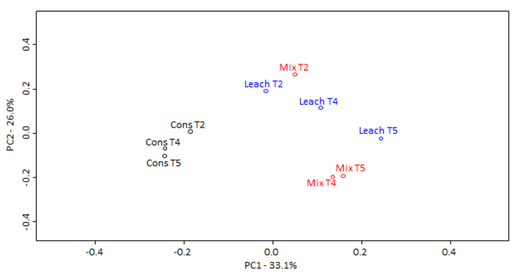


Figure 6 Distribution of samples' metagenomics patterns in 2 dimensional space. PCA is applied to all metagenomics fingerprints to calculate distance between samples. Here, samples are represented according to two first principal components.

	Cons T2	Cons T4	Cons T5	Leach T2	Leach T4	Leach T5	Mix T2	Mix T4	Mix T5
Cons T2	0,00								
Cons T4	0,31	0,00							
Cons T5	0,27	0,14	0,00						
Leach T2	0,44	0,46	0,48	0,00					
Leach T4	0,40	0,45	0,49	0,44	0,00				
Leach T5	0,52	0,54	0,52	0,52	0,36	0,00			
Mix T2	0,44	0,50	0,50	0,39	0,35	0,47	0,00		
Mix T4	0,47	0,47	0,45	0,49	0,44	0,43	0,52	0,00	
Mix T5	0,47	0,48	0,46	0,49	0,43	0,39	0,52	0,26	0,00

Table 2 Euclidian distance between different microbial populations (metagenomics fingerprint) according to their coordinates in principal components space.

➢ **Cytometry fingerprinting allows identification of population dynamics → highlights stabilisation of « consortium » and « mix » microbiomes along the process**

⇔ Metagenomics analysis of different microbiomes confirm cytometric results about population stabilisation.

➢ **Cytometry fingerprinting does not allow distinction of populations with different species composition ⇔ No correlation between cytometric and metagenomics results**

Conclusions

➢ **Addition of isolated consortium improves cellulolytic potential of leachate.**

➢ **Cytometry fingerprinting as an efficient and rapid tool for population dynamics identification.**

These works fit in GreenWin project ''Minerve'' funded by Région Wallonne of Belgium.
Leachates come from landfill site of Mont-Saint-Guibert in Belgium managed by Shanks SA.