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Usefulness of Molecular Biology Tools in the Operation and Control of the Anaerobic Digestion Process

Jean-Philippe Steyer
Laboratoire de Biotechnologie de l’Environnement, INRA Narbonne
Usefulness of Molecular Biology Tools

- Microbial Resource Management towards Ecological Engineering?

From « Who is present? » « Who is doing what? » « With whom? »....

... to « why are they doing it together? »
Study and control of electro-assisted fermentation in mixed cultures:
The role of engineering of microbial interactions

Javiera Toledo Alarcón
PhD Candidate

Supervisor: Nicolas Bernet
Co-Supervisor: Eric Trably
Metabolic Pathways

- **H₂ to release e⁻ excess**

- **Acetate route**

  \[ \text{Glucose} + 2H₂O \rightarrow 2\text{Acetate} + 2CO₂ + 4H₂ \]

- **Acetate + ethanol route**

  \[ \text{Glucose} + H₂O \rightarrow \text{Ethanol} + \text{Acetate} + 2CO₂ + 2H₂ \]

- **Butyrate route**

  \[ \text{Glucose} \rightarrow \text{Butyrate} + 2CO₂ + 2H₂ \]

- **Lactate route**

  \[ \text{Glucose} \rightarrow 2\text{Lactate} + H^+ \]
Electro-Fermentation

“Novel process that consists of electrochemically controlling microbial metabolism with electrodes”

- Voltage applied on working electrode
- **Anodic EF**: partial electron sink
- **Cathodic EF**: additional electron source
- Microbial interaction between species and electrode surface
- Microbial interaction between species
Experimental methodology

Control
(n=5)

Electro-Fermentation

Glucose (5 g.l\(^{-1}\)) & Other Nutrients: Starkey

pH\(_{\text{initial}}\): **6.0** (MES Buffer)

Temperature: **37 °C**

Operation: **Batch x 20 h**

Inoculum: **HT anaerobic sludge sampled from a lab-scale AD treating sewage sludge**

- **0.9 V vs SCE** (n=2)
- **0.4 V vs SCE** (n=2)
+ **0.4 V vs SCE** (n=3)
+ **0.9 V vs SCE** (n=3)
H₂ production during Electro-Fermentation (Not correlated with the voltage applied)

H₂ production (mol H₂/mol Glucose)

Control: 0.74
-0.9 V: 1.49
-0.4 V: 1.80
0.4 V: 1.34
0.9 V: 1.81

(Anova, F=20.68, p=0.001)

Small current → High impact
Metabolite distribution

\[ \text{Glucose} \rightarrow 2\text{Lactate} + H^+ \]

\[ \text{Glucose} \rightarrow \text{Butyrate} + 2\text{CO}_2 + 2\text{H}_2 \]

\[ \text{Glucose} + 2\text{H}_2\text{O} \rightarrow \text{Ethanol} + \text{Acetate} + 2\text{CO}_2 + 2\text{H}_2 \]
Microbial community

![Bar chart showing the family distribution of bacterial communities under different voltages.](chart)

- **Streptococcaceae**
- **Enterobacteriaceae**
- **Clostridiaceae**

### Family Distribution (%)
- **Control**: Clostridiaceae 0.2%, Enterobacteriaceae 95%, Streptococcaceae 4.8%
- **-0.9 V**: Clostridiaceae 0.1%, Enterobacteriaceae 95%, Streptococcaceae 4.8%
- **-0.4 V**: Clostridiaceae 0.1%, Enterobacteriaceae 95%, Streptococcaceae 4.8%
- **0.4 V**: Clostridiaceae 0.1%, Enterobacteriaceae 95%, Streptococcaceae 4.8%
- **0.9 V**: Clostridiaceae 0.1%, Enterobacteriaceae 95%, Streptococcaceae 4.8%

### Notes
- Others (<4.0%)
Analysis of Metabolic patterns & microbial community

- **Streptococcaceae**
  - Lactate
  - H2

- **Clostridiaceae**
  - Butyrate
  - H2
  - Lactate

- **Enterobacteriaceae**
  - Acetate
  - Ethanol
  - Succinate
Usefulness of Molecular Biology Tools

Could these tools help us to optimize process performances?
Anaerobic digestion

Complex ecosystem – High diversity

SSCP fingerprint
Anaerobic digestion

Under specific operating conditions (pre-heating, low pH, short HRT)

No biogas but bioH₂ and biomolecules
Anaerobic digestion

- complex polymers
  - cellulolytic and other hydrolytic bacteria
- monomers
  - fermentative bacteria
  - succinate, propionate, butyrate, alcohols
  - $H_2$-producing fatty acid oxidizing bacteria (syntrophs)
- acetate
  - Homoacetogens
- $H_2 + CO_2$
  - Hydrogenotrophic methanogens
  - Acetoclastic methanogens

One major species

Few minor species: roles?
Ecological Engineering for biotic control

In each reactor, same operating conditions (feed=glucose, HRT=10h, T=37°C, pH=5.5)

In steady state in each reactor (after several HRTs), only one major specie (difference only in minor species)
Ecological Engineering for biotic control

Identical major bacteria, so identical performance, isn’t it?....

NO !!!

Relative Abundance of bacteria

Link between structure and function of the ecosystem

Sub-dominant bacteria as keystone species in microbial communities producing bio-hydrogen

Yan Rafafi*, Eric Trably*, Jérôme Hamelin*, Eric Latrille*, Isabelle Meynial-Salles*, Saida Benomar*, Marie-Thérèse Giudici-Orticoni*, Jean-Philippe Steyer*
Ecological Engineering for biotic control

To act on the structure of the ecosystem to influence the function

Biotic control of the metabolism

✓ PERFORMANCES

✓ STABILITY
Ecological Engineering for biotic control

Study of the interactions: a model

$Clostridium$ sp.: $H_2 \rightarrow$

$Desulfovibrio$ sp.: $H_2 = \emptyset$

$\Rightarrow$
Ecological Engineering for biotic control

influences the metabolism of !!!

[H₂] x 2.5 ! And faster !

Change in metabolic flux
Ecological Engineering for biotic control

Study of the interactions: a model

Clostridium sp.
H2 ↗

Desulfovibrio sp.
H2 = ∅

Aggregation of the two organisms
Ecological Engineering for biotic control

In addition....

A physical contact is mandatory!
Ecological Engineering for biotic control

New microbial interactions!

**ARTICLE**

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**Nutritional stress induces exchange of cell material and energetic coupling between bacterial species**

Saïda Benomar1, David Ranava1, Maria Luz Cárdenas1, Eric Trably2, Yan Rafai2, Adrien Ducret3, Jérôme Hamelin2, Elisabeth Lojou1, Jean-Philippe Steyer2 & Marie-Thérèse Glédi-Orticoni1
Usefulness of Molecular Biology Tools

Is microbial diversity an advantage?
Role of the inoculum and microbial diversity

- 17 different inocula (from real digesters réels or natural environments)
- Same complex substrate for all digesters
- Same stable conditions for hundreds of days
Role of the inoculum and microbial diversity

Occasional substrate addition as fed-batch

The 17 inocula: soil, compost, freshwater sediments, digestors...
Hypothesis: Divergence of ecosystem performance

moved up in rank

weeks 1 – 3

moved down in rank

weeks 10 – 12
Role of the inoculum and microbial diversity

occasional substrate addition as fed-batch

feeding/withdrawing every two day

final substrate pulse(s) in batch, BMP-like

Adaptation phase

Continuous mode

Final performance

Initial inoculum

Six months

three months

The 17 inocula: soil, compost, freshwater sediments, digestors...
Role of the inoculum and microbial diversity

From 17 continuous processes to 256 batch reactors!
Role of the inoculum and microbial diversity

The 17 ecosystems alone

After mixing of the 17 ecosystems

No substrate

“Overyielding”
Role of the inoculum and microbial diversity syntrophic networks with different community structures
Usefulness of Molecular Biology Tools

How to integrate all the information in mathematical models?
Modeling of microbial ecosystems
Microbial diversity or functional distribution?

Modelling competition between multiple populations: emergence of the "redox tower of microbial metabolism"
Microbial diversity or functional distribution?

Growth rate formulation:

From \( \mu = \mu_{\text{max}} \frac{S}{K_S + S} \) to \( \mu = \mu_{\text{max}} \prod_{i=1}^{\text{card}(C)} e^{\frac{\nu_i^{\text{MET}}}{V_h[C]_i}} \)

with

\[ \mu_{\text{max}} = \frac{k_B T}{h} f(T, \Delta S, k_B, h) \]

\[ \nu_i^{\text{MET}} = \nu_i^{\text{AN}} + \lambda(C, T) \cdot \nu_i^{\text{CAT}} \]

\[ \lambda(C, T) = \frac{\Delta G_{\text{an}}(C, T) - \Delta G_{\text{dis}}(NoC_{C_s}, \gamma_{C_s})}{-\Delta G_{\text{cat}}(C, T)} \]

Only tuning parameter! \( V_h \): harvest volume

\([C]_i\): concentration of chemical specie #i
Microbial diversity or functional distribution?

Comparing with classical Monod equation

- Complex particulate waste and inactive biomass
  - Carbohydr.
  - Proteins
  - Fats
  - MS
  - AA
  - LCFA
  - Propionate
  - HVa, HBu
  - Acetate
  - H2
  - CH4, CO2
  - Inert particulate
  - Inert soluble
  - Death
Usefulness of Molecular Biology Tools

Examples of remaining open questions
Never forget Mother Nature!

Treated Pollution (kg COD/m^3·j)

- Min
- Max

0 50 100 150 200 250 300 350 400

70 220 400
Never forget Mother Nature!
From the analysis of 190 digestive tracts
Never forget Mother Nature!

Batch reactor

Continuous stirred tank reactor (CSTR)

CSTRs in serie

Plug-flow reactor
Never forget Mother Nature!

In terms of volume

Readily Biodegradable Substrates

Herbivorous

Omnivores

Carnivorous

enzymes

Assimilation (sugar, acetate, etc.)

<table>
<thead>
<tr>
<th>Carnivorous</th>
<th>Herbivorous</th>
<th>Omnivores</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>30%</td>
<td>20%</td>
</tr>
<tr>
<td>20%</td>
<td>50%</td>
<td>50%</td>
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<tr>
<td>0%</td>
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</tbody>
</table>

Never forget Mother Nature!
Never forget Mother Nature!

The ‘herbivorous’ configuration

Lama

Hoazin

Cow

Kangaroo
New opportunities for wastewater treatment?
New opportunities for wastewater treatment?

- Granules settle very well: Largely reduce size of settler (space and operation = $$$)
- Phototrophs produce oxygen: reduced need for external oxygen supply (aeration = $$)
- When digested, phototrophic granules produces 30% more CH$_4$ ($$$)
Usefulness of Molecular Biology Tools

As a conclusion...
Usefulness of Molecular Biology Tools

Demonstrated at the industrial scale!
Thank you for your attention

http://www.montpellier.inra.fr/narbonne

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