Valorisation of agroindustrial waste for the production of energy, biofuels and biopolymers

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Biomass: is the oldest and most promising source of energy

- Biomass Organic and animal wastes, wastewaters, energy crops, agricultural and industrial residues
- Biomass: is the oldest and most promising source of energy
Valorization of agroindustrial wastes

- Agricultural residues
- Agroindustrial wastes
- Food waste and by-products

Bioprocessing

Added-value products and energy

Biofuels

Biopolymers

Energy
Biomass utilization for energy

• First generation (energy crops)
  – Serious concern as it replaces food resources
• Second generation (residues, mainly lignocellulosic)
• Third generation (algal biomass)
Biomass Conversion to Biofuels

Technologies:

- Chemical (biodiesel)
- Thermal (direct combustion, pyrolysis, gasification)
- Biological (anaerobic digestion, hydrogen fermentation, alcoholic fermentation etc)

An emerging new possibility:

Direct Electricity Generation: Microbial fuel cells (MFCs)
Anaerobic metabolism

Glucose

Phosphoenolpyruvate → Oxalocetate → Succinate → Propionyl-CoA → Propionate

Pyruvate → Acetyl-CoA → Acetaldehyde → Ethanol

Acetyl-CoA → Butyryl-CoA → Butyrate → Butanol

Lactate

Acetate
What will be produced depends on:

1. Feedstock characteristics
2. Microbial species present (pure and mixed cultures)
3. Operating conditions
Anaerobic digestion

☑ One of the most important biochemical processes for biomass conversion
☑ CH$_4$ and CO$_2$ are produced from organic substrates via mixed microbial consortia under anaerobic conditions

Overall reaction:

\[
\text{Organic substrates + H}_2\text{O} \rightarrow \text{CH}_4 + \text{CO}_2 + \text{NH}_3 + \text{new cells}
\]

Advantages:

• Methane production (gaseous biofuel)
• Suitable for wastes of high organic load
• Digestate can be composted
Biological macromolecules: carbohydrates, proteins, lipids

Monomers: sugars, amino acids, fatty acids

Hydrolysis

Acidogenesis

\[ \text{H}_2 + \text{CO}_2 \rightarrow \text{Acetic acid} \]

Acetogenesis

Acetic acid

Lactic acid

Ethanol

Propionic and Butyric acid

Acetogenesis

\[ \text{H}_2 + \text{CO}_2 \rightarrow \text{Acetic acid} \]

Acetogenesis

\[ \text{H}_2 + \text{CO}_2 \rightarrow \text{Acetic acid} \]

Methanogenesis

\[ \text{METHANE} \]
Virtually AD is the only feasible biological process for treating agroindustrial wastewaters (e.g. dairy, piggery and olive-mill)

- high organic load
- seasonal nature
- small distributed units

Normally high retention times are needed in standard CSTR-type reactors

High-rate reactors such the UASBR and the ABR have been developed
Periodic Anaerobic Baffled Reactor (PABR)

... based on the simple ABR configuration ...

... it was made flexible to alternate its operation between the ABR (compartmentalized) and UASBR (homogenized) operation mode by directing the influent into all compartments successively.

The PABR compartments are arranged in a circular manner:
The experimental PABR
Feedstocks & Conditions – CH₄

- Sorghum extract
  - 20d, 15d, 10d
- Olive Mill Waste
  - 20d, 15d, 10d
- Dairy wastewater
  - 20d, 10d, 4.4d
## Yields and rates – CH$_4$

<table>
<thead>
<tr>
<th>HRT (h)</th>
<th>CH$_4$ (%)</th>
<th>Specific rate (L biogas/L/d)</th>
<th>Specific rate (L CH$_4$/L/d)</th>
<th>Yield (L CH$_4$/kg TS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>64.4 ± 2.0</td>
<td>0.34 ± 0.03</td>
<td>0.21 ± 0.02</td>
<td>25.2</td>
</tr>
<tr>
<td>15</td>
<td>64.9 ± 1.8</td>
<td>0.53 ± 0.02</td>
<td>0.35 ± 0.02</td>
<td>31.2</td>
</tr>
<tr>
<td>10</td>
<td>63.1 ± 1.2</td>
<td>0.93 ± 0.02</td>
<td>0.59 ± 0.01</td>
<td>35.2</td>
</tr>
<tr>
<td>20</td>
<td>66.2 ± 2.0</td>
<td>0.64 ± 0.05</td>
<td>265</td>
<td>79.5</td>
</tr>
<tr>
<td>15</td>
<td>65.0 ± 2.0</td>
<td>0.79 ± 0.05</td>
<td>245</td>
<td>73.6</td>
</tr>
<tr>
<td>10</td>
<td>67.0 ± 2.0</td>
<td>1.13 ± 0.08</td>
<td>234</td>
<td>70</td>
</tr>
<tr>
<td>20</td>
<td>74.9 ± 1.0</td>
<td>1.7 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>26</td>
</tr>
<tr>
<td>10</td>
<td>73.6 ± 3.0</td>
<td>3.5 ± 0.5</td>
<td>2.6 ± 0.3</td>
<td>26</td>
</tr>
<tr>
<td>4.4</td>
<td>71.8 ± 2.2</td>
<td>6.9 ± 0.6</td>
<td>5.0 ± 0.6</td>
<td>22</td>
</tr>
</tbody>
</table>
Fermentative hydrogen production

Biopolymers: carbohydrates, proteins, lipids

Monomers: sugars, amino acids, fatty acids

**Hydrolysis**

**Acidogenesis**

- Acetic acid
- H₂ + CO₂

**Acetogenesis**

- Acetic acid
- Propionic acid, Butyric acid, lactic acid, Ethanol

**Methanogenesis**

- H₂ + CO₂
- Acetic acid

METHANE
Why hydrogen?

- A clean and environmentally friendly fuel which produces water instead of greenhouse gases, when burned.

- Possesses the highest specific energy yield (122kJ/g).

- Could be used to produce electricity through fuel cells.

- Can be produced by renewable raw materials, such as biomass, through biological or thermochemical processes.
Fermentative hydrogen production

ACETIC ACID PRODUCTION
• $\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 2\text{CO}_2 + 4\text{H}_2$

BUTYRIC ACID PRODUCTION
• $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{CO}_2 + 2\text{H}_2$

PROPIONIC ACID PRODUCTION
• $\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{H}_2 \rightarrow 2\text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O}$

LACTIC ACID PRODUCTION
• $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{CH}_3\text{CHOHCOOH}$

ETHANOL PRODUCTION
• $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{CH}_3\text{CH}_2\text{OH} + 2\text{CO}_2$

The absence or presence of hydrogen consumers in mixed consortia also affects the final distribution of metabolites and H$_2$ yields.

Mixed microbial consortia need to be pretreated so as hydrogen consumers to be suppressed and hydrogen producers to dominate the consortium.

Thermal pretreatment (100°C, 15min)
Parameters affecting fermentative hydrogen production

- pH
- $\text{H}_2$ partial pressure
- Hydraulic Retention Time (HRT)
- Temperature
- Nutrients concentration
- Initial carbohydrates concentration
- Organic loading
Hydrogen production from wastewaters, solid wastes and energy crops

In this framework several bioreactors of CSTR (Continuous Stirred Tank Reactor), SBR (Sequential Batch Reactor) and UFCR (Up-flow Column Reactor) type are used for the exploitation of:

- Dairy wastewater
- 3-phase and 2-phase olive mill wastewater
- Sweet sorghum biomass
- Glycerol
- Food wastes
- Wastepaper sludge
Reactors & Conditions – \( \text{H}_2 \)

- CSTR type, 0.5 or 3 L
- \( T=35^\circ \text{C} \)
- HRT differs according to substrate
  - **Sorghum extract**
    - 24h, 12h, 8h, 6h, 4h
  - **Olive mill wastewater**
    - 30h, 14.5h, 7.5h
  - **Dairy wastewater**
    - 24h

- Column bioreactor, 1.5L
- Immobilization of cells on ceramic beads
- \( T=35^\circ \text{C} \)
- HRT 36h
  - glycerol
### Yields and rates – H₂

<table>
<thead>
<tr>
<th>HRT (h)</th>
<th>H₂ (%)</th>
<th>Specific rate (L H₂/L/d)</th>
<th>Molecular yield (mol H₂ / mol gluc)</th>
<th>Yield (L H₂/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>30.4 ± 1.2</td>
<td>0.82 ± 0.08</td>
<td>0.37 ± 0.02</td>
<td>4.9</td>
</tr>
<tr>
<td>12</td>
<td>39.9 ± 1.2</td>
<td>3.48 ± 0.20</td>
<td>0.86 ± 0.04</td>
<td>10.4</td>
</tr>
<tr>
<td>8</td>
<td>40.5 ± 1.9</td>
<td>4.14 ± 2.40</td>
<td>0.75 ± 0.05</td>
<td>8.4</td>
</tr>
<tr>
<td>6</td>
<td>39.2 ± 0.5</td>
<td>5.10 ± 0.18</td>
<td>0.70 ± 0.02</td>
<td>7.6</td>
</tr>
<tr>
<td>4</td>
<td>35.0 ± 1.5</td>
<td>4.36 ± 0.30</td>
<td>0.41 ± 0.02</td>
<td>4.3</td>
</tr>
<tr>
<td>30</td>
<td>26.4 ± 1.7</td>
<td>0.26 ± 0.01</td>
<td>0.81</td>
<td>1.9</td>
</tr>
<tr>
<td>14.5</td>
<td>26.7 ± 1.4</td>
<td>0.39 ± 0.05</td>
<td>0.61</td>
<td>1.3</td>
</tr>
<tr>
<td>7.5</td>
<td>29.1 ± 1.6</td>
<td>0.47 ± 0.05</td>
<td>0.504</td>
<td>0.8</td>
</tr>
<tr>
<td>24</td>
<td>29.3 ± 1.6</td>
<td>2.51 ± 0.43</td>
<td>0.9 ± 0.1</td>
<td>(L H₂/L) 2.49</td>
</tr>
<tr>
<td>36</td>
<td>37.1 ± 1.2</td>
<td>0.35 ± 0.01</td>
<td>0.13 ± 0.0</td>
<td>3.1</td>
</tr>
</tbody>
</table>
The effluent of hydrogen generating reactors is used directly as substrate for further stabilization and valorisation in continuous anaerobic digesters for methane recovery.

- Stabilization of waste
- Minimization of COD
- Maximum energy generation

- Dairy wastewater
- 2-phase olive mill wastewater
- Sweet sorghum biomass
- Glycerol
- Food wastes
Production of polyxydroxyalkanoates

- PHAs accumulated by numerous bacteria as reserve of carbon and energy source in the form of inclusion bodies (granules) with a diameter of 0.2-0.9 μm.

![PHB intracellular granules formed in *Ralstonia eutropha* (Yu J., 2002)](#)

**General structure of PHAs:**

![General structure of PHAs](image)

**Most common PHAs:**

- $R = \text{CH}_3 \Rightarrow \text{PHB poly-3-hydroxybutyrate}$
- $R = \text{C}_2\text{H}_5 \Rightarrow \text{PHV poly-3-hydroxyvalerate}$
PHAs Biosynthesis

Chen, 2010, Microbiology Monographs 14: 17-37
Alternating nutrient limitation (carbon & nitrogen) leads to PHA formation when nitrogen availability is limited
SBR phases:
a) growth phase, N supply (no or limited carbon supplied),
b) biomass settling phase (no aeration and stirring)
c) supernatant withdrawal (2/3 of $V_w$),
d) PHA accumulation phase, C supply (nitrogen limitation)
e) withdrawal of 2/3 of the working volume under agitation (for PHAs extraction)

Wastes:
- Olive-mill wastewater
- Industrial glycerol
Recovery of PHAs from Cells

Collecting dry biomass by centrifugation, freezing and lyophilization

Extraction with chloroform

Purification: Mixing the extract with methanol for precipitation of bioplastic and then filtration

Collection of the solid and re-dissolution in chloroform to obtain bioplastic films
A fuel cell is an electrochemical energy conversion device that produces electricity from external supplies of fuel (on the anode side) and oxidant (on the cathode side).

In the microbial fuel cell electricity is produced via microorganisms.

Substrates studied:

**Pure substrates:**
- Glucose, lactose
- Glycerol

**Wastes:**
- Dairy wastewater
- Municipal wastewater
The working principle of an MFC

Chemistry of MFC: As an example, glucose is used as an organic substrate.

Anode: \[ C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \]

Cathode: \[ 24H^+ + 24e^- + 6O_2 \rightarrow 12H_2O \]
Two-chamber MFC

- Two bottles connected via a glass tube.
- Anode electrode: carbon fiber paper
- Cathode electrode: carbon cloth coated with a Pt catalyst
- Proton exchange membrane (Nafion 117)
Experiments using (diluted) cheese whey as substrate

- Diluted cheese whey (dilution 1:100) at a final concentration of 0.73 g COD/L

- After addition of fresh medium: the MFC voltage increased rapidly, reaching a constant value within only a few hours (approximately 50 mV for an external load of 100 Ω)

- COD removal completed within 50 hours, accompanied by rapid decrease in voltage

- Diluted cheese whey (dilution 1:100) at a final concentration of 0.73 g COD/L
Varying the external load from 0.1 to 1000 kΩ

Maximum power density 18.4 mW/m²
## Experiments with cheese whey

<table>
<thead>
<tr>
<th>Initial concentration (mg COD/L)</th>
<th>% COD removal</th>
<th>Duration of each cycle (h)</th>
<th>Maximum power output (mW/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>350</td>
<td>95</td>
<td>68</td>
<td>40.5</td>
</tr>
<tr>
<td>700</td>
<td>96</td>
<td>110</td>
<td>39.9</td>
</tr>
<tr>
<td>1500</td>
<td>96</td>
<td>213</td>
<td>39.8</td>
</tr>
<tr>
<td>2700</td>
<td>96</td>
<td>335</td>
<td>38</td>
</tr>
<tr>
<td>6700</td>
<td>96</td>
<td>851</td>
<td>42</td>
</tr>
</tbody>
</table>
Duration as a function of initial concentration

\[ y = 0.12 \times \]

\[ R^2 = 0.999 \]
Single cell chamber
Innovative single chamber MFC

- A single cylindrical plexiglas chamber with four plexiglas tubes placed, in a concentric arrangement, inside the chamber.

- The tubes inside the cell were homogenously drilled with holes.

- Anode electrode: graphite granules.

- Cation Exchange Assembly: GORE-TEX ® cloth covered with graphite conductive paint and electrolytic manganese oxide, MnO₂.
Continuously operated Single chamber performance using glucose as substrate
Thank you for your attention!