VALORIZATION OF GAS FERMENTATION ACETATE-RICH STREAM INTO VALUABLE MICROALGAL BIOMASS

G.Proietti Tocca, F. Regis, S. Fraterrigo Garofalo, V. Agostino, B. Menin, T. Tommasi, D. Fino

giacomo.proietti@polito.it
ACETATE IS A PRODUCT OR BY-PRODUCT OF DIFFERENT BIOTECHNOLOGICAL PROCESSES

**FERMENTATION TWO STEP**
- Alcoholic fermentation: Sugars (glucose) → Ethanol
- Acetic fermentation: Ethanol → Acetate

**ACIDOGENIC FERMENTATION AND DARK FERMENTATION**
- Organic wastes and effluents
- Acidogenesis: Hydrolysis → Acidogenic fermentation nVFAs
- Acetogenesis: Digestate VFAs
- Methanogenesis: Biogas and digestate
- Methanogenesis: Anaerobic digester
- Acetogenesis: Digestate VFAs

**GAS FERMENTATION**
- Chemical industries, biogas upgrading, biorefineries
- Syngas fermentation with acetogens
- Stirred tank reactors
- Water splitting

**THERMOCHEMICAL CONVERSION OF BIOMASS**
- Plants
- Plants cells
- Cellulose, Hemicellulose, Lignin
- HTC
- HTL
- Organic waste

**ARTIFICIAL PHOTOSYNTHETIS**
- Sunlight
- CO₂
- H₂O
- O₂
- Cell
- CO₂
- CH₃COO⁻

**MICROBIAL ELECTROSYNTHESIS (MES)**
- Water splitting
- Electrical power
- H₂O
- O₂
- CH₃COO⁻
GAS FERMENTATION WITH ACETOGENS - CHALLENGES AND LIMITS

Limits in using acetogens as biocatalysts:

1. PRODUCTS VARIETY; 2. LOW ECONOMIC VALUE OF ACETATE.

- Two fermenters in series (different culture conditions)
- Acetate from C1 gases has been utilized by
 Saccharomyces cerevisiae, Yarrowia lipolytica, Ralstonia eutropha etc.

Wood-Ljungdahl pathway of CO$_2$ fixation

Valorization of C1 gases to value-added chemicals using acetogenic biocatalysts
Jiyun Bae, Yoseb Song, Hyeonsik Lee, Jongoh Shin, Sangrak Jin, Seulgi Kang, Byung-Kwan Cho

Recycling carbon for sustainable protein production using gas fermentation • Esteban Marcellin, Largus T Angenent, Lars K Nielsen and Bastian Molitor

Current Opinion in Biotechnology
MIXOTROPHIC AND HETEROTROPHIC MICROALGAL GROWTH USING ACETATE AS CARBON SOURCE

- Advantage compared to photoautotrophic cultivation: higher biomass productivity and economic sustainability!

- Heterotrophy:
  - complete independence of light;
  - easy sterilization of fermenters (hard using tubular photobioreactor in mixotrophy);
  - valorization of waste and effluent as carbon feedstock;
  - high productivities
VALORIZATION OF GAS FERMENTATION ACETATE-RICH OUTFLOW INTO VALUABLE MICROALGAL BIOMASS

\[ \text{STR} \]  
\[ \text{P}_{\text{TOT}} = 2 \text{ bar} \quad \text{CO}_2 : \text{H}_2 = 1 : 1 \]

**Thermoanaerobacter kivui**

\[ \text{H}_2 \quad 400 \text{ rpm} \quad \text{pH} = 6.5 \]

\[ \text{CO}_2 \]

**Dark**

\[ \text{T} = 35^\circ \text{C} \quad \text{pH} = 7.5 - 8 \]

150 rpm

**Sumatant rich in ACETATE + M-8 medium salts**

**Tubular bioreactors 250 mL each**

\[ \text{Chlorella sorokiniana SAG 211-8k} \]

\[ \text{PROTEINS} \]

\[ \text{LIPIDS} \]

\[ \text{Starch} \]

\[ \text{Pigments} \]

Air = 100 mL/min

**POTENTIAL VALORIZATION FOR ANIMALS FEED**

\[ \text{CO}_2 : \text{H}_2 = 1 : 1 \]
To evaluate microalgal growth, 4 acetate concentrations were tested, diluting the gas fermentation effluent in the M-8 medium.
Test using gas fermentation effluent without any dilution (13.3 g L\(^{-1}\) of acetate), adding M-8 medium salts directly in the effluent.
PROCESS PARAMETERS AND BIOMASS CHARACTERIZATION

- **Process parameters**

  Specific growth rate and duplication time

<table>
<thead>
<tr>
<th>Condition</th>
<th>( \mu_{\text{MAX}} ) (h(^{-1}))</th>
<th>( t_d ) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterotrophy</td>
<td>0.1153 ± 0.0187</td>
<td>6.12 ± 0.86</td>
</tr>
<tr>
<td>Photoautotrophy</td>
<td>0.0558 ± 0.005</td>
<td>12.4 ± 0.9</td>
</tr>
</tbody>
</table>

- **Biomass characterization**: no differences between the different heterotrophic conditions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Proteins (%)</th>
<th>Lipids (%)</th>
<th>Carbohydrates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterotrophy</td>
<td>53.1 ± 1.1</td>
<td>21.8 ± 3.2</td>
<td>14 ± 2.1</td>
</tr>
<tr>
<td>Photoautotrophy</td>
<td>45.4 ± 2.8</td>
<td>21.1 ± 1.9</td>
<td>13 ± 3.5</td>
</tr>
</tbody>
</table>

Biomass productivity:

<table>
<thead>
<tr>
<th>Conditions</th>
<th>( r_{\text{END}} ) (g L(^{-1})day(^{-1}))</th>
<th>( r_{\text{MAX}} ) (g L(^{-1})day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterotrophy (1.1 gL(^{-1}))</td>
<td>0.227 ± 0.023</td>
<td>0.417 ± 0.009</td>
</tr>
<tr>
<td>Heterotrophy (2.2 gL(^{-1}))</td>
<td>0.429 ± 0.009</td>
<td>0.662 ± 0.013</td>
</tr>
<tr>
<td>Heterotrophy (3.3 gL(^{-1}))</td>
<td>0.607 ± 0.029</td>
<td>0.750 ± 0.001</td>
</tr>
<tr>
<td>Heterotrophy (5.5 gL(^{-1}))</td>
<td>0.684 ± 0.023</td>
<td>0.882 ± 0.052</td>
</tr>
<tr>
<td>Heterotrophy (no dilution - 13.3 gL(^{-1}))</td>
<td>1.32 ± 0.11</td>
<td>1.61 ± 0.12</td>
</tr>
<tr>
<td>Photoautotrophy</td>
<td>0.101 ± 0.005</td>
<td>0.189 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>Heterotrophy</td>
<td>Photoautotrophy</td>
</tr>
<tr>
<td>------------</td>
<td>--------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.54 ± 0.34</td>
<td>1.63 ± 0.20</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.47 ± 0.38</td>
<td>3.67 ± 0.45</td>
</tr>
<tr>
<td>Leucine</td>
<td>8.22 ± 1.65</td>
<td>8.93 ± 1.91</td>
</tr>
<tr>
<td>Lysine</td>
<td>4.15 ± 0.83</td>
<td>2.72 ± 1.4</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.28 ± 0.29</td>
<td>1.73 ± 0.41</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.2 ± 0.03</td>
<td>0.1 ± 0.03</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.01 ± 0.58</td>
<td>5.30 ± 1.0</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3.32 ± 0.21</td>
<td>3.65 ± 0.44</td>
</tr>
<tr>
<td>Valine</td>
<td>5.45 ± 1.32</td>
<td>6.01 ± 1.11</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.24 ± 0.31</td>
<td>4.84 ± 0.33</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>7.67 ± 0.25</td>
<td>7.77 ± 0.31</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>9.55 ± 0.61</td>
<td>9.54 ± 1.12</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.25 ± 0.56</td>
<td>5.56 ± 0.77</td>
</tr>
<tr>
<td>Glycine</td>
<td>4.68 ± 0.32</td>
<td>5.38 ± 0.69</td>
</tr>
<tr>
<td>Alanine</td>
<td>7.11 ± 0.23</td>
<td>7.18 ± 0.27</td>
</tr>
</tbody>
</table>

- No significative differences between the microalgal biomass obtained in heterotrophy and photoautotrophy;
- The content of essential amino acids is higher compared to the content of the essential amino acids of common vegetable proteins (soy) and animals proteins (beef).

[^a]: FAO 1970

*Essential amino acids
PROTEINS CHARACTERIZATION: ESSENTIAL AMINO ACIDS PROFILE

<table>
<thead>
<tr>
<th></th>
<th>mgAA gProt.⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heterotrophic Chlorella (this study)</td>
</tr>
<tr>
<td>Hystidine</td>
<td>15.4 ± 3.4</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>34.9 ± 3.8</td>
</tr>
<tr>
<td>Leucine</td>
<td>82.2 ± 15.8</td>
</tr>
<tr>
<td>Lysine</td>
<td>45.5 ± 8.3</td>
</tr>
<tr>
<td>Valine</td>
<td>54.5 ± 13.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>42.4 ± 3.2</td>
</tr>
<tr>
<td>Sulfuric containing amino acids</td>
<td>15.4 ± 2.9</td>
</tr>
<tr>
<td>Aromatic amino acids</td>
<td>53.3 ± 4.4</td>
</tr>
</tbody>
</table>

Sulfuric containing amino acids: methionine + cysteine
Aromatic amino acids: phenilalanine + tyrosine

LIPIDS CHARACTERIZATION: FATTY ACID PROFILE

**Fatty acids profile**

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Heterotrophy</th>
<th>Photoautotrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipid (%)</td>
<td>21.8 ± 3.2</td>
<td>21.1 ± 1.9</td>
</tr>
<tr>
<td>Saturated fatty acids (%)</td>
<td>40.9 ± 2.5</td>
<td>45.2 ± 1.8</td>
</tr>
<tr>
<td>Unsaturated fatty acids (%)</td>
<td>59.1 ± 1.4</td>
<td>54.8 ± 2.7</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids (%)</td>
<td>49.5 ± 1.2</td>
<td>45.1 ± 1.1</td>
</tr>
</tbody>
</table>
CONCLUSIONS

- *Chlorella sorokiniana* can grow heterotrophically on real gas fermentation effluent using acetate as carbon source also at high concentrations, observing the complete conversion of acetate into valuable microalgal biomass;

- The heterotrophic specific growth rate and the biomass productivity are high and satisfactory, especially when compared to the photoautotrophic ones;

- The heterotrophic biomass is rich in proteins and essential amino acids: the amount of them overcome the FAO requirments;

- The heterotrophic biomass is rich in polyinsaturated fatty acids, including good percentage of alpha-linolenic acid omega-3;

- The high heterotrophic biomass quality is confirmed by the absence of significative differences with the photoautotrophic biomass; this results indicates also an absence of contamination.
Mixotrophy using acetic acid

Continuing the acetic acid valorisation for mixotrophic cultivation of *Galdieria sulphuraria*

**NEXT STEPS**

![Diagram](image)

*Doubling of Microalgae Productivity by Oxygen Balanced Mixotrophy*  
Fabian Abiusi, Rene H. Wijffels, and Marcel Janssen

**Mixotrophy guarantees higher biomass yields: all (or almost) the carbon in the organic acids is converted into microalgal biomass, without CO₂ losses.**

*G. Sulphuraria* is the perfect strain to grow mixotrophically:

- It grows at very acid pH (1-2) → avoiding the contamination from competitive or predatory bacteria...

  …the sterilization of the photobioreactor is not necessary.
THANK YOU FOR YOUR KIND ATTENTION

Giacomo Proietti Tocca
giacomo.proietti@polito.it
APPENDAGES
BIOTECHNOLOGICAL PROCESSES EXPLOIT MICROORGANISMS AS BIOCATALYSTS

**Bacteria**

**Microalgae**

**Yeasts**

**AUTOTROPHY**
Carbon and energy from inorganic (and different) substances:
- \( \text{CO}_2 \)
- \( \text{CO} \)
- Light

**HETEROTROPHY**
Carbon and energy from organic substances:
- Sugars or volatile fatty acids
- Waste or wastewaters

**MIXOTROPHY**
Both inorganic and organic substances as sources of carbon and energy: a sum of autotrophy and mixotrophy

Carbon + Energy = Waste or wastewaters
<table>
<thead>
<tr>
<th>Process</th>
<th>Acetate - product type</th>
<th>Chemical composition</th>
<th>Production cost (€/kG)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol carbonylation</td>
<td>Main Product</td>
<td>Pure acetic acid</td>
<td>0.32</td>
<td><a href="https://doi.org/10.1021/acs.est.6b02101">https://doi.org/10.1021/acs.est.6b02101</a></td>
</tr>
<tr>
<td>Aerobic fermentation</td>
<td>Main Product</td>
<td>Acetic acid 66 - 106 g L⁻¹ Other potential compounds: propanoic acid, butyric acid, butanone, ethyl acetate, formic acid, microbial biomass, inorganic salts, colloids, CO₂, organic non-electrolytes</td>
<td>0.85 - 1.45</td>
<td><a href="https://doi.org/10.3311/PPhc.11004">https://doi.org/10.3311/PPhc.11004</a>; DOI: 10.1080/15422119.2016.1185017</td>
</tr>
<tr>
<td>Pyrolysis</td>
<td>By-product (acqueous phase of bio-oil)</td>
<td>Acetic acid: 5 - 157 g L⁻¹ Other potential compounds: glycoaldehyde 10-137 g L⁻¹, acetal 26-86 g L⁻¹, levoglucosan 30 - 65 g L⁻¹, other minor organic compounds (propanoic acid, non-aromatic aldehydes, furans, formic acid, acetone, formaldehyde)</td>
<td>//</td>
<td>DOI: 10.1002/bbb.2273; doi:10.1016/j.fuproc.2007.05.002</td>
</tr>
<tr>
<td>Hidrotermal treatments (HTC and HTL)</td>
<td>By-product: residual process water</td>
<td>Acetic acid: 0.7 - 33 g L⁻¹ Other potential compounds: formic acid 0.13-2.45 g L⁻¹, 1 - 4.5 g L⁻¹ lactic acid, proionic acid 0.14 - 0.42 g L⁻¹, glycolic acid 1.57 - 6.82 g L⁻¹, levulinic acid 0.37 - 1.44 g L⁻¹; phenol 1.5 - 4.5 g L⁻¹,</td>
<td>//</td>
<td><a href="http://dx.doi.org/10.1016/j.biombioe.2015.01.011">http://dx.doi.org/10.1016/j.biombioe.2015.01.011</a>; <a href="http://dx.doi.org/10.1016/j.rser.2018.09.008">http://dx.doi.org/10.1016/j.rser.2018.09.008</a>; <a href="http://dx.doi.org/10.1016/j.biortech.2013.05.098">http://dx.doi.org/10.1016/j.biortech.2013.05.098</a></td>
</tr>
<tr>
<td>Acidogenic fermentation (AF)</td>
<td>By - product: AF effluents</td>
<td>Acetic acid: 0.3 - 29 g L⁻¹ Other potential compounds: butyric acid: 0.33 - 32 g L⁻¹; propionic acid: 0.2 - 11.7 g L⁻¹; valeric acid: 0.26 - 5.66 g L⁻¹; iso-valeric acid: 0.16 - 21.8 g L⁻¹</td>
<td>//</td>
<td><a href="https://doi.org/10.1007/s11157-021-09566-0">https://doi.org/10.1007/s11157-021-09566-0</a></td>
</tr>
<tr>
<td>Dark fermentation (DF)</td>
<td>By - product: DF effluent</td>
<td>Acetic acid: 0.06 - 12.2 g L⁻¹ Other potential compounds: butyric acid 0.05 - 14.8 g L⁻¹; propionic acid 0 - 2.8 g L⁻¹ lactic acid 0.1 - 0.9, ethanol 0 - 0.9 g L⁻¹ Other potential nutrients: TN (mainly ammonium) 0.1 - 3.46 g L⁻¹, TP (mainly orthophosphate) 0.02 - 0.38 g L⁻¹</td>
<td>//</td>
<td>doi:10.1016/j.jhydene.2008.07.057; <a href="http://dx.doi.org/10.1016/j.procbio.2016.03.018">http://dx.doi.org/10.1016/j.procbio.2016.03.018</a>; <a href="http://dx.doi.org/10.1016/j.rser.2012.11.030">http://dx.doi.org/10.1016/j.rser.2012.11.030</a></td>
</tr>
<tr>
<td>Anaerobic digestion (AD)</td>
<td>By - product of Liquid Digestate</td>
<td>VFAs: 0.1 - 1.0 g L⁻¹ Other potential nutrients: NH₄ - N (ammonium nitrogen) up to 12 g L⁻¹; P (phosphorus) up to 5.8 g L⁻¹</td>
<td>//</td>
<td><a href="https://doi.org/10.3390/app11031056">https://doi.org/10.3390/app11031056</a>; doi:10.1039/c5ee01633a</td>
</tr>
<tr>
<td>Gas fermentation (for acetate production)</td>
<td>Main Product</td>
<td>Acetic acid: 1.9 - 59.2 g L⁻¹ Other potential compounds: formic acid 1.12 - 4.8 g L⁻¹; ethanol 0.03 - 0.17 g L⁻¹; butyrate 0.05 - 0.14 g L⁻¹; Ions that can be present: Mg²⁺, Cu²⁺, Ca²⁺, Mn²⁺, Zn²⁺, Ni²⁺, Co²⁺, Fe²⁺</td>
<td>4.68 (starting from CO)</td>
<td><a href="http://dx.doi.org/10.1016/j.jbiotec.2016.04.032">http://dx.doi.org/10.1016/j.jbiotec.2016.04.032</a>; <a href="https://doi.org/10.1016/j.jcej.2023.141555">https://doi.org/10.1016/j.jcej.2023.141555</a>; <a href="https://doi.org/10.1021/acs.est.6b02101">https://doi.org/10.1021/acs.est.6b02101</a></td>
</tr>
<tr>
<td>Bio-electrosynthesis</td>
<td>Main Product</td>
<td>Acetic acid: 0.002 - 11 g L⁻¹. Other potential compounds: 2-oxobutyrate 0.001 - 0.0051 g L⁻¹ ; formic acid 0.00023 - 0.59 g L⁻¹; butyrate 0.046 - 0.74; propionate 0.007 - 0.16; ethanol in traces</td>
<td>1.76</td>
<td>Referenze 6-7-8-13-16 di Bioelectrosynthesis of acetate</td>
</tr>
<tr>
<td>Artificial photosynthesis</td>
<td>Main Product</td>
<td>Acetic acid: 0.05 - 45 g L⁻¹ Other by-products in minor concentrations: propionate, n-propanol, ethanol, ethylene</td>
<td>?</td>
<td><a href="https://doi.org/10.1038/s43016-022-00530-x">https://doi.org/10.1038/s43016-022-00530-x</a></td>
</tr>
</tbody>
</table>
ADVANTAGES AND LIMITS OF HETEROTROPHIC CULTIVATION OF MICROALGAE

Commonly cultivated in:
• Open pounds

• Photobioreactor

• Heterotrophic fermenters:

Advantages:
• Indipendent of light;
• Costs of feedstock exploiting agro-industrial waste;
• Control;
• Major growth, yields and productivity

Limit:
• Cost of carbon feedstock
ACETATE AND $pH$

![Graph showing the fraction of CH$_3$COOH and CH$_3$COO$^-$ as a function of pH.](image)

![Graph showing the concentration of CH$_3$COOH as a function of pH for different molar concentrations.](image)
Biomass production costs:
• Het: 4.00 €/Kg
• Auto: 3.50 – 5 €/Kg

BUT

Higher productivities and the integration with waste or wastewaters treatment (zero or negative cost feedstock) can strongly decrease the biomass production costs: in a potential scenario, 1.08 €/Kg
PROCESS PARAMETERS

• Specific growth rate and duplication time:

\[ \mu_{MAX} = \frac{\ln(X_f) - \ln(X_{0,Exp})}{t_f - t_0} \]

\[ t_{DUPL.} = \frac{\ln 2}{\mu_{MAX}} \]

• Biomass productivity:

\[ r_X = \frac{(X(t) - X_0)}{t} \]

• Biomass yield:

\[ Y_{X/S} = \frac{\Delta X}{\Delta S} = \frac{(X(t) - X_0)}{(S_0 - S(t))} \]
PROTEIN CONVERSION FACTOR

Proteins = 16% nitrogen  →  100g of proteins = 16g of nitrogen  →  100/16 = 6.25

\[ K_A = \frac{\sum E_i}{\sum D_i} \]

- \( E_i \) = sum of the amino acids residues;
- \( D_i \) = sum of %N found in these amino acids (including N lost during hydrolysis)

\( K_A \) is an upper bound

\[ K_P = \frac{\sum E_i}{N} \]

- \( N \) = Total nitrogen, including non-protein nitrogen (NPN)

\( K_P \) is a lower bounds

**Ideal K for real samples: Average between \( K_A \) and \( K_P \)**
ACETATE METABOLISM IN ALGAE

**Acetate Metabolism**

- **Acetate** enters the cell through MCT and is deprotonated to Acetate.
- **Acetic acid** is converted to Acetate by the acetate kinase (ACS) enzyme.

**Glyoxysome**

- **Citrate** is converted to Isocitrate by the Citrate lyase (ICL) enzyme.
- **Oxalacetate** and **Malate** are synthesized from Isocitrate.
- **Succinate** and **Fumarate** are generated from Malate.
- **Glyoxylate** is produced from **2 Acetyl-CoA**.

**ER**

- **Phospholipids** are synthesized from **Acetyl-CoA**.
- **Triacylglycerides** and **Lysophosphatidic acid** are synthesized.

**Mitocondria**

- **Pyruvate** is converted to **Acetyl-CoA** by the pyruvate dehydrogenase complex (PDC).
- **Oxalacetate** is generated from **Malate**.
- **Fumarate** and **Malate** are synthesized from **Succinate**.

**Calvin cycle**

- **Carbon dioxide** is fixed by the Rubisco enzyme to form **3-phosphoglycerate**.
- **Glucose** is synthesized from **3-phosphoglycerate**.

**Plastid**

- **Starch and cellulose biosynthesis**.
- **Amino acids biosynthesis**.

**TCA cycle**

- **Citrate** is converted to **Isocitrate** by the Citrate lyase (ICL) enzyme.
- **Oxalacetate** and **Malate** are synthesized from **Isocitrate**.
- **Succinate** and **Fumarate** are generated from **Malate**.