FROM WASTE TO BIOPLASTIC

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COMPLEX BIOWASTE

**Municipal and commercial waste**
1 kg of household waste is generated per citizen and day

**Sludge derived from urban water treatments**
In 2003-2006, about 10 million tons DM (Dry Matter) of sewage sludge were produced in the EU

**Agricultural waste**
The EU estimated that its member states produce 700 million tons of agricultural waste annually
FROM WASTE TO BIOPLASTIC

SYNPOL aims to develop an industrial process for sustainable bioconversion of waste into biopolymers

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BACTERIAL STRAINS

METABOLIC ENGINEERING

GENETIC ENGINEERING

SUSTAINABLE PHA PRODUCTION

METABOLIC ENGINEERING

SYNGAS FERMENTATION

• Municipal solid waste
• Agricultural residues
• Sewage sludge

BIOPLASTICS

DOWNSTREAM PROCESSING

BIOPOLYMERS FROM SYNGAS FERMENTATION

RECYCLING

SUBSTRATES

SYNGAS

CO

H₂

SYNGAS

CO₂

CH₄
BACTERIAL POLYESTERS-PHAs

Bioplastics

Biodegradable

Biomaterials

Renewable sources
SYNGAS-FERMENTING BACTERIA

ANAEROBIOsis

ACETOGENIC

PHOTOTROPHIC

CARBOXIDOTROPHS

AEROBIOsis

Clostridium

Rhodospirillum

Oligotropha
Rhodospirillum rubrum: BIOCATALYST IN THE BIOCONVERSION OF SYNGAS INTO PHA

- Naturally produces PHB
- High metabolic versatile: auto or heterotrophically, photo or chemotrophically growth with or without $O_2 \rightarrow$ It can grow with SYNGAS

ELECTRON MICROSCOPY IMAGE

CIB, CSIC (Spain)
SYNGAS FERMENTATION

CO  CO2  H2  N2
10% 40% 40% 10%

SYNGAS

....doing vacuum inside the bottle ....

....purging with syngas....
SYNGAS FERMENTATION

- **CO** 40%
- **CO2** 10%
- **H2** 40%
- **N2** 10%

**SYNGAS**

- **OD600** 1.5
  - RRNCO + SYNGAS

- **OD600** 0.03
  - RRNCO - SYNGAS

**Kerby R.L. *et al.*, 1995**

**Graphs:**

- Red line: **LIGHT**
- Orange line: **DARKNESS**

**OD600** vs **t(h)**

0, 10, 100, 200
OPTIMIZING SYNGAS FERMENTATION: non-fermentable acids

- Acetic Acid
- Malic Acid

Growth Rate $\mu$ (h$^{-1}$)

**Acetate**
- Dark: 0.04
- Light: 0.06

**Malate**
- Dark: 0.02
- Light: 0.04

PHB (%/CDW)

**Acetate**
- Dark: 50
- Light: 20

**Malate**
- Dark: 10
- Light: 0
SYNGAS CONSUMPTION

CO + H₂O → CO₂ + 2H⁺ + 2e⁻ → CO₂ + H₂

SYNGAS

GC-TCD
(Gas chromatograph equipped with a thermal conductivity detector)

% GASES CONSUMPTION

CO
CO₂
BIOREFINERY

Feedstock

- MSW
  - Organic
  - Inorganic
  - Others

Pyrolysis by microwave heating

- SYNGAS
  - CO
  - H₂

BioProducts

Value added product: BIOPLASTICS

Fermentation

- Biomass
  - Rhodospirillum
  - Clostridium

BioProducts

- CHAR
- TAR
MICROWAVE INDUCED PYROLYSIS (MIP)
MICROWAVE INDUCED PYROLYSIS (MIP)

Growth Rate (h-1)

- Synthetic
  - LIGHT: 0.035
  - DARKNESS: 0.02
- MIP
  - LIGHT: 0.035
  - DARKNESS: 0.02
SYNGAS CONSUMPTION

% of CO Conversion

mmol of CO

Synthetic vs MIP at t0 and t48/72 h
BIOPLASTIC FROM MSW

PHB

- Synthetic
- MIP

- LIGHT
- DARKNESS

Graph showing PHB content comparison between Synthetic and MIP under LIGHT and DARKNESS conditions.
CO METABOLISM
CO METABOLISM

\[ CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^-\rightarrow CO_2 + H_2 \]

CODH
CO METABOLISM

CO + H₂O → CO₂ + 2H⁺ + 2e⁻ → CO₂ + H₂
CO METABOLISM

CO + H₂O → CO₂ + 2H⁺ + 2e⁻ → CO₂ + H₂
CO METABOLISM

CO + H₂O → CO₂ + 2H⁺ + 2e⁻ → CO₂ + H₂
TCA: Tricarboxylic acid
PPP: Pentose phosphate pathway
CBB: Calvin-Benson-Bassham Cycle
CM: Citramalate Pathway
EMCoA: EthylmalonylCoA Pathway

CO + H_2O → CO_2 + H_2 + 2e^- → CO_2 + H_2
REVEALING CO METABOLISM

- Stable Isotope Labelling Experiments
- Gene Expression Analysis → qPCR
- Enzyme Activity
STABLE ISOTOPE LABELLING EXPERIMENTS

SYNGAS FERMENTATION

12C

12CO2

10%

40%

40%

10%

13C Acetate


Intracellular Metabolites

13C/12C

13C dilution

12CO2 assimilation
CO METABOLISM

\[
CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^- \rightarrow CO_2 + H_2
\]
CO + H₂O → CO₂ + 2H⁺ + 2e⁻ → CO₂ + H₂
GENOMIC ORGANIZATION

CENTRAL CARBON METABOLISM

PHB METABOLISM

CO METABOLISM

PHB

3-Hydroxybutyryl

Acetoacetyl-CoA

3-Hydroxybutyryl-CoA

PhaZ

PhaC

PhaB

CO

H₂O

2H⁺

CO₂ + 2e⁻

CODH

PHB METABOLISM

CO METABOLISM

CENTRAL CARBON METABOLISM

3933 orf
4 352 825 bp

332 bp

40%

10%

40%

10%
CO + H₂O → CO₂ + 2H⁺ + 2e⁻ → CO₂ + H₂

CODH

CO METABOLISM
CO + H₂O → CO₂ + 2H⁺ + 2e⁻ → CO₂ + H₂

CO METABOLISM
Specific activity (nmol min$^{-1}$ mg$^{-1}$)

**A**

- Control
- Syngas_light
- Syngas_darkness

**B**

- Acetyl-CoA $\rightarrow$ Pyruvate
- CO$_2$

**Reaction Important for Syngas:**

- CCR
- PFOR
- Ethylmalonyl-CoA
- Crotonyl-CoA
- CO$_2$
CO METABOLISM

\[
\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{CO}_2 + \text{H}_2
\]
CO + H₂O → CO₂ + 2H⁺ + 2e⁻ → CO₂ + H₂
TAKE-HOME MESSAGES

- MSW derived syngas can be used as feedstock for PHB production.
- Syngas fermentation by *R. rubrum* is independent of the syngas composition, increasing the industrial interest of this process.
- SYNGAS cannot be used as a single carbon source. Acetate is needed for PHB production.
- CO metabolism is actively channeling CO into CO$_2$ and finally into PHB.
- Pyruvate Carboxylase and Crotonyl-CoA Carboxylase are potential targets to optimize strains by Metabolic Engineering Strategies.
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The diagram illustrates the metabolic processes involving various compounds. Notable reactions include:

- **1,3-BPG (1,3-Phosphoglycerate)**
- **2/3-PG (2/3-Phosphoglycerate)**
- **PEP (Phosphoenolpyruvate)**
- **RUBISCO**
- **PHB (Polyhydroxybutyrate)**
- **TCA (Tricarboxylic Acid)**
- **Cit (Citrate)**

Key reactions:

1. **1,3-BPG** formation involves a carbon dioxide (**CO2**-13) incorporation.
2. **2/3-PG** formation is indicated by a light source, suggesting a light-dependent process.
3. **PEP** is involved in the Calvin cycle, a key component of photosynthesis.
4. **RUBISCO** is highlighted with a carbon dioxide incorporation (**12CO2/13CO2**).
5. **PHB** is synthesized from carbon dioxide and 4-carbon compounds.
6. **TCA** cycle is depicted with a hydrogen (**H2**-40) and nitrogen (**N2**-10) incorporation.

The diagram uses various colors to differentiate between different conditions or compounds, such as **DARK** and **LIGHT** conditions.
CO + H₂O → CO₂ + 2H⁺ + 2e⁻ → CO₂ + H₂

CODH

CO METABOLISM