Operations with syngas bioreactors
– Process control

Daniel Egger & Manfred Zinn, 31.10.2014
Agenda

About Plastics

The Challenges

Our Approach within the Synpol Project

Conclusions
Plastics are great materials

- Super cheap
- Easy to process and shape
- Light weight
- Transparent but easy to color
- Resistant to many chemicals
- Odorless
- Durable
Global plastic production (2012) ≅ 288 Mtons y⁻¹

Consumption of plastic kg capita⁻¹, 1980 to 2015*

- NAFTA
- Western Europe
- Central Europe and CIS
- Latin America
- Middle East, Africa
- Japan
- Asia (excluding Japan)
- Rest World total

Source: PlasticEurope (PEMRG) / Consultic and www.statista.com

*estimated
World plastic production 1950 - 2012

Includes:
thermoplastics, polyurethanes, thermosets, elastomers, adhesives, coatings and sealants, and PP-fibers

Not included:
PET-, PA- and polyacryl-fibers

Source: PlasticEurope (PEMRG) / Consultic
Plastics raw material – Good news!?

"The oil prize has been dropping recently"

Source: Alex Munton, Middle East analyst (Wood Mackenzie)
Plastics raw material – Good news!? 

Source: www.oil-price.net/
No plastic - No Halloween
Plastics raw material

"The easy oil is coming to an end"

Source: Alex Munton, Middle East analyst (Wood Mackenzie)
Plastics

“Made to last forever, designed to throw away”
Drowning in plastic: The Great Pacific Garbage Patch is twice the size of France

There are now 46,000 pieces of plastic per square kilometre of the world’s oceans, killing a million seabirds and 100,000 marine mammals each year. Worse still, there seems to be nothing we can do to clean it up. So how do we turn the tide?
Plastic ban: Will BBMP be successful this time?

By Prajwala Hegde | ENS - BANGALORE
Published: 26th September 2012 08:30 AM
Last Updated: 26th September 2012 12:06 PM

The BBMP has set a deadline for segregation of garbage this time, which many corporators opine, will play a vital role in imposition of plastic ban too. (Express Photo)
Some People Are Really Upset About the Los Angeles Plastic Bag Ban

JOHN METCALFE  JUN 20, 2013  24 COMMENTS
Fate of most plastics world-wide: Landfills

Incineration in Switzerland:
650’000 t y\(^{-1}\) (65%)

http://www.mtholyoke.edu/~hart22a/classweb/econ_203_waste/waste%20management.html

http://www.industcards.com/wte-switz.htm
The solution: «Bioplastics»?
Different types of bioplastics...

- Bioplastics
  - Renewable raw material
    - Biodegradable
  - Fossil and renewable raw materials
    - Biodegradable
  - Fossil raw materials
    - Biodegradable
Global production capacities of bioplastics

Source: European Bioplastics | Institute for Bioplastics and Biocomposites (December 2013)
### Biopolymers production capacity 2015 (by type)

<table>
<thead>
<tr>
<th>Biopolymer Type</th>
<th>Capacity (metric tons)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-PE</td>
<td>450,000</td>
<td>26%</td>
</tr>
<tr>
<td>Bio-PET</td>
<td>290,000</td>
<td>17%</td>
</tr>
<tr>
<td>PLA</td>
<td>216,000</td>
<td>13%</td>
</tr>
<tr>
<td>PHA</td>
<td>147,100</td>
<td>9%</td>
</tr>
<tr>
<td>Biodegradable Polyesters</td>
<td>143,500</td>
<td>8%</td>
</tr>
<tr>
<td>Biodegradable Starch Blends</td>
<td>124,800</td>
<td>7%</td>
</tr>
<tr>
<td>Bio-PVC</td>
<td>120,000</td>
<td>7%</td>
</tr>
<tr>
<td>Bio-PA</td>
<td>75,000</td>
<td>5%</td>
</tr>
<tr>
<td>Regenerated Cellulose(^1)</td>
<td>36,000</td>
<td>2%</td>
</tr>
<tr>
<td>PLA-Blends</td>
<td>35,000</td>
<td>2%</td>
</tr>
<tr>
<td>Bio-PP</td>
<td>30,000</td>
<td>2%</td>
</tr>
<tr>
<td>Bio-PC</td>
<td>20,000</td>
<td>1%</td>
</tr>
<tr>
<td>Others</td>
<td>22,300</td>
<td>1%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1,709,700</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

\(^1\) only hydrated cellulose foils

Source: European Bioplastics | University of Applied Sciences and Arts, Hanover
What are polyhydroxyalkanoates (PHAs) and how are they biosynthesized?
Different types of polyhydroxyalkanoates

Short-chain-length PHA (scl-PHA)

- Thermoplasts (Tm: 120 – 180°C)
- High crystallinity (40-80%)
- Density >1.2 g cm⁻³
- Elongation at break 6-10%
- Industrial production: China, U.S.A. and Brasil

Medium-chain-length PHA (mcl-PHA)

- Elastomeric, thermoplastic, duroplastics or fluidoplastic properties (Tm: n.d. – 80°C)
- Low crystallinity (0-40%)
- Density: ca. 1.05 g cm⁻³
- Elongation at break: Adjustable (>100%)
- Industrial research: Ireland, Canada
Production of poly(3-hydroxybutyrate) (PHA)

Fermentation broth → Centrifugation → Wet Biomass → Freeze-drying → Dry biomass → Extraction with organic solvents, filtration → PHA in solution → Precipitation, drying under vacuum → Pure PHA

Challenges for PHA

- Price of Substrate
- Quality
- No competition to food sources
- Sustainability
EU Project: Synpol

- 1 Vision
- 8 Countries
- 14 Partners
- 48 Months

www.synpol.org
EU Project: Synpol
Fermenter design & Syngas fermentation

INFORS HT

• Building a flexible & powerful research platform for syngas fermentations (Batch, Fed-Batch and (two-stage)-Chemostat)

HES-SO

• Process optimization of syngas fermentations with *Rhodospirillum rubrum*
The Universities of applied sciences with activities in Life Sciences

École d'ingénieurs et d'architectes de Fribourg
- Chemistry

École d'ingénieurs de Changins
- Oenology

Haute école du paysage, d'ingénierie et d'architecture
- Natural Sciences

HES-SO//Valais, Haute Ecole d’ingénierie
- Life Technologies
Institute of Life Technologies at HES-SO Valais//Wallis

- 19 Professors (Food Technology, Biotechnology, Analytical Chemistry)
- 36 Scientific collaborators (PhD, MSc, BSc, Engineers) and technicians
- 4 PhD students
- 12 Apprentices
Projects are carried out by research groups of principal investigators and senior research associates.
Research activities of Life and Bioresource Technologies and my contributions

- **Bioresources**
  - White and Red Biotechnology
  - Liquid and gaseous substrates

- **Bioanalytics**
- **Bioengineering**
  - Bioprocess design
  - Scale-up from shake flasks to 300 L

- **Chemical Biotechnology**
  - Chemical Synthons

- **Biomaterials**
  - Biopolymesters (biocompatible and biodegradable)
Our company is true to its roots and to our traditional Swiss values. We combine perfection with passion. We work on each solution with scrupulous care and imaginative flair until everything fits like clockwork. We are instinctively quality-conscious and place a high value on top-quality materials and expertly trained employees. Our stable corporate culture ensures that we remain firmly grounded and determinedly independent. To us, our Swiss heritage means that we also act sustainably – in environmental, social and economic terms.
1965
- Founding of Hawrylenko Technique by Alexander Hawrylenko, 1st Patent

1967
- First Shaker for high-speed applications

1968
- First 75-litre bioreactor

2014
- Complete bioprocess equipment and software portfolio < 1mL – 1000 L
- Microbial, Cell Cultures, Biofuel 2nd and 3rd gen. and specialized bioprocess platforms
Strong bioprocess platforms enhanced by powerful software
Synpol challenge no. 1

**Strain and growth conditions**

- Cell physiology of strain is flexible (aerob, anaerob, (non)-photothroph, autotroph)

- Syngas composition is a function of the origin of organic matter
Production strain: *Rhodospirillum rubrum*

- Gram negative, spiral-shaped, motile bacteria
- Found in aquatic environments (e.g. ponds, lakes), in mud and sewage
- Purple, non-sulfur, photosynthetic bacteria
- Non pathogenic
- Genome sequenced (1 chromosome + 1 plasmid)
Rhodospirillum rubrum PHA production

Up to 30% PHA production with fructose/acacetate as carbon and energy source under anaerobic conditions

Photo: CIB, CSIC (Spain)
Cell physiology of *R. rubrum*

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>Metabolism</th>
<th>Carbon source</th>
<th>Energy source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark /Light</td>
<td>Aerobic respiration</td>
<td>Heterotrophic</td>
<td>Chemotrophic</td>
</tr>
<tr>
<td>Dark</td>
<td>Anaerobic alcoholic fermentation</td>
<td>Heterotrophic</td>
<td>Chemotrophic/CO</td>
</tr>
<tr>
<td>Light</td>
<td>Anaerobic anoxogenic photosynthesis</td>
<td>Heterotrophic/Autotrophic</td>
<td>Phototrophic</td>
</tr>
<tr>
<td>Dark/Light</td>
<td>CO Water-shift reaction (CO + H₂O → CO₂ + H₂)</td>
<td>CO</td>
<td>CO</td>
</tr>
</tbody>
</table>

![Diagram 1](image1.png)

![Diagram 2](image2.png)
Synpol challenge no. 2

Safety and tight project timeline

- Syngas is very toxic and explosive
- Dependence on other project partners
- Tailor made bioprocess unit
Safety issues: Fermentation with Syngas

**Inflammation values**

- H₂: 4 - 76 vol% (air, 1013 mbar, 20°C)
- CO: 10.9 - 76 vol% (air, 1013 mbar, 20°C)

**Toxicity values**

- CO₂
  - 6 % = prominent respiratory stimulus,
    exaggerated respiratory response to exercise,
    beginnings of mental confusion
- CO
  - LC = 1’500 ppm for 1 h or
  - LC = 40’000 ppm for 2 min
Safety aspects linked to Syngas

Prevent Syngas (H₂/CO/CO₂) leakage
- Reduce manipulations
- Work in fume hoods

Detect Syngas (H₂/CO/CO₂) leakage
- Specific sensors in fume hoods and lab

React in case of leakage
- Alarms
- Stop of syngas flow
- Ventilation / Dilution
CIP & SIP for more safety and research speed

LabCIP for CIP & SIP of the Labfors 5 Bioreactor
Synpol challenge no. 3

Bioprocess
- Low solubility of syngas (low yields)
- Very slow growth rates and co-feeds
- Different fermentation strategies
- Quantity of biopolymer

Our goal: optimal control of syngas supply for PHA production
- Efficient biosynthesis of biomass from syngas
- High PHA content
- Avoid intracellular PHA degradation
CO transfer challenge

Oxygen Transfer Rate

\[ OTR = k_La(c_{O2}^* - c_{O2}) \]

In analogy we can formulate a CO transfer rate:

\[ COTR = k_La(c_{CO}^* - c_{CO}) \]

- \( k_La \): Volumetric mass transfer coefficient \([h^{-1}]\)
- \( c_{CO}^* \): (liquid phase) CO saturation concentration \([mg L^{-1}]\)
- \( c_{CO} \): Actual measured (liquid phase) CO concentration \([mg L^{-1}]\)

\[ c_{CO}^* = He \ p_G \]

- \( c_{CO}^* \): Saturation concentration of CO in liquid phase \([mol L^{-1}]\)
- \( H_G^* \): Henry coefficient for CO in liquid phase \([mol L^{-1} Pa^{-1}]\)
- \( P_G \): Partial pressure of CO in gas phase \([Pa]\)
CO transfer challenge

\[ \text{COTR} = \text{COUR} = \frac{1}{Y_{X/CO}} \cdot \mu \cdot x \]

- \( Y_{X/CO} \): Biomass yield coefficient on \( \text{[g g}^{-1}\] )
- \( \mu \): Specific growth rate \( \text{[h}^{-1}\] )
- \( x \): Biomass concentration \( \text{[mg L}^{-1}\] )

\[ c_{CO}^* = ? \text{ and } Y_{X/CO} = ? \]
How can we increase COTR?

• Increase of the reactor pressure to 2-3 bar:
  - $c_{CO}$ and therefore $\Delta c$ increases.

• Enrichment of gas flow with pure CO
  - $c_{CO}^*$ and therefore $\Delta c$ increases.

• Use a lower process temperature:
  - $c_{CO}^*$ and therefore $\Delta c$ increases.
How can we increase COTR?

• Increase of the stirrer speed = increase of the specific power input:
  - Shear stress reduces the gas bubble size.
  - Relative velocity between gas bubbles and fluid phase increases.

• Increase of the gas flow rate (Caution: High gas rates could lead to impeller flooding!)
  - Number of gas bubbles per unit volume increases.
  - Reduction of CO-depletion in the gas bubbles.

• Establish a gas recycling system

→ Need for a powerful and flexible PAT research platform
Process control and automation

We decided to monitor the bioprocess by mainly 3 tools:

**Off-gas analysis by mass spectrometry**
- Gas balance and RQ
- Parameters for carbon balance (CO, CO₂)
- Detection of unknown gaseous side products (e.g. H₂S)

**Redox probe**
- Determination of change from aerobic to anaerobic growth conditions

**Online flow cytometer**
- Cell number and growth kinetics
- PHA biosynthesis vs. degradation
- Quantification of PHA content
Fermentation design (Automation of liquid handling)

- INFORS HT bioreactor
- Liquid handling system
- Online flow cytometer

Aseptic sampling → Staining of cells → Dilution to $10^6$ cells mL$^{-1}$
OD600
mean fluorescence

0 2 4 6 8 10 12 14 16 18
time after inoculation [h]

OD600
mean fluorescence

SYTO 62 (DNA)

BODIPY (PHA)

counts

Photo: CIB, CSIC (Spain)
The image contains a graph showing the change in OD600 and mean fluorescence over time after inoculation. The graph is labeled with the following:

- OD600
- Mean fluorescence

The graph also includes data points for SYTO 62 (DNA) and BODIPY (PHA) counts. The fluorescence graphs for BODIPY (PHA) show a peak at around 10^6 counts.
SYTO 62 (DNA)

BODIPY (PHA)

Counts

OD_600

mean fluorescence

time after inoculation [h]

OD_600

mean fluorescence

SYTO 62 (DNA)

BODIPY (PHA)
SYTO 62 (DNA)
BODIPY (PHA)

OD
mean fluorescence

0 10 20 30 40
OD
mean fluorescence

0 150 450 750
0 450 750

0 2 4 6 8 10 12 14 16 18

time after inoculation [h]

BODIPY (PHA)
SYTO 62 (DNA)

counts

0 300 600 900
1 2K

10^{-2} 10^{2} 10^{3} 10^{4} 10^{5} 10^{6} 10^{7}

Q1 Q2 Q3 Q4

60.3 39.1 0.65
SYTO 62 (DNA)

BODIPY (PHA)
**Graphs and Data**

- **Graph 1:** Time course graph showing OD<sub>600</sub> and mean fluorescence over time.
- **Graph 2:** Scatter plot showing the relationship between SYTO 62 (DNA) and BODIPY (PHA) counts with Q1-Q4 quadrants.
- **Graph 3:** Histogram showing the distribution of mean fluorescence in time.

**Legend:**
- Blue triangles: OD<sub>600</sub>
- Green squares: mean fluorescence

**Axes:**
- Time after inoculation [h]
- OD<sub>600</sub>
- Mean fluorescence

**Counts:**
- Q1: 0
- Q2: 92.9
- Q3: 6.61
- Q4: 0.52
**SYTO 62 (DNA)**

**BODIPY (PHA)**

**OD600**

- 0
- 10
- 20
- 30
- 40

**Mean Fluorescence**

- 0
- 150,000
- 450,000
- 750,000

**Time after inoculation [h]**

- 0
- 2
- 4
- 6
- 8
- 10
- 12
- 14
- 16
- 18

**BODIPY (PHA)**

- Q1: 6.7E-3
- Q2: 95.3
- Q3: 4.33
- Q4: 0.38

**Counts**

- 0
- 300
- 600
- 900
- 1.2K
SYTO 62 (DNA)

BODIPY (PHA)
SYTO 62 (DNA)

BODIPY (PHA)

counts

OD600

Mean fluorescence

time after inoculation [h]

0 2 4 6 8 10 12 14 16 18

0 150000 450000 750000

OD600

0 10 20 30 40

0 10 20 30 40

BODIPY (PHA)
SYTO 62 (DNA)

BODIPY (PHA)

mean fluorescence
SYTO 62 (DNA)

BODIPY (PHA)
Correlation between fluorescence and PHA content

\[ y = 1.1 \times 10^{-4}x + 0.33 \]

\[ R^2 = 0.97 \]
Proof of concept: Syngas fermentation with *R. rubrum*
Conclusions & Outlook

- Bioplastics could be a solution for the plastic “problem”
- Holistic & interdisciplinary projects like Synpol are needed
- Up-cycling is the future approach
- Flexible and easy expandable research platforms are mandatory
- Sophisticated syngas platform has been established
  - PAT needed for understanding and control of complex processes
  - Ready for gas fermentation research
Acknowledgement

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Obrigado, Dank U, Merci, mahalo, Köszí, cnacu6o, Grazie, Thank you, mauuruuru, Takk, Gracias, Dziêkuje, Dêkuju, danke, Kiitos
1. Fume hoods and ventilation system

2. Gases stored outside the lab and brought to the fume hoods with installed pipes equipped with electro valves

3. Emergency kill switch (switches-off electro valves)

4. Detectors for H2 and CO automatically switching off the electro valves if triggered

5. Alarm panels + sound warnings inside and outside the lab
15-30% is mono ethylene glycol from sustainable resources.

70% is petrol-based terephthalic acid.

Bio-PET can be recycled like normal PET…

…however, by no means biodegradable!
European plastic demand

Sources:
- Data: PlasticEurope (PEMRG) / Consultic