Fermentation and purification of lactic acid using membrane processes
Our mission:

Our research is aimed at sustainable intensification. We analyze, model and evaluate bio-economic production systems. We develop and integrate new technologies and management strategies for a knowledge-based, site-specific production of biomass, and its use for food, as bio-based materials and fuels - from basic research to application.
The National Research Strategy Bioeconomy 2030 was published under the direction of the Federal Ministry of Education and Research (BMBF), together with six additional ministries.

- the strategy involves **five key fields of action**

  - Ensuring sustainable agricultural production
  - Developing renewable energy carriers from biomass
  - Developing raw materials and energy carriers for industry
  - Using renewable biomass-based energy carriers
  - Securing healthy and safe food and feed

Cascading use of biomass / Biorefinery concepts
Research Program
„Material and energetic use of biomass“

Coordination: Dr. Joachim Venus

Consideration of the entire value chain - System‘s approach

**Biomass provision**
(Cultivation, harvest, storage... e.g. short rotation wood, hemp)

**Material use**
(Fibers, insulation, biotechnological products)

**Energetic use**
(Biogas, wood pellets, biochar)

Valorization of residues, sidestreams etc.
Biobased products and processes from renewable resources not only help preserve the environment and climate, but also make a significant contribution to the structural change from a petrochemical to a biobased industry, with related opportunities for growth and employment. Industrial biotechnology, also known as white biotechnology, is an important driving force in this transition.
BIOREFINERIES
RESOURCE EFFICIENCY EXEMPLIFIED
Biotransformation of lignocellulosic materials into value-added products—A review

M. Bilal, M. Asgher, H.M.N. Iqbal, H. Hu, X. Zhang
Building blocks that could be produced via fermentation

Numbers next to biochemicals designate the total annual production in thousands of t

The market for lactic acid is growing as it is largely used in various industrial applications such as in biodegradable polymers, food & beverages, personal care products, and pharmaceutical industries. The lactic acid market is mainly driven by its end-use industries.

In 2013, Biodegradable polymers formed the largest application for lactic acid, followed by food and beverages. The lactic acid market is estimated to grow at a CAGR of 18.8% from 2014 to reach $3,577.5 million by 2019.
The processes for producing lactic acid from biomass/residues include the following 4 main steps:

(1) Pretreatment - breaking down the structure of the feedstock matrix

(2) Enzymatic hydrolysis - depolymerizing biopolymers like starch, cellulose etc. to fermentative sugars, such as glucose (C6) and xylose (C5), by means of hydrolytic enzymes

(3) Fermentation - metabolizing the sugars to lactic acid, generally by LAB

(4) Separation and purification of lactic acid - purification of lactic acid to meet the standards of commercial applications
Biorefinery-concept for (1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd}...?) biomass feedstocks

BIOCONVERSION

(Different) Composition & Behaviour of (lignocellulosic) Biomass

M.A. Abdel-Rahman et al. Journal of Biotechnology 156 (2011) 286–301


N. Mosier et al. / Bioresource Technology 96 (2005) 673–686
Beyond Petrochemicals: The Renewable Chemicals Industry

P. N. R. Vennestrøm, C. M. Osmundsen, C. H. Christensen, and Esben Taarning

Table 1: Overview of chemicals that are currently produced, or could be produced, from biomass together with their respective market type, size of the market, and potential biomass feedstock. Major players involved are also given.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Market type</th>
<th>Market size (Mty⁻¹)</th>
<th>Major player(s)</th>
<th>Feedstock</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetic acid</td>
<td>existing</td>
<td>9.0</td>
<td>Arkema, Cargill/Novozymes</td>
<td>ethanol</td>
</tr>
<tr>
<td>acrylic acid</td>
<td>existing</td>
<td>4.2</td>
<td>Arkema, Cargill/Novozymes</td>
<td>glycerol or glucose</td>
</tr>
<tr>
<td>C₄ diacids</td>
<td>emerging</td>
<td>(0.1–0.5)</td>
<td>BASF/Purac/CSM, Myriant</td>
<td>glucose</td>
</tr>
<tr>
<td>epichlorohydrin</td>
<td>existing</td>
<td>1.0</td>
<td>Solvay, DOW</td>
<td>glycerol</td>
</tr>
<tr>
<td>ethanol</td>
<td>existing</td>
<td>60</td>
<td>Cosan, Abengoa Bioenergy, ADM</td>
<td>ethanol</td>
</tr>
<tr>
<td>ethylene</td>
<td>existing</td>
<td>110</td>
<td>Braskem, DOW/Crystalse, Bobalys</td>
<td>glucose</td>
</tr>
<tr>
<td>ethylene glycol</td>
<td>existing</td>
<td>20</td>
<td>India Glycols, Dacheng Industrial</td>
<td>glucose or xylitol</td>
</tr>
<tr>
<td>glycerol</td>
<td>existing</td>
<td>1.5</td>
<td>ADM, P&amp;G, Cargill</td>
<td>vegetable oil</td>
</tr>
<tr>
<td>5-hydroxymethylfurfural</td>
<td>emerging</td>
<td>–</td>
<td>–</td>
<td>glucose/ fructose</td>
</tr>
<tr>
<td>3-hydroxypropionic acid</td>
<td>emerging</td>
<td>(≥0.5)</td>
<td>Novozymes/Cargill</td>
<td>glucose</td>
</tr>
<tr>
<td>isoprene</td>
<td>existing/emerging</td>
<td>0.1 (0.1–0.5)</td>
<td>Danisco/Goodyear</td>
<td>glucose</td>
</tr>
<tr>
<td>lactic acid</td>
<td>existing/emerging</td>
<td>0.3 (0.3–0.5)</td>
<td>Cargill, Purac/Akema, ADM, Galactic</td>
<td>glucose</td>
</tr>
<tr>
<td>levulinic acid</td>
<td>emerging</td>
<td>(≥0.5)</td>
<td>Segetis, Maine Bioproducts, Le Calorie</td>
<td>glucose</td>
</tr>
<tr>
<td>oleochemicals</td>
<td>existing</td>
<td>10–15</td>
<td>Emery, Croda, BASF, Vantage OIcochemicals</td>
<td>vegetable oil/fat</td>
</tr>
<tr>
<td>1,3-propanediol</td>
<td>emerging</td>
<td>(0.1–0.5)</td>
<td>Dupont/Tate &amp; Lyle</td>
<td>glucose</td>
</tr>
<tr>
<td>propylene</td>
<td>existing</td>
<td>80</td>
<td>Braskem/Novozymes</td>
<td>glucose</td>
</tr>
<tr>
<td>propylene glycol</td>
<td>existing/emerging</td>
<td>1.4 (≥2.0)</td>
<td>ADM, Cargill/Ashland, Senegy, Dacheng Industrial</td>
<td>glucose/glycerol or sorbitol</td>
</tr>
<tr>
<td>polyhydroxyalkanoate</td>
<td>emerging</td>
<td>(0.1–0.5)</td>
<td>Metabolix/ADM</td>
<td>glucose</td>
</tr>
</tbody>
</table>

[a] Market size of an existing market is given as its current size including production from fossil resources; for emerging markets the expected market size is reported in parenthesis.
Data Gaps
Scale-up of lactic acid production would require clean, cheap sugars from lignocellulosic biomass to compete with commodity sugar and starch substrates. There is a lack of data about lactic acid production and purification from biomass hydrolysates, including issues of C5 sugar utilization, although it appears work has started to address some of these issues.

This report is available at no cost from the National Renewable Energy Laboratory (NREL) at www.nrel.gov/publications
Fermentation feedstocks already tested:

- **Starchy materials**: (cereals, industrial grade corn/potatoe starch, tapioca)
- **Green biomass**: (alfalfa, grass juice, lupine, sweet sorghum, forage rye, silage, coco juice)
- **Lignocellulosics**: (wood/straw hydrolysates, 2ndG sugars, bagasse)
- **Residues & By-products**: (oilseed cake/meal, thick juice, molasses, whey, coffee residues, waste bread, waffle residues, algae biomass, fruit residues, rice bran, meat & bone meal, OMSW...)

Process steps for the manufacture of lactic acid

1. **Raw material storage**
   - 5 m³ (Trevira® UV-silo, HIMEL Maschinen GmbH & Co. KG)

2. **Hydrolysis**
   - 1 m³ stirred vessel; 0.5 m³ storage tank (Apparate und Behältertechnik Heldrungen GmbH)

3. **Pre-, Microfiltration**
   - 0.8 mm coarse filter (Sommer & Strassburger GmbH & Co. KG),
     Microfiltration (ZrO₂-TiO₂ CeRAM® INSIDE, TAMI Industries France)

4. **Sterilization of the nutrient broth**
   - 2 x 400 L, 2 x 250 L stirred vessels (Apparate und Behältertechnik Heldrungen GmbH)

5. **Fermentation with cell retention**
   - Pilot fermentor Type P, 450 L (Bioengineering AG)
     MOLSEP® Hollow fibre PES membrane
     (FS10-FC-FUS50E2, MICRODYMNADIR GmbH/Daicen Membrane Systems Ltd.)
The Copenhagen Declaration for a Bioeconomy in Action

... The conference also underlined the need for new pilot and demonstration plants and scaling up facilities, in particularly biorefineries. It was stressed, that the development of these facilities requires smart integration of various funding sources, including the Common Agricultural Policy, the Common Fisheries Policy, the Cohesion Policy, the Renewable Energy Policy, Horizon 2020, and private investments.

...
Scale-up of bioprocesses

Process development up to xx m³ ??

“Got a few problems going from lab scale up to full-scale commercial.”
Pilot plant facility

• **pilot facility for production of lactic acid** at the ATB consequently fills a gap in the various phases of bioprocess engineering

• **provision of product samples** is intended to open up the possibility of interesting **partners in industry with specific product requirements** in various applications

**scale up**

BIOSTAT® Bplus (Sartorius BBI Systems GmbH, Germany) equipped with a digital control unit DCU for the continuous fermentation with cell recycling


Continuous fermentation process with cell retention


"...Unbelievably, a cheap product such as lactic acid has until recently been manufactured by batch processes, although continuous production is the only economically viable way of producing it..."
Continuous mode fermentation with cell retention by hollow fibre membranes

Example coffee residues: residues from the coffee production

Mass balance from coffee pulp to lactic acid (*downstream processing was not optimized). All figures are based on dry weight.

Example agro-residues: Sugarcane bagasse

Figura 1 – Fotos de bagaço da cana de açúcar: (a) sem tratamento térmico; (b) 180°C; (c) 200°C e (d) 220°C por 5, 10 e 15 minutos (da esq. para dir.).

Figura 4 – Produção de ácido lático e consumo de açúcares presentes no meio MRS modificado contendo hidrolisado de bagaço (glicose 33 g l⁻¹, xilose 19 g l⁻¹, arabinose 0,4 g l⁻¹, extrato de levedura 15 g l⁻¹, K₂HPO₄ 2 g l⁻¹, MgSO₄ 0,1 g l⁻¹ e MnSO₄ 0,04 g l⁻¹).

http://www.unicamp.br/unicamp/ju/540/conversao-de-bagaco-da-cana-abre-frente-para-producao-de-polimero-verde

Hidrólise Térmica de Bagaço da Cana-de-açúcar para Produção Homofermentativa de L-Ácido Lático

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Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V., ATB
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Max-Eyth-Allee 100
D-14469 Potsdam

- **Targets of Russian Partner (INBI):**
  - Enzymes for lignocellulosic feedstocks degradation and production of sugars (C6, C5) and value added products

- **Targets of German Partner (ATB):**
  - Conversion of simple sugars (C6, C5) to added value products - fuels, solvents, organic acids, biopolymers, misc. organics

Example green biomass:
Grass processing with a screw press into juice and pellet


Typical time course of a batch lactic acid fermentation supplemented by conventional nutrients (open symbols), and green juice (solid symbols) as an alternative source.
Example agri-residues: Rapeseed cake/meal

SynRg®

A cluster of 17 partners will explore the recycling of vegetable raw materials along the complete value chain

Start of project: July 1st, 2009

2nd phase: July 1st, 2012 – June 30th, 2014

http://www.synrg-cluster.de/index.html

WP2: Technologies and Processes for the harvest, pre-treatment, and purification, in particular: utilization of (oilseed) residues


Example wheat straw: Sugar uptake & product formation

- Fermentation ended after 50-60 hours with a yield of nearly 100% and 64 g/L (top left)
- (Total) Sugars (firstly Glucose followed by Arabinose/Xylose with residues of Disaccharides) have been used completely in the same time (bottom left)
- (Max) Lactate productivity (>5 g·L⁻¹·h⁻¹) is much higher than comparable published results

[Li/Cui: Microbial Lactic Acid Production from Renewable Resources, pp. 211-228. In O.V. Singh and S.P. Harvey (Eds.), Sustainable Biotechnology - Sources of Renewable Energy. Springer, 2010]
Example food waste: Bakery industry


The award ceremony for the best 2015 Life projects took place in the context of "Green Week" (30 May to 3 June) in the Egg Conference Centre in Brussels
### Ongoing projects

<table>
<thead>
<tr>
<th>Project Description</th>
<th>Funding/Agreement Details</th>
<th>Duration</th>
<th>Websites</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProFIT „Polymerisierbare Milchsäure aus alternativen Zuckern“, Antrags-Nr.: 80170179</td>
<td>national: EFRE (FuE) / ILB</td>
<td>2017-2020</td>
<td><a href="#">LXP GROUP</a></td>
</tr>
<tr>
<td>Chemical building blocks from versatile MSW biorefinery — PERCAL</td>
<td>EU/BBI, Grant agreement No: 745828</td>
<td>2017-2020</td>
<td><a href="#">PERCAL</a></td>
</tr>
<tr>
<td>HyAlt4Chem “Säurebasierte Hydrolyse von unbehandelten Altholzrecyclaten zur Bereitstellung von Biochemikalien”</td>
<td>national: BMBF/VDINDE Innovation +Technik GmbH; FKZ: 03VNE1032D</td>
<td>2017-2020</td>
<td><a href="#">ATB</a></td>
</tr>
</tbody>
</table>

[PERCAL](#) - [http://percal.aimplas.es/](http://percal.aimplas.es/)

Fermentation with cell retention

- **Pilot fermentor** Type P, 450 L (Bioengineering AG)
- **MOLSEP® Hollow fibre PES membrane** (FS10-FC-FUS50E2, MICRODYN-NADIR GmbH/Daicen Membrane Systems Ltd.)

**Softening**
- 2 x 135 L PUROLITE, 1.5 m³/h (UIT GmbH Dresden)

**Monopolar/Bipolar Electrodialysis**
- FT-EDR/ED4-15; 7.68 m² monopolar/3.2 m² bipolar (FuMA-Tech GmbH Vaihingen)

**Ion exchange**
- Cationic resin, 50 L; Anionic resin, 2 x 90 L (UIT GmbH Dresden)

**Decolorization**
- Activated carbon, several specific resins

**Evaporation**
- «chem Reactor» CR15 (Büchi AG Uster/Switzerland);
  Rotary Evaporator LABOROTA 20 S (Heidolph Instruments)
Effect of several down-streaming steps on the purity of lactic acid

**Na-Lactate** (Fermentation)

**down-stream processing**
(Filtration, Softening, Electrodialysis, Ion exchange, Evaporation)

![Graph showing the effect of down-streaming steps on lactic acid purity](image)

- **Start_Ferm**
- **End_Ferm**
- **MF**
- **Soft**
- **EDR**
- **EXA133**
- **EXC08**
- **Evap**

**Ions [mg/L]**

- Ca$^{2+}$
- Mg$^{2+}$
- K$^+$
- Na$^+$
- N$_{ges}$
- SO$_4^{2-}$
- Cl$^-$
- PO$_4$-P
- Lactic acid

**Lactate [g/L]**

- Start_Ferm
- End_Ferm
- MF
- Soft
- EDR
- EXA133
- EXC08
- Evap
Process steps for the manufacture of lactic acid

1. Raw material storage: 5 m³ (Trevira®UV-silo, HIMEL Maschinen GmbH & Co. KG)
2. Hydrolysis: 1 m³ stirred vessel; 0.5 m³ storage tank (Apparate und Behältertechnik Heldrungen GmbH)
3. Pre-, Microfiltration: 0.8 mm coarse filter (Sommer & Strassburger GmbH & Co. KG), Microfiltration (ZrO₂-TiO₂ CeRAM®INSIDE, TAMI Industries France), 0.3 µm
4. Sterilization of the nutrient broth/additives: 2 x 400 L, 2 x 250 L stirred vessels (Apparate und Behältertechnik Heldrungen GmbH)
5. Fermentation with cell retention: Pilot fermentor Type P, 450 L (Bioengineering AG), MOLSEP®Hollow fibre PES membrane (FS10-FC-FUS50E2, MICRODYN-NADIR GmbH/Daicen Membrane Systems Ltd.)
6. Softening: 2 x 135 L PUROLITE, 1,5 m³/h (UIT GmbH Dresden)
7. Monopolare/Bipolare Electrodialysis: 10 cat- and 10 anions exchange membranes unit Type 100 (Deukum, Germany)
8. Ion exchange: Cationic resin, 50 L; Anionic resin, 2 x 75 L (UIT GmbH Dresden, Germany)
Conclusions

Scale-up of fermentation processes challenging.

Production of inexpensive compounds in continuous flow cultures reasonable.

Cell retention in continuous flow cultures significantly increases volumetric productivity.

Conventional downstream processing including filtration, dialysis and chromatography appropriate.

Pure lactic acid formulation can be obtained even with complex substrates.
Thank you for your attention!

More information: www.atb-potsdam.de

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