**BioREFINE-2G Workshop:**
**Bioplastics from 2nd Generation Biorefineries**
**5 June 2015, York**

# Downstream processing and process integration

**Biotrend SA**

biotrend
Inovação e engenharia em biotecnologia

This project is co-funded by the European Union within the 7th Frame Programme. Grant Agreement n°613771.
The sole responsibility of this publication lies with the author. The European Union is not responsible for any use that may be made of the information contained therein.

# Presentation outline

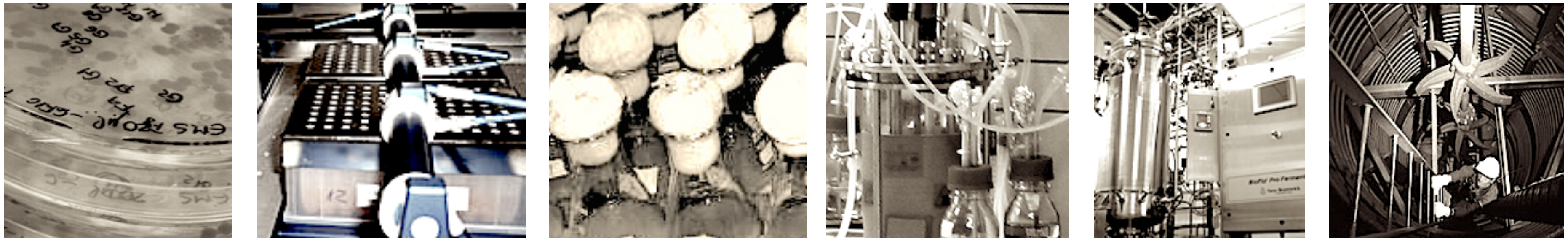
About Biotrend

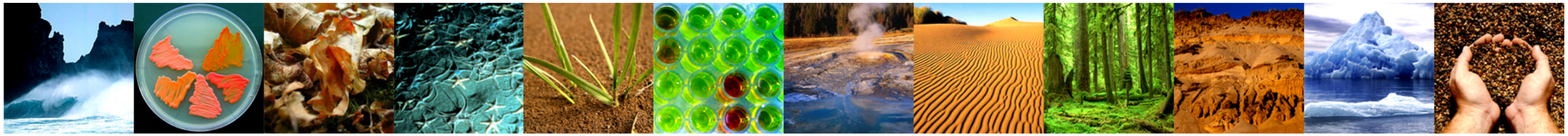
Downstream processing in biorefineries

Purification of diacids

Process integration

# About Biotrend





# Bioprocess development

**Highly qualified and experienced team**

- Staff with international experience
  (Portugal, The Netherlands, Canada, France, Germany, Switzerland)

**State-of-the-art facilities**

- Process development, optimization and integration
- Process scale-up, de-risking and validation
  (2-250L and beyond)


# Advanced services

**Microbial strain screening**

- High-throughput platform optimally designed to screen biocatalysts and assess process conditions.
- Typical screening of process-relevant activities: able to use C5 carbon sources; resist to the presence of inhibitors; low salt concentrations).

**Process development and optimization**

- Cultivation strategies maximizing productivities, yields and titers.
- Process integration opportunities with raw material pre-processing and with downstream processing.

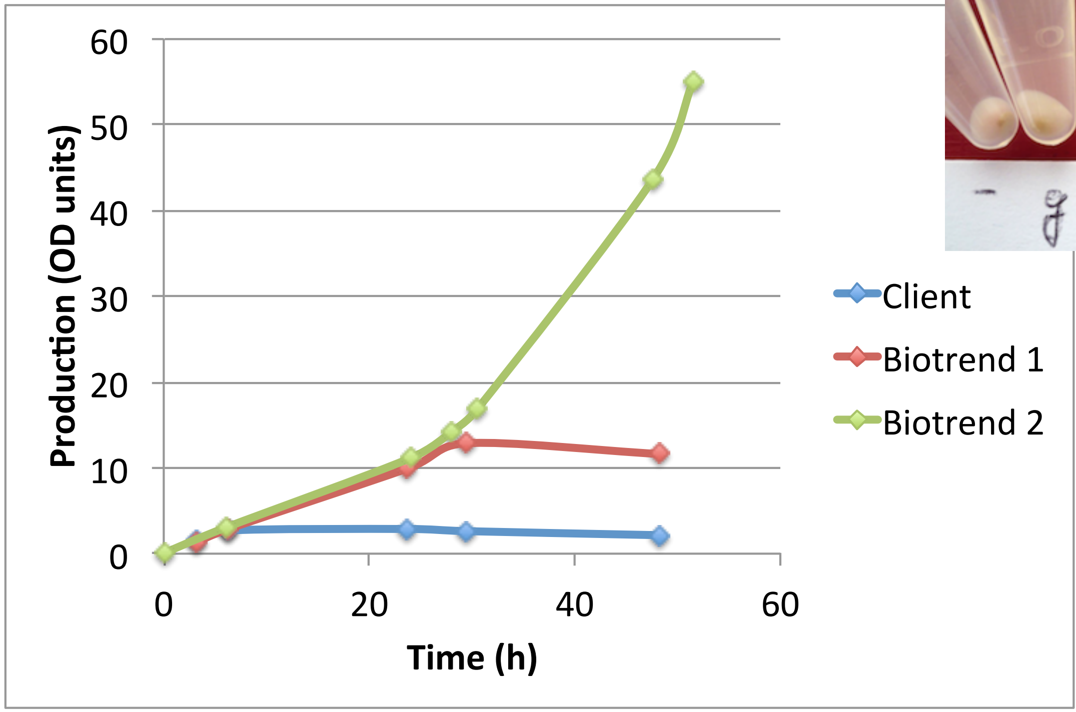
**Process scale-up, de-risking and validation**

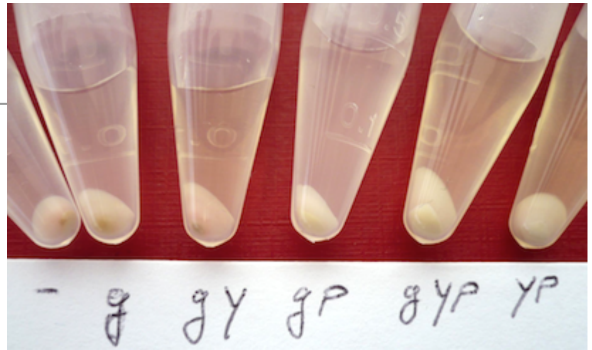
- 2L, 10L, 50L and 250L bioreactors for scale-up and process de-risking.
- Access to facilities from 1,000L to 150,000L capacity.

# Process intensification

**Process intensification ➔ Improve the economics of the process**

- Ex. Marine bacteria extract production



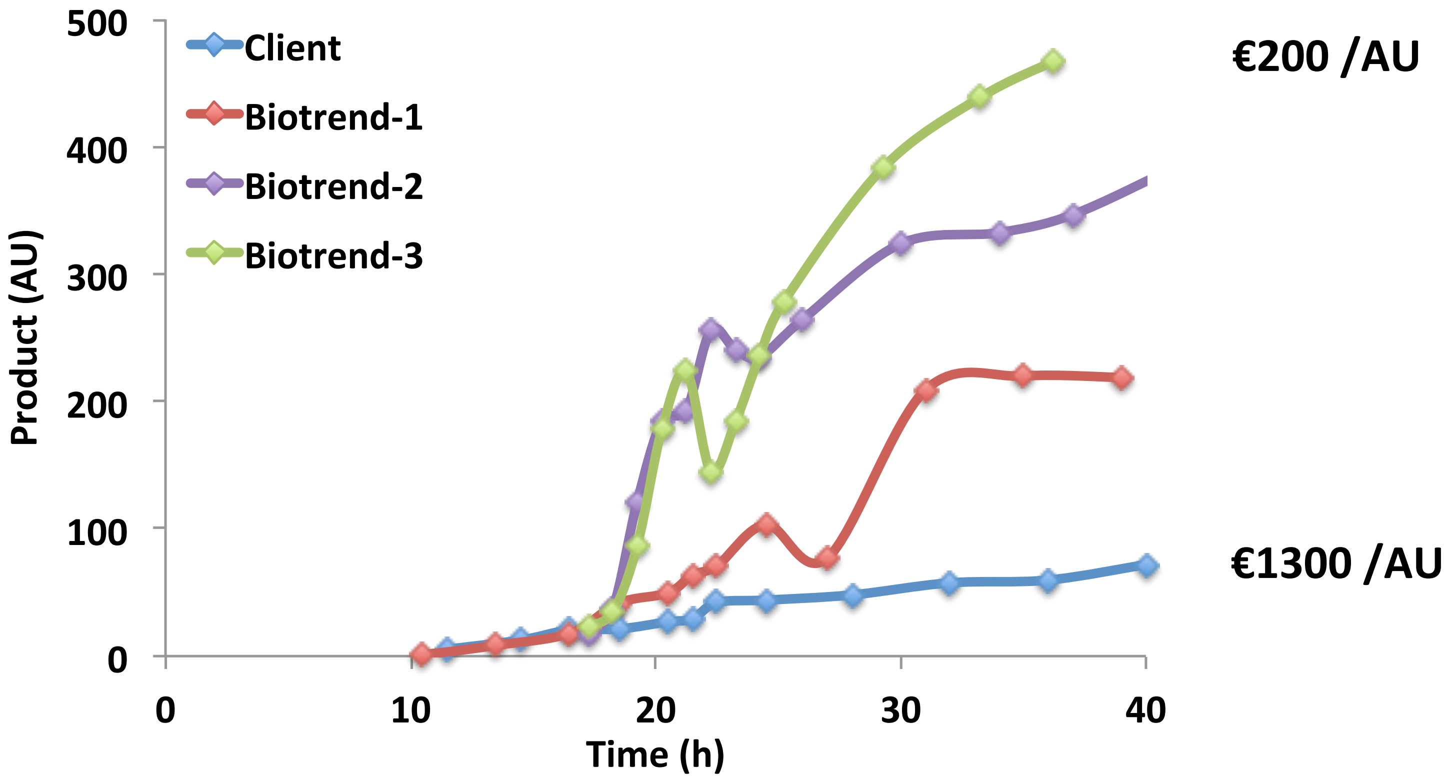



**Clients:** from small start-ups to multi-billion USD multinationals

# Process intensification

**Process intensification → Improve the economics of the process**

- Ex. Industrial enzyme production




**Clients:** from small start-ups to multi-billion USD multinationals

# Example projects for clients

**Large pulp and paper company, world leader in premium office paper (PT):** Fermentation-based valorization of carbon-rich liquid waste streams from pulp and paper mills.

**Multinational chocolate producer and leading plastics research institute (IT/ES):** Deconstruction and transformation of carbohydrate-rich solid residues into materials suitable for packaging applications.

**Innovative enzyme engineering company (FI):** Increase the productivity and reduce the cost of the production of the enzyme.

**Marine biotech start-ups (PT; CH/UK):** Increase the yield and productivity of the production of cell extracts with cosmetic use or biomass for nutrition applications.

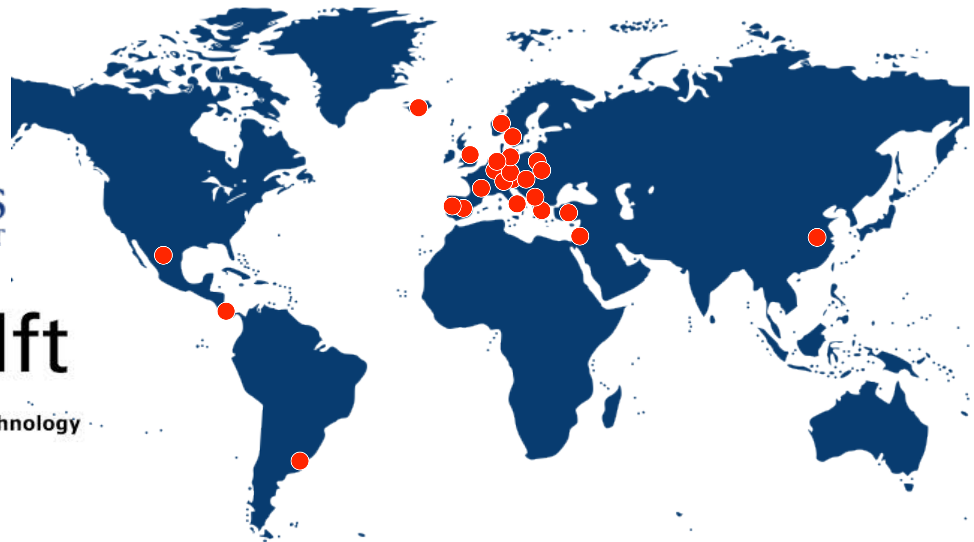
**Beverage company (PT):** Increase the robustness and scale-up of the production of an innovative fermented beverage.

**Biotech start-up (PT):** Process transition and optimization from shake flask to pilot scale of a biofouling control formulation for marine applications.

# Collaborative R&D

**At the forefront of scientific development**

- 7 EU co-funded large collaborative projects with partners across the industrial biotechnology and marine biotechnology value chain
- 49 companies
- 29 universities and RTDs

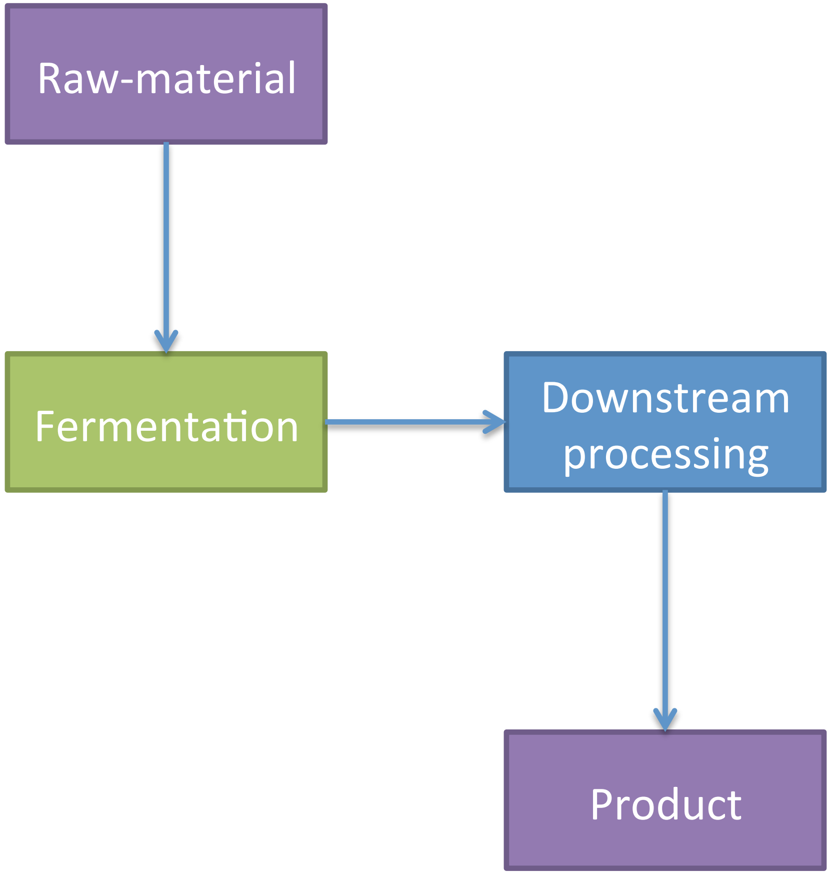


- 25 countries, 3 continents

# Downstream processing in biorefineries

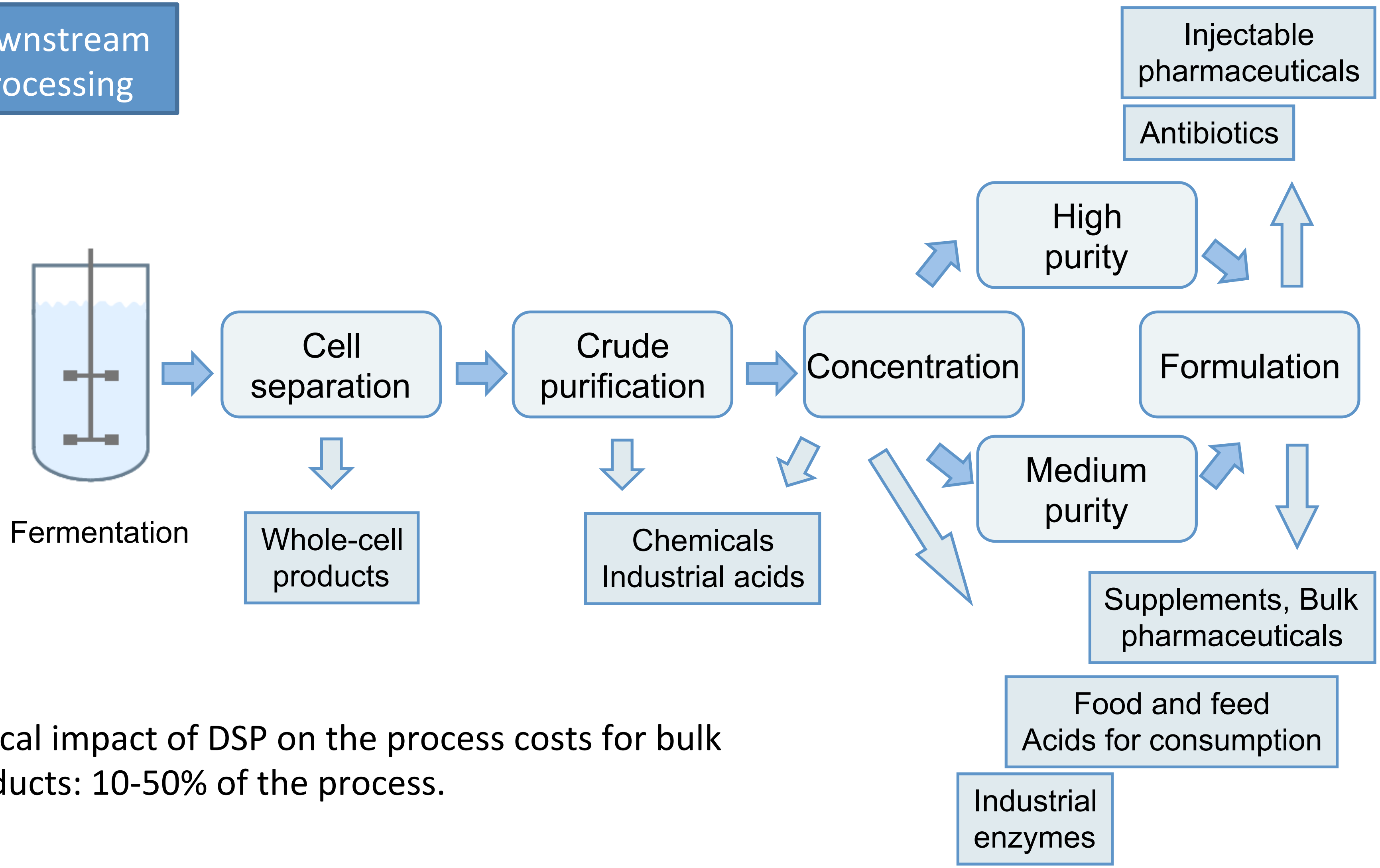
# Typical bioprocess

Fairly defined raw materials
Mostly used "as is" in the process



# Typical bioprocess

Downstream processing

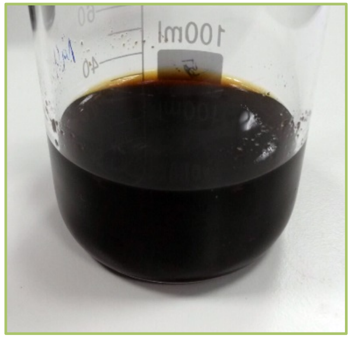



Typical impact of DSP on the process costs for bulk products: 10-50% of the process.

# Biorefineries

- Potato pulp hydrolysate

- Corn Hydrolysate




- Banana press juice

- Spent sulfite liquor

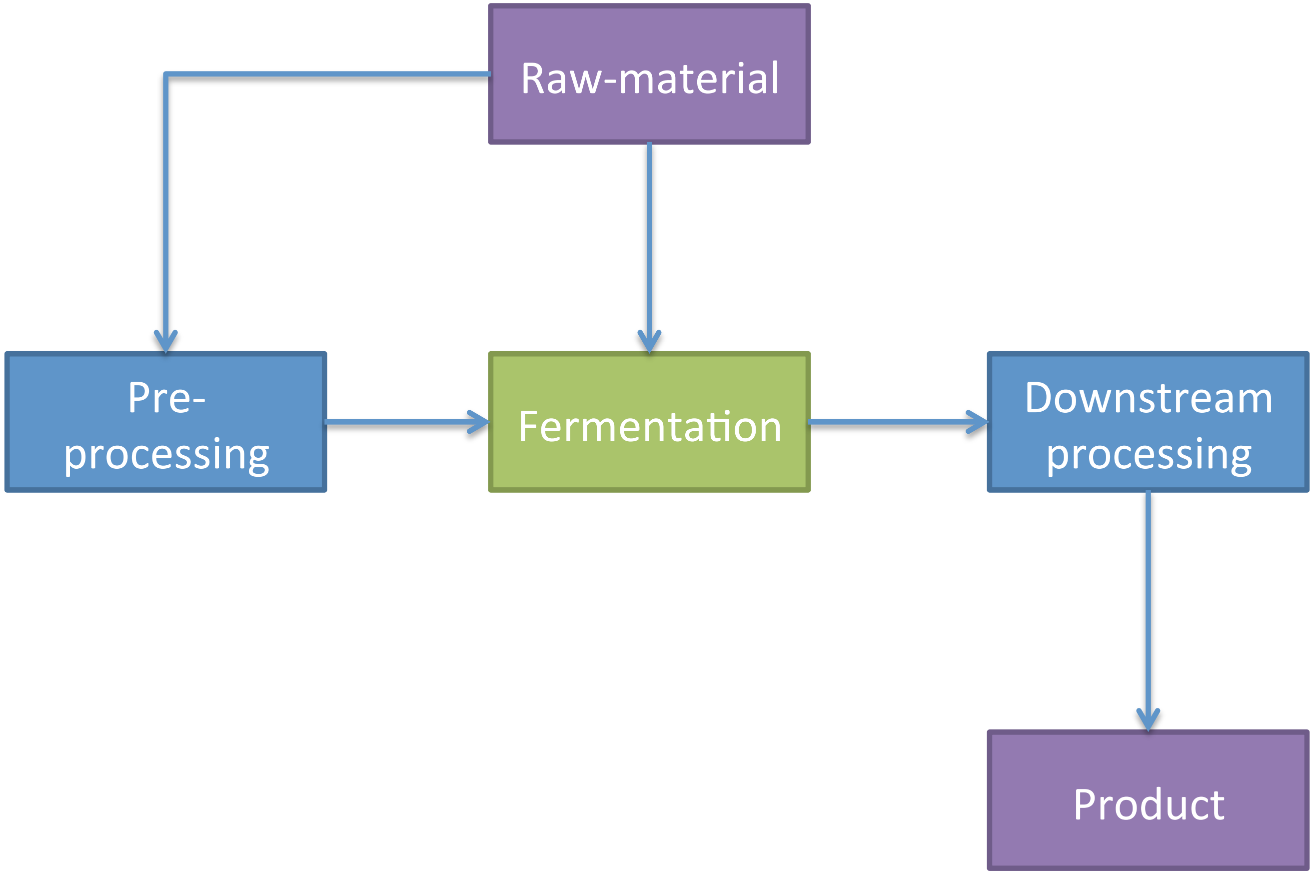
Only a fraction of the components will be consumed in the fermentation
The rest will remain

# Biorefineries

Microbial biomass grown on refined hydrolysate:

Same microbial biomass grown on spent sulfite liquor:

# Biorefineries

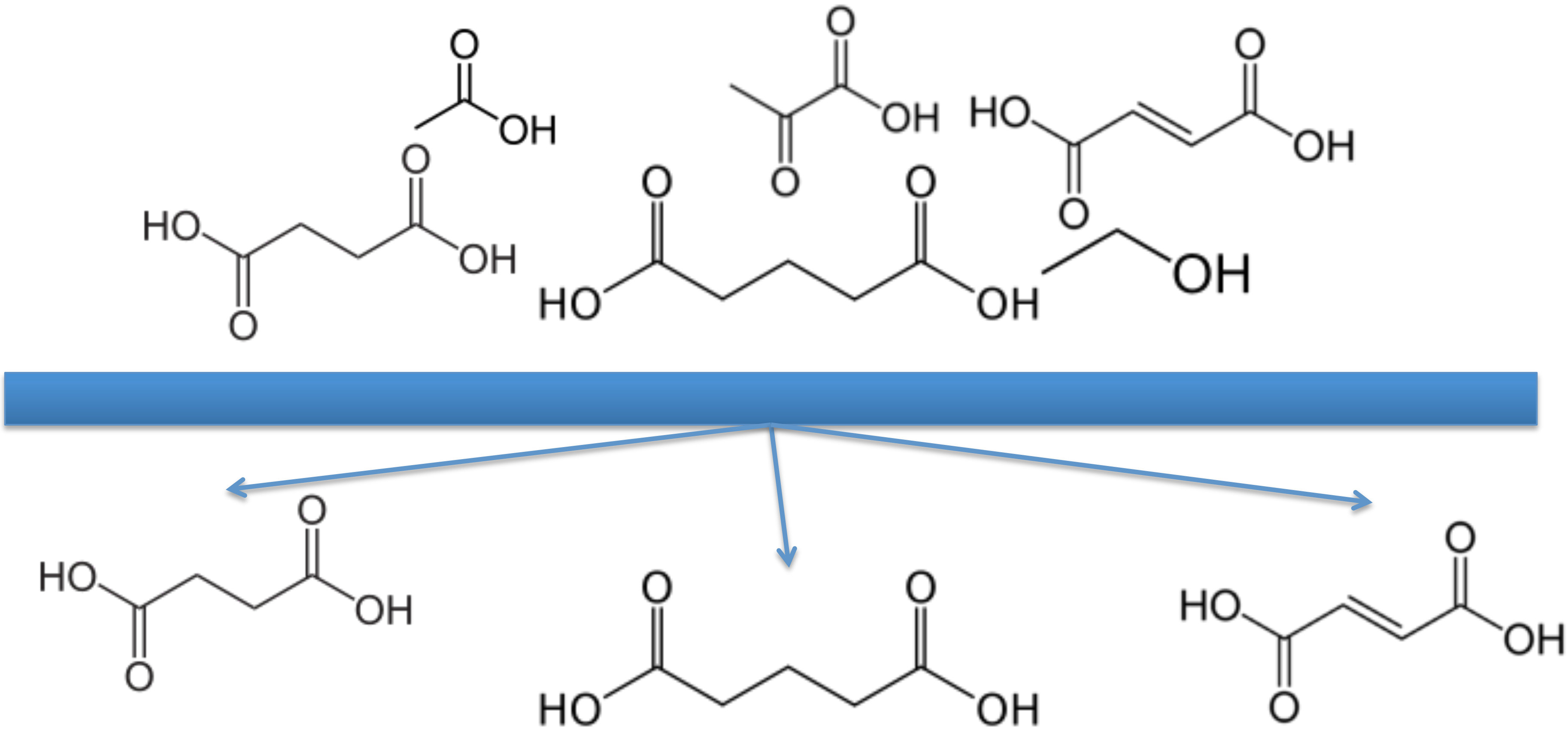



Pre-processing and DSP are closely related, similar operations and goals.

# Design considerations

- **Composition of the raw material**
- **Co-/by-products produced during the fermentation**
- **Required specifications of the end-product**
- **Translatable to industrial operation using industry-standard equipment**

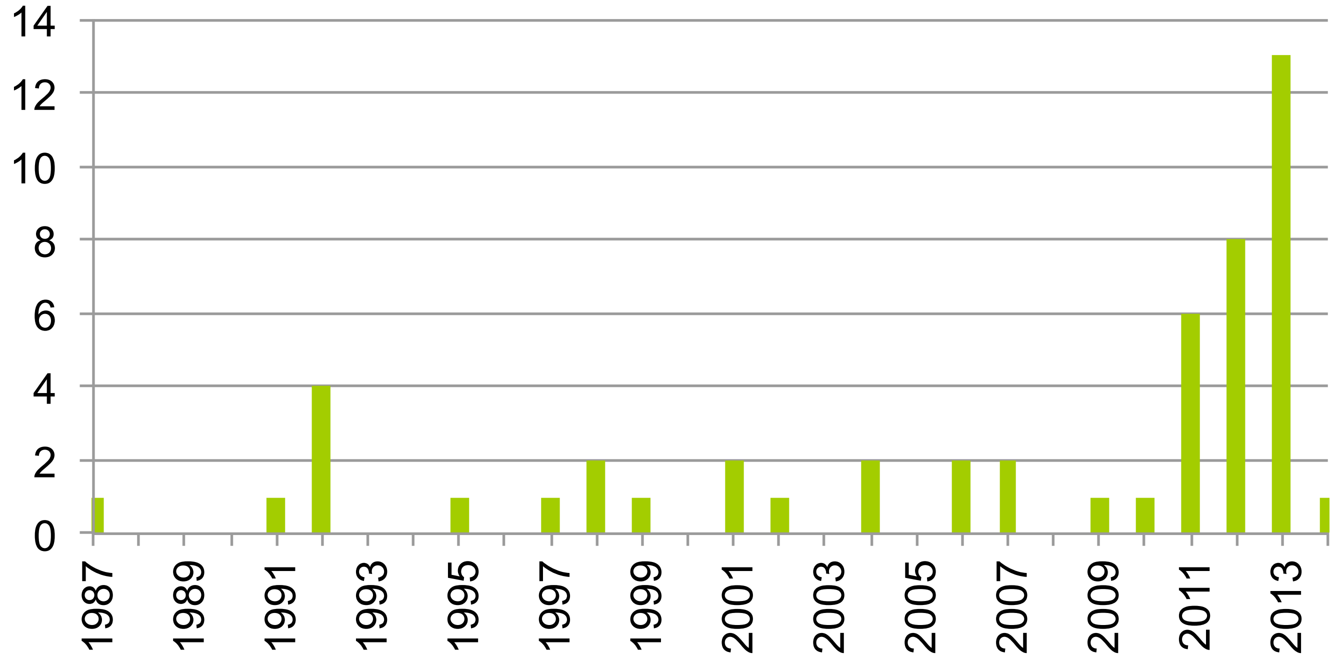
# Purification of diacids



# Diacid purification

**Increasingly hot topic**

- Patents. Most for succinic acid.



# Diacid purification

**Succinic acid**

Succinity = Purac + BASF

EP2360137
WO2013/025105, WO2013/025106
WO2010/092155

Reverdia = DSM + Roquette

EP2504307
US2012/0040422

BioAmber = DNP Green Technology + ARD

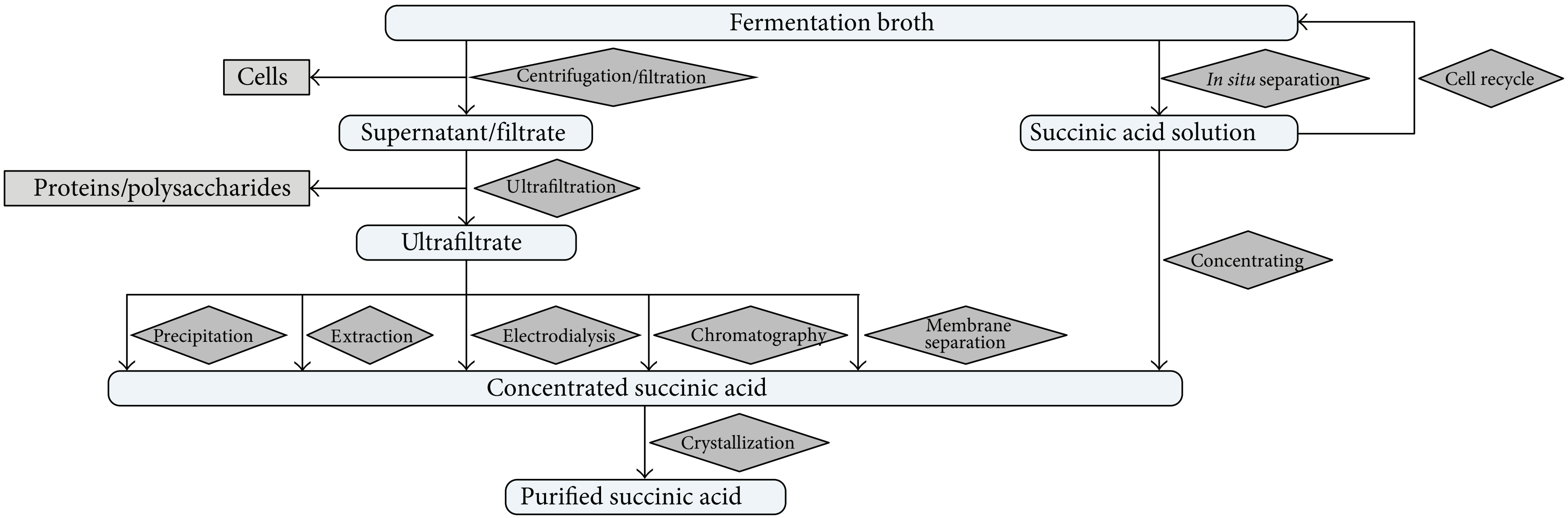
US20110237831
US2012/0021473
US2011/0297527
WO212/158180
US2012/0259138
US2013/0150621
US2013/0072714

Myriant

US2010/06235

# Diacid purification

**Many options (theoretically) possible**



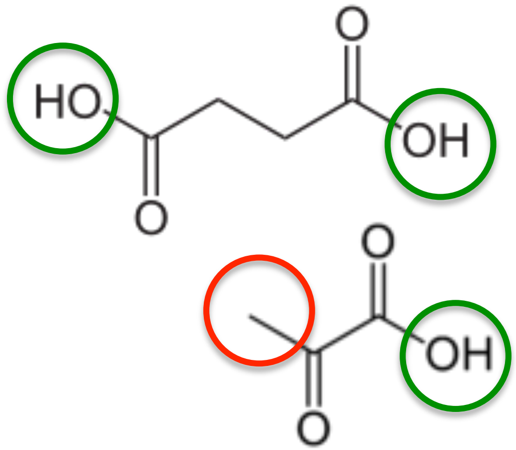

Cao et al., BioMed Research International, 2013, Article ID 723412
http://dx.doi.org/10.1155/2013/723412

# Challenges

**Specifications for final use, ex. polymerization**

Diacids required for polymerization

Monocarboxylic acids stop the reaction due to the lack of the functional group Required for the polymerization reaction to go on




Some cations will dramatically reduce the lifetime of the polymerization catalysts

# Challenges

**Raw material - SSL**

Salts, including $Na^{+}$ and $Ca^{2+}$

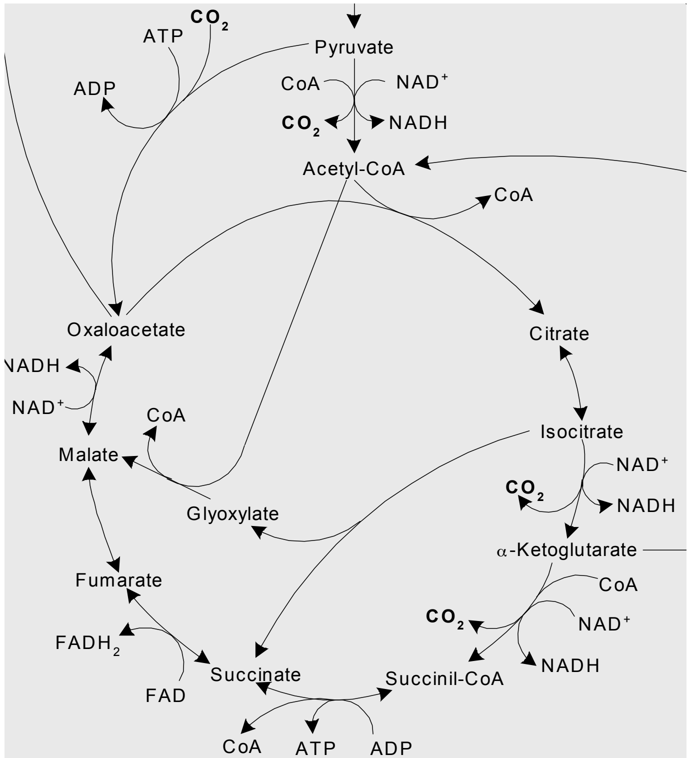
Monocarboxylic acids, ex. acetic, formic

Lignosulphonates

**Fermentation**

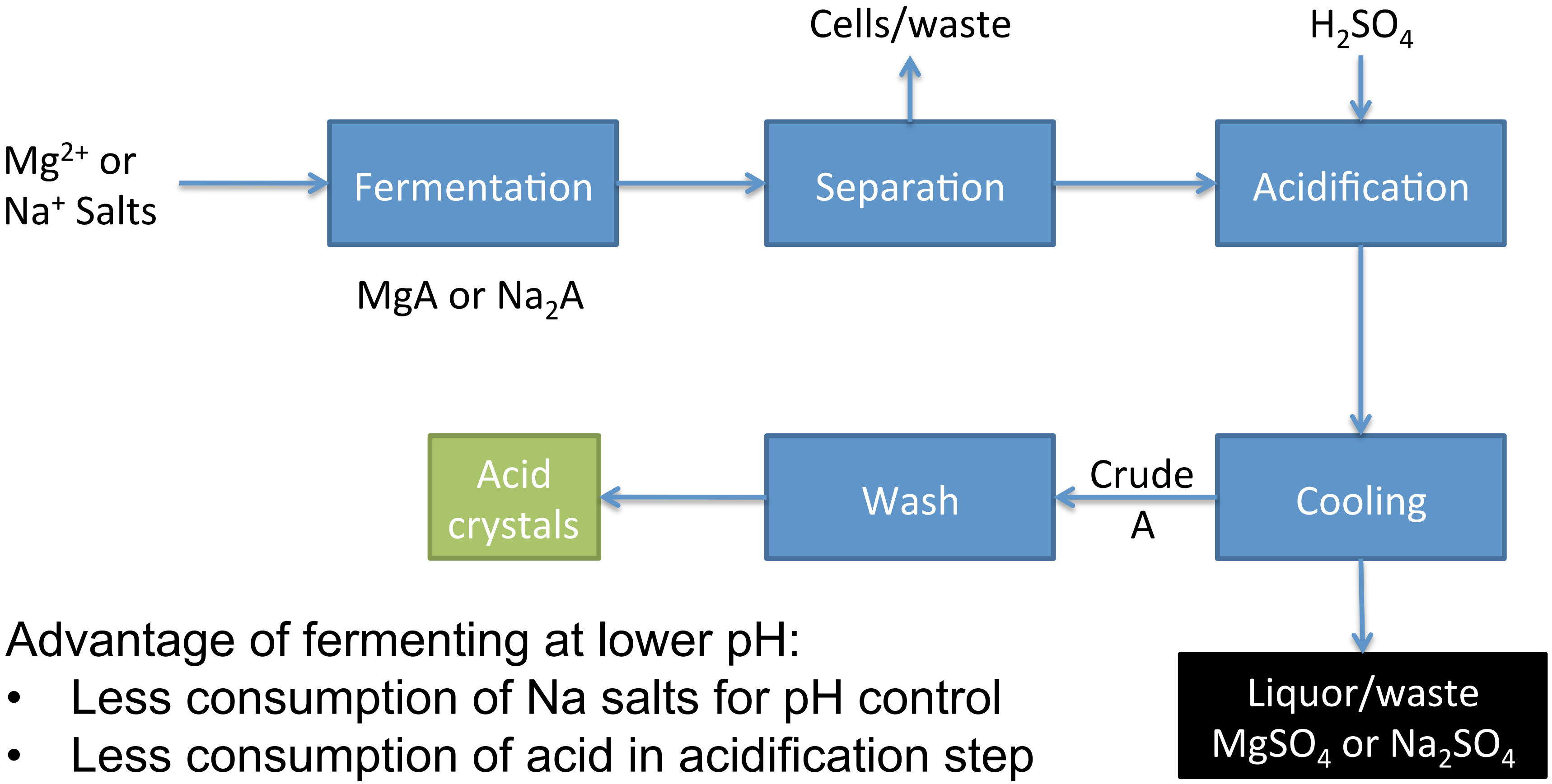
Other dicarboxylic and monocarboxylic acids

Other metabolites



# Traditional process

**Precipitation**




Advantage of fermenting at lower pH:

- Less consumption of Na salts for pH control
- Less consumption of acid in acidification step
- Less waste generated

# Alternatives: Electrodialysis

- Separation of charged species through charged membranes
- Normally preceded by stringent polishing steps, ex. nanofiltration

| Advantages: | Disadvantages: |
|---|---|
| ▫ Modular (linear scale-up), | ▫ Requires very low concentrations of divalent cations |
| ▫ Can be applied in-situ, | ▫ Prone to fouling |
| ▫ Regeneration of pH control agent, | ▫ High energy consumption |
| ▫ Relatively mild conditions. | ▫ High capital investment |

- Despite its potential, due to the very complex nature of the raw materials, may be challenging.

# Alternatives: Extraction

**Extraction**

- Different affinity between aqueous medium and organic phase:
  - The solvent reduces the solubility of the dicarboxylic acid on the liquid phase causing its precipitation, or
  - The dicarboxylic acid is more soluble in the organic phase and is thereby removed from the fermentation medium.

| Advantages: | Disadvantages: |
|---|---|
| ▫ Low technological barriers,<br>▫ Customization to the molecule of interest,<br>▫ Possibility of solvent recycling. | ▫ May involve high amounts of organic liquids<br>▫ Toxicity issues: impact on solvent containment and effluent treatment |

- Unless "green solvents" can be used, it will be avoided.

# Alternatives: Extraction

**Extraction – Amine complex formation**

- The formation of a dicarboxylic acid – amine complex soluble on the organic phase may increase the efficiency of the extraction of the undissociated acid (“reactive extraction”).
- pH, pKa1, concentration of carboxylic acid and amine, polarity of the diluent, the operating temperature and regeneration of the acid are factors requiring optimization.
- Can be implemented as membrane reactive extraction and in-situ separation could be envisaged (but technologically challenging).

# Alternatives: Adsorption

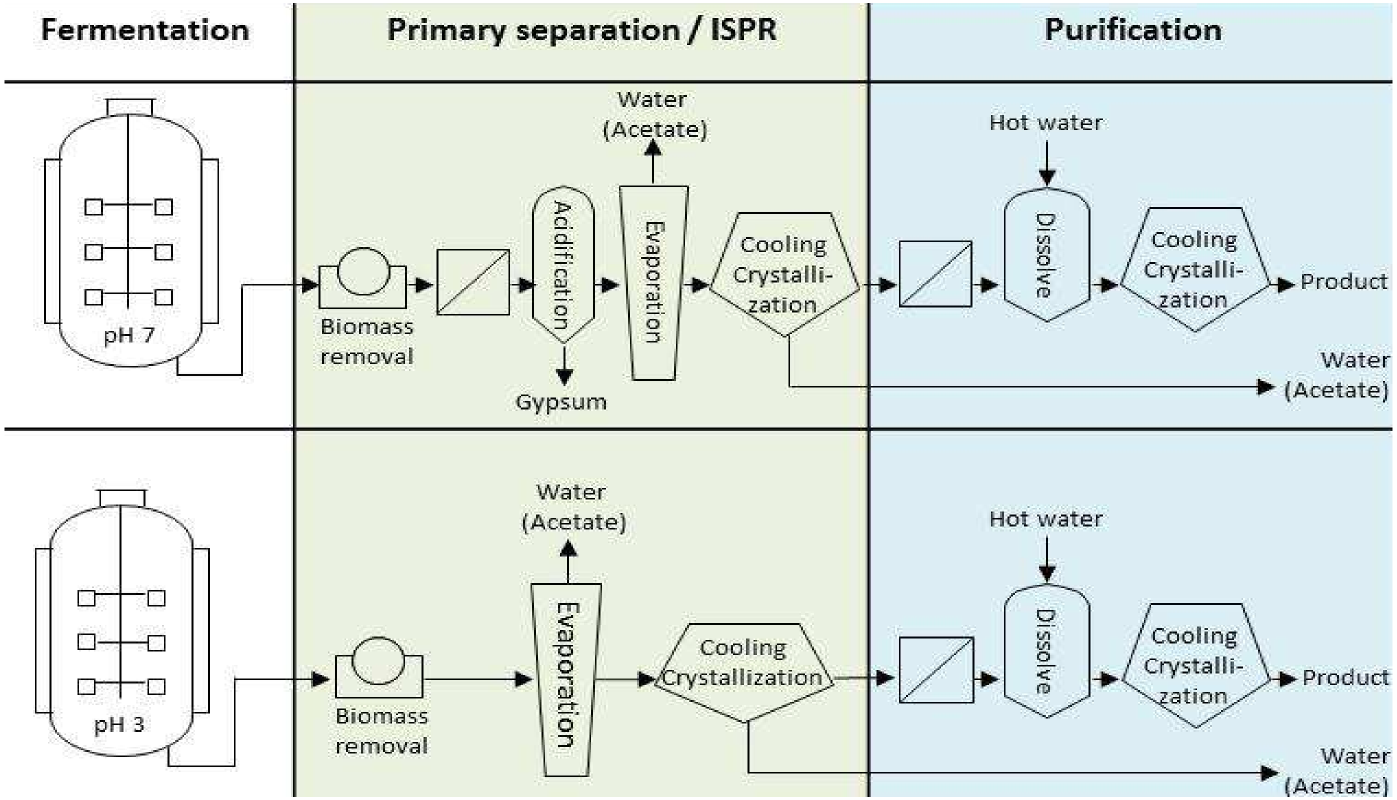
## Adsorption

- Use of an adsorbent (various interactions possible) in a fixed bed column through which the dicarboxylic acid solution is circulated.
- High capacity, specificity and low cost of regeneration are needed.

| Advantages: | Disadvantages: |
|---|---|
| ▫ In situ application,<br>▫ Specificity,<br>▫ Less by-products. | ▫ Normally the regeneration produces high salt effluents<br>▫ Generally high capital investment |

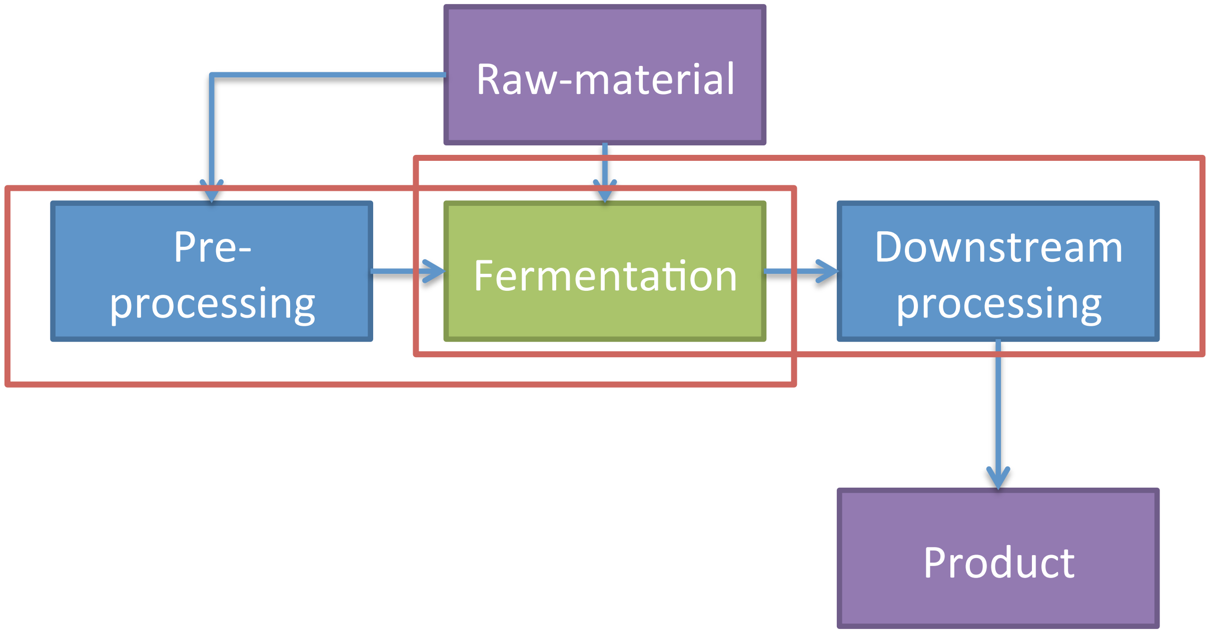
- It is normally used as a polishing step at the end of the DSP.

# Multiple operations




Bio-Con-Sept: D5.1 Technology roadmap for the purification of platform chemicals and side products, 24-3-2014

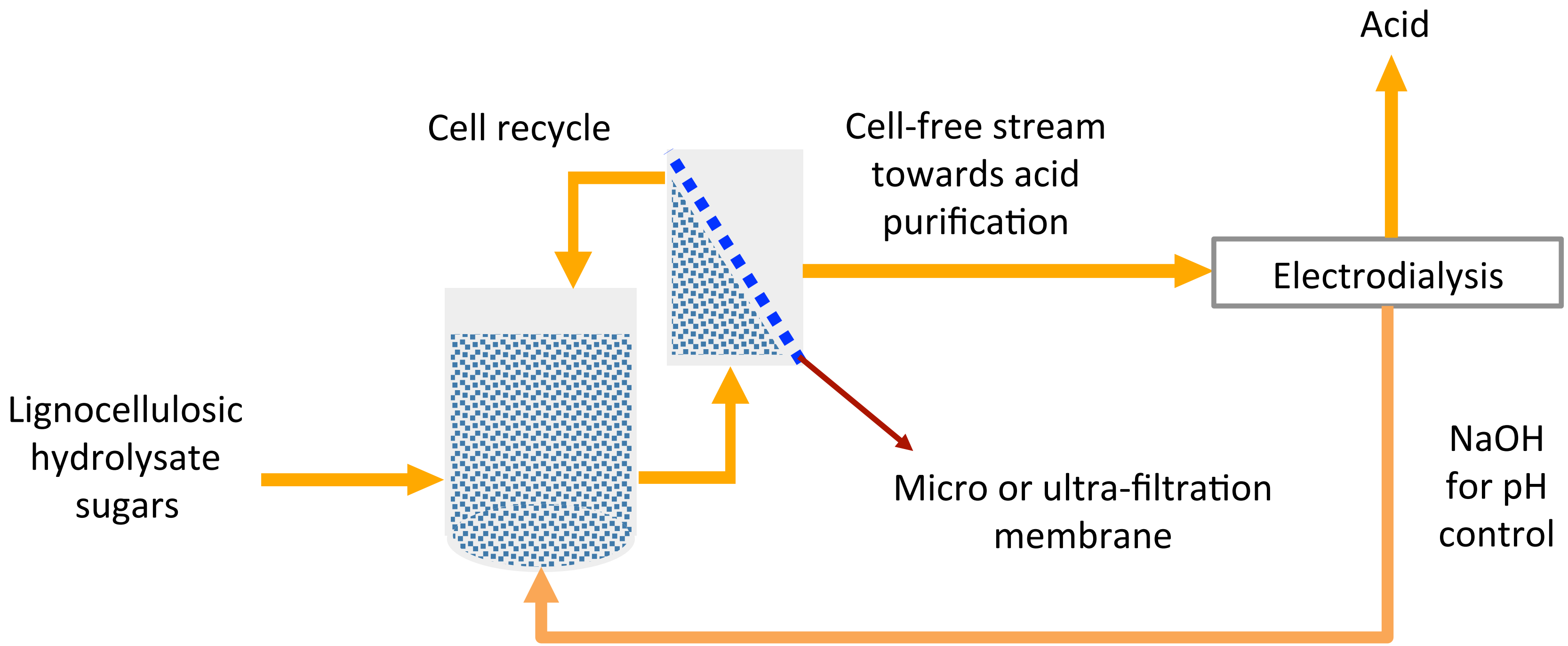
# Process integration



# Fermentation-separation

**Acid from lignocellulosic residues**

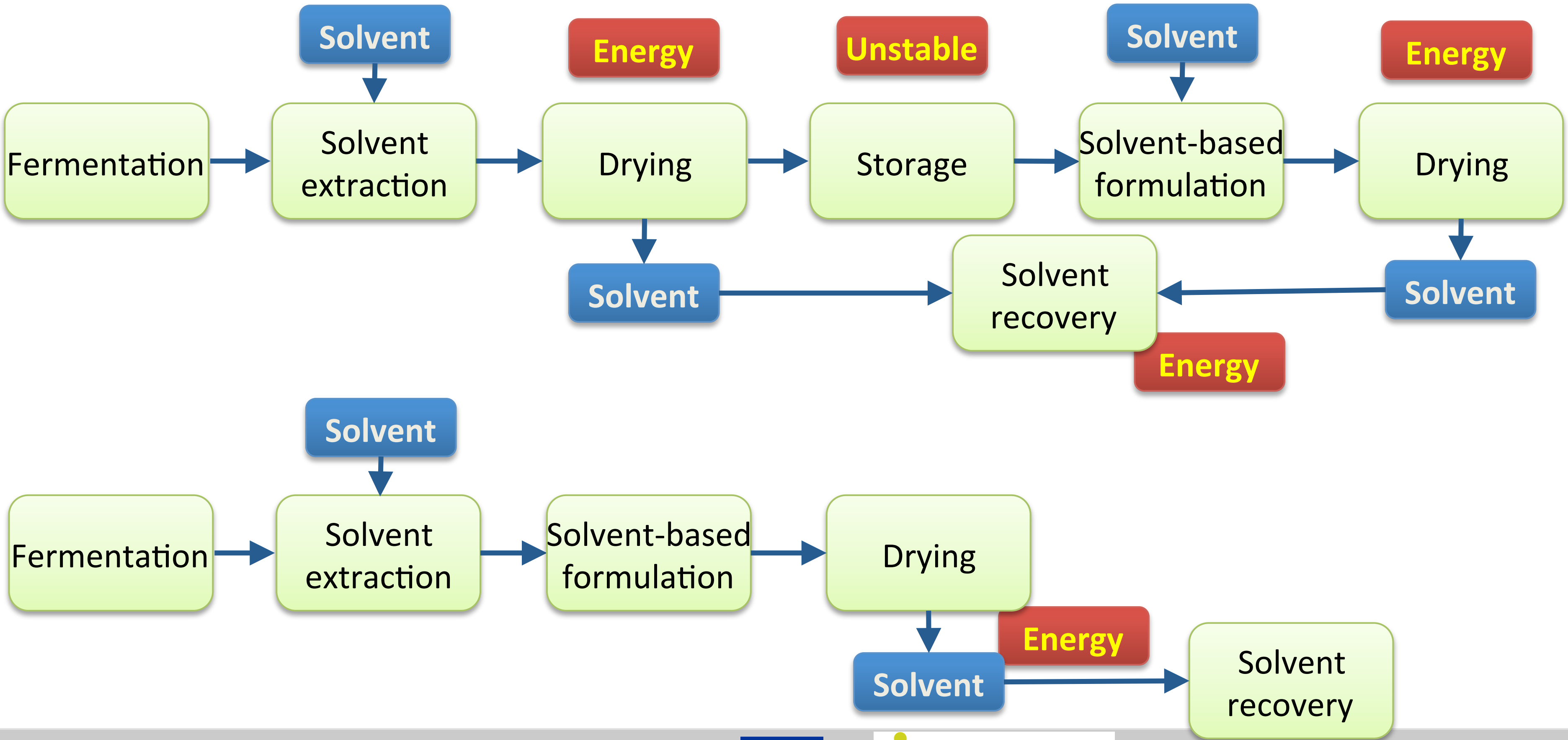
- In-situ cell recycle: cell-free product stream and keep a high concentration of the biocatalyst within the bioreactor (higher productivities)
- Reagent reuse: NaOH used in the pH control of the fermentation recovered in the electrodialysis step converting sodium carboxylate to carboxylic acid (lower operational costs)



# Purification-formulation

**Carotenoid purification and formulation:**

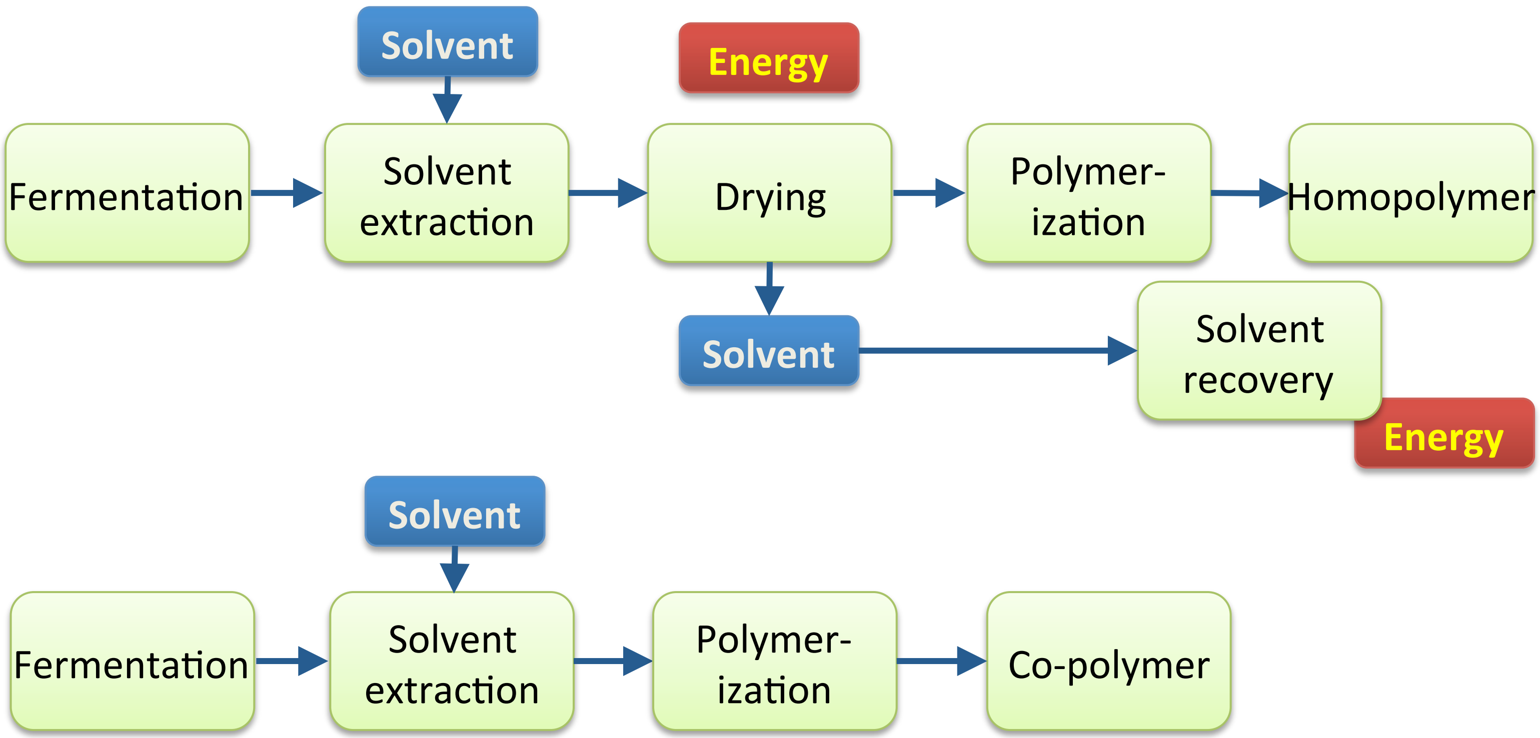
- Less operations, less energy requirements, safer operation



# Purification-application

**Diacid extraction and polymerization**

- Less operations, less energy requirements, safer operation
- However, no buffering between steps.



BIOREFINE-2G


**Bruno Ferreira: bsf@biotrend.biz**

**Frederik van Keulen: fvk@biotrend.biz**

**Diogo Figueira: diogo.figueira@biotrend.biz**


biotrend
Inovação e engenharia em biotecnologia



This project is co-funded by the European Union within the 7th Frame Programme. Grant Agreement n°613771.
The sole responsibility of this publication lies with the author. The European Union is not responsible for any use that may be made of the information contained therein.