Section II. Developments in biorefinery: feedstocks, processes and products (I)

Production of bio-based chemicals and polymers from industrial waste and by-product streams

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Main research interests

Agricultural University of Athens

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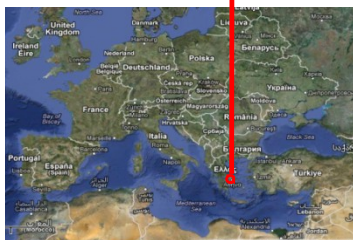

- Biorefinery development using agri-industrial waste and by-product streams
- Separation of value-added co-products
- Bioprocess development using entirely renewable resources for the production of platform chemicals, biopolymers and microbial lipids
- Biorefinery and bioprocess design

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Valorization of industrial waste and by-product streams *via* fermentation for the production of chemicals and biopolymers

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Approach on industrial waste/by-product biorefinery development The case of food waste

Extraction of value-added co-products

Food Wastes

Upstream Processing

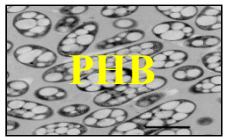
Enzymatic Hydrolysis

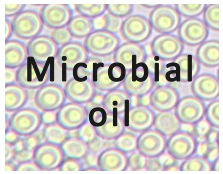
Fermentation

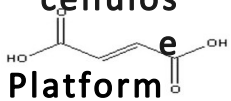
Downstream processing

Filtration
L-L Extraction
Distillation
Crystallisation
Drying

Bio-based products

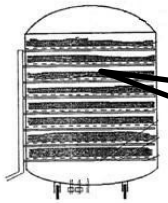




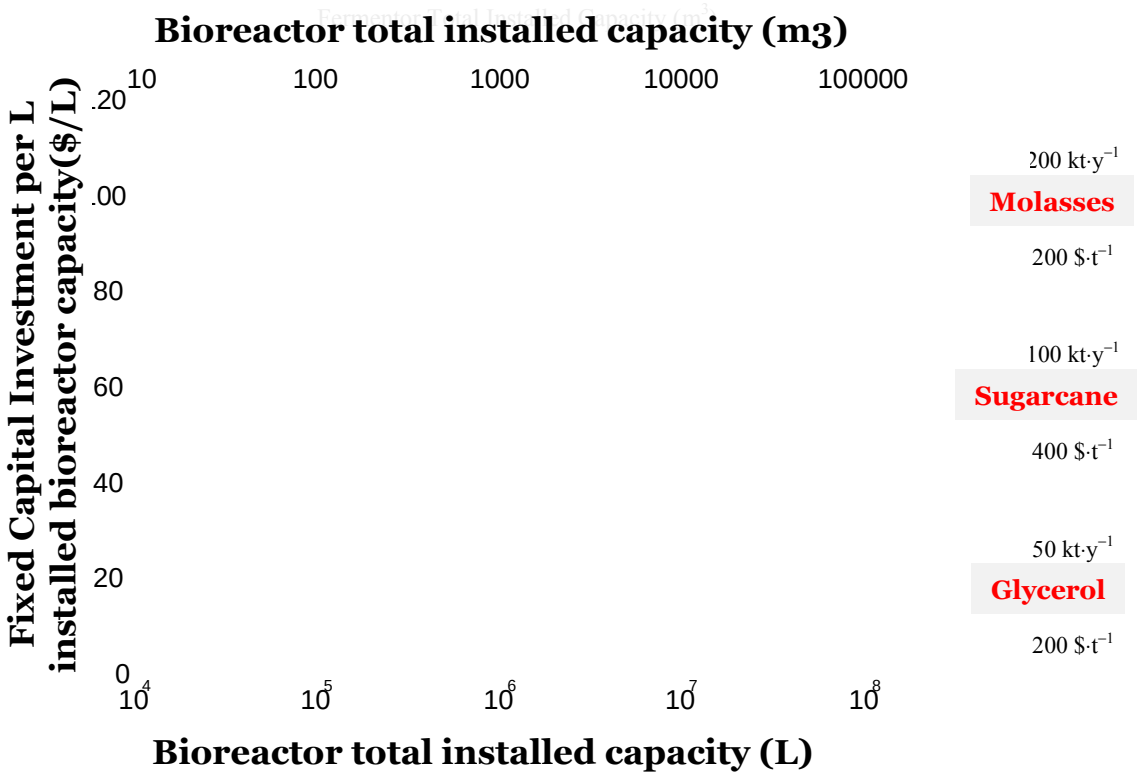


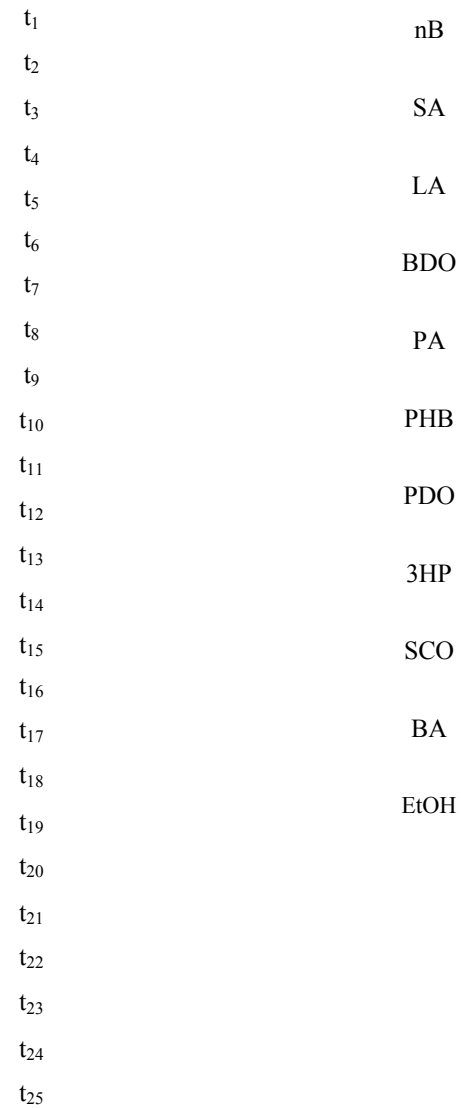
Platform chemicals

Solid State Fermentation



Biorefinery/bioprocess design and techno-economic evaluation





Dheskali et al. 2017. Bioresource Technology 224:509-514

Bonatsos et al. 2016. Biochemical Engineering Journal 116:157-165

Koutinas et al. 2016. Bioresource Technology 204:55-64

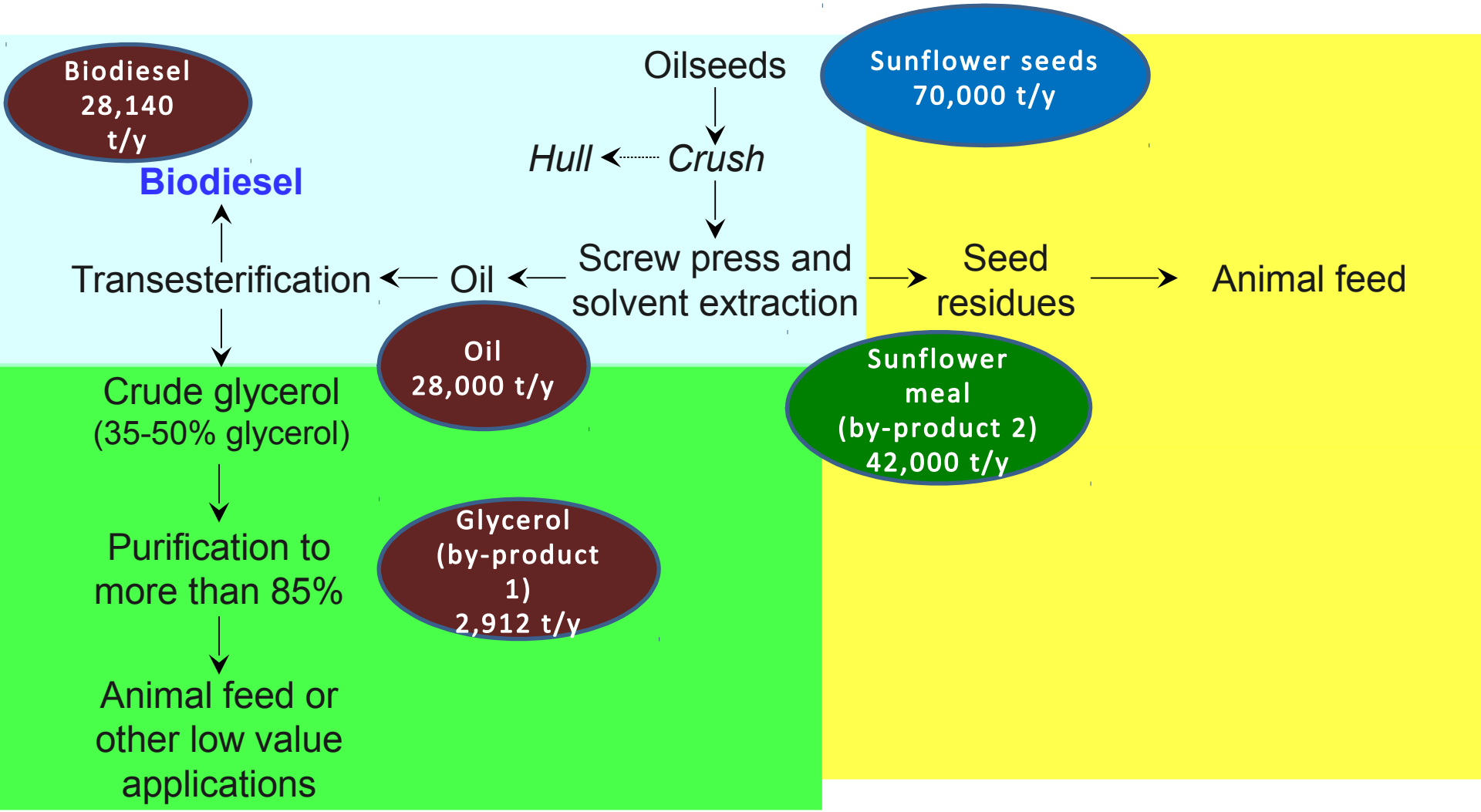
Dimou et al. 2016. Biochemical Engineering Journal 116:157-165

Koutinas et al. 2014. Fuel 116:566-577

Biorefinery development based on the valorisation of biodiesel industry by-products

for the production of antioxidant-rich fraction, protein isolate and poly(3-hydroxybutyrate)

Current biodiesel production process from oilseeds



Base-case processing scenario

Sunflower seed

Mechanical pressing and hexane extraction

Sunflower oil

Transesterification

Biodiesel

Crude glycerol

Carbon source

Sunflower meal

Aspergillus oryzae

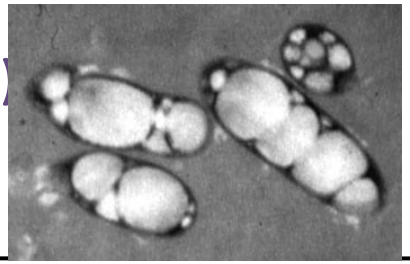
Solid state fermentation

Enzymatic hydrolysis

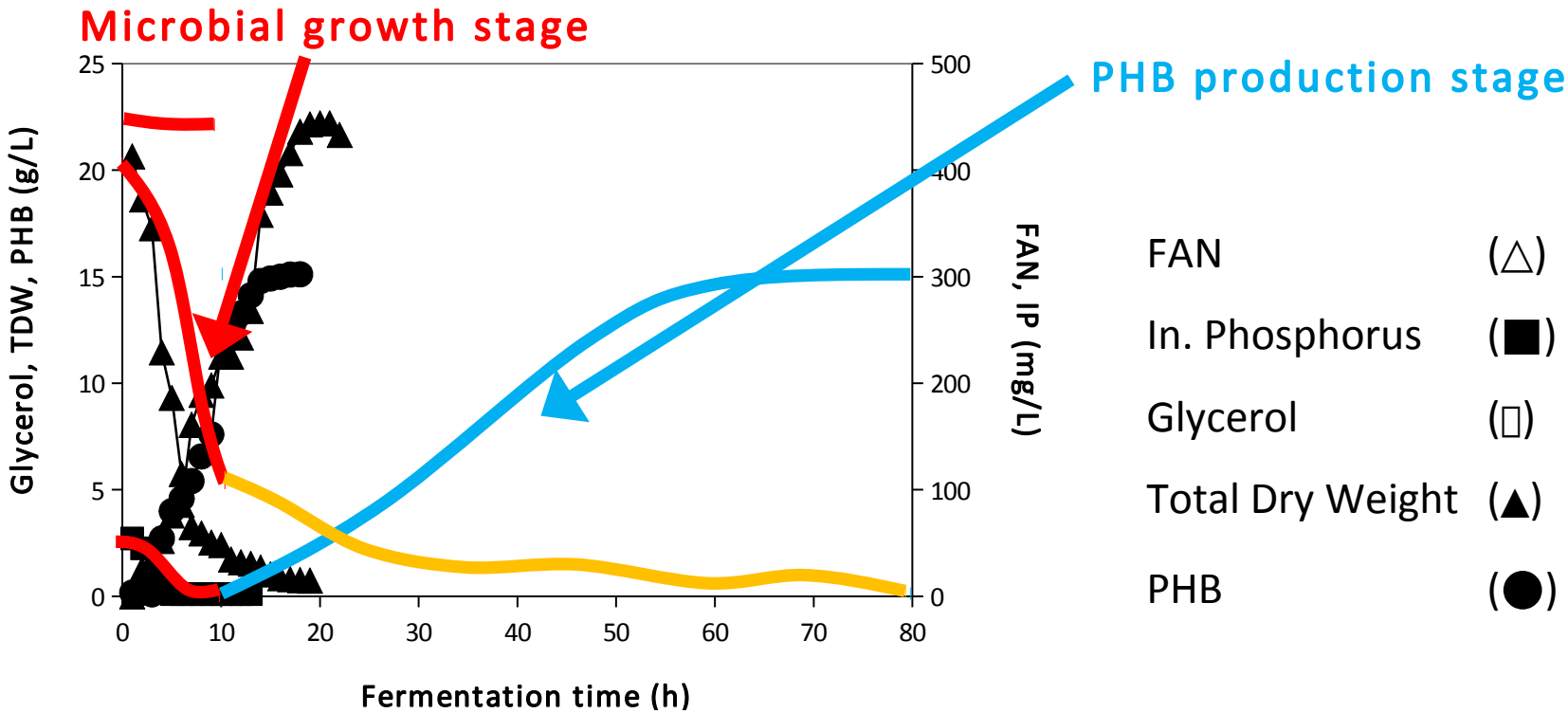
Nutrient-rich supplement

Microbial fermentation

Poly(3-hydroxybutyrate)



PHB production from whole sunflower meal hydrolysate (SFM) and crude glycerol



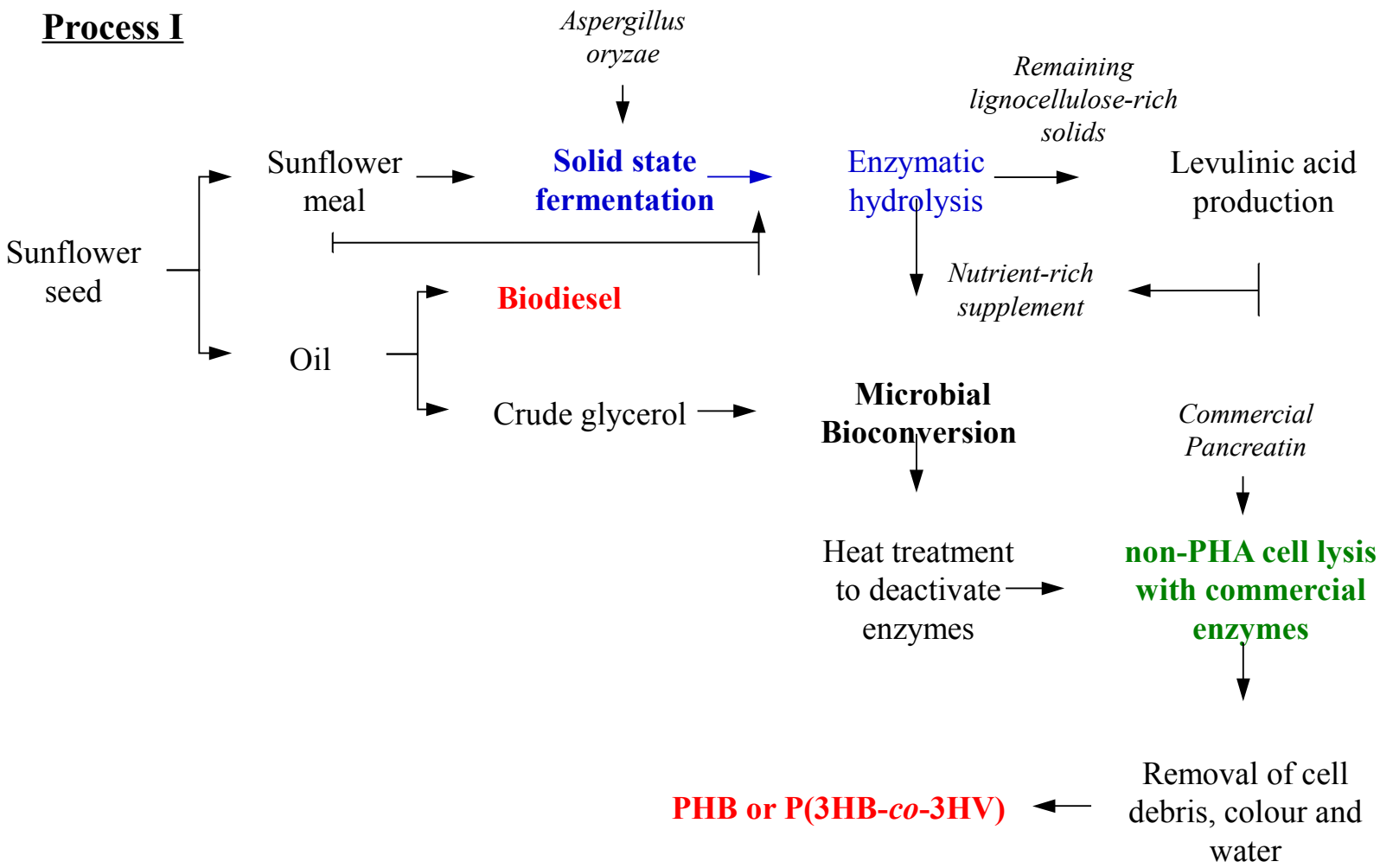
Crude glycerol

Sunflower meal hydrolysate

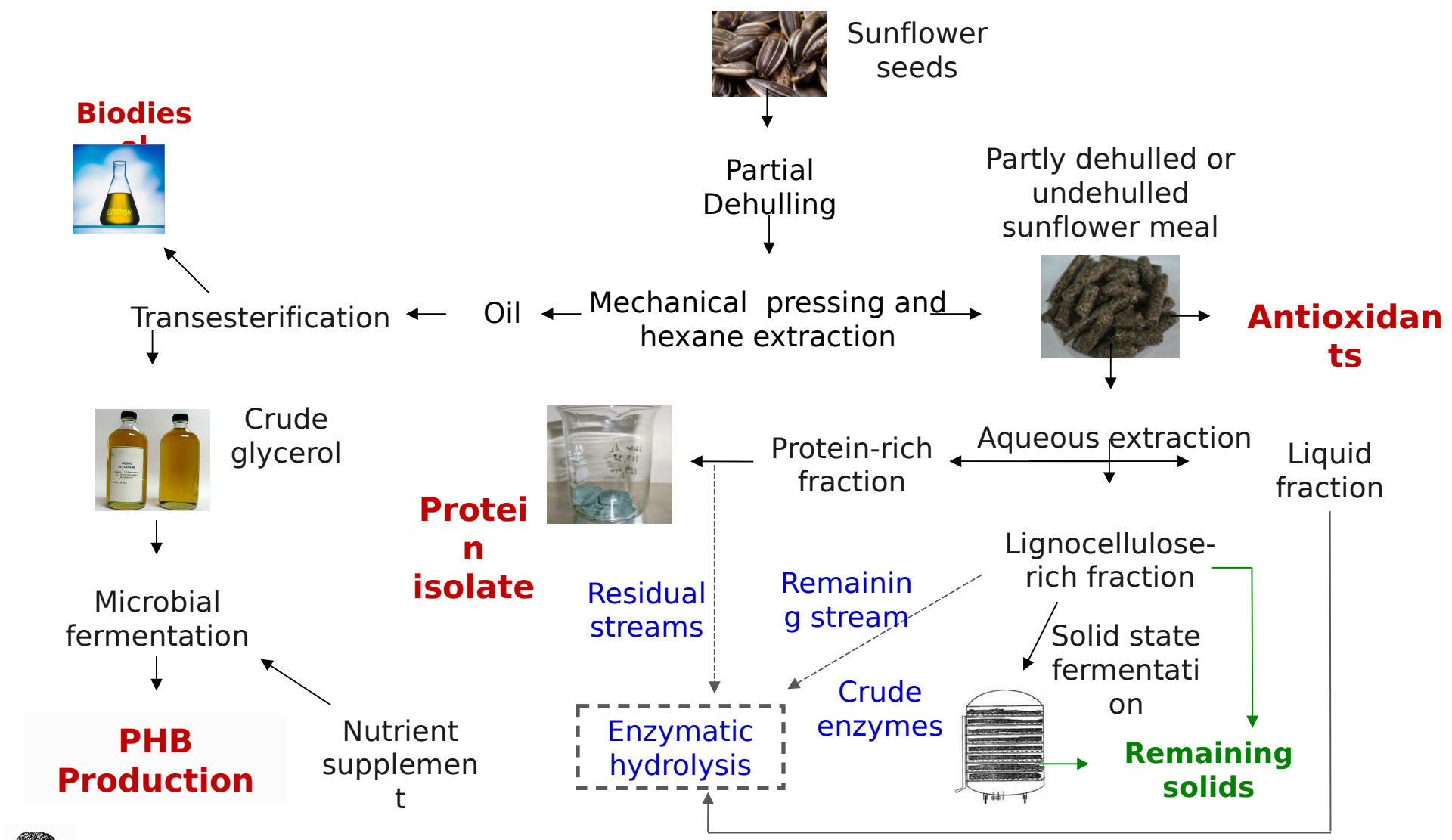
→ Microbial fermentation → **Polyhydroxybutyrate**

Kachrimanidou et al. 2014. Bioresourse Technol. 172:121–130

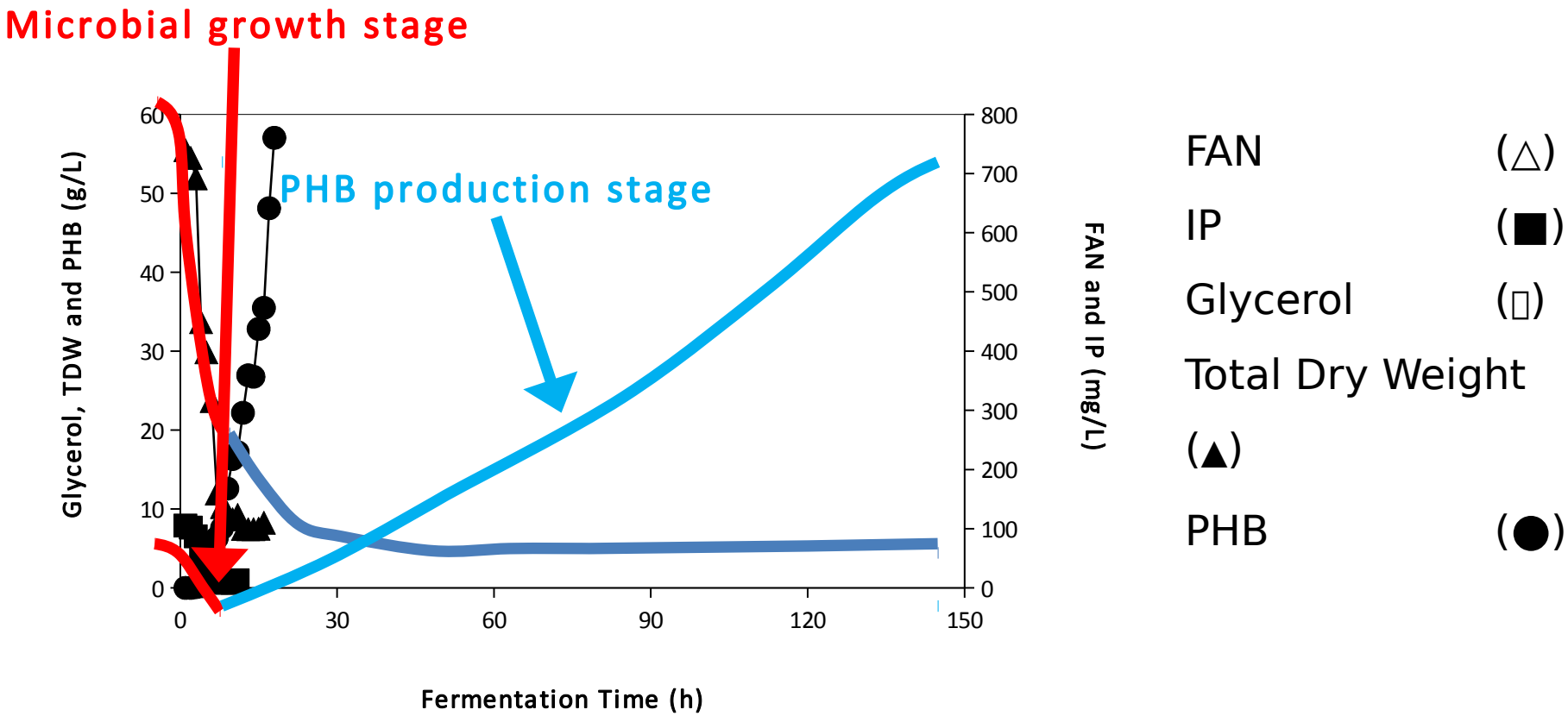
Purification of PHAs using commercial enzymes



Advanced sunflower-based biorefinery



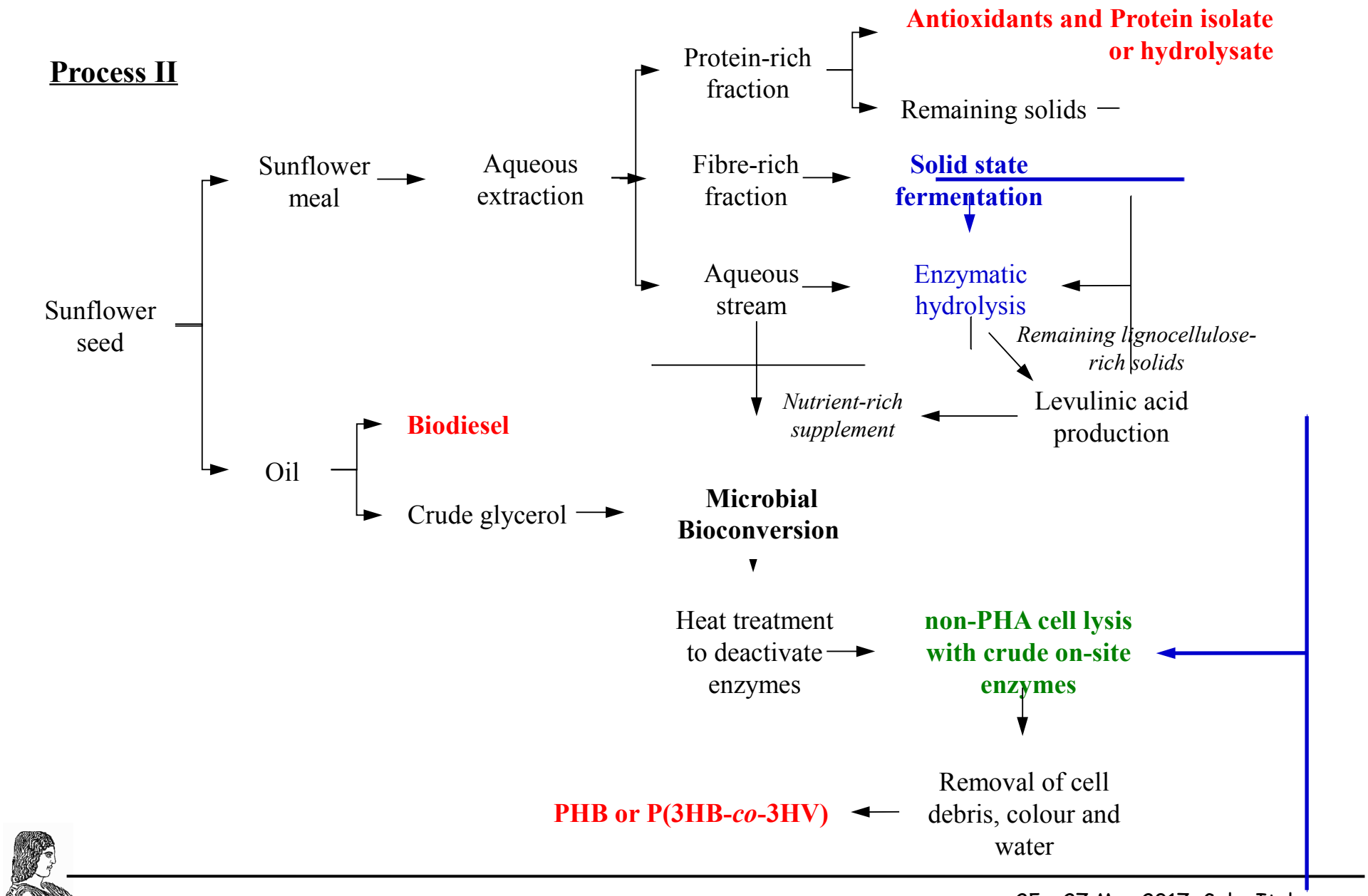
PHB fermentation using the hydrolysate produced when all residual streams are employed



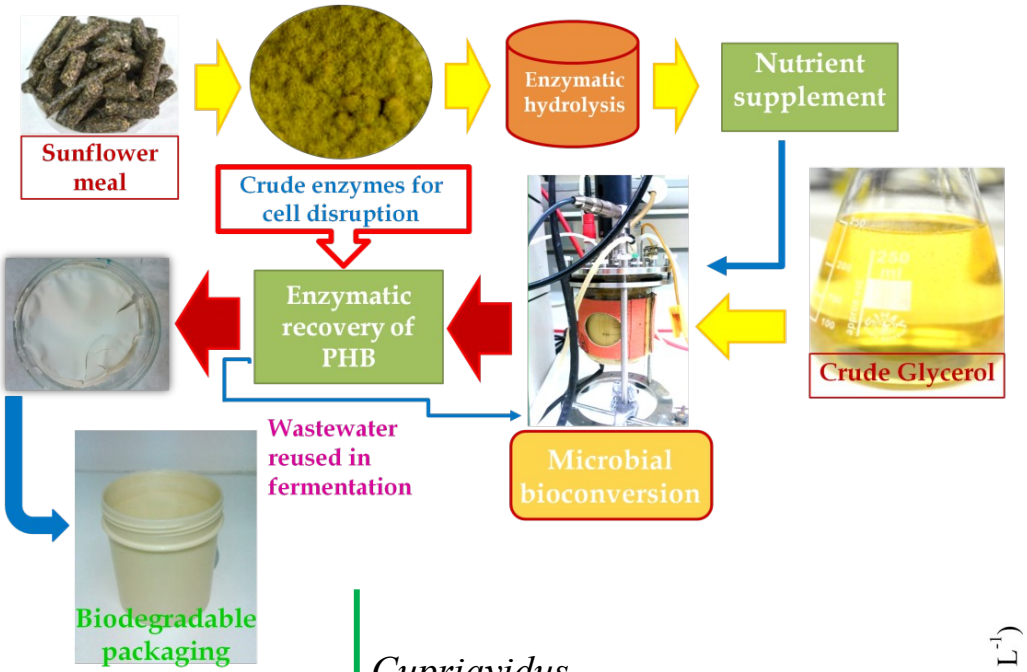
This medium is efficient for enhanced PHB production

Kachrimanidou et al. 2015. Ind. Crops Prod. 71:106–113

Integration of multipurpose usage of crude enzymes produced on-site



Evaluation of hydrolyzed solution of bacterial cells as fermentation substrate for PHB production

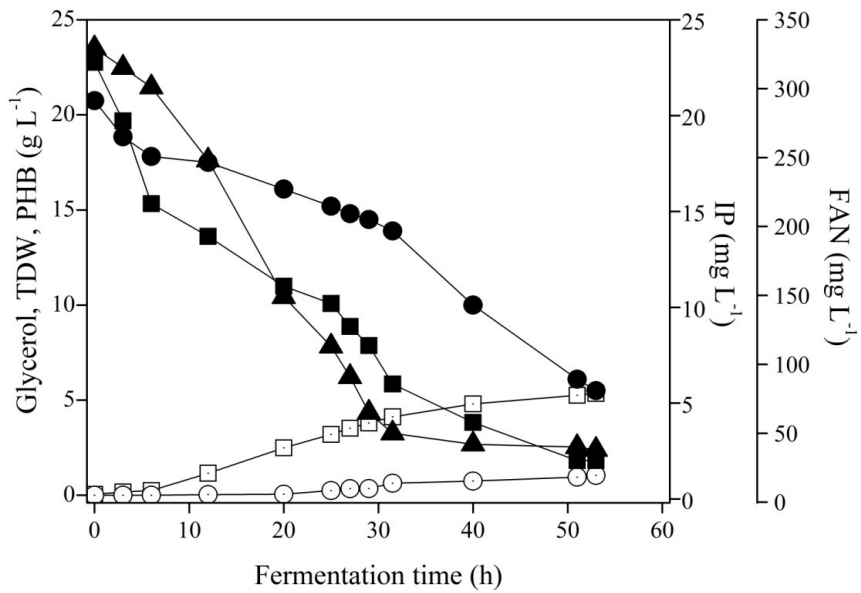


The lysate from enzymatic non-PHB cell lysis could be used as fermentation feedstock supplemented with phosphate salts

Cupriavidus necator DSM 7237

FAN: 329 mg/L
Glycerol: 20 g/L

TDW: 5.75 g/L
PHB content : 20%



Profitability assessment of different processing scenarios

Process type	**Net Present Value 10 year (million $)**
<u>Current practice</u> - utilisation of SFM and crude glycerol as animal feed	**31.5**
<u>Process I</u> - PHB production from crude glycerol and part of SFM - SFM utilisation as animal feed	**Lower than 31.5**
<u>Process II</u> (low range of market prices for biorefinery products) - fractionation of SFM to produce protein isolate and antioxidants - PHB production from crude glycerol and remaining SFM fractions	**Lower than 31.5**
<u>Process III</u> (high range of market prices for biorefinery products) - fractionation of SFM to produce protein isolate and antioxidants - PHB production from crude glycerol and remaining SFM fractions	**Higher than 31.5**

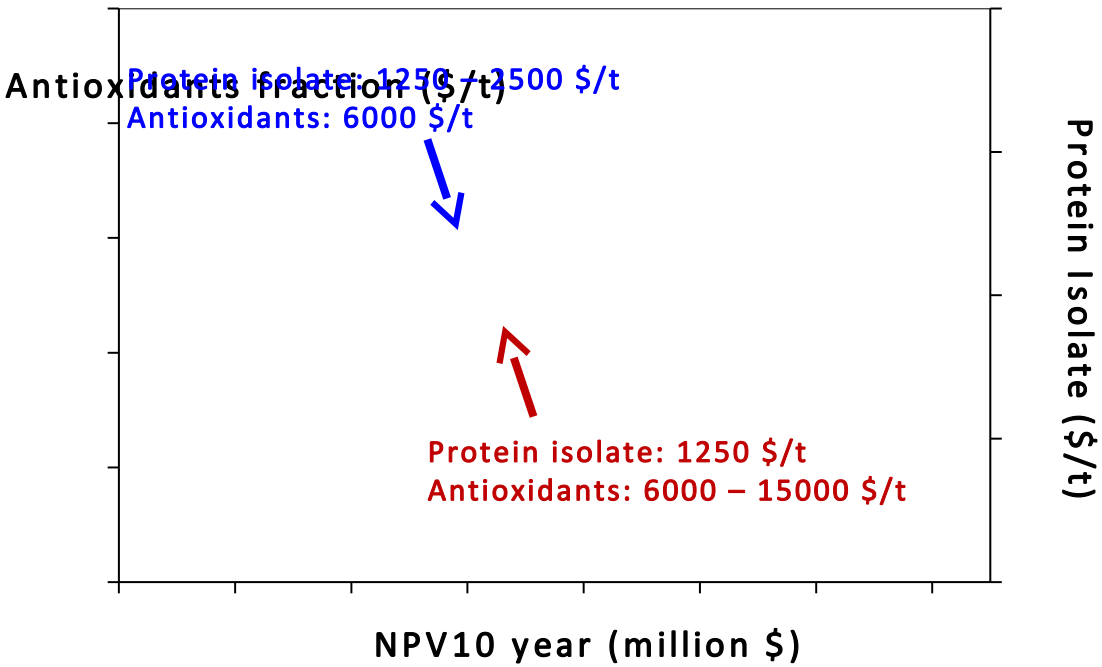
NPV range estimated based on low and high market prices for the protein isolate (1,250 – 2,500 $/t) and antioxidants (6 – 15 $/kg)

Current Practice

NPV = 31.5 million $

Low NPV value estimated when the protein isolate and antioxidants market prices are 1,250 $/t and 6 $/kg, respectively — **Lower than 31.5 million $**

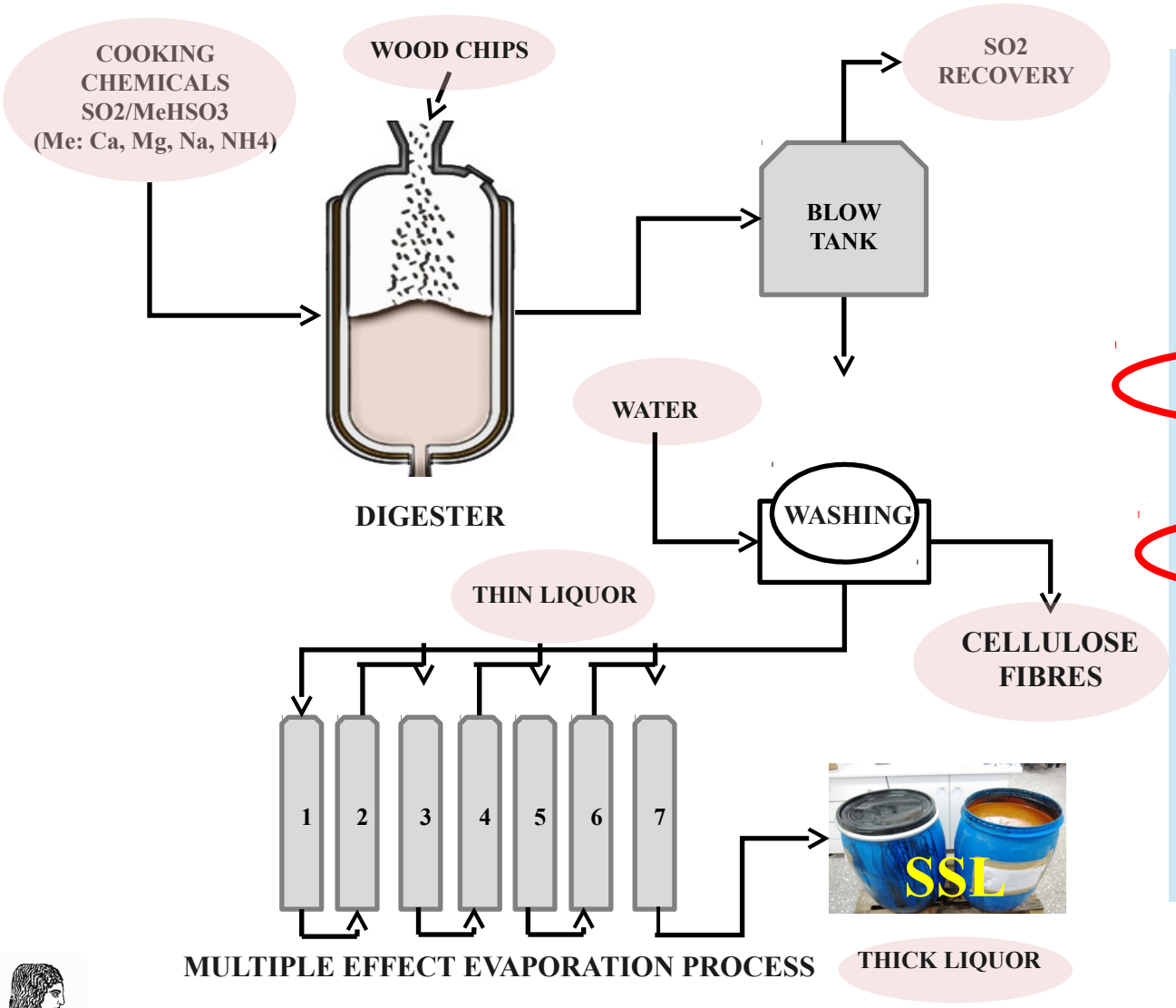
High NPV value estimated when the protein isolate and antioxidants market prices are 2,500 $/t and 15 $/kg, respectively — **Higher than 31.5 million $**



Biorefinery development based on the valorisation of spent sulphite liquor

for the production of lignosulphonates, succinic acid and antioxidant-rich fraction

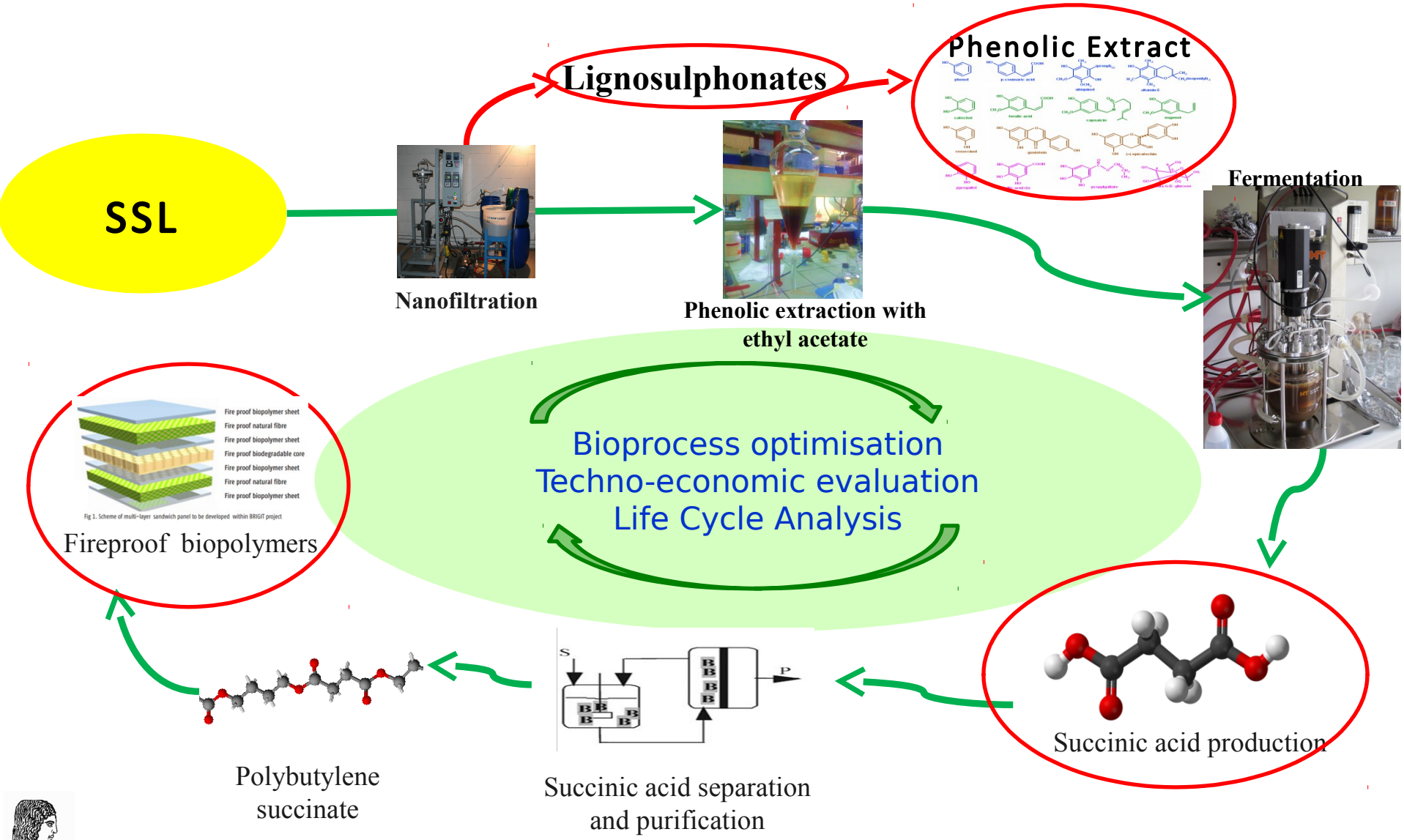
Spent sulphite liquor (SSL) production in the sulphite wood pulping process



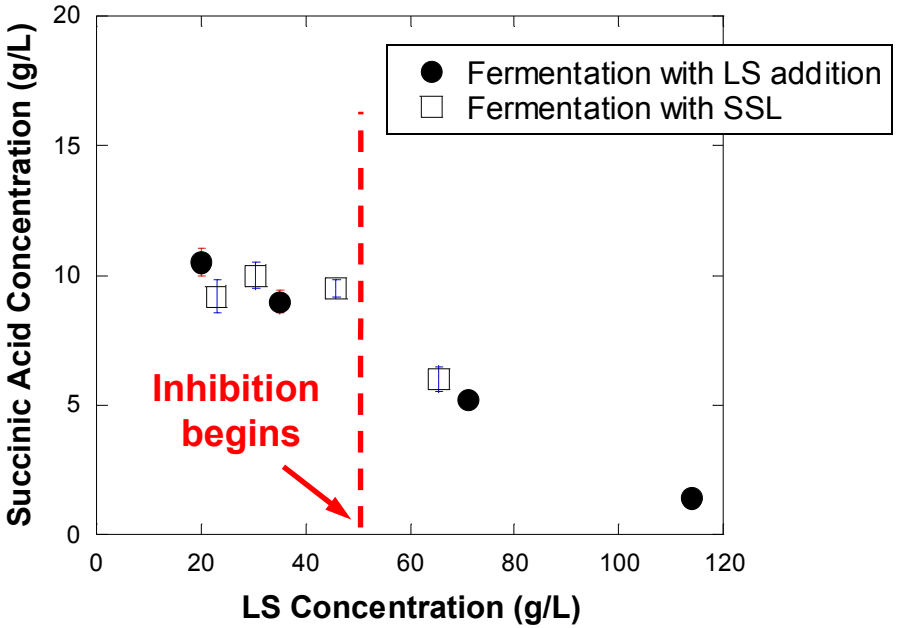
SSL Characterisation

	Value	St Dev
pH	2.7	
Density (g/mL)	1.277	0.007
Viscosity (cP)	552	167
Dry Matter (g-DM/L)	816.5	0.6
Lignosulphonates (g/L)	458.8	2.7
Ash % (g/g-DM)	8.62	0.55
Phenolics % (g/g-DM)	1.55	0.04
Carbohydrates (g/L)	176.41	
Xylose (g/L)	128.08	0.59
Galactose (g/L)	21.47	5.50
Glucose (g/L)	19.27	0.39
Mannose (g/L)	7.41	1.30
Arabinose (g/L)	0.18	0.05
Acetic Acid (g/L)	6.91	0.49

Integrated biorefinery based on current pulp and paper mills

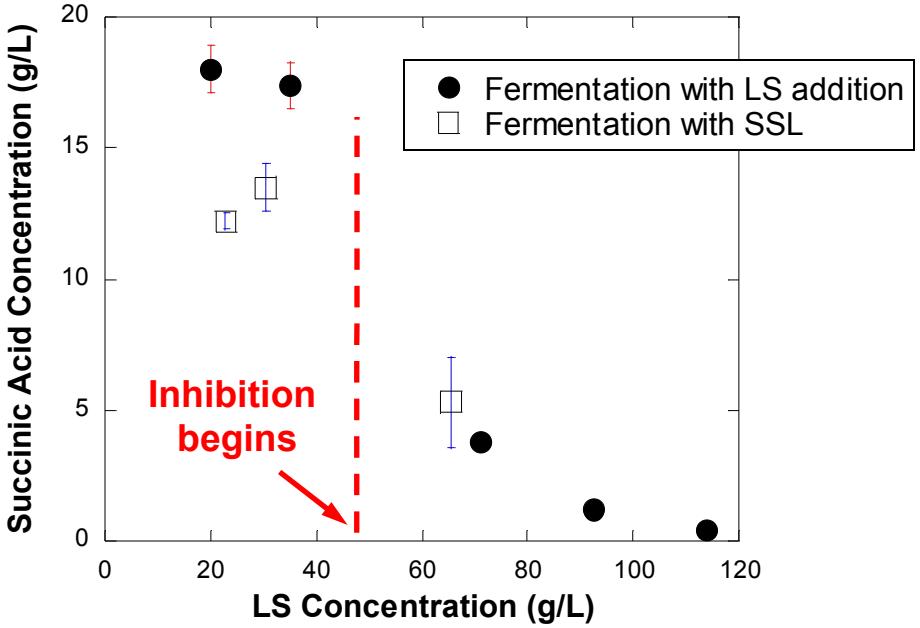


Effect of SSL and extracted lignosulphonate (LS) concentration on succinic acid production



Actinobacillus succinogenes

Basfia succiniciproducens



Pretreatment of spent sulphite liquor (SSL)

Nanofiltration

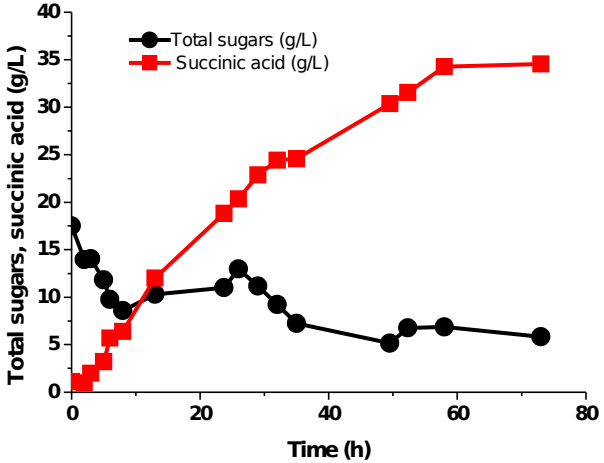
Phenolic extraction with ethyl acetate

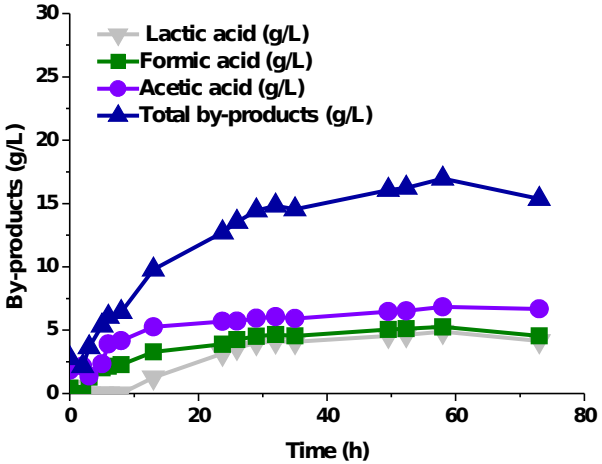
Fermentation

Fed-batch fermentations in bench-top bioreactor

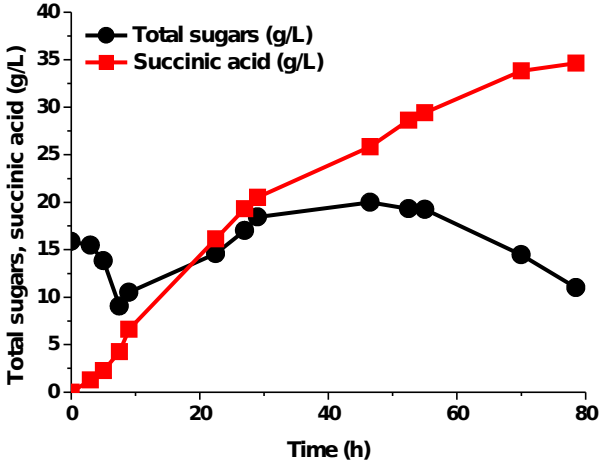
B. succiniciproducens

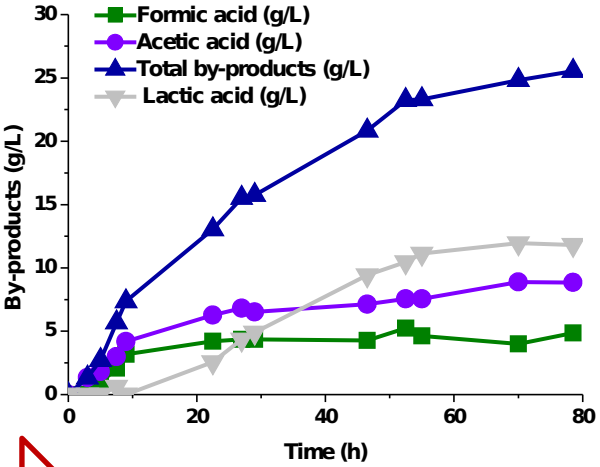
Pure mixed sugars





Nanofiltrated SSL





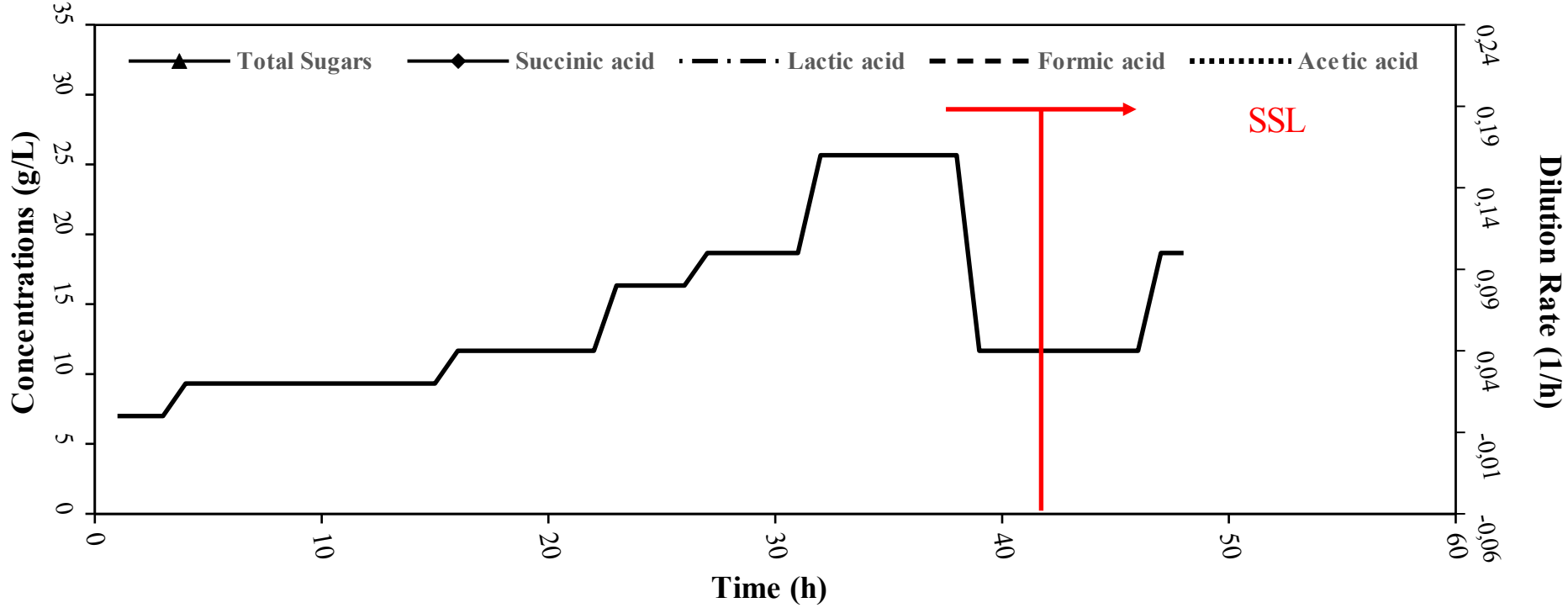
Pure mixed sugars

Final SA concentration: 34.2 g/L
Yield: 0.65 g/g
Productivity: 0.59 g/L/h

Nanofiltrated SSL with 0.5 kDa MWCO

Final SA concentration: 33.8 g/L
Yield: 0.58 g/g
Productivity: 0.48 g/L/h

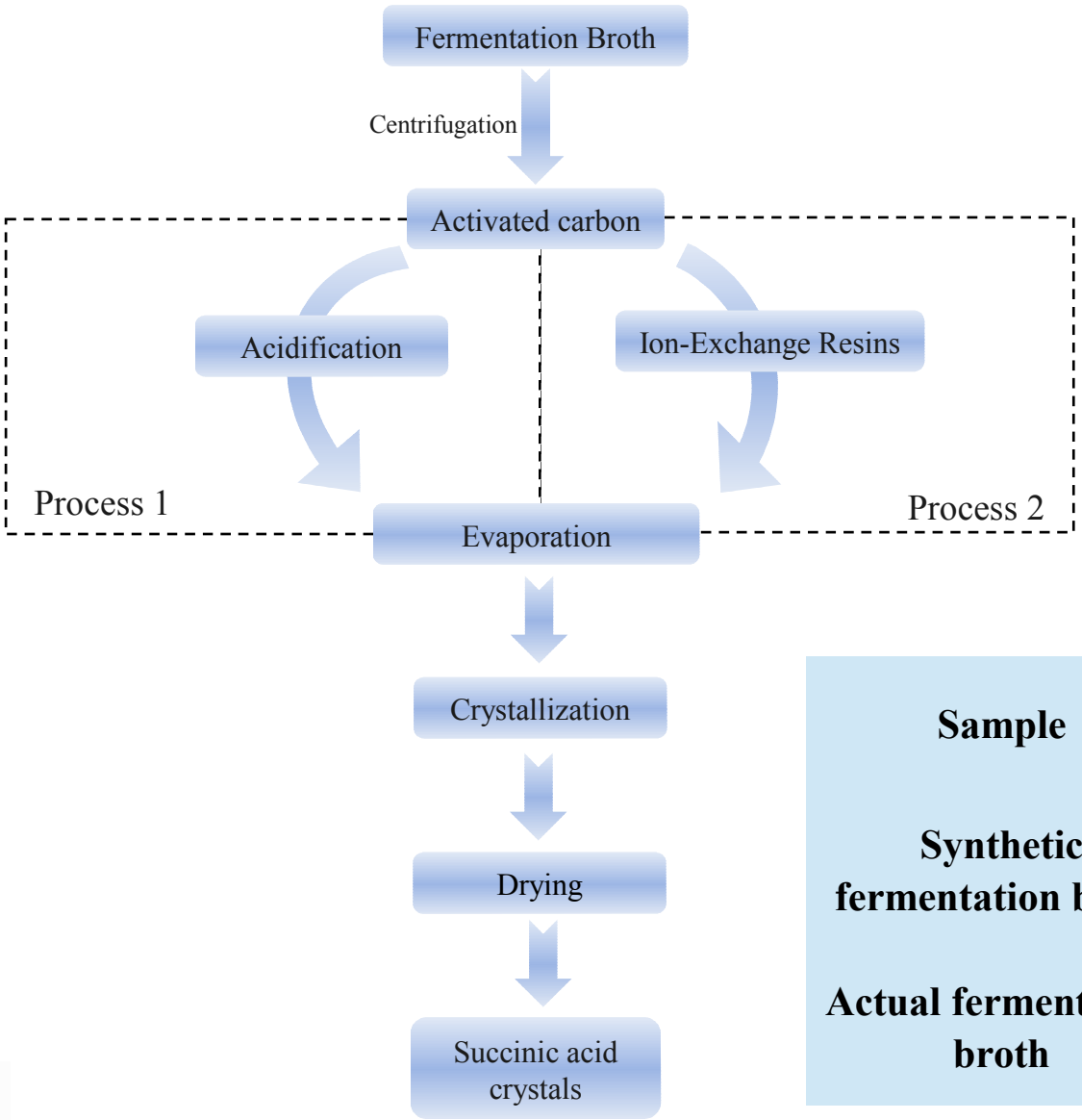
ontinuous cultures for the production of succinic acid from SS



Observations

- Succinic acid concertation varied between 20-25 g/L when synthetic xylose was consumed.
- Xylose accumulation was observed when dilution rate was increased
- The optimum dilution rate observed was 0.04 h-1 when pretreated SSL was used
- The maximum succinic acid concentration achieved was 20.7 g/L at dilution rate 0.04 h-1.

Direct crystallization scheme for succinic acid purification



Sample	Yield (%)	Purity (%)
Synthetic fermentation broth	76	98
Actual fermentation broth	up to 80	up to 99.7

Cost evaluation of bio-based succinic acid production

Succinic acid production via fermentation using spent sulphite liquor as substrate

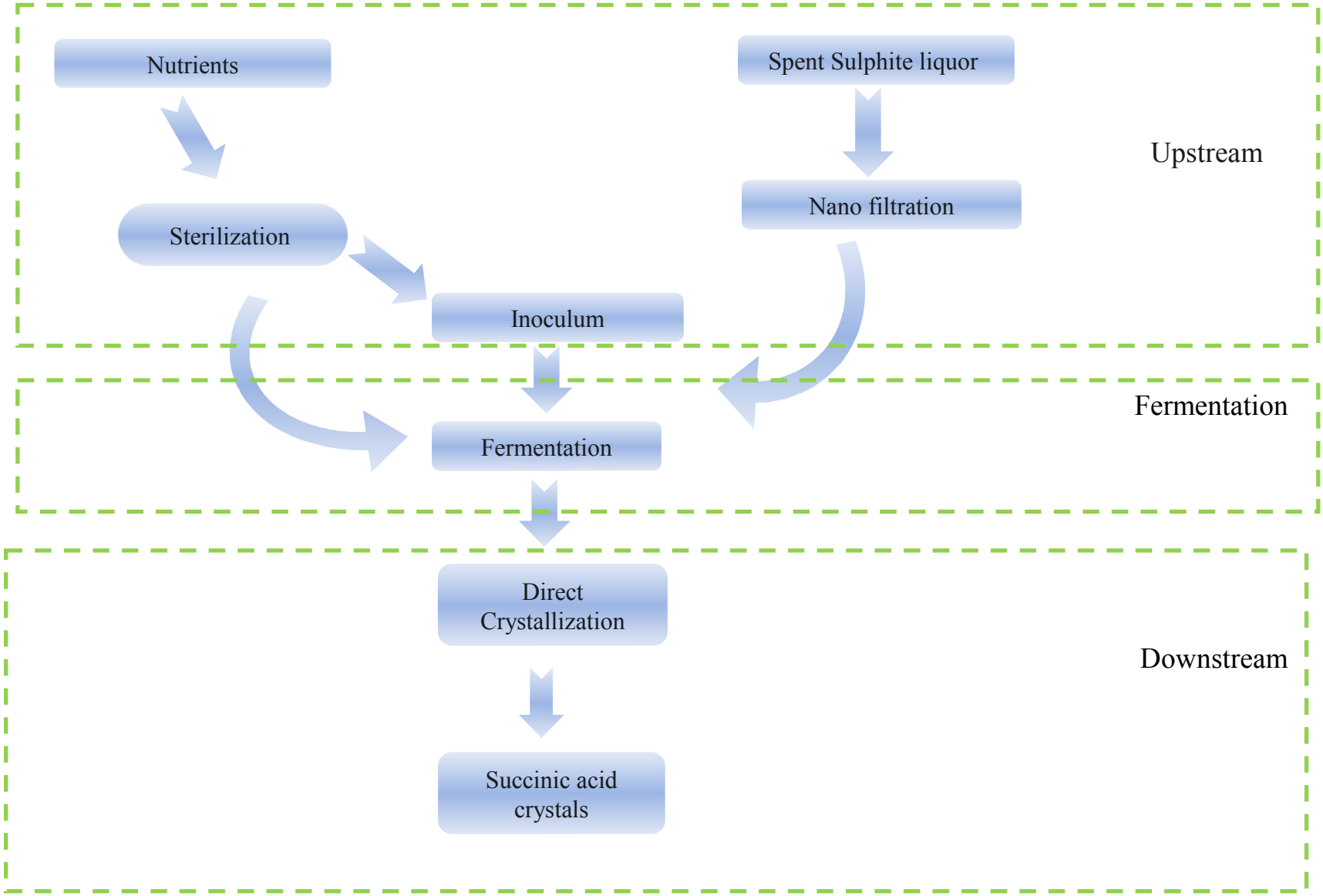
Two different fermentation strategies

- Continuous
- Fed-batch

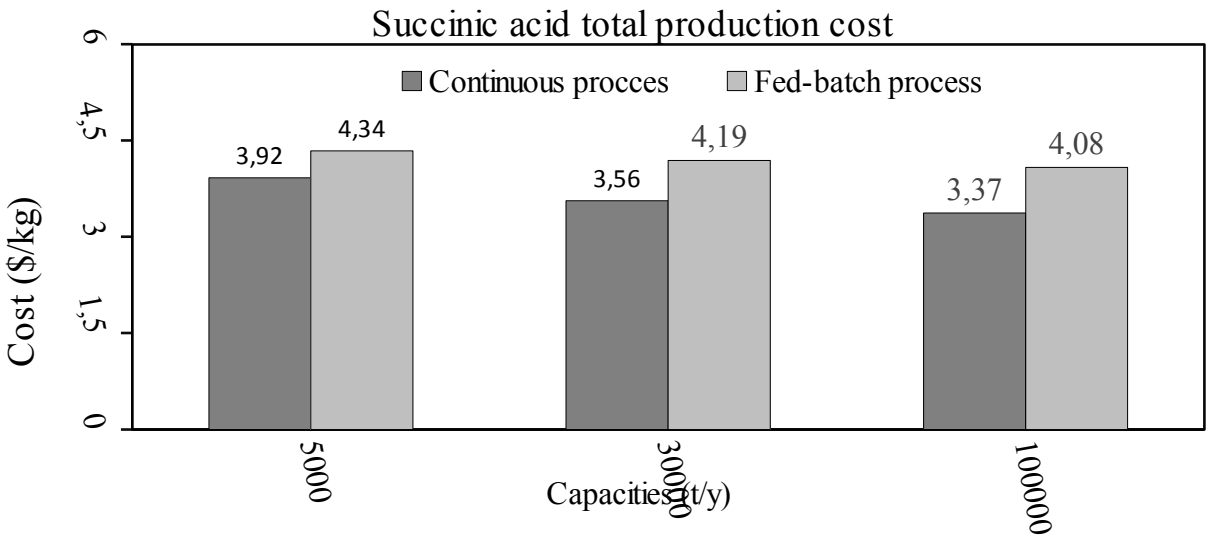
Three production capacities

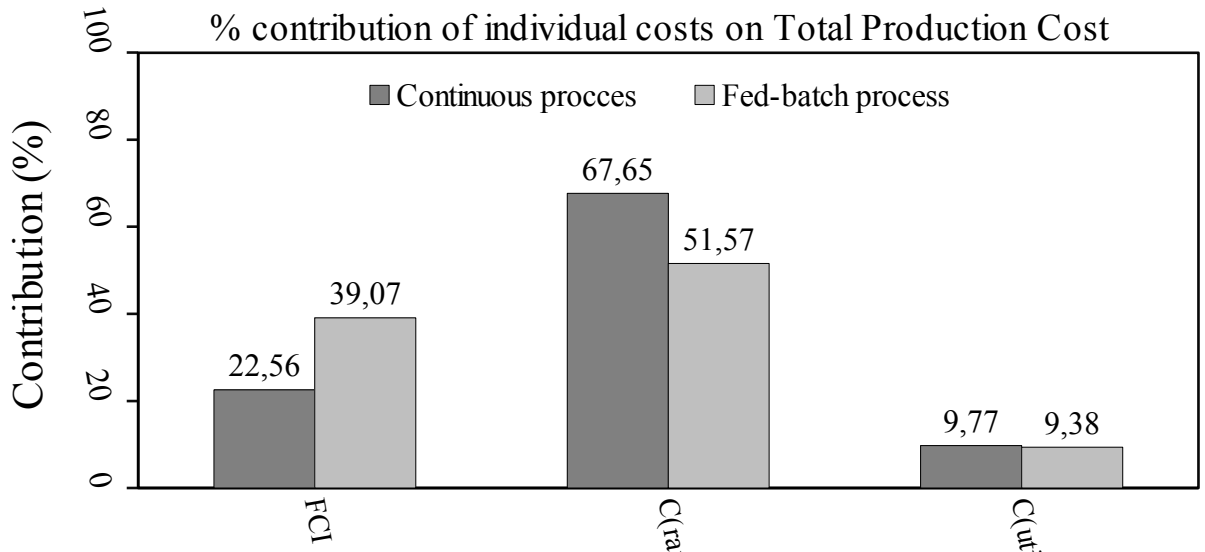
- 5000 (t/y)
- 30000 (t/y)
- 100000 (t/y)

Succinic acid production flow diagram



Cost evaluation of bio-based succinic acid production





Thank you for your attention