Valorisation of pulp and paper industry waste streams for the production of succinic acid

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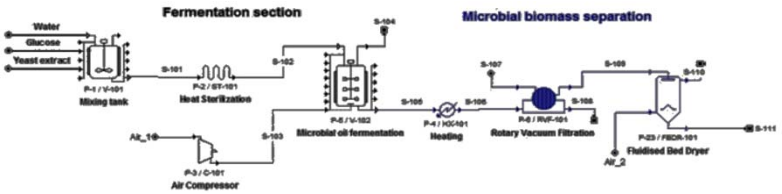
Biorefinery development based on renewable resources

Valorisation of renewable resources

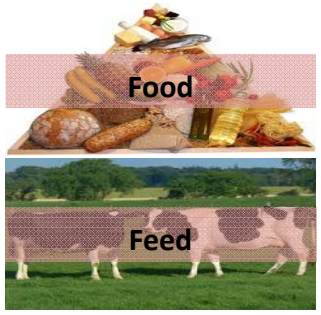
Agricultural crops and residues	Industrial wastes and by-product streams	Food waste and by-products
		

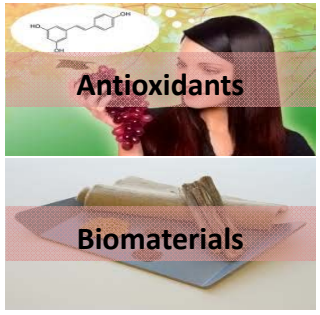
Biorefinery development

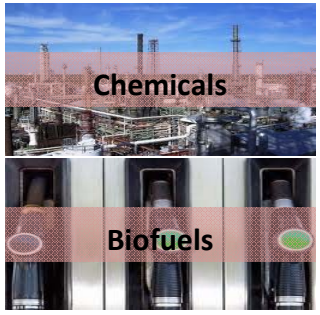


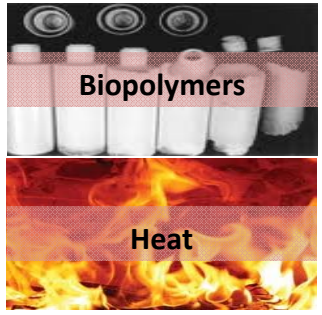
- White biotechnology
- Bioprocess / biorefinery engineering
- Bioprocess / biorefinery design
- Bioprocess optimisation

Added-value products

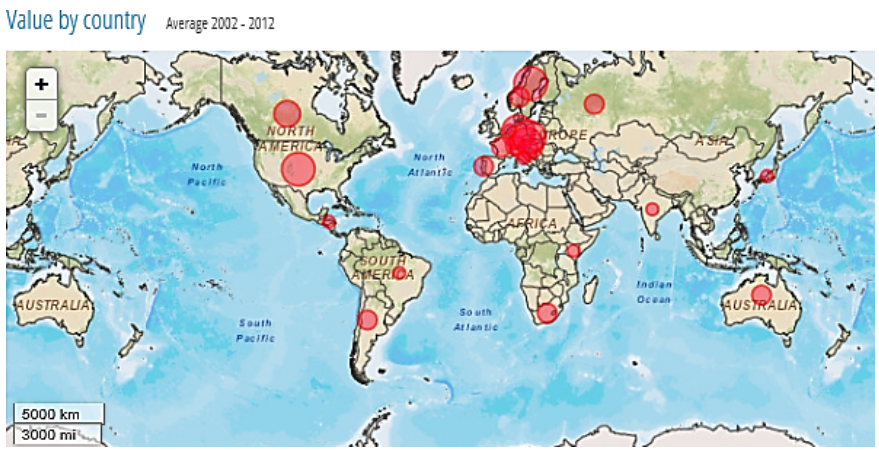


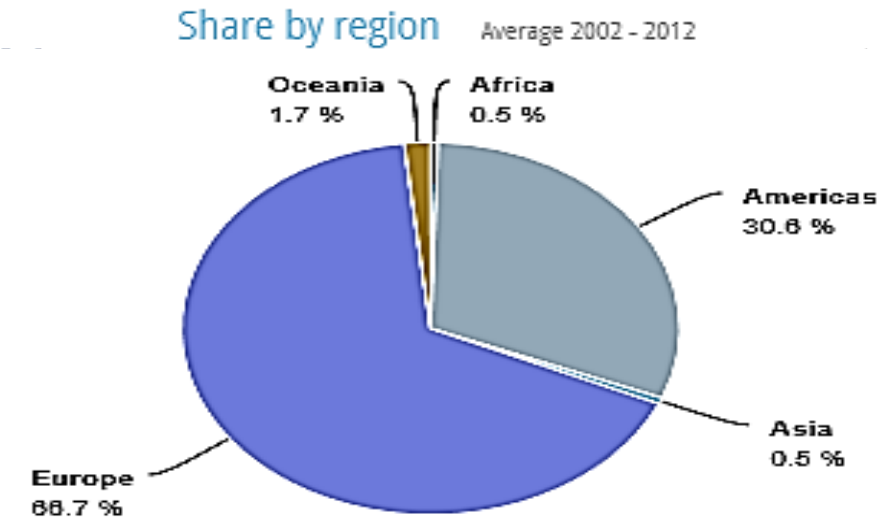






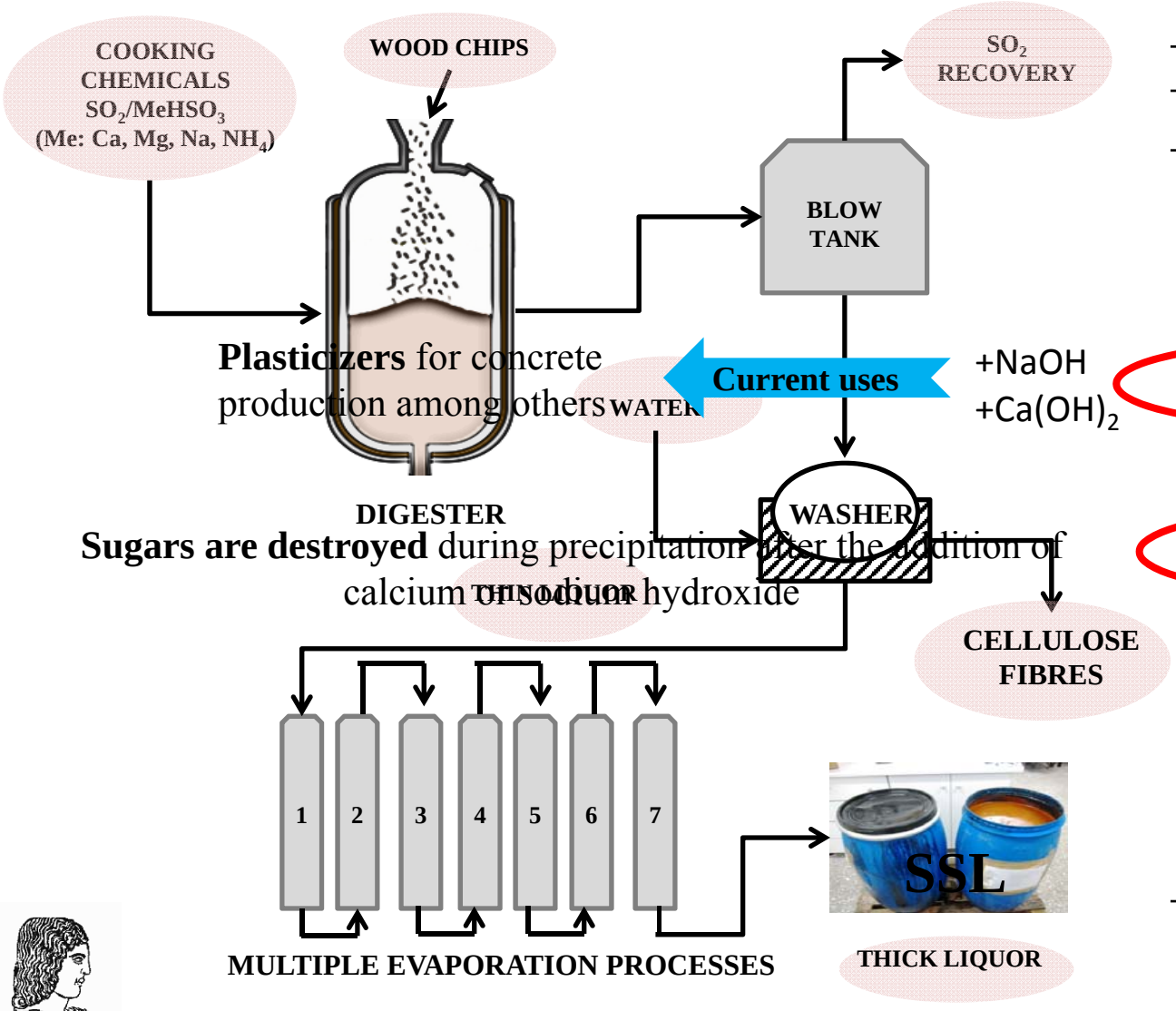
Worldwide production quantity of bleached sulphite pulp in the last decade





- Global **annual production** of bleached sulphite pulp: 3.6 Mt/yr (FAO, 2012) →14% increase since 2009
  - Annual production in the USA: 1 Mt/yr (FAO, 2012) → 21% increase since 2009
  - Annual production in South America: 0.21 Mt/yr (FAO, 2012) → 74% increase since 2009
  - Annual production in the European region: 2 Mt/yr (FAO,2012) → 0,01% increase since 2009

SSL Production in Pulp and Paper Industry



Plasticizers for concrete production among others

Sugars are destroyed during precipitation after the addition of calcium or sodium hydroxide

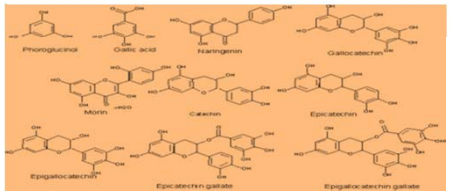
SSL Characterisation		
	Value	St Dev
pH	2.7	
Density (g/mL)	1.277	0.007
Viscosity (cP)	552	167
Dry Matter (g-DM/L)	816.5	0.6
Lignosulphonates (g/L)	458.8	2.7
Ash % (g/g-DM)	8.62	0.55
Phenolics % (g/g-DM)	1.55	0.04
Carbohydrates (g/L)	176.41	
Xylose (g/L)	128.08	0.59
Galactose (g/L)	21.47	5.50
Glucose (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

Formation of Sugars & Inhibitors During the Process

Lignin

Lignosulphonates

Phenolic compounds



Cellulose

glucose

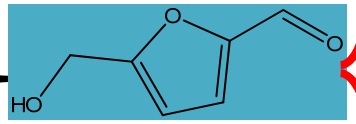
Hemicellulose

galactose

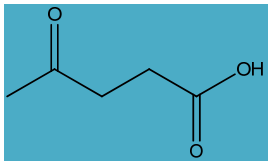
mannose

xylose

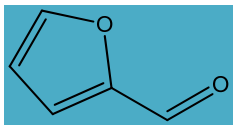
arabinose



5-HMF

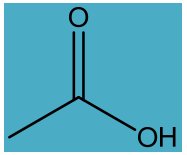


Levulinic acid



Furfural

Formic acid



Acetic acid

Integrated biorefinery based on current pulp and paper mills

SSL

LS

N_2

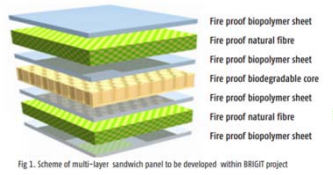
Ultrafiltration

Phenolic Extract

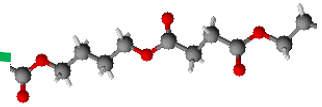
Phenolic Extraction with Ethyl acetate

Fermentation

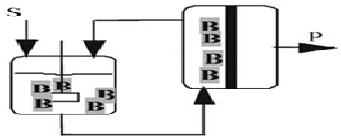
Bioprocess optimisation
Techno-economic evaluation
Life Cycle Analysis



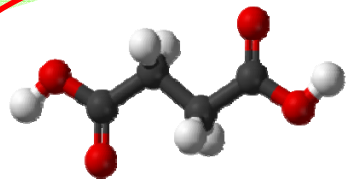
Fireproof biopolymers



Polybutylene succinate

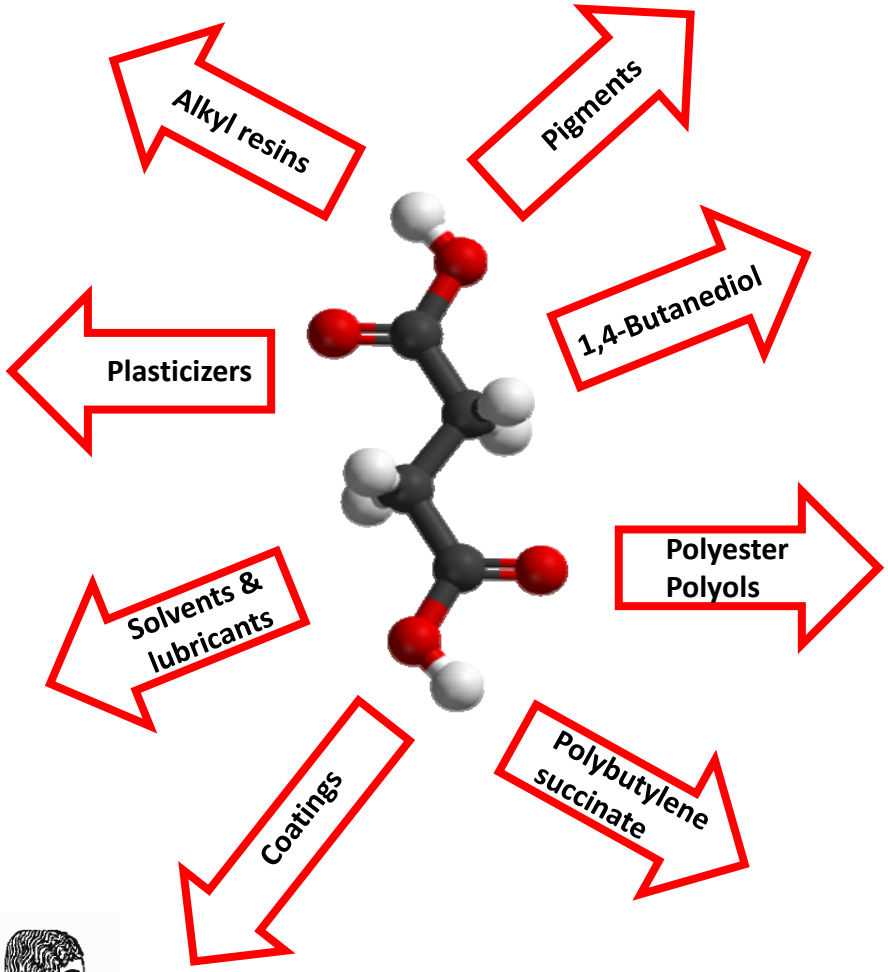


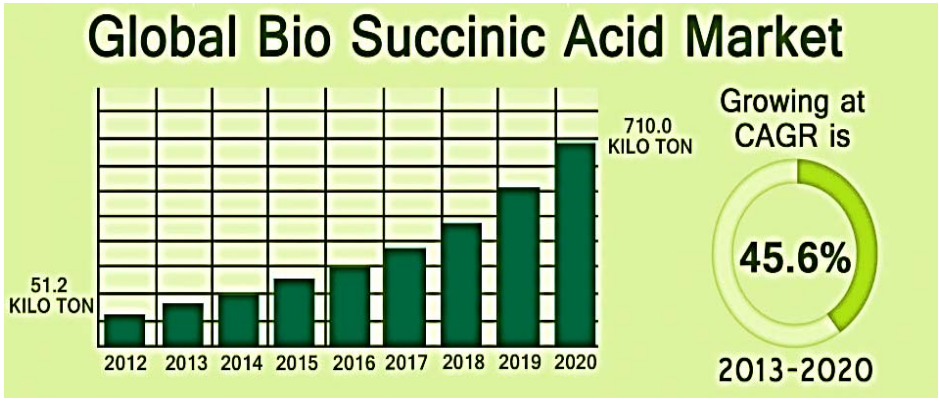
Succinic acid Separation and Purification



Succinic acid production

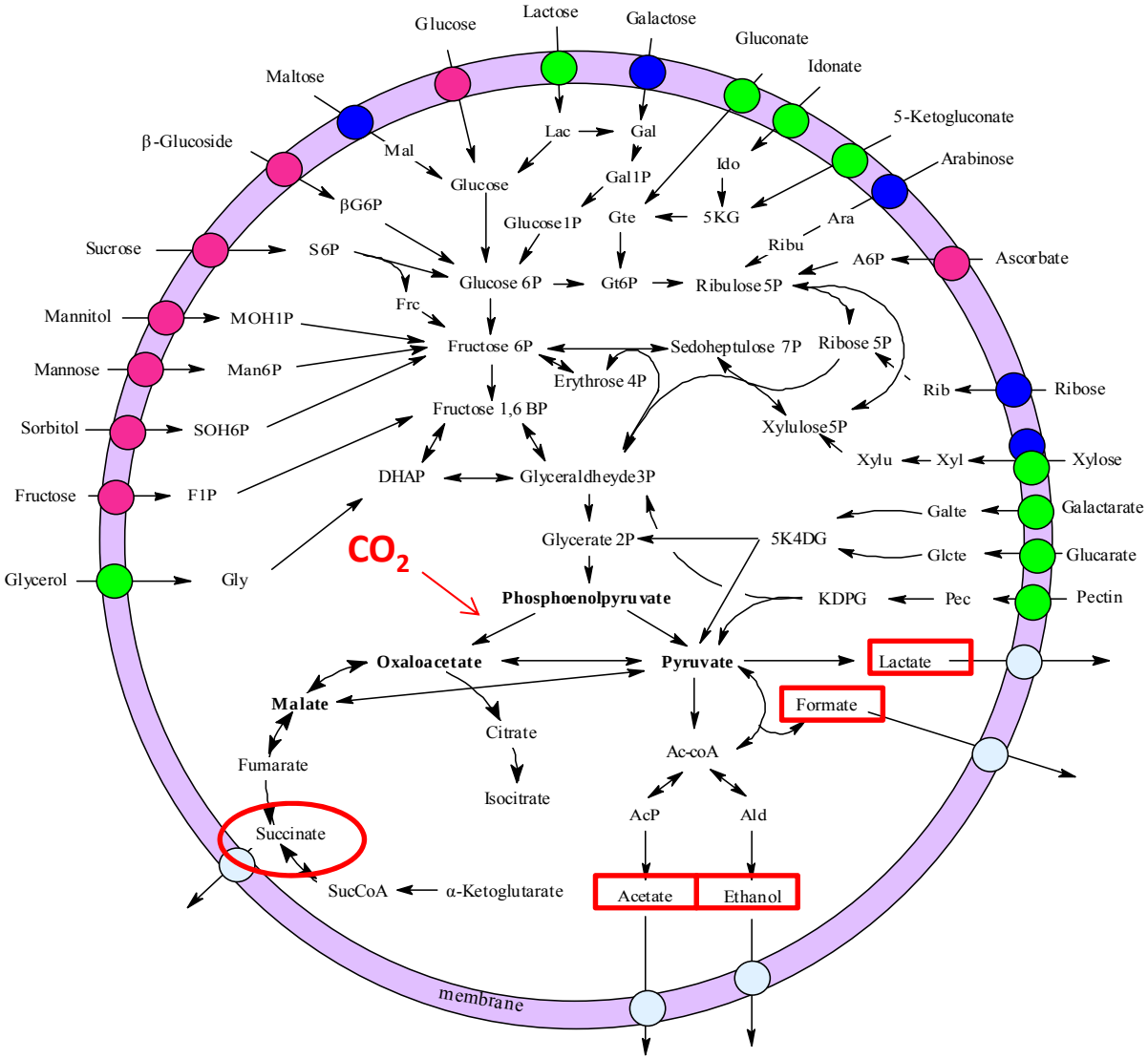
Succinic acid applications



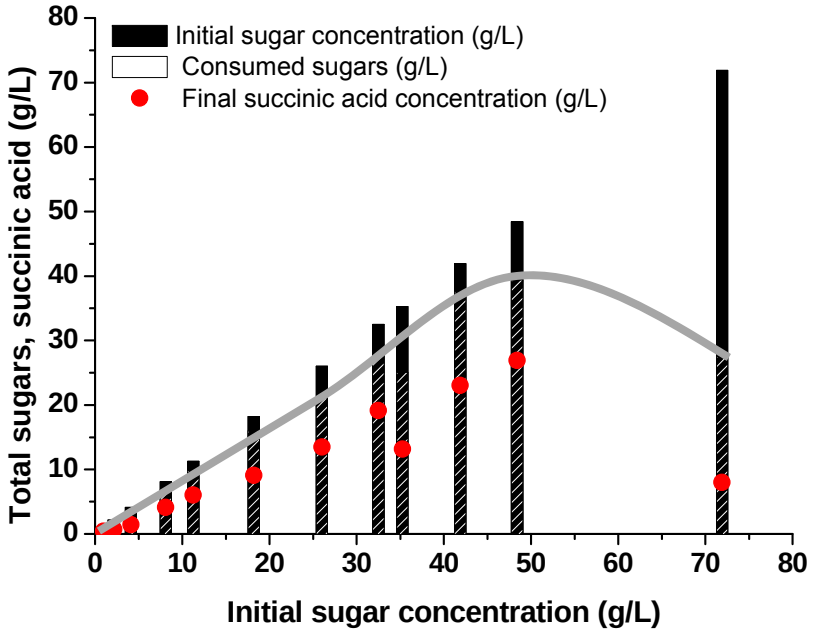


Allied market research, 2013

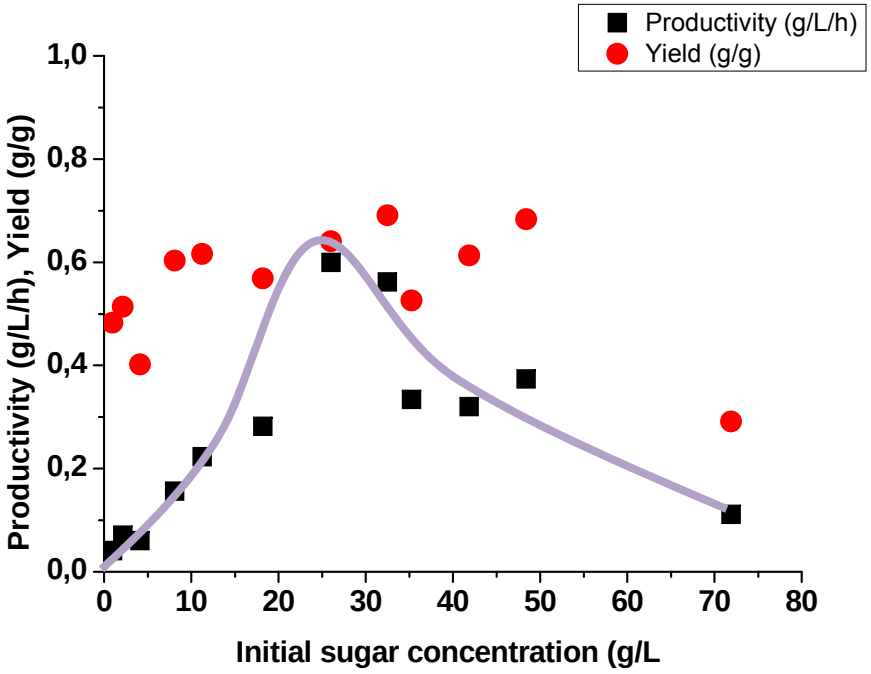
- Among top-10 most promising platform chemicals
- Can be produced via bioprocesses
- Utilisation of renewable resources

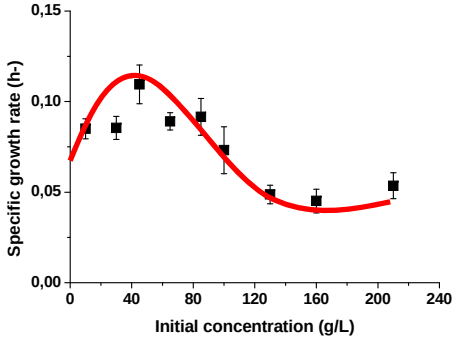
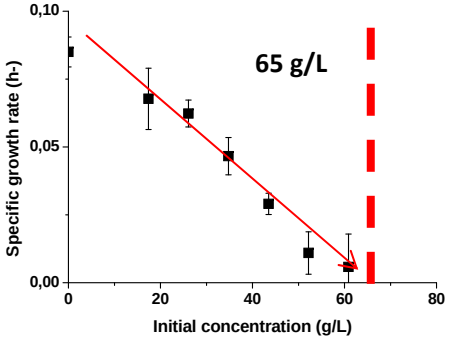
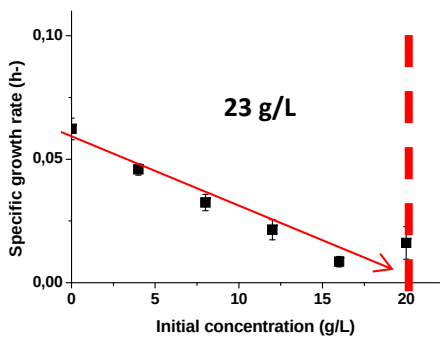
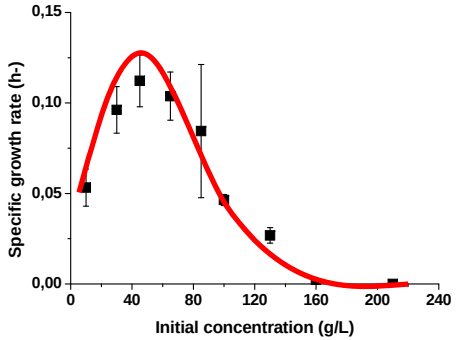
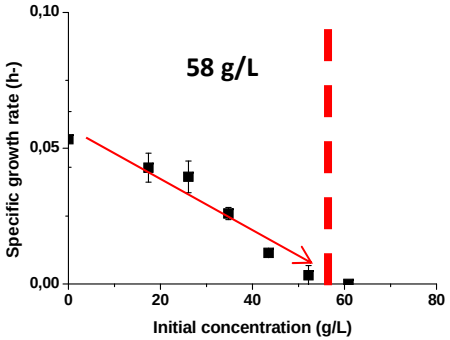
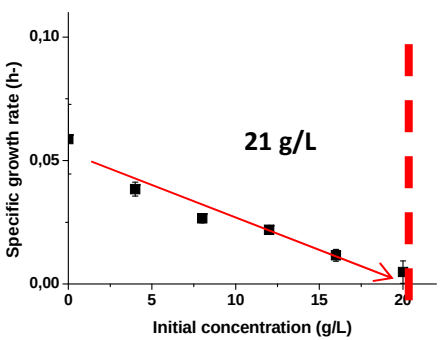
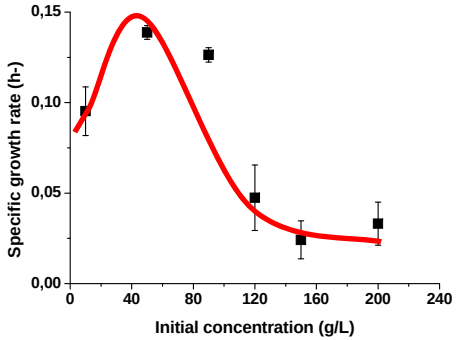
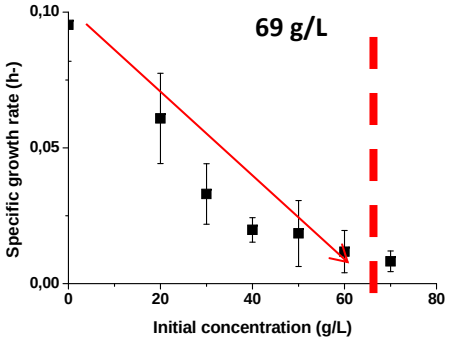
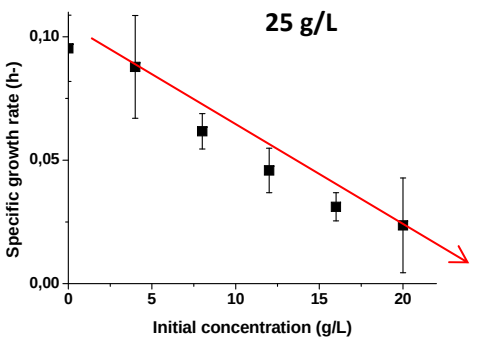

Glucose
Lactose
Galactose
Gluconate
Idonate
5-Ketogluconate
Arabinose
Ascorbate
Maltose
β-Glucoside
Mal
Lac
Gal
Gal1P
Ido
5KG
Ara
Ribu
A6P
βG6P
Glucose1P
Gte
Sucrose
S6P
Glucose 6P
Gt6P
Ribulose 5P
Frc
Mannitol
MOH1P
Fructose 6P
Sedoheptulose 7P
Ribose 5P
Mannose
Man6P
Erythrose 4P
Rib
Ribose
Sorbitol
SOH6P
Fructose 1,6 BP
Xylulose5P
Xylu
Xyl
Xylose
Fructose
F1P
DHAP
Glyceraldheyde3P
Galte
Galactarate
Glycerate 2P
5K4DG
Glcte
Glucarate
Glycerol
Gly
CO2
Phosphoenolpyruvate
KDPG
Pec
Pectin
Oxaloacetate
Pyruvate
Lactate
Malate
Formate
Citrate
Ac-coA
Fumarate
Isocitrate
AcP
Ald
Succinate
SucCoA
α-Ketoglutarate
Acetate
Ethanol
membrane
PTS
Facillitated transporter
ATP dependent transporter
Antiporter

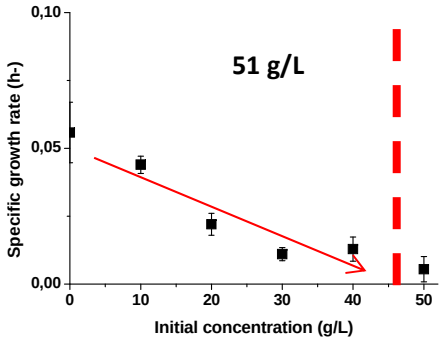
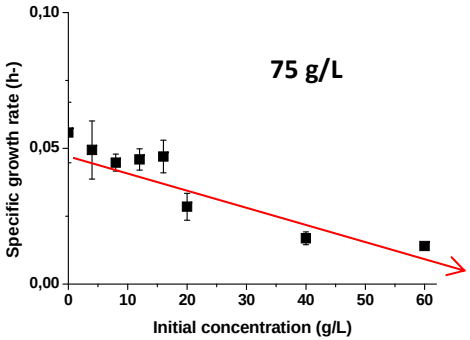
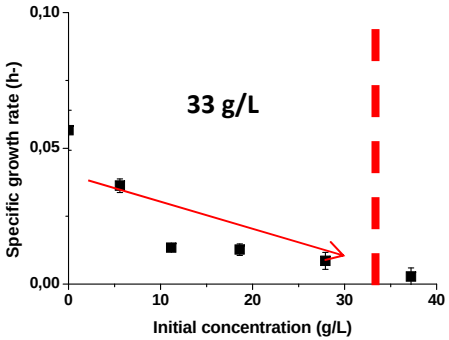
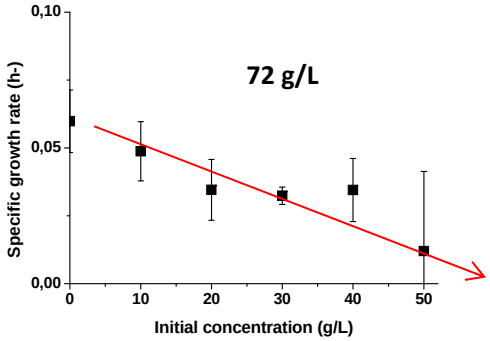
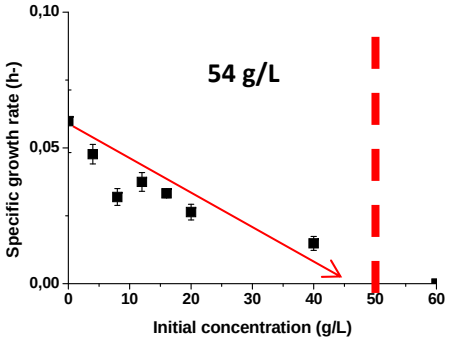
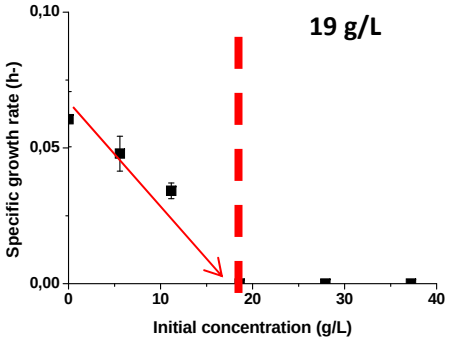
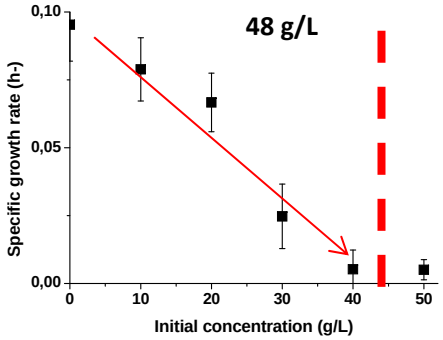
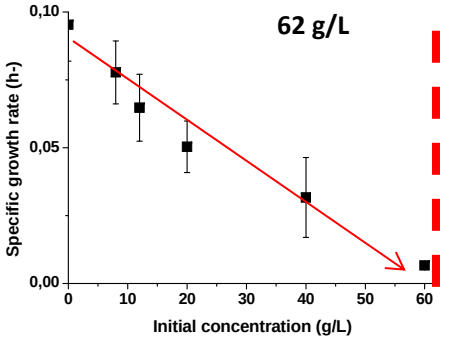
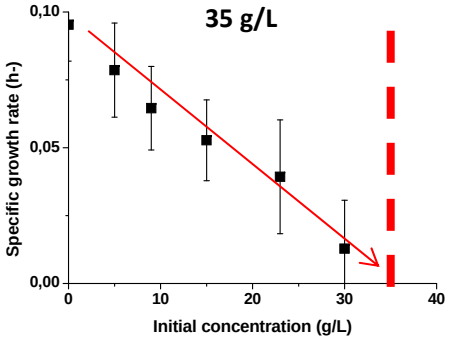
Initial substrate tolerance and initial product inhibition



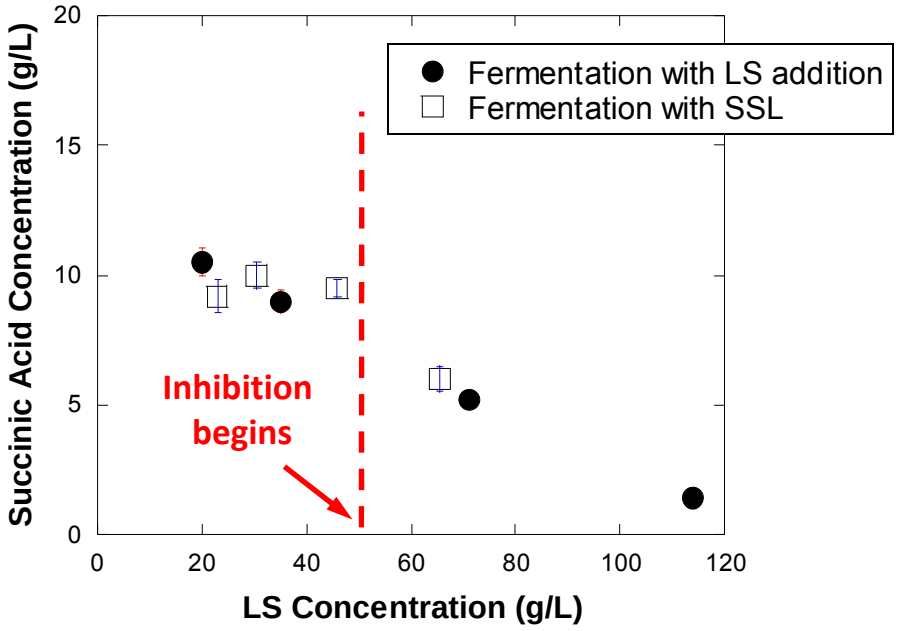
Actinobacillus succinogenes



	Initial substrate	Initial succinate	Initial formate
A. succinogenes 130 Z			
A. succinogenes CCUG 3343			
Basfia succiniciproducens JF 4016			

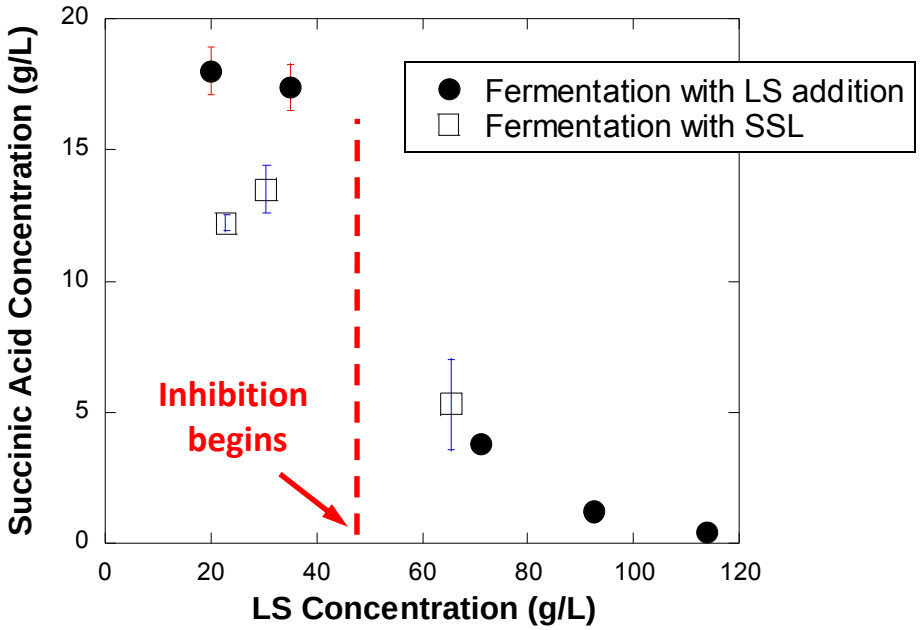
	Initial acetate	Initial lactate	Initial mixed acids
A. succinogenes 130 Z			
A. succinogenes CCUG 3343			
Succinivibrio dextrinosolvens JF 4016			

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



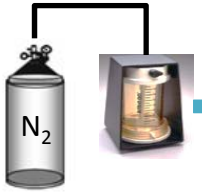
Actinobacillus succinogenes

Basfia succiniciproducens



Pretreatment of SSL

SSL



Ultrafiltration

Phenolic Extraction with Ethyl acetate

Immobilisation on alginate beads

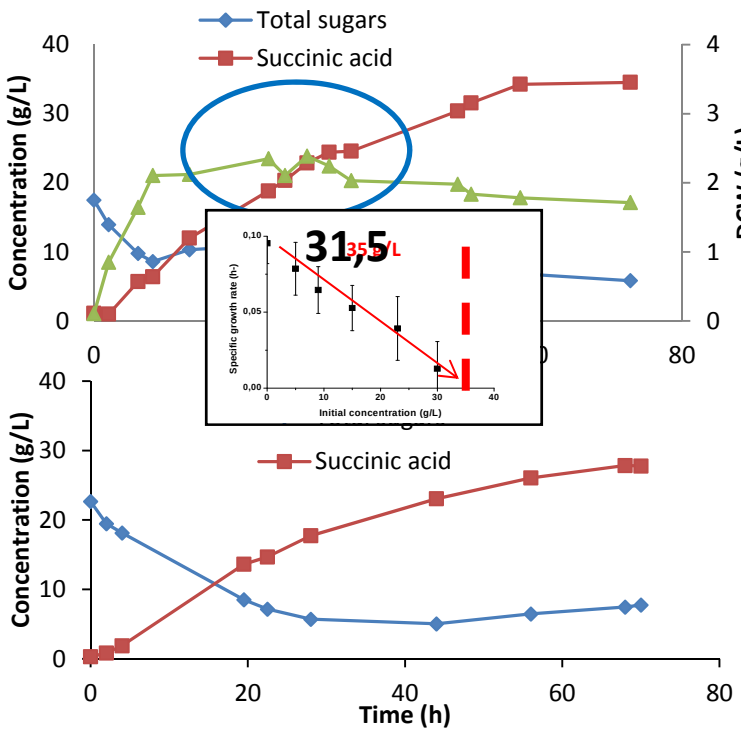
Immobilisation on alginate beads

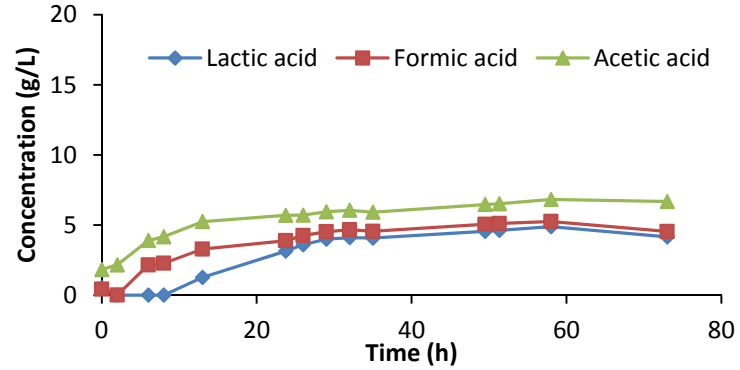
Fermentations

Fed-batch fermentations in bench-top bioreactor

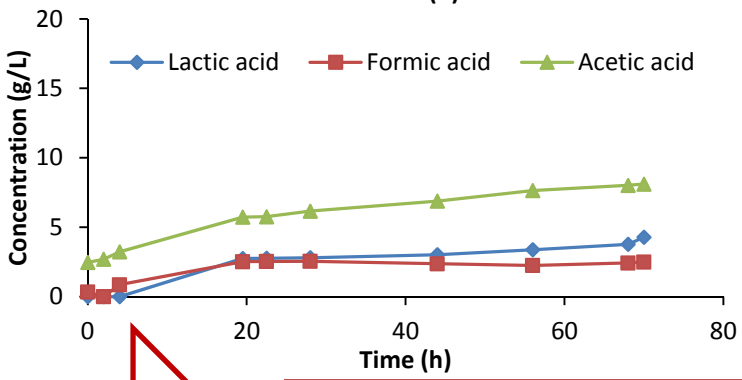
B. succiniciproducens

Pure sugars





Filtrated SSL



Pure sugars

Final SA concentration: 34.3 g/L at 58 h
Yield: 0.54 g/g at 58 h
Productivity: 0.57 g/L/h

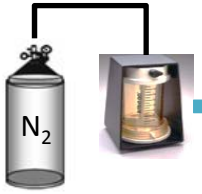
Filtrated SSL with 3 kDa MWCO

Final SA concentration: 27.8 g/L at 68 h
Yield: 0.59 g/g at 68 h
Productivity: 0.46 g/L/h at 56 h

	LA:SA Ratio	FA:SA Ratio	AA:SA Ratio	Total by-product:SA Ratio
Pure sugars	0.13	0.15	0.15	0.43
Filtrated SSL	0.14	0.09	0.20	0.43

Pretreatment of SSL

SSL



Ultrafiltration

Phenolic Extraction with Ethyl acetate

Immobilisation on alginate beads

Immobilisation on alginate beads

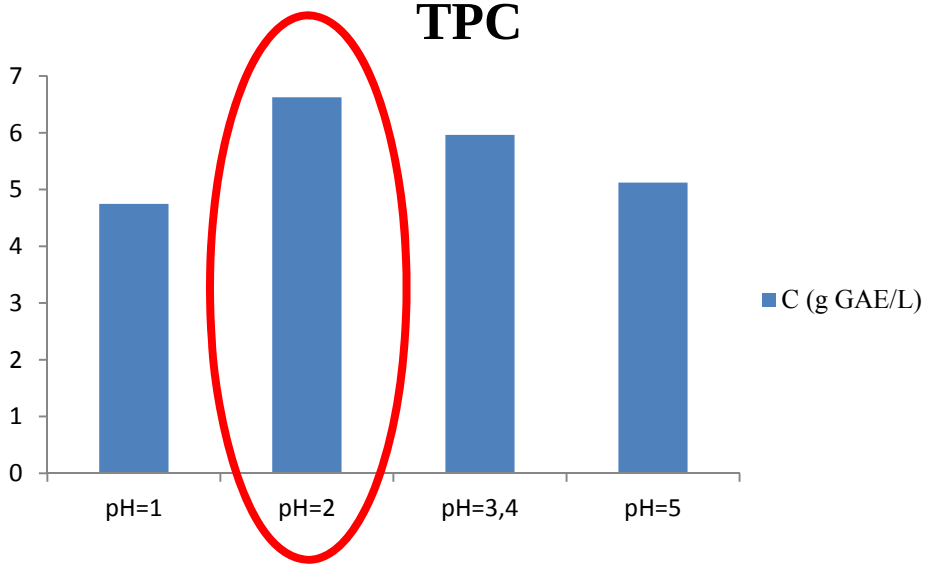
Fermentations

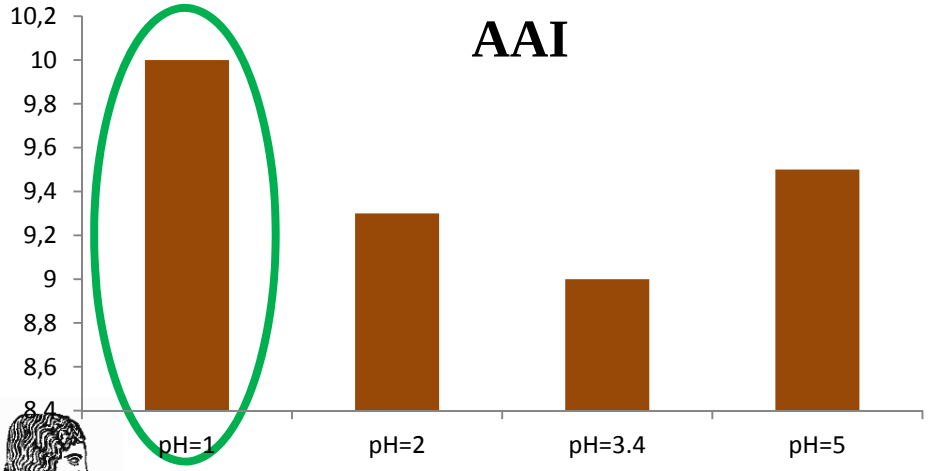
Optimisation of solvent extraction - Effect of pH

- Extraction in 4 different pH values (SSL-to-solvent ratio 1:3 (v/v), 30 min extraction time)
  - pH =1
  - pH = 2
  - pH = 3.4
  - pH = 5

Folin-Ciocalteu- TPC

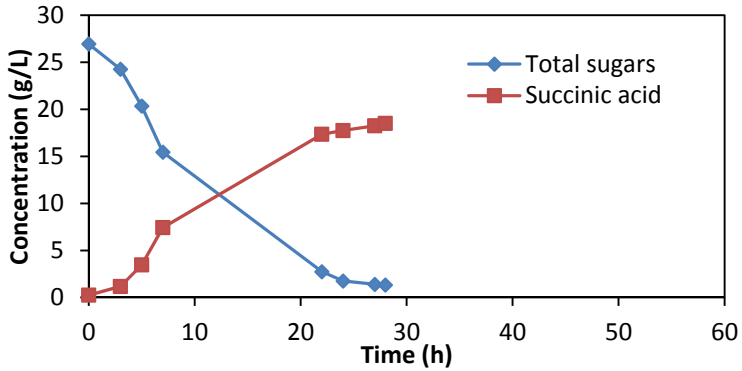
DPPH - AAI

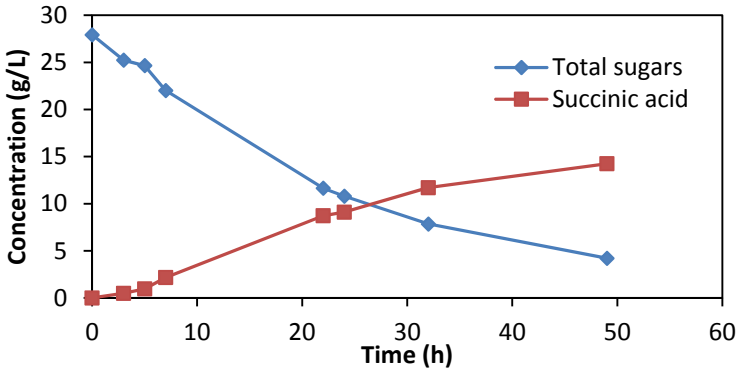


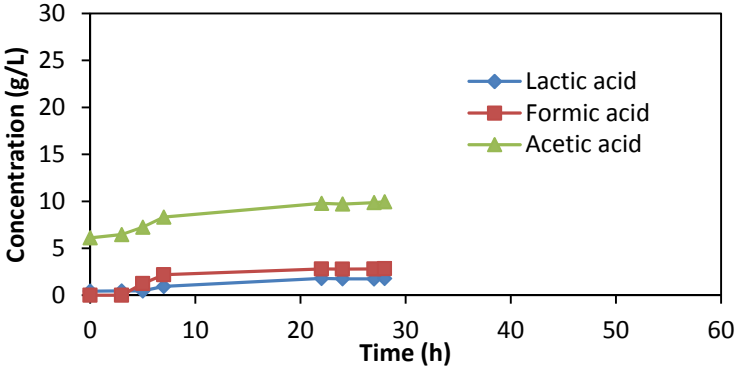


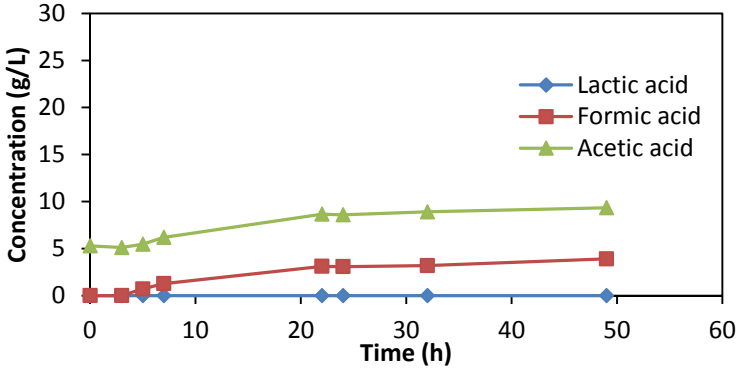
- ✓ **Poor antioxidant activity : AAI < 0.5**
- ✓ **Moderate antioxidant activity : 0.5 < AAI < 1.0**
- ✓ **Strong antioxidant activity : 1.0 < AAI < 2.0**
- ✓ **Very strong antioxidant activity : AAI > 2.0**

Filtrated-Extracted SSL - Batch Fermentations









Basfia succiniciproducens

18.2 g/L SA
SA yield 0.71 g/g
28 h
Productivity 0.65 g/L/h

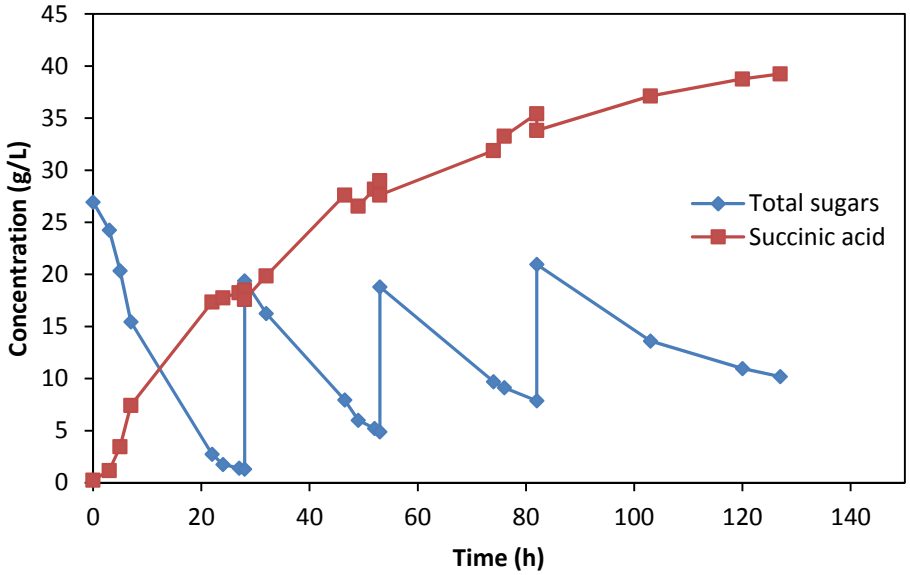
LA:SA = 0.07
FA:SA = 0.15
AA:SA = 0.21

Actinobacillus succinogenes

14.2 g/L SA
SA yield 0.60 g/g
49 h
Productivity 0.29 g/L/h

FA:SA = 0.27
AA:SA = 0.28

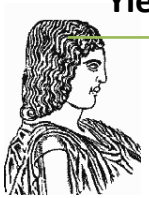
Filtrated-Extracted SSL in fed-batch fermentation



- After the batch culture with filtrated-extracted SSL, the fermentation was continued by feeding with pure sugars
- 39 g/L final SA concentration achieved with 0.63 g/g SA yield
- Low by-product formation
- SA productivity 0.31 g/L/h

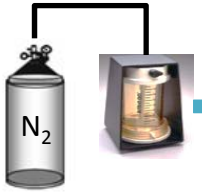
	SA	LA	FA	AA	Total product-yield
Produced (g/L)	**39**	**10.7**	**2**	**3.5**	**-**
Yield (g/g TS)	**0.63**	**0.17**	**0.03**	**0.06**	**0.89**

Ratio LA:SA = 0.27
Ratio FA:SA = 0.05
Ratio AA:SA = 0.09
Ratio Total by-prod:SA = 0.41



Pretreatment of SSL

SSL



Ultrafiltration

Phenolic Extraction with Ethyl acetate

Immobilisation on alginate beads

Immobilisation on alginate beads

Fermentations

Immobilisation

Microorganism: *Actinobacillus succinogenes*

- Dilution of *A. succinogenes* cells in ringer solution (0.9% NaCl)
- Mixture of the ringer solution with sodium alginate solution (Sigma Aldrich)
- Addition of this solution in 2% $CaCl_2$ solution drop by drop in order to form beads

A. succinogenes immobilized cells on alginate beads

Fermentation substrate:

- Repeated batch fermentations were carried out with 7 times diluted SSL
- Fed-batch fermentations were carried out with ultrafiltrated SSL using 5 kDa MWCO membranes and nanofiltrated SSL from 800 Da MWCO membranes
- In all cases, CO_2 flow was 1 vvm and pH was controlled by the addition of 5M NaOH solution

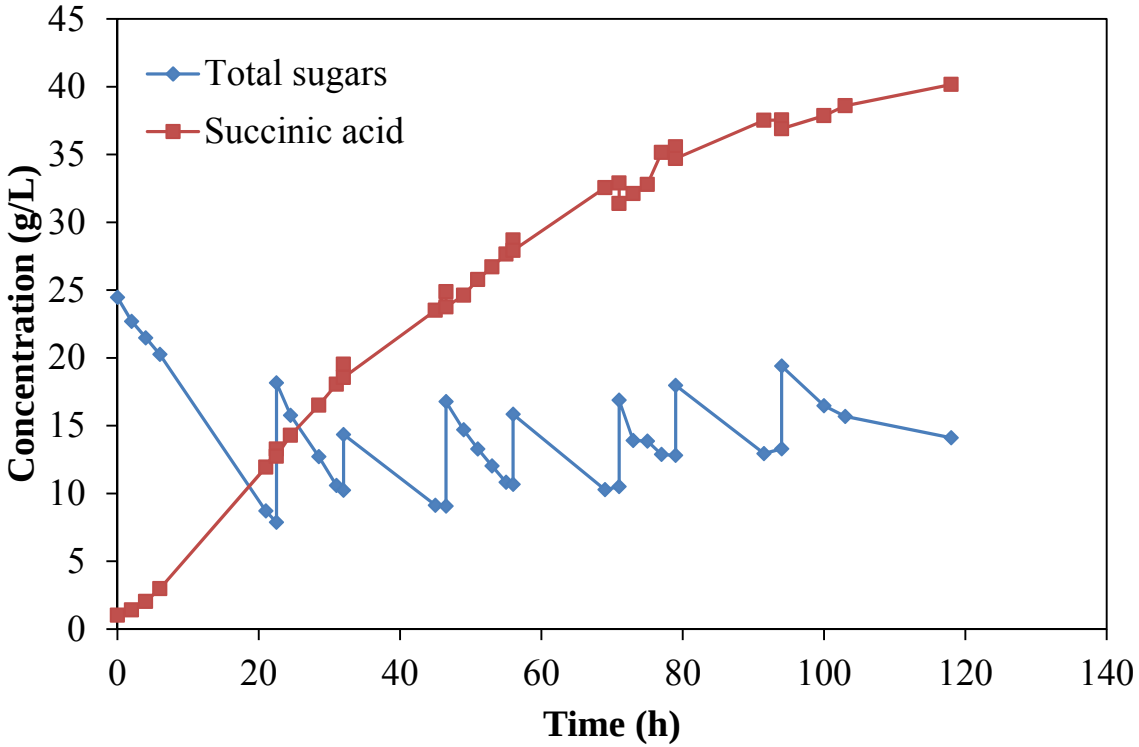
Immobilization: Repeated Batch

	SA produced (g/L)	SA yield (g/g TS)	Productivity (g/L/h)
Batch 1	9	0.58	0.49
Batch 2	10.8	0.63	1.13
Batch 3	10.9	0.61	0.56
Batch 4	11.2	0.64	0.43
Batch 5	12.3	0.71	0.51
Fed batch	39.1	0.71	0.33

Higher SA productivity in 2nd batch

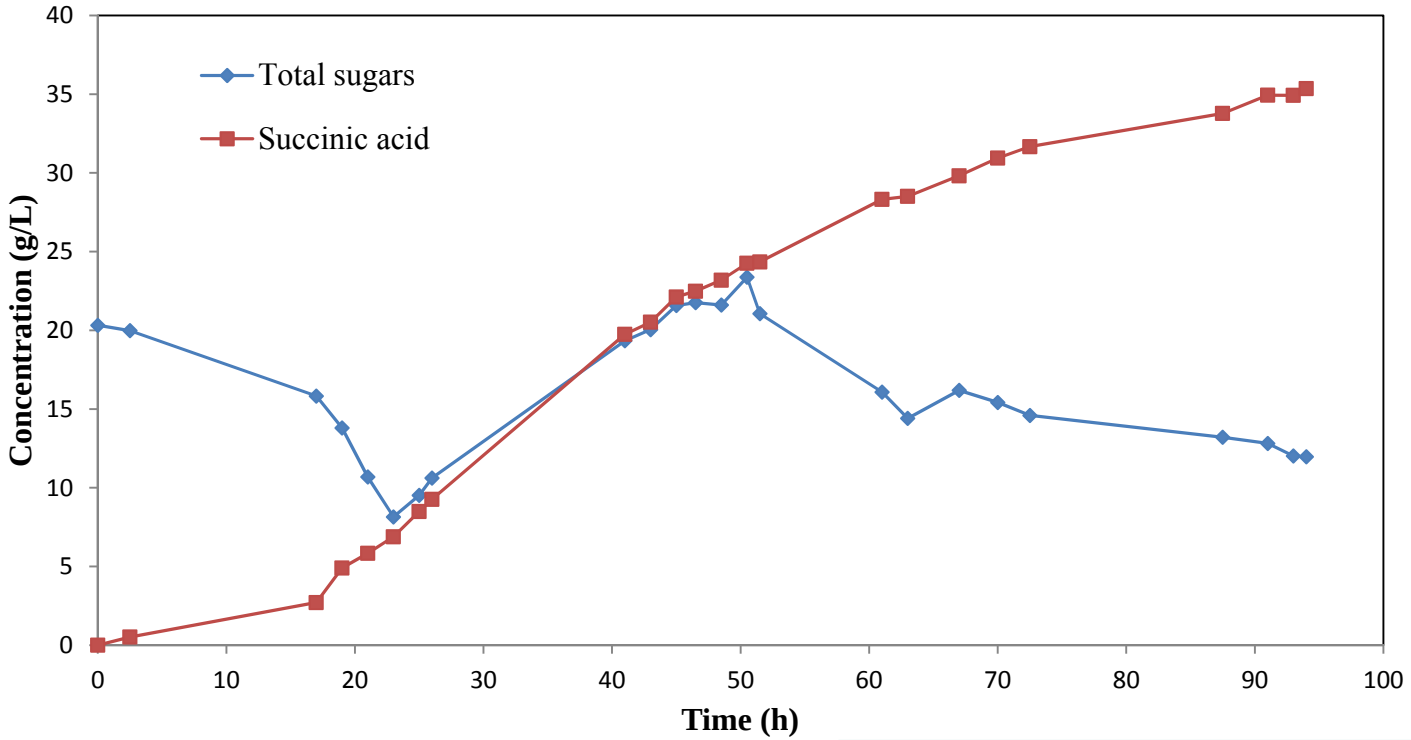
After 5 batches the immobilized cells are still able to ferment SSL

Immobilisation: Fed-Batch in ultrafiltrated SSL



- **7 times diluted SSL filtrated with Ultrafiltration membrane (~25 g/L TS and 35 g/L LS)**
- Feeding: concentrated filtrated SSL (~200 g/L TS)
- **39.1 g/L SA**
  0.71 g/g yield
  0.4 g/L/h productivity
- Very low by-product production (by-products yield 0.2 g/g TS, mainly AA)

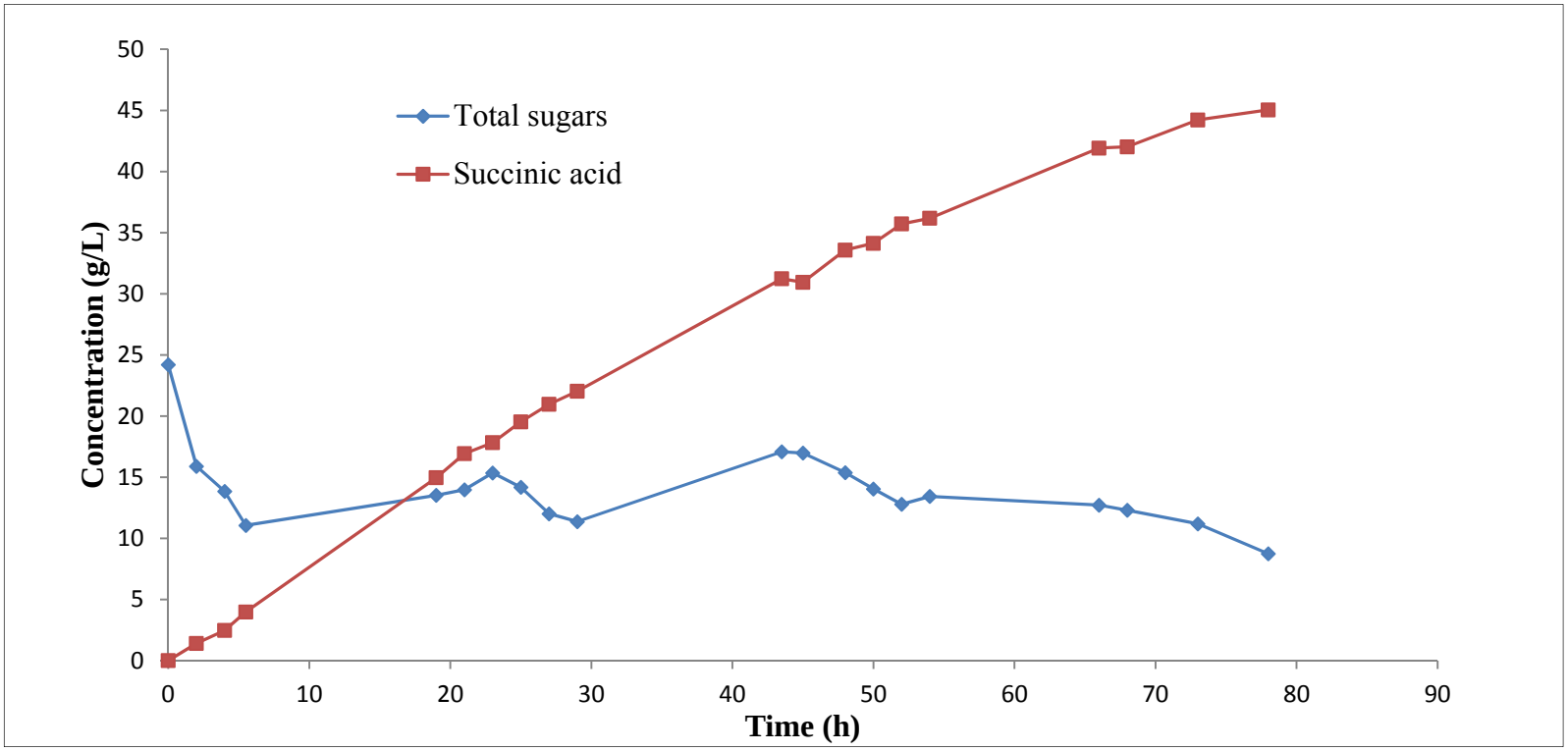
Immobilisation: Fed-Batch in nanofiltrated SSL with *Actinobacillus succinogenes*



- **Nanofiltrated SSL from 800Da membranes (initial LS ~ 5 g/L)**
- **Continuous feeding of concentrated nanofiltrated SSL (~ 400 g/L TS)**

- ✓ **Final SA : 35.4 g/L**
- ✓ **SA yield : 0.62 g/g**
- ✓ **SA productivity : 0.38 g/L/h**
- ✓ **Total by-products : 13.1 g/L**
- ✓ **Total by-product yield : 0.23 g/L/h**

Immobilisation: Fed-Batch in nanofiltrated SSL with *Basfia succiniciproducens*



- **Nanofiltrated SSL from 800Da membranes (initial LS ~ 5 g/L)**
- **Continuous feeding of concentrated nanofiltrated SSL (~ 400 g/L TS)**

- ✓ **Final SA : 45 g/L**
- ✓ **SA yield : 0.66 g/g**
- ✓ **SA productivity : 0.58 g/L/h**
- ✓ **Total by-products : 14.9 g/L**
- ✓ **Total by-product yield : 0.22 g/g**

Conclusions

- SSL contains phenolic compounds with strong antioxidant activity that could be considered as value-added co-product
- Ultrafiltration of SSL enhance both microbial growth and SA yield.

➢ ***Basfia succiniciproducens***

- ✓ Inhibitors removal is necessary
- ✓ **Filtration - Phenolic Extraction** resulted in higher final succinic acid concentration and yield, with similar by-product formation as in the case of pure sugar utilisation
- ✓ Immobilization in alginate beads led to the highest SA production

➢ ***Actinobacillus succinogenes***

- ✓ Lower succinic acid production than *Basfia succiniciproducens* in pure sugars
- ✓ **Filtration - Phenolic Extraction** enhance succinic acid yield with lower by-product formation
- ✓ **Immobilisation** increased significantly the tolerance of the biocatalyst to inhibitors

Thank you for your attention

The AUA team

BRIGIT

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