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Biorefinery development for the production of succinic acid and value-added coproducts from sunflower meal

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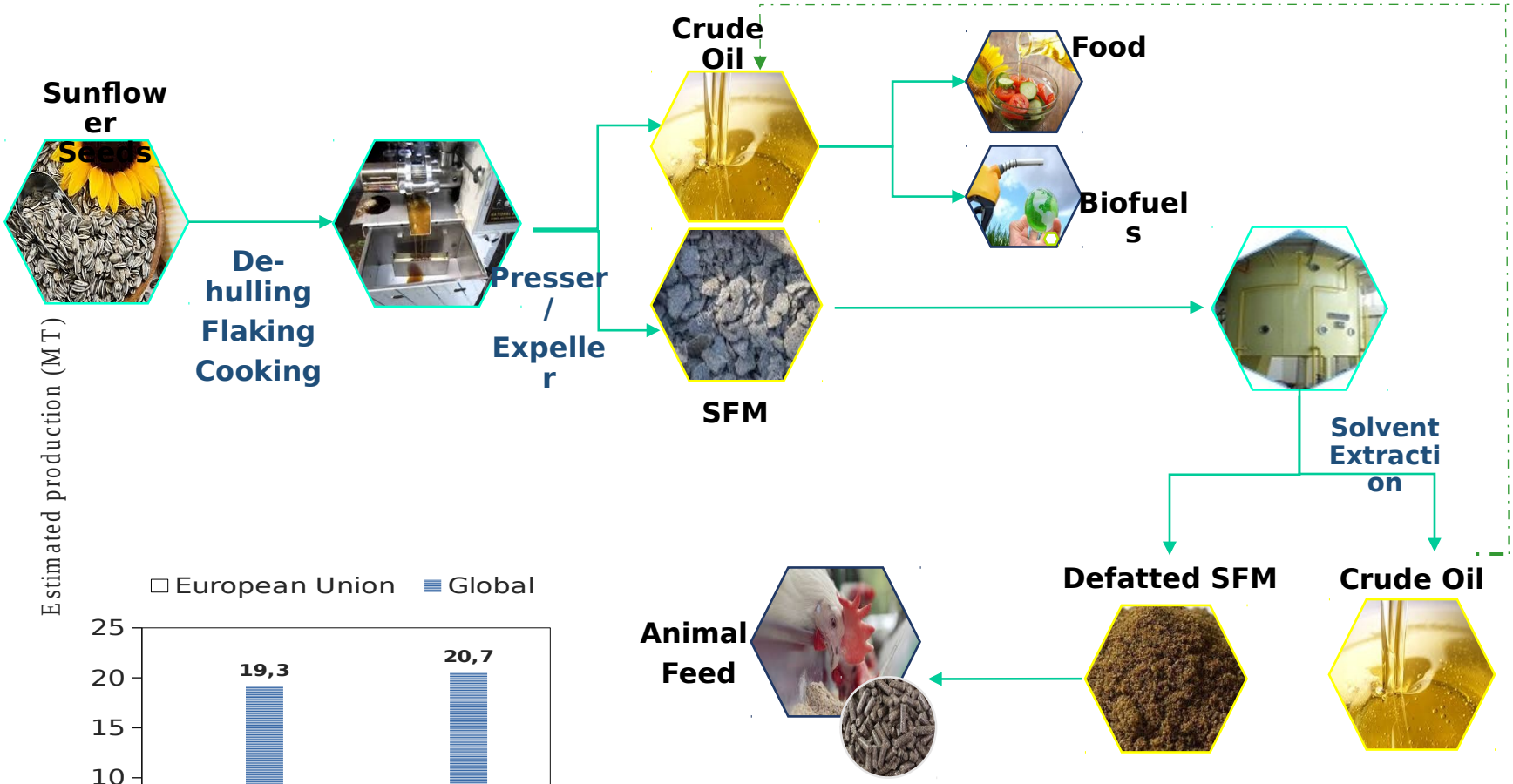
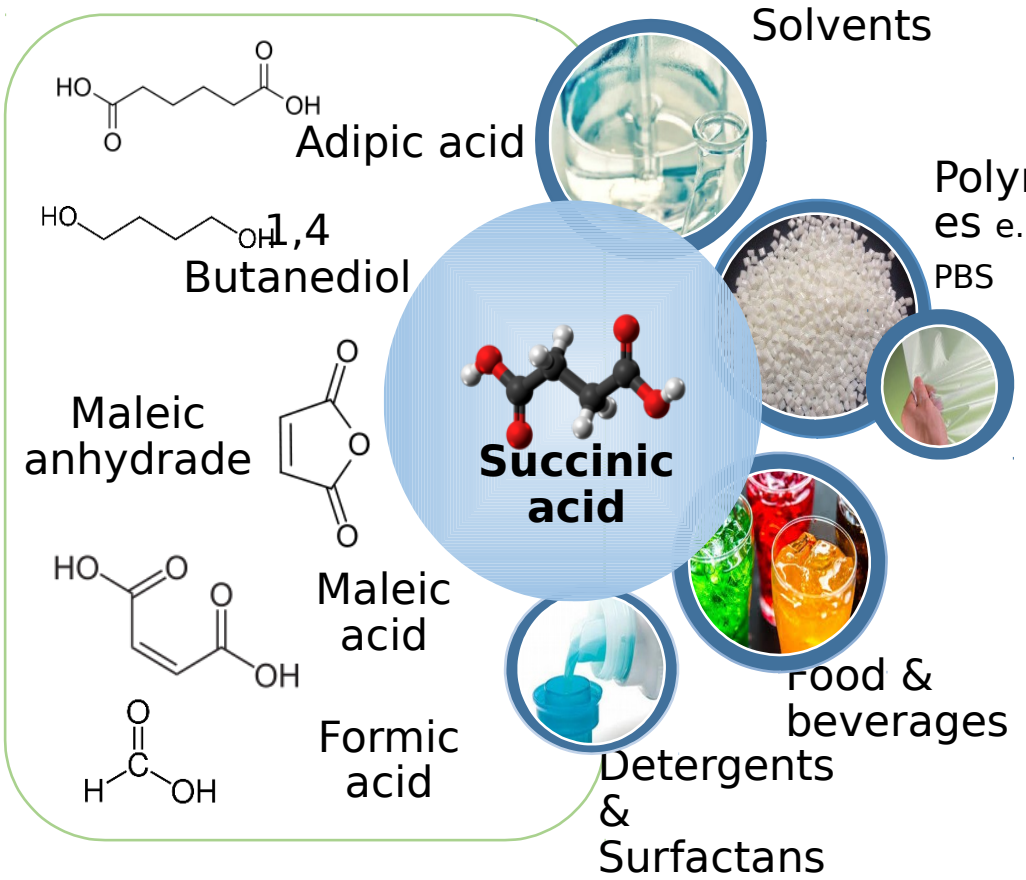
Sunflower Meal (SFM) Production



Source:https://apps.fas.usda.gov/psdonline/app/index.html#/app/advQuery

Succinic acid

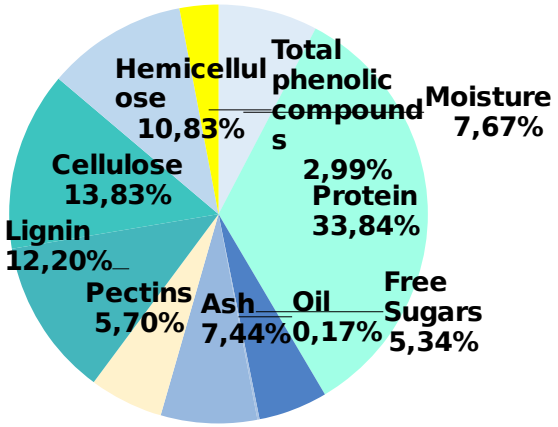
- C4-dicarboxylic acid
- Conventional production via hydrogenation of maleic anhydride
- Bio-based production is 50% of the total
- Glucose syrup and glycerol are the main carbon sources used in industry
- *Basfia succiniciproducens*, engineered *Escherichia coli* and yeast are the main strains used
- Market size expected to reach 48.7 Million US $ by 2024



Composition of sunflower meal

Composition (g/g % db)	This Study	Literature
Moisture[a]	7.6 ± 0.03	3.7-10.4
Total Solids	92.4 ± 0.03	89.6-96.3
Protein	33.7 ± 0.50	26.6-41.8
Total phenolics (CAE)	3.0 ± 0.04	2.4-4.7
Oil	0.1 ± 0.06	0.9-2.0
Ash	7.4 ± 0.11	4.3-9.0
Pectins	5.7 ± 0.20	4.9
Lignin	12.2 ± 0.03	5.9-11.4
Cellulose	13.8 ± 1.83	14.9-23.0
Hemicellulose	10.8 ± 1.92	7.5-12.9
Total Free Sugars	5.3±0.03	-
Sucrose	4.6 ± 0.02	-
Glucose	0.5 ± 0.04	-
Xylose	0.1 ± 0.01	-
Galactose	0.2 ± 0.10	-
IP[b]	63.6 ± 2.33	-
FAN[b]	28.8 ± 0.12	-

[a] wet basis
[b] mg/100g db



Biorefinery approach

SFM
100 g

Ultrasound Phenolic Extraction
EtOH: H_2O 1:1
1/10 *w/v*

Crude Phenolic Extract
8.2 g

Total Phenolic content
33% (CAE)

Protein rich Solid Residue
91.8 g

Protein Extraction & Precipitation

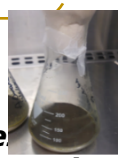
Protein Concentrate

Recovery
17%
Purity 80%

Solid Residue
72.7 g

Pretreatment
H2SO4 1.25% w/v
NaOH 2% w/v

Cellulose rich Solid Residue
19.6 g



Hydrolysis
Crude enzyme Aspergillus awamori

Sugar rich Hydrolysate

Total Sugar concentration
15.7 g/L

Fermentation
Actinobacilus succinogenes

Succinic acid

Concentration 40 g/L
Productivity 0.48 g/L/h

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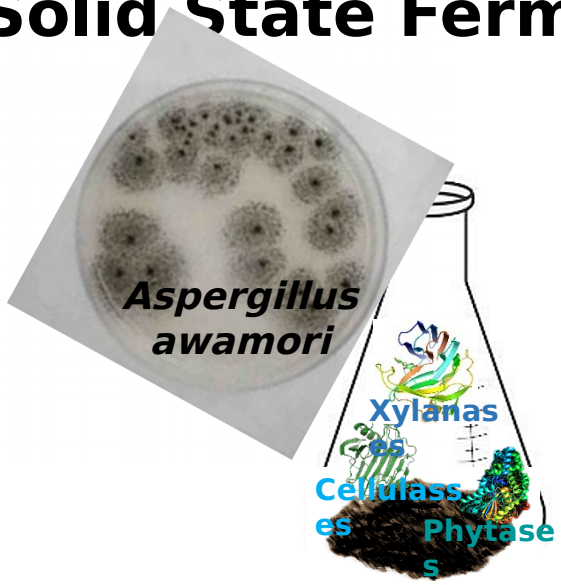
Solid State Fermentation - Enzyme Activity



Enzymes

- Cellulases – CMC 1 % w/v, pH 5, 50 ºC, 30 min
- Xylanases – Xylane 0.25 % w/v, pH 5, 50 ºC, 60 min
- Proteases - Casein 0.75% w/v, pH 7, 50 ºC, 30 min
- Phytases - Phytic acid 0.2 % w/v, pH 5, 50 ºC, 15 min

Conditions

- 5 g SFM
- 1.0×10^6 spores/g solid
- 30 ºC
- Initial moisture content 50, 55, 60 & 65 %

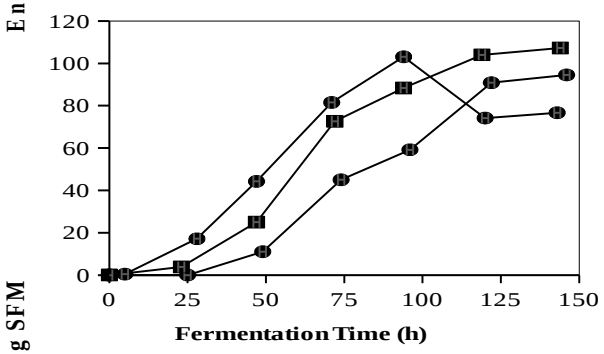
Definition of enzyme activity units

- Proteases & Phytases:
  amount of enzyme needed for the production of 1 µg Free Amino Nintrogen (FAN) or Inorganic Phosphorus (IP) per minute

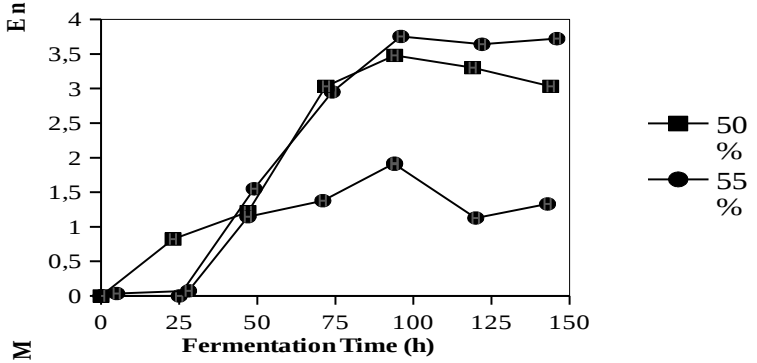
- Cellulases & Xylanases
  amount of enzyme needed for the production of 1 mg Glucose or Xylose per minute

Solid State Fermentation - Enzyme Activity

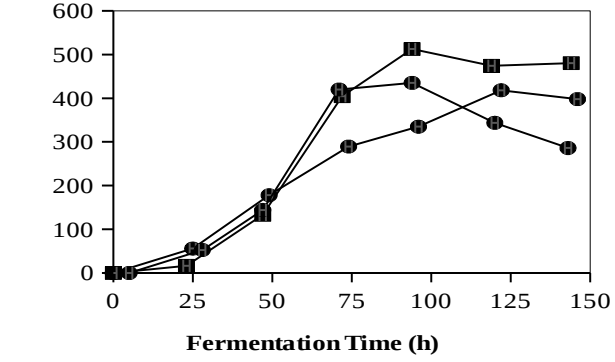
- ✓ Proteases
- ✓ 107.2 U/g after 94 h
- ✓ Moisture content 60%



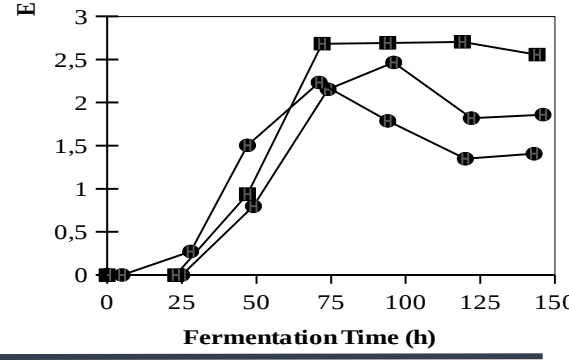
- ✓ Cellulases
- ✓ 3.7 U/g after 94 h
- ✓ Moisture content 65%



Phytases
512.7 U/g after 94 h
Moisture content 60%



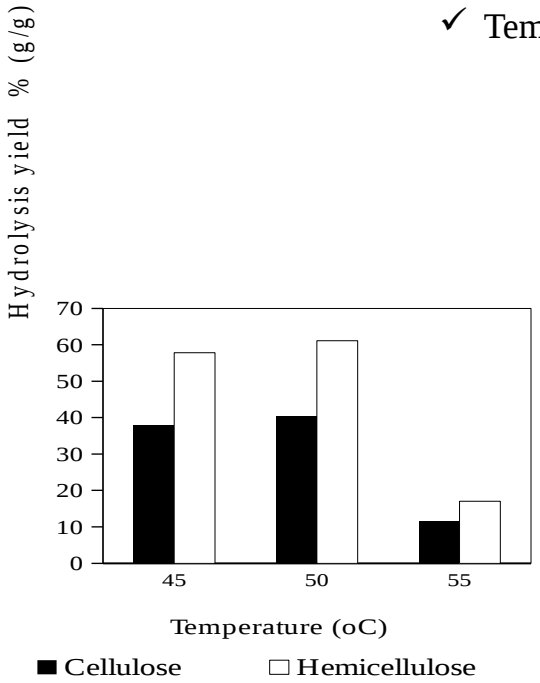
- ✓ Xylanases
- ✓ 2.7 U/g after 94 h
- ✓ Moisture content 60%

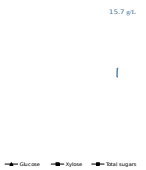


Enzymatic Hydrolysis - optimization

Effect of Temperature

- ✓ Hydrolysis yield: 40.5% Cellulose; 61.1% Hemicellulose
- ✓ Temperature 50 °C



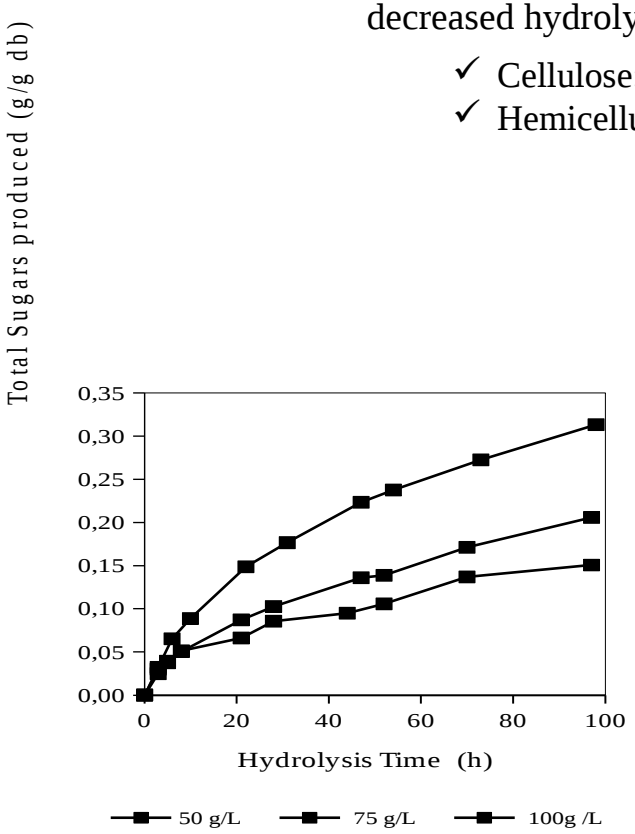


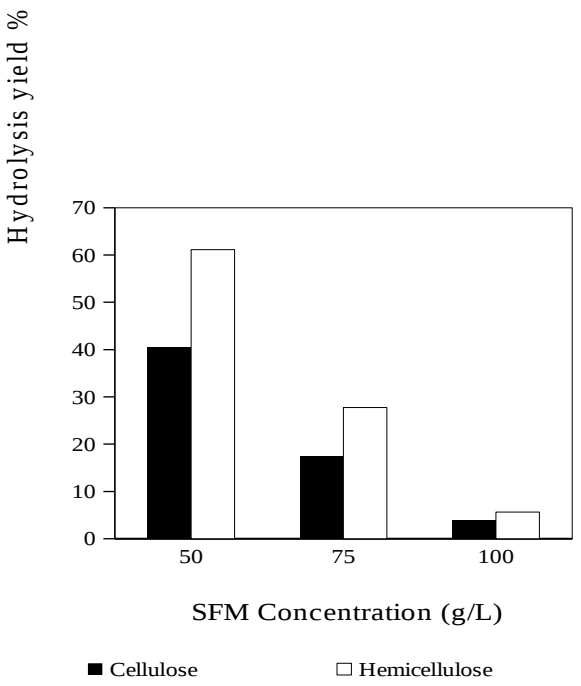
Enzymatic Hydrolysis - optimization

Effect of initial SFM concentration

Increasing concentrations of SFM led to decreased hydrolysis yields :

- ✓ Cellulose: 17.4 – 3.9 %
- ✓ Hemicellulose: 27.7 – 5.6 %





Fed-batch fermentation for succinic acid production

Conditions

- ✓ *Actinobacillus succinogenes*
- ✓ Temperature: 37 ^{o}C
- ✓ Initial sugar concentration : 60 g/L
- ✓ Feeding up to 30 g/L

- ✓ Succinic acid concentration: 40 g/L
- ✓ Yield: 0.53 g succinic acid/g consumed sugars
- ✓ Productivity: 0.48 g/L/h

Total Sugars Succinic

Conclusions

Sunflower meal was effectively valorized through the development of an advanced biorefinery concept for the production of proteins, phenolic compounds and succinic acid:

- ✓ Phenolic rich extract (33% CAE)
- ✓ Protein Concentrate (80 % purity)
- ✓ Succinic acid production
  - ➢ 40 g/L
  - ➢ Yield: 0.53 g succinic acid/g consumed sugars
  - ➢ Productivity: 0.48 g/L/h

Acknowledgements

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Thank you
for
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attention!!

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MINISTRY OF
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SPECIAL SECRETARY FOR ERDF & CF

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