Bioprocess development for the production of succinic acid from orange peel waste

Maria Patsalou[1], Chrysanthi Pateraki[2], Marlen Vasquez[1], Chryssoula Drouza[3] and Michalis Koutinas[1]

[1]Department of Environmental Science & Technology, Cyprus University of Technology
[2]Department of Food Science and Human Nutrition, Agricultural University of Athens
[3]Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology

Limassol, 2016

Citrus fruits

- 88 x 10^6 tn worldwide production
- Industrial OPW generation: 15 x 10^6 tn/ y

- Oranges: 82 % of the total production
- 50% of fruit is peel waste

- Animal feed
- Disposal in landfills

Orange peel waste

- Peels
- Seeds
- Segment membranes

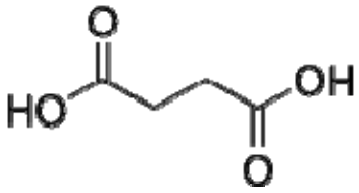
- Composition of peel*

Components	% Dry mass
Soluble sugar	16.90
Starch	3.75
Cellulose	9.21
Hemicellulose	10.50
Lignin	0.84
Pectin	42.50
Ash	3.50
Fat	1.95
Protein	6.50
Others	4.35

*Lopez *et al.*, Crit Rev Biotechnol. , 2010; 30(1): 63-69

Succinic acid

- Di-carboxylic acid



- Important biobased platform chemicals
  - Polybutylene succinate (PBS)
  - Polybutylene succinate-terephthalate (PBST)
  - Polyester polyols
  - Food industry
  - Pharmaceutical industry
  - Production of resins, coatings and pigments

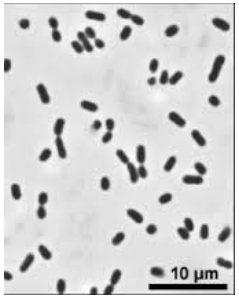
- Chemical technologies
  - Catalytic hydrogenation
  - Paraffin oxidation
  - Electrolytic reduction of maleic acid or anhydride

- High theoretical yield
- Environmental friendly impact

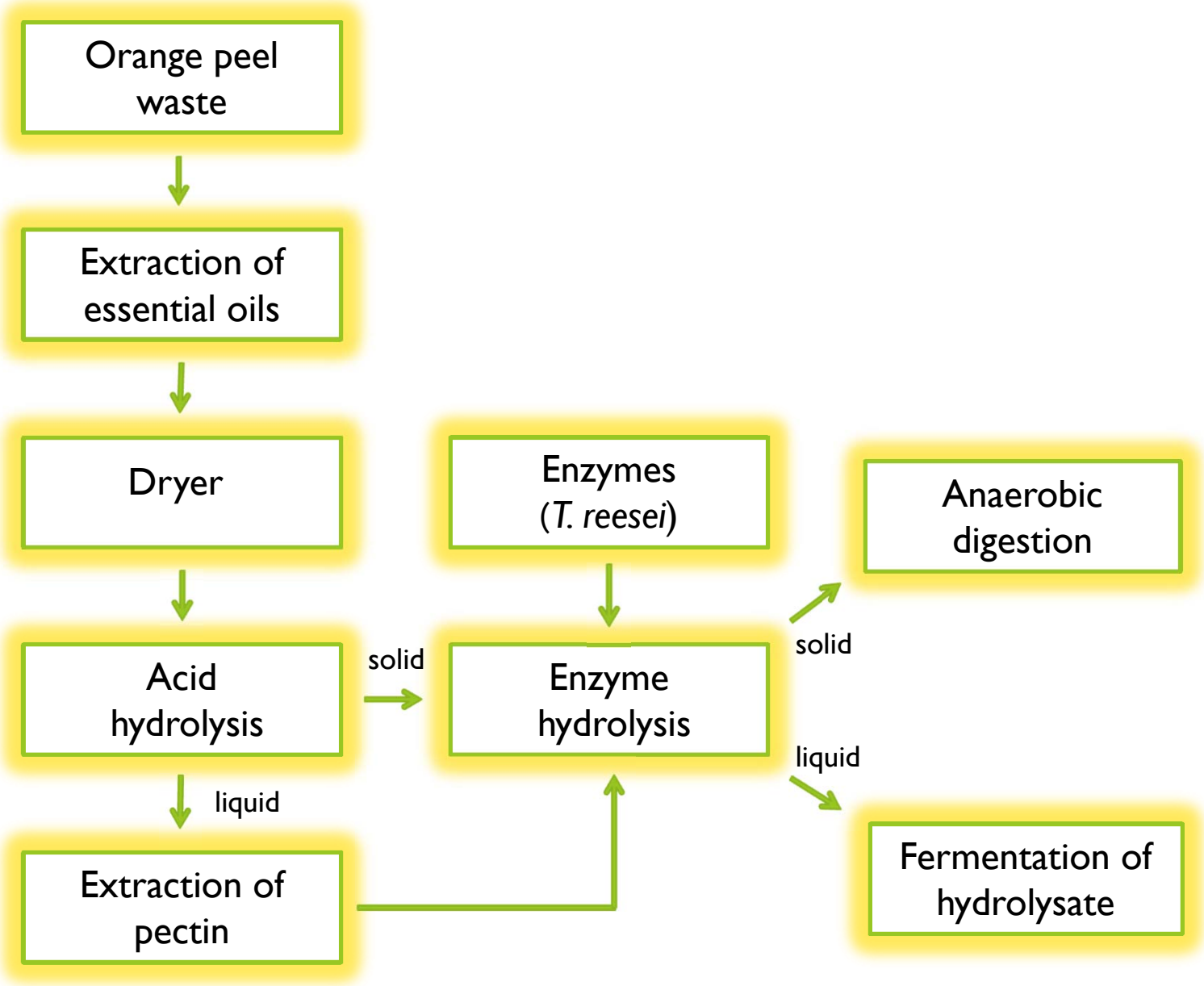
Succinic acid producers

- *Mannheimia succiniciproducens*
- *Anaerobiospirillum succiniciproducens*
- *Basfia succiniciproducens*

- *Actinobacillus succinogenes*
  - Isolated from bovine rumen
  - Capnophilic
  - Mesophilic
  - CO_2



Proposed Flow Diagram

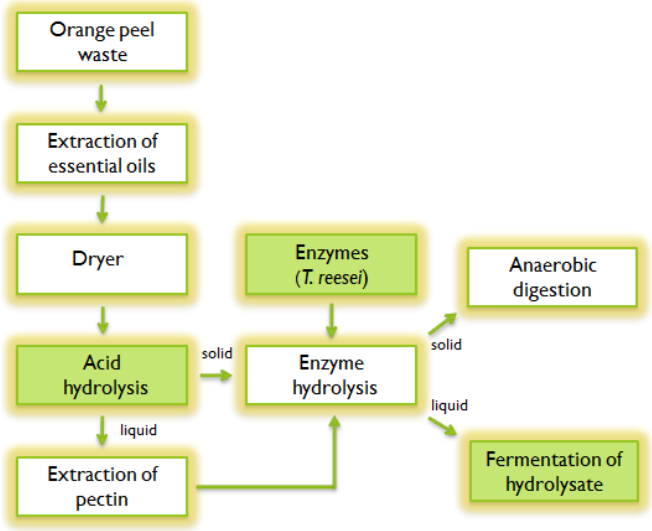


Aim and Objectives

- Preliminary study for the development of an OPW bio-refinery to produce succinic acid

1. Release of metal ions after acid hydrolysis and acid/enzyme hydrolysis

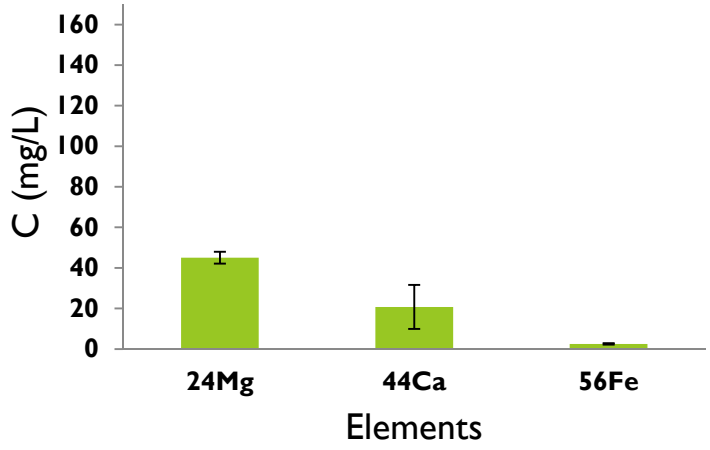
2. Dilute-acid hydrolysis conditions
   - SA Fermentations, simple sugars
   - Selection of conditions
   - Presence of HMF in hydrolysates
   - SA Fermentations, dilute-acid hydrolysates

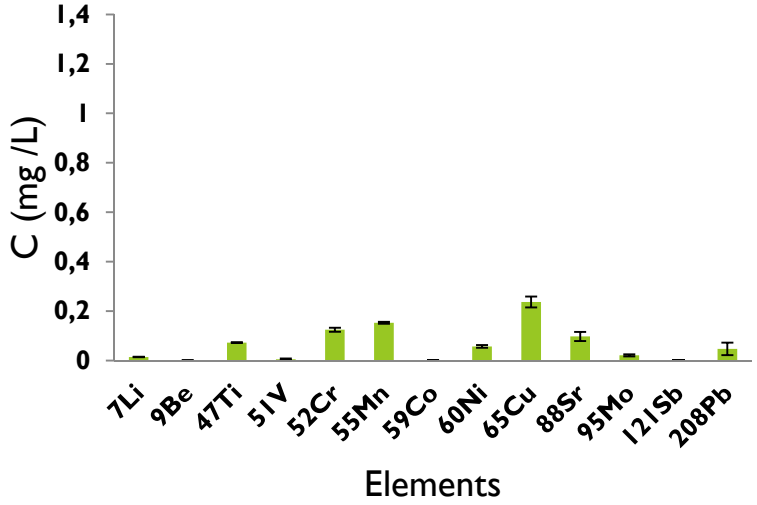


3. Optimal cultivation time for cellulolytic enzyme production by *T. reesei*

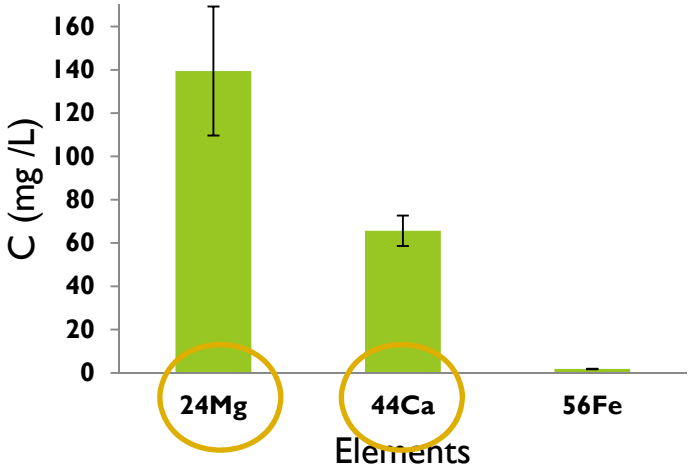
Elemental analysis (ICP-MS) of Hydrolysates

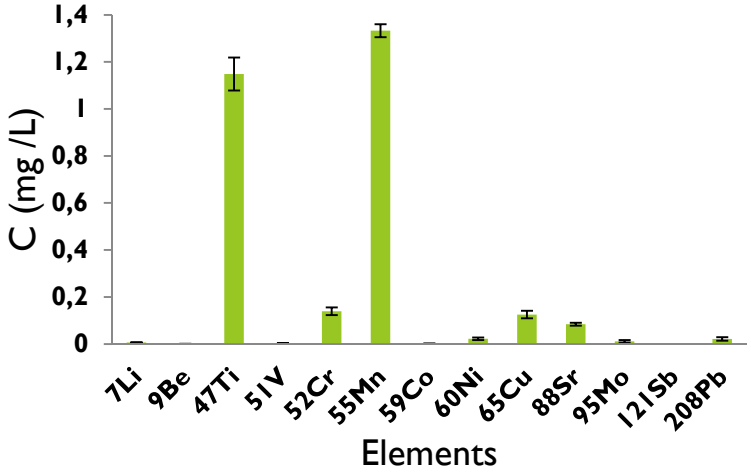
Acid hydrolysis



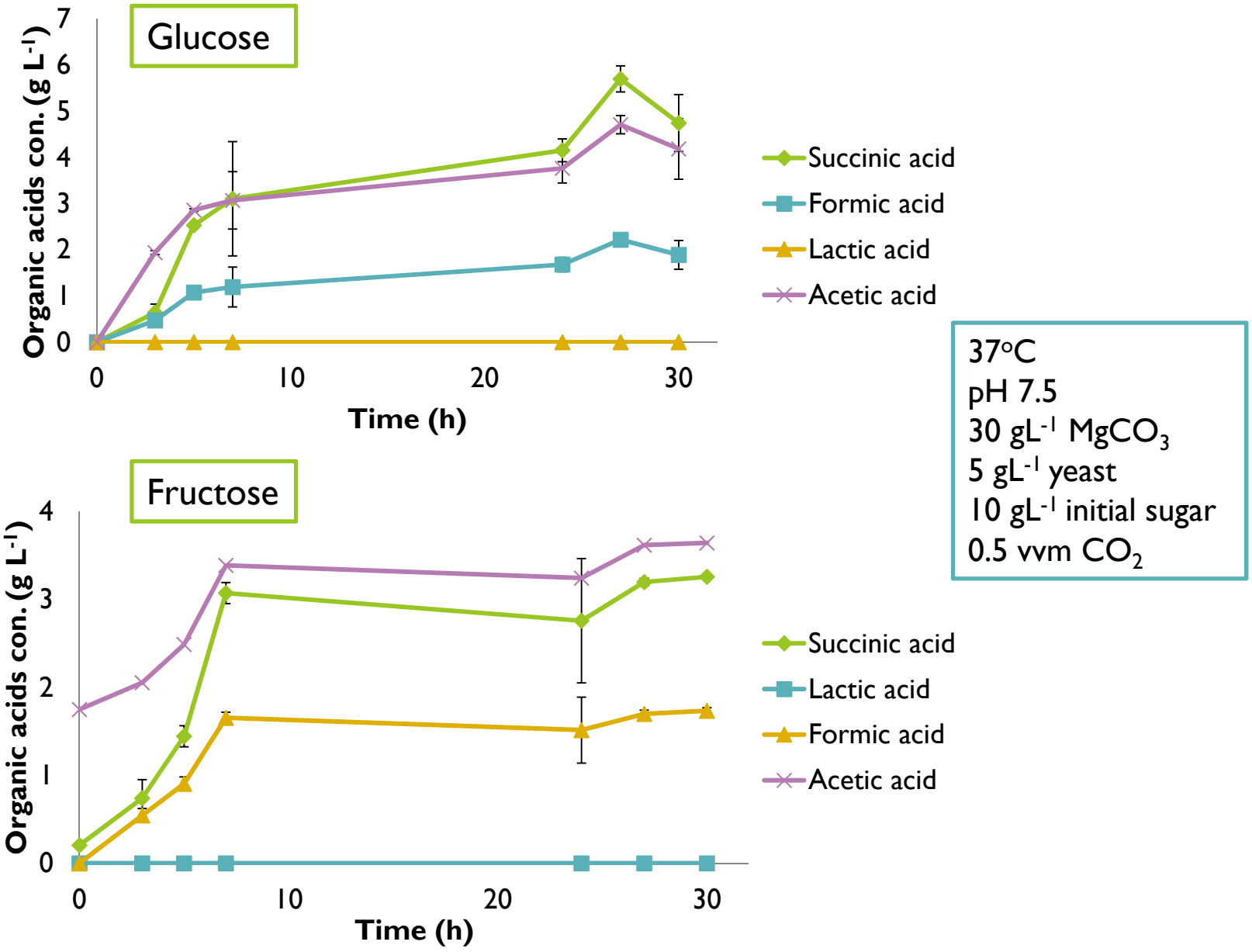


Acid and enzyme hydrolysis

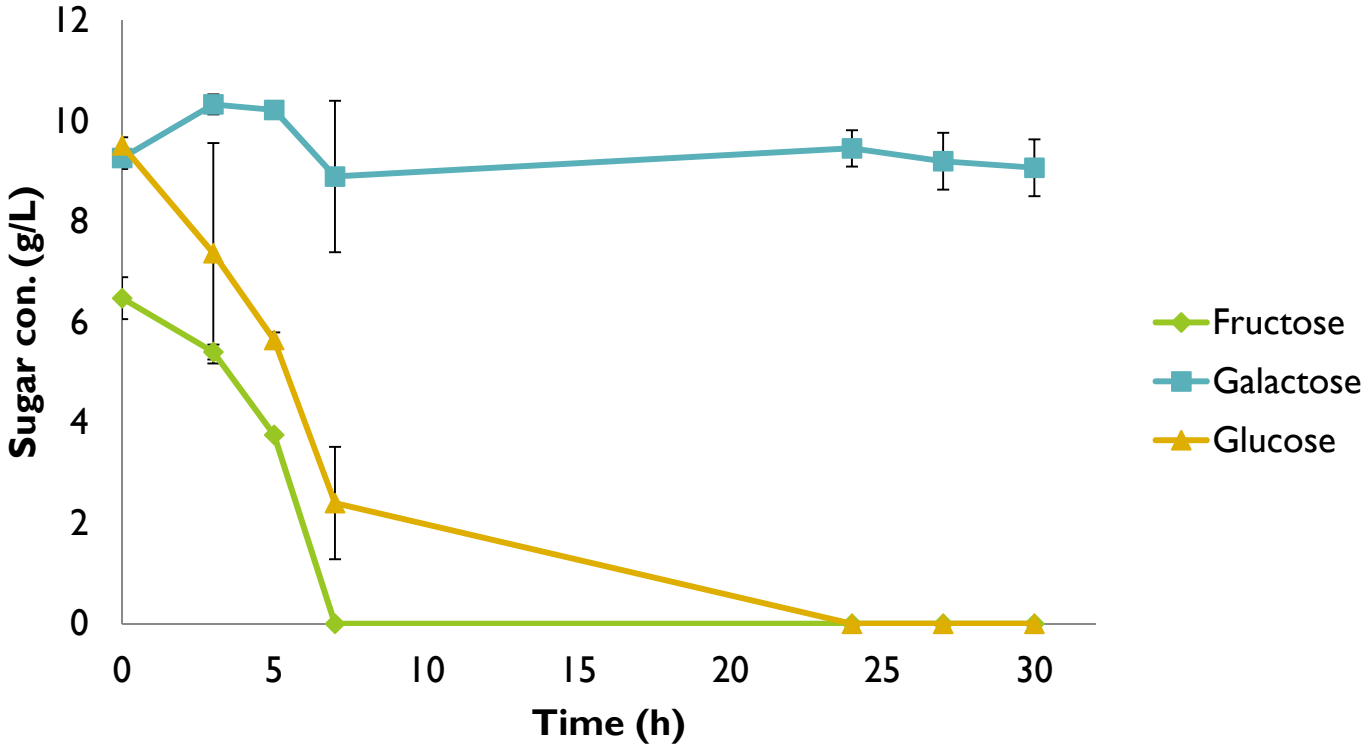




Succinic acid production from simple sugars fermentation



Consumption of each simple sugar, Yields of fermentations



Sugar	SA Yield (g_p/g_s)	OA Yield (g_p/g_s)
Glucose	0,57	1,26
Fructose	0,33	1,10
Galactose	-	-
Glucose (Bioreactor)	0,66	1,10

Dilute-acid hydrolysis conditions

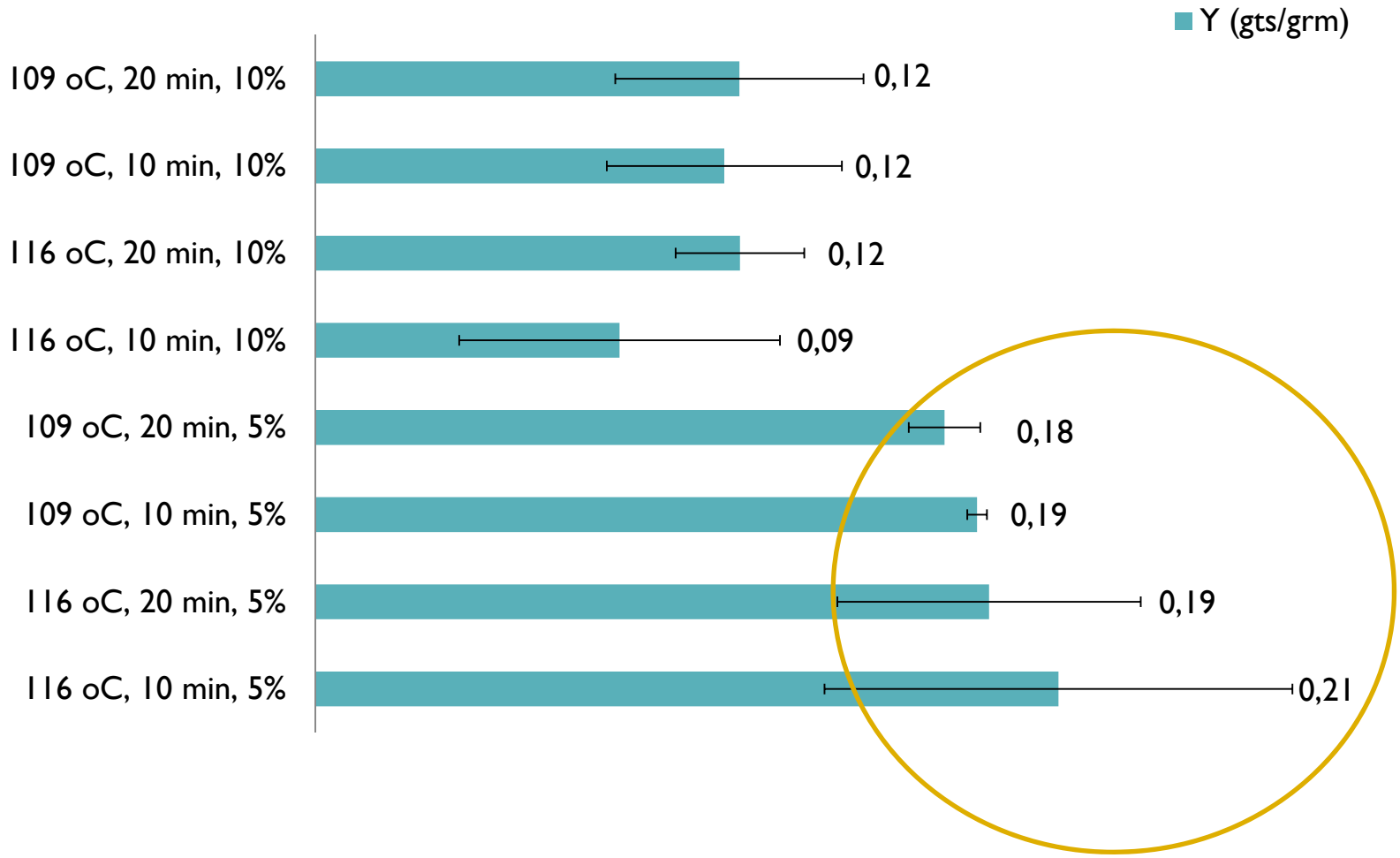
- 100-120 °C, fructose*
- >120 °C, arabinose and galactose*

- 116°C, 10min, 5%
- 116°C, 20min, 5%
- 109°C, 10min, 5%
- 109°C, 20min, 5%

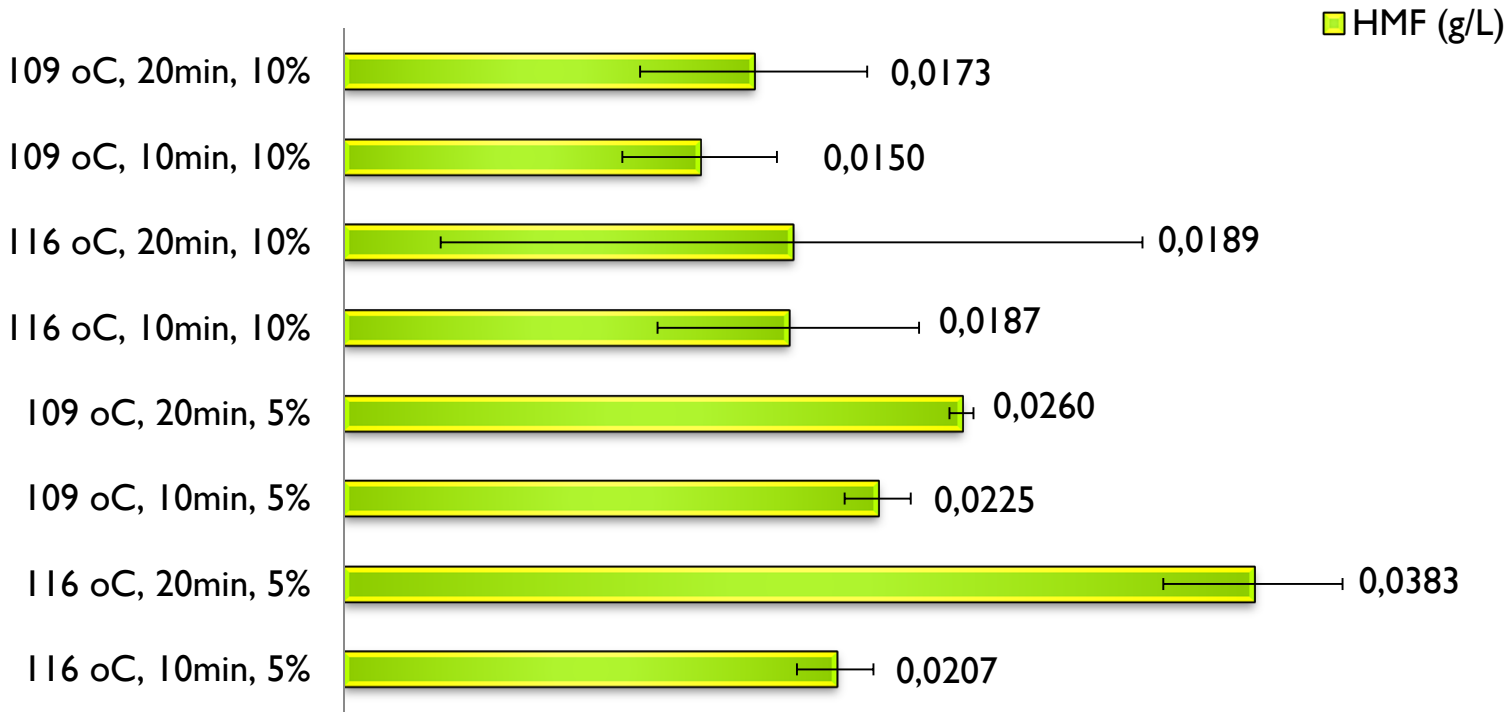
- 116°C, 10min, 10%
- 116°C, 20min, 10%
- 109°C, 10min, 10%
- 109°C, 20min, 10%

*Grohmann *et al.*, Bioresour. Technol., 1995; 54: 129-141

Release of sugar of dilute-acid hydrolysis



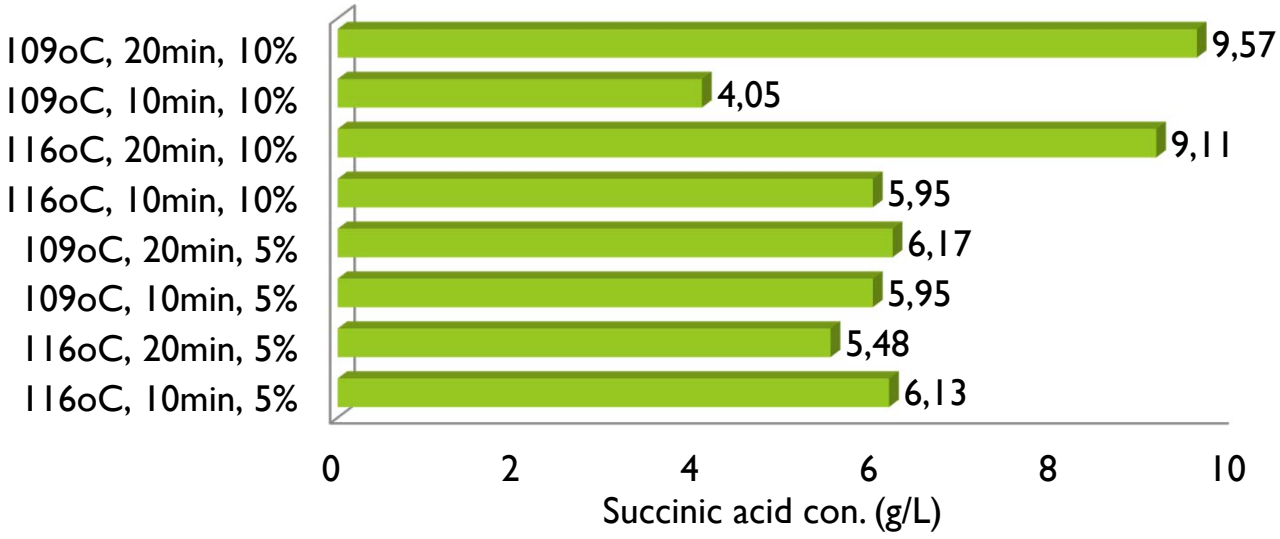
Concentration of inhibitors of dilute-acid hydrolysate

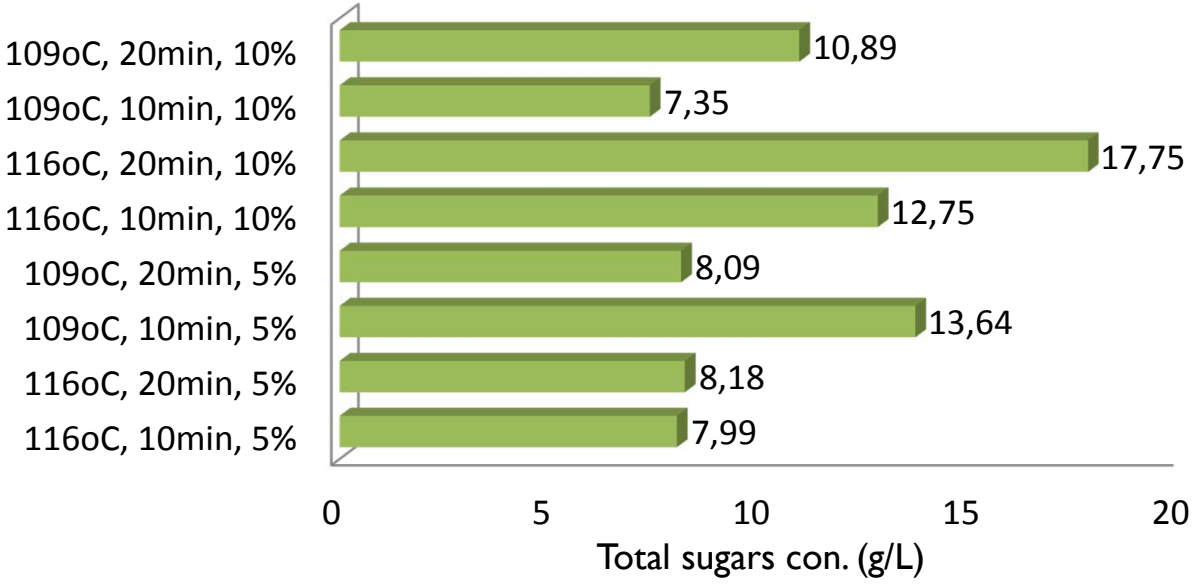


0,15-0,35 gL^{-1} *

*Gunnarsson *et al.*, Bioresour. Technol., 2015; 182: 58-66

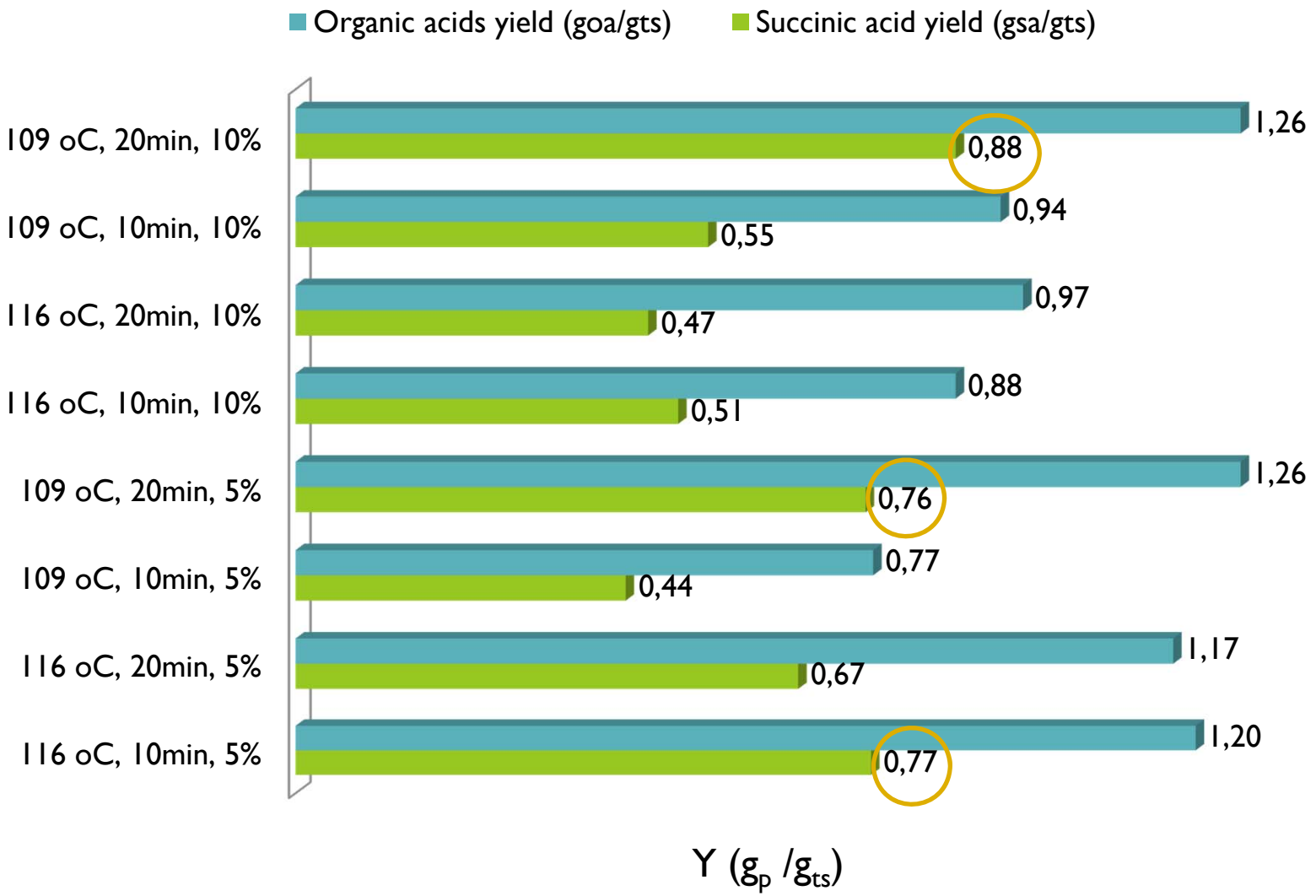
Succinic acid production and consumption of total sugars





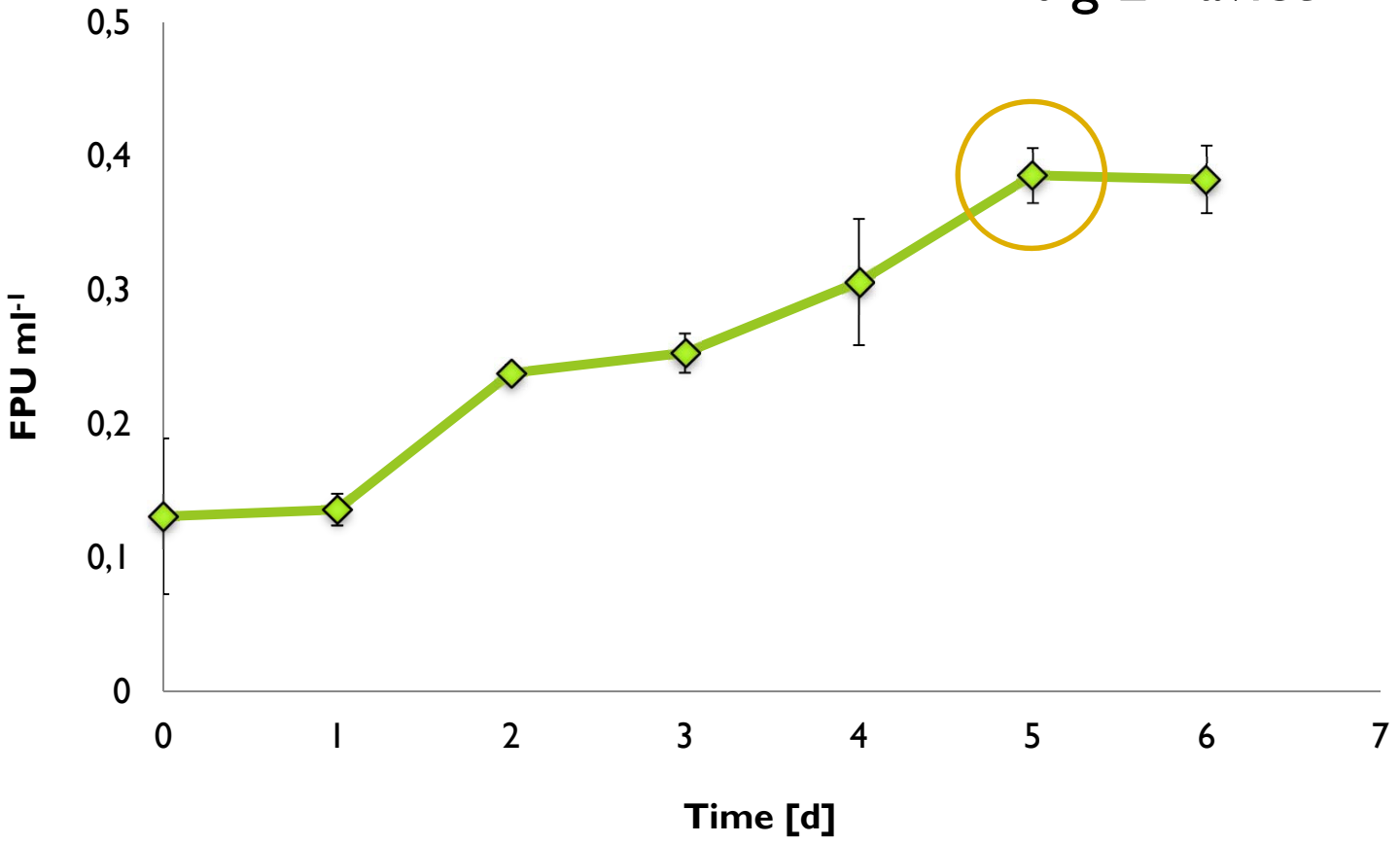
37°C
pH 7.5
30 gL^{-1} $MgCO_3$
5 gL^{-1} yeast extract
0.5 vvm CO_2

Yields of fermentations of dilute-acid hydrolysate



Cellulase production

- Fermentation *T. reesei*
  - 28 °C, pH 5.5, 180 rpm
  - 40 g L^{-1} wheat bran, 10 g L^{-1} avicel



Succinic acid bio-production

Raw material	Nitrogen source	Gas supply, Fermentation, Total volume, Working volume	Succinic acid (g /L)	Y (g_{SA}/g_{ts})	Ref.
Glycerol	YE (10)	Anaerobic, fed-batch, bioreactor 2 L -1.5 L	49.62	0.64	Carvalho et al., 2014
Wheat hydrolyzate	YE (5) / Vit	Anaerobic, batch, bioreactor 1.8 L, 0.5 L	62.1	1.02	Dorado et al., 2009
Bread hydrolyzate	BH (200 mg/L FAN)	Anaerobic, batch, bioreactor 2.5 L, n.d.	47.3	nk	Leung et al., 2012
Cotton stalk hydrolyzate	YE (30) / Urea (2)	Anaerobic, batch SSF, bottles 500 mL, n.d.	63	0.64	Li et al., 2013
Macroalgal hydrolyzate	YE (16.7)	Anaerobic, batch, bioreactor 3L, 1.5L	33.78	0.63	Morales et al., 2015
Rapeseed meal	YE (15)	Anaerobic, fed-batch SSF[a], bioreactor 3 L, 1.2 L	23.4	0.115	Chen et al., 2011
Whey	YE (5) / Pep (10)	Anaerobic, batch, bioreactor 2.5L, 1.2L	22.2	0.57	Wan et al., 2008
Acid hydrolysis of OPW	-	Anaerobic, batch (*Fibrobacter succinogenes*), serum bottles 125ml, 25ml	1.9	0.12	Li et al., 2010
Dilute-acid hydrolysis of OPW	YE (5)	Anaerobic, batch, bottles 100mL, 100mL	6.17 and 6.13	0.76 and 0.77	Current study

Conclusions

- Elemental analysis on hydrolysates
  - Mg^{2+}, Ca^{2+}
- Dilute-acid hydrolysis conditions
  - 109 °C, 20 min, 5% (w/w), y=0,76 (g_{sa}/g_{ts})
  - 116 °C, 10 min, 5% (w/w), y=0,77 (g_{sa}/g_{ts})
- Cellulase production
  - 5 days incubation

Future Work

- Ultrasound
  - Frequency
  - Duration
- Enzyme hydrolysis
  - Enzyme units
  - Duration

Thank you!