The importance of pretreatment on butanol production from agrofood wastes

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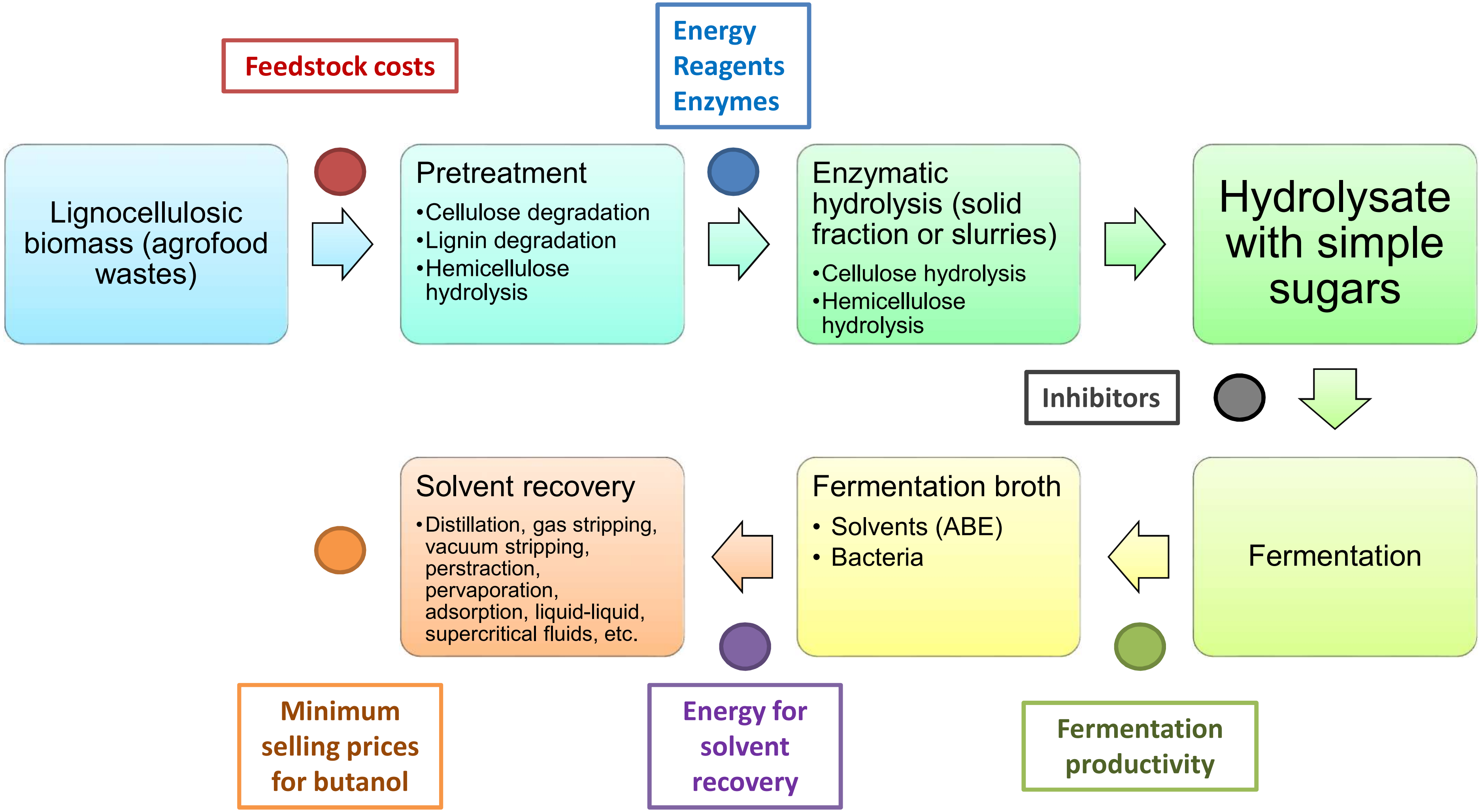
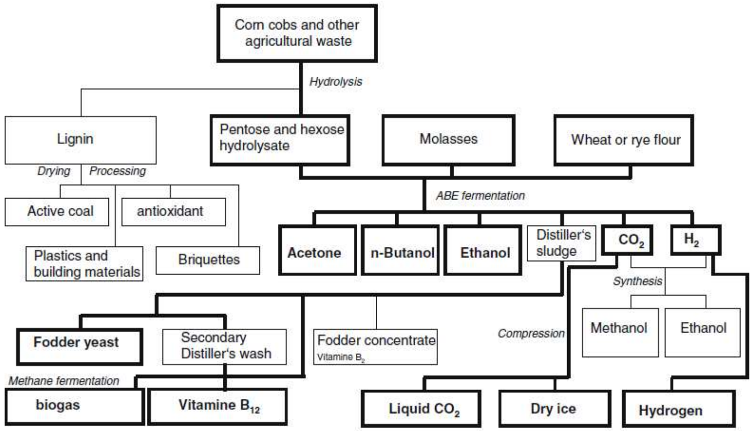
Introduction

Conventional process for the fermentation of lignocellulosic biomass



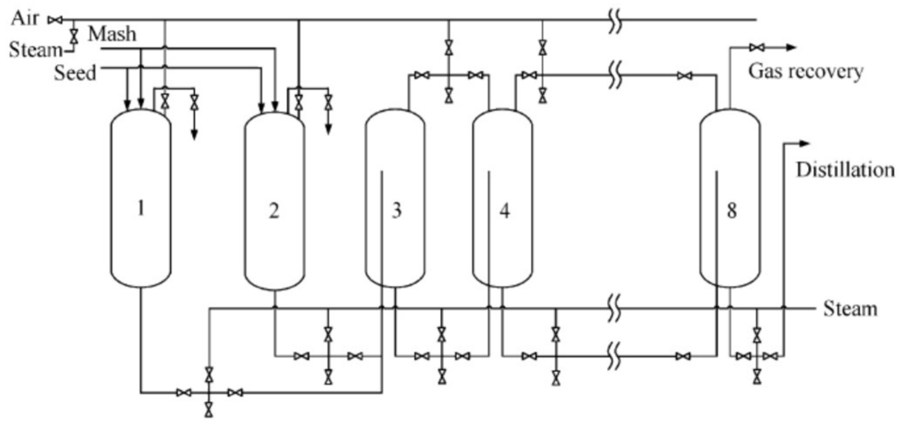
Proposals applied at industrial scale

Recovery of various products



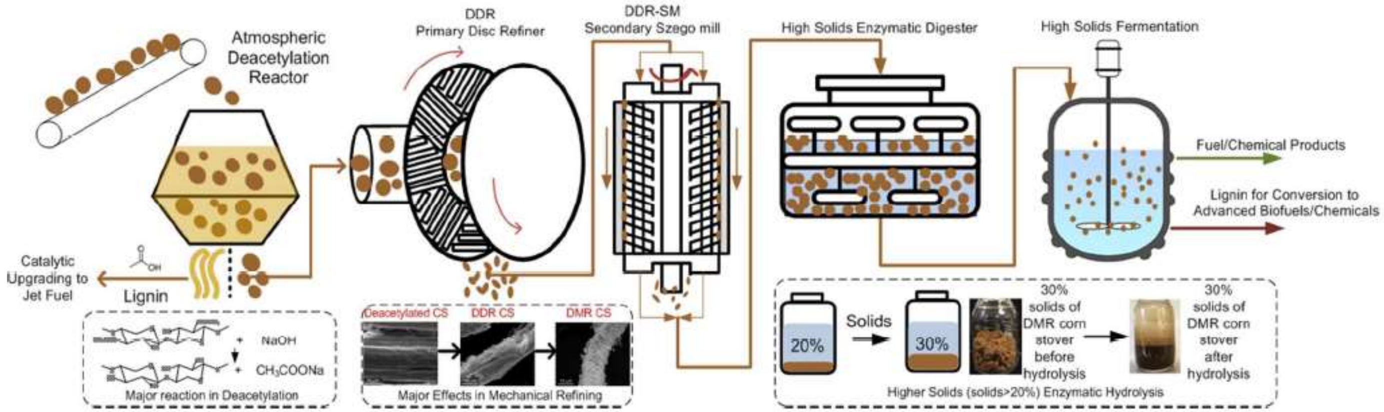
Zverlov et al. Appl Microbiol Biotechnol (2006) 71: 587–597.

Continuous fermentation



Ni & Sun. Appl Microbiol Biotechnol (2009) 83:415–423.

NREL pretreatment (deacetylation and mechanical refining)



Chen et al. Energy Environ. Sci., 2016, DOI:10.1039/C5EE03718B.

Proposals at developmental stage

Genetic engineering

- Modification of strains.
- Enhance solvent production.
- Improve butanol tolerance.
- Increase the ratio butanol:solvents.
- Allow strain to grow on complex cellulose substrates.
- Use of hosts for butanol production.

Advanced fermentation techniques

- Cocultures.
- High cell concentrations in continuous fermentation.
- Multi-stage continuous fermentation.
- Addition of organic acids.
- Addition of electron carriers.
- Simultaneous saccharification and fermentation.

Clean fractionation

- Organsolv process originally applied to woody feedstocks.
- Separation of biomass components into three streams (cellulose, hemicellulose, lignin).
- Upgrading processes for the three enriched fractions.

Mechanical pretreatment

- Mechanical size-reduction of biomass after the chemical pretreatment (and not before it).
- Reduce energy requirements.

Lignocellulosic biorefinery

- Heat, electricity, synthesis gas, biogas.
- Hydrogen, methanol, higher alcohols.
- Aromatics, hydrocarbons, coke.
- Furfural, 5-HMF, levulinic acid.
- Acetone, butanol, ethanol.
- Alkanes.
- Liquid fuels.

Genetic engineering	Advanced fermentation techniques	Clean fractionation	Mechanical pretreatment	Lignocellulosic biorefinery
Gu et al., 2011. Zheng et al., 2015.	Modified from: Zheng et al., 2015. Jiang et al., 2018.	Katahira et al., 2014.	Zhu et al., 2010.	Maity, 2015.

Pretreatments applied to lignocellulosic biomass

Conventional pretreatments	Alternative pretreatments
• Steam explosion • Wet oxidation • Autohydrolysis (liquid hot water) • Dilute/concentrated acid pretreatment • Alkaline pretreatment • Organic solvents • Surfactants (usually with acids)	• Ammonia fibre explosion (AFEX) • Ammonia recycle percolation (ARP) • Ionic liquids • Subcritical and supercritical treatment • Microbial cocultures • Microwaves • Ultrasounds • Ligninolytic enzymes • Deep eutectic solvents

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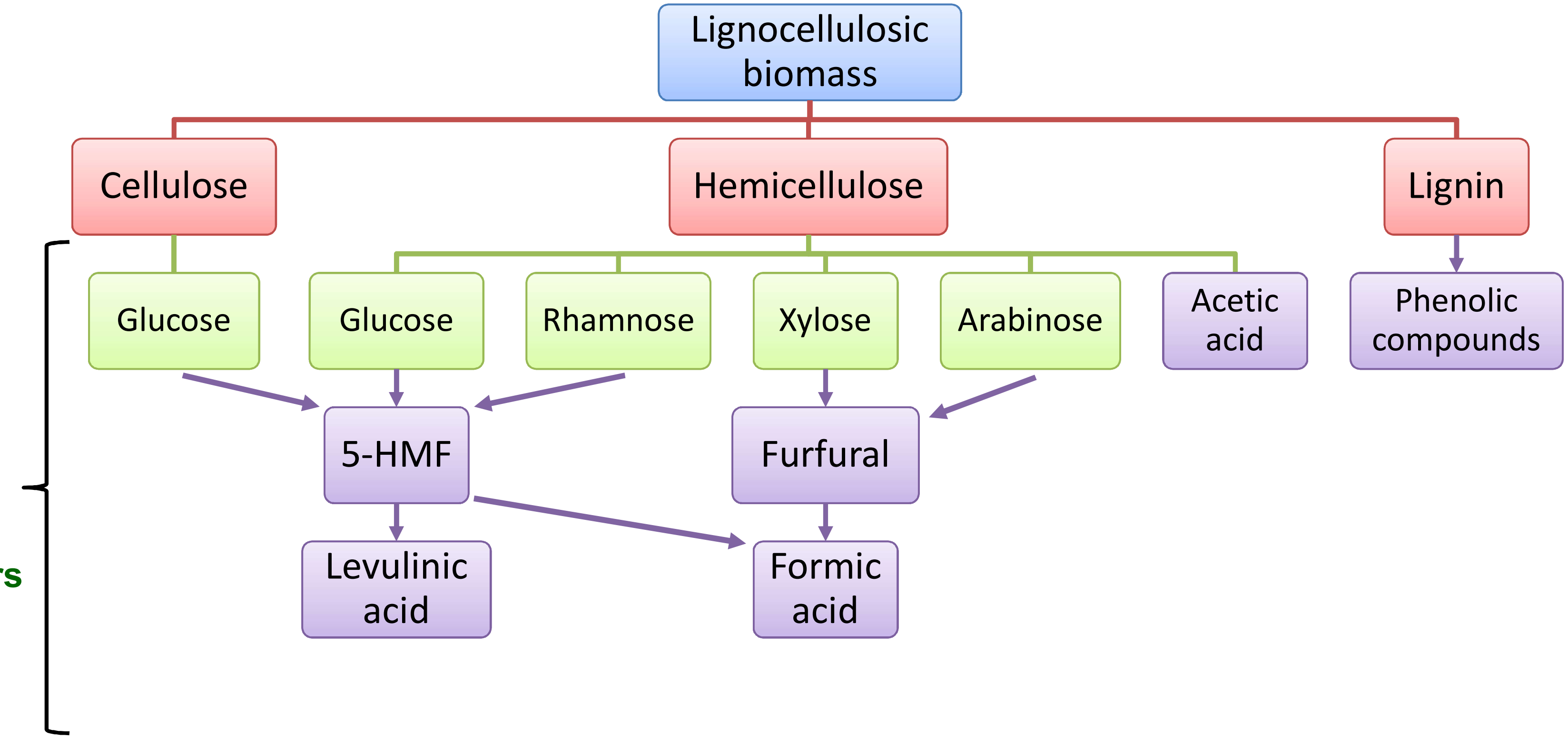
Modified from: de Jong & Gosselink (2014) Bioenergy Research: Advances and Applications, 277–313.

Degradation of lignocellulosic wastes by physicochemical pretreatments

PRETREATMENT
- **Temperature**
- **Pressure**
- **Reagents**

Simple sugars

Inhibitors



Palmqvist & Hahn-Hägerdal (2000) Bioresource Technology 74: 25-35.

Main limitations of conventional pretreatments for agrofood wastes

Physico-chemical pretreatments

- Technical difficulties to operate at high solid-to-solvent ratios (> 10-20%).
- Generation of fermentation inhibitors (especially for acid or high-temperature pretreatments).
- Cost of reagents.
- Energy consumption (milling, heating, shaking).

Enzymatic hydrolysis

- Cost of enzymes.
- Long treatment times (energy consumption).

Result

- Relatively low sugar concentrations in the hydrolysate.
- Toxicity for fermenting bacteria.

Objectives

Agrofood wastes as feedstocks for biobutanol

Physicochemical pretreatments

- Reduce the use of reagents
- Reduce power consumption
- Reduce waste generation
- Reduce water consumption
- Reduce the generation of inhibitors
- Increase sugar release
- Maximise butanol production
- Simplify the global process

Experimental section

Agrofood wastes used

WASTE2FUELS

Selected AFWs:
- Potato peel and wastes
- Fruit residues (apple juice industry)
- Brewers' spent grain
- Coffee silverskin

↓

Drying

↓

Grinding

↓

Sieving
(0.50-1.00 mm)

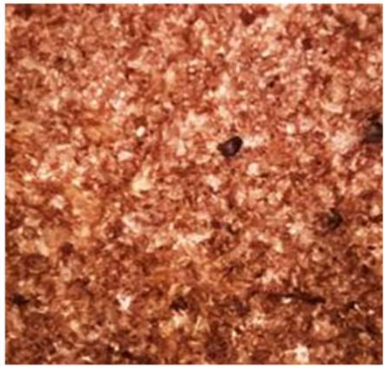
↓

STORAGE
In a cool, dry and dark place

Potato peel
43% carbohydrates

Apple pomace
56% carbohydrates

Brewers' spent grain
40% carbohydrates



Coffee silverskin
30% carbohydrates

Agrofood wastes used

	Potato peel	Apple pomace	Brewers' spent grain	Coffee silverskin
Total carbohydrates (%)	43.20	55.86	39.76	30.37
Soluble carbohydrates (%)	0.43	15.55	0.12	0.40
Cellulose (%)	8.3	21.22	11.90	10.33
Hemicellulose (%)	7.41	14.75	18.59	9.64
Starch (%)	23.01	n.a.	4.87	7.15
Lignin (%)	32.88	18.50	20.47	29.91
Protein (%)	10.73	4.87	19.8	14.43
Fats (%)	2.45	1.42	5.25	4.97
Ash (%)	7.45	1.31	3.43	5.87
Moisture (%)	5.26	6.56	12.79	4.81
Total phenolic compounds (mg/g)	2.5	3.5	1.0	8.0

n.a. Not analysed.

Pretreatments employed

PHYSICOCHEMICAL PRETREATMENTS

- **Autohydrolysis**
- **Alkalis (NaOH, KOH, NH_4OH)**
- **Acids (H_2SO_4, HCl, HNO_3)**
- **Organic solvents (acetone, ethanol, methanol)**
- **Surfactants (Tween 80, PEG 6000, CTAB)**

Biomass ratio 10% (w/w)

Pretreatment slurry (solid biomass + liquid hydrolysates)

ENZYMATIC HYDROLYSIS

Citrate buffer 50 mM, pH 5.0, 50°C, 180 rpm, 72 h

Enzymes and doses	
Cellic CTec2	15 mg protein/g glucan
Spirizyme Fuel (for potato peel)	16.7 µl/g glucan

CHEMICAL ANALYSIS

- **Fermentable sugars:** glucose, xylose, rhamnose, cellobiose and arabinose.
- **Inhibitors:** organic acids (acetic, formic and levulinic acids), furans (HMF and furfural) and phenolic compounds.

Experimental procedure

Select the most appropriate physicochemical treatment for each waste

Autohydrolysis?	Acids?	Alkalis?	Organic solvents?	Surfactants?

↓

Select the best reagent within each group

H_2SO_4?	HCl?	HNO_3?

↓

Determine the optimal conditions for the physicochemical pretreatment (RSM)

Temperature, time, reagent concentration	Maximum sugar concentration, minimum inhibitor concentration

↓

Fermentability test

Toxic? Fermentable?	Detoxification step?

↓

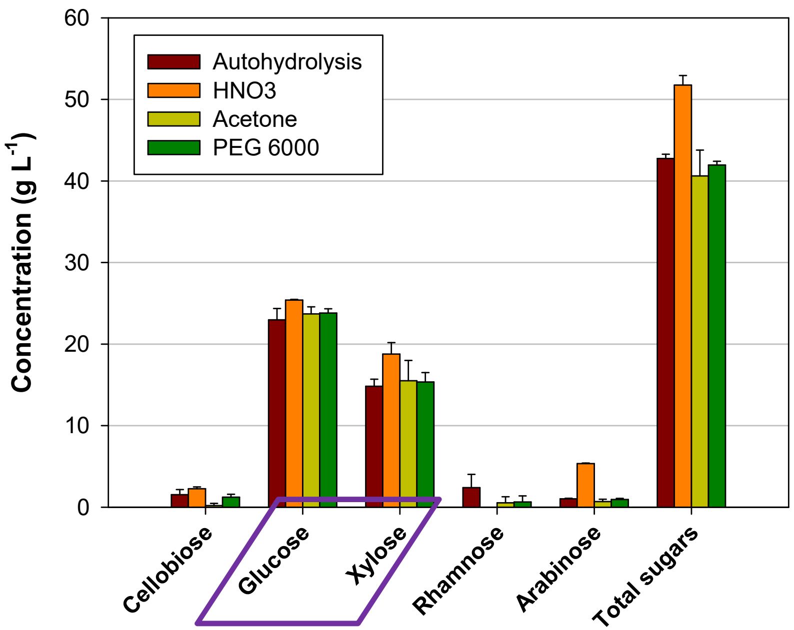
Bacterial strain screening

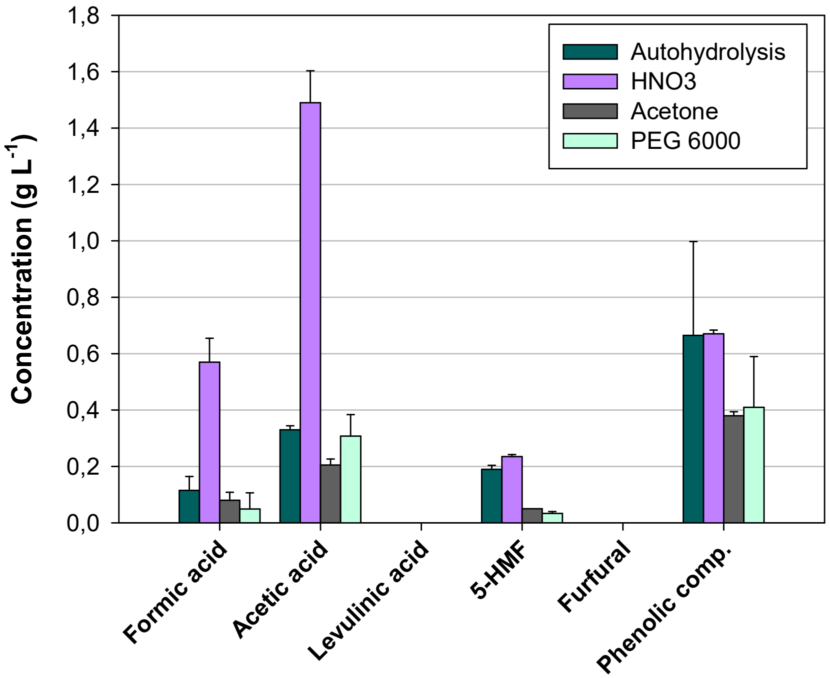
Selection of a suitable strain

Results

Apple pomace

		Physicochemical treatment optimal conditions (RSM)			Hydrolysate composition	
Pretreatment	**Selected substance**	**T (°C)**	**t (min)**	**Reagent (%, w/w)**	**Total sugars (g/L)**	**Total inhibitors (g/L)**
Autohydrolysis	-	142.4	12.0	-	42.7 ± 0.53	1.30 ± 0.38
Acid	HNO_3	124.2	7.3	1.83	51.8 ± 1.17	2.97 ± 0.02
Alkali	Not efficient	-	-	-	-	-
Solvent	Acetone	112.1	5.0	10	40.6 ± 3.16	0.72 ± 0.06
Surfactant	PEG 6000	100.2	5.0	1.96	42.0 ± 0.46	0.80 ± 0.17



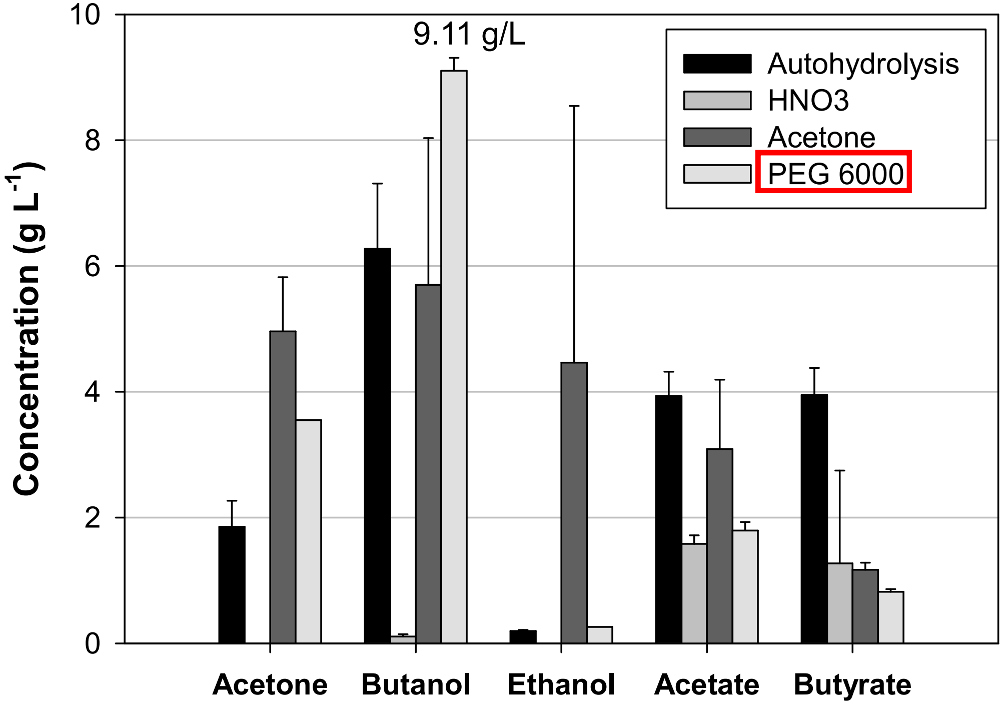


Hijosa-Valsero et al. (2017) Appl Microbiol Biotechnol 101: 8041–8052.

Apple pomace

Fermentability test

Clostridium beijerinckii CECT 508 (=NCIMB 8052), 96 h, additional nutrients

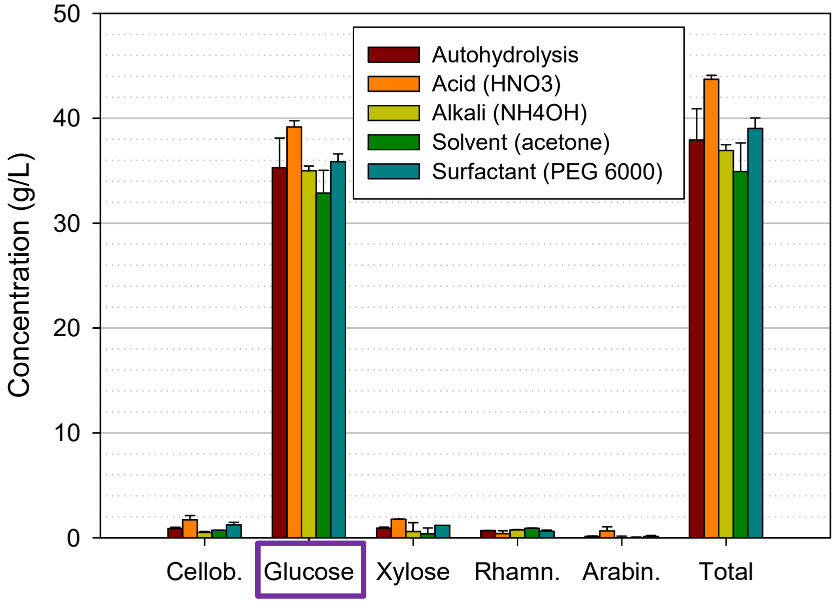


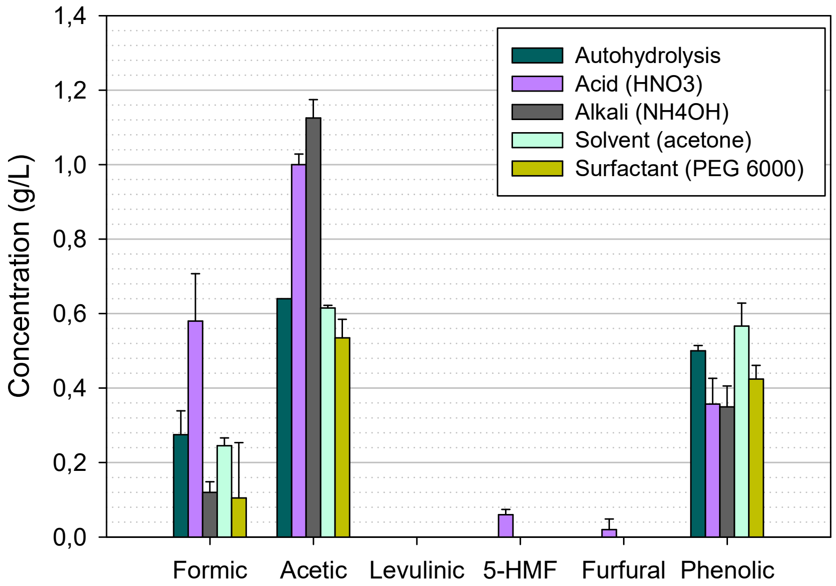
Sugar consumption (%)			Yield	Productivity
Hexoses	Pentoses	Total	$Y_{B/S}$ (g/g)	W_B ($g\ L^{-1}\ h^{-1}$)
99 ± 0	94 ± 0	91 ± 0	0.28 ± 0.01	0.095 ± 0.002

Hijosa-Valsero et al. (2017) Appl Microbiol Biotechnol 101: 8041–8052.

Potato peel

		Physicochemical treatment optimal conditions (RSM)			Hydrolysate composition	
Pretreatment	**Selected substance**	**T (°C)**	**t (min)**	**Reagent (%, w/w)**	**Total sugars (g/L)**	**Total inhibitors (g/L)**
Autohydrolysis	-	140.2	56.1	-	37.9 ± 2.99	1.41 ± 0.08
Acid	HNO_3	109.6	83.4	1.81	43.7 ± 0.37	2.02 ± 0.24
Alkali	NH_4OH	111.8	29.0	0.50	36.9 ± 0.55	1.59 ± 0.03
Solvent	Acetone	127.7	85.1	10.0	34.9 ± 2.74	1.43 ± 0.05
Surfactant	PEG 6000	145.6	5.0	1.92	39.0 ± 1.00	1.06 ± 0.06



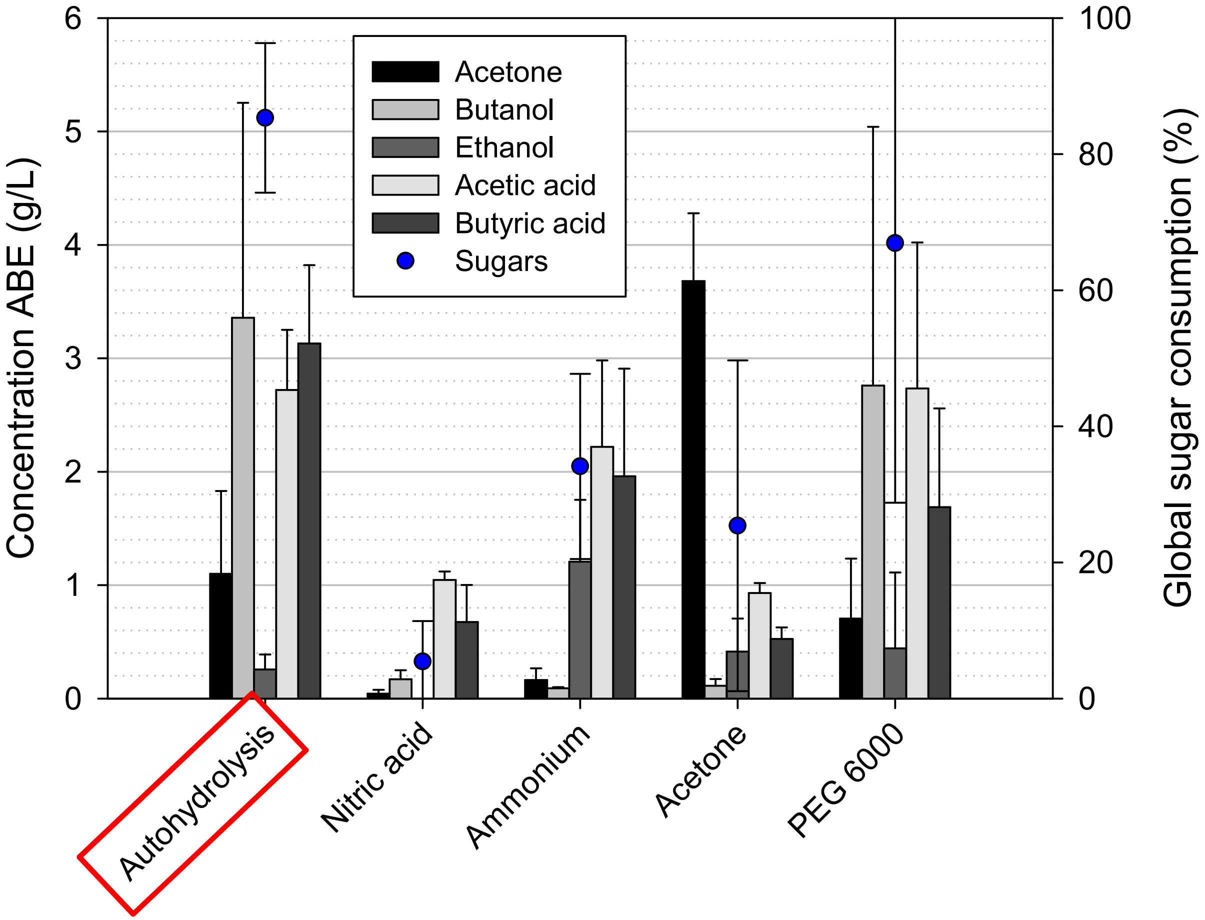


Hijosa-Valsero et al. (2018) New Biotechnology 46: 54-60.

Potato peel

Fermentability test

Clostridium beijerinckii CECT 508, 96 h, additional nutrients

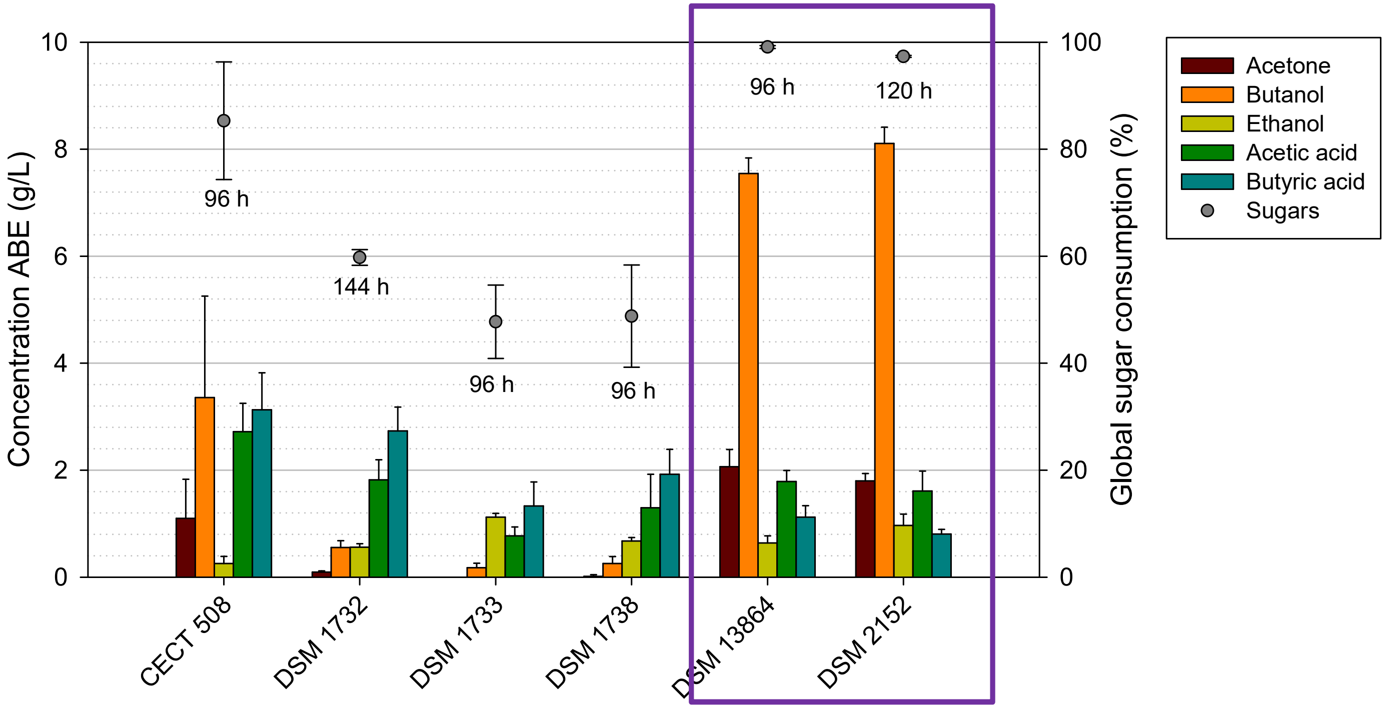


Hijosa-Valsero et al. (2018) New Biotechnology 46: 54-60.

Potato peel

Strain comparison

Autohydrolysis, *Clostridium* strains, additional nutrients



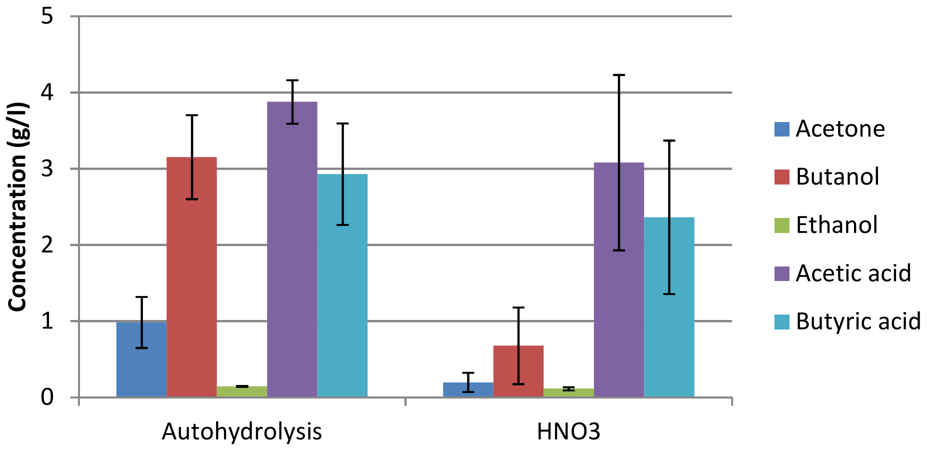
	Sugar consumption (%)			Yield	Productivity
Strain	**Hexoses**	**Pentoses**	**Total**	$Y_{B/S}$ **(g/g)**	W_B **(g L^{-1} h^{-1})**
DSM 13864	99.2 ± 0.2	94.2 ± 5.2	99.1 ± 0.3	0.186 ± 0.007	0.079 ± 0.003
DSM 2152	98.5 ± 0.2	55.2 ± 3.2	97.3 ± 0.2	0.203 ± 0.008	0.068 ± 0.003

Hijosa-Valsero et al. (2018) New Biotechnology 46: 54-60.

Brewers' spent grain

		Physicochemical treatment optimal conditions (RSM)			Hydrolysate composition	
Pretreatment	Selected substance	T (°C)	t (min)	Reagent (%, w/w)	Total sugars (g/L)	Total inhibitors (g/L)
Autohydrolysis	-	185.4	21.8	-	27.09	3.56
Acid	HNO_3	114.2	11.0	2.08	32.01	1.77
Alkali	Not efficient	-	-	-	-	-
Solvent	Not efficient	-	-	-	-	-
Surfactant	Not efficient	-	-	-	-	-

Clostridium beijerinckii **CECT 508**

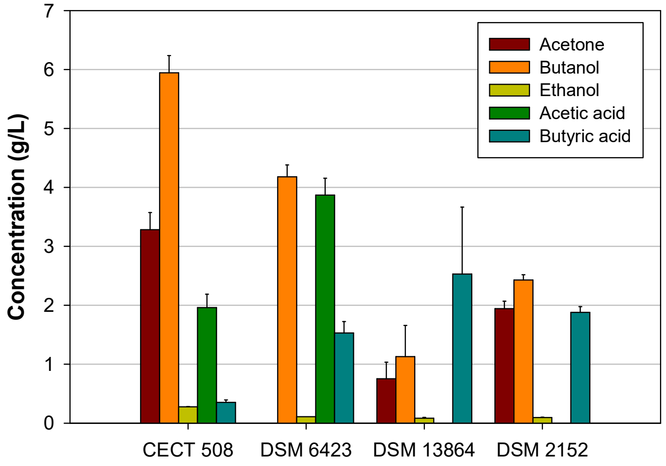


- The hydrolysates are not readily fermentable.
- The pretreatments applied are not efficient for brewers' spent grain.

Coffee silverskin

		Physicochemical treatment optimal conditions (RSM)			Hydrolysate composition	
Pretreatment	**Selected substance**	**T (°C)**	**t (min)**	**Reagent (%, w/w)**	**Total sugars (g/L)**	**Total inhibitors (g/L)**
Autohydrolysis	-	163.8	5.0	-	13.03	2.04
Acid	H_2SO_4	123.6	53.8	1.47	24.08	3.13
Alkali	Not efficient	-	-	-	Too low	-
Solvent	Not efficient	-	-	-	-	-
Surfactant	Not efficient	-	-	-	-	-

		Physicochemical treatment optimal conditions (RSM)			Hydrolysate composition	
Pretreatment	**Biomass (%, w/w)**	**T (°C)**	**t (min)**	**Reagent (%, w/w)**	**Total sugars (g/L)**	**Total inhibitors (g/L)**
Autohydrolysis	20	170	20	-	34.39 ± 2.61	5.26



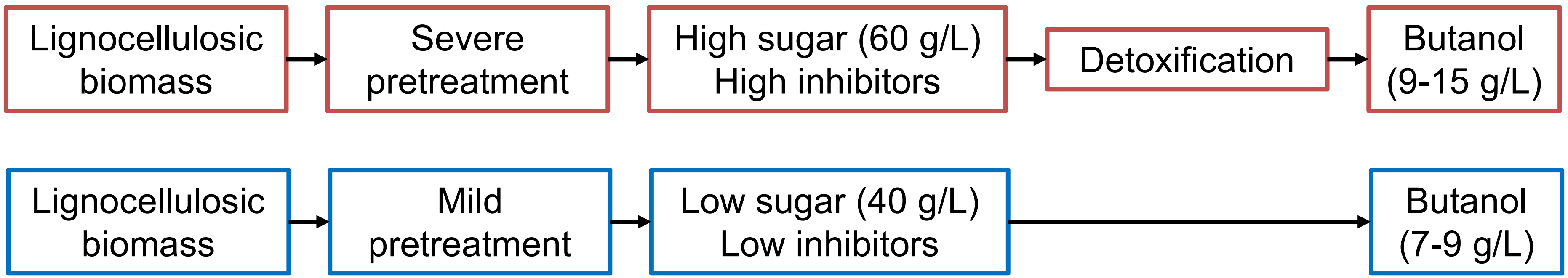
Hijosa-Valsero et al. (2018) Microbial Cell Factories 17: 154.

Conclusions

5. Conclusions

- Simple physicochemical pretreatments can be efficient.
- Each agrofood waste needs a specific pretreatment.
- Pretreatment conditions (T, t, reagent concentration) must be optimised.
- Pretreatments should focus on sugar maximisation and inhibitor minimisation in order to avoid detoxification steps.
- However, this leads to low sugar concentrations in the hydrolysate (40 g/L).
- These hydrolysates can produce broths with 7-9 g/L butanol after fermentation.
- Strain screening is essential to improve butanol production from lignocellulosic biomass.

- Change of paradigm?



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www.waste2fuels.eu

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