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Evaluation of cold plasma treatment for enhanced the enzymatic hydrolysis of lignocellulosic waste stream

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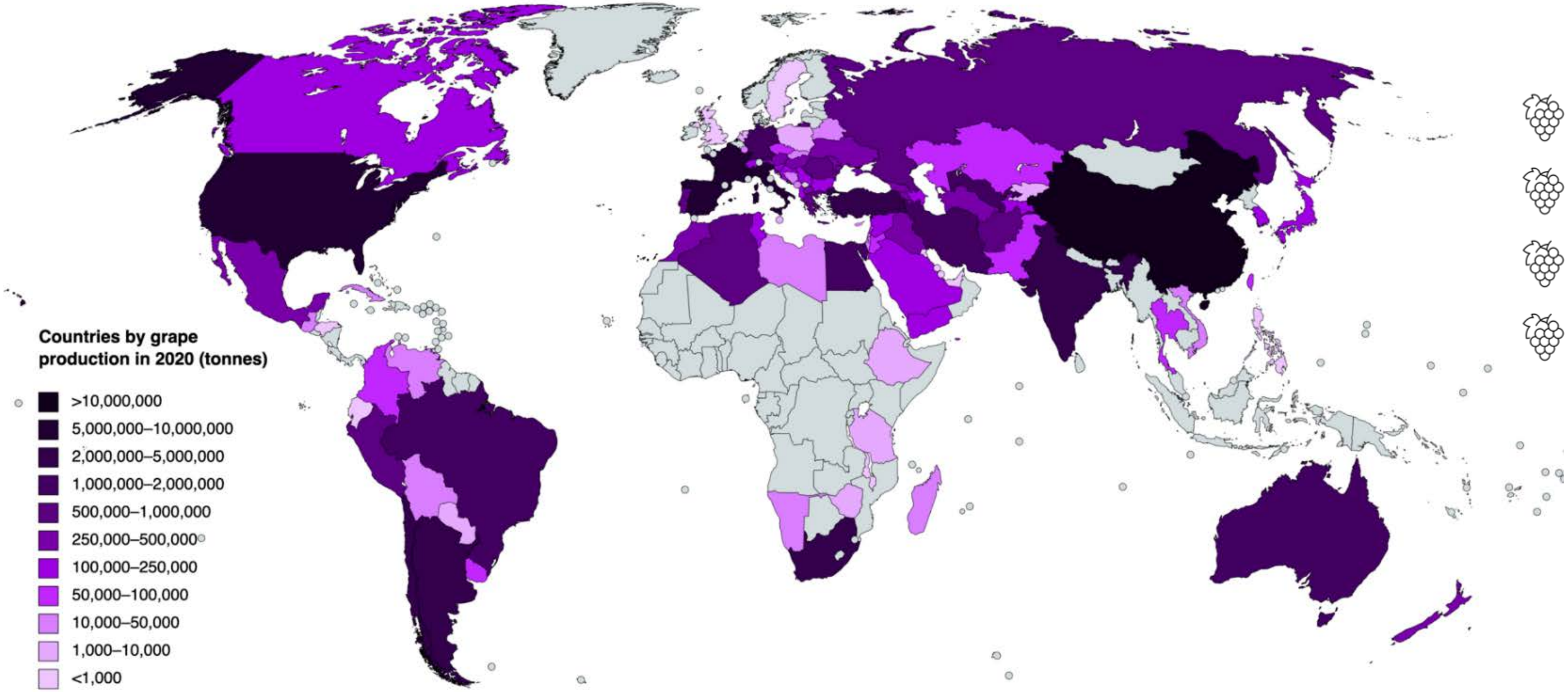
Objectives

- Characterisation of raw material
- Pretreatment with non-thermal plasma
- Enzymatic hydrolysis
- Production of biobased succinic acid

Global grape production



- Fresh
- Dried as resins
- Jams, jellies & juices
- **Wine (57% of grapes)**

The estimated total grape production worldwide in 2020 was 78,03 million t (FAOSTAT)

Grape stalks → 5% of grapes → 3.9 million t grapes stalks

Lignocellulosic biomass

Hernández-Beltrán, Javier Ulises, et al. "Insight into pretreatment methods of lignocellulo biomass to increase biogas yield: current state, challenges, and opportunities." *Applied sciences* 9.18 (2019): 3721.

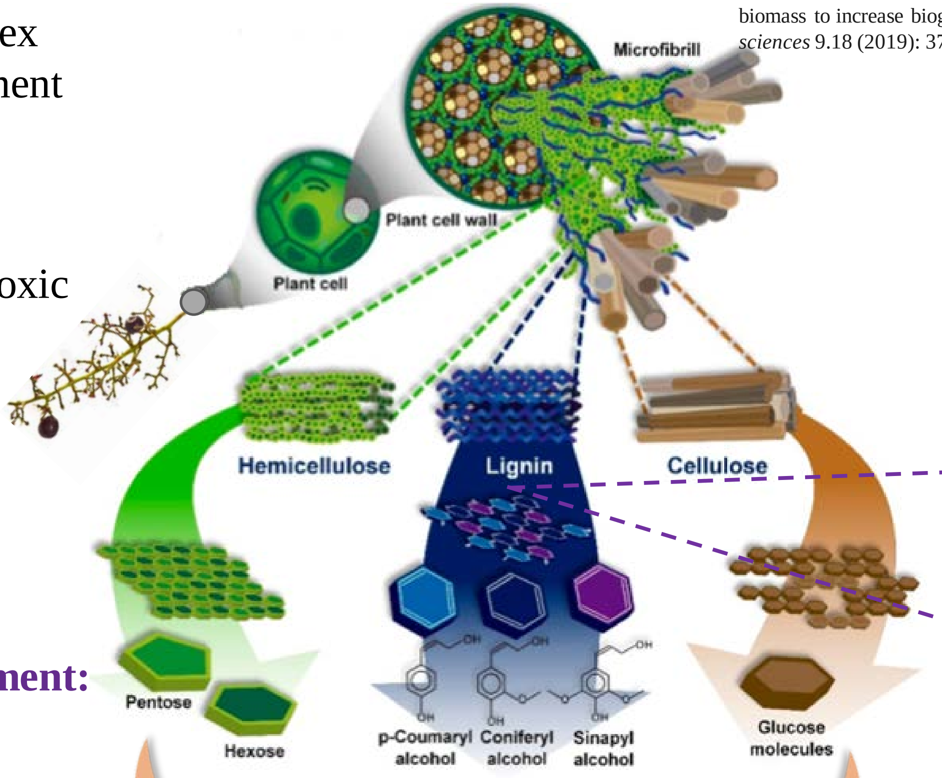
One of the most common way to break down the complex structure of lignocellulosic biomass is chemical pretreatment

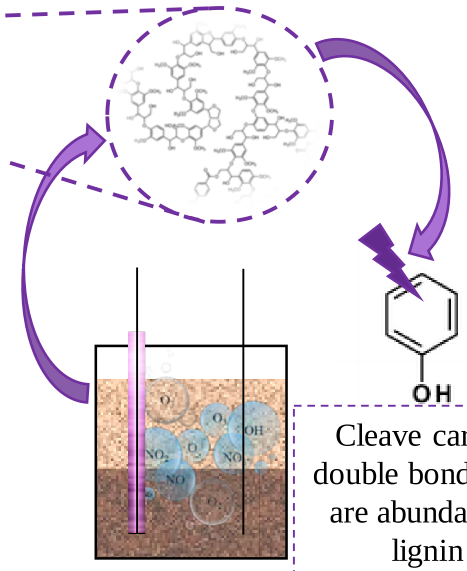
Acid pretreatment

- ✓ high sugar yield
- ✓ increase the formation of inhibitors resulting in a toxic reaction mixture unsuitable for microbial growth

Alkali pretreatment

- ✓ high lignin removal
- ✓ reduces the residual solid concentration





Cleave carbon double bonds that are abundant in lignin

Could be bio-converted through microbial fermentation in several added-value products

Advantages of plasma treatment against chemical treatment:

- Environmentally friendly and sustainable alternative to conventional thermochemical processes
- Ambient temperatures and pressures
- Dry gases instead of chemicals and solvents
- Maximize the efficiency of enzymatic hydrolysis

Disrupt linkages within lignocellulosic matrix

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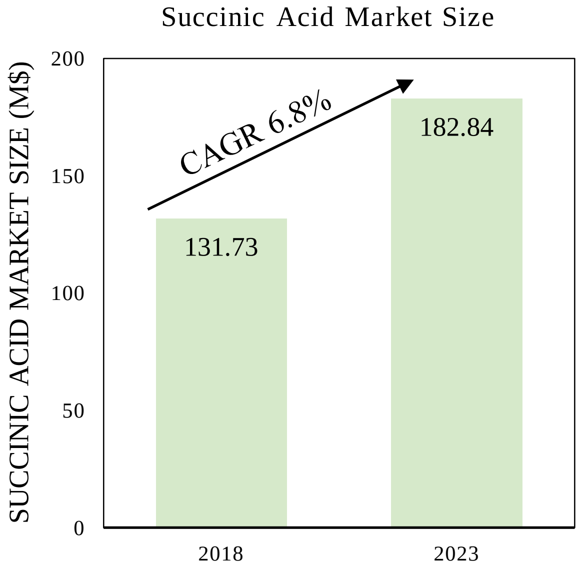
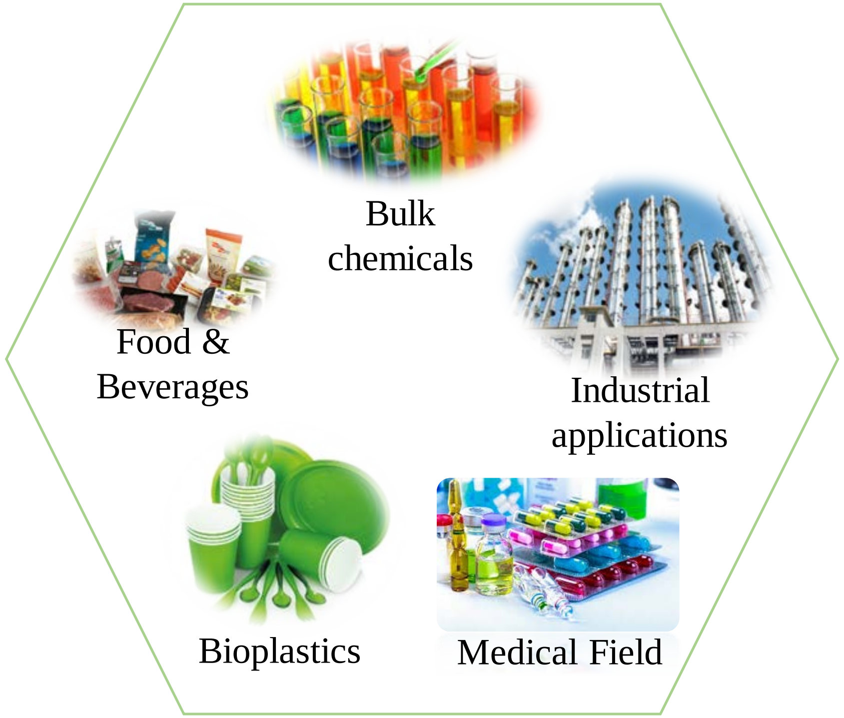
Bio-based Succinic Acid

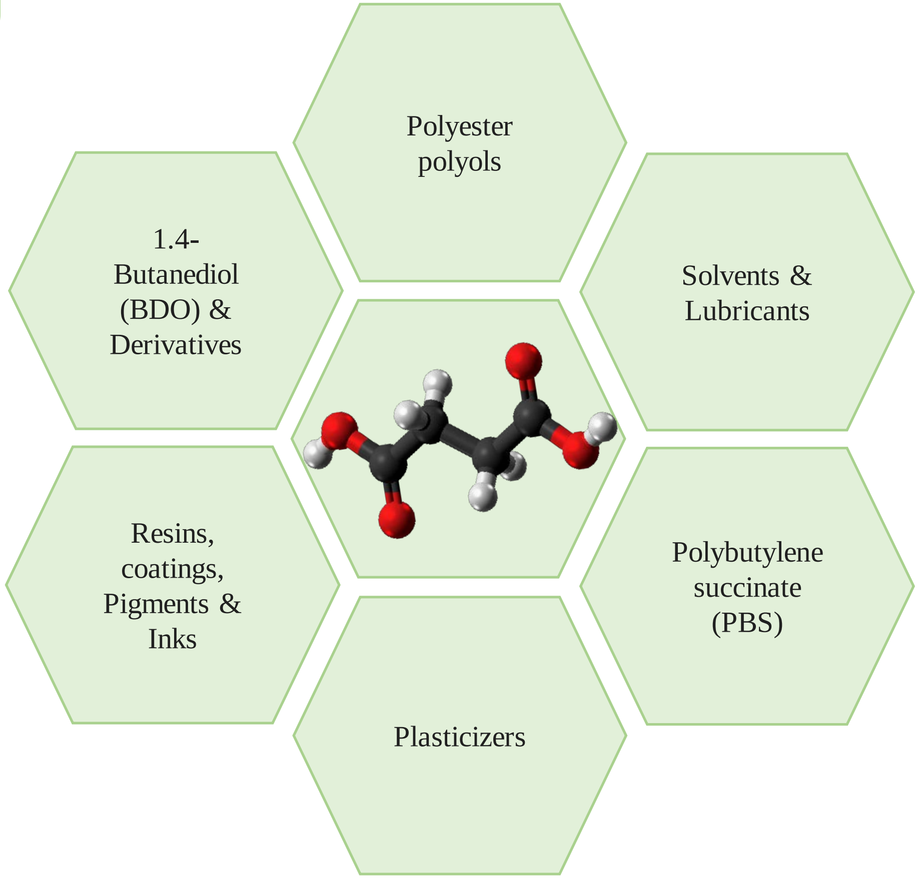
Succinic acid is one of the top 12 high-value-added bio-based chemicals
Intermediate in several chemical processes



Source: Markets and Markets



Industrial Applications



*CAGR: Compound annual growth rate

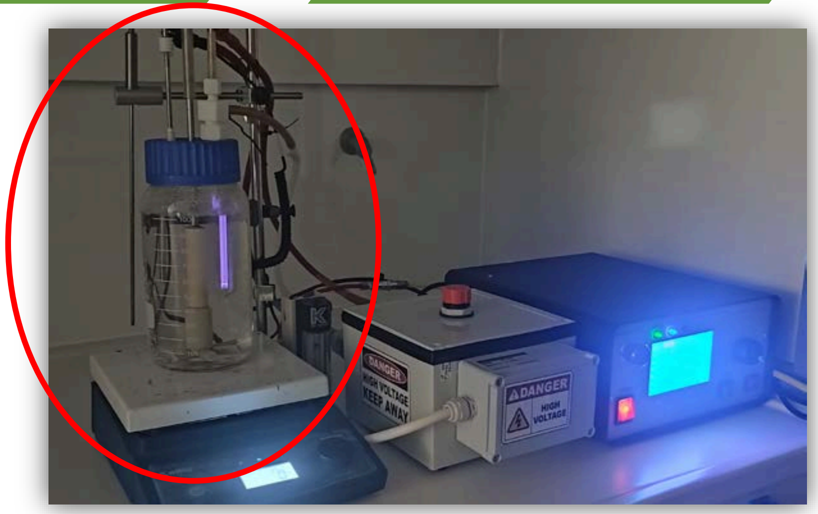
Methodology

Grape Stalks → Non- Thermal Plasma Treatment → Enzymatic hydrolysis → Succinic acid production

Pretreatment of grape stalks with non-thermal plasma

Conditions that remain constant in all cases

- Working volume: 1 L
- Ambient temperature
- Mechanical stirring: 350 rpm
- Voltage: 200 V
- Duty cycle: 250 μs
- Frequency: 60 Hz



Evaluate different pretreatment parameters such as:

- Pre-treatment durations (30, 60, 120, 180, 360 min)
- Atmospheric air supply (1, 2.5 vvm)
- Discharge frequencies (500, 1000 Hz)
- Initial solids concentrations (50, 100, 150 g/L)

Methodology

Grape Stalks → Non- Thermal Plasma Treatment → Enzymatic hydrolysis → Succinic acid production

Enzymatic hydrolysis

Pretreated solids were subjected to enzymatic hydrolysis using commercial enzyme cocktail composed of:

- cellulase, hemicellulase and β-glucosidase

The enzymatic hydrolysates were carried out:

- At 50°C
- For 48 h
- pH: 5
- 100 g/L solid concentration
  - Deionised water (ddH_2O)
  - Liquid obtained after plasma treatment

Succinic acid production

- Batch Fermentation
- Strain: *Actinobacillus succinogenes*
- Working volume: 500 mL
- Carbon source: Enzymatic hydrolysate produced from grape stalks pretreated with non-thermal plasma
- Nitrogen source: 5 g/L yeast extract
- Temperature: 37°C
- pH: 6.7
- Aeration: 0.1 vvm CO_2

Grape stalks composition

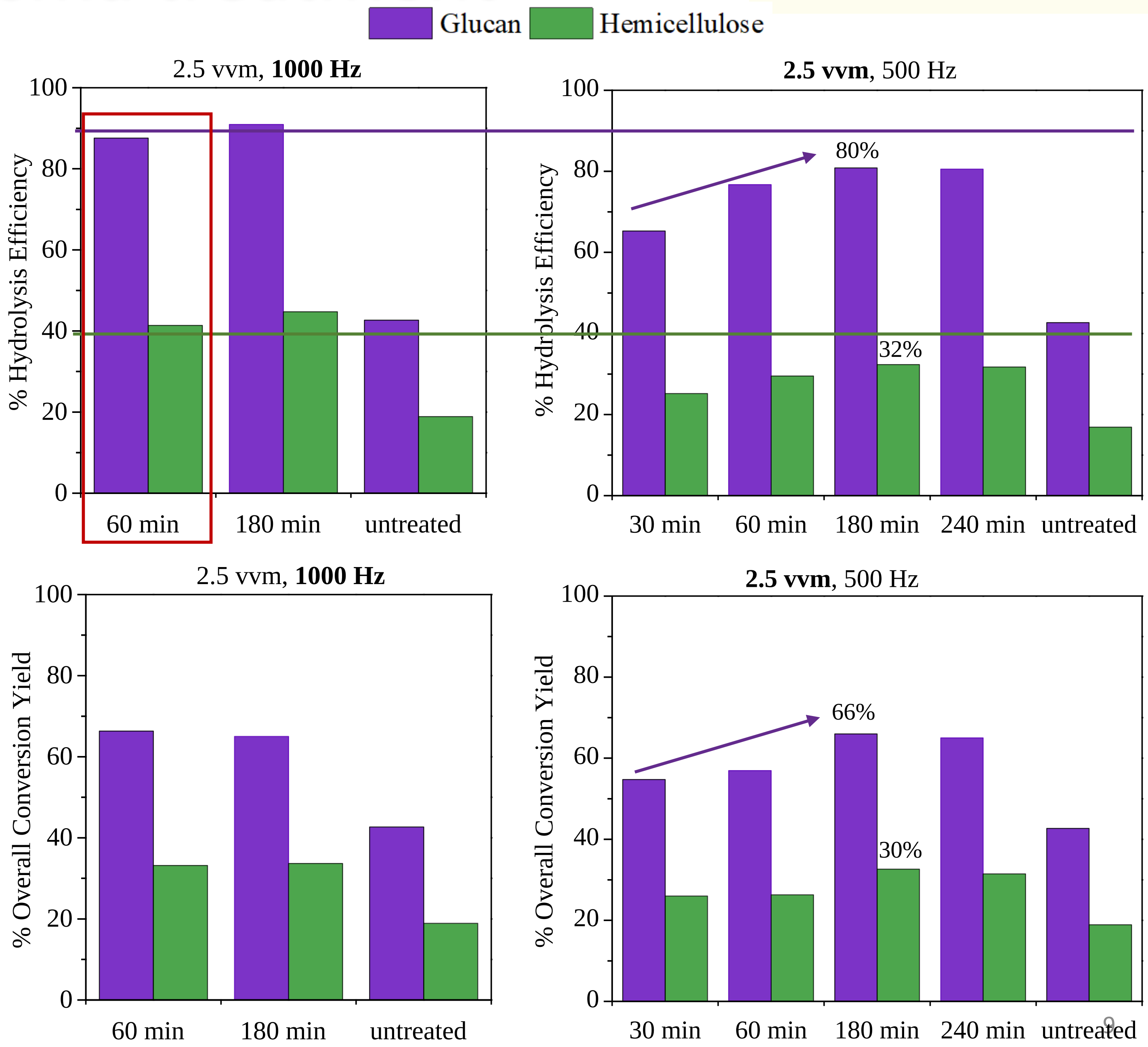
Determination of Structural Carbohydrates and Lignin in Biomass proposed by the National Renewable Energy Laboratory (NREL)

Composition (%, dry basis)	This Work	Literature
Lignin	30.2	17.4 – 40.6
Cellulose	22.4	20.8 – 36.3
Hemicellulose	12.4	11.5 – 24.5
Xylan	4.8	4.2 – 8.5
Galactan	2.6	1.3 – 2.8
Arabinan	2.4	2.0 – 3.1
Mannan	2.6	1.5 – 3.6
Protein	9.2	6.1 – 11.8
Lipids	9.7	4.7 – 10.3
Free sugars	4.8	1.3 – 5.4
Ash	5.2	3.9 – 7.7

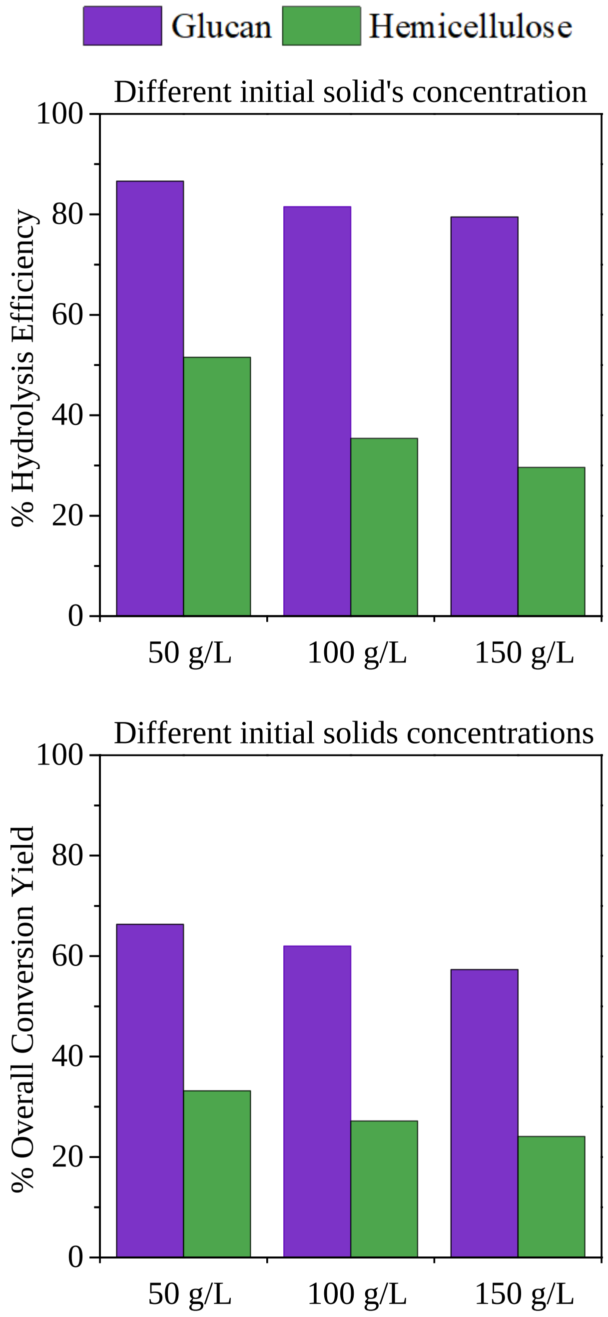
Plasma treatment

Influence of aeration applied in non-thermal plasma treatment, in the enzymatic hydrolysis of the pretreated solids in different pretreatment durations

Conditions	
Particle size	0.25 mm
Applied Voltage	200 V
Frequency	60 Hz
Duty Cycle	250 μs
Frequency discharge	**500, 1000 Hz**
Aeration supply	**2.5 vvm**
Solids concentration in plasma treatment	50 g/L
Solids concentration in enzymatic hydrolysis	100 g/L



Plasma treatment

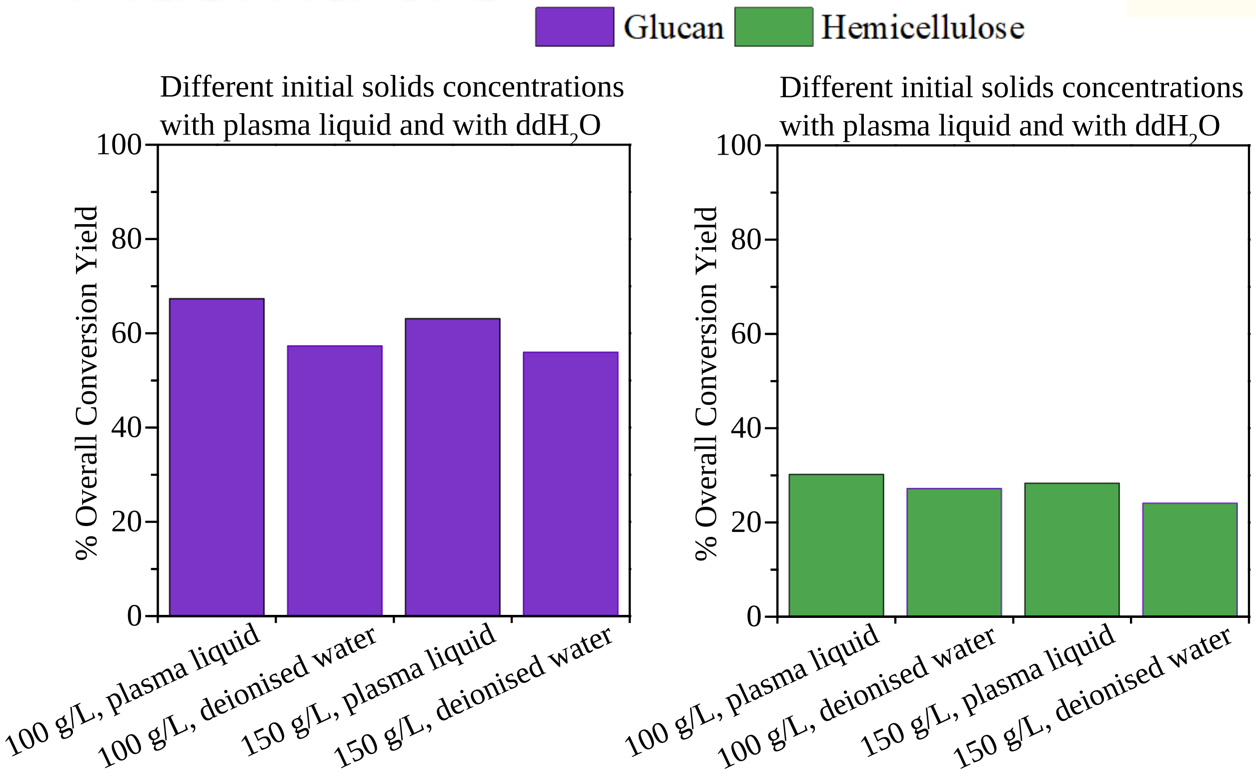


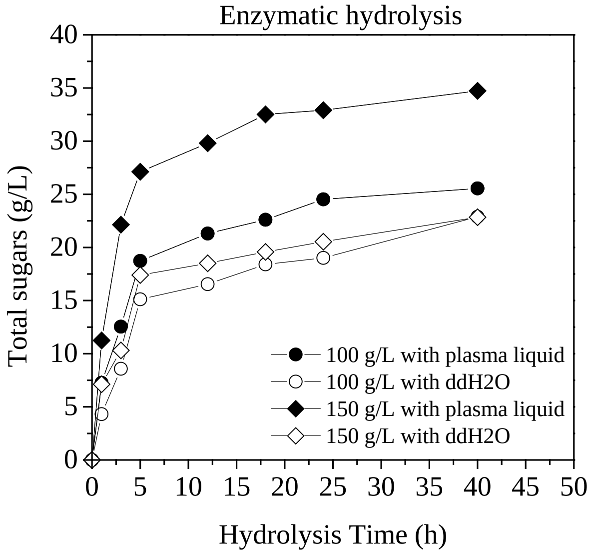
Conditions	
Particle size	0.25 mm
Applied Voltage	200 V
Frequency	60 Hz
Duty Cycle	250 μs
Frequency discharge	**1000 Hz**
Aeration supply	**2.5 vvm**
Solids concentration in plasma treatment	**50, 100, 150 g/L**
Solids concentration in enzymatic hydrolysis	100 g/L

The increase of the initial solid concentrations in non-thermal plasma treatment, significantly reduces the yield of hemicellulose, but a little effect presented in the case of glucan

Plasma treatment

Conditions	
Particle size	0.25 mm
Applied Voltage	200 V
Frequency	60 Hz
Duty Cycle	250 μs
Frequency discharge	1000 Hz
Aeration supply	2.5 vvm
Solids concentration in plasma treatment & enzymatic hydrolysis	**100, 150 g/L**
Hydrolysis Liquid	**ddH_2O, plasma liquid**



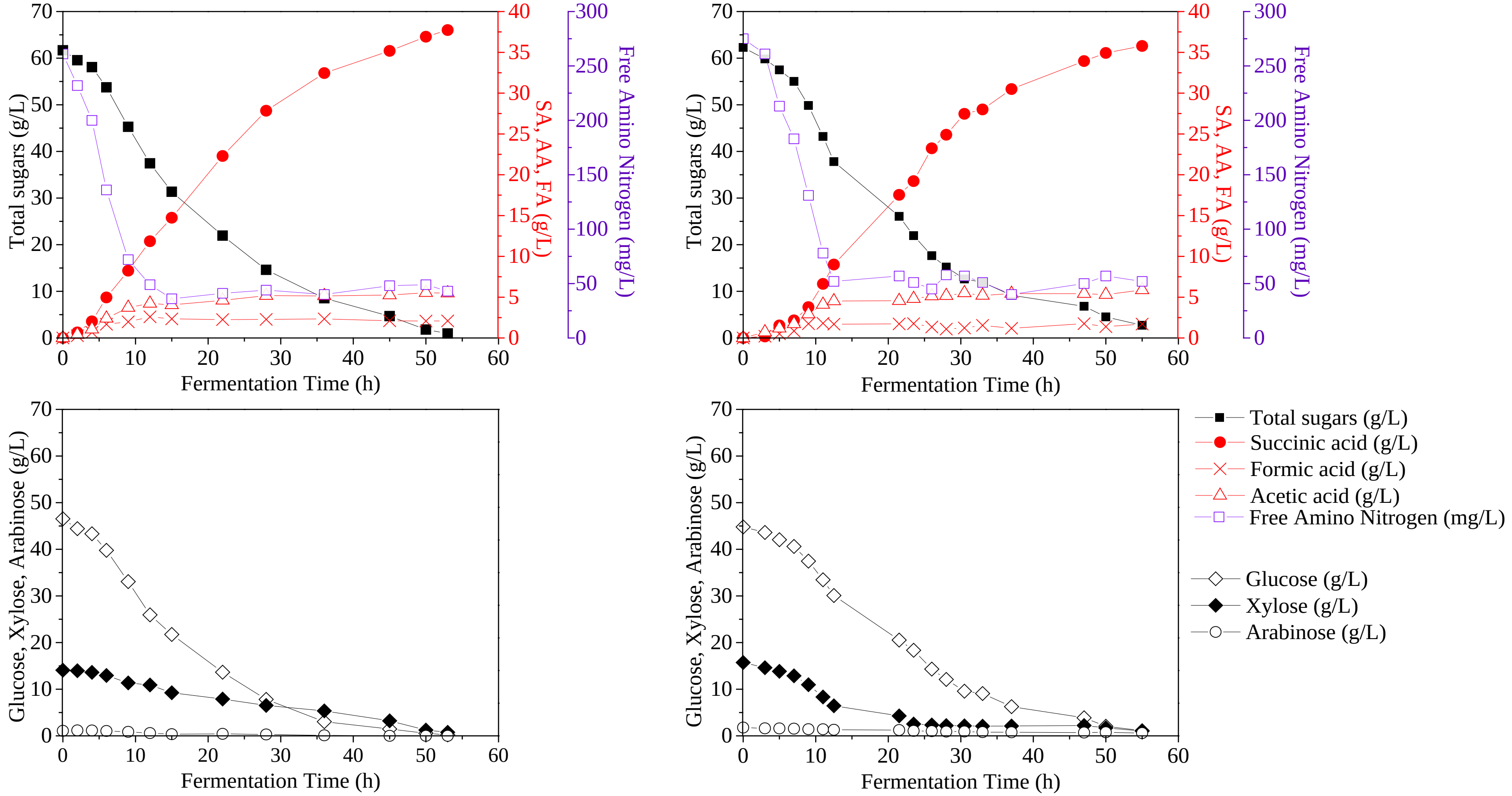


The overall conversion yields were higher when hydrolysis performed directly after non thermal plasma treatment

Succinic Acid Production

Simulated commercial sugars

Hydrolysate produced after plasma treatment



	Fermentation Time (h)	Succinic acid (SA) (g/L)	By-products: SA ratio (g/g)	Yield gSA/gtotal sugars	Productivity (g/L/h)
Commercial sugars	53	37.7	0.21	0.66	0.71
Hydrolysate after plasma	50	34.9	0.21	0.64	0.70
Hydrolysate after alkali treatment	51	28.8	0.65	0.42	0.57

Conclusions

- The higher atmospheric air supply (2.5 vvm) has a positive effect in the enzymatic hydrolysis
- The increase of frequency discharge to 1000 Hz results in higher hydrolysis efficiency yield for both glucan (90%) and hemicellulose fraction (up to 40%)
- The increase of the solid concentration in plasma treatment gave similar results in the case of glucan hydrolysis yield but seems to affect the hemicellulose hydrolysis
- The hydrolysis conducted directly after plasma treatment (using both solid and liquid fraction) resulted in higher yields comparing with that obtained with deionised water
- The effectiveness of non-thermal plasma treatment was further confirmed with the efficient production of bio-based succinic acid

Thank you for your attention!

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