Chemical pretreatment of *Hippophae rhamnoeides* prunings towards their biotechnological exploitation through anaerobic digestion

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1. Theoretical background

1.a Lignocellulosic biomass

Lignocellulosic biomass

Rich and abundant source of nutrients

High exploitation potential for renewable energy production

Various sources (residues & by-products)

Agro-industrial residues

- Forestry
- Livestock
- Municipal solid waste
- Other

Consist of three structural polymers

Cellulose | Hemicellulose | Lignin

Sugars' polymers (Cellulose, Hemicellulose)

Phenols' polymer (Lignin)

Various types of exploitation processes

- Thermal
- Biochemical
- Chemical

Production of platform chemicals, value added products

Conversion into solid, liquid, gas fuels

1.b Wood-related residues

Various sources
Forestry
Agro-industrial sources
Municipal solid waste

Various forms
Sawdust
Shavings
Twigs, leaves
Prunings from crops
Gardening residues

- **High environmental impact due to their improper treatment**
- **Unexploited waste streams**

Agro-industrial sources
- Prunings from tree cultivations e.g., olive trees, citrus fruit trees etc.
- Production at an annual base
- Improper ways of treatment are usually applied

Combustion

- Greenhouse effect (CO_2)
- Fires → Loss of valuable forests
- Non-recovery of nutrients and energy

Deposition in landfills

- Non-recovery of nutrients and energy
- High environmental impact

Urgency for sustainable treatment techniques

Shaving and deposition in fields

- Soil fertilizer
- Deteriorates quality of soil, since microbial growth (fungi) is enhanced → Devasting for crops

1. c Sustainable solutions and impediments

Energy recovery

Thermal processes
(pyrolysis, gasification etc.)

Biochemical processes
- aerobic composting
- anaerobic digestion

Nutrient and energy recovery

Anaerobic digestion

A complex sequence of biological processes where organic matter is turned into **biogas** (mixture of $\mathbf{CH_4}$ and CO_2) under anaerobic conditions.

Biogas can be turned into heat and/or electrical energy

Lignocellulosic materials are hard to depolymerize due to their complex nature

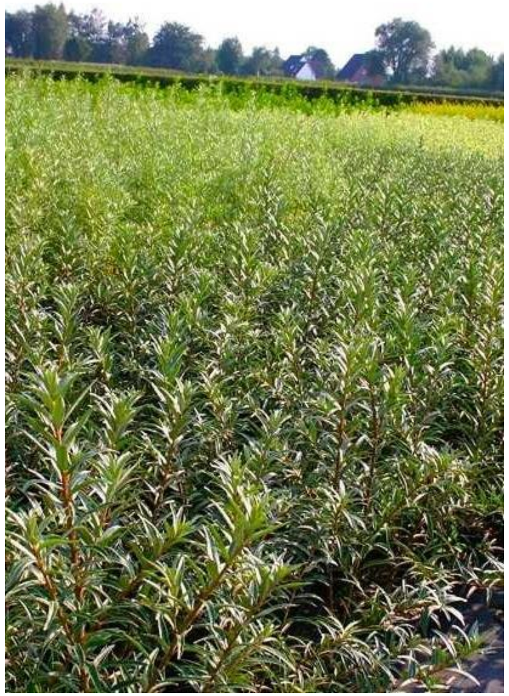
Pretreatment methods are usually applied to make cellulose and/or hemicelluloses more easily accessible by microorganisms

Several types of pretreatment each with a different effect on polymers structure

Chemical pretreatment with dilute acid (H_2SO_4, HCl, H_3PO_4 etc.). Affects hemicellulose structure

Easy
Fast
Reliable method
Production of inhibitors (furfural, HMF etc.)

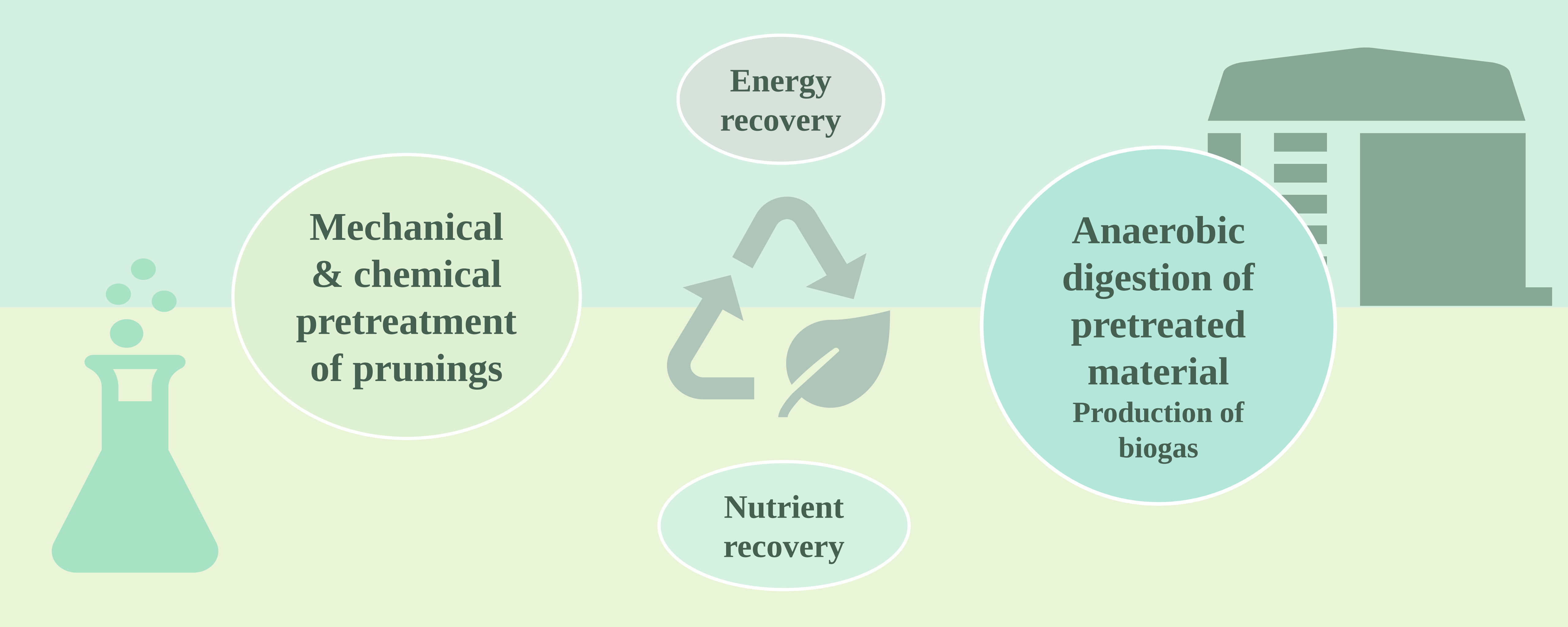
1.d Hippophae rhamnoeides (Sea-buckthorn) prunings: An emerging type of wood-related lignocellulosic residue



- **Sea-buckthorn**, scientifically known as ***Hippophae* sp**. is a plant cultivated mainly in Europe for its little, orange fruits, which are considered as **superfoods**, due to their high nutritional value.
- **Prunings** occur as by-products during the harvesting process of the fruits.
- Rising interest of consumers for superfoods → Increase in sea-buckthorn crops → Increase in agro-industrial by-products, such as prunings.
- Data towards the quantities produced annually are hard to evaluate.

2. Our project:
Chemical pretreatment and anaerobic digestion of *H. rhamnoeides* prunings

2. Chemical pretreatment and anaerobic digestion of H. rhamnoeides prunings



3. Areas of research

3. *Areas of research*

Mechanical and chemical pretreatment of prunings

How to maximize sugars depolymerization and solubilization from material to enhance biodegradation in anaerobic digestion.

- **Mechanical pretreatment**
  Decrease volume, break down bonds, ease handling
- **Chemical pretreatment with dilute acid (H_3PO_4)**
  Depolymerize hemicellulose, ease access to cellulose

Anaerobic digestion of pretreated material

Discover the potential of the material for methane production and evaluate the pretreatment effect.

- **BMP tests**

4. Experimental methods

4. Experimental methods

Mechanical pretreatment

Separation of leaves & twigs from fruits

Shredding & Freeze-drying

Milling

Mechanically pretreated material
Mean particle diameter < 0.04 μm

Chemical pretreatment with dilute H_3PO_4

Addition of H_3PO_4 to milled material for an adequate time period and under desirable conditions

Thermochemically treated material (hydrolyzed)

LIQUID FRACTION
Rich in monomeric sugars due to hemicellulose hydrolysis

SOLID FRACTION
More easily accessible cellulose

4. Experimental methods

Parameters tested

- Temperature
- Duration of pretreatment
- Acid concentration
- Feedstock loading

Parameter tested	Ranges of values tested
Temperature	100 - 131^{o}C
Duration of pretreatment	15 – 120 min
Acid concentration	1 – 4 % v/v
Feedstock loading	4 – 40 % w/v

The operational parameters affecting hydrolysis that were tested, were evaluated based on:

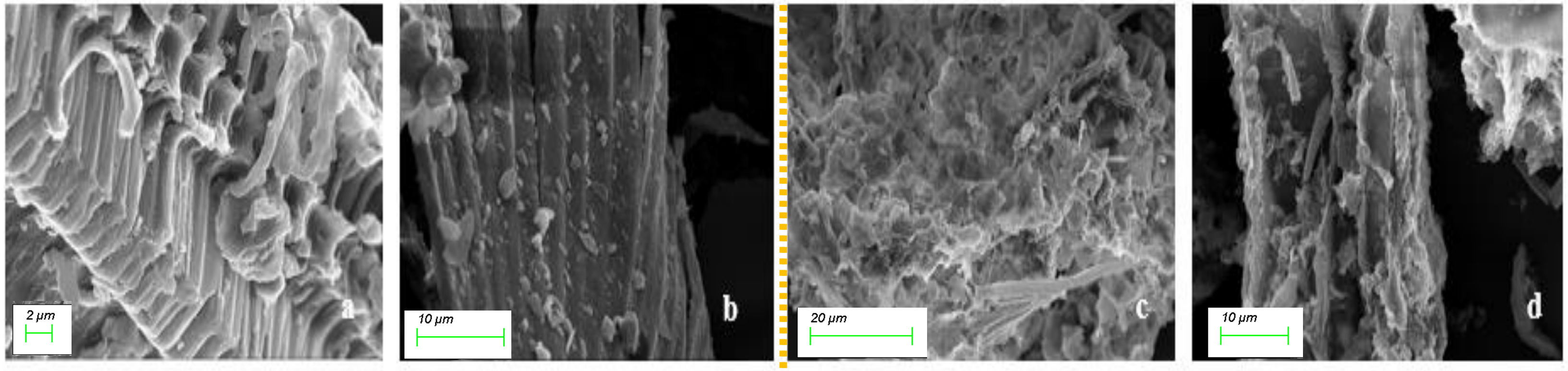
- Maximizing the sugar content on the liquid fraction of hydrolysate
- Minimizing the potential inhibitor content on the liquid fraction of hydrolysate

5. Results

5. Results

Physicochemical properties of milled material	
Cellulose (%dry)	36.51 ± 1.10
Hemicellulose (%dry)	17.04 ± 0.34
Lignin (%dry)	39.45 ± 2.12
Ash (%dry)	0.67 ± 0.09

The high lignin content means depolymerization of holocellulose is facing a barrier.



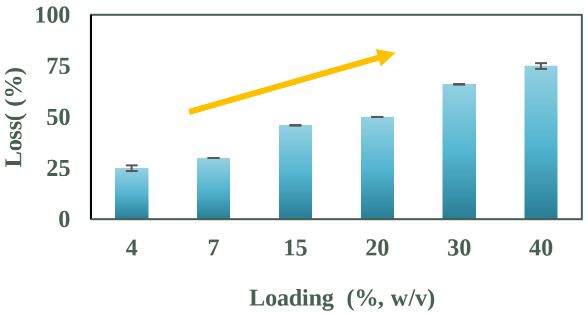
Before hydrolysis

After hydrolysis

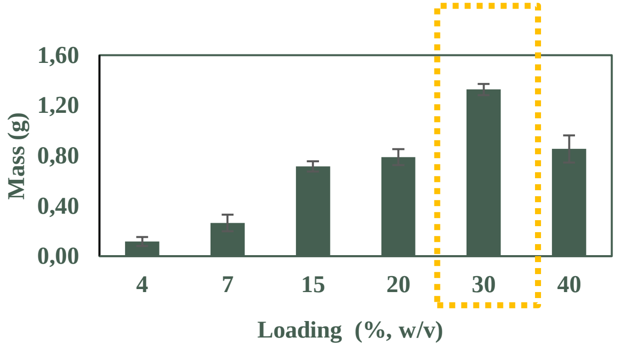
- Comparing the SEM images of the untreated and treated material, after hydrolysis the material is **more porous**, has **more cavities** and its **surface is rougher**.
- Optical confirmation that chemical pretreatment has effect on material's structure.

5. Results

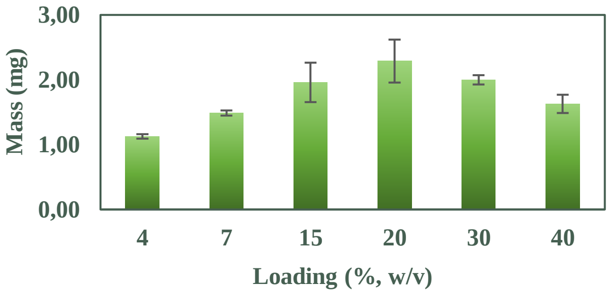
Effect of feedstock loading



Volume loss



Carbohydrates

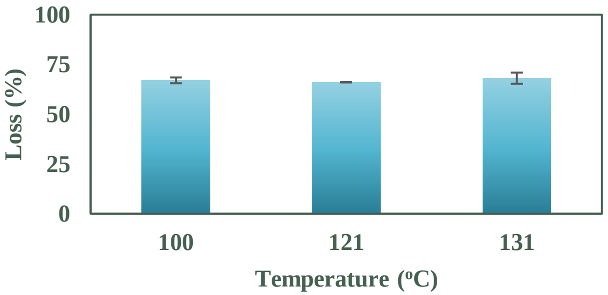


NH_3-N

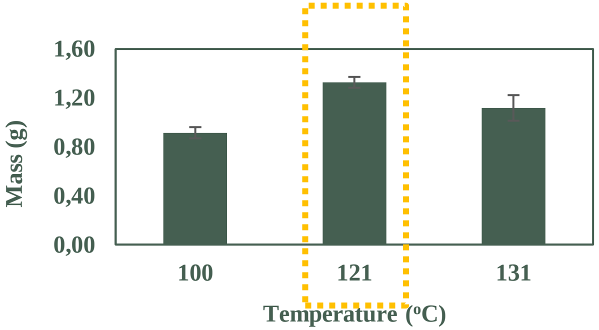
- Volume loss almost proportional with loading.
- Higher mass of sugars achieved at 30% loading.

Effect of temperature

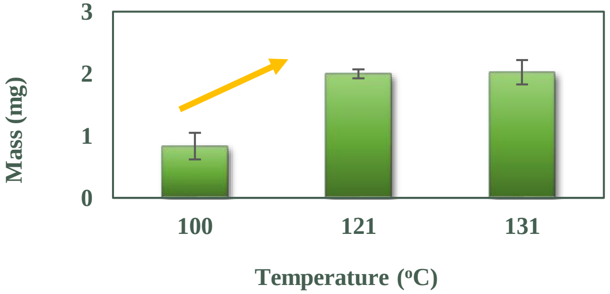
- Volume loss unaffected by temperature.
- Mass of sugars maximized at 121°C.
- Mass of NH_3-N stable after 121°C.



Volume loss



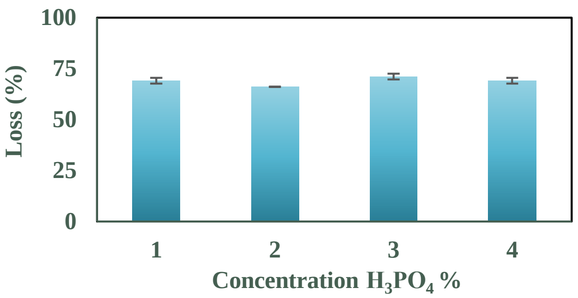
Carbohydrates



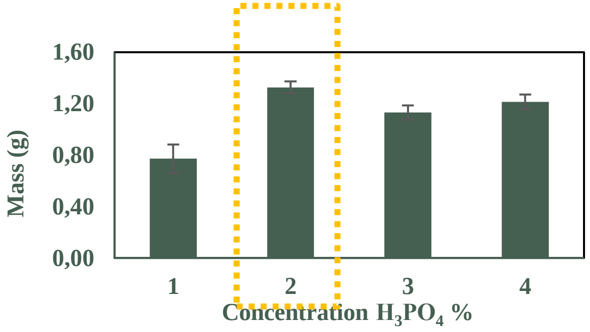
NH_3-N

5. Results

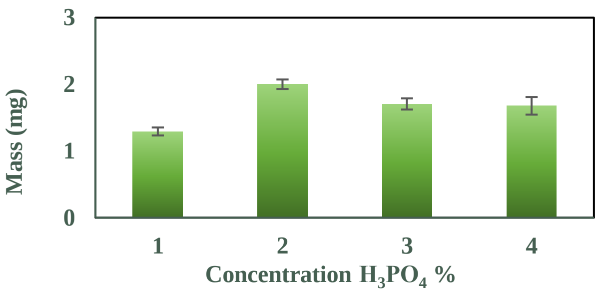
Effect of acid concentration



Volume loss



Carbohydrates

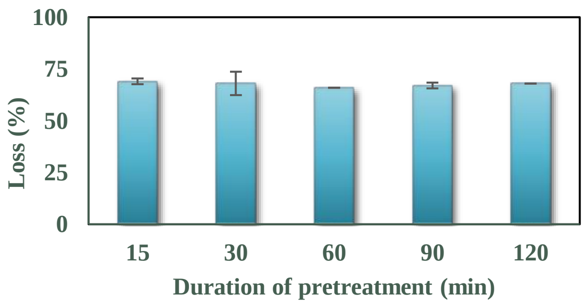


NH_3-N

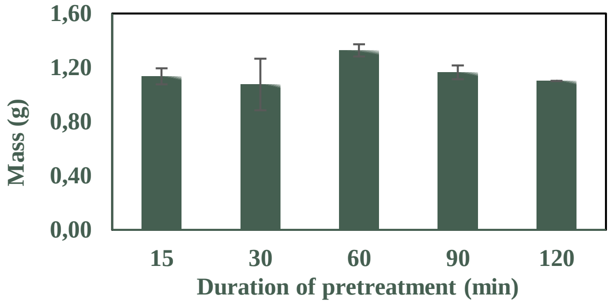
- Volume loss almost unaffected by acid concentration.
- Mass of sugars stable after 2% H_3PO_4.

Effect of duration of pretreatment

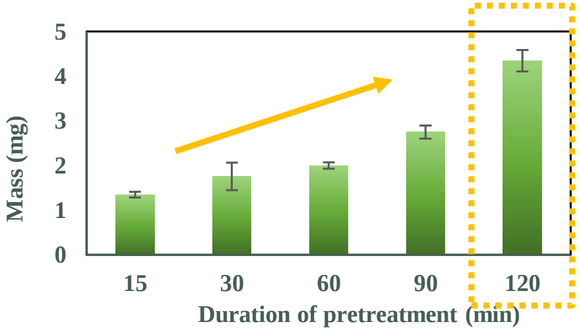
- Volume loss and mass of sugars almost unaffected by duration of pretreatment.
- Mass of NH_3-N increasing and reaching maximum at 120 min.



Volume loss



Carbohydrates



NH_3-N

6. Conclusions & Future research

6. Conclusions & Future research

- H_3PO_4 was effective towards hemicellulose saccharification. As expected, cellulose was not affected by dilute acid hydrolysis.
- Feedstock loading is an important parameter of hydrolysis, affecting volume loss and thereby, the composition of the liquid fraction of hydrolysates.
- The higher mass of sugars is achieved at a high loading (30%, w/v), yet higher yield of sugars is achieved at lower loadings (4%, w/v).
- Volume loss is not affected by the other parameters. Temperature has the bigger impact on sugars and duration of pretreatment on NH_3-N.
- The optimum hydrolysate was obtained under **121^{o}C, 120 min, 30 %, w/v, 2%, v/v H_3PO_4.**

- BMP tests could be done to verify potential of substrate towards biogas production.
- Study of hydrolysis with experimental design.

Thank you!

7. Acknowledgments

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