Biorefinery strategies
based on Room Temperature Ionic Liquids, hydrolases and their synergism with other pretreatments

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BIOREFINERY CONCEPT

Toward the valorization of the whole plant

Hemicellulose (20 - 30 %)
Lignin (15 - 30 %)
Cellulose (30 - 45 %)
Others (5 %)

Proteins, lipids, polyphenols, etc.

Glucose/cellobiose as PLATFORM MOLECULES or FERMENTABLES SUGARS

Synthesis of sugar esters from C₅ mono/oligomers: SURFACTANTS

Synthesis of ester derivatives of lignins: partially biosourced COMPOSITES


Lignocellulose constituents: some examples of application

Innovative and ecological strategies for valorization


https://www.valbran.eu/fr/
Poster no 22
Hemicellulose

Cellulose

Lignin

**Simplified view of recalcitrant LCB**

**PRETREATMENT : THE KEY TO UNLOCKING**

*Disorganization, compositional or structural changes?*

- **Example no 1**
  - Single disorganization

- **Example no 2**
  - Disorganization + fractionation
  - *Ex: delignification*

- **Example no 3**
  - Disorganization + fractionation + structural changes of biopolymers
  - *Ex: delignification*

ROOM TEMPERATURE IONIC LIQUIDS (RTILs)

Organic salt: organic cation and organic or inorganic anion

Interesting properties: non-volatile solvent, low melting point, low toxicity, capacity to solubilize various biomolecules, recycling, etc.

Solubilization of biopolymers

Eco-friendly properties

Corrosive effect

Biocompatibility

Hydrophilic\(^a\)

Imidazolium-based\(^b\)

(alkyl chain length < 4C)

No\(^c\)

Yes\(^d\)

Cellulases / Xylanases / S. cerevisiae / K. marxianus

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1-ethyl-3-methylimidazolium acetate

\([\text{C2mim}][\text{OAc}]\)

1-ethyl-3-methylimidazolium methylphosphonate

\([\text{C2mim}][\text{MeO(H)PO}_2]\)

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Lignocellulosic biomass

Incubation in RTIL
Temperature – duration

Regeneration step by adding water

Filtration and washing

Solid fraction

Enzymatic hydrolysis

Sugar monomers/oligomers-rich liquid fraction

RTIL-water mixture*

Water evaporation

Recovered RTIL*

Lignin extraction (patent protection)

*Containing lignin and others compounds
FLOWCHART

Schematic of the overall sequential process

Lignocellulosic biomass

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Mild conditions

110 °C
40 min
Schematic of the overall sequential process

1. **Lignocellulosic biomass**
   - Incubation in RTIL (Temperature – duration)
   - Regeneration step by adding water
   - Filtration and washing

2. **Solid fraction**
   - Enzymatic hydrolysis
   - Sugar monomers/oligomers-rich liquid fraction

3. **RTIL-water mixture**
   - Water evaporation
   - Recovered RTIL*

4. **Lignin extraction** (patent protection)

*Containing lignin and others compounds

**Mild conditions**

110 °C 40 min

**Characteristics**

- Structural properties (ssNMR, FTIR, XRD)
- Morphological & textural properties (ESEM)
- Chemical composition (Van Soest method, NREL, etc)
- Characterization, separation and/or quantification (HPAEC-PAD or UV)
APPLICATION TO LIGNOCELLULOSIC BIOMASSES

Agricultural residues

- Wheat straw
- Rape straw (pellets)
- Sunflower seed shells
- Wheat bran

Forest residues

- Spruce sawdust (Softwood)
- Oak sawdust (Hardwood)

Dedicated crops

- Miscanthus

Large representative panel of lignocellulosic biomasses
PRODUCTION OF GLUCOSE

Toward fermentable sugars or platform molecules

Significative improvement of enzymatic saccharification [C2mim][OAc] as promising RTIL for efficient pretreatment

Glucose yield (g / 100 g of dry matter)*

*Yield relative to cellulose content (%)
[C2mim][OAc] RECYCLING AND LIGNIN ACCUMULATION

Toward fermentable sugars or platform molecules

Focus on Spruce sawdust

Recycling and reuse at least until 7 times without loss of performances in spite of significative accumulation of lignin in the RTIL

*Obtained with new RTIL

Impact of RTIL-pretreatment: fractioning or disorganization?

**Chemical Composition of Solid Fraction**

Specific interest of RTIL to apply soft pretreatment on biomass in minimizing fractioning and depolymerization of polysaccharidic fractions

IMPACT ON STRUCTURAL PROPERTIES OF BIOPOLYMERS

What is happening about changes?

- XRD
- FTIR
- ssNMR

**Cristallinity index (%)**

**Glucose yield (g / 100 g of dry matter)**

- Spruce sawdust
- Oak sawdust
- Rape straw
- Miscanthus
- Wheat straw
- Sunflower seed shells
IMPACT ON STRUCTURAL PROPERTIES OF BIOPOLYMERS

What is happening about changes?

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**XRD**

**FTIR**

**ssNMR**

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![Graph showing crystallinity index and glucose yield](image)

- **Spruce sawdust**
- **Oak sawdust**
- **Rape straw**
- **Miscanthus**
- **Wheat straw**
- **Sunflower seed shells**

**Cristallinity index (%)**

**Glucose yield (g / 100 g of dry matter)**
IMPACT ON STRUCTURAL PROPERTIES OF BIOPOLYMERS

What is happening about changes?

Digestibility of cellulosic fraction not exclusively related to Crl depending on biomasses RTIL pretreatment: toward the preservation of each polymer

IMPACT ON TEXTURAL PROPERTIES OF SOLID FRACTION

SEM analyses for a better understanding

Focus on Miscanthus

A complex organization: highly fibrillar morphology of strongly agglomerated sub-micrometric particles

Disorganization resulting in an expanded material with irregular and more porous texture

RTIL pretreatment for a drastic disorganization of lignocellulosic matrix: toward a better accessibility for enzymes

**RTIL, Xylanases and Cellulases**

**Production of C₆ & C₅ sugar monomers**

- Wheat straw
  - [C₂mim][OAc] pretreatment (110 °C – 40 min)
  - Solid fraction no1
    - Xylanase⁹-catalyzed hydrolysis
      - Xylose-rich liquid fraction
        - $\gamma_{\text{xylose}} \sim 98\%$
    - Solid fraction no2
      - Cellulaseᵇ-catalyzed hydrolysis
        - Glucose-rich liquid fraction
          - $\gamma_{\text{glucose}} \sim 98\%$
  - RTIL recycling
  - Xylanases

**Complementarity of [C₂mim][OAc] xylanase and cellulase for total depolymerisation of carbohydrate polymers**

⁹Endo-xylanases from *Thermobacillus xylanilyticus* (UMR FARE)
ᵇCellulases from *Trichoderma reesei* (commercial)
RTIL, Xylanases and Cellulases in One Pot

Production of C₆ & C₅ sugar monomers

**Wheat straw (2% w/v)**

- Incubation in RTIL 110 °C – 40 min
- Adding aqueous buffer
- Xylanase & Cellulase
- Xylose and Glucose-rich liquid fraction

_Simultaneous process_

_A compromise: beneficial effects of H-bond network disruption of LCB versus detrimental effect of hydrolases denaturation_


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RTIL concentration in hydrolysis medium (% v/v)

Glucose or solubilized xylans yields (%)
RTIL & SUBCRITICAL WATER COMBINATION

Application to lignocellulosic wastes from oleaginous crops

Sunflowers seed shells or rape straw (from 2% to 10% w/v)

Sunflower seeds and rape straw were pretreated in a reactor with RTIL [C2mim][OAc] or [C2mim][MeOHPO2] under conditions of 200°C and 120 min, with a pressure range of 5-10 bars. The reactor volume was 560 mL.

Solid fraction no1

Cellulase-catalyzed hydrolysis

Glucose-rich liquid fraction

Y_{glc} = 78% vs 67% (single RTIL)

Liquid fraction no1

Water evaporation

Recovered RTIL

Lignin extraction (patent protection)

C_{5} sugars from hemicellulosic fraction

CONCLUSIONS - PERSPECTIVES

Versatility toward the varieties of lignocellulosic biomasses

Optimization: Factorial experimental design

POSTER no 23

C$_6$ / C$_5$ sugars from cellulosic and hemicellulosic fractions

Enzymatic processing or co-fermentation

Enriched-lignin fraction for further valorization

Biofuel, Bioproducts and Biomaterials
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