

OUTLINES

Wednesday 24th June



TO Kim Anh (SBFT)

~60min

Screening cellulosic fungi in term of hydrolysis ability-secretom.

COMA Veronique(LCPO)

~60min

Intermediate compounds during hydrolysis: identification and quantification.

Lunch time (CEMES)

FILLAudeau L., NGUYEN Tien Cuong & LE Tuan (LISBP)

Hydrolysis of pretreated lignocellulosic matrixes: focus on physical insight

~90min

LABORATOIRE D'INGÉNIERIE
DES SYSTÈMES BIOLOGIQUES
ET DES PROCÉDÉS





Véronique Coma

Laboratoire de Chimie des Polymères Organiques

IPB/ENSCBP, UMR 5629,

Université de Bordeaux France

veronique.coma@enscbp.fr

université
de **BORDEAUX**

UNIVERSITÉ
DE
BORDEAUX

4 Thematic Research Teams

Fields of expertise:

Polymer Chemistry

Physico-Chemical Properties of Polymers

Functional Polymer Materials

**Polymerization
Catalyses and
Engineering**

Prof. D. Taton

**Polymers Self-
Assembly and Life
Sciences**

Prof. S. Lecommandoux

**Advanced Functional
Polymer Materials**

**Biopolymers
and Bio-Sourced
Polymers**

Prof. H. Cramail

**Polymer Electronic
Materials and Devices**

Prof. G. Hadzioannou

Advanced functional polymer materials

Controlled
Polymerizations
Macromolecular
Engineering

Physical-
chemistry of
soft matter
(bulk and
solution)

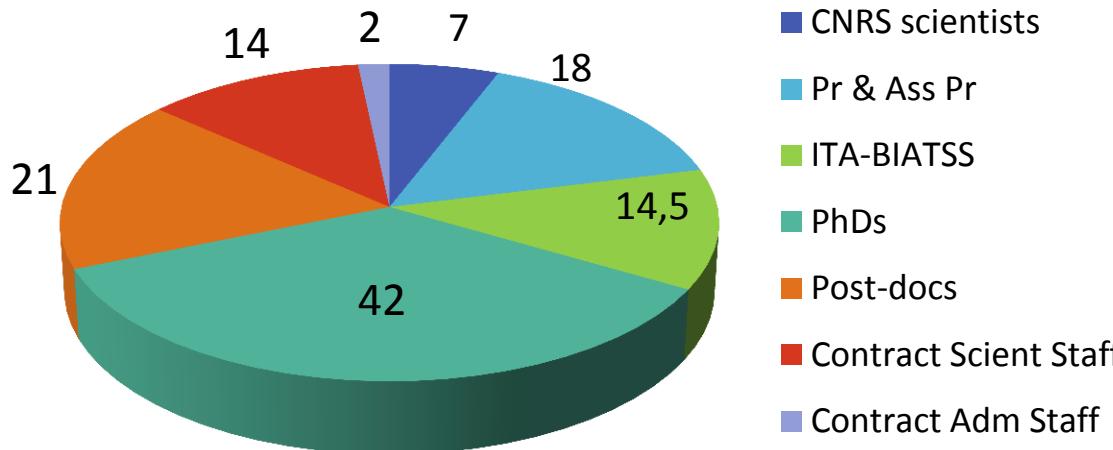
Green
catalysis &
Biomass
valorization

Advanced Polymer
Materials in the
fields of

Life Sciences
Energy
Sustainable
Technologies

All the people - June 2014

**120-140 people
devoted to
Polymer Science**



14.5 ITA-BIATSS

25 Scientists

Permanent staff : 39
Non permanent staff : 79
+ Master degree: 19

Main Equipments within LCPO – Polymer Chemistry



POLYMERIZATION REACTORS

- Supercritical reactor (scCO₂)
- Reactor for ethylene & propylene
- High pressure reactors (gas)
- Synthesis reactor
- Microwave reactor
- Glove boxes



LIGNOCELLULOSIC FIBER BIREFINERY

- Digester with circulation/Rotary
- Cutter mill grinder
- Ultracentrifugal mill

AQUEOUS SOLUTIONS AND SOLVENTS

- Solvent Purification System (Mbraun)
- Water Purification System (Millipore, Elga)

MOLECULAR STRUCTURE

- 2 NMR: ¹H, ¹³C & other nuclei (400 MHz)
- Fluorimeter
- UV-Vis-Fluorescence Spectrometer
- Access to 3 Mass spectrometers (1 MALDI-TOF)

MOLECULAR DIMENSIONS

- Osmometer (Osmomat 090 Gonotec)
- Tonometer
- Capillary viscometer (CK 300, Schott)
- SEC (H₂O, THF, DMF,) RI, UV & LS detectors
- GC (FID)

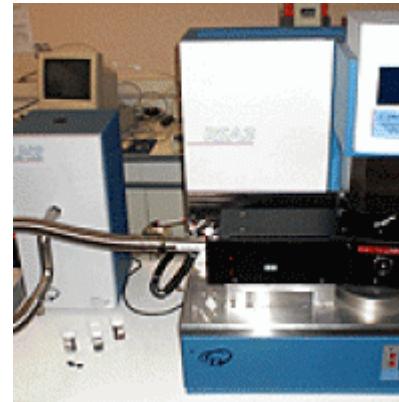
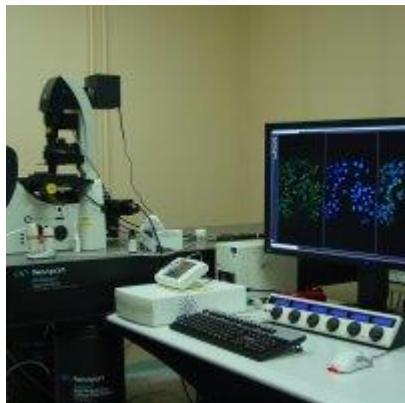


CPER 2007-2013 1.5 M€

Main Equipments within LCPO – Physical/materials testing

MICROSCOPIES

Dimension Icon AFM
 Fluorescence/DIC inverted microscope
 Inverted microscope
 Confocal microscope (Leica SP5)
 2 Bright-field upright micropscopes



COLLOIDAL STATE PROPERTIES

Multi-angle goniometer for SLS and DLS (ALV)
 DLS detector(Malvern, Cordouan)
 Zetameter
 Pendant and sessile drop tensiometer



THERMOMECHANICAL PROPERTIES

DSC Q100 TA Instruments
 DMA Imposed strain RSA 3 TA Instruments
 Imposed stress Rheometers
 Dynamometer (Instron)



ANTIMICROBIAL PROPERTIES

Antibacterial
 Antifungal

TRANSFER PROPERTIES AND INTERACTIONS

20MHz proton relaxometer
 Water vapor and oxygen permeability

Main Equipments within LCPO – Biology

Novel ‘Biotechnology’ platform

BIOCHEMISTRY & MOLECULAR BIOLOGY

Thermostated centrifuge
Ultrasonic processor
Freeze dryer
Automatic peptide synthesizer



CHROMATOGRAPHY

Gas Chromatography
HPLC



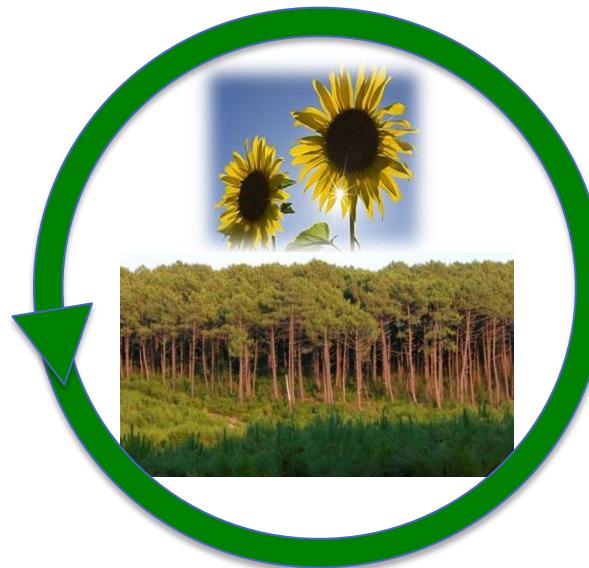
MICROBIOLOGY

Incubator
Sterilizer
Laminar flow hood
Freezer (-80° C) and climatic chamber
Fermentors



Biopolymers and Bio-Sourced Polymers

Team 2 created january 2011



Biopolymers and Bio-based Polymers Team

Objectives & Strategy

Objectives: Valorization of biomass for the design of high value-added bio-sourced 'synthons' and polymers
 Design of functional bio-based polymeric Materials with original features for sustainable technologies

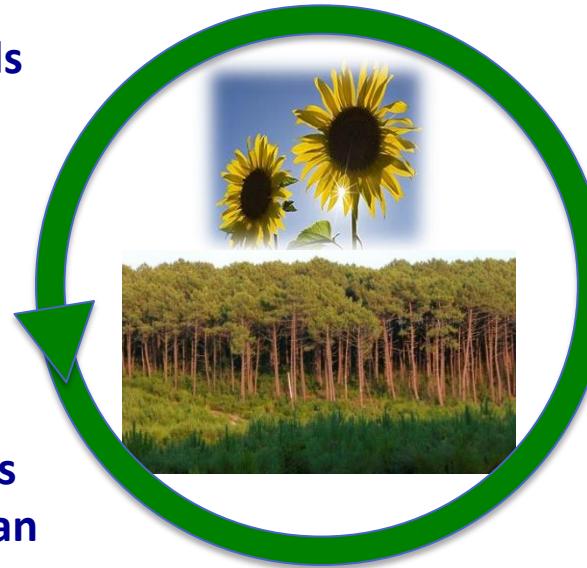


Bio-based Polymers from fatty acids & vegetable oils

*PU, NIPU, polyesters
 epoxy resins, PLA additives,
 Hyperbranched systems,
 waterborne latexes*

Advanced functional Materials based on cellulose and chitosan

*Nanofibrils, nanocrystals as emulsion stabilizers,
 porous materials, reinforcing agents.
 Bio-based materials with
 antimicrobial, anti-oxidative and barrier properties*



Biomass deconstruction & controlled depolymerization of lignocellulose

*Cellulosic fibers
 Oligosaccharides
 Phenolic derivatives*

Specific Analysis & purification

- *HPLC, GC/MS*
- *Preparative chromatography*
- *Fermenter*
- *Microbiology equipment*
- *Fiber morphology analyzer*

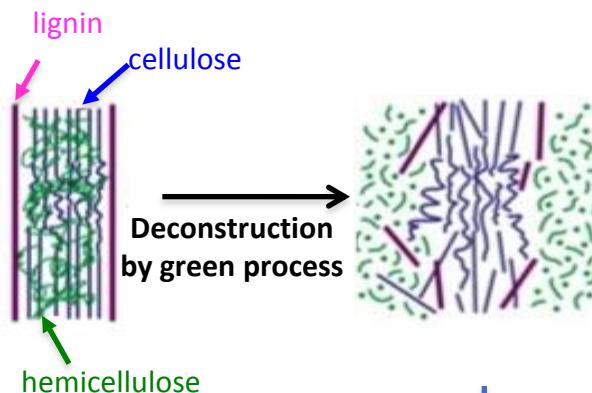
A- Biomass deconstruction & controlled depolymerization of lignocellulose



XYLOFOREST-XYLOCHEM

Platform “Forest-Wood-Fibre-Biomass for the Future”

A technical hall equipped with reactors for biomass deconstruction and fragmentation of biomass.
Fermenters for enzymatic modification of biopolymers



CELLULOSE →
OLigomers
SOLUBILITY

FERMENTESIBLE
SUGARS



Enzyme-based
processes

Well-controlled oligomers

New polysaccharides-based bio-oligomers with specific properties....for new bio-based materials

Wood

Cello-
oligosaccharides

Chitin

Cello-
chitooligosaccharides

- 🔥 Renewable feedstock
- 🔥 Abundant

HYDROPHILIC
POTENTIALLY BIOACTIVE
(chitin derivatives)
EASIER TO FUNCTIONALIZE
SOLUBLE

KEY POINT
CONTROL OF THE
DEPOLYMERIZATION TO
PRODUCE SELECTED dp

ACTIVE BIO-BASED AGENTS
for food, materials, or
biopesticides

Building block for
NEW BIOPOLYMER-BASED
MATERIALS

Crossing the biomass Resources....

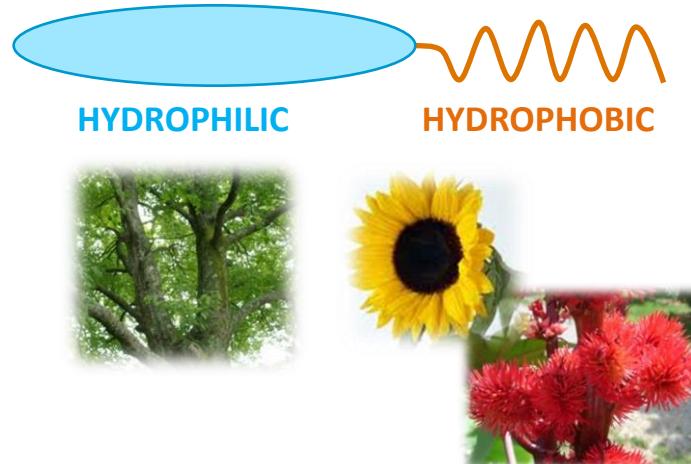
Oligosaccharide moiety linked to another oligo moiety or to a fatty chain

Source of polyssaccharides

Cello-oligosaccharides

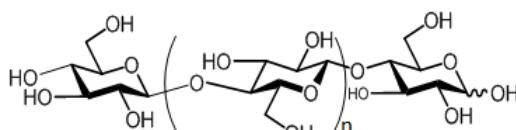
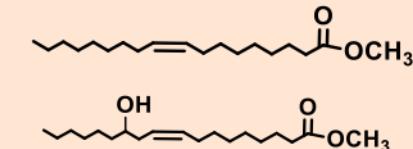
Xylo-oligosaccharides

Chito-oligosaccharides

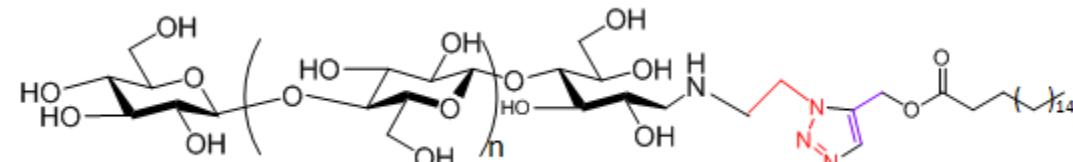


Oil

Fatty esters



Cellulose oligomers



Cellulose oligomers-*b*-stearic acid

OLIGOCELL



**Cellulose oligomer preparation
by enzymatic depolymerization
for the synthesis of new bio-
based and hybrid copolymers**

*Laboratoire de Chimie des Polymères Organiques
(UMR5629, Université Bordeaux 1-IPB-CNRS, 33607 Pessac)*

Elise BILLES

Supervisors: Frédéric PERUCH, Stéphane GRELIER and Véronique COMA



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POLYMIÈRES
ORGANIQUES
lcpo

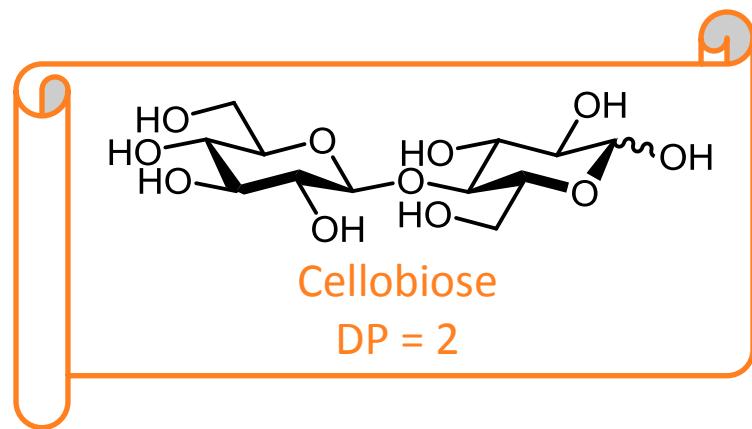
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Goals

1st goal

To obtain monodisperse water soluble cellulose oligomers

- DP \leq 10 soluble in water
- Not commercially available except DP 1 and 2
- Separation by solubilization processes in specific solvents



2nd goal

To use them to create block-copolymers totally bio-based or hybrid

- Properties of both blocks + some from their association
- Auto-assembly

Context / Goals

Most relevant results
to obtain cellulose
oligomers

Acidic hydrolysis

85 wt% H_3PO_4 for 20h at
 50° C

68% – DP = 7,5 / PDI = 1,7 [1]

Other

Pyrolysis, plasma radiation,
phosphorolytic, pivaloylisis,
etc.

- [1] Heinze et al., *Macromol. Symp.*, **2008**, 262, 140.
- [2] Gupta & Lee, *Biotechnol. Bioeng.*, **2009**, 102, 1570.
- [3] Kobayashi et al., *J. Am. Chem. Soc.*, **1991**, 113(8), 3079.
- [4] Nakatsubo et al., *J. Am. Chem. Soc.*, **1996**, 118, 1677.

CELLULOSE OLIGOMERS

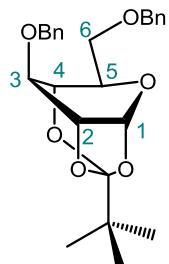
Enzymatic hydrolysis

Cellulase mixture, after 72h
15% – DP = 2-7 [2]

Chemical synthesis

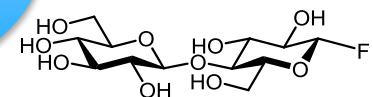
ROP, Ph_3CBF_4 , CH_2Cl_2 , 2h,
RT

93% – DP $\downarrow n$ = 10,5 [4]

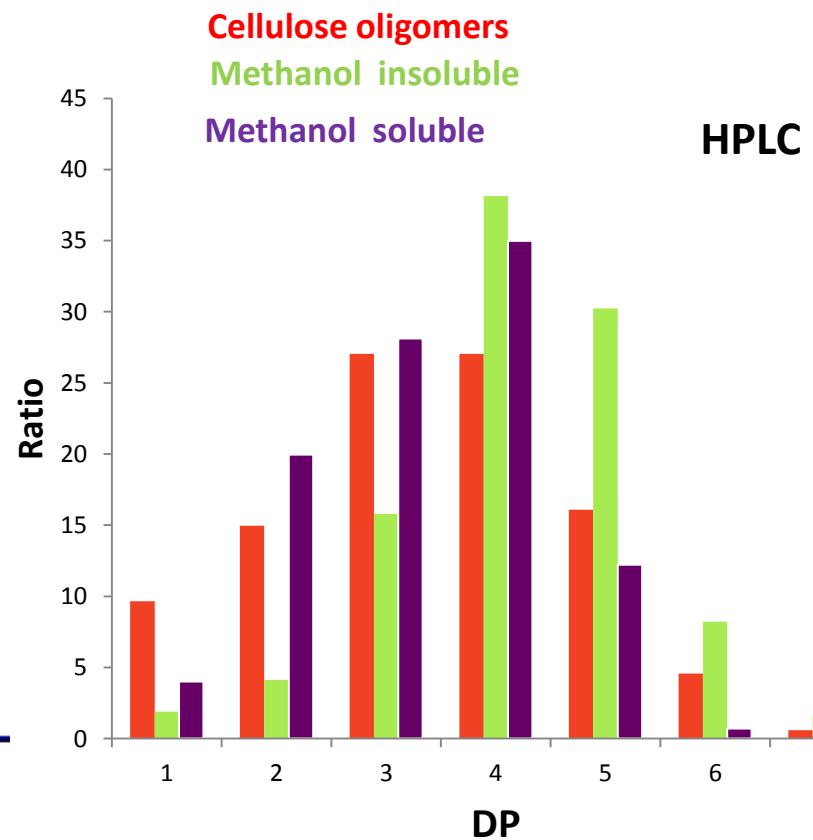
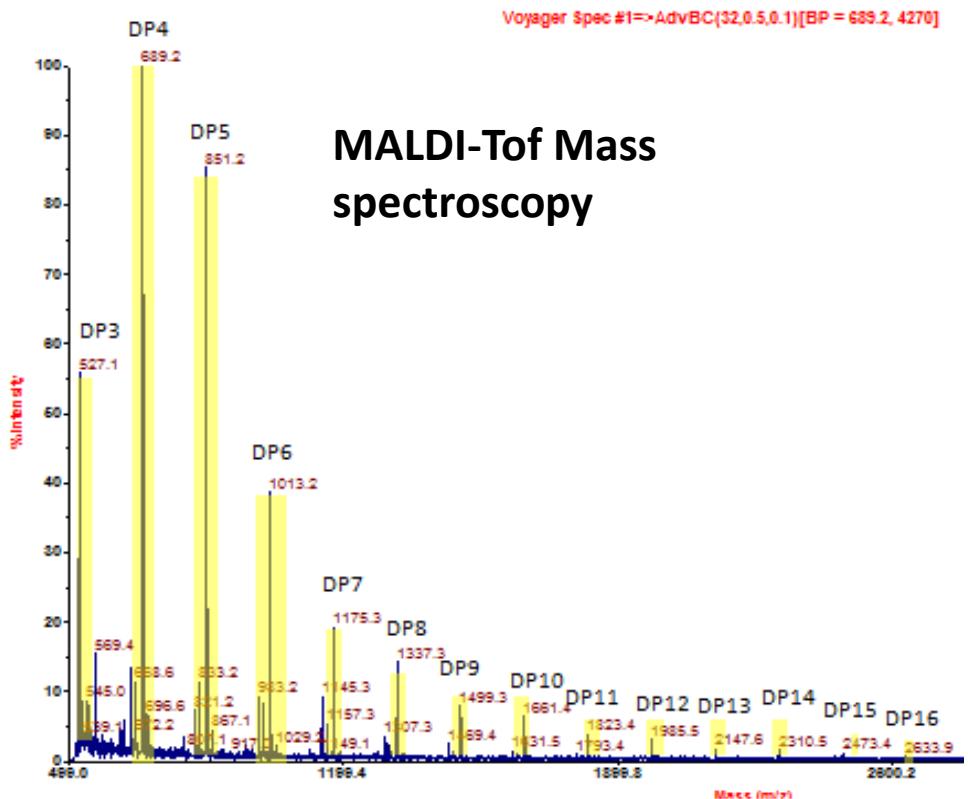


Enzymatic synthesis

β -D-celllobiosyl fluoride
polymerization by cellulase in ACN/
acetate buffer at 30° C for 12h
64% – DP = 2-8 [3]



Cellulose oligomers after phosphoric acid treatment

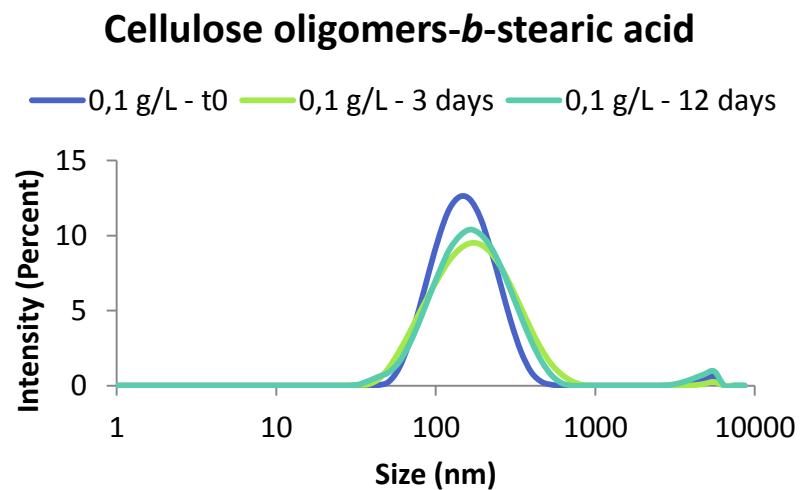
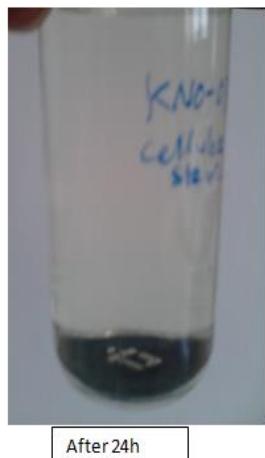
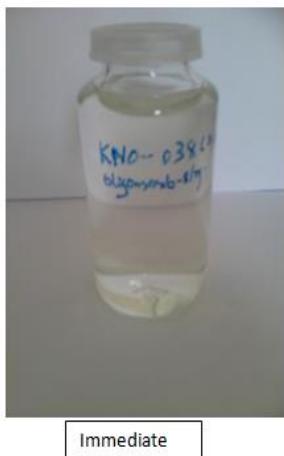
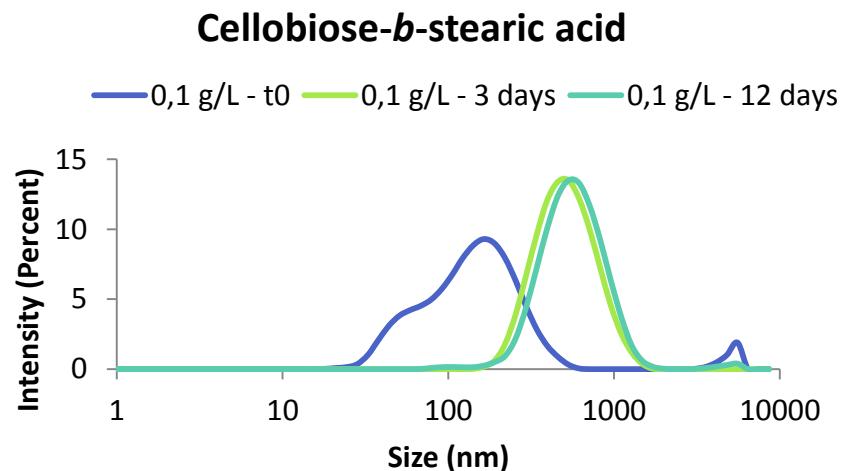
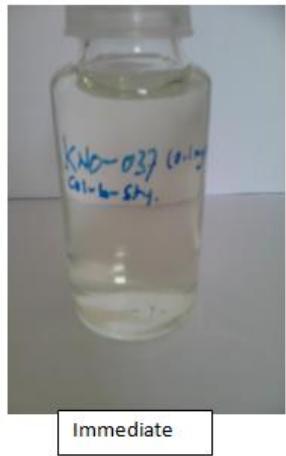


LOWER DISPERSION
DP 3-5

Cellulose oligomers-*b*-stearic acid

Size evolution and stability studies

DLS (MALVERN)





Lignocellulosic biomass characterization before and after enzymatic treatment

LISBP collaboration

PhD Tuan LE



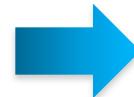
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Biomass

Paper pulp
Bagasse
Whatman paper



INITIAL CHARACTERIZATION

Enzyme-based treatment

Treated Biomass

Residual Paper pulp
Residual Bagasse
Residual Whatman paper

**CHARACTERIZATION OF THE
INSOLUBLE FRACTION**

Composition

Cellulose fraction
characterization (dp,
cristallinity)

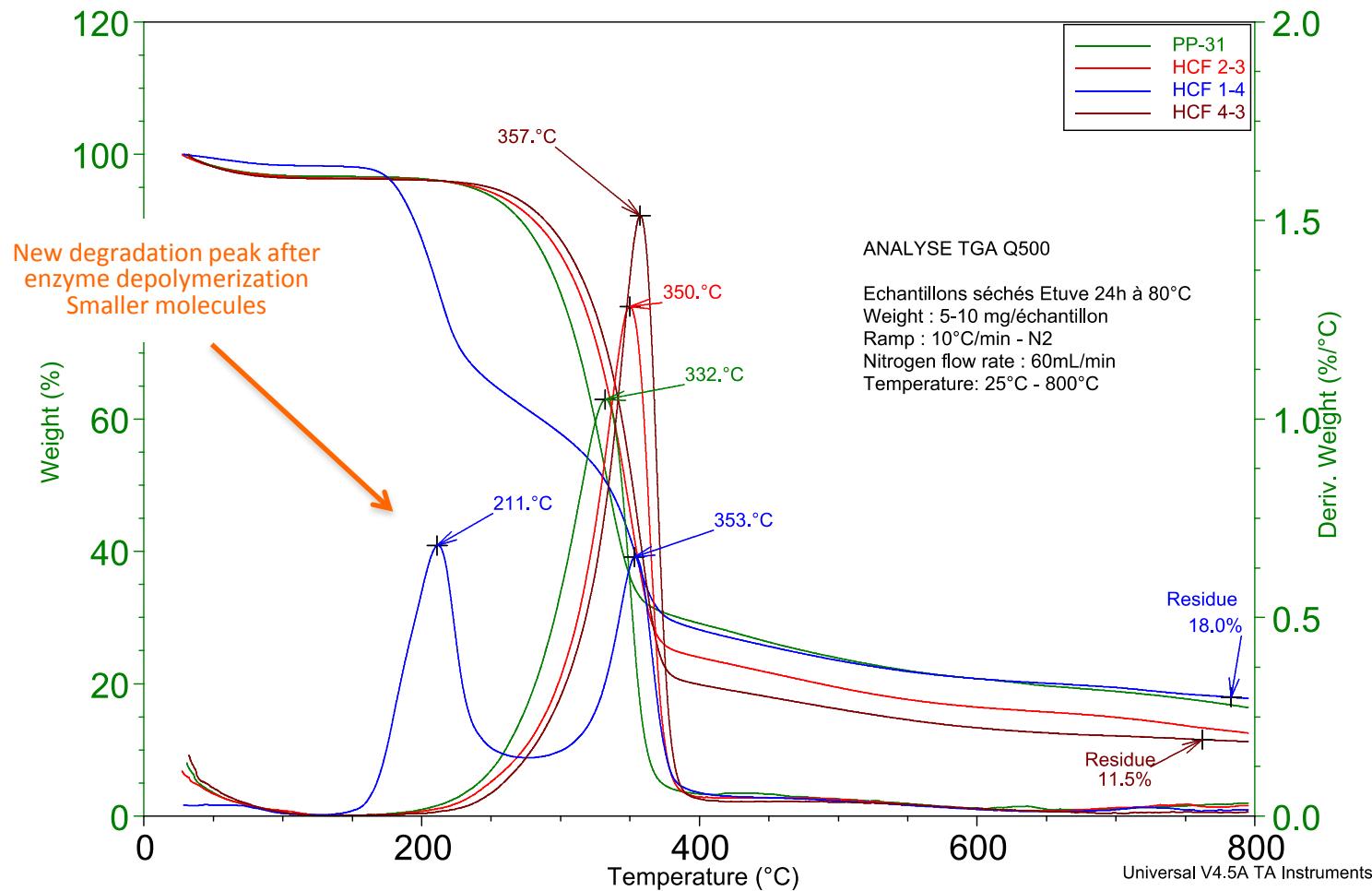
Porosity, morphology

Thermal properties (TGA)

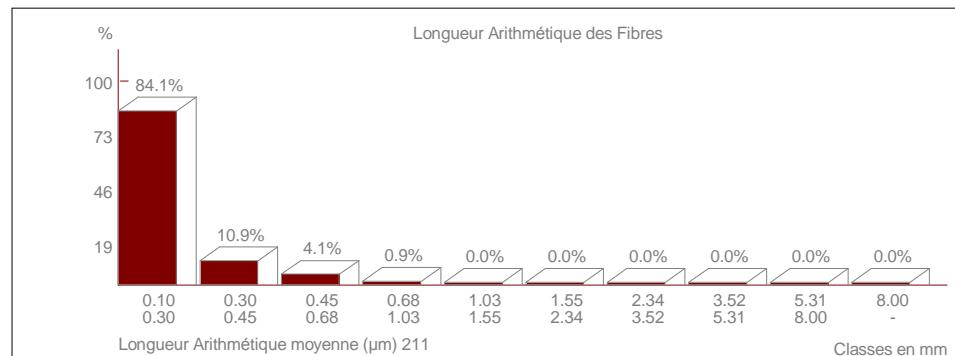
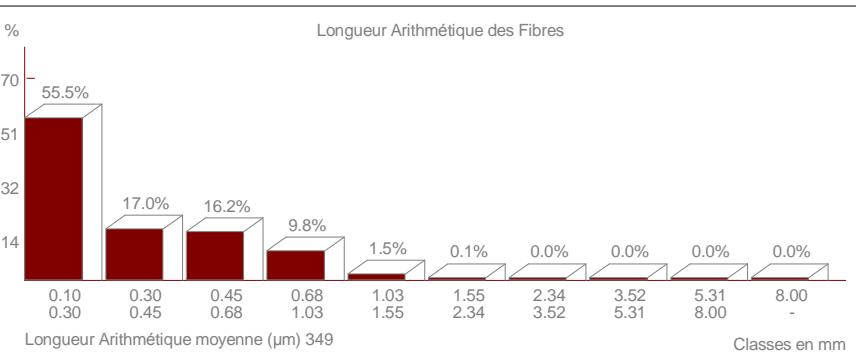
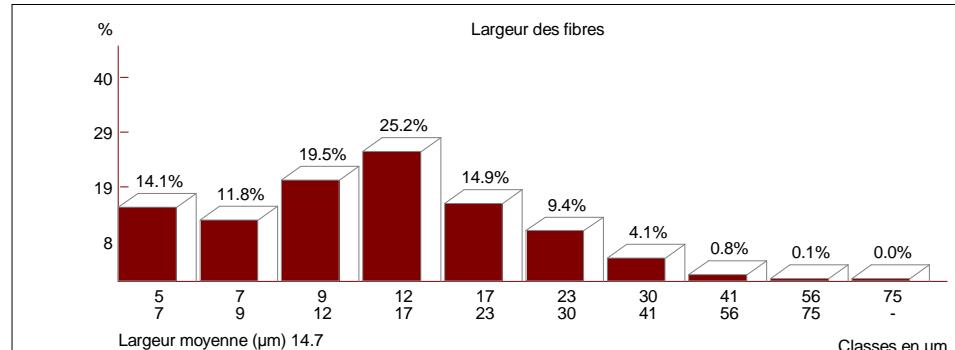
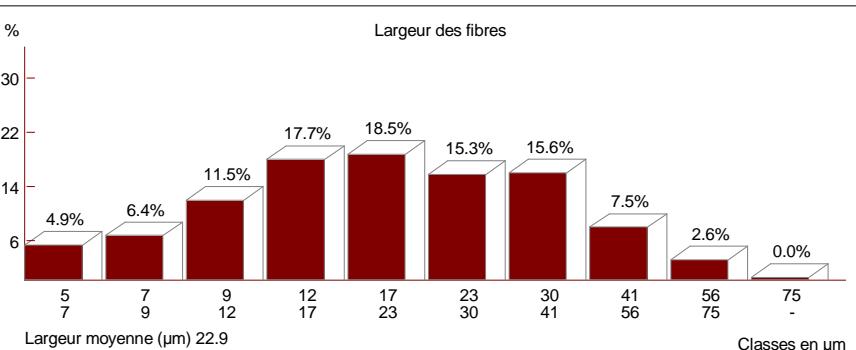
**Water interactions (Water
retention value,sorption
isotherms, NMR
relaxometry, etc)**

**CHARACTERIZATION OF THE
SOLUBLE FRACTION?**
Mw

Thermal analysis of paper pulp



Fiber morphology of paper pulp

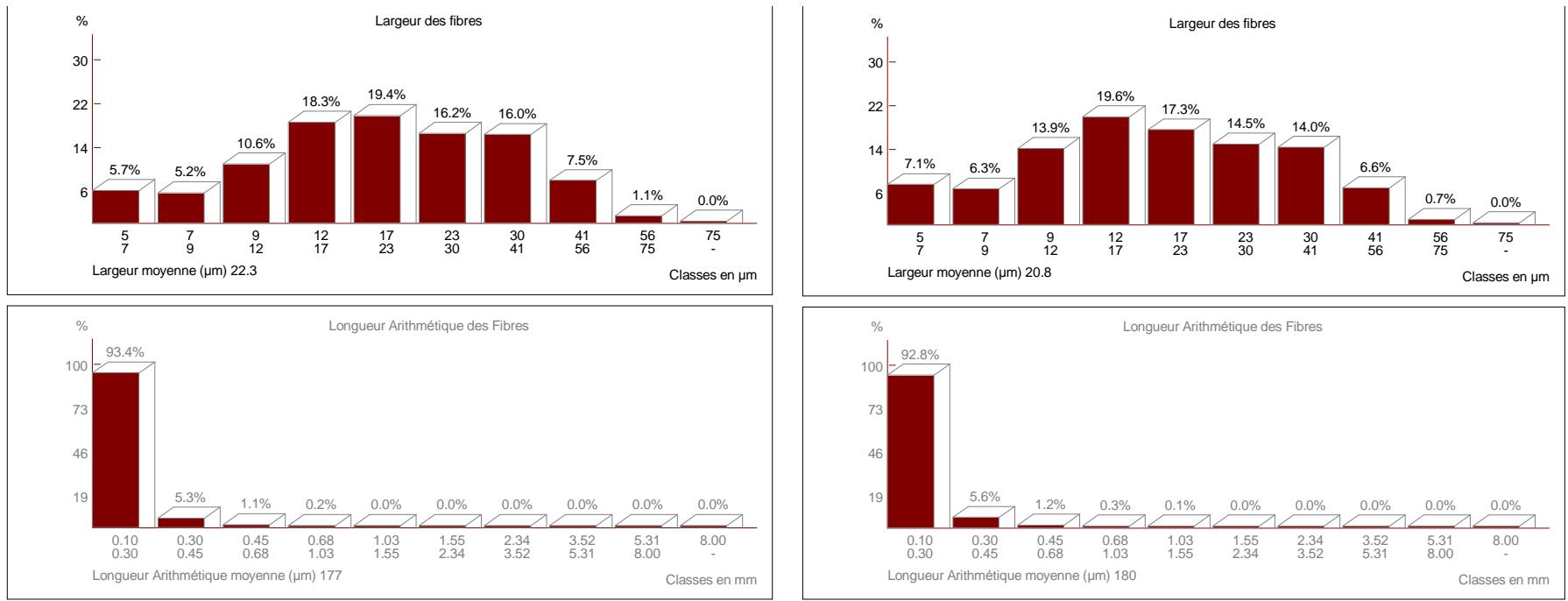


Paper pulp (PP-31) before the hydrolysis

Paper pulp (PP-31) after 24h hydrolysis by commercial enzymes (CTec2 (HCF 1-4)

→ Effect on the fiber length and section

Fiber morphology of pretreated bagasse



Pretreated bagasse (extruded 150122) before hydrolysis

Pretreated bagasse (extruded 150122) after 24h hydrolysis by commercial enzymes (CTec2 (HCF 1- 3)

→ Slight Effect on the fiber section

SIMILAR RESULTS WITH WHATMAN PAPER

Water Retention Value

| Substrates | AVERAGE WRT | Standard deviation |
|-----------------------------------|-------------|--------------------|
| PAPER PULP | 153,7 | 3,0 |
| Paper pulp + Ctec2 | 203,0 | 1,4 |
| Paper pulp + Endo | 155,9 | 4,6 |
| Paper pulp + (Endo + Exo + betaG) | 143,7 | 5,7 |
| BAGASSE | 210,6 | 7,3 |
| Bagasse + Ctec2 | 187,7 | 6,9 |
| Bagasse + Endo | 187,2 | 5,4 |
| Bagasse + (Endo + Exo + betaG) | 183,4 | 10,7 |

PP31
Higher WRT
after
treatment
with
commercial
enzyme
cocktail

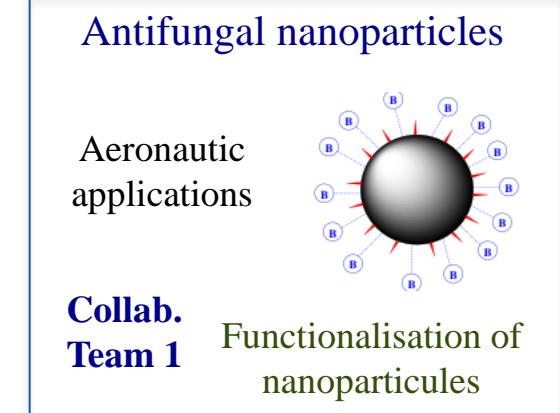
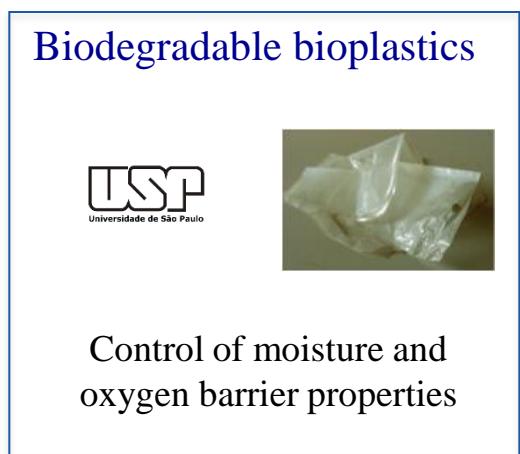
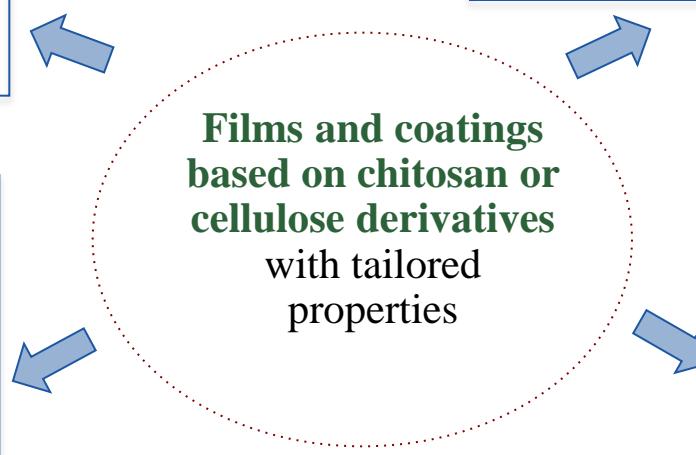
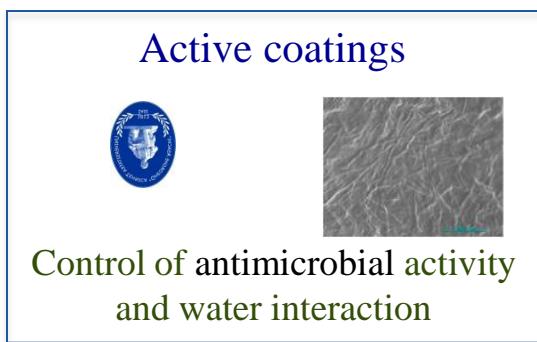
Lower WRT
afetr
treatment
with the
LISBP blend

First « fresh » results regarding the polymerization degree

| | tendency |
|----------------------------------|----------|
| BAGASSE 2 (150410) | |
| Bagasse + CTec2 | ↓↓↓ |
| Bagasse + Endo | ↓ |
| Bagasse + (Exo + beta) | Stable |
| Bagasse +(Endo + Exo + beta) | ↓ |
| PAPER PULP | |
| Paper pulp + (CTec2) | ↓↓↓↓ |
| Paper pulp +(Endo) | ↓↓ |
| Paper pulp + (Endo + Exo + beta) | ↓↓↓ |

B- Design of innovative functional materials with controlled properties

Advanced functional materials based on cellulose and chitosan



Some Key Publications:

- Biomacromolecules*, 11, 88 (2010),
- Cellulose*, 18, 699 (2011),
- Food Additives and Contaminants*, 13, 1 (2011),
- J. Agric. Food Chem.* 60, 10516 (2012),
- Carbohydrate Polymers*, 110, 374 (2014)
- Food hydrocolloides* (2015, *in press*)

Strategies to create active packaging materials

Use of inherently active polymer OR
Chemical/enzymatic modification of
polymers



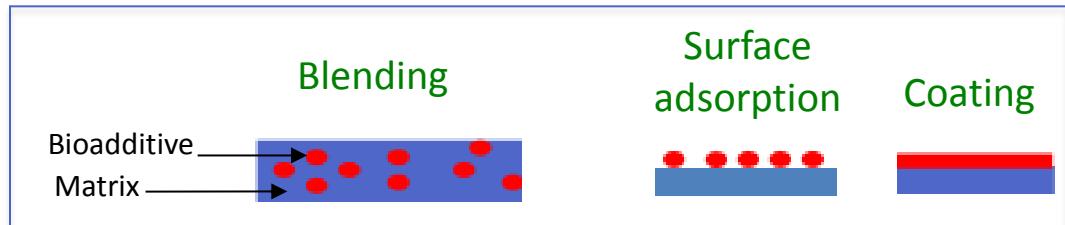
Grafting of active
agent, quaternary
ammonium, etc

Multi-component engineering

Association with biomolecules having active properties
Surface adsorption, coating or blending

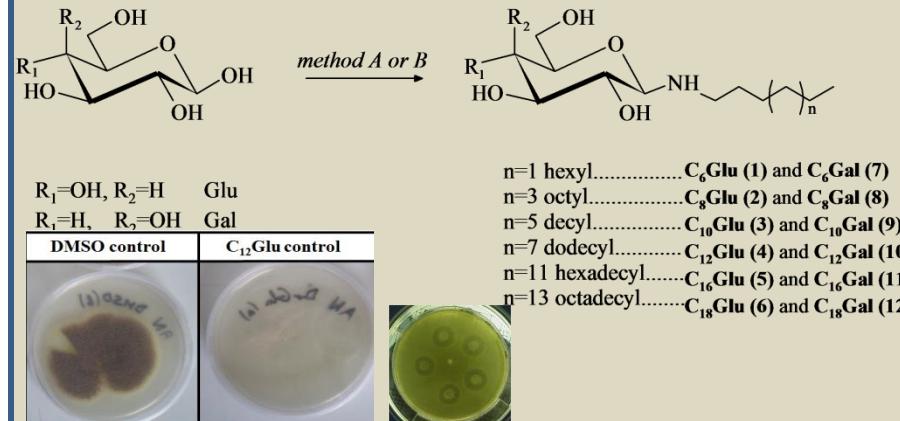


Combination

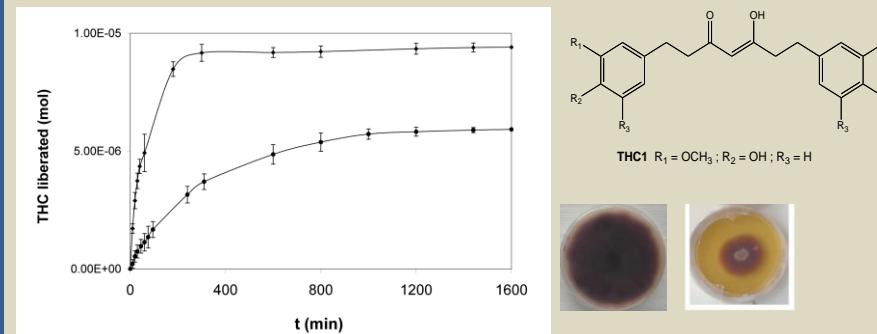


Biopolymers and bio-based polymers

Highly antimicrobial and barrier chitosan/amino sugars-based materials



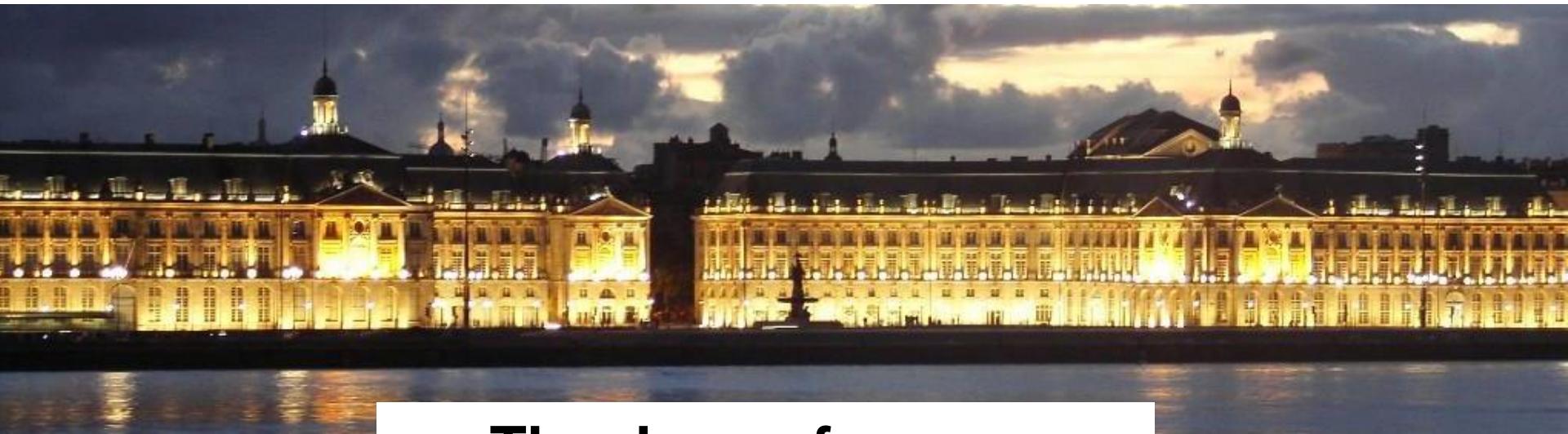
Functional antimycotoxinogen polysaccharide-based coatings: controlled release of curcumin derivatives used as bioactives



Carbohydrate polymers, 76, 578-584 (2009)
Biomacromolecules, 11, 88-96 (2010)
JAFC 60, 10516-10522 (2012)



FUTURE
WORK
TOGETHER?



**Thank you for your
attention and welcome to
Bordeaux**

