IFPB 2018

10TH INTERNATIONAL CONFERENCE ON FIBER AND POLYMER BIOTECHNOLOGY

PROCEEDINGS

APRIL | 24-27/2018

Balneário Camboriú | SC | Brazil

FINANCIAL SUPPORT

CAPES CNPq FAPESC Golden

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PROMOTION

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PREFACE

The Organizing Committee of IFPB-2018, the 10th International Conference on Fiber and Polymer Biotechnology, was very pleased to welcome you in Balneário Camboriú, Santa Catarina, Brazil, to discuss the most recent advances in textiles and fiber and polymer biotechnology. The tenth edition of this conference was held between April 24 and 27 in the Mercure Hotel.

The proposal of the International Conference on Fiber and Polymer Biotechnology is to promote the scientific, professional and social exchange between researchers, students, research institutes, governmental entities and industries who are active in the field of textiles and fiber and polymer biotechnology. Its objective is to contribute to the growth and consolidation of the scientific community and the technological development in these fields through scientific education and formation of a critical mass of graduate and post-graduate students and future professionals in this area, by means of dissemination and publication of scientific academic and technological works.

The scientific program offered 5 plenary lectures, 7 keynote lectures and 24 oral presentations. The program included also a short course about Biocatalysis Applied to Fibre and Polymer Science and two poster sessions with more than 30 presentations. Presentations covered a wide variety of topics and disciplines related to textiles and fiber and polymer biotechnology currently under investigation.

The conference IFPB 2018 brought together 100 researchers and participants from 15 different countries, including Mexico, United States of America, China, Japan, Portugal, Spain, Italy, Austria, Switzerland, Slovenia, The Netherlands, United Kingdom, Ireland, Sweden, Israel and Brazil.

The organizing committee would like to express its profound gratitude to the funding agencies *Coordination for the Improvement of Higher Education Personnel* (CAPES), *National Council for Scientific and Technological Development* (CNPq), *Santa Catarina Research Foundation* (FAPESC) and *The 13th Regional Council of Chemistry* (CRQ XIII), without whose financial support this conference would not be possible. We would also like to give our special thanks to the invited lecturers who accepted the challenge, the members of the scientific committee, the sponsor Golden Technology, the Convention and Visitors Bureau of Balneário Camboriú, to Izabela Toledo and the staff of Jocintra Eventos, who did a spectacular organizing work, to Ana Blanco for supporting and making the right contacts from the very beginning, to Instituto Gene for accepting to help us with the financial issues,

to the Regional University of Blumenau (FURB-Universidade Regional de Blumenau) for promoting and supporting this event and last but not least to all participants who made their way to Balneário Camboriú to make this conference feasible.

The Regional University of Blumenau, represented by the Department of Chemistry and the Post-Graduation Program in Chemistry (PPGQ-FURB) feels honored for having had the opportunity to promote the 10th International Conference on Fiber and Polymer Biotechnology.

We immensely acknowledged your presence and your contribution to IFPB 2018 and we hope that you had a wonderful time during your stay with us in Balneário Camboriú.

On behalf of the Organizing committee

Jürgen Andreaus (Chairman of IFPB 2018)

GENERAL INFORMATION

Executing and promoting institution: FURB - Universidade Regional de Blumenau / FURB-Regional University of Blumenau

Areas: Polymers, Enzymology, Proteins, Bioprocesses, Textiles, Chemistry of macromolecules

Date: April 24 to 27, 2018

Venue: Mercure Hotel, Balneário Camboriú

Keywords: polymers, textile fibers, biocatalysts, biofunctionalization, nanobiocatalysis and nanostructured materials, sustainable processes, biotechnology, enzymes, biocatalysis, technological application, biopolymers

Editoring: Jürgen Andreaus

Venue

Mercure Hotel

Av. Atlântica, 2010

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SUMMARY

The 10th International Conference on Fiber and Polymer Biotechnology (IFPB 2018) programmed for the period from April 24 to 27, 2018, in the Mercure Hotel in the city of Balneário Camboriú, Santa Catarina, is giving continuity to the series of events held since 2000 in biennial form. IFPB 2018 focuses on research and development of biotechnology and enzyme technology applied to fibers and polymers. It is an internationally consolidated Congress, which is in its Tenth Edition and has been carried out so far in eight countries and three continents. The use of biotechnology and biocatalysts in processes related to fibers and polymers has grown a lot in the last 25 years and proved to be a fundamental tool in the improvement and modernization of processes and products of the textile industry and other industries involving polymers, detergents, cosmetics etc. The application of biocatalysts and biotechnology is critical to the development of greener and sustainable processes with less consumption of harmful chemical reagents, milder procedure conditions (neutral pH, lower temperatures), lower consumption of energy and water, and lower generation of effluents and residues. The use of biotechnology also allowed the development of innovative and high-quality products for clean technologies, in line with the technological requirements, and environmental preservation. IFPB 2018 is a multidisciplinary event and thematic technical sessions will be focused on the following themes: Industrial Enzymes, Natural and Bio-based Polymers and Fibers, Biofunctionalization of Synthetic Materials, Sustainable Processes, Smart Materials through Nano bio-catalysis, Nano/Bio-materials and Applications. The region of the Itajaí Valley, an industrially and economically important area nearby Balneário Camboriú, is considered one of the largest textile centers in Brazil and Latin America and has various industries sectors using bioprocesses involving biocatalysts. The IFPB 2018 is an exceptional opportunity for the discussion of research advances and prospects of biotechnology applied to the processing of polymers and fibers. Plenary lectures, technical sessions, panel sessions and short courses will stimulate the exchange of information between participants from universities, research institutes, government agencies and industries. In addition, the event will promote the participation and interaction of Brazilian undergraduate and postgraduate students, especially from FURB, event promoter, with national and international researchers and disseminate the Regional University of Blumenau-FURB more in the academic and business world.

HISTORICAL - PREVIOUS CONFERENCES:

Year	Name of the conference	Organizer	City	Country
2000	1st International Conference on Textile Biotechnology	Uminho	Póvoa de Varzim	Portugal
2002	2nd International Conference on Textile Biotechnology	University of Georgia	Athens	USA
2004	3rd International Conference on Textile Biotechnology	Technische Universität Graz	Graz	Austria
2006	4th International Conference on Textile Biotechnology	Kitech	Seoul	South Korea
2007	5th International Conference on Textile Biotechnology	Jiagnan University	Wuxi	China
2009	6th International Conference on Textile and Polymer Biotechnology	Ghent University	Ghent	Belgium
2011	7th International Conference on Polymer and Textile and Biotechnology	Stazione Sperimentale per la Seta	Milan	Italy
2014	8th International Conference on Polymer and Fibre Biotechnology	Uminho	Braga	Portugal
2016	9th International Conference on Fiber and Polymer Biotechnology	Osaka Seikei Colleage	Osaka	Japan

CONFERENCE TOPICS

1. *Industrial Enzymes*
    a. Novel biocatalysts for specific applications
    b. Robust enzymes for polymer and textile bioprocessing
    c. Extremozymes
2. *Natural and Bio-based Polymers and Fibers*
    a. Emerging natural
    b. Genetic engineered
    c. Biomass/fermentation-derived polymers and fibers
    d. Biodegradable polymers
    e. Self-assembling polymers
    f. Functionalization of (bio)polymers
    g. Renewable sources of polymers and chemicals; valorization of waste materials
3. *Biofunctionalization of Synthetic Materials*
    a. Surface modification
    b. Functionalization of synthetic polymers and fibers through biocatalysis
    c. Biologically active surfaces and interfaces
4. *Sustainable Processes*
    a. Bio-catalytic process design
    b. Upscaling of bioprocesses to industrial level
    c. Combination of biotechnological, chemical and physical processes
    d. Low health and environmental impact processes
    e. Textile bioprocessing
    f. Wastewater treatment (bio-adsorption/biodegradation)
5. *Smart Materials through Nano bio-catalysis*
    a. Immobilization/incorporation of enzymes into nanostructured materials
    b. Applications (proteomics, biofuel cells, antifouling, bioconversion, biosensing, bioremediation)
6. *Nano/Bio-materials and Applications*
    a. Bio-inspired, bio-mimicking approaches to material design Nanostructured materials
    b. (particles, fibers, etc.)
    c. Regenerative medicine
    d. Drug/actives encapsulation and delivery
    e. Cosmetics
    f. Detergency

FINAL PROGRAM

The final program previews one Short-course, 5 Plenary Lectures, 7 Key Note Lectures, Technical Sessions with oral presentations with a duration of 20 minutes and two Poster Sessions on April 25 and 26 in the afternoon during the coffee-break.

Time	April 24 Tuesday	Time	April 25 Wednesday	April 26 Thursday	April 27 Friday
8:30 – 10:00		**9:00 - 10:00**	Plenary Lecture 1 (Opening) (Georg Guebitz)	Plenary Lecture 3 (Artur Cavaco-Paulo)	Plenary Lecture 5 (Ulyana Shimanovich)
		10:00 - 10:30	Key Note Lecture 1 (Marcio Poças)	Key Note Lecture 4 (Gianluca Ciardelli)	Key Note Lecture 7 (Pedro Henrique H. Araújo)
10:00 – 12:00		**10:30 – 11:00**	Coffee Break	Coffee Break	Coffee Break
		11:00 – 12:20	Session 1	Session 4	Session 6
12:00 – 14:00	Registration	**12:20 – 14:00**	Lunch	Lunch	Closing Ceremony, Lunch
14:00 – 15:45	Short-Course Part 1	**14:00 - 15:00**	Plenary Lecture 2 (Richard Gross)	Plenary Lecture 4 (Airton Martin)	
15:45 -16:15	Coffee-Break	**15:00 – 15:30**	Key Note Lecture 2 (Luiz Pereira Ramos)	Key Note Lecture 5 (Madalena Martins)	
16:15 – 18:00	Short-Course Part 2	**15:30 – 16:30**	Session 2	Lecture (Kenzo Koike) Key Note Lecture 6 (Silgia Aparecida da Costa)	
18:00 – 18:30	Free	**16:30 – 17:30**	Coffee Break and Poster Session	Coffee Break and Poster Session	
18:30 – 19:00	Opening Ceremony	**17:30-18:00**	Key Note Lecture 3 (Sérgio Henrique Pezzin)	Session 5	
19:00 – 20:00	Amélia Malheiros (Project presentation)	**18:00-19:10**	Session 3		
20:00	Welcome Cocktail	**After 19:10**	Free	Social Program	

Detailed program

The final program is composed of one Short-course, 5 Plenary Lectures, 7 Key note lectures, Technical Sessions with presentations with a duration of 20 minutes and Poster Sessions.

Short Course

Title: Biocatalysis Applied to Fibre and Polymer Science

Georg Guebitz, Institute of Environmental Biotechnology, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

Richard Alan Gross, Rensselaer Polytechnic Institute, Department of Chemistry and Chemical Biology, New York, EUA

Oral presentations

Oral presentations have a duration of 20minutes and should be prepared in PowerPoint (Office Microsoft).

Poster sessions

Posters might have a maximum size of 1.20 m (height) x 1.00 m (width). They have to be prepared to be hanged with a cord or wire.

Posters should be fixed on April 24 in the afternoon or April 25 in the morning and shall be exposed during the whole conference. There will be a special Poster Session on April 25 and 26 in the afternoon (simultaneously with an extended coffee break).

Schedule of Presentations

Tuesday, April 24, 2018

12:00-14:00	***Registration***
14:00-15:45 ***Short Course***	Richard Alan Gross, Rensselaer Polytechnic Institute, Department of Chemistry and Chemical Biology, New York, USA Short Course Biocatalysis Applied to Fibre and Polymer Science Part 1
15:45-16:15	*Coffee Break*
16:15-18:00 ***Short Course***	Georg Guebitz, Institute of Environmental Biotechnology, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria Short Course Biocatalysis Applied to Fibre and Polymer Science Part 2
18:00-18:30	Free
18:30-19:00	***Opening Ceremony***
19:00-20:00	Amélia Malheiros – Hering S.A., Brazil *Trama Afetiva* (Affective Weft) – A collaborative experience through sustainable design
20:00–21:00	***Welcome Cocktail***

Wednesday, April 25, 2018

Session Chair	Gianluca Ciardelli, Politecnico di Torino, Italy Jürgen Andreaus, Universidade Regional de Blumenau, Brazil
Opening Lecture ***Plenary Lecture 1*** 9:00-10:00	Georg Guebitz, Institute of Environmental Biotechnology, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria Designing enzymes for future polymer and fibre processing
10:00-10:30 ***Key Note Lecture 1***	Marcio José Poças Fonseca, University of Brasilia, Brasilia, Brazil The impact of DNA methylation on Humicola grisea var. thermoidea enzyme activities and on the glucose-mediated gene repression
10:30-11:00	*Coffee Break*
Session Chair	Pedro Henrique H. Araújo, Federal University of Santa Catarina, Brazil Tzanko Tzanov, Universitat Politècnica de Catalunya, Spain
Session 1 11:00-11:20	Alessandro Pellis, Department of Chemistry, Green Chemistry Centre of Excellence, University of York, York, United Kingdom Enzymatic tools for the green synthesis of clickable polyesters
11:20-11:40	Jürgen Andreaus, Department of Chemistry, Universidade Regional de Blumenau (FURB), Blumenau, Brazil Ultrasound - a green tool to boost enzyme reactions in lignocellulosic biomass exploitation
11:40-12:00	Pramod Agrawal, Saxion University and Agrawal-Ecolabs, Enschede, The Netherlands Hydrophobic bio-functionalization of pure PLA and PLA/Jute bio-composite by surface activation with diverse esterase enzymes and coupling with Alkyl Ketene Dimer
12:00-12:20	Daniela Bresolin, Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil A green polyol as support for the immobilization of lipase NS 40116 in polyurethane foam
12:20-14:00	*Lunch*
Session Chair	Artur Cavaco-Paulo, Universidade do Minho, Portugal Kenzo Koike, Kao Corporation, Japan
14:00-15:00 ***Plenary Lecture 2***	Richard Alan Gross, Rensselaer Polytechnic Institute, Department of Chemistry and Chemical Biology, New York, USA Leaf Branch and Compost Cutinase and Ultra-Thin Bacterial Cellulose

15:00-15:30 ***Key Note Lecture 2***	Luiz Pereira Ramos, Department of Chemistry, Federal University of Paraná (UFPR), Curitiba, Brazil Confocal laser scanning microscopy of cane bagasse before and after steam explosion and alkaline delignification
Session 2 15:30-15:50	Qiang Wang, College of textiles and clothing, Jiangnan University, Wuxi, China Highly efficient and eco-friendly degradation of wool by L-Cysteine-assisted Esperase
15:50-16:10	José Domingos Fontana, Federal University of Technology – Paraná (UTFPR), Curitiba, Brazil Insights on bacterial nanocellulose for food and non-food applications
16:10-16:30	Simona Bronco, IPCF-CNR, Pisa, Italy Valorization of food by-products as starting materials for bioplastics
16:30-17:30	***Coffee Break and Poster Session***
Session Chair	Luiz Pereira Ramos, Federal University of Paraná, Brazil Simona Bronco, IPCF-CNR, Italy
17:30-18:00 ***Key Note Lecture 3***	Sergio Henrique Pezzin, State University of Santa Catarina (UDESC), Joinville, Brazil Development of biocomposites derived from biodegradable polyesters
Session 3 18:00-18:20	Camila Utsunomia, Institute of Life Tchnologies, HES-SO Valais Wallis, Sion, Switzerland Biocatalytic synthesis of polyhydroxyalkanoates block-copolymers: Challenges and novel approaches
18:20-18:40	Felipe Andre Pavan, Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil Influence of key production parameters in the Poly(hydroxybutyrate) production cost
18:40-19:00	André Lourenço Nogueira, Post Graduation Program in Process Engineering, UNIVILLE, Joinville, Brazil PMMA/Nanocrystalline Cellulose Nanocomposites Produced by in situ Suspension Polymerization

Thursday, April 26, 2018

Session Chair	Georg Guebitz, University of Natural Resources and Life Sciences, Austria Marcio José Poças Fonseca, University of Brasilia, Brazil
9:00-10:00 ***Plenary Lecture 3***	Artur Cavaco-Paulo, Centre of Biological Engineering, Universidade do Minho, Braga, Portugal Polymer and Fibre Biocatalysis
10:00-10:30 ***Key Note Lecture 4***	Gianluca Ciardelli, Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy Design of fibrous and injectable platforms for the release of therapeutic ions and drugs in chronic skin wounds treatment
10:30-11:00	*Coffee Break*
Session Chair	Airton Abrahão Martin, University Brazil - São Paulo, Brazil Madalena Martins, Universidade do Minho, Portugal
Session 4 11:00-11:20	Guillem Ferreres, Universitat Politècnica de Catalunya, Terrassa, Spain Bactericidal hybrid metal-enzyme nanoparticles with polysaccharide biofilm eradication ability
11:20-11:40	Tzanko Tzanov, Universitat Politècnica de Catalunya, Terrassa, Spain Freestanding layer-by-layer membranes incorporating antibacterial biopolymer-capped silver nanoparticles
11:40-12:00	Kristina Ivanova, Universitat Politècnica de Catalunya, Terrassa, Spain, Multifunctional hyaluronic acid based hydrogel with enzymatically embedded silver/lignin nanoparticles

12:00-12:20	Jeddah Marie Vasquez, Vornia Biomaterials Ltd., Synergy Center, Institute of Technology – Tallaght, Dublin, Ireland Honey-mimetic Antibacterial ROS in situ forming Hydrogel Wound Dressing
12:20-14:00	*Lunch*
Session Chair	Kristina Ivanova, Universitat Politècnica de Catalunya, Spain Ulyana Shimanovich, Weizmann Institute of Science, Israel
14:00-15:00 ***Plenary Lecture 4***	Airton Abrahão Martin, Instituto Científico e Tecnológico da Universidade Brasil – University Brazil - São Paulo, São Paulo, Brazil In vivo Confocal Raman Spectroscopy Applied to Cosmetic Science
15:00-15:30 ***Key Note Lecture 5***	Madalena Martins, Centre of Biological Engineering, Universidade do Minho, Braga, Portugal Changes of the shape of keratin based fibers
15:30-16:00	Kenzo Koike, Kao Corporation, Tokyo, Japan Biotechnology in Cosmetics. - Application of enzymes in hair care products.
16:00-16:30 ***Key Note Lecture 6***	Silgia Aparecida da Costa, School of Arts, Sciences and Humanities, University of São Paulo (USP), São Paulo, Brazil Biopolymers applied in the development of medical textiles
16:30-17:30	***Coffee Break and Poster Session***
Session Chair	Montserrat E. Sanchez , Universidad Autónoma Metropolitana, Mexico Sergio Henrique Pezzin, State University of Santa Catarina, Brazil
Session 5 17:30:17:50	Kristina Ivanova, Universitat Politècnica de Catalunya, Terrassa, Spain Electrical monitoring of enzymatic infection biomarkers using antibody and peptidoglycan-modified nanoporous membranes
17:50-18:10	Kazuya Sawada, Osaka Seikei College, Osaka, Japan Preparation of the fibrous bio-scaffold utilizing supercritical fluid extraction
18:10-18:30	Laura Morgan, De Montfort University, Leicester, United Kingdom Innovative Technologies for Sustainable Textile Coloration and Surface Design
18:30-18:50	Vanja Kokol, Institute of Engineering Materials and Design, University of Maribor, Maribor, Slovenia Biochemical modification and functionalization of nanocellulose, and its application potentials
18:50-19:10	Richard Cassio Oliveira Amorim, Faculty of Technology of Praia Gande, Praia Grande, Brazil Nanocellulose extraction from banana pseudo-stalk for the production of bioplastic

Friday, April 27, 2018

Session Chair	Pramod Agrawal, Saxion University and Agrawal-Ecolabs, The Netherlands Richard Alan Gross, Rensselaer Polytechnic Institute, USA
9:00-10:00 ***Plenary Lecture 5***	Ulyana Shimanovich, Department of Materials and Interfaces, Weizmann Institute of Science, Rehovot, Israel Protein self-assembly in bio-inspired materials
10:00-10:30 ***Key Note Lecture 7***	Pedro Henrique Hermes Araújo, Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil Enzymatic ring-opening polymerization and functionalization of macrolactones
10:30-11:00	*Coffee Break*

Session Chair	Kazuya Sawada, Osaka Seikei College, Japan Vanja Kokol, University of Maribor, Slovenia
Session 6 11:00-11:20	Manuel Eduardo Martínez-López, Biotechnology Department, Universidad Autónoma Metropolitana, Mexico City, Mexico Removal of heavy metals from contaminated water using an extruded matrix of biodegradable polymers
11:20-11:40	May Kahoush, Textile Materials Technology, Department of Textiles, University of Borås, Borås, Sweden Bio-Electro-Fenton for the Treatment of Textile Wastewater
11:40-12:00	André Lourenço Nogueira, Post Graduation Program in Process Engineering, UNIVILLE, Joinville, Brazil Antibacterial Efficiency of Cellulose Microparticles Functionalized with Silver Nanoparticles for Water Purification
12:00-12:20	Montserrat Escobar Sanchez, Universidad Autónoma Metropolitana, Mexico City, Mexico Volumetric oxygen transfer coefficient (kLa) and Reynolds number (Re) as scaling-up criteria for the production of Î²-N-acetylhexosaminidase of *Lecanicillium lecanii*
12:20-12:40	***Closing Ceremony***
12:40-14:00	*Lunch*

Poster Presentations

PO1 Amanda Bueno, Department of Chemical Engineering, Universidade Regional de Blumenau (FURB), Blumenau, Brazil

Dyeing of Polyester Fabric in High Temperature with Natural Dye Annatto

PO2 Amanda Marina Agustini, Department of Chemistry, Universidade Regional de Blumenau (FURB), Blumenau, Brazil

Microwave assisted synthesis of furfural and 5-hydroxymethylfurfural from glucose, fructose and sucrose

PO3 Andrea Cristhiane Krause Bierhalz, Federal University of Santa Catarina (UFSC), Blumenau, Brazil

Release kinetics of sodium diclofenac from alginate films cross-linked with calcium ions

PO4 Andrea Cristhiane Krause Bierhalz, Federal University of Santa Catarina (UFSC), Blumenau, Brazil

Effect of cross-linking on swelling degree and mass loss of alginate membranes from different polymeric structures

PO5 Andreza Lopes, Department of Cell Biology, University of Brasilia, Brazil

Establishment of enzymatic cocktail for hydrolysis of biomass lignocellulosis

PO6 Ângela Graziela Lechinski da Luz Andrade, Department of Chemistry, State University of Santa Catarina (UDESC), Joinville, Brazil

Synthesis of nanocellulose ε-caprolactone biocomposites via in situ polymerization

PO7 Bernardo Dias Ribeiro, Department of Biochemical Engineering, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

Suberin as an Inducer for Enzymes Production by Yarrowia lipolytica IMUFRJ 50682

PO8 Bernardo Dias Ribeiro, Department of Biochemical Engineering, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

Green Biphasic System for Enzymatic Polymerization of 11-Aminoundecanoic Acid

PO9 Bruna Lyra Colombi, Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil

Laccase production of white rot fungus grown on SBS paperboard coated with PET, aiming the bioadsorption strategy

PO10 Carolina Zulian Boeira, Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil

Crude glycerin and vinasse as feedstock for PHA production by engineered Cupriavidus necator

PO11 Carolina Zulian Boeira, Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil

Characterization of poly-hydroxybutyrate from engineered Cupriavidus necator grown on glycerol and glucose

PO12 Cristian de Oliveira Romera, Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil

Enzymatic esterification using Novozym® 435 to obtain a diene with posterior application in polymerization

PO13 Diandra Albuquerque Lopes Costa, Department of Biology, University of Brasilia, Brazil

Production of mannanase isoforms by Clonostachys byssicola cultivated in soybean hulls

PO14 Felipe Andre Pavan, Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil

Economic Assessment of Poly(hydroxybutyrate) production

PO15 Francielle Schmitz, Department of Chemistry, Universidade Regional de Blumenau (FURB), Blumenau, Brazil

Preparation and characterization of nanocomposites with zein and quantum dots of ZnO

PO16 Jacinto Gonçalves, Department of Chemistry, Universidade Regional de Blumenau (FURB), Blumenau, Brazil

Enzymatic hydrolysis of lignocellulosic biomass assisted by ultrasound irradiation

PO17 Kazuya Sawada, Osaka Seikei College, Osaka, Japan

Keratin Scaffold made by Animal Fiber Protein

PO18 Laís Feltrin Sidou, Department of Chemistry, Universidade Regional de Blumenau (FURB), Blumenau, Brazil

Can ionic liquids enhance textile dyeing? - Assessing color fixation differences in disperse dyeing when adding ILs in small concentrations

PO19 Marcia Margarete Meier, Department of Chemistry, UDESC – State University of Santa Catarina, Joinville, Brazil

Development of Biofunctional Bacterial Cellulose Membrane

PO20 Mariana Quintana-Quirino, Biotechnology Department, Universidad Autónoma Metropolitana, Mexico City, Mexico

Comparison of Gluconacetobacter xylinus cellulose produced by submerged and solid cultures

PO21 Natália Santos Nascimento, Department of Biotechnology, Federal University of Paraíba, João Pessoa, Brazil

Preparation of Thermo-Responsive Hydrogels Containing Carvacrol Encapsulated in Nanoparticles

PO22 Patrícia Raquel Silva Zanoni, Embrapa Forestry, Colombo, Brazil

Laccase immobilization on nanofibrillated cellulose for use in lignin refinery

PO23 Roberta Karoline Morais Ferreira, Department of Chemistry, Regional University of Goias, Anapolis, Brazil

Conductive Monolithic Polymers for Peroxidase Immobilization

PO24 Sabine Hillesheim, Department of Chemistry, Universidade Regional de Blumenau (FURB), Blumenau, Brazil

Activity of β-glucosidase enzyme under ultrasonic irradiation

PO25 Sidnei Emilio Bordignon, Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil

Kinetic analysis on cell growth and poly-hydroxybutyrate production by parental and recombinant Cupriavidus necator strains

PO26 Sidnei Emilio Bordignon, Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil

Kinetic and respiration parameters of engineered Cupriavidus necator during poly-hydroxybutyrate production

PO27 Tania Maria Costa, Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil

α-Glucosidase inhibition by extracts of *Ganoderma lipsiense* mycelium.

PO28 Taisei Takeuchi, Department of Biomedical Engineering, Osaka Institute of Technology, Osaka, Japan

In vitro mouse embryo culture on decellularized uterus tissue

PO29 Thalles Canton Trevisol, Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil

Effect of CaCl2 crosslinking on mechanical properties of polysaccharide-based membranes

PO30 Thalles Canton Trevisol, Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil

Water behavior properties of films made by different alginate and carboxymethyl cellulose proportions

PO31 Vanja Kokol, Institute of Engineering Materials and Design, University of Maribor, Maribor, Slovenia

Effect of peptide binding on antibacterial activity and cytotoxycity of protein-based substrates

PO32 Xuerong Fan, Key Laboratory of Science and Technology of Eco-Textile, Jiangnan University, Wuxi, China

Synthesis, characterization, reactivity ratios and properties of starch-g-poly (acrylic acid-co-methyl acrylate) triggered via enzyme

PO33 Roziana Cunha C. Jordão, Centre of Sciences and Technology, Catholic University of Pernambuco – UNICAP, Recife, Brazil

Biosurfactant producing species evaluating several substrates for application in decontamination by petroderivatives

PO34 Roziana Cunha C. Jordão, Centre of Sciences and Technology, Catholic University of Pernambuco – UNICAP, Recife, Brazil

Production of Levan by *Bacillus subtilis var. Natto* in Bioreactor

PO35 Karina Alves, Department of Chemistry, Universidade Regional de Blumenau (FURB), Blumenau, Brazil

Valorization of cotton waste using deep eutectic solvents

PO36 Dagoberto de Oliveira Silva, Department of Chemistry, Universidade Regional de Blumenau (FURB), Blumenau, Brazil

Influence of deep eutectic solvents on the enzymatic hydrolysis of cellulose

PO37 Camila Utsunomia, Institute of Life Tchnologies, HES-SO Valais Wallis, Sion, Switzerland

Bioconversion of syngas into biodegradable plastics using *Rhodospirillum rubrum*

PLENARY LECTURES

(PL1) Designing enzymes for future polymer and fibre processing

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Keywords: synthetic fibres, polyesterases, oxidoreductases, functionalization

INTRODUCTION

In recent years, the enzymes are increasingly used for polymer and fibre processing in line with current trends to render industrial processes more efficient and environmentally friendly. Besides specific surface functionalization and coating strategies, the potential of enzymes for recycling of blended materials has also been demonstrated. Modern approaches to improve enzymes for conversion of non-natural substrates include engineering of the active site architecture and of sorption properties, incorporation of non-canonical amino acids as well as increasing process stability and reducing end-product inhibition. Apart from recent achievements for hydrolyses like polyesterases, this paper will also review strategies for the improvement of oxidoreductases for polymer processing.

RESULTS AND DISCUSSION

To improve sorption to hydrophobic polyesters, hydrophobic binding modules were attached to various polyesterases from different origin (Fig.1b). These involved a binding module from a PHA depolymerase which is designed by nature to assist adsorption of PHA depolymerases to the hydrophobic polyester PHA (1,2). In another approach, fungal hydrophobins were fused to polyesterases (Fig.1c) likewise improving the sorption process (3). Apart from the fusion to binding modules, replacement of amino acids located on the enzyme surface (Fig.1d) was also effective (4). In all cases, improved sorption as demonstrated by using various analysis methods like quartz crystal micro balance analyses also led to improved hydrolysis of PET. Interestingly, in case of certain esterases truncation (Fig.1e) led to improved hydrolysis of PET (5). Apart from engineering of sorption, modifications of the active site architecture (Fig.1f) was also successful while the incorporation of non-canonical amino acids has also been suggested (6). Further strategies related to enzyme engineering for improved PET hydrolysis will be reviewed including reducing end-product inhibition or increasing enzyme stability (7).

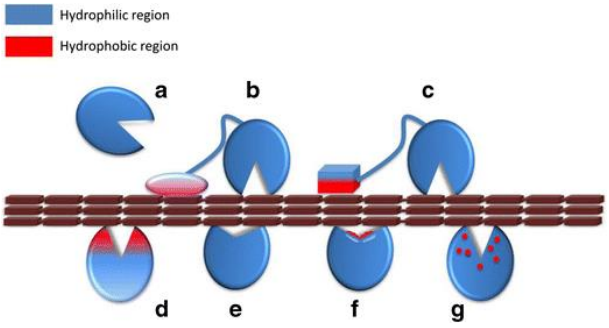

Figure 1. Schematic diagrams of various strategies for surface engineering of polyesterases Reprinted by permission from Springer Nature, Appl. Microbiol. Biotechnol. Surface engineering of polyester-degrading enzymes to improve efficiency and tune specificity, Biundo et al. 2018

ACKNOWLEDGEMENTS

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(PL2) Leaf Branch and Compost Cutinase and Ultra-Thin Bacterial Cellulose

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Keywords: cutinase, glycosylation, bacterial cellulose, ultra-thin films, anti-reflection coatings

INTRODUCTION

Cutinases: Nature provides powerful biocatalysts that can be deployed towards developing green polymer chemistry processes. In this context, one of several areas where biocatalysis has strong potential is for polymer recycling and surface modification reactions. Poly(ethylene terephthalate), PET, and cellulose acetate, CA, constitute a large volume of polymers employed for textile applications. Insert your text here. Cutinases are esterases secreted by phytopathogenic fungi that hydrolyze cutin (biopolyester coating found on leaves and fruits) which gives them access to interior plant tissues. They also are powerful hydrolases that can cleave ester bonds of PET and even side chain ester bonds of polyvinyl acetate and CA

Bacterial cellulose (BC) is an extracellular nanoscale matrix that consists of high crystallinity inter-connected fibers of self-assembled poly(β-(1→4)-D-glucose), e.g. cellulose. The process involves bacterial export through multi-enzyme complexes in the plasma membrane of sub-elementary fibers that, due to their close proximity, form microfibrils and ultimately cellulose ribbons (diameters ~2, 3-4 and 30-100 nm, respectively.[5] Thus, BC combines the extraordinary material properties of cellulose with the unique features of nanomaterials. Aerobic bacteria such as Gluconacetobacter, Rhizobium, Agrobacterium, and Sarcina are known to directly produce high-purity and high-crystallinity nanocellulose fibers in static fermentation processes from sugar feed-stocks. The biosynthesis of BC is eco-friendly and could be scaled up for commercial manufacturing.

RESULTS AND DISCUSSION

Cutinase: Given the extraordinary potential of Leaf Branch Compost Cutinase (LCC) for PET and CA hydrolysis, this paper discusses the thermodynamics and kinetics of LCC conformational and colloidal stability.[1] Aggregation emerged as a major contributor that reduces LCC kinetic stability. In its native state, LCC is prone to aggregation owing to electrostatic interactions (**Figure 1**). Further, with increasing temperature, perturbation of LCC's tertiary structure and corresponding exposure of hydrophobic domains leads to rapid aggregation. Glycosylation was employed in an attempt to impede LCC aggregation. Owing to the presence of three putative N-glycosylation sites, expression of native LCC in Pichia pastoris resulted in the production of glycosylated LCC (LCC-G). LCC-G showed improved stability to native state aggregation while increasing the temperature for thermal induced aggregation by 10°C. Furthermore, stabilization against thermal aggregation resulted in improved catalytic PET hydrolysis both at its optimum temperature and concentration (**Figure 2**).

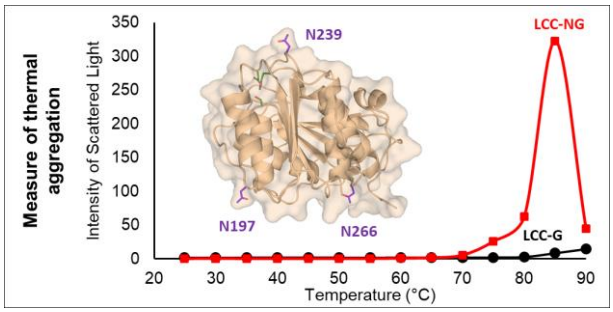

Figure 1. Glycosylation of LCC protects the cutinase from aggregation that leads to denaturation. .

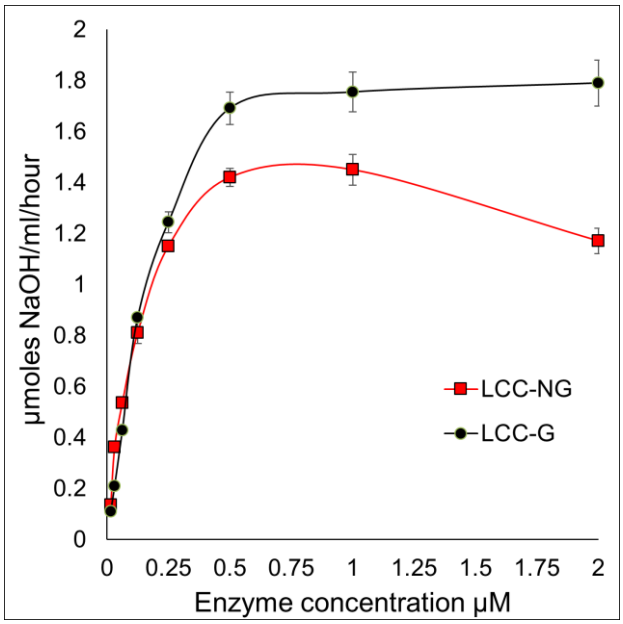

Figure 2. PET hydrolysis activity of LCC-NG and LCC-G at predetermined enzyme concentrations (70 °C in 5 mM tris buffer, pH 8 and 2 cm^2/mL substrate concentration).

Bacterial Cellulose: A facile and effective method is described for the biosynthesis of ultra-thin bacterial cellulose (BC) mats, which are green,

inexpensive, light weight and flexible. BC mat thickness was varied by controlling the depth of the culture broth so that films with predictable thickness, between 113 and 1114 nm, were produced. These BC films have similar fiber morphology but increased surface area (75 $m^2 \cdot g^{-1}$) then corresponding mm thick BC films prepared under static culture conditions (**Figure 3**). Analysis of BET isotherms for the 328 nm thick BC film shows that its pore size distribution is 12-30 nm, consistent with pore size values of thick BC films (22 nm) (**Figure 4**). To increase BC film hydrophobicity, surface trihexylsilylated BC (THSBC) mats with DS_{avg} 0.015 were prepared (**Figure 3**). Both native and THSBC mats were investigated as antireflection coatings for silicon substrates. The 328 ± 42 nm thick BC mat demonstrated broadband, interference type antireflection over a spectral range of 500 to 1800 nm. Different reflection properties were obtained as a function of BC film orientation revealing that engineered density gradients can be used to manipulate BC optical properties. Thus, high performance, mechanically strong and environmental friendly ultra-thin BC films are promising biomaterials for next-generation optoelectronic devices.

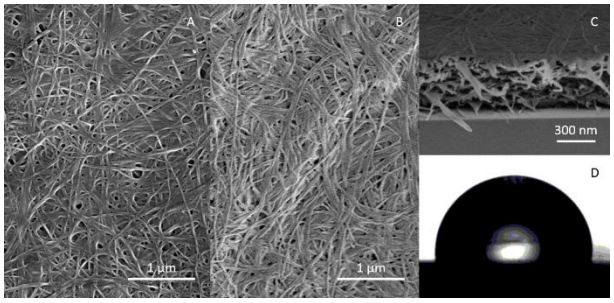

Figure 3. SEM images of the a) top of native (unmodified) BC b) top of trihexylsilane modified BC (THSBS) c) cross-section of a 330 nm thick native BC mat prepared from a broth depth of 0.26 cm. c) water drop on THSBC from which the water contact angle (98±2º) was measured.

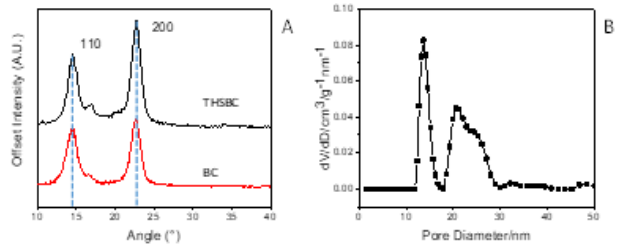

Figure 4. a) Wide angle X-ray diffraction pattern obtained for multiple layers of 328 nm thick bacterial cellulose (BC) and chlorotrihexylsilane modified BC (THSBC). b) b) pore size distribution of the 328 nm ultra-thin BC films

CONCLUSIONS

Cutinases: (LCC-NG) displays high global conformational stability based on T_m values determined by CD and fluorescence thermal scans (83ºC and 75ºC, respectively). However, LCC-NG is highly prone to aggregation. Indeed, electrostatic interactions resulted in its precipitation even at room temperature and low concentrations (10-20 µM). Hence, the purification and storage of LCC-NG requires use of salts at concentrations that vary with protein concentration. Further, efficient PET hydrolysis requires LCC-NG to be active at or above 70ºC, which is very close to the onset tertiary structure loss. At this temperature, LCC undergoes rapid aggregation that is a function of its concentration. This behavior imposes limitations on its practical applications such as for catalysis of PET hydrolysis. LCC's bacterial origin results in its non-glycosylated native state. However, biosynthesis of glycosylated LCC inhibits LCC thermal-induced aggregation. Consequently, glycosylated LCC (LCC-G) enables facile purification and high concentration storage without salt. Further, the onset of thermal induced aggregation temperature for LCC-G is 10ºC higher than LCC-NG. Moreover, the rate of aggregation for LCC-G is slow compared to the rapid aggregation of LCC-NG at or above 70 ºC. Stabilization of LCC by glycosylation also enables an improvement in the catalytic performance of LCC for PET hydrolysis. That is, LCC-G is significantly more active than LCC-NG at higher temperature (above 65ºC) while retaining activity at higher concentrations relative to its non-glycosylated counterpart. Hence, glycosylation of LCC is a powerful stabilization strategy that can be applied to other aggregation prone proteins regardless of their bacterial origin.

Bacterial cellulose: In summary, this study is the first report of an ultra-thin bacterial-grown nanoscale interconnected fibrous cellulose network, which is light, foldable, surface-functionalizable, mechanically strong, as well as environmental friendly. The ultra-thin BC films are fabricated as antireflection coatings (ARCs), that can be used to increase light capture in silicon and perhaps other optoelectronic devices. Additionally, hydrophobic THSBC is fabricated as ARCs on silicon substrates, which exhibit favorable hydrophobicity, and similar optical properties. This work builds a platform for biologically grown optical coatings, which avoids conventional energy intensive ARC fabrication routes. The process is simple, inexpensive, amenable and industrial-compatible.

ACKNOWLEDGEMENTS

This work was funded by the National Science Foundation Partnerships for International Research and Education (PIRE) Program (Award #1243313).

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(PL3) Polymer and Fibre Biocatalysis

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Keywords: *Laccase; esterases; polyethylene glycol; immobilization; PEGylation; Molecular modelling studies*

GENERAL ABSTRACT

In this lecture an overview is done about the last results produced with the BBRG group at the University of Minho. Laccase and esterase enzymes were intensively used for the production or polymers.

Laccases were used for the production of poly(catechol). This polymerization was studied in presence of PEG in solution, after pegylation of the enzyme and with different forms of enzyme immobilization. Enzyme mobility, linkers and mixing of the reaction product with PEG are key paramenters on the production of poly(catechol). This reaction was also studied with intensification methods like high pressure homogenization ultrasound. The precipitated polymers were characterized by Nuclear Magnetic Resonance spectroscopy (^{1}H NMR) and MALDI-TOF spectrometry.

Esterase enzymes were studied for the production of polyesters. The reagents were chosen that a volatile reaction product was obtained and final vacuum step could “push” the reaction forward. All polyesters were deeply characterized by Nuclear Magnetic Resonance spectroscopy (^{1}H NMR) and MALDI-TOF spectrometry. The obtained PES was used to coat cotton fibres and properties were measured. Coupling reactions promoted by enzymes (mostly with estereases) for various application are also mentioned.

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(PL4) In vivo Confocal Raman Spectroscopy Applied to Cosmetic Science

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Keywords: human skin, human hair, antiaging, collagen, infrared, blue light

INTRODUCTION

Many cosmetic or dermocosmetic formulations are not appropriate because of the natural barrier of human skin, the stratum corneum (SC), and several topical agents have a small percentage of bioavailability. The interaction and possible adsorption of cosmetic formulation components in human skin is highly dependent on the formulation. The problem of understanding the barrier function of the skin and the evaluation of the permeation mechanisms is difficult by conventional methods. There is currently no universal predictive model for skin permeation or interaction, particularly for cosmetic formulations and the modulation of barrier function, and the establishment of a model is complex because of the variability found in conventional diffusion studies. There is a need to provide a molecular view of the mechanisms of interaction of cosmetic formulations with the skin. The use of conventional biophysical techniques such as Franz cell or tape-stripping does not provide biochemical modulation information or spatial resolution for determining the distribution of different domains and compounds in the skin. This information is crucial for understanding the interaction of cosmetic formulation in the skin, determining its efficacy and factors influencing this interaction, including possible permeants and retainers of formulation compounds through and under the skin. In this regard, the confocal Raman spectroscopy technique has provided excellent results in the characterization of dermal delivery and biochemical modulation resulting from this process. Unlike traditional methods, confocal Raman Spectroscopy (CRS) has been recently used to provide, in a non-invasive, real-time manner, biochemical, molecular and structural changes in human skin resulting from the effects of aging, photo aging, chronological aging, permeation and effect of solar radiation or air pollutants on the skin.[1,2]

In this talk we will show how Raman information can be used by the cosmetic industry to develop new cosmetic formulations or optimize their product by finding potentially enhancer to increase their penetration and to identity the most efficient nano-encapsulating strategies to support their claim. It will be shown that CRS can be used *in vivo* to determine the efficacy of products against the effect of exposure to visible, IR and light blue radiation from laptops, cell phones and tablets.

RESULTS AND DISCUSSION

The *in vivo* non-invasive characterization of the skin was performed using confocal Raman spectrometer (River Diagnosis, 3510) with an inverted microscope (Figure 1). Raman spectra of skin dermis (Figure 2) shows the presence of collagen features at 815 cm^{-1} (C-C stretching), an intense Raman bands was observed at 875 cm^{-1} assigned to hydroxyprolin ring, two Raman bands at 856 and 920 cm^{-1} assigned to proline ring and another band at 938 cm^{-1} assigned to C-C stretch vibration of backbone formed by Gly-X-Y sequences.

Figure 1. Confocal Raman measurement of the IR exposed area

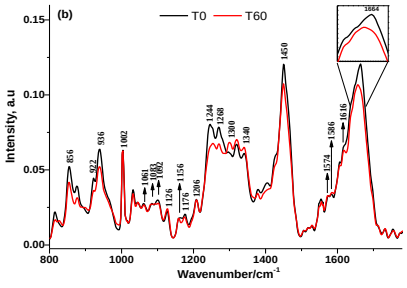

Figure 2. Averaged *in vivo* Raman spectra of the skin dermis before (T0) and after IR irradiation (T60).

The changes found in the Raman spectra of Figure 2, could be understood that the amount of protein decreased and more specifically, the amount of collagen decreased after the IR irradiation. It has been reported that, IR radiation induces the

production of matrix metalloproteinases (MMPs) without an increase in the respective inhibitor TIMP-1.[3] These MMPs contributes to the degradation of collagen and abnormal activity of MMPs were considered as the important factors responsible for the degradation and aging of skin. The red shift observed at 1664 cm^{-1} for T60 indicates the change in (Amide I) α helical structure.

CONCLUSION

Based on our study, it may be concluded that confocal Raman spectroscopy is an efficient tool to monitor minor changes and the IR dose that the persons receives in a day during summer, in a tropical country causes changes in the skin structure.

ACKNOWLEDGEMENTS

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(PL5) Protein Self-Assembly in Bio-Inspired Materials

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Keywords: protein self-assembly, nanofirils, microfluidics

ABSTRACT

Self-assembly is the autonomous organization of components into patterns or structures without human intervention. A number of different assemblies can be formed by proteins. A particularly interesting example of protein self-assembly is a formation of highly ordered, nearly one-dimensional fibrillar structures. This high-level, long-range ordering is relatively independent of the molecular identity of the protein monomers. Interestingly, in nature, such structures can perform either beneficial roles or appear as aberrant protein aggregation, which is in a latter case results in the development of neurological disorders. The main objective of our research is to understand the evolution of protein complexes in the context of both biological function and malfunction as well as to draw the links between structure and properties of self-assembling materials based on natural polypeptides.

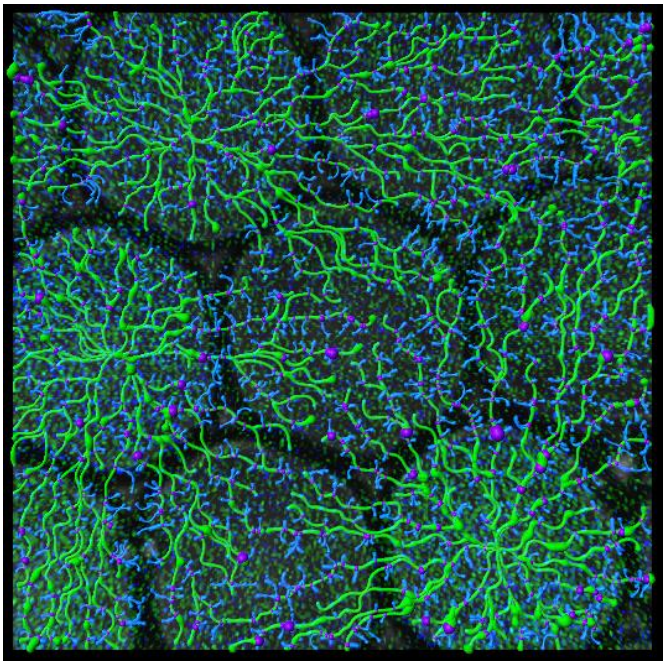

Figure 1. Three-dimensional reconstruction of nanofibrilar protein-based capsules from confocal z-stacked images.

ACKNOWLEDGEMENTS

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KEYNOTE LECTURES

(KL 1) The impact of DNA methylation on *Humicola grisea* var. *thermoidea* enzyme activities and on the glucose-mediated gene repression

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Keywords: 5-aza-2′-deoxycytidine, DNMTs inhibition, epigenetic regulation, glycosyl hydrolases, *Humicola grisea* var. *thermoidea*

INTRODUCTION

Humicola grisea var. *thermoidea* (Hgvt) is a thermophilic ascomycete that produces lignocellulolytic enzymes and it is proposed for the conversion of agricultural residues into useful byproducts. Drugs that inhibit the DNA methyltransferases (DNMTs) activity are employed in epigenetic studies but nothing is known about a possible effect on the production of fungal enzymes. We evaluated the effect of 5-aza-2′-deoxycytidine (5-Aza; a chemical inhibitor of DNMTs activity) on the secreted enzyme activity and on the transcription of cellulase and xylanase genes from Hgvt grown in agricultural residues and in glucose.

RESULTS AND DISCUSSION

Concentrations ranging from 10 to 100 µM of 5-aza-2-deoxycytidine did not affect growth or sporulation on potato-dextrose agar medium, thus indicating that the drug was not cytotoxic to the fungus. *H. grisea* was then grown up to 96 h in liquid minimal medium supplemented with 25 µM of the DNMTi and with wheat bran, sugar cane bagasse or ground hay, as enzyme-inducing carbon sources, or with glucose, as repression condition. Although, in general, secreted celullase and xylanase activities were lower than for the control condition upon growth on inducing conditions, a striking increase of transcript accumulation for *cbh1.1 and cbh1.2 (*cellobiohydrolase)*, and xyn2 (xylanase) genes was observed upon growth on glucose*

CONCLUSION

In this pioneer study, we demonstrated that DNA methylation inhibition overcomes the glucose-mediated transcription repression mechanism we previously observed for these genes (Mello-de-Sousa et al., 2011) and that an epigenetic approach can be used to improve Hgvt potential as a producer of biotechnology inputs

ACKNOWLEDGEMENTS

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Fundação Universidade de Brasília (FUB); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Fundação de Apoio à Pesquisa do Distrito Federal (FAP-DF)

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(KL 2) Confocal laser scanning microscopy of cane bagasse before and after steam explosion and alkaline delignification

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Keywords: cane bagasse, pretreatment, confocal microscopy, lignin fluorescence

INTRODUCTION

Pretreatment of lignocellulosic materials are crucial for developing biomass conversion technologies[1]. For this, it is also critical to develop methods for evaluating the effect of pretreatment on fiber morphology and chemical composition, both being extremely important for predicting surface properties and accessibility to processes such as enzymatic hydrolysis. In this work, confocal laser scanning microscopy (CLSM) and fluorescence lifetime imaging microscopy (FLIM) using one- and two-photon excitation[2] were used to visualize the lignin distribution within bagasse fibers before and after pretreatment with mineral acids at equivalent combined severity factors. The effect of delignification on fiber properties was also investigated, aiming at visualizing lignin distribution in the cell wall by analyzing changes in lignin fluorescence after the two-photon laser excitation.

RESULTS AND DISCUSSION

CLSM/FLIM was performed by measuring the fluorescence spectra of lignin and its fluorescence decay times[3]. Untreated and steam-exploded cane bagasse fibers, before and after alkaline delignification, were suspended in water and dropped on cover-slides for microscopic analysis. CLSM images were obtained as an average of three scans using a Plan-Apochromat objective lens (20x) and a Zeiss LSM 780 confocal microscope with a Coherent Chameleon laser (Tisapphire) as a source for two-photons (2P) for the excitation experiments. The optical resolution of the lens was about 300 nm. For FLIM analysis, the 2P laser was pulsed at 80 MHz with a time response limited to about 100 ps at 800 nm and an average power of 15 mW.

Images were obtained in the spectral mode in which each pixel contains the information of the emission spectrum of lignin. The combination of spectra in each pixel generates colors that are faithful to the spectral region being emitted. The untreated sample basically emits in blue and little spectral variation is observed. This means that the molecular arrangement and the chemical composition of lignin are practically the same throughout the sample specimen. The samples treated by steam explosion presented significant changes compared to the untreated fibers. Low-energy red emissions were associated to the formation of lignin-carbohydrate complexes and molecular aggregates, as well as to the accumulation of lignin on the fiber surface. This demonstrates a profound deconstruction in the structural arrangement of lignin in the plant cell wall, as already observed by others[2,3]. Therefore, as a result of pretreatment, lignin was removed from the inner layers of the cell wall while being chemically modified to be adsorbed on the fiber surface as clusters, observed not only in red but also in green emissions. The interior of the fibers contained less lignin aggregates, suggesting that lignin migrated to the outer layers of the fiber surface. This technique was also applied to native and pretreated fibers that were delignified by alkali. These fibers emitted predominantly in blue, which indicates the effective dissolution of lignin aggregates from the fibrous material. However, for fibers that were delignified to a lesser extent, a thin layer of lignin was observed on the fiber surface and this partially explains the recalcitrance of such fibers to conversion processes such as enzymatic hydrolysis.

Native and pretreated materials, delignified or not, were also characterized using several analytical methods and the results obtained by CLSM were tentatively correlated with their corresponding chemical composition and accessibility to processes such as enzymatic hydrolysis.

CONCLUSION

CLSM and FLIM revealed structural features and molecular organization of lignin within the cell wall of cane bagasse, before and after steam explosion and alkaline delignification.

ACKNOWLEDGEMENTS

CAPES, CNPq, UFPR, INCT of Bioethanol.

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(KL 3) Development of biocomposites derived from biodegradable polyesters

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Keywords: biocomposites, biofibers, biodegradable, polyesters, renewable resources

INTRODUCTION

Biocomposites consist of a biodegradable polymer as matrix material and (bio)fibers as reinforcements, which enhance the strength and stiffness of the matrix. For biomedical applications, for example, a number of composite biodegradable scaffolds have been evaluated for the development of tissue engineering [1], while elastomeric networks have been investigated for the development of artificial microvasculature, nerve guidance and drug delivery devices [2].

Two polymers successfully used as reinforcements are poly(hydroxybutyrate) (PHB) electrospun fibers, with potentially interesting applications due to its biodegradability, bioresorbability and biological synthesis from renewable sources, and nanocellulose (NC) fibers, which is produced from a very abundant natural polymer.

Different biodegradable polyesters can be used as matrices. In our research group (GRUPOL-UDESC), we have been working with poly(glycerol sebacate) (PGS) and poly(mannitol sebacate) (PMS), which are tough elastomeric biodegradable polymers with very promising features to be used for in vivo blood vessel engineering, due to its biocompatibility and mechanical properties, as well as copolymers produced from L-lactide and ε-caprolactone (PLLA-co-CL) and resveratrol which can be synthesized using monomers from natural renewable resources.

In this work, we will present some recent developments of GRUPOL-UDESC in biocomposites and polyesters from renewable resources.

RESULTS AND DISCUSSION

Electrospinning of PHB fibers

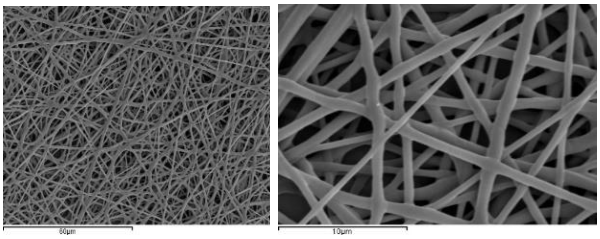

Figure 1. SEM images illustrating the morphology of electrospun PHB fibers at the best experimental conditions: Concentration: 10% (m/v) in CF / DMF (9/1); Flow rate: 5.8 mL / h; Voltage: 17 kV; with addition of sodium dodecyl sulfate (SDS)

Preparation of Biocomposites Reinforced with Electrospun PHB Fibers (MSc. Mariana Bertoncini)

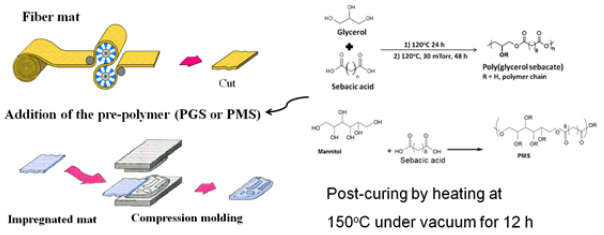

Figure 2. Scheme for the preparation of biocomposites reinforced with electrospun PHB fibers.

Condensation polymerization of trans-resveratrol with adipic acid (MSc. Lidiane Mendes)

Resveratrol is considered a promising monomer in polymer chemistry, once its hydroxyl groups can be used in esterification reactions. Thus, adipic acid was employed as a short chain carboxylate source. Polymerization reactions between resveratrol and adipic acid were tested via two distinct methods. At mild conditions employing heating in acetic anhydride only an oligomer was obtained. In the second method, adipic acid was converted into acid chloride, a highly reactive intermediate, and then reacted with resveratrol yielding the desired polymer.

PLLA-co CL/NC composites
(DSc. Katiusca Wessler Miranda)

Ring opening polymerizations L-lactide and ε-caprolactone by bulk polymerization and solution polymerization, in order to evaluate the influence of initiator concentration (Sn octoate), reaction time and reaction temperature on the yield and molecular weight of both polymers. It was possible to functionalize CNC particles *in situ*, using 80/20 LLA/ε-CL systems, with Sn octoate as catalyst.

CONCLUSION

Biocomposites reinforced with electrospun PHB fibers or NC were successfully prepared by *in situ* techniques. The results show interesting thermal and mechanical behavior and very good adhesion between polymer and fibers.

ACKNOWLEDGEMENTS

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(KL 4) Design of fibrous and injectable platforms for the release of therapeutic ions and drugs in chronic skin wounds treatment.

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Keywords: injectable thermo-sensitive hydrogels, electrospun fibers, therapeutic ions, gelatin, polyurethane

INTRODUCTION

More than 40 million people suffer from chronic wounds worldwide (8 million in EU and 6.5 million in US in 2009), most commonly caused by poor blood supply to the lower limbs. Chronic wounds represent a frequent and very severe problem in patients with diabetes mellitus, a pathological condition involving around 285 million people globally. Hence, their treatment is a growing burden for healthcare systems due to the increasing costs, the population aging and the rise in diabetes and obesity incidence. The ideal wound dressing should (i) provide a moist environment, (ii) protect from external agents, (iii) remove exudate, and (iv) enhance tissue regeneration. However, no existing treatment fulfils all these demands.[1] Hence, the design of innovative, smart and effective wound dressings represents a current and important challenge. In this contribution, we developed two different approaches for the treatment of chronic skin wounds, i.e. antibacterial gelatin nanofibrous membranes and injectable thermosensitive polyurethane-based hydrogels releasing copper ions and/or ibuprofen from mesoporous glasses.

RESULTS AND DISCUSSION

Gelatin nanofibres loaded with silver nanoparticles (AgNPs) and gentamicin sulphate (GS) were successfully obtained by electrospinning starting from aqueous solution to avoid denaturation phenomena.[2] The antibacterial properties of the optimized fibrous matrices were tested against four pathogenic bacteria isolated from infected wounds (*Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus Epidermidis, Pseudomonas aeruginosa*) and high efficiency against tested strains was observed. Furthermore, the biocompatibility of the developed nanofibres was confirmed using Neonatal Normal Human Dermal Fibroblasts (NHDF-Neo).

Injectable thermosensitive hydrogels were designed by solubilizing an amphiphilic polyurethane (PU), based on Poloxamer P407, 1,6-hexamethylene diisocyanate and 1,4-cycloexane dimethanol, in physiological solution (0.9g/l NaCl).[3] Injectable hydrogels for the targeted release of anti-inflammatory drugs and/or ions with antibacterial properties were designed by loading PU-based sol-gel systems with different amounts of mesoporous bioactive glasses releasing copper ions and/or ibuprofen. Tube inverting, gelation time and rheological tests showed the ability of 15%w/v concentrated PU solutions to undergo a sol-to-gel transition at about 27°C within few minutes. Encapsulation of different amounts of mesoporous particles (within the concentration range 1-20mg/ml) did not significantly affect the thermosensitive behaviour of PU-based hydrogels. A slight decrease in gelation kinetics was observed with increasing particle content, but no hydrogel lost its capability to undergo a sol-to-gel transition in physiological conditions. The release kinetics of Cu^{2+} and ibuprofen were assessed by Inductively Coupled Plasma Atomic Emission Spectrometry Technique (ICP-AES) and High Performance Liquid Chromatography (HPLC), respectively.

CONCLUSION

The treatment of chronic wounds requires the design of multifunctional dressings combining antibacterial features with tissue regeneration potential. Two promising approaches for this challenging application have been here reported, based on gelatin-based nanofibres loaded with AgNPs and GS, and PU-based hydrogels carrying in situ mesoporous and multifunctional particles.

ACKNOWLEDGEMENTS

This work was supported by the Horizon 2020 European Union funding for Research & Innovation project "MOZART" (MesopOrous matrices for localiZed pH-triggered releAse of theRapeuTic ions and drugs) (H2020-NMP6-2015).

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(KL 5)Changes of the shape of keratin based fibers

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Keywords: keratin, disulfide bonds, hydrogel coating

INTRODUCTION

Keratin fiber is a biocompatible and huge network polymer material. Its structure consists in a cuticle, cortex and cell membrane complex. This protein composes the soft keratins as structural component of skin (e.g. from epidermis), and the hard keratins as a major component of hair, epidermis, wool, horns and also feathers, claws, and beaks of birds and reptiles. Various studies related to wool keratin structure have been extended to studies related with human hair keratin since its microstructure is analogous to wool keratin.

Haircare industry has developed a plethora of products to modify hair characteristics being the hair straightening one of the most popular processes. Many of these products contain toxic or environmentally hazardous chemicals such as strong alkaline agents among others, including thioglycolates, sodium or lithium hydroxide, guanidine or even formaldehyde. These chemicals have high toxicity, potential to generate poisonous gas and have a negative impact on human health.

Common chemical styling processes such as coloring, perms, straightening or bleaching make permanent changes to the hair's cortex, destroying parts of its structure. These processes tend to weaken and dry hair, making it brittle. As a result of the damage, hair loses some of its properties, concretely strength and elasticity. Excessive or repeated hair chemical treatments produce irreversible changes in hair texture, and can result in a fracture of the hair fibre once the cuticle is removed and the cortex exposure leads to it breakage. Disulphide bonds disruption and formation are known to be associated with the changes of shape in hair.

Based on hydrogels preparation methods, they can be classified as homopolymer gels or copolymer gels, and can be prepared from synthetic or/and natural polymers. Hydrogels as three-dimensional crosslinked hydrophilic polymer networks have capacity of swelling or de-swelling reversibly in water and retaining large volume of liquid in swollen state_ENREF_13. Photopolymerization of hydrogels is of particular interest in biomedical applications because of its in situ and non-toxic gelation. Light-activated polymerization is typically carried out with an appropriate wavelength, whereby free radicals attack vinyl groups on precursor macromolecules, leading to the formation of covalent bonds that crosslink the hydrogel network under UV light exposure.

There is an ongoing need to develop new products for hair care cosmetic use as changing its morphology characteristics. In this context, the aim of this study was the development of a hydrogel formulation in order to coat the surface of wool yarn, and test it capacity of stretching and curling induced by humidity. In a cosmetic point of view, the hydrogel coating on wool yarn could have high potential interest in hair care use since it explores dimensional changes of the hair fiber under humidity conditions [1]. Here, we also explore the use of peptides as substitutes of alkaline and thio-relaxers which are commonly used for hair straightening. Our goal is to present a novel green approach to validate decapeptides sequences as eco-friendly alternatives for the control of hair shape changes. These peptides were selected from a previous broad study which analyzed over one thousand different peptides containing two to five cysteines based on the human hair genome of keratin and KAPs. It is known that cysteine has been applied as a reducing agent for the substitution of environmentally harmful chemicals. The peptides origin, their high cysteine content, and hydrophobicity are intended to improve hair properties, enabling the restructuring and reinforcement of hair fiber structure. A deep characterization of the peptides was established regarding their ability as hair straightening agents and ability to recover and/or increase hair tensile strength and elasticity. Herein, we explore the application of peptides as innovative ecological and healthy alternatives for hair straightening, without compromising hair integrity, opening an innovative way to change the shape of the hair fiber [2].

RESULTS AND DISCUSSION

HUMIDITY INDUCES CHANGES IN THE DIMENSIONS OF HYDROGEL-COATED WOOL YARNS

The wool yarns were coated by different hydrogel formulations and were evaluated under length, elongation and strength variation. The hydrogel formulation containing Al2O3-AD was investigated in the frame of this work since the strength of hydrogel-coated wool yarn was favoured when two monomers were used (AA+DMAA) with the nano-alumina particles as crosslinking agent.

Additionally, was analysed the effect of different pre-treatments before the hydrogel coating on wool yarns. The length variation of the hydrogel-coated wool yarns at wet and dry state was studied based on different pre-treatment conditions in order to achieve the best formulation under humidity conditions, for further characterization. It was found that increasing the concentration of sodium hydroxide in the presence of urea lead to the increase of the length variation of wool yarns, for five cycles of dry-wet cycles. Polar solvents such as urea and alkaline pH solutions are well known because of their swelling and penetration promoting properties. They disturb inter- and intra-molecular hydrogen bonds, weak the hydrophobic interaction between polypeptides, thus leading to an exposure of more polypeptides chains of keratin to the solvent. From Figure 1 it is perceptible that the length variation of the yarn increases as the increasing of concentration of the NaOH.

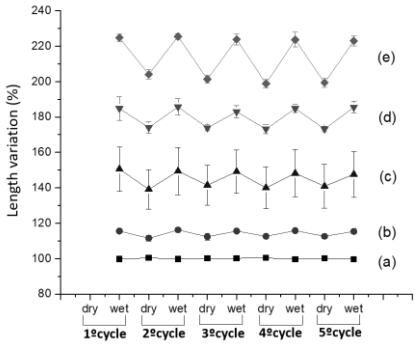

Figure 1. Length variation of hydrogel-coated wool yarns at wet and dry state during five consecutives cycles. Wool yarns were pre-treated with different concentrations of urea and sodium hydroxide: a) 8 M urea, b) 8 M urea with 0.0025 M NaOH, c) 8 M urea with 0.005 M NaOH, d) 8 M urea with 0.025 M NaOH, and e) 8 M urea with 0.05 M NaOH during 2 hours; after pre-treatment of yarns, UV light was used to trigger the polymerization on the surface of wool yarns forming the hydrogel. Formula of the hydrogel: 10% nano-Al2O3, 1% AA, 9% DMAA, 0.1% photo-initiator.

The production of the hydrogel network was formed by free-radical polymerization of multifunctional vinyl monomers (AA+DMAA). Each of these monomers contains a carbon double bond through which an active centre may propagate to produce polymer chains. The method to generate active centres depends on the specific monomers, solvents and the reaction conditions employed. Herein, it was based on UV light by the use of photo-initiator Irgacure 2959. Photoinitiator has an essential role in the photopolymerization process, since it is excited under UV radiation which leads to the formation of the free radicals in the initiation step of the polymerization. The photo-initiator, Irgacure 2959, has been identified as extremely reactive, once been irradiated, it generates benzoyl radicals (higher reactivity than alkyl radicals) to initiate the polymerization, and also present high thermal stability _ENREF_19. It has also been suitable in achieving high cell viability during the photopolymerization reaction of cell-encapsulating hydrogels. The hydrogel adhesion of the hydrogel coating remains stable up to 2 months.

Metal oxide nanoparticles including alumina (Al2O3) can provide magnetic properties which can be suitable for biomedical applications. The magnetic nanoparticles form covalent bonds with the polymer network due to the intrinsic strong negative charge of the acrylic acid [3]. This magnetic hydrogel network is suitable for humidity induced dimensional changes of wool yarns, allowing the modification of elastic properties and changes in the shape of the sample.

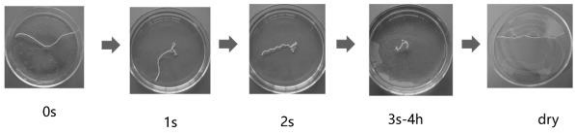

Figure 2. Images of sequential addition of water on hydrogel-coated wool yarns.

The dimensions of the hydrogel-coated wool yarns were changed after addition of water. These dimensional changes are well perceptible from Figure 2. After few seconds the wool yarns quickly curled after the addition of water and when dried elongate again. The molecular distribution of the hydrogel formulation was obtained by GPC/SEC. The molecular weight distribution (polydispersity: Mw/Mn) was about 1.72, which indicates a moderate molecular weight distribution. The hydrogel was characterized by FT-IR spectroscopy [1]. The presence of functional groups in the hydrogel had a crucial effect on it water holding capacity. Consistent with literature discussed, the disappearance of the characteristic vinyl bands at 1680 cm-1 in spectra indicates that all monomeric groups were involved in the polymerization reaction [4-5]. The thermal analysis allowed to observe that wet hydrogel is more stable than dry hydrogel since remains steady with a large scale of temperature [1].

The modification of the shape of the hair it was also studied based on peptides which have the ability to bind to hair proteins, be absorbed by the hair fibre, improve damaged hair mechanical properties and aid on the straightening of curly hair.

CHANGING THE SHAPE OF HAIR WITH KERATIN PEPTIDES

The straightness of African curly hair was assessed by the incorporation of peptides into a serum formulation. The formulation containing the peptides was applied in hair tress. The hair tresses were combed straight and mechanically straightened with an iron flat. The shape of the hair tresses was analysed for each peptide after treatment. The penetration of the peptides towards hair fibre was facilitated through their incorporation into the serum formulation containing benzyl alcohol, propylene glycol and ethanol. The hair tresses were treated with two concentrations (0.01% and 0.1% (w/v)) of the selected peptides: PepE, PepG and KP. The selection of these peptides was based on the straightening efficiency in the three conditions of the single straightening hair treatments. Figure 3 shows the qualitative and quantitative analysis of these treatments. The inclusion of the peptides into a serum formulation was done in order to simulate the effect of the peptides as straightening agents in a commercial formulation. The development of a cosmetic product as a green alternative to the chemical relaxing treatment was the motivation behind the work here presented. Thus, it was essential to evaluate the straightening effect of the peptides when included in a formulation.

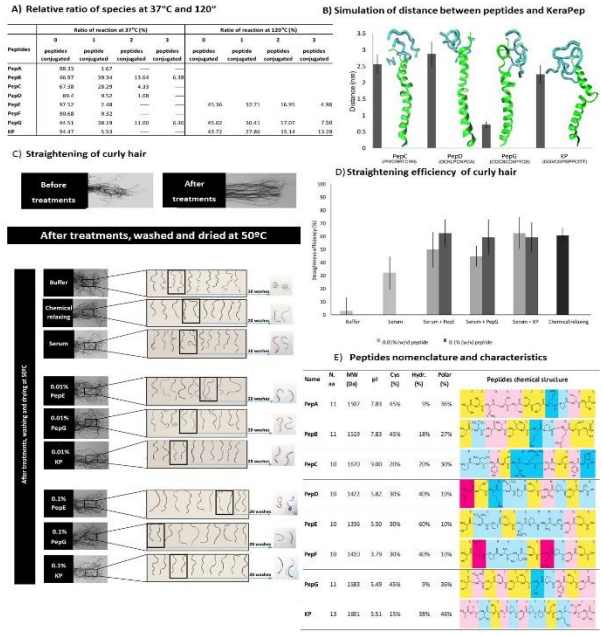

Figure 3. (A) Relative ratio of the reaction of the peptides and the KeraPep at 37°C and at 120°C obtained by MALDI-TOF/TOF; **(B)** Molecular dynamics simulation of the average distance on the last 10 ns of simulation between dimers of the KeraPep and the peptides PepC, PepD, PepG, and KP. The images reflect the final state of the simulations; **(C)** Straighten curly hair tresses before and after peptide treatments: images on top; After treatments the hair tresses were washed and dried at 50°C: the bottom images present the samples treated with the serum formulation containing peptides (PepE, PepG, KP) at 0.1% (w/v), the images at the middle present the samples treated with the serum formulation containing peptides (PepE, PepG, KP) at 0.01% (w/v) and on the top the samples treated without peptides: buffer, chemical and base serum formulation treatment. **(D)** Straightening efficiency of peptides PepE, PepG and KP in serum formulation; **(E)** Peptides nomenclature and characteristics: name, number of amino acids (N.a.a), molecular weight (MW), isoelectric point (pI), percentage of cysteines (Cys), percentage of hydrophobic (Hydr.) and polar amino acids in the peptide sequence, and peptides chemical structure.

Each peptide has a specific amino acidic sequence which was crucial for its behaviour towards hair proteins. Due to the conformation of each peptide the input in the final results is highly dependent on the reactivity of each cysteine and its accessibility; on the residues in the vicinity of the cysteine; on the charge-charge interactions and on the location of the cysteine. Basically, the peptide mechanism involves the breakage of the disulphide bonds between hair keratin filaments and then the re-modulation of the hair occur by the disulphide bonds rearrangements between hair proteins and peptides. The formation of the disulphide bonds between the cysteine thiols presented in the peptides with the hair keratin or KAPs is due to the reduction of the hair protein disulphide bonds and the reformation of these disulphide bonds with the thiol of the peptides and even with themselves. Despite the lower ability of the sulphur atoms of cysteines, they may also be linked by hydrogen bonds or electrostatic interactions.

PepG and KP present net negative charge, however, their ability to form disulphide bonds with the hair fibre were more effective. Their conformation and cysteines accessibility was imperative for the higher conjugation with hair keratin which leads to an effective recovery of chemically damaged hair and efficient straightness of African hair. The effect of the treatment with these peptides is sustained for, at least, 20 washes with shampoo, confirming the long durability of the treatment. These results open an innovative way to repair damaged hair fiber from severe and repetitive treatments (e.g. coloration, permanent and waving) and to straight curly hair avoiding conventional chemicals, preserving the integrity of the hair structure.

CONCLUSION

Although conventional chemical treatments are the most popular methods to change the style of the hair, they represent a threat for hair, user and for the environment.

The modification of the keratin fiber morphology based on hydrogel coating was successfully achieved. Coating of wool yarns by a polymeric hydrogel based on acrylic acid and N,N dimethylacrylamide was successfully achieved by photopolymerization reaction. The reaction was conducted with nanoparticles of alumina as

crosslinker and triggered by UV radiation. The success of crosslinking reaction was confirmed by FTIR analysis and the suitable polymer molecular weight distribution was established by GPC analysis. The length of hydrogel-coated wool yarns varied in the presence of water and once dried elongate again. These results revealed that hydrogel coating change the dimensions of wool yarns under humidity conditions.

The repetitive use of conventional cosmetic chemical treatments can severely affect hair physicochemical properties. Eight engineered small peptides based on fragments of human hair keratin and KAPs were designed to restore hair properties and to straighten curly hair. These peptides, when applied to chemically over-bleached hair and curly African hair, showed to penetrate the hair fibre into the cortex and to bind to the hair proteins. Due to this binding, they induced a significant recovery in tensile strength and elasticity of severely damaged hair. Of the eight tested peptides pepE, pepG and KP, showed higher ability to interact with a keratin peptide model. Based on these the three peptides were explored as modulators of hair shape. When applied to curly hair, these peptides were shown to enable a high straightening efficiency without the use of harsh chemicals. Peptide treatment at neutral pH involves the formation of intra and inter molecular disulphide bonds mechanism between cysteine based peptides and hair proteins showing great potential for cosmetics use as modulators in the shape of the hair. The new method here proposed based on peptide formulations is environmentally friendly and constitute a real alternative to the conventional chemical treatments, opening a new chapter for a green haircare cosmetic industry.

Note: Reproduced from articles already published: "Humidity induces changes in the dimensions of hydrogel-coated wool yarns" in Polymers and "Changing the shape of hair with keratin peptides" in RSC Advances.

ACKNOWLEDGEMENTS

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(KL6) Biopolymers applied in the development of medical textiles

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Keywords: Natural polymers, Medical textile

INTRODUCTION

Several types of natural polymers have been studied in recent years as they offer advantages such as being biocompatible, biodegradable, non-toxic and of great abundance in nature. Natural polymers can be divided into: protein-based polymers (eg collagen, albumin, gelatin) or polysaccharides (eg starch, agarose, alginate, carrageenan, chitosan and chitin)[1,2].

The objective of this work is to present applications of natural polymers such as cellulose from sugarcane bagasse and chitosan (agroindustrial residues) and alginate (algae polymer) in the development of fibers, membranes, nanofiber membranes, scaffolds and microcapsules with drugs with the objective of medical applications.

RESULTS AND DISCUSSION

Cellulose was obtained from sugarcane bagasse and used to produce textile fibers Figure1 and Figure 2. It was also converted to cellulose triacetate and produced as nanofiber membranes Figure 3. The alginate, chitosan and hybrids (alginate/chitosan) textile fibers were produced by the same extrusion method of bagasse cellulose fibers and characterized. Figure 3 shows the scaffolds and Figure 4 the microcapsule.

Figure 1. (a) Sugarcane bagasse and (b) cellulose

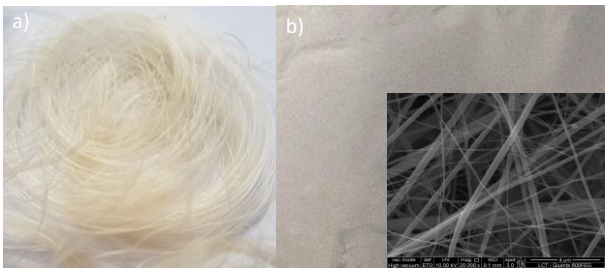

Figure 2. (a) Textile fibers of cellulose sugarcane bagasse and (b) nanofibers membranes of cellulose acetate.

Figure 3. Scaffolds of fibers hybrid (chitosan/alginate)

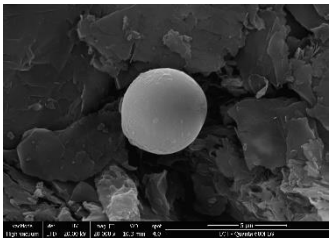

Figure 4. Microcapsules observed from SEM

CONCLUSION

The results were promising for obtaining fibers from bagasse cellulose, alginate, chitosan and alginate / chitosan hybrids. The bagasse cellulose was converted to cellulose acetate, which was used in the production of nanofiber membranes, all the materials developed showed no toxicity and the mechanical properties were adequate.

ACKNOWLEDGEMENTS

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(KL 7) Enzymatic ring-opening polymerization and functionalization of macrolactones

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Keywords: Cyclic macrolactones, Lipase, e-ROP, polymer functionalization

ABSTRACT

During the last decades, many efforts have been made in the development of new biocompatible and bioresorbable polymeric devices for biomedical application, aiming to improve the quality of life of the patients. Polyesters are one of the most studied polymers to these applications, due to its capacity of being bioresorbed/biodegraded, besides being biocompatible. The ring opening polymerization reaction of lactones to produce polyesters may be catalyzed by organic and organometallic catalysts, as well as by biocatalysts as enzymes. The use of enzymes as catalysts is promising, since unlike organic and organometallic catalysts, it does not generate toxic residues and the reaction can be carried on under mild conditions, in an efficient way[1]. In our research group, the polymerization and copolymerization of macrolactones, unsaturated globalide and ω-pentadecalactone (ω-PDL), was performed by enzymatic ring-opening polymerization in different solvents[2], including supercritical carbon dioxide[3], as well as in aqueous miniemulsion[4] to produce both saturated and unsaturated aliphatic polyesters. The effect of different lipases and concentration on the polymerization yield and polymer molar masses were evaluated[5], as well as particle size distribution, when the reactions were performed in miniemulsion. The copolymerization of globalide (Gl), an unsaturated macrolactone, with ω-PDL or ε-caprolactone resulted in a linear polyester containing unsaturations in the main polymer chain that were functionalized with different chemical groups through thiol-ene reactions, adding to the copolymers characteristics such as lower crystallinity, higher hydrophilicity and affinity for different human tissues.

ACKNOWLEDGEMENTS

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ORAL PRESENTATIONS

(OP 1) Enzymatic tools for the green synthesis of clickable polyesters

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Keywords: Enzymatic synthesis, functional polyesters, Candida antarctica lipase B, green polymer chemistry

INTRODUCTION

The unique selectivity of enzymes, along with their remarkable catalytic activity, constitute a powerful tool for transforming renewable feedstocks and adding value to a wide array of building blocks produced by the emerging bio-based chemistry sector[1].

RESULTS AND DISCUSSION

In this work, Candida antarctica lipase B (CaLB) was used to catalyze the synthesis of functional polyesters based on dimethyl itaconate (DMI) and various aliphatic diols. The same enzymatic preparation was also used for the terminal coupling of poly(hydroxyalkanoates) (PHAs) with a) DMI in order to introduce reactive side chain vinyl and b) biocompatible poly(ethylene glycol) (PEG) in order to tune the polymer's hydrophilicity properties[2]. Moreover various immobilization methods, ranging from simple adsorption to covalent binding, were tested on CaLB and on cutinase 1 from Thermobifida cellulosilytica (Thc_cut1) and highly active preparations were obtained and characterized. The immobilized biocatalysts were therefore successfully tested for the green synthesis of bio-based aliphatic polyesters[3].

CONCLUSION

This work sheds light on the possibility of synthesizing, via enzymatic catalysis, functional, clickable oligoesters that can be further cross-linked or coupled with end-chain moieties in a 2nd reaction step.

ACKNOWLEDGEMENTS

Alessandro Pellis thanks the FWF Erwin Schrödinger fellowship (grant agreement J 4014-N34 for financial support).

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(OP 2) Ultrasound - a green tool to boost enzyme reactions in lignocellulosic biomass exploitation

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Keywords: Ultrasound; Enzymes; Biomass; lignocellulosic;

INTRODUCTION

Biobased energy, fuels and platform chemicals are the cornerstones of a sustainable society. Renewable sources such as lignocellulosic biomass have to be exploited according to the biorefinery concept and the 12 principles of green chemistry. The major challenge encountered for lignocellulosic biomass is their recalcitrance that has to be overcome with efficient pretreatment and fractionation procedures and enzymatic hydrolysis to obtain monosaccharides and other important constituents[1].

Ultrasound is the result of vibrational waves on an elastic medium leading to cycles of compression and expansion that finally result in the effect of cavitation. Ultrasound has shown to be an efficient green technology to enhance chemical and enzymatic reactions[1,2]. Our group has been studying the effect of ultrasonic irradiation on different hydrolytic enzymes involved in biomass hydrolysis and its effect on hydrolysis itself. Major results will be presented and challenges and limits of ultrasound utilization are also discussed

RESULTS AND DISCUSSION

The effect of ultrasound on enzyme activity and stability and the hydrolysis of lignocellulosic materials was carried out in test tubes in an ultrasonic bath with 25 and 37 kHz. Control experiments were performed in the absence of ultrasound without or with mechanical agitation (100 rpm). The used enzymes were the *T. reesei* based enzymes *Cellic ctec2* (Novozymes) and *T. crude* (Rhoem Enzymes). Enzymes concentrations varied between 10 and 20 FPU.g^{-1} substrate. Substrates were filter paper Whatman Nº 1 for cellulase, cellobiose for beta-glucosidase and pretreated sugar cane bagasse. We also investigated the energy input and the sonochemical effect of ultrasonic irradiation with a standard chemical reaction such as KI oxidation in the ultrasonic bath[3].

The effect of ultrasound irradiation was found to vary significantly throughout the ultrasonic bath and the definite result depends much on the exact position of the reaction vessel (glass tube or Erlenmeyer) in the bath. This is true for simple KI oxidation as well as reducing sugar formation during enzymatic cellulose hydrolysis. In contrast ultrasound irradiation leads to an almost constant and quite uniform temperature increase in the ultrasonic bath. Stability of cellulases and beta-glucosidase seem to be only slightly affected. Ultrasound irradiation showed to have a significant effect on cellulose hydrolysis and differences in cellulase activities of different cellulase preparations could be diminished by the use of ultrasound.

CONCLUSION

Low frequency ultrasound (25 and 37 kHz) showed to be an efficient tool to boost the activity of enzymes involved in the hydrolytic degradation of liognocellulosic biomass. However, ultrasonic baths are not the most adequate apparatus as they provide a very heterogenous sonochemical effect concerning bath geometry. Another problem is overheating, which may lead to serious equipment breakdown.

ACKNOWLEDGEMENTS

CAPES CNPq novozymes FURB UNIVERSIDADE REGIONAL DE BLUMENAU

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(OP 3) Hydrophobic bio-functionalization of pure PLA and PLA/Jute bio-composite by surface activation with diverse esterase enzymes and coupling with Alkyl Ketene Dimer.

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Keywords: Surface activation, esterase, Alkyl Ketene Dimer (C18-AKD), hydrophobicity, PLA, bio-composite

INTRODUCTION

Bio-composite faces severe issue of surface erosion and lose structure integrity because of moisture, contact with water and other chemicals. Therefore, surface hydrophobicity is an important attribute for such bio-composite materials. The question is how can the surface of pure PLA and Jute/PLA bio-composite be activated and functionalized with the help of C18-AKD to increase the surface hydrophobicity. A systematic approach has been adopted to address this challenge. The activation of PLA surface for getting free hydroxyl groups have been conducted with various selected enzymes such as (L1-Lipase from *Thermomyces Lanuginosus*, L2- Lipase from *Candida sp. Recombinant expressed in Asperaillus oryzae*, L3-Lipase *Candida sp. Recombinant expressed in Asperaillus niger*, Novozymes L5-Lipase NS59030 and L6-Novozymes Cutinase NS29061) and with 1M NaOH. Followed by covalent coupling of selected C18-AKD (17.1-18.1% solids) on PLA and Jute/PLA bio-composite surface. The surface characterizations have been conducted using FTIR, contact angle measurements in advancing mode and microphotographs.

RESULTS AND DISCUSSION

PLA activation and coupling with C18-AKD: Three different PLA vis., PLA2003D (high crystalline), PLA4060D (amorphous) and equal ratio of PLA2003D and PLA4060D have been used to produce thin sheet (0.5mm) using hot press at 180°C at 150kN for 3min. All three PLA sheets have been subjected to surface activation with enzymes L1, L2, L3, L5, L6 for 2 hrs, with 2500U enzyme concentrate, pH 8.0 using 0.1M Tris-HCl buffer, at 60°C and 1M NaOH for 2hrs at 60°C. The spraying of C18-AKD on PLA surface was carried out with air brush at 2 atm pressure at room temperature (±20°C). The coupling reaction between C18-AKD and PLA thin sheet was conducted at 60°C for 24hrs in hot air oven. Evaluation with FTIR and contact angle measurement suggest that PLA4060D shows better surface activation and AKD coupling. The best results are for the L1 and NaOH activated PLA surface followed by C18-AKD treatment as reflected in terms of higher contact angle of Θ = 130.5 vs Θ = 113.5 for the blank sample. From FTIR spectra of AKD coupled PLA4060D surface, it is clear that high intensity peaks in the region of 2900-3000 cm-1 for C-H stretching as well as high intensity multi peaks at around 1700-1800cm-1 confirming the covalent bonding of AKD to PLA surface. The pure PLA has single peak in the region of 1700-1800cm-1. But the AKD coupled PLA have at least two peak showing coupling (spectra are not shown here).

PLA/Jute bio-composite activation and coupling with C18-AKD: PLA4060D has been selected as a choice of matrix material for preparing two layers of bio-composite with used woven jute bags from Starbucks. The bio-composite samples were produced using hot press technique (100 kN at 180°C for 3min). The produced bio-composite samples were treated with enzymes L1, L3, L5, L6 and 1M NaOH for the surface activation followed by coupling with C18-AKD as described earlier. Results show that the AKD coupling on L1 and NaOH activated bio-composite samples have a contact angle of Θ = 180 compared with Θ = 106.5 for blank sample. The microphotograph shows that the surface activation with NaOH causes the structural damage to the bio-composite. FTIR results also confirms the attachment of C18-AKD on Jute/PLA surface as described earlier. The better coupling of C18-AKD on bio-composite could be attributed to more free hydroxyl groups available for C18-AKD attachment from jute surface as well.

CONCLUSION

Under chosen conditions, L1- Lipase from *Thermomyces Lanuginosus* shows the best surface activation of pure PLA4060D surface and PLA4060D/Jute bio-composite. The C18-AKD has been covalently coupled to the activated surface. The superiority of enzyme activation over NaOH has been proven on the basis of structural integrity of the bio-composite.

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(OP 4) A green polyol as support for the immobilization of lipase NS 40116 in polyurethane foam

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Keywords: Polyurethane. Green polyol. Lipase NS 40116.

INTRODUCTION

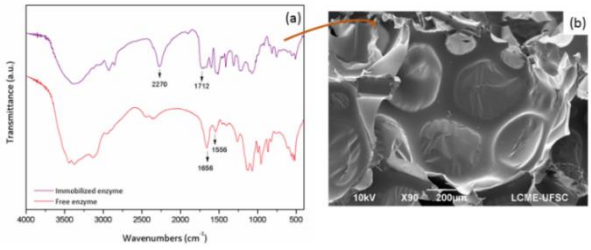
Lipases are important biocatalysts in the industry, mainly by the characteristics of high catalytic activity, selectivity, specificity and high activity under mild environmental conditions. These catalysts can be in free and immobilized forms. The use of an enzyme in free form presents some disadvantages, as instability in reactional medium and reuse inability. Thus, the immobilization of these proteins becomes attractive from a commercial point of view[1]. Polyurethane is a porous polymeric matrix that presents a wide applicability for the enzyme immobilization. This polymer is obtained by reaction of a diol (OH) and diisocyanate (NCO) in a polyaddition polymerization and when employed as support to immobilize enzymes, entrapment method is most commonly used. The objective of the present study was to use the product of enzymatic glycerolysis, between castor oil and commercial glycerol – monoacylglycerol (MAG) and diacylglycerol (DAG) – as green polyol to obtain polyurethane foam and to immobilize the lipase NS 40116 via entrapment method.

RESULTS AND DISCUSSION

The green polyol was obtained using enzymatic glycerolysis reaction, as previously described by Valério et al. (2010)[2] and MAG and DAG determination were carried out in a GC Shimadzu 2010 following the ASTM D6584-13 (2014)[3]. The MAG and DAG corresponded to a fraction of 64.52±2.32% the products. The free lipase NS 40116 was concentrated using collagen membranes, according to dialysis methodology. The content of the membranes was lyophilized and immobilized using the entrapment technique through bulk polymerization of the polyurethane foam. The reactions were carried out in room temperature with manual stirring for 1 minute, the molar ratio NCO:OH used was 1:1 using 1% of water as blowing agent, according to the total mass of the monomers and the amount of purified enzyme used was 15 wt% (w/w).

The FTIR analysis were performed on apparatus prestige-21 (Shimadzu) and presented in Figure 1a. The stretching in the region 2924 cm^{-1} and 2844 cm^{-1} was to the presence of asymmetric and symmetric methyl, respectively. The region at 1750–1700 cm^{-1} can be attributed to the bond linkage between the enzyme and the functional group (NH) from polyurethane. Free lipase showed a typical spectrum with proteins absorption bands associated with the amino group (CONH), primary, and secondary amino groups between 1580 and 1650 cm^{-1}.

Figure 1. (a) Fourier transform infrared spectra (FTIR) of free and immobilized Lipase NS 40116; (b) SEM Micrographs of immobilized lipase NS 40116 in PUF support.



With the SEM analysis (Figure 1b) it was possible to perform the count of the mean number of cells. The cells were observed in their large part of the closed profile with medium size of 748.6 ± 0.2 µm. Enzyme activity was determined according to Chiou et al. (2004)[4] and the free lipase presented enzyme activity of 5.71±0,98 U/g. After immobilization, the enzyme derivative presented an activity 6.62 ± 0.36 U/g. The results showed the positive effect of the immobilization on the lipase activity.

CONCLUSION

In this study, lipase NS 40116 was immobilized in polyurethane foam using a green polyol from enzymatic glycerolysis via entrapment technique. The high presence of MAG and DAG in this biopolyol enables the obtainment of a rigid support with closed and uniform cells. The immobilization of the enzyme did not result in loss of activity.

ACKNOWLEDGEMENTS

The authors thank the financial support of CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico and Laboratory of Electronic Microscopy (LCME) of Federal University of Santa Catarina for the SEM analyses.

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(OP 5) Highly efficient and eco-friendly degradation of wool by L-Cysteine-assisted Esperase

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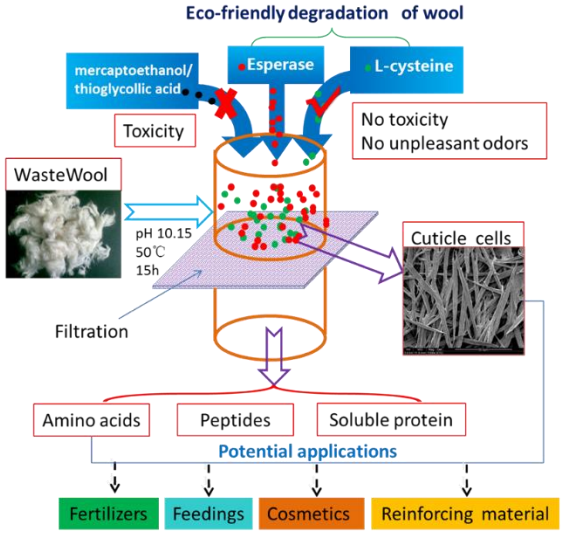
Keywords: wool degradation, L-cysteine, protease, disulfide bonds, peptide bonds, hydrolysate

INTRODUCTION

Although wool fiber contains about 99% protein, its complete degradation cannot be easily achieved by common proteases due to its high cysteine content. The work described provided a sustainable and high efficient chemicobiological method for wool degradation, in which L-cysteine was used to assist Esperase hydrolysis by breaking down disulfide bonds. The degradation efficiency of different combination of L-cysteine, Esperase and urea were investigated first. In addition, the solid residues resulted from different treatments were characterized by scanning electron microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), and amino acid analysis. Moreover, the resulting hydrolysates were analyzed by gel permeation chromatography (GPC) to examine the molecule weight of peptides.



Scheme 1 degradation and reutilization of wool

RESULTS AND DISCUSSION

The degradation efficiency of different combination of L-cysteine, Esperase and urea were investigated first. In addition, the solid residues resulted from different treatments were characterized by scanning electron microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), and amino acid analysis. Moreover, the resulting hydrolysates were analyzed by gel permeation chromatography (GPC) to examine the molecule weight of peptides.

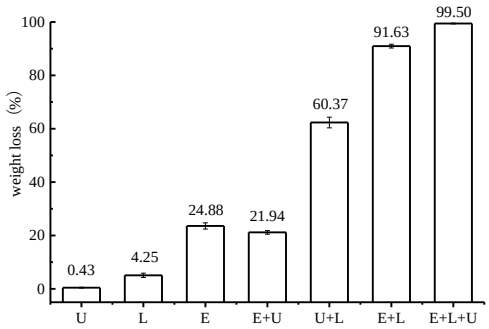


Figure 1. Weight loss of wool treated by different combination of 8 M Urea (U), 0.165 M L-cysteine (L) and 400μL Esperase (E) in Borax-sodium hydroxide buffer (0.02 M, pH 10.15) at 50 ℃ for 15 h

Treatment with combined Esperase and Urea caused weight loss slightly decreased than that of enzymatic treatment. Percent weight loss of 60.37% was obtained from combined L-cysteine and urea, while that of 91.63% was obtained from combined Esperase and L-cysteine. The number reached 99.50% when all three were combined, and the solid residues of which were precipitated to the bottom after centrifugation at 4000 rpm.

CONCLUSION

Combined L-cysteine and Esperase treatment could almost completely degrade the wool fiber with cortex cells remained. The main products in the hydrolysate were amino acids, oligopeptides with molecular lower than 3000. With no chemical residue, these products can be further used in fertilizer, feedstuffs or hair-care products. This work not only presents a green and efficient alternative method for wool degradation, but also discusses the solid residues and products in hydrolysates from wool treated with different combinations, which provides significant guideline in wool utilization.

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(OP 6) INSIGHTS on BACTERIAL nanoCELLULOSE for FOOD and NON-FOOD APPLICATIONS

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Keywords: bacterial cellulose; skin burns healing; innovations; composites

INTRODUCTION

Bacterial cellulose (BC), now subnamed as nano-BC, was initially reported by us[1] as a convenient biological dressing to accelerate skin burns healing as well as a barrier to avoid the wound infection. Concerning physical and chemical properties, this peculiar polymer, biosynthesized in a pure form by *Glucoacetobacter xylinus; G(a)x (*formerly *Acetobacter xyli*num; nowadays *Komagataeibacter xylinus)* , is quite different from plant cellulose whose fabrication demands extremely harsh chemical treatment to eliminate hemicelluloses and lignin. BC is now a medico-pharmaceutical good intensively used in some hospitals following the manufacturing in a larger scale and technology transfer to a private company (ITB – TUL, Technological University of Lodz, Poland; S. Bielecki group). BC recent advances and applications were reported at Gdansk, Poland in 2015[2]. There is a large room for innovations on BC (bio)technology and particularly in the S&T segment of its composites. Here in it is highlighted some novelties and prospections on some of these heteromaterials with emphasis on blends incorporating colored natural antioxidant compounds (oxycarotenoids, flavonoids and anthocyanins) as well one case of cell wall of glycan(s). In some cases, edible BC composites were further equilibrated with 5% FOS-Fructoligosacharies (ex-OPA-hydrolysed inulin) for sweetness improvement by the mild acid catalyst and better nutraceutical bioactivity[3].

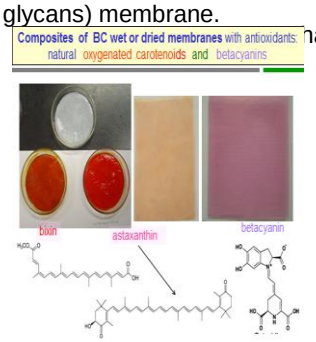
RESULTS AND DISCUSSION

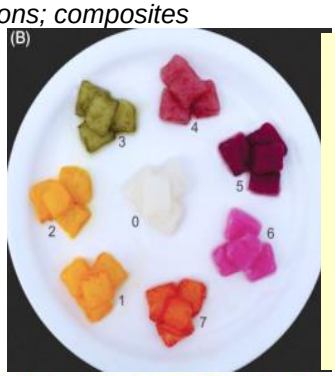
The hydrophobic antioxidant pigments (e.g., cartotenoids) were extracted from their sources (*Bixa orellana; Haematococcus pluvialis; Carophyl Red)* using absolute ethanol or acetone without mild acidification in the case of phenolics (e.g., *Calendula officinalis*). Anthopigments (betacyanin; red dragon fruit; *Hylocereus monocanthus*) was extracted with 80% ethanol at pH 2.

BC membranes were obtained as previously reporte[1]. They were kept wet, equilibrated to saturation with each desired pigment, quickly washed with water and sliced in small cubes in the case of the nutraceutical foods.

Antioxidant assays with the BC composites were carried with the usual DPPH and ABTS procedures.

Anesthetized mice were dorsally shaved and a 1 cm skin squares were surgically removed and immediately covered with each dry BC composite of the same area (yeast cell crude glycans) membrane.

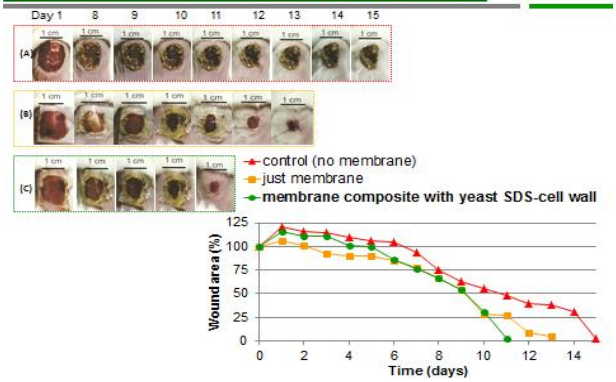




0. control BC; 1, bixin from *Bixa*
2, turmeric extract from *Curcuma*
3. spinach chlorophyll from *Spinacia*
4. açaí paste from *Euterpe oleracea*;
5 . red grape juice from *Vitis vinifera*;
6. cyaniding from *Hylocereus*
7. astaxanthin from

BC dressing covering the dorsal wound

Mice wound healing bioassays : summary



CONCLUSION

BC-based colored & antioxidant food composites were stable and display pleasant mouth feel. BC/yeast cell wall glucan proved helpful on animal skin wound healing.

ACKNOWLEDGEMENTS

Thanks to: CNPq, CAPES, FA-SETI-PR, UTFPR for research funding and graduate and undergraduate students for lab support.

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(OP 7) Valorization of food by-products as starting materials for bioplastics

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Keywords: Food by-products, vegetable fibers, composites, polyesters, bio-lacquer

INTRODUCTION

The public awareness on sustainability and the implementation of environmental regulations especially regarding waste disposal which limit the use of petroleum based plastics in applications where recycling is not easy, push the industry to find new alternative to such materials. According new market demands, bio-based plastics have found wider acceptance in the industry because they lead the reduction of greenhouse emission and reduce the dependence on crude oil, however the concern for the use of raw materials competitive with the food have increased.

In the last years, the interest is focused on the the possibility to find more sustainable and renewably sources for the plastic industry with the valorisation of by-products from the food processing industry which are currently discarded to be used in the preparation of materials for agriculture, packaging and automotive applications. Their *valorization* has to be considered *previous to the waste consideration* according to the Directive 2008/98/CE.

RESULTS AND DISCUSSION

The main purpose of the study concerned the valorization of by-products by the industry of vegetables to be used as starting compounds for the preparation of materials for agriculture applications and in the packaging and automobile industries.

By-products from different families of legumes (peas, green beans, lentils and Borlotti beans in LEGUVAL Project) and from industrial tomato processes (Biocopacplus and AGRIMAX projects) were investigated. Anyway, in the case of legumes, pea by-products were selected due to the simplicity of the matrix, good processability, availability and quantitative yield as raw material. Therefore, SSICA developed and optimized innovative methods to extract proteins at pilot scale from by-products (with a purity degree close to 80% in the case of pea wastes) and fibers and similarly cutins, the main component of the tomato skins. After the extraction, different approaches were used for their transformation in the final bio-based material.

In particular, proteinaceous components from legumes were used as starting compounds for the preparation of materials that could be turned into films through wet and dry processes (mixtures with polymer matrices).

The residual fraction resulting from the extraction process, which is enriched in fibers components was used as an additive in the production of compostable composites. The compostability of the material becomes therefore a strategic feature and it was verified on the products of the projects. The fibers were chemically characterized in terms of polysaccharides, lignin, pectin and waxes content, morphology and thermal properties. Their processability, also in the presence of plasticizers was tested in the preparation of composites at lab and pilot plant level (Fig. 1).

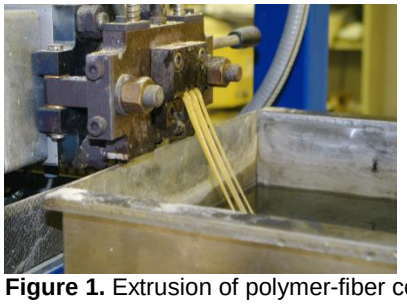

Figure 1. Extrusion of polymer-fiber composite.

Chemical approaches were also tested on the fibrous component with the aim to prepare cellulose-enriched nano- or micro-fibers and the investigation of their behavior in the dispersion in polymer matrices. Among the different types of fibers obtained from the legume wastes treatment, fibers from green beans were chosen because their higher content in cellulose as quantified by chemical fractioning with respect to the other legume fibers. In Fig. 2 one SEM image collected on micro-fibers is shown as an example.

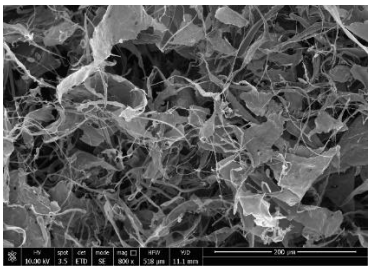

Figure 2. SEM image of cellulose-enriched fibers after chemical and physical treatments.

The fibers were used as filler in PLA-based composites and the influence of the preparation conditions on the properties of the materials were also investigated (Fig.3).
The attention was focused in particular on the nucleating effects of the fibers on the polymer matrix (Fig. 4). A very similar behavior was already investigated in details by the research group in PLA-based composites with hemp fibers.

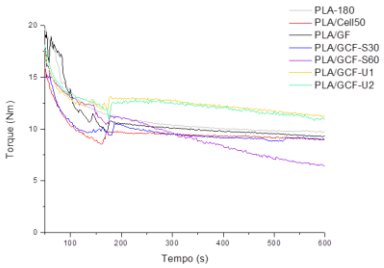

Figure 3. Torque profile during the mixing in the molten state.

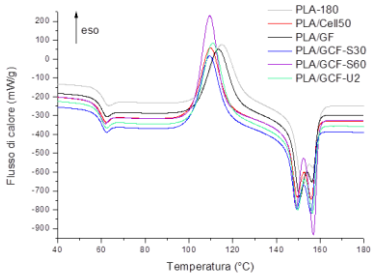

Figure 4. DSC profile of PLA-green beans fibers composites.

It is interesting to report here that industrial approach developed and used for legume by-products was applied also on tomato by-wastes and cutin from tomato skins was obtained (Fig. 4), after the optimization of the extraction conditions. Cutin consists of omega hydroxy acids and their derivatives, which are interlinked via ester bonds, forming a polyester polymer of indeterminate size.

Figure 3. Some examples of extracted cutins from tomato skins.

From the characterization of the material extracted, the differences observed in terms of molecular weight (Fig 4), thermal properties (Fig 5) with the process conditions used in the industrial process were correlated.

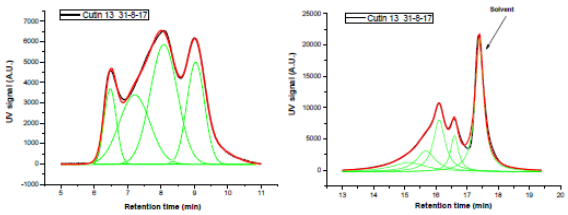

Figure 4. An example of deconvolution on the GPC signals of two different cutin samples.

The behavior of cutins during polymerization reactions was also tested with the aim to produce bio-lacquers to be used as new strategies in terms of safety and sustainability of the coating for food contact in the packaging sector.

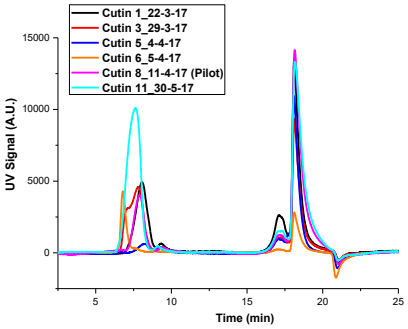

Figure 5. Comparative analysis of the DCSC traces of different cutin samples.

CONCLUSION

The goal of this work was to prove and demonstrate the viability of innovative technologies for the valorization of bio-wastes from food industries of legume and tomato. The fibrous component can be used in the production of biodegradable and compostable composites and/or chemically treated to obtain micro- or nano-fibers enriched in cellulose able to improve the final properties of the biocomposite. Cutins obtained from tomato represent an interesting material that can be easily processed to produce bio-lacquers.

ACKNOWLEDGEMENTS

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(OP 8) Biocatalytic synthesis of polyhydroxyalkanoates block-copolymers: Challenges and novel approaches

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Keywords: Block-copolymers, enzymatically catalyzed reaction, bioprocess

INTRODUCTION

Polyhydroxyalkanoates (PHAs) are microbial polyesters synthesized from renewable carbon sources and generally are biodegradable and biocompatible. To improve the competitiveness of PHAs facing petroleum-based polymers, achieving block-copolymer structures is a promising approach for the development of new and favorable polymer properties. Block copolymers are polymer chains that contain blocks of different monomers that are covalently linked together. Such structures potentially undergo microphase separation to form periodic nanostructures and can lead to physical properties that cannot be obtained by random copolymerization, polymer blending, or filler-adding techniques. In the present work we aim at the enzymatically catalyzed modification of various PHAs to form novel and well-defined block-copolyesters. To this end, two challenging approaches will be employed: The all *in vivo* synthesis and the *in vitro* enzymatically catalyzed conversion of biosynthesized homo- and random copolymers (Figure 1). Here we describe the preliminary results and perspectives of this recently initiated project.

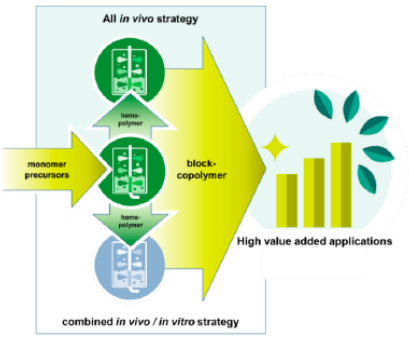

Figure 1. This work approaches the production of well-defined block-copolyesters.

RESULTS AND DISCUSSION

As a starting point, we attempted to synthesize diblock-copolymers consisting of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) and poly(4-hydroxybutyrate) (P4HB), designated as PHBV-*b*-P4HB, via enzymatic transesterification similar to previously published results [1]. PHBV was produced by *Cupriavidus necator* and subjected to chain end modification with ethylene glycol to generate PHBV-diol with weight-average molecular weight (M_w) of 6700 g/mol (Table 1). A recombinant *Escherichia coli* [2] produced P4HB with broad and multimodal molecular weight distribution (Table 1). To synthetize the block copolymers, excess PHBV-diol, P4HB and lipase B from *Candida antarctica* (Novozyme 435) were mixed together in previously dried toluene. The reaction was conducted at 70 °C under inert nitrogen atmosphere and continuously stirred for 67 h. GPC analysis of the resulting material revealed a new molecular weight distribution (Table 1). Further peak molecular weights (M_p) substantially different from the starting material were observed. Especially, a new distribution with M_p at 990 × 10^3 g/mol indicated a significant molecular weight increase. Yet, besides the fact that the remaining molecular mass distribution shows unreacted starting material, a low molecular weight distribution with M_p at around 1000 g/mol suggests competitive hydrolysis reaction during these very first trials.

Table 1. GPC results of polymers analysed in chloroform.

	M_p (10^3 g/mol)	M_w (10^3 g/mol)	PDI
P4HB	557.4 /150	704.8	6.4
PHBV	5.9	6.7	2
P4HB-*co*-PHBV*	990 /1	663.1	12.1

*Residues from the starting material were also present with M_p at 150, 550 and 6 × 10^3 g/mol.

PERSPECTIVES

Further optimization of the reaction between lipase B and different PHAs will be carried out to improve the reaction yield and the conversion to higher molecular weights. Analysis of the polymer microstructure will be performed to verify the formation of block-copolymers. For the all *in vivo* synthesis, our goal is to produce block-copolymers via optimized bioprocesses. Thus, diverse bacterial strains and cultivation systems, e.g. fed-batch or continuous cultivation, will be evaluated in future.

ACKNOWLEDGEMENTS

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(OP 9) Influence of key production parameters in the Poly(hydroxybutyrate) production cost

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Keywords: Poly(hydroxybutyrate); Cupriavidus necator; Economic assessment; Production cost

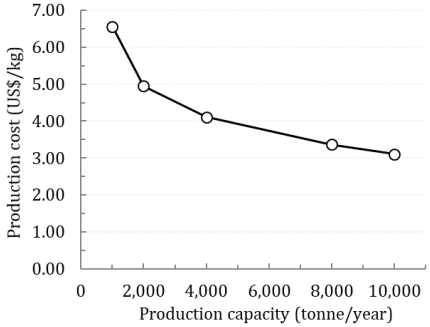
INTRODUCTION

Poly(hydroxybutyrate) (PHB) is a polyester synthesized by bacteria and intracellularly accumulated as carbon storage material. Although it has higher production cost than petroleum-based plastics, efforts are being made in order to make these biopolymers commercially attractive[1]. Thereby, the purpose of this work is to evaluate the production cost of PHB when some main production parameters are changed.

RESULTS AND DISCUSSION

Through laboratory-scale experiments data it was possible to simulate and economically evaluate the entire PHB industrial production process by *Cupriavidus necator* DSM 545, using propylene carbonate as extraction solvent coupled with thermal biomass pretreatment (global extraction capacity of 92.1%)[2] and citric molasses as carbon source. The base-case scenario considers the design of a 2,000 tonnes per year facility's production capacity with a cultivation phase final cell concentration of 62 $g.L^{-1}$ with 69% of intracellular PHB[3]. It was taken into account the raw materials and utilities usage as well as the sizing of the necessary equipment of each unit operation, allowing the estimation on the capital investment, operational costs and revenue surveys[4]. The costs were indexed to February 2018 value using the IGP-M index and exchange rate of R$3.24/US$. The base-case study provided a production cost of US$ 4.95/kg and it was wondered what would happen if the production capacity increased or if the final cell concentration at the end of the cultivation phase were higher. In this context, the variation in the equipment acquisition cost was estimated according to the power law expression and the obtained results are shown in Figure 1 and 2.

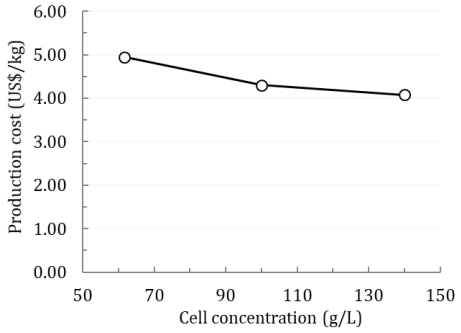
Figure 1 - Influence of scaling on the cost of production



In the production capacity scenario of 10,000 tons per year, the unitary cost is US$3.10/kg, with further declining trend with increasing scale. However, this new production would consume almost all of the citrus molasses generated in the State of São Paulo, therefore must be the acquisition and transport of molasses from other orange-processing plants, thus the polymer production cost will undergo an increase over the estimated value.

Fixing the plant annual production, the increase in the cellular concentration leads to reduction in the capacity of, mostly, upstream equipment leading to final product cost reduction, as shown in Fig. 2. If the final cell concentration of 140 $g.L^{-1}$ is achieved that would represent almost a 20% cost decrease.

Figure 2 - Influence of cell concentration on the PHB production cost.



CONCLUSION

In the economic evaluation of PHB production, biomass concentration and production capacity are key economic elements and have direct influence on equipment size and raw materials consumption, which can lead to reduction in production costs making this biopolymer production more attractive.

ACKNOWLEDGEMENTS

We thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support and Laboratório Nacional de Ciência e Tecnologia do Bioetanol (CTBE/CNPEM) for the technical assistance.

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(OP 10) PMMA/Nanocrystalline Cellulose Nanocomposites Produced by *in situ* Suspension Polymerization

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Keywords: nanocrystalline cellulose, functionalization, suspension polymerization, methyl methacrylate

INTRODUCTION

Developments of nanocomposites by using natural compounds are constantly growing because of the concerns regarding environmental issues. Cellulose, the more abundant natural polymer on the planet has drawn attention mainly on the nanometric scale due to its good mechanicals properties[1]. However, the high polarity and, consequently, its hidrophilicity hinder the dispersion of this natural compound in non-polar polymeric matrices. In this way, chemical modifications of the cellulose surface are necessary to avoid such difficulties[2]. In order to contribute to the development of sustainable technologies for the production of nanocomposites, firstly, the present study chemically modified the surface of the nanocrystalline cellulose (NCC) by using the bifunctional organosilane 3-(trimethoxysilyl)propyl methacrylate (MPS). Hereafter, the functionalized NCC was incorporated in poly(methyl methacrylate) through the *in situ* suspension polymerization technique.

RESULTS AND DISCUSSION

The NCC was produced by the acid hydrolysis of a commercial microcrystalline cellulose (Blanver, 50 µm). As seen in Figure 1.a, nanoparticles of crystalline cellulose were obtained (~200 nm). The crystalinity of the nanocellulose was evaluated by X-ray difratometry (DRX – Figure 1.b).

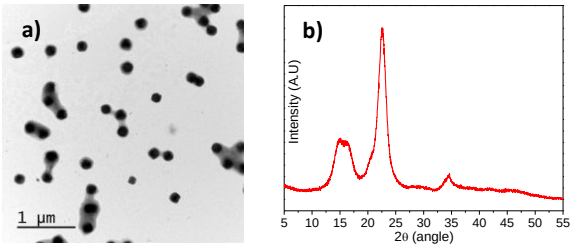

Figure 1. NCC characterization: a) image taken by transmission electron microscopy; b) DRX spectrum of the non-functionalized CNC.

The NCC was functionalized with different amounts of MPS (5, 10 and 15% wt related to the NCC mass) in a solution of ethanol:water (80:20 v/v) with pH=4,0. The dispersion was stirred for 24 hours and hereafter dried and cured at 120°C for 150 minutes. The results of the first derivative of the mass loss obtained from thermogravimetric analysis (TGA) of the pure and functionalized NCC samples revealed a small increase of the thermal stability of all the functionalized samples comparing to the pure cellulose. Observing closely the curves in the range from ~360 to ~385°C, it is noticeable that the thermal degradation profile presented two events. The first one is related to the cellulose degradation while the second one is assigned to the presence of the MPS adsorbed onto the surface of the cellulose nanoparticles.

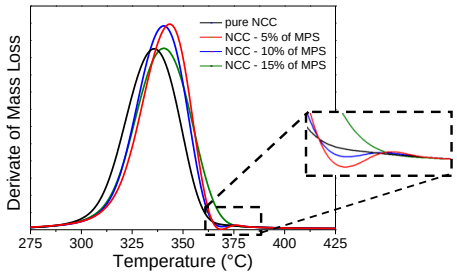

Figure 2. DTG results for the pure and functionalized NCC samples.

Comparing the TGA results with those one obtained from potentiometric titrations (used to estimate the molar concentration of hydroxyls [OH] contained on the surface of the NCC samples), it was observed that the higher was the degree of functionalization (*i.e.* the lower was the [OH] remained on the cellulose surface) the higher was the maximum degradation temperature (T_{max}). The findings obtained by potentiometric titration corroborated the behavior of the second peak observed in the DTG curves. In this case, the lower was the MPS amount used in the functionalization the higher was the amount of this organosilane adsorbed onto the NCC surface (Table 1).

Sample	[OH] (µmol.g^{-1} NCC)	T_{onset} (°C)	T_{max} (°C)
Pure NCC	174.30 ± 11.45	317.3	335.5
NCC – 5% MPS	30.42 ± 22.20	325.0	342.9
NCC – 10% MPS	57.86 ± 5.93	323.1	340.8
NCC – 15% MPS	72.26 ± 6.01	322.1	340.2

Table 1. Comparison between the amount of the remaining hydroxyl concentration [OH] and the thermal degradation temperatures for each NCC sample.

The functionalized NCC were dispersed in the monomer (MMA) in distinct proportions (0.0, 0.1, 0,25 and 0.5 % wt). Hereafter, the MMA was polymerized by using the suspension technique, considering a holdulp of 0.2. Benzoyl peroxide was used as initiator (0.374%) and poly(vinyl pyrrolidone) with M_w=11.000 g/mol as stabilizer. The NCC functionalized with 15% of MPS was not used in the polymerizations due to the lower silylation degree.

The PMMA particles produced by the suspension polymerizations were sieved and the results showed an increase of the Sauter mean diameter (d_{32}) as the amount of nanocrystalline cellulose dispersed in the monomer was increased (Table 2). Such findings might be related to a change in the interfacial tension of the system due to the increase of the amount and silylation degree of the NCC.

NCC Content Dispersed in MMA (%)	Sauter Mean Diameter – d_{32} (mm)	
	CNC – 5% MPS	CNC – 10% MPS
0.50	1.468 ± 0.232	1,468 ± 0,190
0.25	1.286 ± 0.300	0,880 ± 0,284
0.10	0.856 ± 0.311	0,735 ± 0,275
pure MMA (0.0)	0,619 ± 0,336	

Table 2. Sauter mean diameter (mm) and the respective standard deviation of the PMMA particles produced with different quantities of NCC functionalized with different amounts of MPS (5 and 10%).

In general, according to the results of TGA obtained for the produced PMMA particles shown in Figure 3, the addition of NCC in the polymerization promoted a subtle increase of the polymer thermal stability (comparing to the pure PMMA). When the NCC functionalized with 5% of MPS was used (Figure 3.a), there was a reduction of the thermal stability of the polymer. When 0.1 and 0.25% of the CNC functionalized with 10% of MPS was used (Figure 3.b), the thermal stability of the polymer remained almost the same of the pure PMMA.

The molar mass of the pure PMMA and their nanocomposites were evaluated by gel permeation chromatography. In general, the weighted molecular weight (M_w) reduced around 20% in comparison to the pure PMMA by adding the NCC in the polymerization system (results not shown). However, the values did not follow a tendency by varying the amount or even the silylation degree of the NCC. Based on the molecular properties obtained for the nanocomposites, it is clear that the presence of NCC in the system influenced the reaction kinetics, probably due to the C=C bond presented in the MPS molecule grafted onto the surface of cellulose nanoparticles. More experiments are necessary in order to better understand the effect of the presence of silylated NCC on the molecular and thermal properties of the produced PMMA-NCC nanocomposites.

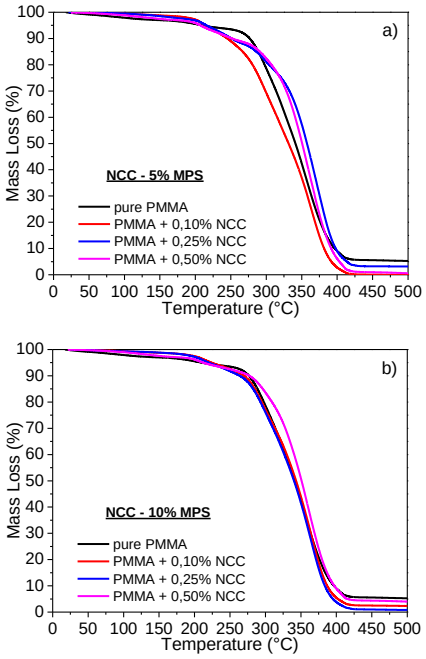

Figure 3. TGA of pure PMMA and their nanocomposites produced with different quantities of the NCC functionalized with distinct amounts of MPS: a) 5% of MPS; b) 10% of MPS.

The images presented in Figure 4 clearly demonstrated the successful incorporation of the functionalized NCC in the PMMA matrix by using the *in situ* suspension polymerization technique.

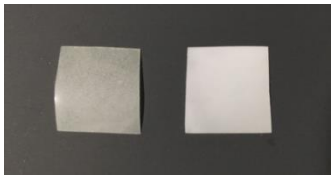

Figure 4. Image of the films produced with the pure PMMA (left) and the PMMA-NCC nanocomposite (right) synthesized with 0.5% of NCC functionalized with 5% of MPS.

CONCLUSION

This work demonstrated that the nanocrystalline cellulose (NCC) were successfully chemically modified by using the 3-(trimethoxysilyl)propyl methacrylate (MPS). In addition, the study proved that it is possible to produce PMMA-NCC nanocomposites by using the *in situ* suspension polymerization technique.

ACKNOWLEDGEMENTS

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(OP 11) Bactericidal hybrid metal-enzyme nanoparticles with polysaccharide biofilm eradication ability

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Hybrid Nanoparticles, silver, α-amylase, biofilm reduction, bactericidal

INTRODUCTION

The emergence of drug-resistance microbial pathogens is creating a worldwide healthcare threat already responsible for 700 thousand deaths annually with an associated huge financial loss. In order to fight antimicrobial resistance (AMR) infections researchers are developing bactericidal ways that avoid evolutionary pressure towards resistance mechanisms. Among them, bactericidal metal nanoparticles are promising materials, their simultaneous mode of action (oxidative damage, metal ion release, and non-oxidatives mechanisms[1]), avoids appearance of resistance mechanisms and cause bacterial death through destruction of cell membranes, blockage of cellular pathways and DNA damage. In parallel, there is an increasing interest on developing antimicrobial strategies that prevent bacterial virulence mechanisms, in particular biofilm, thus enhancing clearance by the host. Bacterial biofilms are structured, coordinated communities with distinct architectures and properties. Microbial biofilms are at the root of many chronic and recurrent infections and have been related with the 80 % of all microbial infections currently treated in hospitals. Different enzymes have been successfully used for prevention and removal of stablished biofilms[2].

In this study, we synthesized hybrid enzyme-metal nanoparticles combining the synergistic activities of different antimicrobial agents. The enzyme in the hybrid nano-entitiesacts eliminating and inhibiting the formation of biofilm. On the other hand, the metal counterpart provides the biocidal properties. This antimicrobial approach could be applied in the form of coatings on surfaces such as hospital textiles, water treatment membranes and implantable medical devices, ensuring a safer environment for both patients and healthy population.

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RESULTS AND DISCUSSION

The hybrid nanoparticles (NPs) were produced in a two step method. First, the protein nanoparticles were synthesized by desolvating with ethanol a mixture of α-amylase and gallic acid which is further oxidized by laccase for NPs crosslinking and stablilization. The resulting NPs were incubated with silver acetate which is incorporated into the protein NPs to form the final hybrid nanostructure. The DLS analysis showed an increase of size between the final hybrid complex and the protein nanoparticles (320vs 260 nm), indicating the silver incorporation. The zeta potential analysis shows the stability of the hybrid-composite with values between -30 and -35 mV.

The antimicrobial properties of these nano-complexes were tested in their capacity to inhibit microbial growth. The bacteria chose for these experiments were *Escherichia coli* as a gram negative, and *Staphylococcus aureus*, as a gram positive and the growth inhibition properties of different concentrations of hybrid nanopartices was tested in Müeller-Hinton medium growth. For both, gram positive and gram negative, the hybrid nanoparticles reduced the amount of bacteria from the initial growth by 3 logs, indicating a biocidal effect which has been widely described in silver materials[3].

To assess the ability of the hybrid NPs for biofilm removal stablished biofilms were exposed to the hybrid nanoparticles for 24 h, and the remaining biofilm was quantified through crystal violet staining. The nanostructures were able to reduce more than 85 % of the bacterial extracellular matrix biomass formatted by *E. coli* and *S. aureus* (Figure 1).

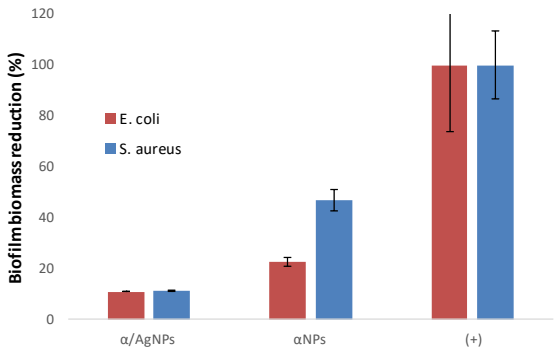

Figure 1.Comparison of biofilm biomass reduction between amylase NP and hybrid NP. The NPs were assessed for removal of biofilm from *E. coli* (red) and *S. aureus* (blue). Treatment with, α/Ag NPs (hybrid NPs), αNPs (protein NPs), (+) biofilm without treatment.

CONCLUSION

The synthesized hybrid nanoparticles are able to reduce the microbial population and the biofilm formation of both, gram negative and gram positive bacteria, showing a dual functionality due to the

integration of the two nanoparticle components, the α-amylase and the silver nanoparticles.

ACKNOWLEDGEMENTS

The research leading to these results has received funding by E.C. within the Framework of H2020T

PROTECT - Pre-commercial lines for production of surface nanostructured antimicrobial and anti-biofilm textiles, medical devices and water treatment membranes, H2020 - 720851

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(OP 12) Freestanding layer-by-layer membranes incorporating antibacterial biopolymer-capped silver nanoparticles

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Keywords: silver nanoparticles, aminocellulose, chitosan, layer-by-layer, antibacterial membranes, biofilm inhibition

INTRODUCTION

Engineering biocomposites through non-covalent assembly leads to robust construct materials with controllable functionality for variety of biomedical and biotechnological applications. Advancements in the nanobiocomposites fabrication, where one of the building phases shows a nanometer range dimension, are of particular interest due to the unique properties of nanoparticles (NPs) imparting exceptional chemical, physical and biological characteristics.[1]

One of the most rapidly growing and versatile technologies for incorporating nanoscaled components into materials is layer-by-layer (LbL)[2,3] assembling. The material build-up relies on the electrostatic interactions between oppositely charged components, which are alternately deposited on the supporting substrate to create versatile biomaterials. In case of a low number of deposited layers, usually less than 20, the coatings must be firmly anchored on the supporting substrate that maintain their mechanical properties and shape, behaving as an integrated macroscopic system.[4,5] By contrast, building higher number of layers allows for detaching the multilayer system from the substrate and use it as a freestanding multilayer composite.

In this study, antibacterial freestanding multilayer nanobiocomposite membranes were obtained by the sequential deposition of biopolymer-capped silver nanoparticles (AgNPs) and the biocompatible anionic hyaluronic acid (HA). Innovatively, stable membranes were obtained from polycations in nanoparticulate form and polyanions in bulk form, where the polycation-capped AgNPs play the dual role of an active agent and a structural element in the membranes. The goal of assembling biopolymers and antibacterial AgNPs is to fabricate a safe by design biocompatible material able to inhibit the planktonic growth and biofilm formation of the clinically relevant *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) pathogens. Silver was chosen as a largely recognized efficient antimicrobial agent, especially in its nanoform.[6,7] These particles, however, also present some drawbacks, such as nanotoxicity and complicated fabrication. The methodology we used for their synthesis and simultaneous capping with biopolymers resolves both issues since it is technologically simple, relatively fast (3 hours) and ecologically acceptable compared to the conventional chemical reduction of $AgNO_3$ employing the toxic sodium borohydrate over long reaction times. Our approach yields stable, highly concentrated and biocompatible hybrid biopolymer-AgNPs dispersions using aminocellulose (AC) or chitosan (CS) as reducing and stabilizing agents.[6,7] The cationic biopolymer-AgNPs were then embedded in the membranes as both active antibacterial agents and building elements of the freestanding multilayer membranes. Their LbL assembling with the antifouling HA aimed to restrict bacterial growth and prevent biofilm formation.

RESULTS AND DISCUSSION

A two-step manufacturing approach was adopted for the fabrication of the freestanding membranes, comprising: i) the synthesis of concentrated dispersions of AgNPs capped with the cationic antimicrobial biopolymers CS or AC via high-intensity ultrasound (**Fig. 1, I**), and ii) the LbL assembling of the membranes on a template silicone surface by the alternating deposition of positively charged CS- or AC-capped AgNPs and negatively charged HA (**Fig. 1, II**).

The concentrated biopolymer capped AgNPs dispersions were sonochemically synthesized in a 3-hours process at 60 ºC. Hybrid biopolymer-capped AgNPs with size smaller than 100 nm were formed as confirmed by transmission electron microscopy and UV-Vis spectroscopy. Then, the obtained ACAgNPs and CSAgNPs (as polycations) were sequentially deposited on the surface of APTES-functionalized silicone strips using HA as an alternate polyanion to generate nanobiocomposite multilayer coatings/membranes of 10, 50, 100 and 200 bilayers. Unlike the 10 bilayers, the 50 and especially the 100 and 200 bilayer membranes, could be easily detached from the silicone substrate and handled for analyses (**Fig. 1 III**). The detachment of the nanobiocomposite membranes confirms the feasibility of the LbL technique for production of freestanding NPs-containing membranes.

The LbL build-up and deposition of HA and ACAgNPs or CSAgNPs was further confirmed *in situ* with a quartz microbalance (QCM-D). The variations in the frequency (Δf) and dissipation (ΔD) are more pronounced in the constructs where CSAgNPs are present, which is correlated with the formation of thicker layers. The deposition of HA-ACAgNPs and HA-CSAgNPs was also evidenced by: i) the appearance of several new bands in FTIR spectra compared to the aminated silicone control surfaces and ii) the SEM images taken at the cross-section of the LbL materials.

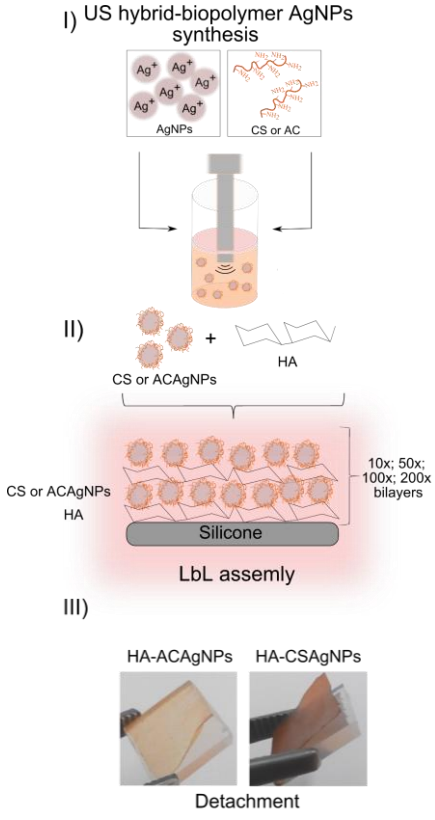

Figure 1. The adopted approach for fabrication of multilayer freestanding membranes from HA-ACAgNPs or HA-CSAgNPs (I and II), and their detachment from silicone (III). AgNPs are oversized in the scheme to illustrate the concept for the multilayer build-up.

Cumulative release profiles of silver ions from the HA-ACAgNPs and HA-CSAgNPs membranes incubated in phosphate buffered saline at 37 ºC comprising 100 and 200 bilayers were further obtained with ICP-MS. The developed membranes exhibited a burst initial release over the first 24 h followed by sustained release during the whole tested period. After 7 days of incubation, the higher silver release was reached for HA-CSAgNPs in comparison with HA-ACAgNPs membranes.

The antibacterial efficiency of LbL coatings and freestanding membranes was evaluated against clinically relevant *S. aureus* and *E. coli* pathogens. All LbL freestanding membranes, i.e. HA-ACAgNPs or HA-CSAgNPs membranes with ≥ 50 bilayers, displayed full kill (100 % viability reduction) for both bacterial strains. The adhesion of *S. aureus* and *E. coli* and subsequent establishment of bacterial biofilms on the freestanding membranes was further assessed by fluorescence microscopy and viable cell counts. The nanobiocomposites inhibited the biofilm growth to a different extent as compared to pristine silicone, on which the individual cells expectedly agglomerated into robust sessile bacterial communities (**Fig. 2**). Although the LbL coatings with 100 bilayers of HA-ACAgNPs or HA-CSAgNPs significantly reduced *S. aureus* and *E. coli* biofilms compared to the pristine silicone, their surface was still colonized with bacterial clusters (**Fig. 2**). The build-up of 200 bilayers brought about the total prevention of the *S. aureus* and *E. coli* biofilm formation on HA-CSAgNPs (**Fig. 2**), reducing the viable cells on the surface by 7 logs and 6 logs, respectively.

The biocompatibility of the developed nanobiocomposite membranes was also evaluated with skin fibroblasts. After one week of contact with freestanding membranes, the cultured cells were metabolically active with no significant difference in cell viability (above 95 %) observed among the experimental groups.

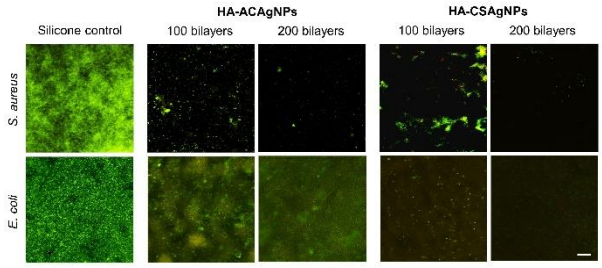

Figure 2. Antibiofilm activity of freestanding membranes. Fluorescence microscopy images (x10 magnification) after Live/Dead staining of *S. aureus* and *E. coli* biofilms on silicone control and on HA-ACAgNPs and HA-CSAgNPs multilayer nanobiocomposites. The scale bar corresponds to 100 μm.

CONCLUSION

In this study, the LbL approach was exploited to fabricate surface coatings and freestanding membranes with antibacterial and antibiofilm

activities. Their antibacterial efficiency is due to the polycation-decorated silver nanoparticles embedded alternately between the polyanion HA layers. The NPs had both i) a structural role to stabilize the multilayer bionanocomposite, and ii) a functional role as a source of antibacterial silver ions release. Prior to their inclusion into bionanocomposite membranes, the NPs were synthesized using sonochemistry to complete the overall environmentally friendly approach for the fabrication of functional membranes. High antibacterial effect towards planktonic bacteria and the ability of the membranes to inhibit the adhesion of clinically relevant bacterial species and the consequent biofilm formation is further demonstrated. These features, coupled to their excellent biocompatibility pave the way for their biomedical application for wound healing and as drug delivery systems.

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(OP 13) Multifunctional hyaluronic acid based hydrogel with enzymatically embedded silver/lignin nanoparticles

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Keywords: silver nanoparticles, biomedical application, hyaluronic acid, hydrogels

INTRODUCTION

Silver nanoparticles (Ag-NPs) are the most commonly used nano-materials in health care for e.g. diagnosis, medical skin treatment, drug delivery, and coating of medical devices and textiles. Ag-NPs possess antibacterial, antifungal, antiviral and anti-inflammatory activities. On the other hand, hyaluronic acid (HA), an immunoneutral polysaccharide ubiquitously present in the human body, is an attractive biocompatible matrix material tuneable into many physical forms such as viscoelastic solutions, soft or stiff hydrogels or even nanoparticulate fluids for a wide range of biomedical applications. Bio-nanocomposites formed by the combination of HA and Ag-NPs would offer the properties inherent to both materials.

In the present work, one-step enzyme-assisted approach was used to synthesize multifunctional hydrogels of thiolated hyaluronic acid (HA-SH) and lignin capped silver nanoparticles (Ag-Lig NPs) as therapeutic platform for treatment of chronic wounds. “Green”, solvent-free, synthesis of AgNPs was performed using the biopolymer lignin as reducing and capping agent. The multiple phenolic groups present on the Ag-Lig NPs surface, were then enzymatically oxidised into reactive quinones able to crosslink with the thiol and amino groups from HA leading to gelation. The morphology, swelling properties, and rheology of the Ag-Lig NPs-embedded HA hydrogels as well as the control over the major chronicity factors in the wounds such as MMPs and MPO, cellular reactive oxygen species and bacterial contamination were investigated. Finally, the toxicity and anti-inflammatory activity of silver containing hydrogels were addressed to explore the potential of these new materials for biomedical applications.

RESULTS AND DISCUSSION

HA-SH polymers were synthesized from different molecular weight HA (200 KDa, 700 KDa and 1.8 MDa), in a two-steps procedure.[1] Adipic acid dihydrazide grafted HA was prepared and then modified into HA-SH with 2-imminothiolane. HA-SH preparations with different degree of substitution were obtained varying the ratio between the reagents.

Highly stable Ag-Lig NPs with ζ-potential above -40 mV and average size of 72 nm were obtained. They demonstrated strong antibacterial activity against a panel of multi-drug resistant Gram-positive and Gram-negative bacteria. The particles were further used as starting gelation nucleus for one-step laccase assisted hydrogels formation. The laccase-catalysed crosslinking reaction between HA-SH and Ag-Lig NPs yielded hydrogels with tuneable

physico-mechanical properties depending on the HA-SH/Ag-Lig NPs ratio.

The hydrogels presented self-healing properties, did not affect the viability of human cell fibroblasts and revealed above 90 % antioxidant activity. They reduced the growth of medically relevant *S. aureus* and *P.* aeruginosa by 2 and 5 logs, respectively. Importantly, the developed dressing materials demonstrated inhibitory activity on MPO and MMPs *in vitro* and *ex-vivo*.[2]

CONCLUSION

Multifunctional hydrogels containing covalently embedded antimicrobial NPs were enzymatically prepared from HA-SH and Ag-Lig NPs. The biocompatible hydrogel showed bioactivities beneficial for chronic wound healing.

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(OP 14) Honey-mimetic Antibacterial ROS in situ forming Hydrogel Wound Dressing

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Keywords: Hyperbranched polymers, Click-chemistry hydrogels, Wound healing, Antibacterial materials

INTRODUCTION

Due to increasing antibiotic resistance, alternative antimicrobial strategies are under exploration, such as the use of plant based products like honey[1]. This study aims to mimic the ability of honey to produce antibacterial Reactive Oxygen Species (ROS) in the form of hydrogen peroxide (H_2O_2) within the hydrogel[1,2]. This was achieved using two components found in honey: the glucose oxidase enzyme and glucose, which were incorporated into the quick forming hydrogel[3].

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RESULTS AND DISCUSSION

For this study Hyperbranched Polyethylene Glycol Diacrylate (HB PEGDA) was synthesized using Reversible Addition Chain Transfer Fragmentation (RAFT) polymerization. With the mechanism of thiol-ene click chemistry, HB PEGDA is able to form a hydrogel with thiolated Hyaluronic Acid (HA-SH).

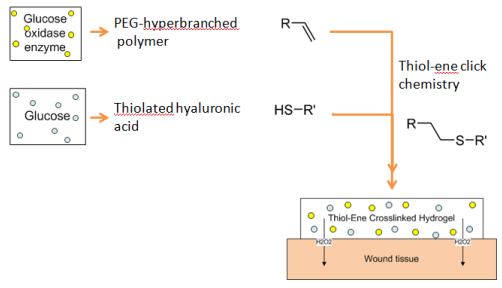

Figure 1. Thiol-ene click chemistry to form the hydrogel with continuous production of hydrogen peroxide

For a hydrogel with a composition of 5 w/w% HBPEGDA and 1 w/w% HA-SH, an average gelation time of 585±45 seconds was obtained. For the 10 w/w% HBPEGDA and 1 w/w% HA-SH gelation time was 41±4 seconds.

The study characterized the chemical characteristics of the polymer and the hydrogels' rheological properties, swelling and degradation properties, and enzyme encapsulation properties.

The ROS produced with varying glucose and enzyme concentrations at different time points were quantified.

These same enzyme and glucose concentrations were then tested for their biocompatibility in the 3T3 mouse fibroblast cell line using an Alamar Blue Assay, and were found to have positive viability.

CONCLUSION

Given its quick forming property, biocompatibility, and ability to continuously produce antibacterial ROS, this type of hydrogel system makes a promising material for wound dressing applications and should be further characterized and studied.

ACKNOWLEDGEMENTS

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(OP 15) Biotechnology in Cosmetics - Application of enzymes in hair care products

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Keywords: hair, enzymes, color, repair

ABSTRACT

Recently, natural and organic cosmetics have been popular and common not only in Japan but also in the world. Therefore, biological substances are widely used in the cosmetics, such as plant extracts which are applied like medicines as effective ingredients in the cosmetics products. For example, extracts of *Swertia japonica* (SENBURI) and *Eucalyptusis* leafs are typical additives in a scalp lotion of our products. Henna, dried leafs of *Lawsonia inermisis*, is used as a coloring material for gray hair.

As other approach, enzymes were tried to use in the cosmetics. Papain, a protease, is used in face washes. Oxidation enzymes, such as Uricase and Laccase, were attempted to use in hair color products. These enzymatic hair color systems are superior to conventional hair dyes because of no usage of H_2O_2 causing hair damages. Laccase system is thought to better in the point of views; complete exclusion of H_2O_2 and color vibrancy. If the enzyme costs were reasonable for the commodity products, they could be used in hair dyes. It is too expensive to be usual. As another utilizing approach of the enzyme reaction, we tried to make natural dye stuffs by biotechnology, that is the same reaction as in nature. By using oxidation enzymes such as Laccase and Tyrosinase, melanin precursors can be produced in a bioreactor. Mainly dihydroxyindole (DHI) is used for hair dye. DHI automatically change to melanin by oxygen in air, that means no need to add oxidants in the product, such as H_2O_2. The hair dye using this bio-DHI has been launched and sold in Japan.

As another topic, to avoid hair damage, we tried to decompose the melanin pigment in the hair by peroxidase (POD). This POD was very special, Mn-POD, can degrade synthetic melanin easily. That mechanism was also examined, so that oxidized Mn ions is a key in the reaction. For practical usage, more studies will be needed.

As different biotechnology for hair care, human keratin proteins can be produced by recombinant technology. It is useful for repairing the damaged hair.

As described above, biotechnology is very useful for cosmetics.

(OP 16) Electrical monitoring of enzymatic infection biomarkers using antibody and peptidoglycan-modified nanoporous membranes

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Keywords: Nanopore, nanochannel, biosensor, enzyme, bacteria

INTRODUCTION

Bacterial hyaluronidases produced by a number of pathogenic Gram-positive bacteria catalyze the degradation of hyaluronic acid (HA), initiating infections at the skin or the mucosal surfaces. It's known that *streptococcus*, *staphylococcus*, *streptomyces* or *clostridium* bacteria between others, use this enzyme as a virulence factor to destroy the polysaccharide that holds animal cells together, making easier for the pathogen to spread through the tissues of the host.[1] The interest in the detection of this enzyme is related to two different aspects: i) the evaluation of the secreted levels of enzymes for different bacterial species would allow to discriminate between Gram-positive and Gram-negative bacteria and also to classify them in terms of virulence and ii) the evaluation of the enzyme secretion inhibition would allow to propose novel antimicrobial/antivirulence agents.

On the other hand, lysozymes are antimicrobial enzymes produced by the immune system of animals. Its mechanism of defense consists in degrading the peptidoglycans in the cell walls of Gram-positive bacteria, being thus considered as a wound infection biomarker.[2] Therefore its detection in wound fluids is of great interest for the monitoring of bacterial infection processes.

However, the current available tools for the detection of these enzymes are quite limited. They are very small proteins (molecular weight of 60 kDa for hyaluronidase and 15 KDa for lysozyme) which difficult their detection using traditional immunoassays, typically radioimmunoassays (RIA) and enzyme-linked immunosorbent assays (ELISA) that are expensive, time consuming and need hazardous label reagents.

In this context, biosensors in general and the ones based on nanoporous platforms in particular, overcome most of these limitations, since they are rapid, cheap and allow label-free detection. Nanopores and nanochannels-based platforms stand out from the variety of nanostructured materials used for biosensing applications. The use of arrays of solid-state nanochannels has opened the way to different and versatile sensing systems ranging from electrochemical to optical detection devices.[3,4] In particular, anodized aluminum oxide (AAO) nanoporous membranes have been shown as excellent platforms for protein[5,6] and DNA[7] electrical detection, based on the nanochannel blockage upon the biomolecule recognition by specific receptors, which is electrically monitored through the evaluation of standard red-ox indicators.

We propose here a novel methodology for enzyme detection on AAO nanoporous membranes based on both immunoassays and enzymatic assays. In the case of hyaluronidase, specific antibodies are immobilized in the inner walls of the nanochannels, being monitored the blocking generated by the immunocomplex formation. In parallel, lysozyme detection is proposed as proof-of-concept of enzymatic assay monitoring. In this case, peptidoglycan substrate is immobilized in the membranes, being its degradation by the enzyme monitored through the unlocking of the nanochannels upon peptide degradation.

RESULTS AND DISCUSSION

AAO nanoporous membranes with pore sizes of 20 nm were used as sensing platforms after their silanization. The receptors were immobilized through the peptide bond formed between the amino groups in the membrane and the carboxylic groups present in the constant region of the anti-hyaluronidase antibody and in the L-alanine and D-glutamine residues of the peptidoglycan. After the incubation with the corresponding enzyme (30 μL; 2h; room temperature) the membranes were assembled with an Indium tin oxide/polyethylene terephthalate (ITO/PET) electrode and the nanochannel blocking degree was evaluated by monitoring the voltammetric signal of hexacyanoferrate (III) red-ox indicator (Ag/AgCl and Pt were used as reference and counter electrodes). The diffusion of the $[Fe(CN)_6]^{4-}$ through the membrane to the electrode is affected by steric/electrostatic force changes upon enzyme recognition. The voltammetric peak corresponding to oxidation of $[Fe(CN)_6]^{4-}$ to $[Fe(CN)_6]^{3-}$ at approximately +0.35 V is considered as the analytical signal.

The results of the studies for hyaluronidase immunodetection are shown inFigure 1.

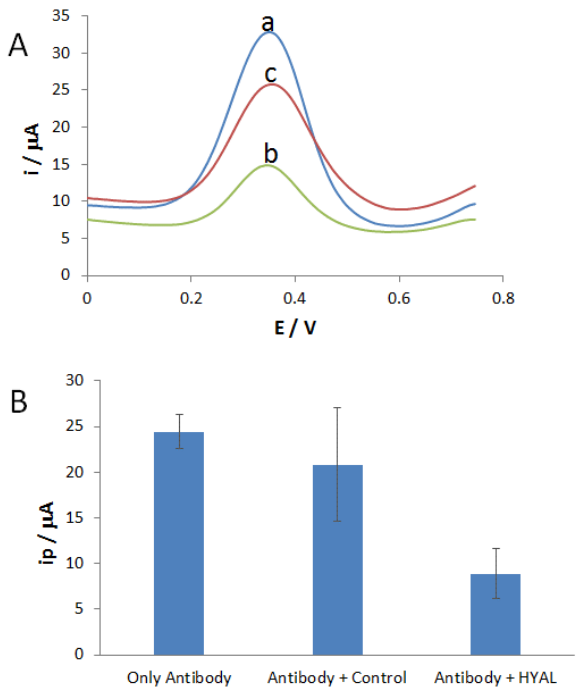

Figure 1. Hyaluronidase immunodetection. (A) Differential pulse voltammetric (DPV) scans from 0 V to +0.75 V (step potential 10 mV, modulation amplitude 50 mV, scan rate 33.5 mV/s, non-stirred solution) recorded for membranes modified with only antibody (a), and with antibody after incubation with: hyaluronidase (b) and protease V8 (control) (c). (B) Peak intensity values corresponding to three replicates of each assay. Hyaluronidase concentration: 473 UI/mL. Protease V8 concentration: 2 mg/mL. Electrolyte: 1 mM $K_3[Fe(CN)_6]$ / 0.1M KCl.

When only the antibody is present, a high voltammetric signal of around 25 µA is observed, evidencing a partially open nanochannel (Figure 1A(a)). After the interaction with the enzyme, a decrease in the voltammetric peak current of around a 60% is detected, as result of the partial blockage of the nanochannel (Figure 1A(b)). The restoring of the signal observed for protease V8 (evaluated as control) demonstrates the specificity of the assay (Figure 1A(c)). The summary of voltammetric peak values shown in Figure 1B evidences the good reproducibility of our method.

Lysozyme studies are shown in Figure 2. Peptidoglycan receptor immobilization produces a 50% decrease in the voltammetric peak current (Figure 2A(b)) when compared with the bare membrane (Figure 2A(a)). A total restoring of the signal is observed by the action of the lysozyme (Figure 2A(c)), evidencing the specific degradation of the peptidoglycan and the unblocking of the nanochannel. The summary of results obtained by triplicate demonstrates the high reproducibility of the method, especially for the open membranes (Figure 2B).

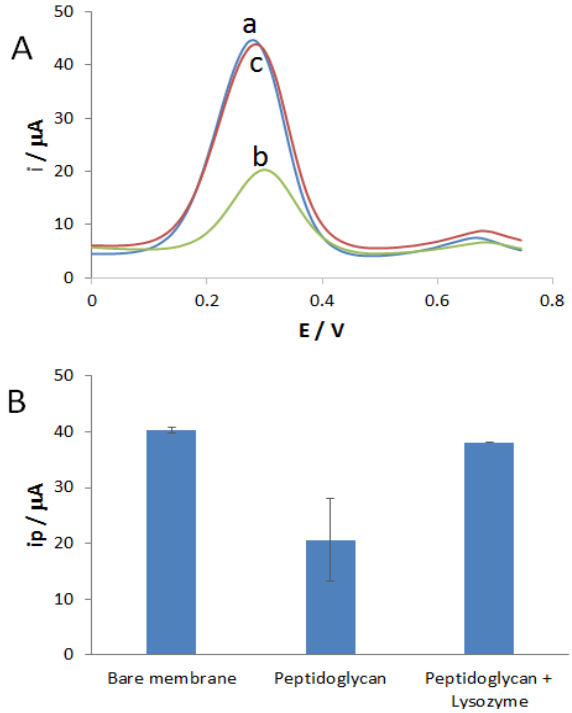

Figure 2. Lysozyme enzymatic detection. (A) Differential pulse voltammetric (DPV) scans from 0 V to +0.75 V (step potential 10 mV, modulation amplitude 50 mV, scan rate 33.5 mV/s, non-stirred solution) recorded for bare membranes (a), membranes modified with the peptidoglycan before (b), and after incubation with lysozyme (c). (B) Peak intensity values corresponding to three replicates of each assay. Lysozyme concentration: 1 mg/mL. Electrolyte: 1 mM $K_3[Fe(CN)_6]$ / 0.1M KCl.

CONCLUSION

The proposed analytical method based on the electrical monitoring of specific nanochannels blocking/unblocking has been shown as a useful tool for the detection of hyaluronidase and lysozyme through immunoassays and enzymatic assays, respectively. This label-free method is rapid and cheap, avoiding sandwich assays and the use of labels. Preliminary results open the way to future applications for virulence evaluation of enzymes as well as for monitoring bacterial infection processes.

ACKNOWLEDGEMENTS

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(OP 17) Preparation of the fibrous bio-scaffold utilizing supercritical fluid extraction

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Keywords: Supercritical fluid, Extraction, Scaffold

INTRODUCTION

Decellularization of animal tissue is recently noticed as a novel method for preparation of the scaffold for tissue engineering. The most representative method for decellularization would be the use of surfactants, such as SDS and TritonX-100. These surfactants show strong detergency and consequently the cells in the tissue appear to be effectively washed out. However, the complete removal of the toxic surfactants from the tissue is difficult even after repeated rinsing, since they show high affinity to extracellular matrix. In addition, cross-linking of tissue with a suitable aldehyde is generally performed after surfactant treatment to maintain mechanical properties of the tissue. In order to overcome these problems, we examined a novel method for decellularization of animal tissue utilizing supercritical fluid (SCF) extraction. This method has the potential to extract the cells effectively with a single extraction medium by controlling the operating pressure [1,2]. In this study, we selected carbon dioxide (CO_2) or fluoroform (CHF_3) as the extraction medium and applied to dermal tissue, uterine, blood vessel and breast. Because carbon dioxide and fluoroform have moderate critical conditions (carbon dioxide: TC = 32°C, PC = 7.38 MPa, fluoroform: TC =25.74°C, PC =4.84MPa), the mechanical properties of the tissue are unlikely to be lost on the treatment under supercritical conditions. In addition, carbon dioxide and fluoroform in the tissue will return in the gaseous state after the treatment and will naturally diffuse toward the outside of the tissue. As a result, there is no possibility of remaining any chemicals in the tissue and the decellularized tissue would be very safe to the body after implantation.

RESULTS AND DISCUSSION

Figure 1 shows photographs of HE-stained rat dermal tissue after SCF extraction. As shown in Figure 1, cells in the SCF treated tissue seemed to be removed though the remaining of cells around epidermis regions was observed. Penetration of supercritical fluid into epidermis regions where cells are intensively localized may be insufficient. The effects of cell extraction by fluoroform seem to be higher than that by carbon dioxide. Further, cell extraction effect was markedly improved by an addition of ethanol as entrainer in each medium. From these data, extraction efficiency of high polar cells seems to be improved by an increase in the apparent permittivity of the medium. As is generally known, apparent permittivity can also be varied by changing operating pressure. In addition to the selection of the extraction medium and entrainer, screening and determination of suitable operating pressure may improve easy access of the medium to the epidermis regions. As a result, dissolution and extraction of cells into the medium may be improved.

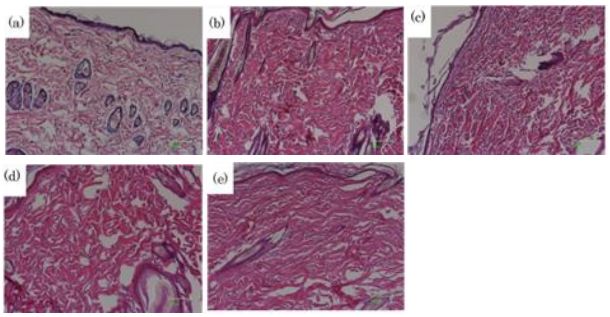

Figure 1. HE-stained dermal tissue after supercritical fluid extraction.

(a) Control, (b) Treated in CO_2, (c) Treated in CO_2 + C_2H_5OH (d) Treated in CHF_3, (e) Treated in CHF_3 + C_2H_5OH

CONCLUSION

Decellularization of animal tissue was examined utilizing SCF extraction method. Supercritical carbon dioxide containing a small amount of ethanol was a suitable medium to extract cells from animal tissue. Further, supercritical fluoroform which has higher permittivity than carbon dioxide was found to be more effective extraction medium. Compared to other decellularization methods, removal of cells with SCF extraction process can be attained under gentle experimental conditions without remaining toxic chemicals. Further detail investigation would raise the potential of decellularization by SCF.

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(OP 18) Innovative Technologies for Sustainable Textile Coloration and Surface Design

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Keywords: Textile Design; Enzyme Biotechnology; Laser Technology; Sustainable Textiles; Coloration

ABSTRACT

The environmental impact of textile dyeing and finishing is of paramount concern in the textile industry. Research into two emerging textile processing technologies, laser processing and enzyme biotechnology, were investigated as a means of applying new surface design and coloration techniques with a focus on improving the efficiency and sustainability of existing textile design and finishing methods. Each technique considered a reduction in energy, dye chemicals, and subsequent waste water effluent for sustainable textile processing. Through industrial stakeholder engagement and cross-disciplinary research involving textile design, fibre and dye chemistry, biotechnology and optical engineering, the work resulted in a catalogue of new coloration and design techniques including:

- Laser enhanced dyeing for wool and wool blend textiles
- Peri-dyeing: a laser dye-fixation method for textiles
- Laser moulding for synthetic stretch textiles
- Laser fading linen
- Laccase catalysed coloration for textile fibres
- Laccase catalysed decoloration to achieve decorative surface patterning
- Enzyme generated surface patterning
- Enzyme assisted printing for decorative surface patterning

ACKNOWLEDGEMENTS

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(OP 19) Biochemical modification and functionalization of nanocellulose, and its application potentials

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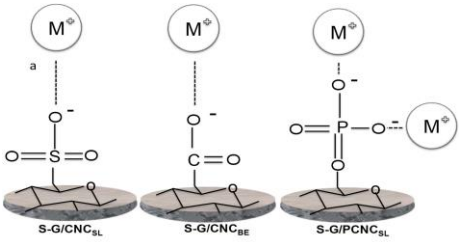
Keywords: nanocellulose, carboxylation, phosphorylation, hydrophobization.

INTRODUCTION

Cellulose nanomaterials (i.e. cellulose nanofibers / CNFs and nanocrystals / CNCs) have been receiving a great importance in the last decade due to their specific aspect ratio and huge surface area as well as being renewable, nontoxic, sustainable and biodegradable nanomaterial. Moreover, the chemical character of the cellulose molecule enables the creation of new functional groups or even introduction of new molecules, giving them higher added-value and thus additionally govern the final material properties and broadens their application.

In the contribution, some bio-catalytically induced strategies for surface modification and functionalization of nanocellulose / NC will be presented, as i) specific glucosidic-bond hydrolysis by endo-cellulase to modify the NC surface area (size, shape) vs. reactivity (–OH groups), ii) the introduction of aldehyde *vs.* carboxyl functional groups by laccase/TEMPO systems (1,); iii) the phosphorylation using hexokinase-mediated modification (2,3,4), and iv) hydrophobical functionalization using acetyc anhydride and lipase (5). The functional properties and application potentials will be shown.

RESULTS



Comparisons of sorption capacities between nanoCelluloses and other adsorbents						
	Equil. sorption capacity (*mg/g*)			Theoret. max sorption capacity of REF adsorbers (*mg/g*)		
Metal ions	CNC	Phos-CNC	Phos-CNF	Modified chitosan	Nano TiO2	Montmorillonite
Ag^{+}	56	136	120	510	128	–
Cu^{2+}	20	117	114	70	9.9	28.8
Fe^{3+}	6.3	115	73	72	71.3	28.9

Fig. 1 Comparison of metal ions sorption capacity of used nanocellulose membranes with other adsorbents.

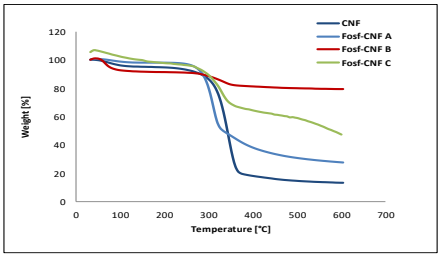


Fig. 2 Comparison of weight loss of native and differently phosphorylated CNFs, showing on an improved thermal stability.

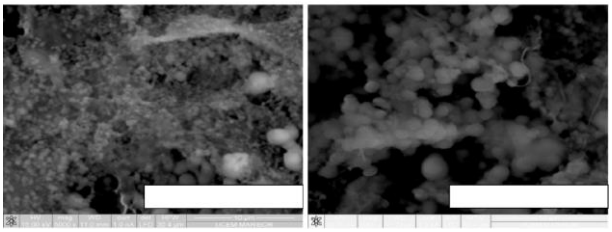


Fig. 3 SEM images of a native (left) and phosphorylated-CNF (right) samples after 10 days of culturing in 1.5 SBF, showing a biomimetic growth of CaP crystals on phosphorylated-CNF.

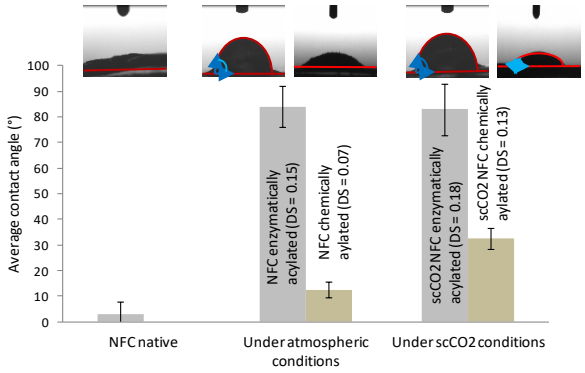


Fig. 4 Contact angle measurements of pressed films from native, enzymatically and chemically-acetylated NFCs after freeze-drying, showing on a surface hydrophobization.

ACKNOWLEDGEMENTS

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(OP 20) Nanocellulose extraction from banana pseudo-stalk for the production of bioplastic

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Keywords: Banana, Nanocellulose, Bioplastic

INTRODUCTION

Today we live in a globalized world characterized by the process of production and mass consumption, factors such as: population growth, industrialization, agricultural modernization gives rise to a notorious concern with environmental issues. Research indicates that the unbridled consumption of society has led to greater environmental degradation, where there is a close and strong relationship between consumerism and the environment. This is because to meet the demand of production and consumption is necessary to remove raw materials from nature, manufacture and transport materials, make great use of electricity, water, among others. All this generates emission of polluting gases, degradation and environmental devastation, general pollution and, consequently, the destruction of ecosystems. And in this context, it is necessary to search for materials that reduce the environmental impact and add value to sustainability. In this context, Brazil presents several sources, especially after harvesting in large crops. In the case of banana farming, there is a large generation of waste due to the plant's own life cycle, when stem and leaves degradation occurs after fruit removal. These residues, when not used in composting or handicrafts, are inadequately deposited, attracting vectors of disease or polluting the environment. The objective of this work was to evaluate the possibility of producing bioplastic through the use of banana pseudo-stalk, being the main objective the extraction of nanocellulose as an alternative to minimize the accumulation of this organic residue. As expected results, both in the chemical processes and in the mechanics, is the obtaining of the nanocellulose films. This makes its use possible as a possible socially sustainable alternative. Concluding the relevance of this study as an alternative to substitute raw material in the production of plastics. And a route to a replacement of an organic residue that after its useful life becomes garbage, transforming and aggregating values a biodiversity.

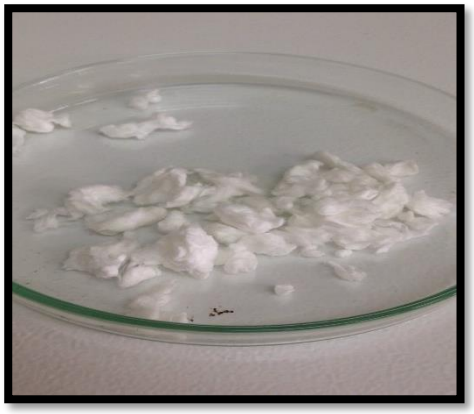
RESULTS AND DISCUSSION

This work elaborated two types of methodology, one chemical and another mechanic. Both were experimental.
Two distinct processes were used for comparative purposes: 1) Chemical Method: It uses specific chemical agents to cook under pressure, the material. The processes may be basically acids (sulfite) or alkaline (sulfate and soda). Fully employed in the pulp and paper industry, having as a disadvantage, the formation of highly polluting waste. 2) Mechanical Method: They use only mechanical energy, not involving the use of chemical reagents, but the cost is high ground in a hammer mill and a further classification on an electromagnetic sieve shaker with meshes of 250, 180, 75, 59, 0.125 ppm. After this step the sample was divided into two: 1) one underwent the mercerization process; 2) another suffered the mechanical process.

The pseudo-stalk was ground in a cane grinder and the obtained fibers were weighed and had an irregular size between 5 and 40 cm. The sample was dried by an air recirculating incubator at 50 ° C for 7 days and

1- Disintegration of banana pseudo-stalk fibers
The proposed methodology for the mercerization of the banana pseudo-stalk, through NaOH solution and subsequent bleaching with the peroxide and hydroxide was efficient, thus reaching the results. In figure 3, the fiber obtained after this process is observed.
With this alkali solution (NaOH) it was possible to carry out a first disintegration of the material and with the bleaching, the lignin chains were broken, resulting in the cellulose.

Figure 1- fiber obtained after bleaching



Source: Author (2016)

2- Nanocellulose

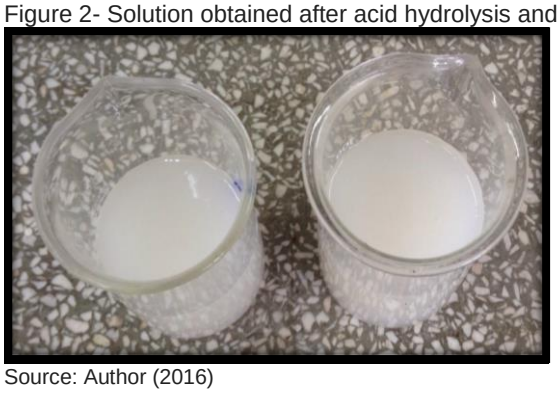
The two methods proposed were efficient and produced nanocelluloses. Visually no difference was observed between the two methodologies, the hydrolysis and the defibrillator mill, according to figure 4.

It was observed that the suspension produced is similar to that described by other authors, indicating that this suspension has nanocellulose structures (VIANA, 2013).

However, it is necessary to perform a characterization by Electronic Transmission Electron Microscopy (MET), in this way it is possible to measure the size of the nanoparticles, that is, nanofibers and verify on the nanometric scale if there were significant differences among the methodologies proposed in this work.

In terms of waste generation, the methodology through mechanical processing, mill, is more advantageous. For, there is no generation of chemical residues as happens in hydrolysis. In the national scenario, an industrial-scale defibrillation mill has already been implanted in a paper mill. Thus, it can make this research of laboratory character in an alternative for the industry.

Figure 2- Solution obtained after acid hydrolysis and mil



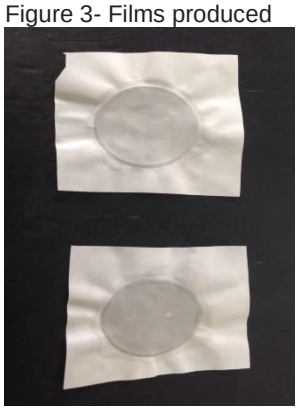
Source: Author (2016)

3- Bioplastic (films)

Figure 3 shows the visual aspect of the films produced.

The Films being prepared for the tests of water absorption, being the top of the hydrolysis and under the mechanical process.

Figure 3- Films produced



Source: Author (2016)

The produced nanocellulosic films are similar to those described in the literature Viana (2013), Wang et al., (2013), but these references are from films produced with wood fibers. Visually there was no difference between the products of the two proposed methodologies. As a result of the tensile tests: the top of the hydrolysis being: 15.5 ± 3.1 (Mpa), and absorption: 46.26 ± 5.3 (g / m²); and from the mechanical process: 18.6 ± 2.7 (Mpa), and at absorption: 51.02 ± 6.9 (g / m²), and thus on average as shown by the literature results. According to Pereira (2010), it was possible to extract nanocellulose by acid hydrolysis, using as raw material fibers of the banana pseudo-stalk. Confirmation will only be possible by scanning electron microscopy. Once proven, the extraction of nanocellulose from the pseudo-stalk of the banana tree proved to be an alternative for the use of agroindustrial residues, thus avoiding waste and contamination, and at the same time, obtaining a product of high added value.

CONCLUSION

After the experiments carried out and the analysis of the obtained results it is concluded that it is possible to obtain nanocellulose through acid and mechanical hydrolysis, having as a raw material fibers of the banana pseudo-stalk, but the confirmation is only possible by scanning electron microscopy and for the crystallinity of the nanofilms it is necessary an X-ray diffractometer that will be made in future works.

The proposed methodology for mercerization proved to be efficient. The fibers extracted from the banana pseudo-stalk exhibit a high content of extractives and ashes, which are largely removed by mercerizing and bleaching, purifying the fiber.

In the mechanical process the number of pulp passes through the defibrillator mill results in a decrease in the degree of crystallinity of the nanofibrillated cellulose, thus banana fibers represent an additional source of renewable biomass with attractive properties, in addition to having a low cost.

The two procedures for the production of nanocellulose were adequate, however by the mechanical treatment it produced more quickly and without generating chemical residues. Banana nanocellulose bioplastics have been similar to those produced with wood fibers and have a high resistance. At the end of this research the great potential of this raw material is observed. Besides taking advantage of the banana residue, it is possible to produce a sustainable material.

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We thank God first for life and for everything that has been pro-rated to this present moment! Then our teacher Me. Janara de Camargo Matos, the Federal University of Paraná for the opportunity and availability of the laboratories, and especially proifessor Dr. Mayara Elita Carneiro.

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(OP 21) Removal of heavy metals from contaminated water using an extruded matrix of biodegradable polymers

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Keywords: Chitosan, Poly(ε-caprolactone), Heavy Metals, Sorption

INTRODUCTION

Chitosan (Ch) is a natural copolymer obtained by the deacetylation of chitin, wich is commonly found in the exoskeleton of crustaceans. The presence of a significant number of amine groups distributed along its polymeric chain substantiates Ch in the form of a polyanion to neutralize and remove various metallic ions from acid effluents.[1] The poly-ε-caprolactone flexibility and biodegradability are important properties and additionally it can be mixed with other polymers to improve characteristics as solubility and degradability.[2]

RESULTS AND DISCUSSION

Chitin was extracted by the lactic-acid fermentation of shrimp waste followed by alkali and acid treatments to remove residual ash and proteins. Ch was obtained by alkaline treatment of the purified chitin.[3] PCL was synthesized by enzymatic polymerization of ε-caprolactone with immobilized lipase B of *Candida antarctica* in liquid 1,1,1,2-tetrafluoroethane media.[4] Both materials were mixed in ratio 2:1 (Ch: PCL).[5] Then were extruded as cylindrical pellets.

The point of zero charges (pH_{pzc}) of the extruded materials (MCP) were determined by potentiometric titrations dispersing the samples in a solution of NaCl (0.1M) then acidifying the medium with HCl (0.1M) untill pH 3 and then titrating with NaOH (0.1M).[6] The results show the pH_{pzc} at pH=6.7. The data from the potentiometric titration also help us to determine the pKa of the several surface groups present in the MCP.

Three pKas were determined in agreement to the presence of ester, amine and hydroxyl groups in the chemical structure of the polymers[7] (**Fig.1**). The molecular structure is preserved after the thermal treatment in the extrusion as corroborated by FTIR analyses. These groups displayed the characteristic band in the spectra **(Fig. 2)** at 3284 cm^{-1}, 1558 cm^{-1} assigned to the stretching and flexion of the amine group vibrations . Signal at 1723 cm^{-1} is assigned to the stretching band of the carbonyl ester group.

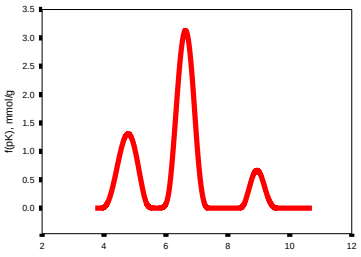

Figure 1. pKa distribution in the MCP

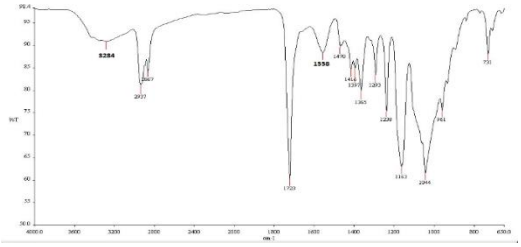

Figure 2. Representative FTIR spectrum for MCP

CONCLUSION

The pH_{pzc} shows that the MCP allows to work with slightly acidic aqueous samples with the capacity to remove positively charged metals ions. The FTIR analyses corroborate that PCL does not react with the Ch during the extrusion thus keeping the free amine moieties. These amine groups play an essential role in the adsorption of heavy metals.

ACKNOWLEDGEMENTS

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(OP 22) Bio-Electro-Fenton for the Treatment of Textile Wastewater

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Keywords: Bio-Electro-Fenton, Electrode, Textile Wastewater Treatment

INTRODUCTION

Textile industry is responsible for almost 20% of freshwater pollution globally (Maxwell, D. McAndrew, L. Ryan, 2015). Various methods are used to treat the effluents on large scale, some of these main methods are chemical Advanced Oxidation Processes related approaches (AOPs). Despite their advantages and efficiency in the effluent treatment, they are still many technical issues. Bio-Electro-Fenton (BEF) is a new sustainable method for degrading persistent organic pollutants from textile industry, which is rich in color and additives. In the anodic compartment in a BEF system, the biological activity will cause electrons to be released. These electrons are transferred from the anode to the cathode through an external electrical circuit. Consequently, Hydrogen peroxide is generated by the reduction of oxygen on the cathode. Furthermore, Fe^{2+} ions are *in-situ* generated due to the reduction of the iron substances existing in the cathodic compartment or directly on the cathode. As a result, HO· radicals are generated sustainably *in-situ* to achieve the advanced oxidation by Fenton reaction to degrade pollutants in textile effluents as shown in Figure1 adapted from (Kahoush et al., 2017), and the following equations taking acetate as a model substrate (Du et al., 2007).

$CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^-$

$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$

$Fe^{3+} + e^- \rightarrow Fe^{2+}$

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$

$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH^{\bullet} + H^+$

This method has many advantages, like *in-situ* generation of the radicals needed in the Fenton's reaction. This reduces the amounts of added chemicals, which reduces the costs of the operation. In addition, no power consumption is needed since the process is self-dependent. In recent years, studies focused on the degradation of different types of dyes using BEF systems with microbial populations (Feng et al., 2010a, 2010b; Fu et al., 2010; Zhuang et al., 2010; Liu et al., 2012). In this paper, BEF system with an enzyme-modified bio-anode have been studied, and the system's capacity of treating textile wastewater have been tested.

RESULTS AND DISCUSSION

The color removal efficiency and the organic pollutants degradation are depending on many factors in BEF system including: the concentration of both hydrogen peroxide and ferrous ions, in addition to temperature, pH and the power density generated in the system, due to the biological activity in the anodic chamber and other factors.

The efficiency of BEF system can be calculated from the Oxidation Current Efficiency (OCE), where C_{exp} is the experimental value of the pollutant concentration output from the system.

$$OCE = \frac{C_{exp}}{C_{theor}} \times 100$$

Furthermore, Chemical Oxygen Demand (COD) removal percentage can be determined according to the following equation.(Benatti and Tavares, 2012)

$$\eta(\%) = \frac{COD_I - COD_O}{COD_I} \times 100$$

The power density generated in the system can be calculated ($mW.m^{-2}$) from the following equation (Birjandi et al., 2016) where V is the voltage of the cell, I is the current intensity and A is the surface area of the cathode:

$$P = \frac{I * V}{A}$$

The higher the power density generated in the system, the more oxygen will be reduced in the cathodic compartment, which in turn leads to the *in-situ* generation of $OH^{\cdot}$ radicals. Thus, higher degradation efficiency and COD removal of the organic materials in the cathodic chamber is obtained.

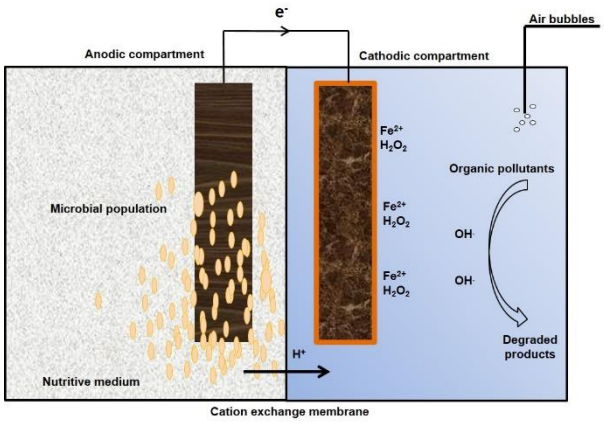

Figure 2. BEF system adapted from Kahoush et al.2017

CONCLUSION

Bio-electro-Fenton is a sustainable promising method for the treatment of textile wastewater. It can improve the safety conditions of the working environment by reducing storage and transfer of hazardous chemicals. In addition to generating power from bio-sources materials, which can reduce the costs of the operation. Nevertheless, these systems should be further studied and tested, to improve their stability, lifetime and power output, to overcome the challenges to their use on industrial large scales.

ACKNOWLEDGEMENTS

This work was financially supported by Erasmus Mundus joint Doctorate Program − Sustainable Management and Design for Textiles (SMDTex), grant number 2015-1594/001-001-EMJD.

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(OP 23) Antibacterial Efficiency of Cellulose Microparticles Functionalized with Silver Nanoparticles for Water Purification

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Keywords: cellulose microparticles, silver nanoparticles, water disinfection, antibacterial activity

INTRODUCTION

In order to make feasible the use of silver nanoparticles to treat water, these nanostructures need to be incorporated in a substrate. The literature reports the use of distinct substrates to immobilize or embed the silver nanoparticles, such as inorganic materials (silica[1], ceramic[2]) and polymers[3,4]. In the present study, cellulose, a natural, renewable, sustainable, nontoxic and "green" compound was used to support the silver nanoparticles (AgNp) were incorporated. The colloidal silver was prepared through the common chemical reduction route by using $NaBH_4$ as reduction agent. The aminoethyl-aminopropil-trimethoxisilane (AES, Dow Corning) was used as stabilizing agent in the synthesis of the AgNp. The AES also acted as coupling agent, allowing the incorporation through covalent bonds of the AgNp on the surface of the microcrystalline cellulose particles (Blanver, 200 µm) used.

RESULTS AND DISCUSSION

The influence of the concentration of AES ([AES]) on the morphological and optical characteristics of the AgNp were investigated. Such results are presented in Figures 1 and 2, respectively. The average diameter of the AgNp (d), showed inside the images, was measured with the software *ImageJ*®. As seen, the average diameter and the standard deviation of sizes reduced by increasing the concentration of AES.

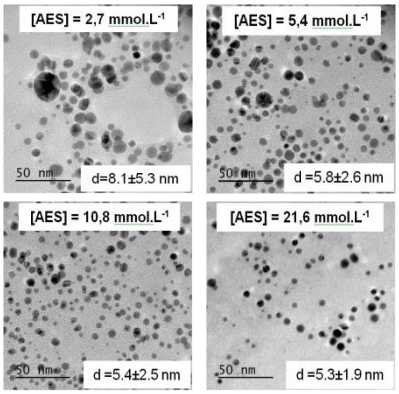

Figure 1. Images taken by transmission electron microscopy of the AgNp produced with different [AES].

UV-Vis spectrophotometry analysis was performed along 10 days to evaluate the stability of the colloidal dispersions produced. The absorbance spectra measured just after the synthesis (results not shown), revealed that the lower was the concentration of AES, the lower was the maximum absorbance (λ_{max}) and the wider was the spectra. These results demonstrated that the colloidal stability diminished as the concentration of AES was reduced. The synthesis carried out with the 21.6 $mmol.L^{-1}$ of AES presented a significant reduction of λ_{max}, as observed in Figure 2. Such findings might be a consequence of the sensibility of the system to the pH value, which increased as the concentration of AES was raised. As seen in Figure 2, the synthesis performed with 10.8 $mmol.L^{-1}$ of AES presented the highest concentration of AgNp and a good stability along the period of analysis (10 days).

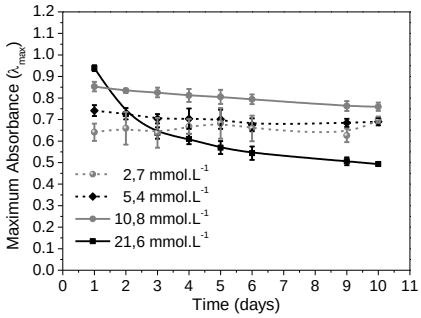

Figure 2. Maximum absorbance (λ_{max}) along the time for the AgNp dispersions produced with distinct [AES].

The functionalization of the cellulose microparticles was performed by mixing 1.0 g of the cellulose with distinct volumes of the aqueous dispersion of AgNp (10, 20 and 100 mL) produced with different concentrations of AES. After mixing the suspensions for 1.0 hour and let them rest for 72 hours, the supernatant was removed and the particles were dried, resulting in products like those shown in Figure 3.

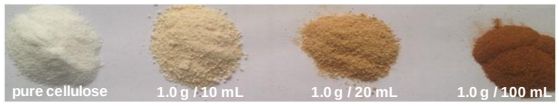

Figure 3. Images of the cellulose microparticles functionalized with distinct volumes of the aqueous dispersion of AgNp.

The total amount of AgNp, synthesized with distinct concentrations of AES, contained in the samples produced with the aqueous colloidal dispersions in the proportions of 1:10, 1:20 and 1:100 ($g.mL^{-1}$) were measured by ICP-MS. According to the results displayed in Table 1, the samples prepared with the colloidal dispersion produced with 10.8 $mmol.L^{-1}$ of AES presented the highest amounts of AgNp regardless the proportion of cellulose mass and the volume of AgNp dispersion used. These results corroborate with those one presented in Figure 2.

Table 1. ICP-MS results for all the functionalized cellulose microparticles produced.

[AES] ($mmol.L^{-1}$)	*Proportion Cellulose: AgNp ($g.mL^{-1}$)*	*Average AgNp mass concentration ($mg.g^{-1}$)*	*Standard Deviation ($mg.g^{-1}$)*
5.4	1:10	0.68	± 0.0
	1:20	1.69	± 0.1
	1:100	5.35	± 0.4
10.8	1:10	1.10	± 0.1
	1:20	1.87	± 0.1
	1:100	**6.44**	**± 0.3**
21.6	1:10	0.80	± 0.2
	1:20	1.65	± 0.5
	1:100	1.79	± 0.6

Lixiviation tests of the Ag^+ were performed by differential pulse voltammetry using the samples containing the highest proportion between the cellulose mass and the volume of AgNp dispersion. For this test, 0.05 g of the functionalized cellulose microparticles were maintained immersed in a flask with 50 mL of deionized water for 28 days. Once per week, an aliquot was removed from the flasks and analyzed. According to the results presented in Figure 4, the sample containing the highest amount of AgNp was that one which released less Ag^+.

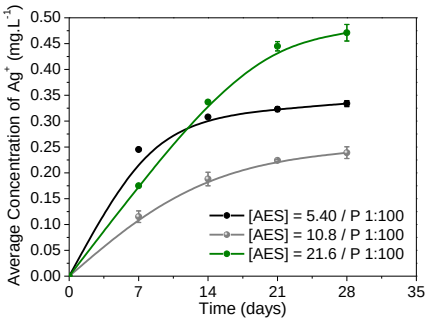

Figure 4. Lixiviation profiles of the Ag^+ for the samples produced with the highest proportion between the cellulose mass and the volume of AgNp dispersion.

Such findings indicated that the AgNp could be suitably incorporated on the surface of the cellulose microparticles. Moreover, it suggests that the incorporation occurred by covalent bonds, and the efficiency of the bonding between the AgNp and the cellulose sites strongly depends on the amount of AES used in the synthesis.

The antibacterial assays were performed according to the standard method ASTM E2149 by using the bacteria Gram positive *Staphylococcus aureus* and the Gram negative *Escherichia coli*. The sample produced with 10.8 $mmol.L^{-1}$ of AES and the proportion of 1:100 between the cellulose mass and the volume of AgNp dispersion was used in such assays due to the highest content of AgNp. Specific amounts of the functionalized cellulose microparticles were used in the inoculum solution in order to achieve the specific concentrations of 1, 3, 5 and 10 ppm of AgNp in such solutions. As seen in the findings presented in Figure 5, the developed product presented excellent antibacterial properties, eliminating 100% of the bacteria in only 15 minutes for the tests with 5 and 10 ppm of NpAg.

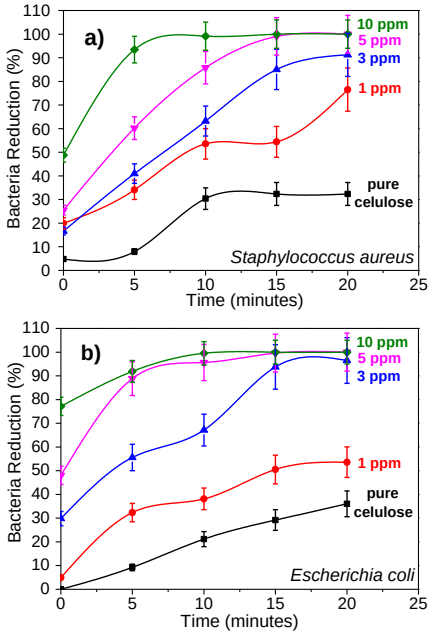

Figure 5. Kinetic profiles of the bacteria reduction as a function of time and AgNp concentration: a) *Staphylococcus aureus*; b) *Escherichia coli*.

CONCLUSION

This study presented a strategy to produce cellulose microparticles with antibacterial properties by incorporating silver nanoparticles onto the surface of such natural substrate. As shown, the low lixiviation of Ag^+ and the excellent antibacterial properties of the developed product against Gram positive and Gram negative bacteria make it a promising filtering element for water purification.

ACKNOWLEDGEMENTS

The authors acknowledge CAPES (Coordination for the Improvement of Higher Level Personnel) for the financial support.

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(OP 24) Volumetric oxygen transfer coefficient (kLa) and Reynolds number (Re) as scaling-up criteria for the production of β-N-acetylhexosaminidase of *Lecanicillium lecanii*

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Keywords: β-N-acetylhexosaminidase, Lecanicillium lecanii, Volumetric oxygen transfer coefficient.

INTRODUCTION

Currently, there is an increasing interest in enzymes with potential industrial applications, such as chitinases due to their uses in biomedical, production of chitin oligosaccharides (GlcNac) [2-7] owing to biological activities such as antibacterial, antitumor and immune enhancing effects. Nevertheless, there are not inexpensive chitinases preparations in the market. Therefore, the present work aimed to determine the K_La and Re of the submerged culture of *Lecanicillium lecanii* for the production of β-N-acetylhexosaminidase.

RESULTS AND DISCUSSION

Spores (10^8 spores/mL) of *L. lecanii* inoculated in a Czapeck medium with added colloidal raw chitin-containing 4.8 % (w/w) and 0.75% (w/w) of residual protein and mineral contents, respectively. The submerged culture was carried out in a bioreactor of 3 L at 25°C and pH 6 up to 120h. The total volume of the bioreactor was employed to determine the total protein content and for estimation of K_La and Re. As well, *β-N-acetylhexosaminidase* activities detected in the broth after biomass removal. The K_La was measured employing the physical replacement method. For this purpose, nitrogen was used to displace the dissolved oxygen in the liquid phase, and deionized water and non-inoculated medium used as controls. The relative viscosity of the medium and its density was employed for estimation of Re **(Table 1)**.

Table 1. Production of Nhasa of *L. lecanii* varying agitation and aeration.

Treatment	Agitation (rpm)	Aeration (vvm)
1	100	1
2	100	2
3	400	1
4	400	2

The type of fluid involved in the submerged culture was Newtonian with a mean relative viscosity value of $5x10^{-4}$ Pas at a constant temperature of 25°C and a laminar regime in a Re number range of 38-154. Maxima activities of chitinases produced by the same fungus are reported using 0.6 vvm, 150 rpm and 1 vvm, 300 rpm respectively. Both works reveal that agitation and aeration could affect dissolved oxygen in the culture and production. [1,2] In the present work, the K_La augmented as the agitation and aeration increased. However, the enzyme productivity was not improved; this might be due to fungal cell damages that consequently changed the cellular metabolism (**Figure 1)**.

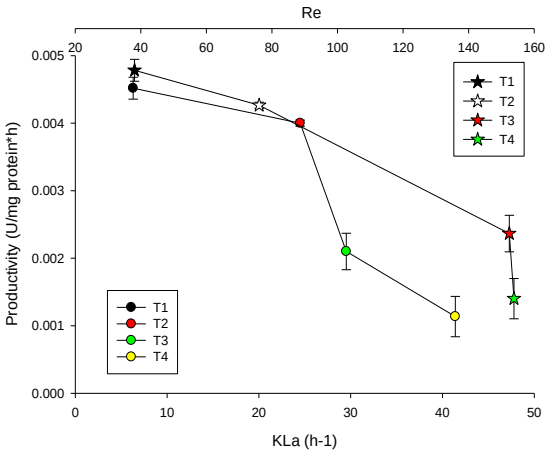

Figure 1. Effect of K_La on Nhasa productivity.

CONCLUSION

The hydrodynamics of the reactor in the range of variation of 100-400 rpm of agitation and 1-2 vvm of aeration is less relevant to the productivity of the process. According to the productivity data, the transfer of oxygen was predominant over all the conditions tested. While in treatments 3 and 4 the productivity became independent of Re since remained constant.

ACKNOWLEDGEMENTS

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POSTER PRESENTATIONS

(PO1) Dyeing of Polyester Fabric in High Temperature with Natural Dye Annatto

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Keywords: dyeing, natural dyes, annatto, polyester.

INTRODUCTION

One of the biggest concerns nowadays is related to sustainability and waste produced by chemical industries that are discarded in the environment. So, studies in this area are increasing in order to minimize these impacts through alternatives that are feasible and beneficial to nature, like the use of natural dye instead of synthetic in the textile industry. [1]

One of the oldest known natural dyes is annatto, extracted from the fruit of a native plant from Central and South America. In the last years, the scientific interest in annatto is increasing due to high biodegradability, low toxicity and compatibility with some textile fibers. [2]

The goal of this study was to evaluate the behavior of the natural colorant annatto in the dyeing of high temperature of polyester (PES) fabric, analyzing parameters and color fastness in order to verify if this is a viable process to be applied industrially.

RESULTS AND DISCUSSION

In Figure 1 are presented the results of apparent color of fabrics dyed at concentrations of 0.5% and 2.5%, at temperature of 130 ºC and pH 5, with the respective values of the colorimetric parameters (L*, a*, b*, C* and h*). The dyeing was done in triplicates, in a laboratory dyeing machine, with fixed time of 60 minutes and bath ratio 1:10.

Figure 1. Apparent color and colorimetric parameters of polyester fabrics dyed at 130 ° C.

	Sample 1 (0,5%)	Sample 2 (0,5%)	Sample 3 (0,5%)	Sample 1 (2,5%)	Sample 2 (2,5%)	Sample 3 (2,5%)
Apparent Color						
L*	73.62	73.48	73.93	65.25	65.28	64.63
a*	31.08	30.82	30.55	38.14	38.32	35.30
b*	35.67	35.40	35.31	46.06	46.31	44.72
C*	47.32	46.94	46.69	59.80	60.10	56.97
h*	48.92	48.93	49.12	50.35	50.37	51.69

The dyeing processes with natural annatto dye showed positive results, presenting good shades of colors and good color equalization in dyed fabrics, which allows to indicate that the dyeing with this natural dye can be viable.

The quality results of dyed fabric regarding color fastness were measured by gray scale readings, which allows to represent by numerical indexes the degree of transfer of the dye to the reference tissue, varying scores from 1 to 5, with 5 being the score given when there is no color difference between the original fabric and the test specimen tested.

Table 1 shows the averages of the scores attributed to the dyed samples.

Tabela 1. Color fastness scoring.

	Wash Fastness (Degradation)	Wash Fastness (Transfer to viscose)	Wash Fastness (Transfer to polyester)	Friction Fastness (Dry)	Friction Fastness (Wet)
Samples Conc. 0,5%	5	5	5	5	5
Samples Conc. 2,5%	5	5	5	5	5

From the scores it is possible to realize that the quality of the dyed fabrics in relation to color fastness was excellent, since all obtained a maximum mark, which means that there was no color transfer from one sample to another. It can also be stated that the scores of color fastness obtained in this study were better when compared to other researches with natural dyes, where the scores of color fastness range from 2 to 5. [3]

ACKNOWLEDGEMENTS

The authors are grateful the Duas Rodas Industrial for donating the dye and CAPES for providing the scholarships.

CONCLUSION

Evaluating the results obtained in this study, it is suggested that the use of annatto dye is a viable technique in dyeing of PES fabric, which provided good quality results of quality and color parameters.

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(PO2) Microwave assisted synthesis of furfural and 5-hydroxymethylfurfural from glucose, fructose and sucrose

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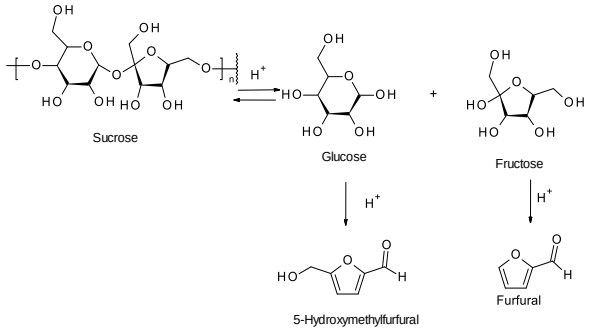
Keywords: Furfural; 5-hydroxymethylfurfural; glucose; fructose; sucrose

INTRODUCTION

Furan derivates, such as furfural and 5-hydroxymethylfurfural (5-HMF), have a great potential as platform chemicals or building blocks in the chemical industry for the production of polymers, solvents, fragrances and drugs. They can be prepared from pentoses or hexoses that originate from renewable sources (Figure 1)[1]. Microwave heating increases acid catalyzed carbohydrate dehydration, because of providing efficiently energy to the reaction[2]. The objective of this study was to compare the conversion of the substrates glucose, fructose and sucrose under different experimental conditions using microwave heating. A factorial design 2^4 with central point was carried out varying H_2SO_4 concentration (0,1 e 0,01 mol L^{-1}), NaCl concentration (0 and 0,34 mol L^{-1}), reaction temperature (140 and 180 °C) and reaction time (2 and 8 min). In all experiments 100 mg of one carbohydrate, a final volume of 5 mL, maximum power of 200 W and maximum pressure of 242 PSI were used. For analyses a HPLC-RI-DAD system with H_2SO_4 0,005 mol L^{-1} as eluent were used.

Figure 1. Acid catalyzed dehydration of sucrose to furfural and 5-HMF[1].



RESULTS AND DISCUSSION

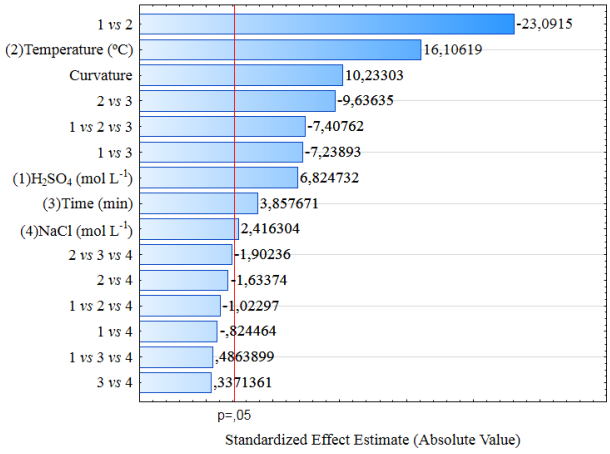
From the three investigated saccharides sucrose showed the highest global yield for furfural and 5-HMF. The best results obtained in this study are shown in Table 1. It can be observed that unequal yields for both products are obtained. This can be eventually attributed to isomerization of pentose to glucose, increasing thus the 5-HMF yield[1]. The influence of the main factors and their interactions on 5-HMF production from sucrose is shown in Figure 1. The most positive effect was observed for reaction temperature, followed by H_2SO_4 concentration, and reaction time. All significant interactions between factors had a negative effect on 5-HMF yield. This should be possible, because the factors provide energy and help on carbohydrates dehydration[1].

Table 1. Maximum yields (%) for 5-HMF and furfural obtained from fructose, glucose and sucrose.

	Fructose	Glucose	Sucrose
5-HMF	45,11 ± 2,34	36,33 ± 2,86	82,88 ± 4,27
Furfural	43,11 ± 3,12	9,34 ± 1,12	33,74 ± 3,53

The influence of NaCl on the dehydration reaction was negative when fructose and glucose were used. Possibly, NaCl diminishes the solubility of the products leading to volatilization and degradation in the headspace[3].

Figure 2. Pareto Chart concerning 5-HMF synthesis using sucrose.



CONCLUSION

The reaction presented the best yields when were used highest levels of temperature, H_2SO_4 concentration, time reaction and lowest NaCl concentration. Sucrose presented the highest yield for 5-HMF, while fructose yielded more furfural.

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(PO3) Release kinetics of sodium diclofenac from alginate films cross-linked with calcium ions

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Keywords: Release, Anti-inflammatory, Films, Alginate, Cross-linking

INTRODUCTION

A recent approach to the treatment of skin lesions consists in using polymeric films containing therapeutic agents to be released to the lesion site acting in the healing process and in pain control. The efficacy of these films is related to the release behavior of the active agent to the wound surface, which should allow the drug to be released for the desired period and in sufficient quantity.[1]

Alginate films promote intense absorption of the wound exudate, aiding in the moisture of the lesion. In addition, alginate is non-toxic polymer from a renewable source and the cross-linking with divalent ions allows the control of release and the modification of film properties.

This research aimed to obtain alginate films with diclofenac sodium cross-linked with different concentrations of calcium ions and to evaluate the release kinetics of the drug in liquid medium.

RESULTS AND DISCUSSION

The films were produced by casting usingmedium viscosity sodium alginate solution at 1% (w/v) containing sodium diclofenac (50 mg/g alginate). After oven drying at 60°C, the films were cross-linked by immersion in aqueous solution of $CaCl_2$ at 3% and 5% (w/v) for 15 min and then washed in distilled water and dried again at room temperature (25°C). For the release assays, samples of the films (3 cm x 1 cm) were immersed in flasks containing 25 mL of water at 25°C under stirring. After predetermined intervals, the concentration of drug released to water was determined by UV/Vis spectroscopy at wavelength of 275.3 nm. The readings were carried out until reaching equilibrium in the concentration variation.

In Figure 1 is shown the kinetics of drug release obtained for alginate films cross-linked with calcium 3 and 5%.

The release kinetics results were similar for the two cross-linker concentrations, with the equilibrium attained around 1000 min. The diffusion coefficient was determined by the Semi-Infinite Solids Model (equation derived from the 2nd Law of Fick)[2] and the mechanism of release by the Law of Power, whose results are presented in Table 1.

The diffusivity of the drug from the film cross-linked with 3% $CaCl_2$ film showed diffusivity slightly higher than the 5% film, which may be related to the lower degree of cross-linking and, consequently, lower compactation of the chains, which facilitates the release of the active agent.

Figure 1. Release kinetic of sodium diclofenac release from alginate films cross-linked with 3 or 5% of calcium ions.

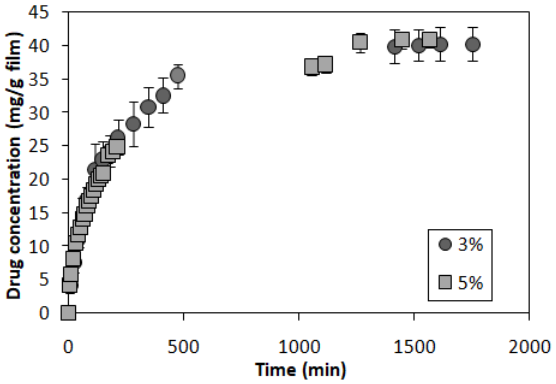

Table 1. Diffusivity (D), diffusional exponent (n) and determination coefficient (R^2) obtained from mathematical models.

$CaCl_2$	D(cm^2/s)	R_D^2	n	R_n^2
3%	1.31×10^{-8}	0.98	0.63	0.98
5%	9.23×10^{-9}	0.99	0.48	0.99

In relation to the mechanism involved in the diffusion process, values of the diffusional exponent (n) of 0.5 indicate that the release mechanism is controlled by diffusion. Values of *n* between 0.5 and 1.0 indicate a anomalous transport, where the swelling of the matrix influences the process.[3] Thus, films with cross-linking in 5% presented a behavior closer to the diffusive when compared to 3%, which had influence of swelling.These results corroborate with the diffusion coefficients.Values of R^2 indicate that models fit well to experimental data.

CONCLUSION

Alginate films with diclofenac sodium cross-linked with 3% $CaCl_2$ presented greater diffusivity coefficient and anomalous release mechanism. The crosslinked sample with 5% $CaCl_2$ showed Fickian behavior, presenting greater potential for drug release in future applications in high performance dressings.

ACKNOWLEDGEMENTS

The authors acknowledge the Conselho Nacional de Desenvolvimento Tecnológico (CNPq) for their support.

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(PO4) Effect of cross-linking on swelling degree and mass loss of alginate membranes from different polymeric structures

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Keywords: Membranes, Cross-linking, Alginate, Viscosity, Absorption

INTRODUCTION

Besides the basic protective function, modern dressings can act actively in the treatment of skin lesions, aiding in the regeneration process and healing by the release of drugs, such as anti-inflammatories at the injury site.

Alginate has potential as a raw material for these dressings because it is biocompatible and biodegradable. It is composed of α-L-guluronic acid residues (G blocks) and β-D-mannuronic acid (M blocks) and distributed in different proportions along the chain.[1]

Cross-linking is very efficient for alginate membranes, since the G blocks of this polysaccharide can react irreversibly with divalent ions, especially with calcium, resulting in benefits to the membrane as mechanical stability and low solubility in water.[1]

The objective of this work was to prepare alginate membranes and to evaluate the effect of the alginate polymer structure (M and G blocks) and cross-linking with calcium ion on the absorption capacity and loss of mass in water.

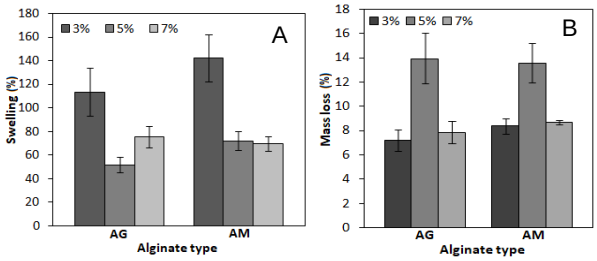
RESULTS AND DISCUSSION

The membranes were prepared by casting method using alginate with high G blocks (61%) and high M blocks (66%), both with high viscosity. Alginate aqueous solutions at 1% (w / v) were oven dried at 60 °C and, after structured, were cross-linking by immersion in 50 mL of aqueous $CaCl_2$ solution at 3, 5 and 7% (w / v) for 15 minutes with subsequent washing in distilled water.

The membranes were submitted to a new drying at room temperature (25ºC). Samples of the pre-weighed membrane with dimensions 3 cm x 1 cm were immersed in water for 24 h at 25 °C. After this period, they were weighed again, and the swelling percentage was obtained. The percentage of mass lost by the samples was determined gravimetrically after 7 days in immersion in water at 25ºC.

The obtained cross-linked membranes were homogeneous and easy to handle. Figure 1 shows the results obtained from swelling and mass loss after 24h and 7 days, respectively.

Figure 2. Swelling in 24h (A) and mass loss in 7 days (B) of the alginate membranes AG (high G blocks) and AM (high M blocks).



The results indicated a reduction of swelling with increasing of reticulant concentration, which is due to the greater compaction of the alginate chains, thus making it difficult for the membrane to absorb water.Non-reticulated membranes were completely disintegrated after a few minutes of contact with the liquid medium. The mass loss was higher for samples with 5% $CaCl_2$, which may be related to a partial and superficial cross-linking. Although cross-linking occurs through the interaction of calcium ions with the G blocks of alginate, no significant differences ($p < 0.05$) were observed between the alginate AG and AM results.

The samples submitted to reticulation with 3% of $CaCl_2$ showed better behavior when aimed at applications as wound dressings. The high swelling (greater than 100%) allows absorption of exudates and the low mass loss indicates that the membrane remains physically stable in media with high humidity.

CONCLUSION

Alginate membranes exhibited swelling above 50% and maximum mass loss of approximately 14%, which indicate good stability in aqueous medium. A tendency to reduce swelling with increasing of reticulant concentration from 3% to 5% was observed. The composition of the alginate did not influence the results of swelling and loss of mass. Cross-linked samples with 3% of $CaCl_2$ presented swelling greater than 100% and low mass loss, with good potential for future applications as biomaterials.

ACKNOWLEDGEMENTS

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico

Universidade Federal de Santa Catarina

ETEX

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(PO5) Establishment of Enzymatic Cocktails for Hydrolysis of Lignocellulosic Biomass

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Keywords: enzyme, cocktail, second generation ethanol

INTRODUCTION

Lignocellulosic biomass is an abundant residue, with a recalcitrant structure that requires a variety of enzymes for its complete degradation. Pools of enzymes with different specificities acting together usually produce an increase in hydrolysis yield. Enzymatic cocktails have been widely studied due to their potential in application for the bioconversion of lignocellulosic biomass by producing biofuels and other products with economic value[1]. The objective of this work is to develop and optimize an enzymatic cocktail that acts efficiently in the degradation of biomass.

RESULTS AND DISCUSSION

For the production of enzymatic cocktails, four fungi and four substrates were selected. *Aspergillus terreus* cultivated in sugarcane bagasse (BCAT) and cane straw (PCAT) and *Trichoderma reesei* cultivated in soybean hulls (CSTR) presented the best performance in different holocellulolitic activities and pH, temperature and thermostability, suitable to enzymatic hydrolysis. Different enzyme loads (0.5, 1, 2, 4 and 8 mg / gram of biomass) were tested and 2 mg of protein / gram of biomass was selected, the load already reported in the literature for sugarcane bagasse hydrolysis[2]. For the preparation of the cocktail, it was used the planning of simplex mixtures.

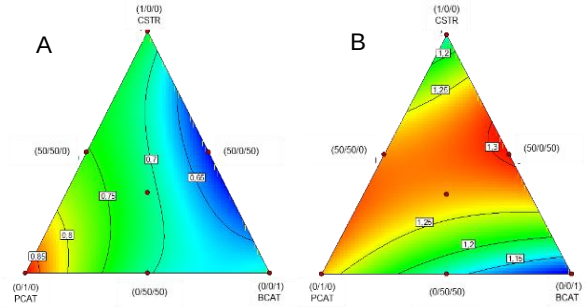

Figure 1. Surface response of simplex blending planning enzymatic hydrolysis of sugarcane bagasse at 48 h of incubation. A - glucose and B - reducing sugars.

In the region with the highest intensity of TRS release (total reducing sugars), new points were scored and the result of hydrolysis can be observed in figure 2. Cocktail 4 (0.45% CSTR, 0.45% BCAT and 0.1% PCAT) presented the best performance.

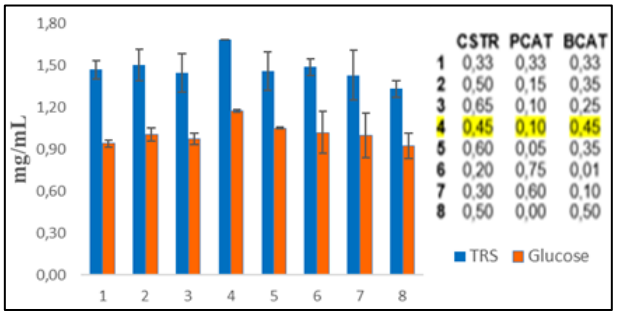

Figure 2. Enzymatic hydrolysis of sugarcane bagasse after 72 h of incubation. Blue - Total reducing sugars and orange - glucose.

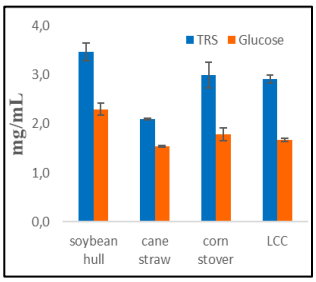

Figure 3. Enzymatic hydrolysis of soybean hull, cane straw, corn stover, lignin-carbohydrate complex after 72 h of incubation using the cocktail 4. Blue - Total reducing sugars and orange – glucose.

The cocktail 4 was efficient when hydrolyzing different biomasses (figure 3), and showed to be thermostable for more than 8 days. This cocktail was tested in the presence of ions and phenolic compounds. Moreover, it was optimized with addition of tween 80 (0.5; 1 and 2%). The best response was when 0.5% of tween 80 was added, which represented a 16% increase in the release of reducing sugars. The next step is to optimize the cocktail with accessory enzymes (expansin and laccase) and compare with commercial cocktails.

CONCLUSION

The elaborated and optimized cocktail 4 is an alternative for the hydrolysis of lignocellulosic biomass.

ACKNOWLEDGEMENTS

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(OP6) Synthesis of nanocellulose ε-caprolactone biocomposites via *in situ* polymerization

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Keywords: Nanocellulose, ε-caprolactone, biodegradable.

INTRODUCTION

There has been a great worldwide interest in the development of "green" technologies that allow the use of products with a lower environmental impact, implying the development of chemical processes and products that lead to a cleaner, healthier and sustainable environment[1].

In this context, it is proposed the use of nanocellulose (NC), which has fibers that can give reinforcing properties to the material and is a very abundant natural polymer.

On the other hand, ε-caprolactone is a cyclic ester of synthetic origin, which ring opening gives rise to poly(ε-caprolactone) (PCL), a biodegradable thermoplastic, soluble in several organic solvents [2].

For the development of this research it was defined to work with the *in situ* polymerization of ε-caprolactone with nanocellulose, using tin octoate ($Sn(Oct)_2$) as the initiator.

RESULTS AND DISCUSSION

NC was prepared from microcrystalline cellulose, by acid hydrolysis followed by washing and centrifugation until pH 7.0 was reached.

X-ray diffraction analysis (XRD) of NC samples presented two peaks: the first one is located between 15 and 17° and the second one is at approximately 32.5°, the latter being the most intense of all. The intensity of the NC peaks is increased in comparison with commercial cellulose, indicating that the crystallinity increases after hydrolysis and suggesting that the amorphous region has decreased considerably during acid hydrolysis [3].

For the polymerization, the tested monomer/ initiator molar ratios (M/I) were 5000 and 3000. Figure 1 shows the infrared spectrum of the PCL-functionalized cellulose and the produced nanocellulose sample. After PCL functionalization with NC, there were small changes in infrared spectra. For a reaction time of 72h, the band of the (-OH) bonds of the cellulose is observed at 3439 cm^{-1}. At approximately 2950 cm^{-1}, the characteristic bands of asymmetric and symmetrical stretches of the methylene group ($-CH_2-$). At 1295 cm^{-1}, the C-O bond stretch and the presence of a band at 1050 cm^{-1} corresponding to the symmetrical and asymmetrical stretching of the C-O-C vibrations are obtained. These bands suggests the functionalization of nanocellulose fibers with PCL.

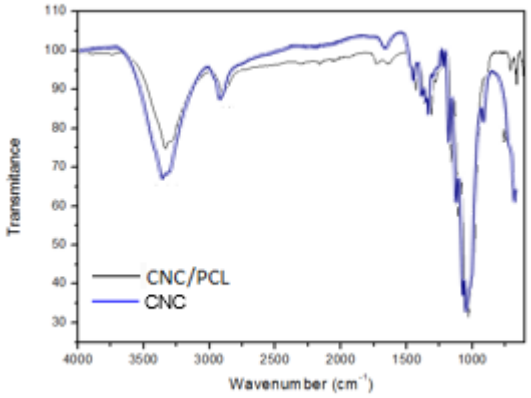

Figure 1. Infrared Spectra of PCL-functionalized cellulose sample for M/I = 3000.

The thermogravimetric analysis for NC functionalized with PCL showed a single stage of degradation of cellulose around 300°C. However, it is observed that for the sample with monomer/initiator ratio of 3000, there was a final mass percentage of about 10%, while for the 5000 molar ratio a mass percentage rate of less than 2% was observed.

CONCLUSION

The results show that a lower monomer/initiator ratio causes a higher efficiency of functionalization (PCL binding in nanocellulose).

ACKNOWLEDGEMENTS

FAPESC, CNPq.

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(PO7) Suberin as an Inducer for Enzymes Production by *Yarrowia lipolytica* IMUFRJ 50682

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Keywords: suberin, cork, solid-state fermentation, enzymes

INTRODUCTION

Suberin is a biopolyester that can be found in some vegetables and in commercial cork[1]. This polymer acts protecting vegetables against pathogens and controlling gas flow, its structure presents mostly aromatic domains, but also aliphatic domains can be found[2–4].

Solid-state fermentation presents many advantages and one of them is the possibility of using residues or by-products as a platform for different metabolites production[5]. *Yarrowia lipolytica* turns to a good choice for solid substrate cultivation due to its dimorphism and for being a well-known enzymes producer[6].

This work aims to evaluate the influence of commercial cork as an inducer for enzymes production by *Yarrowia lipolytica* via solid-state fermentation.

RESULTS AND DISCUSSION

In this study, a wild strain of *Yarrowia lipolytica* (IMUFRJ 50682) isolated from an estuary of Guanabara Bay in Rio de Janeiro was used.
Solid-state fermentation was conducted in 250 mL flasks using soybean bran (d_p < 1.18 mm) and different proportions of commercial cork (0, 5 and 20% w/w) and humidity was adjusted to 55% with an emulsion of water and soybean oil (1.5% w/w).
Lipase activity was determined by titrimetric method and esterase activity was determined spectrophotometrically using p-nitrophenyl butyrate as substrate.
Protein content was determined by Bradford's method.

As it can be seen in table 1, lipase activity reached the highest value (98.61 U/g) when 5% (w/w) of commercial cork was added to the culture media, while esterase activity was higher when 20% (w/w) of commercial cork was added, reaching 5.37 U/g. Protein contents obtained with addition of commercial cork in the culture media were higher than control, when 5% (w/w) was added, this value was almost 2-fold higher.

Table 1. Enzymatic activities obtained by the addition of commercial cork in the culture media.

	Control	5% (w/w)	20% (w/w)
Lipase Activity (U/g)	88.58 ± 3.54	98.61 ± 1.49	58.89 ± 2.91
Esterase Activity (U/g)	2.33 ± 0.01	3.75 ± 1.18	5.37 ± 0.74
Protein Content (g/L)	0.37 ± 0.03	0.73 ± 0.06	0.63 ± 0.03

CONCLUSION

Commercial cork showed high potential to induce lipases and esterases production by *Yarrowia lipolytica* in a solid-state cultivation.

ACKNOWLEDGEMENTS

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(PO8) Green Biphasic System for Enzymatic Polymerization of 11-Aminoundecanoic Acid

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Keywords: lipase, polyamide-11, biphasic system, eutectic solvent

INTRODUCTION

Polyamide 11, known as Nylon-11, is a thermoplastic and aliphatic polymer that presents amide group in its structure chain, with great characteristics as good mechanical properties, thermal stability, low humidity absorption and high solvent resistance, being applied in many industrial sectors as automotive, packaging, electrical, oil and gas, between others. The main production route of this material is the polycondensation reaction of 11-aminoundecanoic acid at high temperatures, which can lead to thermal degradation, cyclization and branching, compromising the product quality. An alternative is the use of enzymes for polymerization in mild reaction conditions and more selectivity. Thus, this work proposal is to produce polyamide 11 by enzymatic polymerization using lipase (Novozym 435) in biphasic system constituted of water and a new eutectic solvent, carvacrol/decanoic acid (4/1 molar ratio). Parameters as monomer quantity, enzyme quantity, temperature, eutectic/water ratio, were evaluated using a central composite design.

RESULTS AND DISCUSSION

After reaction, assay was centrifuged at 6000 rpm for 5 min, material precipitated was separated and washed with water and ethanol, and then lyophilized. Yield obtained was calculated based on product mass and initial monomer ratio.

Table 1. Experimental Design of Enzymatic Polymerization of 11-Aminoundecanoic Acid.

	Monomer (mg)	N435 (mg)	Eutectic/ Water m/m	T (ºC)	Yield (%)
1	100	250	1.0	60	15.8
2	100	250	2.0	60	32.1
3	200	250	1.0	60	8.50
4	200	250	2.0	60	15.1
5	100	750	1.0	60	12.4
6	100	750	2.0	60	47.7
7	200	750	1.0	60	8.30
8	200	750	2.0	60	33.0
9	100	250	1.0	80	23.0
10	100	250	2.0	80	41.8
11	200	250	1.0	80	7.30
12	200	250	2.0	80	5.70
13	100	750	1.0	80	5.40
14	100	750	2.0	80	75.2
15	200	750	1.0	80	5.40
16	200	750	2.0	80	32.7
17	150	500	1.5	50	1.40
18	150	500	1.5	90	56.0
19	150	0	1.5	70	0
20	150	1000	1.5	70	39.3
21	50	500	1.5	70	39.6
22	250	500	1.5	70	30.5
23	150	500	0.5	70	21.0
24	150	500	2.5	70	42.1
25	150	500	1.5	70	25.1
26	150	500	1.5	70	37.9
27	150	500	1.5	70	45.1

CONCLUSION

Increase Eutectic/ water ratio and temperature favoured higher yields. Other analysis are been realized as NMR, FTIR and DSC to confirm structure produced and determined molar mass.

ACKNOWLEDGEMENTS

To Novozymes by enzymes donation

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(PO9) Laccase Production of White Rot Fungus Grown on SBS Paperboard Coated with PET, Aiming the Bioadsortion Strategy

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Keywords: Ganoderma lucidum. Lignocellulosic waste. Laccase.

INTRODUCTION

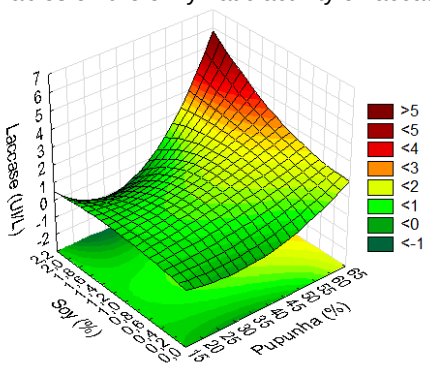
SBS paperboard coated with PET is an example of industrial waste composed of cellulose, hemicellulose and polyethylene terephthalate (PET), which has a chemical structure similar to lignin. Fungi that cause white rot are microorganisms that have the ability to secrete oxidative enzymes, such as laccase, required for the degradation of lignocellulosic materials. In this context, the objective was to evaluate the potentiality of the fungus *Ganoderma lucidum* for producing of laccase, when using SBS paperboard coated with PET as substrate.

RESULTS AND DISCUSSION

A 3² factorial design consisting of nine treatments was applied, varying the source of carbon (pupunha sheath) and nitrogen (soybean meal). Fungal cultures were inoculated with 10 g of paperboard shavings, supplemented and incubated in an oven at 28 ± 1 °C for 30 days. Subsequently, the enzymatic activity of laccase was determined in accordance with a methodology described by Hou et al.[1]

The results obtained (Figure 1) show that the maximum laccase activity was achieved from treatments with higher amounts of carbon and nitrogen. It is evident, by response surface, that the enzymatic production is more sensitive to changes in pupunha concentration than soy, which did not have a significant statistical influence (P=0.2647).

Figure 1. Response surface of the pupunha and soy variables on the enzymatic activity of laccase.



Laccase production was significantly positive in assay 9: 3.68 ± 0.098 UI/L (Table 1). Regina et al.[2], for example, obtained only 0.45 UI/L with the fungus *Lentinula edodes*, in 8 days of cultivation and using eucalyptus sawdust as a lignocellulosic waste. However, better results (13.80 UI/L) were found by Menezes[3], in studies with the same *G. lucidum*, 14 days of incubation and bract matter as substrate.

Table 1. Production of laccase by the fungus *G. lucidum*.

	PUPUNHA SHEATH (%)	SOYBEAN MEAL (%)	LACCASE (UI/L)
1	20	0.1	0.00 ± 0.000
2	40	0.1	0.00 ± 0.000
3	60	0.1	1.35 ± 0.208
4	20	1.0	0.79 ± 0.196
5	40	1.0	1.43 ± 0.196
6	60	1.0	2.46 ± 0.307
7	20	1.9	0.00 ± 0.000
8	40	1.9	0.00 ± 0.000
9	60	1.9	3.68 ± 0.098

CONCLUSIONS

The SBS paperboard coated with PET proved to be a good support for the colonization of the fungus *G. lucidum*. This produced considerable laccases when in the presence of carbon and nitrogen sources in quantities essential for their metabolism.

Aiming at a future application in biotechnology, they represent a promising and sustainable proposal for bioadsorption and bleaching of dyes, for intermittent use in the treatment of textile effluents.

ACKNOWLEDGEMENTS

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(PO10) Crude glycerin and vinasse as feedstock for PHA production by engineered *Cupriavidus necator*

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Keywords: PHB, vinasse, glycerol

INTRODUCTION

Poly-hydroxyalkanoates (PHA), highlighting the poly-hydroxybutyrate (PHB), are biocompatible biopolymers produced intracellularly by several microorganisms. Aiming to reduce its production costs, industrial byproducts have been studied as substrate for PHB production. The bacteria *C. necator* is one of the most widely known PHB producers. Vinasse is the main byproduct from ethanol industry and was already evaluated as a carbon source by *C. necator*[1], as well as the crude glycerin, byproduct from biodiesel[2]. The engineered *C. necator*_glpFK strain was constructed aiming to enhance the glycerol utilization[3]. The objective of this work was to investigate the PHB production by *C. necator*_glpFK with vinasse and crude glycerin as substrates.

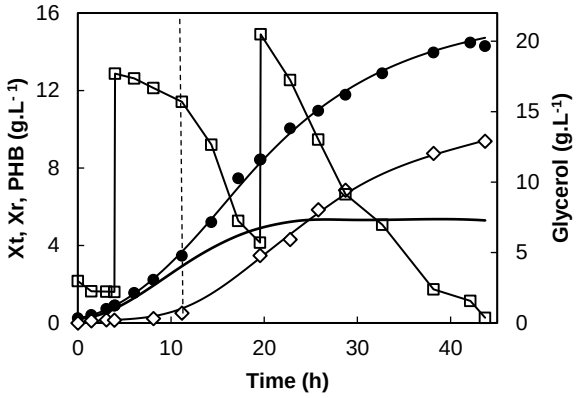
RESULTS AND DISCUSSION

The culture was performed in a 4 L working volume bioreactor equipped with pH, dissolved oxygen and temperature controllers. The cultivation was performed by using two seed cultures. The culture started with vinasse plus mineral medium[4] salts. Crude glycerin (40 $g.L^{-1}$) was added to the system in two pulses, about at 4 and 20 h of cultivation. The biomass concentration was determined by gravimetric analysis. The glycerol concentration was determined using the kit *Triglicérides Liquiform*. The PHB was determined by gas chromatography, according to methanolysis[5]. Residual biomass (Xr) is non-PHB part of total biomass.

The maximum specific growth rate (μ_{Xrmax}) was 0.29 h^{-1}, close to the values reported in the literature for *C. necator* DSM 545 growing on glucose and fructose (0.26 h^{-1})[6] and higher than reported values for glycerol (0.11 h^{-1})[7]. The PHB production started after the nitrogen limitation and it is indicated by the dotted line on Fig 1. The final polymer content was 68% and the productivity was 0.22 $g.L^{-1}.h^{-1}$.The association of substrates is an alternative for the PHB production, because it can foment the cell growth and the biopolymer accumulation. In this work, two low-cost byproducts were used to produce PHB with satisfactory results.

The results of Xt, PHB and Xr production, and the profile of glycerol concentration are presented in Figure 1. A polynomial fit was performed to the experimental data using the software Microsoft Office Excel.

Figure 1. Time courses of total biomass (●), PHB production (◊), residual biomass (-) and glycerol concentration (□), during fed-batch culture of *C. necator*_glpFK.



CONCLUSION

It was possible to produce PHB using vinasse and crude glycerin as substrates by *C. necator*_glpFK. In this study, the accumulation of PHB was 68% with a productivity of 0.22 $g.L^{-1}.h^{-1}$.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support from CNPq and from CAPES, also the donations by Biocapital SA and Usina Iracema.

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(PO11) Characterization of poly-hydroxybutyrate from engineered *Cupriavidus necator* grown on glycerol and glucose

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Keywords: biopolymer, propylene carbonate, PHA.

INTRODUCTION

There is a public and scientific interest towards development and use of biodegradable polymers as an alternative to conventional plastics. Poly-hydroxybutyrate (PHB) is an intracellular biodegradable biopolymer accumulated by several microorganisms. *Cupriavidus necator* is one of the most popular strains for the PHB accumulation. The biopolymer must be recovered from the cells, which may have a different composition depending on the carbon source and the culture conditions employed[1]. Depending on its application, the biopolymer requires different thermo-physical properties. The cost of media contributes most significantly to the overall production cost of PHB. Inexpensive substrates such as glycerol, from biodiesel production, draw the consideration of various researches. In this context, the most efficient use of glycerol by engineered strains is feasible[2]. This work studied the recovery and characterization of PHB by *C. necator*_glpFK[3], using the reused propylene carbonate as solvent.

RESULTS AND DISCUSSION

The biomass of *C. necator*_glpFK with intracellular PHB (65 wt.%) used in this work was obtained in a cultivation with glycerol (10 $g.L^{-1}$) and glucose (20 $g.L^{-1}$) as carbon sources. After the cultivation, the biomass was subjected to heat treatment (150 °C/45 min), centrifugation, drying and grinding, before the extraction. The solvent used was a reused propylene carbonate, with the methodology described by Quines[4]. The PHB was determined by gas chromatography, according to the methanolysis[5].

The biopolymer was characterized by the following analyzes: Fourier transform infrared spectroscopy (FTIR), molar mass, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The studied conditions did not influence the purity and recovery of the biopolymer obtained. The recovery of 73% and purity of 95% were reached.

The biopolymer extracted in this work was confirmed as PHB[6] by characterizing the functional groups of the same by the FTIR analysis.

The FTIR spectra of the PHB indicated absorption bands at 1226 – 1277 cm^{-1} and 2934 – 2975 cm^{-1} corresponding to C-O-C and CH, and at 1381 and 1723 cm^{-1} corresponding to CH_3 and C=O, respectively.

After the analysis of TGA curves, the degradation profile of PHB shows one degradation stage of mass loss. The temperature of onset degradation was 266.4 °C, with a mass loss of 95%.

Table 1. Properties of PHB

Polymer	M_w (Da)	T_m (°C)	T_g (°C)	X_c (%)	Ref.
PHB	5.9 x 10^4	169	-0.8	59.3	This work
PHB	8.8 x 10^4	169.7	-0.8	68.1	4
PHB	5.5 x 10^5	176.4	3.2	65	7

The results of degree of crystallinity (Xc) and melting point (T_m) obtained were compared with the literature by authors who also produced PHB using glycerol as substrate and with values for commercial PHB.

A high recovery of PHB from *C. necator*_glpFK with high purity and physical properties like those reported in the literature for PHB was obtained, with a decrease in the molar mass. Previous studies have also reported that several hydroxyl compounds, including glycerol, decreased molecular weight of PHAs, possibly due to the chain transfer action of glycerol during PHA synthase mediated polymerization[8].

CONCLUSION

It was confirmed that propylene carbonate, a low toxicity solvent, can be used in the extraction process proposed in this work. The biopolymer extracted was confirmed as poly-hydroxyalkanoate. Under the studied conditions, the temperature of onset degradation was 266.4 °C, the degree of crystallinity was 59.3% and the melting point was 169 °C.

ACKNOWLEDGEMENTS

Author thanks to CNPq and CAPES for the fellowship.

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(PO12) Enzymatic esterification using Novozym® 435 to obtain a diene with posterior application in polymerization

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Keywords: green chemistry, biosynthesis, enzyme, 10-undecenoic acid, miniemulsion

INTRODUCTION

The application of enzymes in chemical reactions has shown many advantages as substrate selectivity, mild temperatures, versatility and reduction of hazardous compounds. Among the available enzymes, *Candida antarctica* lipase B, free or immobilized (as Novozym® 435), is remarkable to present a wide list of applications, including esterification, transesterification, aminolysis, aza-Michael reaction and aldol condensation.[1,2]

As renewable resources, plaint oils and its derivatives have been applied as building blocks in the production of green polymers in replacement of petroleum-based derivatives, reducing the impact caused to the environment. Castor oil, a plant oil constituted by about 90% of ricinoleic acid that presents a *cis* double bond (between carbons 9 and 10) and a hydroxyl group on the 12 carbon atom have been highlighted in the last few years as one of the few oils with exclusive and potential use in chemical industry. Pyrolysis reaction of this particular ricinoleate molecule forms 10-undecenoic acid, an interesting building block to obtain monomers from esterification procedures.[3,4,5,6]

The objective of this research is to develop a green process to synthesize a renewable-based monomer from enzymatic esterification between 10-undecenoic acid and 2-hydroxyethyl methacrylate (HEMA) using Novozym® 435 as biocatalyst and posterior thiol-ene polymerization of resulted diene.

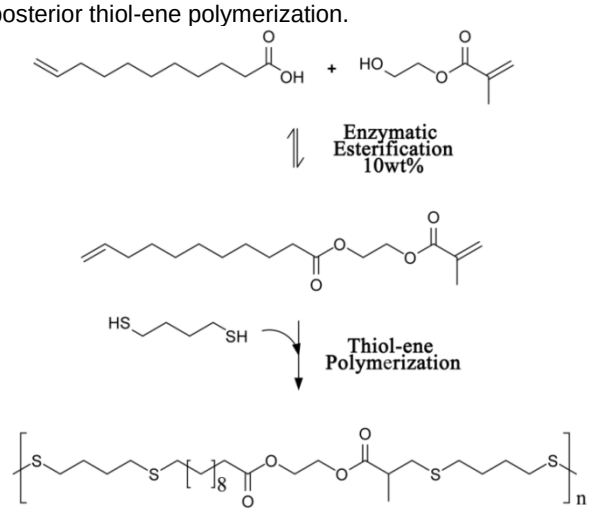
RESULTS AND DISCUSSION

In order to produce monomer from 10-undecenoic acid and HEMA, 10wt% of Novozym® 435 (related to subtrate) was used in a reaction during 72 hours at 50 °C, solubilized in chloroform. which allowed an conversion of 50%. Purity of about 80% was reached, calculated through ^{1}H NMR additional analysis.

Bulk and miniemulsion thiol-ene polymerization reactions were performed with this diene monomer and 1,4-butanedithiol as thiol agent, through initiation by AIBN (1 mol%) at 80 °C.

In terms of weight average molar weight (Mw), bulk system reached (35.1±0.1) kDa, while miniemulsion system, which resulted in particle diameter of (131.6±0.4) nm, presented a gel content of 63%. Tested techniques showed a conversion higher than 95%.

Figure 1. Scheme of enzymatic esterification and posterior thiol-ene polymerization.



CONCLUSION

The use of enzyme as biocatalyst is an important and sustainable tool to reduce by-products during monomer production. Although enzymatic esterification is not able to convert all combination of fatty acids and alcohols in esters, this procedure was useful to convert 10-undenoic acid and HEMA in a diene monomer. Furthermore, product was capable to polymerize, even considering its double bond asymmetry.

ACKNOWLEDGEMENTS

The authors thank the financial support of CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico.

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(PO13) Production of mannanase isoforms by *Clonostachys byssicola* cultivated in soybean hulls

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Keywords: mannanase, Clonostachys byssicola, soybean hulls

INTRODUCTION

Microbial mannanases are enzymes responsible for decomposing the mannan chains present in the hemicellulosic fraction of the plant cell wall and they have several industrial applications, mainly in processes that employ vegetal biomass as feedstock[1]. Mannanases are mainly produced by filamentous fungi, including different species of *Aspergillus* and *Bacillus*. However, there is a need to explore the diversity of fungi for the identification of new strains and consequently the production of mannanases[2], therefore the goal of this research was to produce and characterize mannanases from *Clonostachys byssicola*.

RESULTS AND DISCUSSION

C. byssicola was cultivated in submerged medium containing soybean hulls as carbon source during 7 days. After cultivation the crude concentrate extract (EBC) displayed mannanase activity of 4.0 IU/mL. Electrophoresis and zymogram studies showed that *C. byssicola* produces isoforms of mannanases (Fig. 1). These isoforms apparently cooperate to enhance hydrolysis of complex lignocelluloses[3].

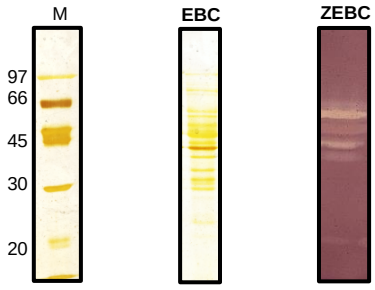

Figure 1. SDS-PAG analyses of isoforms of mannanase. Lane M: molecular marker; Lane EBC: crude extract; Lane ZEBC: zymogram of crude extract showing isoforms of mannanase.

Mannanases from the EBC exhibited maximum activity in acid pH with a relatively high temperature (table 1), and showed half-live of 192 h at 40°C. Moreover, the Co^{2+} ion increased the EBC (15,5%) mananase activity when incubated at a final concentration of 10 mM (fig.2). In addition, HPLC analysis indicated that *C. byssicola* performed an efficient hydrolysis of the soybean hulls, given that large amounts of mannose and mannobiose were released (fig. 3).

Table 1. Optimum pH, temperature and thermostability of mannanases.

pH	5,0
Temperature ºC	55
Thermostability	192 h a 40ºC

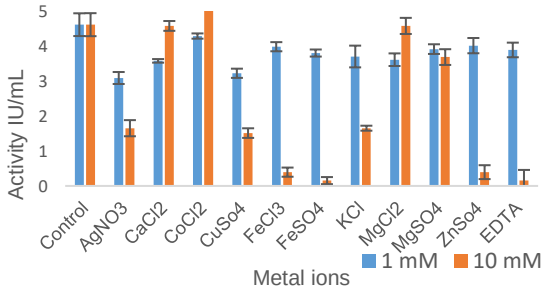

Figure 2. Effect of metal ions at 1mM and 10 mM on mannanase activity from EBC.

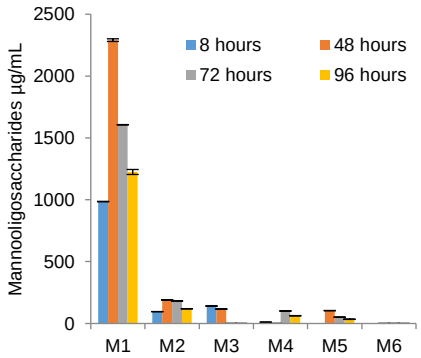

Figure 3. Identification of main saccharides released from 1% soybean hulls following hydrolysis by EBC.

CONCLUSION

Little is known about the ability of *C. bysssicola* to produce hydrolytic enzyme. Since this species have been intensively investigated as biocontrol agent. The characterization of EBC showed that the mannanases secreted by *C. byssicola* are suitable for various industrial applications. However, the mannanase isoforms will be purified for a better understanding of their physicochemical properties.

ACKNOWLEDGEMENTS

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(PO14) Economic Assessment of Poly(hydroxybutyrate) production

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Keywords: Poly(hydroxybutyrate); Cupriavidus necator; Economic assessment; Production cost

INTRODUCTION

Poly(hydroxybutyrate) (PHB) is a biopolymer synthesized by bacteria and intracellularly accumulated as energy reserve material. This polyester is biocompatible and biodegradable and has mechanical properties comparable to polypropylene[1]. However, its higher production cost, when compared to petroleum-based plastics, make these biopolymers commercially unattractive. Thus, in order to reduce PHB production cost, it was observed the development of strategies of accumulation and polymer extraction as well as the use of low-cost substrates[2]. Therefore, the aim of this study is to conduct an economic assessment of the entire PHB industrial production process by *Cupriavidus necator* DSM 545, using propylene carbonate as solvent coupled with mechanical methods of extraction and using citric molasses as carbon source.

RESULTS AND DISCUSSION

The data used in both culture phase and biopolymer extraction and purification are based on laboratory-scale experiments. The cultivation method was the cyclic fed-batch with cell recycle, obtaining a cell concentration of 62 $g.L^{-1}$ with 69% of intracellular PHB[3]. Four different biopolymer extraction routes with different final extraction capacity were economically evaluated: (i) heat treatment followed by solvent extraction (92.2%); (ii) ultrasonication followed by solvent extraction (92.1%); (iii) high pressure (homogenizer) followed by solvent extraction (97.8%); and the fourth, (iv) the use of solvent alone (81.7%)[4]. The base case scenario considers the design of a 2,000 tonnes per year facility's production capacity. Once citric molasses is used as carbon source it is imagined to be annexed to an orange juice processing facility.

The mathematical description of each unit operation that comprises the production and extraction of biopolymer for all the four alternatives was carried out in order to quantify the raw materials and utilities usage and to size the necessary equipment, allowing the estimation on the capital investment, operational costs and revenue surveys[5]. The costs were indexed to February 2018 value using the IGP-M index and exchange rate of R$3.24/US$. Some of the assumptions adopted were: production length of 20 years; running 24 hours per day, 330 days per year; 34% tax rate. The selling price was fixed in US$10,93/kg. Examining the prospects of financial performance over the lifetime of the project it is possible to determine economic indicators setting the best production route from an economic point of view, as shown in Table 1.

Table 1. Economic criteria results for a 2,000 mt/yr plant.

	(i)*	(ii)*	(iii)*	(iv)*
Total capital inv. (MUS$)	40.42	43.57	40.87	43.50
Net Present Value (MUS$)	6.89	1.90	6.61	0.00
Internal Return Rate (%)	14.7%	12.7%	14.6%	12.0%
Production cost (US$/kg)	4.95	5.19	4.94	5.40

*Extraction scenarios: (i) high temperature; (ii) ultrasonication; (iii) high pressure; (iv) only solvent.

The alternative that uses high pressure had the lowest production cost (US$ 4.94/kg). It is noteworthy the production costs similarity between this route and the high temperature alternative (US$ 4.95/kg), even though they have considerably different extraction capacity (97.8% and the latter 92.2%). This is mainly due to the homogenizer's large electric energy consumption. The high cost of the ultrasonication device drove to a high production cost in the scenario (ii). As expected, the route that uses only solvent consumes more resources and has the highest production cost (US$ 5.40/kg) since it presents the lowest extraction yield (81.7%), requiring higher quantities of fermented broth and larger equipment to produce the same amount of biopolymer than the other alternatives. Alternatives (i) and (iii) had similar economic indicators, though the choice of the best process should be given to the high temperature scenario, once its generates more wealth (higher Net Present Value) and it is not so sensitive to electricity price fluctuation.

CONCLUSION

In the economic evaluation of PHB production, the definition of the best production route, not only biomass treatment expenditures and extraction capacity are important, but also market conditions.

ACKNOWLEDGEMENTS

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(PO15) Preparation and Characterization of Nanocomposites with Zein and Quantum Dots Of ZnO

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Keywords: Quantum Dots, Zein, ZnO.

INTRODUCTION

Quantum Dots (QDs) are nanoparticles of semiconductor materials ranging in size from 2 to 10 nm, with distinct properties, such as high absorption, photostability, wide excitation spectrum, narrow emission bands and low photodegradation tendency. In particular, the zinc oxide (ZnO) QDs have received special attention from the scientific community, because it is environmentally friendly, due to its optical, electronic and magnetic properties. In this way, the present study aims to develop methodologies and techniques for synthesis of ZnO nanoparticles further prepare nanocomposites with zein biopolymer.

RESULTS AND DISCUSSION

Figure I shows schematically the way of ZnO QDs preparation and the application in zein films.

Figura 3 Synthes's of ZnO QDs by the sol-gel method and the preparation of films with zein.

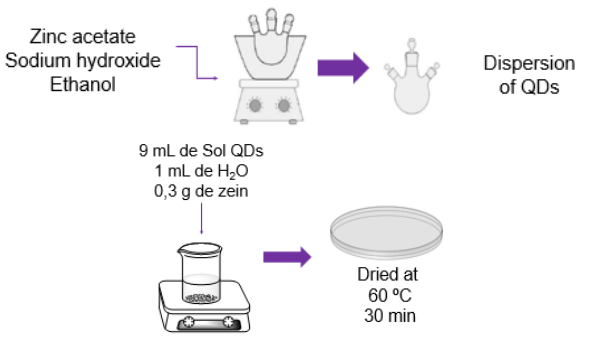


Figura 4 Zein films containing QDs (a) under white light (b) under UV lamp excited at 330 nm

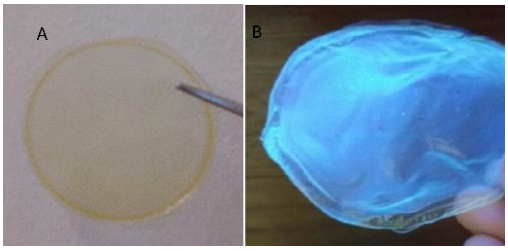
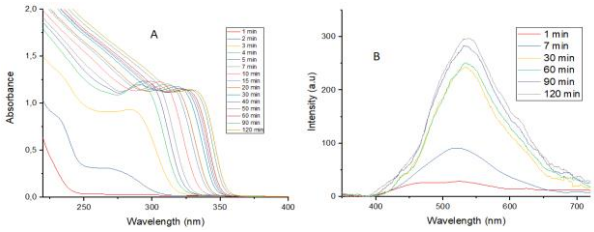


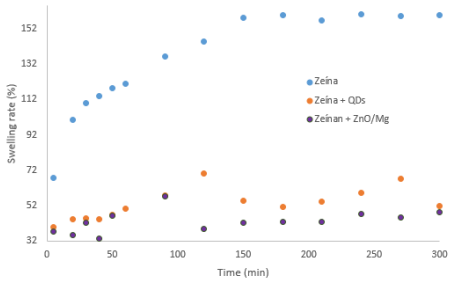
Figure 3 shows the growth of the QDs for 120 min through UV-vis and fluorescence analyzes. In the absorption spectrum of zein, it can be seen that there is an overlap in the absorption region with QDs. From this comes out the possibility of obtaining a nanocomposite with excellent properties UV blocking properties.

Figura 5 Spectra of the colloidal dispersion of ZnO (A) QDs of UV-vis (B) of fluorescence emission, with excitation at 330 nm.



Swelling properties: Zein films containing ZnO QDs were previously dried and then kept in water bath in order to determine the total water gain over the time, as shown in Figure 4.

Figura 6 Swelling rate vs. time



CONCLUSION

The nanocomposite of ZnO QDs with the zein biopolymer is promising, producing films with good textural aspect to be used in several applications that require UV blocker, antimicrobial activities, such as in food packaging and sensing for several analytes, considering the fluorescence optical response of both ZnO and zein.

ACKNOWLEDGEMENTS

Example: FURB, UFPR, FAPESC, Pipe art. 170.

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(PO16) Enzymatic hydrolysis of lignocellulosic biomass assisted by ultrasound irradiation

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Keywords: Ultrasound; Enzymes; Biomass; lignocellulosic;

INTRODUCTION

One of the major challenges for society in 21st century is to find a sustainable eco-friendly renewable liquid fuel for replacing fossil fuels. Bioethanol is one of the most consumable biofuels in the world. Lignocellulosic plant biomass can be an unlimited source of fermentable sugars for significant production of bioethanol. Pre-treatment and enzymatic hydrolysis are amongst the principal bottlenecks for the implementation of this technology[1]. Ultrasound has shown to be an efficient green technology to enhance chemical and enzymatic reactions[2]. Therefore, the aim of this work was to study the effect of ultrasonic irradiation on the enzymatic hydrolysis of lignocellulosic materials to increase the yield of fermentable sugars.

RESULTS AND DISCUSSION

The enzymatic hydrolysis of sulphuric acid pretreated sugar cane bagasse (SCB) was performed in the absence (100 rpm) and presence of ultrasonic irradiation at frequencies of 25 and 37 kHz with pulsation of 100% with an enzymatic concentration of 10, 15 and 20 FPU.g^{-1} of the *T. reesei* based enzymes *Cellic ctec2* (Novozymes) and *T. crude* (Rhoem Enzymes).

Figure 1. Formation of reducing sugars due to hydrolysis of SCB with the enzymes *Cellic ctec2* and *T. crude* without ultrasound irradiation.

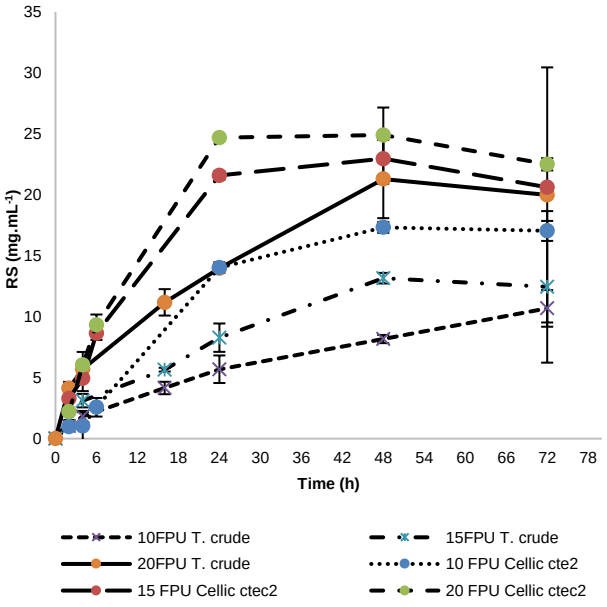
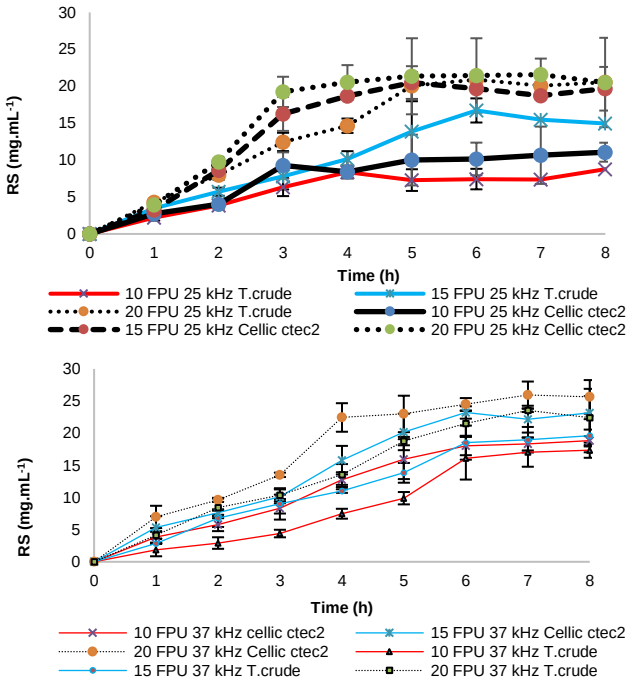


Figure 2 and 3. Formation of reducing sugars from the enzymatic hydrolysis of SCB with the enzymes *Cellic ctec2* and *T. crude* with ultrasound irradiation.



CONCLUSION

The commercial enzyme *Cellic ctec2* has an approximately 5-fold higher activity than the *T. crude* enzyme, however, during ultrasound assisted hydrolysis both enzymes produced similar sugar yields The application of ultrasound at both investigated frequencies (25 and 37 kHz) during enzymatic hydrolysis of pretreated SCB has shown to be an efficient tool to increase the production of fermentable sugars from plant biomass.

ACKNOWLEDGEMENTS

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(PO17) Keratin Scaffold made by Animal Fiber Protein

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Keywords: Keratin, Scaffold, Biomaterial

INTRODUCTION

In recent years, fabrication of the scaffolds for cell culture has been investigated utilizing synthetic polymers and tissue-derived biomaterials. Synthetic polymers like poly lactic acid and poly glycolic acid have an advantage to be able to control its structure and mechanical properties. Unfortunately, synthetic polymers have low affinity to cells. On the other hand, tissue-derived biomaterial such as collagen has high affinity to cells and is widely applied in medical. However, chemical crosslinking with aldehyde is generally performed to collagen for structure stabilization. In addition, collagen derived from an animal has the risk of BSE infection. In order to resolve these problems, we focused on the keratin protein as a scaffold material. Keratin is the major structural fibrous protein contained in hair and nails [1]. Moreover, the presence of cell adhesion sequences such as RGD and LDV contained in keratin protein may accelerate cell adhesion and growth on the keratin substrates [2]. In this study, we evaluated the possibility of keratin scaffolds for cell culture.

RESULTS AND DISCUSSION

Keratin was effectively extracted from wool fiber and human hair without causing reduction of its molecular weight. Molecular mass analysis showed that extracts had average mass in the range of 40-60 kDa and 10-30 kDa which corresponded to α and γ keratose, respectively. Biodegradability of keratin was evaluated by enzymatic hydrolysis with proteases. In the homogeneous system, extracted keratin was completely hydrolyzed by used enzymes within a short time. Then, two dimensional films were prepared and evaluated its physical properties. Keratin films prepared by cast method had smooth surface and showed moderate hydrophilicity. Moreover, it was also possible to enhance strength without causing major hardening by cross-linking. The surface of nanofiber sheets prepared by electrospinning method also showed hydrophilicity. Average diameter of nanofibers could be freely controlled. Following to the evaluation of two dimensional films and nanofiber sheets, three dimensional porous sponges were prepared. Keratin sponges were found to have a cylindrical structure. Further, pore size of sponges depended on the preliminary freezing temperature. As the freezing temperature was lower, the pore of the sponge was small.

Cell culture on keratin films resulted in proliferation in cells with the lapse of days. In addition, the proliferation behaviour was almost the same as those observed on collagen. Three dimensional cell culture on the keratin sponge showed similar results. Cell adhesion was also observed over the whole sponges. When cells were seeded and cultured by using the perfusion bioreactor, cell adhesion and proliferation was remarkable. Figure 1 shows cross section of keratin sponges after cultivation under perfusion bioreactor condition. After cultivation for 3days, cells were infiltrated well in the interior of the sponge.

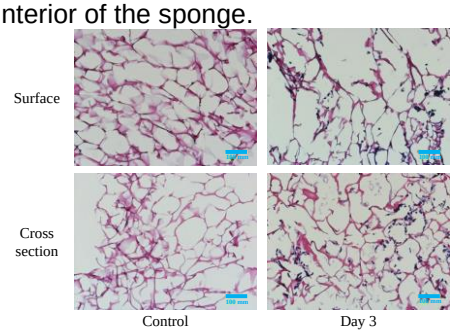

Figure 1 Cross section of the keratose sponges after cultivation in the perfusion bioreactor. (HE staining)

When the keratin sponges were subcutaneously implanted in rats, cellular infiltration was observed without causing remarkable inflammatory. Further, vascularization was also observed within the sponge.

CONCLUSION

Fabrication of the scaffold for cell proliferation was investigated using keratin obtained from wool and human hair. Keratin was found to have good biocompatibility. In addition, cells on the keartin materials were proliferated well.

Keratin is a polymer of biological origin and possible to obtain from human hair. Therefore, if application of human hair becomes realistic, a property to be derived from homogenous origin in addition the non-vascular origin is a quite excellent advantage. It can be concluded that Keratin has a potential to be recognized as a material comparable to collagen.

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(PO18) Can ionic liquids enhance textile dyeing? Assessing color fixation differences in disperse dyeing when adding ionic liquids in small concentrations

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Keywords: ionic liquids, textile dyeing, textile auxiliaries

INTRODUCTION

The textile industry relies on several processes that can be environmentally harsh – not only for the amount of water consumed, but for the chemically contaminated wastewater generated. Ionic liquids (ILs), organic salts with interesting properties such as negligible vapor pressure and high solvatation power and thermostability have been applied to different textile processes[1-3], but their efficiency in dyeing is not yet fully explained. This work means to assess the effect of different types of ILs on disperse dye fixation for natural (wool) and synthetic fibers (nylon and polyester).

RESULTS AND DISCUSSION

One gram fabric samples were dyed at a 1:50 liquor ratio with 0.5 % m/m of the dye C.I. Disperse Red 13 (Sigma-Aldrich) and the following ILs at a concentration of 2.0 $g.L^{-1}$ in the dyebath: choline chloride, 1-ethyl-3-methylimidazolium chloride (EMIM Cl), 1-butyl-3-methylimidazolium chloride (BMIM Cl), 2-hydroxyethylammonium acetate (2HEAA), 1-allyl-2,3-dimethylimidazolium chloride (ADMIM Cl) and 1-hydroxyethyl-3-methylimidazolium chloride (HEMIM Cl). A group of samples was also dyed without ILs to establish comparison to standard procedures. Acetic acid at 4.0 $g.L^{-1}$ was also added to the dyebaths.

For polyester samples, the dyeing processes were carried out at 95 and 130 ºC, both during 60 minutes. Nylon and wool fabrics were dyed at 95 ºC for 60 minutes. All heating steps occurred at a 2.5 $ºC.min^{-1}$ gradient. The samples were then washed using 4 $g.L^{-1}$ standard soap with 1:40 liquor ratio at 60 ºC for 30 min.

The fixation factor (color yield after washing versus color yield before washing ratio) assesses the percentage of dye remaining on the textile substrate after the washing process[5]. The values for wool, nylon and polyester samples are shown in Table 1. On wool and nylon samples, it is safe to assume that the addition of ILs had little to none significant impact on color fixation when compared to the ones without IL. Exceptionally, 2HEAA and HEMIM Cl decreased the dyeing performance for wool by 20.3 and 39.5%, respectively, which might be due to their alkalinizing tendencies[4]. These results contradict those obtained by Bianchini et. al[1]. For the polyester fabric, the best fixation results were found for the dyeing processes at 130 ºC for all ILs tested.

Table 1. Fixation factor (% F) for wool (WO), nylon (PA) and polyester (PES) samples

	Fixation (%)			
Ionic Liquid	**WO**	**PA**	**PES 95 ºC**	**PES 130 ºC**
Choline Cl	> 99.9 *	68.18	88.44	99.37
EMIM Cl	91.82	69.57	86.97	96.28
BMIM Cl	> 99.9 *	70.05	89.71	98.25
2HEAA	70.79	66.12	92.74	96.05
ADMIM Cl	87.93	66.68	88.03	98.28
HEMIM Cl	51.54	69.73	85.31	94.69
Without IL	91.05	70.27	90.46	96.36

*Calculated value was greater than 100%, which indicates irregular color distribution through the sample

However, the difference between those and the control samples at both temperatures was minimal. Since PES dyeing does not rely on chemical interactions between dye and fiber[5], it is not surprising that it was the least affected by the addition of ILs as dyebath auxiliaries.

CONCLUSION

When ILs were used as auxiliaries in the dyeing processes of wool, nylon and polyester, most of the color fixation of the samples was equivalent to those without IL. Further studies are required to determine the nature of interaction between wool keratin and alkalinizing ILs such as 2HEAA and HEMIM Cl.

ACKNOWLEDGEMENTS

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(PO19) Development of Biofunctional Bacterial Cellulose Membrane

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Keywords: bacterial cellulose, controlled release, chlorhexidine

INTRODUCTION

There are several applications of biomedical membrane and most of than needs reasorbable materials as in guided bone regeneration (GBR).
Bacterial cellulose (BC) is a promising natural polymer for such application due to its highly hydrophilic nature that results in a very high liquid loading capacity[1]. However, BC is not enzymatically degraded *in vivo* and this is essentially a limiting factor for its biomedical application. Thus, in order to increase BC bioabsortion, the aim of the present study was modify BC membranes by a regioselective oxidation reaction with sodium metaperiodate and immobilize chlorhexidine digluconate (CHX) in BC to get a modulated controlled release system with antibacterial capacity.
BC membranes were oxidized during 2h (DABC 1) and 6h (DABC2) in sodium metaperiodate (3,0 mol.L^{-1}) solution. CHX were immobilized though membrane immersion in aqueous solutions. The membrane were characterized morphologically by scanning electron microscopy (SEM) examination, bioabsortion at phosphate buffered saline (PBS) solution and CHX release by UV-vis assay.

RESULTS AND DISCUSSION

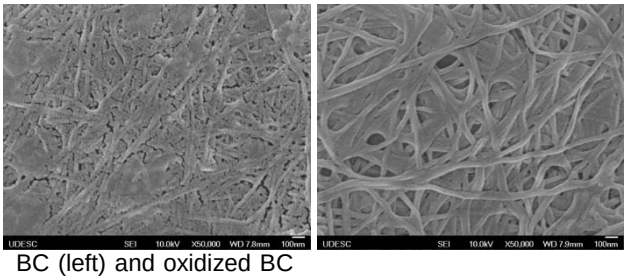
The following table presents the *in vitro* bioabsortion results of BC membranes in phosphate buffered saline (PBS) solution.

Table 1. Weight loss of BC after 2h (DABC1) and 6h (DABC2) of reaction in metaperiodate solution.

Sample	Reaction time (h)	Weight loss (%)
BC (control)	0	3,0
DABC 1	2	27
DABC 2	6	49

The data suggest that oxidized BC was degraded in PBS solution. Periodate oxidation is characterized by the specific cleavage of the C2\C3 bond of the glucopyranoside ring. This cleavage results in the formation of the two aldehyde groups per glucose unit. The dialdehyde cellulose degrades at physiological condition and generates glycolic acid and 2,4-dihydroxybutyric acid[2].

Figure 1. Scanning electron microscopic examination of BC (left) and oxidized BC during 4h (right).



SEM imagens show a fibrillary surface. After oxidation the inter fibrillary material was removed.
Preliminary CHX release from BC and oxidized membrane are shown in Figure 2. All membranes presented a burst effect, releasing high concentration of CHX in the beginning of the experiment. The oxidation processes do not alter significantly the release profile.

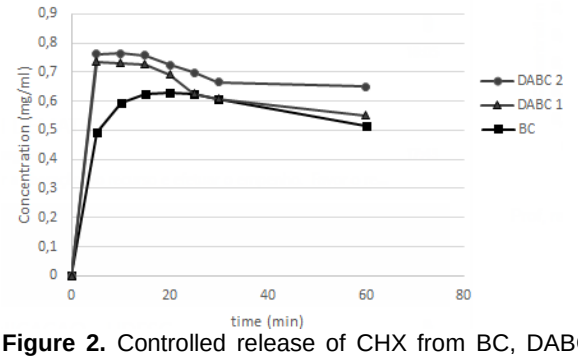


Figure 2. Controlled release of CHX from BC, DABC1 and DABC 2 membranes, at 25ºC.

CONCLUSION

Bioabsorbable bacterial cellulose materials incorporating an antibacterial material have been developed. Further studies are necessary to modify the chemical interaction between CHX and BC to modulated chlorhexidine release.

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(PO20) Comparison of *Gluconacetobacter xylinus* cellulose produced by submerged and solid cultures

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Keywords: bacterial cellulose, submerged culture, solid-state culture.

INTRODUCTION

Bacterial cellulose (BC) is an exopolysaccharide produced for some bacteria that have structural and mechanical properties and obtained easier than plant cellulose[1]. *Gluconacetobacter xylinus* is a well-known cellulose bacteria producer in submerged culture (SmC).[2] This work aimed to compare the output, morphological and chemical characteristics of BC in SmC and solid cultures (SSC).

RESULTS AND DISCUSSION

Production of BC in submerged culture (SmC) was carried out in a bioreactor while solid-state culture (SSC) production was made employing polyurethane foam (PUF) in cubes as inert support. Both experiments were worked with a C/N ratio of 7, initial pH of 6 and Hestrin and Schramm (HS) medium[3] and 5% of inoculum. Bacterial growth (0.175 gBiomass/gISS) and BC production (0.04 gBC/gISS) at 24 h were higher with SSC than in SmC (0.05 gBiomass/gISS and 0.005 gBC/gISS). BC production was carried out efficiently in SSC since it was observed a higher production of 8-fold than that in SmC.

The secretion of cellulose in SmC can be seen as aggregates; while in SSC BC was observed as biofilm covering pores of PUF (Fig. 1).

Fig. 1. BC production in SmC and SSC.

The morphology of BC obtained with both systems of culture was characterized by scanning electron microscopy (SEM). In Fig. 2A, BC is observed as microfibrils, whereas the bacterial growth and the secreted BC deposited on the surface of PUF (Fig. 2B-C). Fluorescence microscopy also evidenced the BC layer formation on the inert support with the interaction between calcofluor white and cellulose secreted in PUF (Fig. 2D).

Samples of purified BC was analyzed by infrared spectroscopy (ATR-FTIR) and X-ray diffraction (XRD).

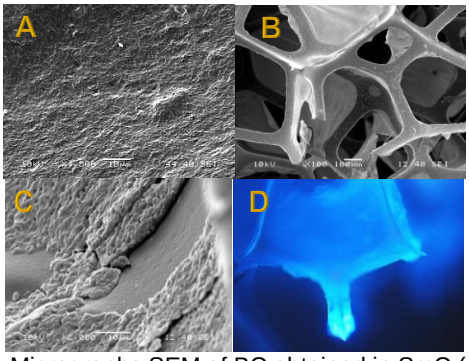

Fig. 2. Micrographs SEM of BC obtained in SmC (A) and SSC (B-C). Fluorescence image of BC in SSC (D).

The spectra showed the characteristic bands of BC in regions near 1160 and 1000 cm^{-1}; 3400 and 2800 cm^{-1} corresponding to the C-O-C and C-O; O-H and C-H groups vibrations, respectively. As well, the band in 1632 cm^{-1} is associated with water, which is absent in the plant cellulose spectrum (CO), just present in BC (Fig. 3). Both samples were obtained with a similar crystallinity index of 86.4% and 95.25% for SmC and SSC, respectively.

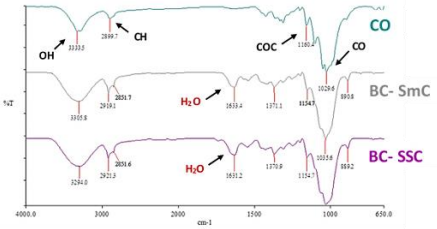

Fig.3. ATR-FTIR spectra of BC-SSC, BC-SmC, and CO.

CONCLUSION

There is greater BC production in SSC than in SmC. The biopolymers presented similar chemical structure. Nevertheless, there was a significant difference on crystallinity with SmC more amorphous structure was determined than that with SSC, as well morphologies were affected for the culture conditions.

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(PO21) Preparation of Thermo-Responsive Hydrogels Containing Carvacrol Encapsulated in Nanoparticles

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Keywords: Hydrogel, Nanoparticles, Carvacrol

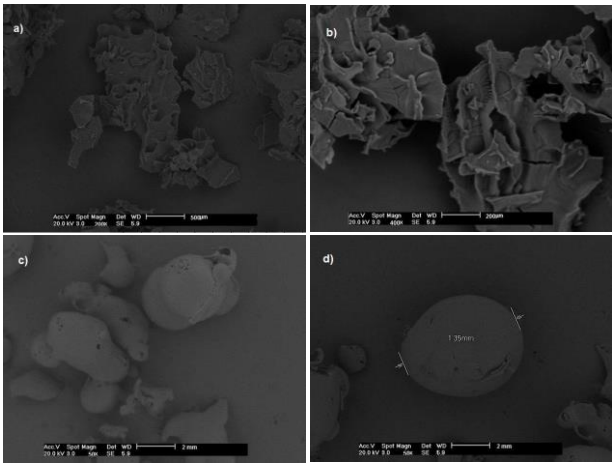
INTRODUCTION

Hydrogels are three-dimensional cross-linked polymer networks that can respond to the fluctuations of environmental stimuli[1]. The Pluronic F-127 is widely used to produce these systems due to its properties of thermal reversibility and nonionic surfactant[2]. The carvacrol possesses a variety of pharmacological benefits, including antibacterial, analgesic and anti-inflammatory properties, but its poor solubility and bioavailability limit its biological activity[3]. Nevertheless, these problems can be overcome by use of drug delivery systems that can increase drug solubility and stability[3]. Nanocapsules are systems able to enhance the solubility and consequently the activity of the drugs. This study reports the preparation of hydrogels thermo-responsive containing carvacrol encapsulated in nanocapsules for topical administration.

RESULTS AND DISCUSSION

The hydrogel, obtained of magnetic stirrer, corresponding of 20% of solubilizing Pluronic F-127 into 25ml of distilled water. After the total solubilization, the hydrogels were stored at 4°C[2]. The hydrogel in its liquid state at 4ºC was placed in a test tube to be subjected to thermal shock with water or Phosphate buffer pH 7.4 at 30°C to 37°C. The hydrogel was efficiently characterized, in the liquid state, at room temperature, the hydrogel is presented as limpid and transparent liquid and at 37°C appears viscous, transparent, clear and translucent. PCL-NP were prepared by nanoprecipitation method. Nanocapsules were prepared by nanoprecipitation method containing 1 mg/mL of carvacrol were prepared. Several batches of nanocacapsules were developed by modifying the concentration of carvacrol. In addition, the liquid-gel transition through thermal shock was analysed. Regarding the amount of nanocapsules containing carvacrol in the hydrogel the better percentage was 4%. Nanocapsules presented mean diameter of 139,76 nm with a polydispersity index of 0.118 and the hydrogel containg carvacrol encapsulated in nanocapsules presented a mean diameter of 195.46 nm with a polydispersity index of 0.281, which indicated a narrow particle size distribution. The characterization study of hydrogel was performed by IR, RAMAN and SEM.

Figure 1. Hydrogel 20% containing nanocapsules imaged by scanning electron microscopy (SEM) showing (a) 200x magnification (b) 400x magnification and Poloxamer (Pluronic F-127) (c) 50x magnification and (d) 50x magnification.



CONCLUSION

It was possible to produce thermo-responsive hydrogels containing carvacrol encapsulated in nanoparticles. The next step will be to evaluate the biological activity of the prepared systems

ACKNOWLEDGEMENTS

PROPESQ Pró-Reitoria de Pesquisa UFPB

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico

UEPB

UFPB

REFERENCES

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(PO22) Laccase immobilization on nanofibrillated cellulose for use in lignin refinery

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Keywords: nanocellulose, enzyme immobilization, enzymatic activity

INTRODUCTION

The lignin present in the black liquor of the cellulose industries has mostly been used for energy generation [1,2]. However, recent research has shown that the potential of lignin is far beyond low-value fuel. To enhance the applications of lignin it is necessary to overcome its heterogeneity by means of processes of fragmentation, purification or modification of the structure [1,3]. This modification can be promoted by enzymes such as laccase, acting as a biocatalyst to add value to lignin products [1,4–7]. However, the use of enzymes in the free form are subject to chemical, physical and biological factors that limit their useful life during use or storage, making it a high-cost product. Many of these undesirable features may be removed or ameliorated by using the enzymes in the immobilized form [8]. The objective of this work was to immobilize laccase on nanostructured cellulose, aiming at future use in biochemical conversions of Kraft lignin.

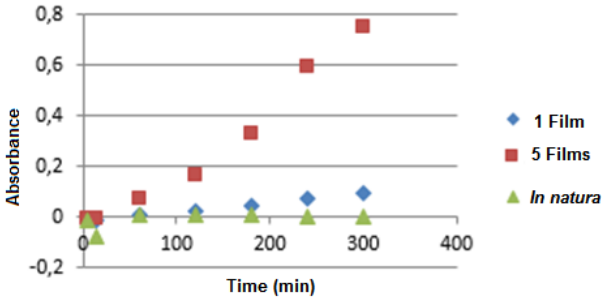
RESULTS AND DISCUSSION

The nanocellulose film (20 $g.m^{-2}$) was prepared from an aqueous suspension of eucalyptus cellulosic pulp previously defibrillated in a colloidal mill. The suspension was filtered through a 60 mesh strainer and nylon mesh, followed by oven drying at 60 °C. The film was oxidized in $NaIO_4$ solution [9]. Immobilization was started incubating 1 g of the oxidized film in sodium acetate buffer (50 mL) containing commercial laccase (0.1 $g.L^{-1}$), for 30 min at 30 °C and 150 rpm, according to Sathishkumar et al. [9]. Subsequent steps of cold incubation and reaction with glutaraldehyde were performed. The films were washed with sodium acetate buffer, left in Tris-HCl buffer, washed again and stored in acetate buffer.

Laccase activity was evaluated for both free and immobilized enzyme using ABTS as a substrate at 30 ° C, spectrophotometer reading at 420 nm. By titration using hydroxylammonium chloride and sodium hydroxide, it was possible to estimate the content of aldehyde groups generated on the films that underwent oxidation. Oxidized films had 18 times more aldehyde groups than those without oxidation, indicating the efficiency of this process for surface preparation prior immobilization.

Analyzing the immobilized laccase on films, the enzymatic activity was calculated to be 0,005 $U.g^{-1}$ of substrate. Despite the low value, it was possible to detect differences between the immobilized films and the original films (*in natura*) – Figure 1. A leakage test was accomplished to discard false positive for immobilized films.

Figure 1. Absorbance *versus* time for immobilized and original films at 420 nm



Considering the activities measured for free laccase solution before and after immobilization, it was possible to estimate the immobilization yield (4.2%). The result was unexpectedly low and some possible reasons could be: low concentration of laccase in the immobilization solution or/and unfavourable characteristics of the film, such as low porosity.

CONCLUSION

Although still insufficient, immobilization of a small fraction of laccase occurred on the nanocellulose film. For greater success, it is proposed to increase the concentration of laccase in the immobilization solution, as well as to use the suspension/gel of nanofibrillated cellulose as substrate, providing a larger surface area.

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(PO23) Conductive Monolithic Polymers for Peroxidase Immobilization

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Keywords: monolithic polymers, polyaniline, peroxidase, immobilization.

INTRODUCTION

Peroxidases are a wide group of enzymes that catalyze the oxidation of a variety of organic and inorganic substrates and can be immobilized into various support[1], the most common of which is polyaniline (PAni), since it presents several advantages over others. However, the PAni has some disadvantages related to its processing as the inability to be processed in high temperatures, since its decomposition temperature is lower than its melting point[2]. One way of solving this and moreover, to improve PAni properties is the incorporation into porous monolithic polymers. In this context, the aim of the present work is to evaluate the immobilization of peroxydase into PAni, incorporated into styrene and divinylbenzene porous monolithic polymers (Sty-DVB)

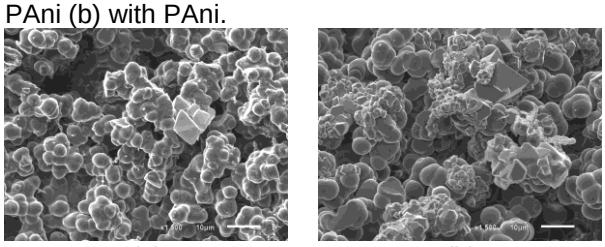
RESULTS AND DISCUSSION

The syntheses of the Sty-DVB monolithic polymers were carried out in cylindrical glass molds by mass polymerization using soybean oil, 1-pentanol and toluene/n-Heptane mixture as diluents under heated conditions. The incorporation of PAni into the polymers was carried out via oxidative polymerization of the aniline in the presence of HNO_3 and HCl. Peroxidase immobilization was performed by the activation of the PAni supported with glutaradehyde followed by direct contact with the crude extract of *Solanum lycocarpum* St. Hil. in different temperature, pH and time conditions. The free and immobilized peroxidase activity was measured under different pH, time and temperature conditions. The polymers were characterized by FTIR-ATR, SEM and measurements of specific surface area, pore volume and average pore diameter.

The monolithic polymers with higher pore volume and specific surface area were obtained with high degree of crosslink with soybean oil or toluene /heptane mixture as diluent. The use of HCl as dopant allowed the distribution of PAni only on the surface, whereas the use of HNO_3 allowed the distribution of PAni on the surface and inside the monolithic polymers.

The FTIR-ATR spectra of the different materials showed characteristic bands of all polymers produced. The immobilization time results suggest that 30 minutes are sufficient and longer intervals can cause process efficiency loss. Both free and immobilized enzyme showed similar optimal pH value, 7.0. The immobilization of peroxidase in polymer supported PAni with high porosity and high specific area produced systems with higher catalytic activity (Figure 1). The peroxidase-immobilized into supported Pani on the different monolithic polymers maintained its catalytic activity unchanged for 10 times use.

Figure 1. SEM of monolithic polymers (a) without PAni (b) with PAni.



(a) (b)

CONCLUSION

The produced Sty-DVB monolithic polymers showed good characteristics for application as a Pani support, such as suitable size and good mechanical strength. After the incorporation of PAni into its structure, the Sty-DVB-PAni presented ideal characteristics for the peroxidase immobilization, once, when immobilized it showed a good catalytic activity.

ACKNOWLEDGEMENTS

We thank FAPEG, Instituto de química da Universidade Federal de Goiás e COREMAL for the polymer providing.

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(PO24) Activity of β-glucosidase enzyme under ultrasonic irradiation

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Keywords: β-glucosidase, Ultrassonic irradiation, Lignocellulosic Biomass, Glucose

INTRODUCTION

Due to the increasing demand for energy and the imminent scarcity of petroleum resources, studies with renewable raw materials such as lignocellulosic biomass are being performed to convert them into bioethanol and other value-added products. The conversion, however, presents numerous challenges due to the chemical and structural complexity of lignocellulosic biomass. Ultrasonic irradiation is considered a green technology and has been shown to permit reduced processing times, operation at lower temperatures and reduction of the use of chemical products.[1,2] In the present work we studied the application of ultrasound during the hydrolysis of cellobiose with a commercial enzyme preparation containing β-glucosidase activity.

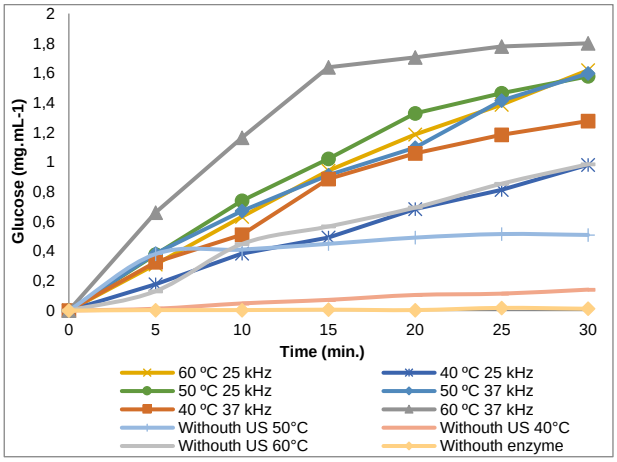
RESULTS AND DISCUSSION

The activity and stability of the β-glucosidase (NS 50010 Novozymes) enzyme was determined according to Ghose (1987)[3], quantifying the glucose released in the cellobiose hydrolysis.

HYDROLYSIS
1 mL enzyme
1 mL cellobiose
pH 5, 40 – 60 ºC
25 or 37 kHz, 0 - 60 min

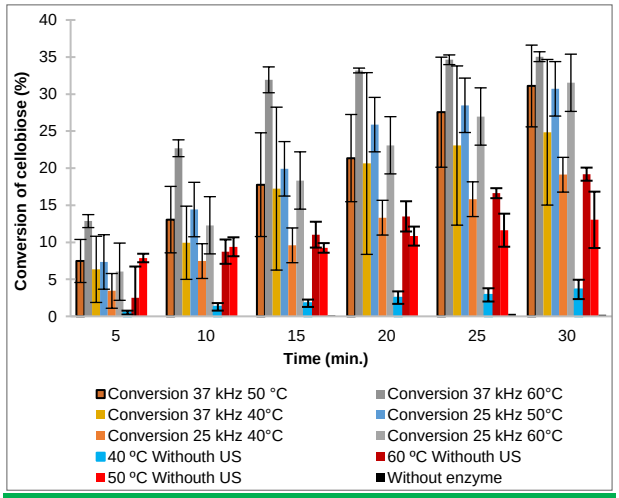
GLUCOSE ANALYSIS
1 mL GOD-POD
37°C, 10 minutes
Absorbance at 500 nm

Figure 1. Glucose formation under 25 and 37 kHz ultrasound irradiation at different temperatures for the β-glucosidase enzyme in the hydrolysis of cellobiose.



A continuous increase of glucose over time was observed (Figure1). The highest conversion was obtained at the frequency of 37 kHz at 60°C followed by the conditions of 25 kHz and 37 kHz at 50°C (Figure 2). Glucose formation increased with increasing temperature. The formation of glucose without the presence of ultrasonic irradiation was lower and increased almost linearly. Ultrasonic irradiation alone without enzyme did not lead to significant glucose formation.

Figure 2. Glucose conversion in cellobioses hydrolysis under 25 and 37 kHz ultrasonic irradiation.



CONCLUSION

Ultrasonic irradiation applied to the enzymatic hydrolysis of cellobiose with β-glucosidase revealed a significant increase in enzyme activity and had a positive influence on the formation of glucose, leading to higher yields in a shorter reaction time.

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FURB UNIVERSIDADE REGIONAL DE BLUMENAU

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(PO25) Kinetic analysis on cell growth and poly-hydroxybutyrate production by parental and recombinant *Cupriavidus necator* strains

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Key words: Glycerol, glucose, PHB.

INTRODUCTION

Poly-hydroxyalkanoates (PHAs) are among the top group of biopolymers that have been intensively investigated and commercialized. They are attractive substitute biopolymers for conventional petrochemical plastics which have similar physical properties to thermoplastics and elastomers. The bacteria *Cupriavidus necator* is a well-known producer of PHAs. PHB is the most studied PHA and its production total costs are related to the microorganism (yields and productivity), carbon and nitrogen sources, culture conditions and downstream process[1]. The use of low-cost substrates, like glycerol, the main coproduct of the biodiesel industry, is feasible[2]. Besides, the most efficient use of substrates by engineered strains could increase the PHB productivity. The aim of this work was to evaluate the kinetic parameters of *C. necator*_glpFK[3] (recombinant) against its parental strain (*C. necator* DSM 545) during the PHB production.

RESULTS AND DISCUSSION

Cultivations were performed as of two seed cultures. In the first one, cells were grown in nutrient broth. The second seed culture was obtained on mineral medium (MM)[4] with glucose and glycerol as carbon sources. The cultivations were performed using a 4 L bioreactor working volume (30 °C/ initial 450 rpm) on MM with glucose (20 $g.L^{-1}$) and glycerol (25 $g.L^{-1}$). The PHB was determined by gas chromatography, according to the methanolysis[5]. Residual biomass (Xr) is non-PHB part of biomass. *C. necator* DSM 545 was able to produce PHB from glycerol and glucose, despite the poor growth ability on glycerol. In the present study, we demonstrated that the DSM 545-based engineered strain possessing *glpFK*$_{Ec}$ produced PHB from glycerol and glucose with higher productivity than the parental strain.

Table 1. PHB production by *C. necator* DSM 545 and *C. necator*_glpFK

Strain	Time (h)	Xt ($g.L^{-1}$)	PHB ($g.L^{-1}$)	% PHB ($g.g^{-1}$)	Productivity ($g.L^{-1}.h^{-1}$)
parental	30*	11.0	4.0	36**	0.13
recombinant	55	20.1	16.3	76.5**	0.29

* Decrease observed in total biomass after 30 h.
** Production phase of 18 h.

On cultivations with *C. necator*_glpFK, when glycerol was used as the sole carbon source, the productivity was about 0.15 $g.L^{-1}h^{-1}$[3]. The present recombinant strain performance and productivity, on glucose and glycerol, was better.

The yields were obtained graphically plotting nutrients concentration as a function of Xr (during the growth phase). Yields of product formation were obtained plotting glycerol concentration as a function of PHB (during the production phase).

Table 2. Yields on biomass and PHB.

Strain	$Y_{Xr/Glucose}$ ($g.g^{-1}$)	$Y_{PHB/Glycerol}$ ($g.g^{-1}$)	$Y_{Xr/N}$ ($g.g^{1}$)
Parental	0.30	0.20	6.10
Recombinant	0.43	0.60	6.90

The PHB yield obtained in this study were higher than the reported for recombinant *Escherichia coli* on fed-batch cultivation (0.21 $g.g^{-1}$), containing glycerol as the sole carbon source[6].

The biomass yield from glucose, for the parental strain, corroborated with literature[7].

The observed difference on $Y_{Xr/Glucose}$, may be due to the concomitant consumption of glucose and glycerol during the growth phase, by *C. necator*_glpFK. *C. necator* DSM 545, in contrast, consumed glycerol only after glucose exhaustion (data not shown).

CONCLUSION

The recombinant strain showed improved performance in PHB accumulation and productivity when compared to the parental one.

ACKNOWLEDGEMENTS

Author thanks to CNPq and CAPES for the fellowship.

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(PO26) Kinetic and respiration parameters of engineered *Cupriavidus necator* during poly-hydroxybutyrate production

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Keywords: Glucose, Glycerol, QO_2, PHB

INTRODUCTION

Poly-hydroxybutyrate (PHB) is a biopolymer, biodegradable, stored as bacterial reserve materials for carbon and energy. The high production costs of PHB still is a bottleneck for its large-scale utilization. One way to reduce the production costs of this biopolymer is the utilization of low-cost raw materials as substrate, like glycerol, a by-product from the biodiesel industry[1]. Besides, genetically modified microorganisms combined with low price carbon sources may be a viable strategy to optimize the production of PHB[2].

There are no reports in the literature about the determination of the respiratory parameters of *C. necator*_glpFK[3]. The objective of the present work was to obtain the kinetic respiratory parameters of *C. necator*_glpFK grown on glycerol and glucose, during the production of PHB.

RESULTS AND DISCUSSION

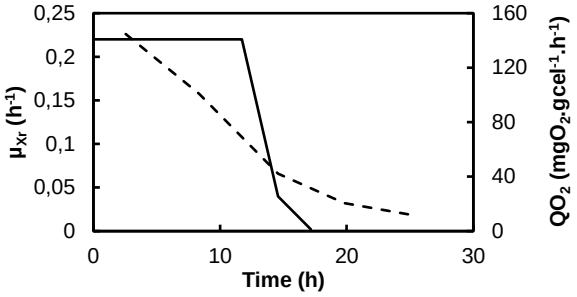
Cultivations were performed as of two seed cultures. In the first one, cells were grown in nutrient broth. The second one, was obtained on mineral medium (MM)[4] with glucose and glycerol as carbon sources. The cultivations were performed using a 4 L bioreactor working volume (30°C/ initial 450 rpm) on MM with glucose (20 $g.L^{-1}$) and glycerol (10 $g.L^{-1}$). The PHB was determined by gas chromatography, according to the methanolysis[5]. Residual biomass (Xr) is non-PHB part of biomass. The specific growth rate (μ_{Xr}) at the exponential growth phase was calculated from the linear fit to the data of ln (Xr) *versus* time. The specific oxygen uptake rate was determined by the Dynamic Method[6].

The final concentration of total biomass and PHB were 14.8 $g.L^{-1}$ and 10.8 $g.L^{-1}$, respectively, around 72wt.% of biopolymer.

Figure 1 shows the profile of μ_{Xr} and QO_2 *versus* time for bioreactor cultivation. In this figure, the maximum value of μ_{Xr} (0.22 h^{-1}) is observed during 11 h of culture, followed by its decrease until the end of the culture.

QO_2 had a virtually linear relation with μ_{Xr}, once the drop in the oxygen consumption rate was higher in the early culture phase, where the specific cell growth rate was constant and maximal. When growth slowed down, QO_2 had a constant behavior.

Figure 1. Profile of specific growth rate (μ_{Xr}) (—) and specific oxygen uptake rate (QO_2) (---).



The value of $Y_{Xr/O}$ was 0.0021 $g_{Xr}.g_{O2}^{-1}$ was the oxygen conversion factors and the cellular maintenance coefficients (m_{O2}) obtained for recombinant *C. necator* was 21.2 $mg_{O2}.g_{Xr}^{-1}.h^{-1}$. The respiratory parameters reported for *C. necator* DSM 545, grown on glucose and fructose, were 0,0048 $g_{Xr}.g_{O2}^{-1}$ ($Y_{X/O}$) and 46.22 $mg_{O2}.g_{Xr}^{-1}.h^{-1}$ (m_{O2})[7]. The difference between kinetic respiratory parameters of *C. necator*_glpFK, when compared to the parental strain, can be attributed to genetic manipulation.

CONCLUSION

It was possible to determine the kinetic and respiration parameters of engineered *C. necator* during poly-hydroxybutyrate production. The knowledge of these parameters can aid in decision-making for scale-up.

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Author thanks to CNPq and CAPES for the fellowship.

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(PO27) α-Glucosidase inhibition by extracts of *Ganoderma lipsiense* mycelium

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Keywords: Enzyme inhibitor, mycelium, G. lipsiense

INTRODUCTION

Interest in α-glucosidase enzyme inhibitors grows in considerably, due the necessity in the control of diabetes mellitus, one of the most common endocrine diseases of the 21st century. Diabetes is a serious metabolic disorder that affects approximately 4% of the population worldwide, with expectative to increase to 5.4% in 2025[1]. Fungi are recognized for their benefits to the prevention and treatment of diseases. The search for new constituents in fungi that reduce α-glucosidase activity, led to the finding of the effects of *Ganoderma* genus in the control of blood glucose levels[2]. *Ganoderma lipsiense* is a scarcely studied fungus in the scientific literature. However, important compounds were identified in their fruiting bodies[3]. Therefore, the objective of the present study was to evaluate the mycelium of *Ganoderma lipsiense* in two culture medium with regard to the inhibition effect of α- glucosidase enzyme.

RESULTS AND DISCUSSION

The α-glucosidase inhibitory assay was evaluated in the mycelium of the *G. lipsiense* (CCIBt 2689 - acquired from Botanic Institute, São Paulo, Brazil) cultivated in red rice broth (**1**) and in synthetic medium (**2**). The rice broth was prepared with 150g of red rice previously boiled at 100 ºC for 30 min with distilled water and ground with 600 mL of distilled water in ball mill and filtrated with lint. The cultivation was performed in cylindrical glass flasks (500 mL) containing 100 mL of rice liquid medium (20 % w/v) in distilled water and autoclaved for 30 min at 121 ºC. The synthetic medium was adapted from Rubel (2006)[4] and constituted of glucose (20 $g \cdot L^{-1}$), yeast extract (2.0 $g \cdot L^{-1}$), KH_2PO_4 (0.833 $g \cdot L^{-1}$) and $MgSO_4 \cdot 7H_2O$ (0.5 $g \cdot L^{-1}$). The static fermentation process was cultivated by 40 days. The mycelium-colonized medium was filtered, and the fungal mycelium was dried in a freeze-dried at - 65 ºC and stored -5 ºC until use. The compounds were extracted by maceration with ethanol during 24 hours at room temperature, filtered and concentrated in a rotary evaporator. The results of enzyme inhibitory activity of mycelium extract (**1**) and (**2**) are summarized in Table 1 and were obtained according to a previously described method[5] with slight modification in the fungal samples dilution. The fungal extract was previously dissolved in MeOH with 1 $mg \cdot mL^{-1}$ concentration. For the IC_{50} assays, different concentrations of the samples, dissolved from main-solution in phosphate buffer (0.1 $mol \cdot L^{-1}$; pH 6.8) were tested. IC_{50} corresponds to the concentration of fungal extracts required to inhibit 50% of the enzyme activity.

Table 1. Inhibitory effect of α-glucosidase by acarbose, mycelium of *G. lipsiense* in red rice broth (**1**) and synthetic medium (**2**)

Test samples	IC_{50} ($\mu g \cdot mL^{-1}$)
(**1**)	250 ± 2.55
(**2**)	400 ± 0.30
Acarbose	193.5 ± 3.82

*Mean ± standard deviation of triplicates

The samples (**1**) and (**2**) showed a strong inhibitory effect on the enzyme (Table 1), having sample (**1**) the best activity. This suggests that the red rice induced the production of compounds with enzyme inhibitory action (250 ± 2.55 $\mu g \cdot mL^{-1}$) by the fungus, when compared to synthetic medium (**2**) (400 ± 0.30 $\mu g \cdot mL^{-1}$). It is important to emphasize that we tested a crude extract and these values were close to the positive control acarbose (193.5 ± 3.82 $\mu g \cdot mL^{-1}$).

CONCLUSION

The *G. lipsiense* mycelium showed potential for the production of compounds with α-glucosidase inhibitory effects. Results indicate that *G. lipsiense* cultivated in red rice broth is a rich source of α-glucosidase inhibitors and their extract could be further explored for the development of novel preventive agents for the treatment of diabetes.

ACKNOWLEDGEMENTS

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(PO28) In vitro mouse embryo culture on decellularized uterus tissue

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Keywords: IVF, endometrium model, SCF

INTRODUCTION

Recent progress of in vitro fertilization technology has much improved the infertility. However, the reason for infertility caused by uterus or after embryo transplantation is still unclear. In this study, in vitro endometrium model has been constructed to investigate embryogenesis mechanism and to improve the infertility treatment in mouse model. In addition, in order to construct an endometrium model, decellularization of the endometrium tissue using a supercritical fluid (SCF) was attempted, and embryogenesis and endometrium structure were observed.

RESULTS AND DISCUSSION

[a] Mouse fertilized eggs were prepared by IVF technique. Ova were collected from 10 to 20-week-old ICR mouse with injection of 0.2mL PMSG and hCG at 48-hour interval. Then the fertilized eggs were cultured in IVC1 and IVC2 medium until blastocysts, embryo implantation, and egg cylinder [1][2]. Embryo adhesion and extension of trophoblasts were observed after 72 hour of culture on polystyrene culture dish and collagen gel. Some blastocysts generated egg cylinders, however they were not same shape as native and did not develop after egg cylinder.

[b] As a control, endometrium tissue of non-pregnant or pregnant mouse were harvested and observed histologically by HE and Picrosirius Red stain. HE and Picrosirius Red staining indicated a change of endometrium collagen type drastically after pregnancy. Adhesion and implantation of embryo onto isolated endometrium cells were also investigated.

[c] We also aimed for co-culture of blastocysts with decellularized uterus tissue to ddevelop after blastocysts. Decellularization of endometrium tissue was examined utilizing SCF extraction method which could decellularize skin tissue in our previous study. Utrine tissues removed from the mice were prefreezed for 2 hours and lyophilized for 24 hours. The uterine tissue was placed in the extraction chamber and extraction was examined at various conditions using carbon dioxide as an extraction medium [3]. Removal of cells was evaluated by HE staining. Figure 1 shows the results of HE staining after SCF extraction. Cells in the tissue were still remained even if extraction condition was varied.

As is generally known, the structure of uterus is composed of three-layer structure which has different function. Compared to simple structural tissue like skin,easy access of the fluid into uterus tissue be insufficient due to complicated structure. Further, solubility parameter of fluid may not be suitable for dissolving cells.

From the results of [a] to [c], it was difficult to develop after the blastocyst by the limited decellularzation of uterus tissue. In order to perform co-culturing, it is necessary to modify the conditions of the SCF extraction method and decellularize the endometrium tissue using a surfactant.

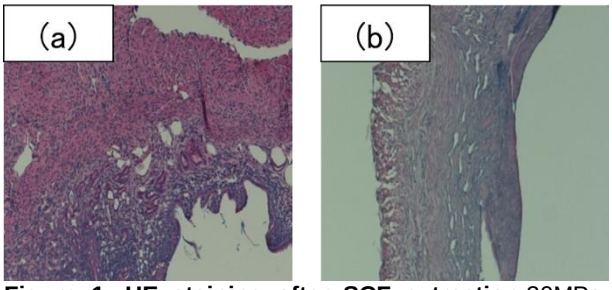

Figure 1. HE staining after SCF extraction;30MPa, 60min, pressure increase rate 5mL/min, pressure reduce rate 30MPa/5min, including 99.5% ethanol 10mL, figure (a) 37℃ (b) 45℃

CONCLUSION

Adhesion of embryo was observed on culture dish and collagen gel. Farther improvement of culture environment using native-like endometrium will be necessary for development after egg cylinder in vitro.

Effect of Decellularzation of endometrium tissue using supercritical carbon dioxide was not sufficient. In order to attain complete removal of cells from uterus tissue, further modification of extraction condition such as pressure control, medium selection and an addition of entrainer, etc., may be necessary.

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(PO29) Effect of $CaCl_2$ crosslinking on mechanical properties of polysaccharide-based membranes

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Keywords: Alginate. Carboxymethyl cellulose. Films.

INTRODUCTION

Alginate and carboxymethyl cellulose (CMC) membranes have been widely explored for different applications due their unique colloidal, biodegradability, biocompatibility, and good film-forming properties[1–3].

The induction of gelation using calcium produces specific and strong interactions between calcium ions and guluronic acid blocks of alginate. A three dimensional network creates interstices by involving the polymer chains. The “egg-box” model formation is the best mechanism to describe this process. Different crosslinkers molecules and concentration can produce unique materials[4].

The aim of the present work is to evaluate the influences of $CaCl_2$ concentration in the second step of crosslinking on mechanical properties of alginate- and CMC-blended membranes.

RESULTS AND DISCUSSION

The casting method was utilized to produce the membranes. Each polymer (1.5 % w/v) was dissolved in distilled water with mechanical agitation of 900 rpm at 50 °C until no particles could be observed. Glycerol was added in both solutions in the concentration of 3 % (w/v). A two-step crosslinking process was utilized. For this, 1 % of $CaCl_2$ aqueous solution was added in the alginate solution

The solutions were mixed in the proportion of 50 % (m/m) of each polymer and, then, aliquots (100 g) were poured in petri dishes of 15 cm of diameter. The drying process occurred during 20 h at 40 °C.

To second process of crosslinking was performed by immersing the dried membranes in 60 mL of $CaCl_2$ aqueous solution (1 or 5 %) during 20 min.

Lastly, the membranes were dried a second time (24 h) at room temperature with its edges fixed by a wooden ring. Before any characterization process, the membranes were kept in a controlled relative humidity environment of 58 %.

The thickness of the membranes was measured using a digital micrometer (MDC-25P, Mitutoyo, Japan).

The ASTM standard method D882[5] was used to determine the mechanical properties of the membranes: tensile strength and elongation at break. The analyses were performed in strips in quadruplicate of each membrane (9 x 2.54 cm^2) in a texturometer (model TA.HD.PLUS, Stable Micro Systems SMD, England). Analysis of variance and the Tukey test ($p < 0.05$) were utilized.

The Table 1 presents the obtained results

Table 1. Effect of different crosslinking agent concentration on mechanical properties of the membrane.

$CaCl_2$ content	Tensile Strength (MPa)	Elongation at break (%)	Thickness (μm)
1 %	5.8 ± 0.6 [b]	51.7 ± 3.1 [a]	122 ± 9 [a]
5 %	22.7 ± 2.5 [a]	42.8 ± 5.8 [b]	127 ± 11 [a]

Average ± standard deviation of experimental determinations. Averages with the same letter, in the same column, indicate no significant differences ($p < 0.05$) by the Tukey test.

The crosslinking concentration did not influenced the membrane thickness ($p < 0.05$). The material obtained with 1 % of $CaCl_2$ had more flexibility and less physical resistance when compared to the one that used 5 % of crosslinking ($p > 0.05$).

The data are in accordance with the literature: the $CaCl_2$ tends to directly affect the tensile strength and elongation at break. The introduction of CMC improved the alginate mechanical properties[1,6].

CONCLUSION

The $CaCl_2$ concentration of 5 % provided more resistant and less flexible membranes. In addition, good mechanical properties were obtained by blending both biopolymers.

ACKNOWLEDGEMENTS

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(PO30) Water behavior properties of films made by different alginate and carboxymethyl cellulose proportions

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Keywords: Biopolymer. Film characterization. Membranes.

INTRODUCTION

In the recent years the production of biodegradable films has grown. These materials can be applied in different industrial areas as food packs, wound dressings, drug delivery systems, etc.[1,2]

Alginates (Alg) is a copolymeric polysaccharide extracted from brown algae which can be cross-linked with divalent ions such Ca^{2+}. Cross-linked alg-based materials presents good mechanical properties, however tend to not have desired water behavior properties[2,3].

The cellulose derivated, carboxymethyl cellulose (CMC) present good water absorption capability. Blending Alg with CMC may achieve the best necessary qualities for the different applications of these materials[1,4].

The aim of the present work was to evaluate the water behavior properties on three different Alg/CMC films.

RESULTS AND DISCUSSION

Alg and CMC filmogenic solutions were separately obtained by each polymer dissolution (1.5 % w/v) at 900 rpm at 50 °C in water containing glycerol as plasticizer (3 % w/v). To the Alg solution, an aqueous solution of $CaCl_2$ (0.075 g of the salt) were added to realize a first crosslinking step.

After obtaining homogeneous solutions, they were mixed in three different Alg:CMC proportions: 3:1; 1:1; and 1:3 in mass. The films were obtained by casting method by drying, during 20 h at 40 °C, 100 g of each solution that was previously poured in petri dishes of polystyrene (d = 15 cm).

The films were released from the molds and crosslinked again by immersion in 60 mL of a $CaCl_2$ water solution (5 % w/v) containing glycerol (3 % w/v) during 20 min. Then, the films were fixed in wooden molds to prevent shrunk and dried by a second time during 24 h at room temperature.

The films were evaluated by their water behavior properties: water vapor permeability (WVP), as the ASTM E96/E96M method[5], maximum swelling degree as described by Rodrigues et al.[3] and moisture content by gravimetric method.

The experiments were realized in quadruplicate and evaluated statistically by Tukey test ($p < 0.05$).

The films produced were homogenous and the presented good polymer distribution in it polymeric matrix.

In the Table 1, a tendency of a higher water attraction was observed with the CMC increase in the films.

Table 1. Different Alg and CMC proportion on water behavior properties of membranes.

Alg:CMC	WVP ($g.mm.m^{-2}.kPa^{-1}.d^{-1}$)	Maximum swelling degree (%)	Moisture content (%)
1:3	7.7 ± 0.9^{ab}	2499 ± 191^{a}	32.3 ± 1.2^{a}
1:1	7.0 ± 0.6^{b}	600 ± 15^{b}	28.9 ± 0.5^{b}
3:1	5.9 ± 0.7^{bc}	259 ± 61^{c}	24.4 ± 2.2^{c}

Average ± standard deviation of experimental determinations. Averages with the same letter, in the same column, indicate no significant differences ($p < 0.05$) by the Tukey test.

The obtained results demonstrated the correlation of water properties between each other. More, the presented data were in accordance when compared by other films made with Alg, CMC or blend of both polymers [1,2,4]. Overall, the introduction of CMC in the Alg enhanced the film-water interactions, it were desired in food packing or medical applications, for example.

CONCLUSION

The higher affinity of CMC with water was demonstrated with increasing this polymer concentration in the films. This enhance on the water behavior properties of the films contributes for use of the materials in different areas.

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(PO31) Effect of peptide binding on antibacterial activity and cytotoxycity of protein-based substrates

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Keywords: wool, gelatin, antimicrobial peptides, cytotoxycity.

INTRODUCTION

Antimicrobial activity of fibrous and polymeric materials is increasingly important in many application areas, but above all in biomedical, therapeutic and hygiene applications, to increase the standard of living and reduce the growing awareness of bacterial, fungal and viral diseases.
Antimicrobial active peptides (AMP) and proteins have been thus recognised as clinically perspective antimicrobial agents, showing high and specific antimicrobial activities at very low (μmol) concentrations, as well as low toxicity to mammalian cells, together with the fact that bacterial resistance to them seems inherently difficult to acquire.
These contribution investigate the conjugation efficacy of different cationic (lysine-based) peptides to a wool fibers andgelatine (GEL) macromolecule: a hydrophilic homopolypeptide ε-poly-L-lysine (ε-PL) and an amphiphilic oligo-acyl-lysyl (OAK) derivative (K-7α_{12}-OH) by using chemical (EDC/NHS; grafting-to vs. grafting-from synthesis routes) and enzymatic (transglutaminase) coupling approach, respectively, and substrates antibacterial activity against Gram-negative *E. coli* and Gram-positive *S. aureus* bacteria after 1–24 h of exposure, as well as their cytotoxicity.

RESULTS

Different spectroscopic (ultraviolet-visible, infrared, fluorescence and electron paramagnetic resonance) and separation techniques (size-exclusion chromatography and capillary zone electrophoresis), as well as zeta potential and potentiometric titration analysis were performed to confirm the covalent coupling of εPL/OAK, and to determine the amount and orientation of its immobilisation.
The highest and kinetically the fastest level of bacterial reduction was achieved with wool/GEL functionalised with εPL/OAK by chemical grafting-to approach. This effect correlated with both the highest grafting yield and conformationally the highly-flexible ″brush-like″ orientation linkage of εPL/OAK, implicating on the highest amount of accessible amino groups interacting with bacterial mebrane. However, OAK`s amphipathic structure, the cationic charge and the hydrophobic moieties, resulted to relatively high reduction of *S. aureus* for grafting-from and the enzymatic coupling approaches using OAK-functionalised GEL.
The εPL/OAK-functionalised GEL did not induce toxicity in human osteoblast cells, even at ~25-fold higher concentration than bacterial minimum inhibitory (MIC) concentration of εPL/OAK, supporting their potential usage in biomedical applications
It was also shown that non-ionic surfactant adsorb strongly onto the wool surface during the process of washing, thereby blocking the functional sites of immobilized εPL and decreases its antibacterial efficiency.

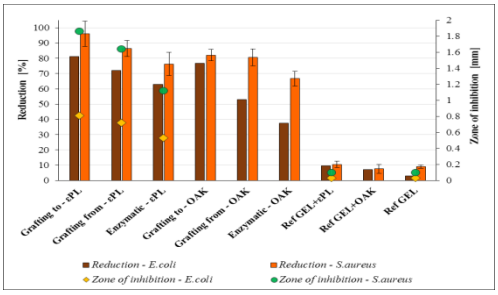

Fig.1 CFU reduction of G- *E. coli* and G+ *S. aureus* bacteria and zone of inhibition after 24 h of exposure.

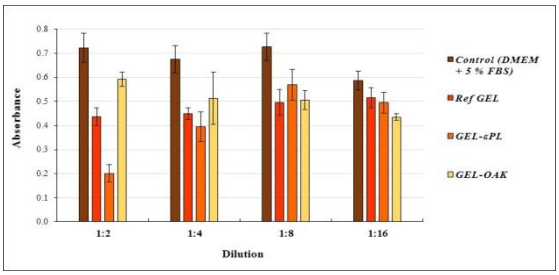

Fig. 2. MTT data for 24h incubation with human osteoblast cells.

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(PO32) Synthesis, characterization, reactivity ratios and properties of starch-g-poly (acrylic acid-co-methyl acrylate) triggered via enzyme

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Keywords: Synthesis, Starch, HRP, Vinyl monomers, Reactivity ratios

INTRODUCTION

In recent years, graft starches with improved properties are gaining increasing attention because of their varied properties, reproducibility, biodegradability and low-cost. The practicability of starch after graft polymerization is exemplified by the fundamental investigations of structure-property relationships and by the wide range of commercial applications [1]. Synthesis, characterization and properties of a biodegradable graft copolymer based on acrylic acid (AA) and methyl acrylate(MA) grafted starch by using horseradish peroxidase (HRP) as initiator were reported. Controllable properties of copolymer were obtained by grafting of PMA and PAA chains onto the starch backbone, simultaneously. Grafting copolymer of starch with different ratio of AA/MA was synthesized via adjusting the feed proportion of monomers in terms of reactivity ratios. Graft starch was characterized by measuring FTIR and 1H NMR spectroscopy. Hydrophilic and mechanical properties of the grafted starch film were also tested.

RESULTS AND DISCUSSION

Although many techniques have been reported for the determination of co-polymer composition and estimating reactivity ratios, 1H NMR spectroscopy has been preferred in this work because of its simplicity and rapidity. The 1H NMR spectroscopy was used for identifying and determining individual monomeric units in the copolymer composition after disposed.The grafted starches with varying feed compositions of AA and MA, were synthesized in distilled water as solvent. The respective signal intensities could be used to obtain the copolymer composition. The relative mole fractions of each monomer bound in the copolymer were estimated from the areas under the appropriate absorption peaks of the spectra in Figure 1. The methyl for the MA bound in the polymer (green peaks) was located at 3.48-3.89ppm. Although the methylene and Methyne in MA-AA copolymer appeared on the continuous area, integral area distinguished that methylene (blue) and Methyne (red) in MA-AA copolymer were located at 1.1-1.9 ppm and 1.9-2.5ppm,respectively.

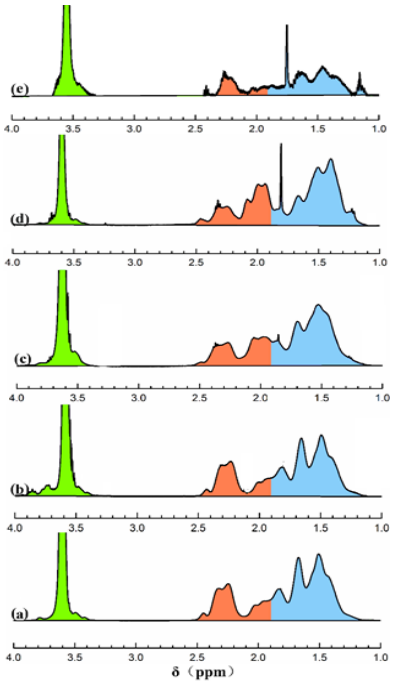

Fig.1 1H NMR spectra of poly (AA-co-MA) after disparate constitution in feed (a) 0.12 ,(b) 0.32,(c) 0.50,(d) 0.73,(e) 0.89

CONCLUSION

In the paper, reactivity ratios of AA and MA under the grafting copolymerization of these monomers and starch in the presence of HRP were determined, which revealed the higher reactivity of AA than MA in free radical initiated polymerization. The structure of starch-g-poly (MA-co-AA) was proofed by 1H NMR and FT-IR analysis. Moreover, the thermal property of grafted starch slightly decreased owing to the poor thermal stability of PAA. With various proportion of MA in starch, grafted starch could display different hydrophilicity and mechanical properties.

ACKNOWLEDGEMENTS

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(PO33) Biosurfactant producing species evaluating several substrates for application in decontamination by petroderivatives

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Keywords: Biotensoactive, Screening, microorganisms, Petrochemical.

INTRODUCTION

Over the years the search for biological alternatives for the removal of petrochemical residues has been increasing. Biosurfactants are amphipathic molecules with properties such as reduction of surface tension, emulsification and dispersion among others [1,2]. Currently, the major market for biosurfactants is the petroleum industry, in which these compounds can be used in the cleanup of oils spills and bioremediation of soil and water [3]. Biosurfactants are mainly produced by aerobic microorganisms which can be obtained in an aqueous medium containing soluble and insoluble substrates as carbon and nitrogen source [4]. In view of the above, the present study aimed to report the selection of microorganisms and the evaluation of different carbon sources as alternatives for the production of biosurfactants and to demonstrate the potential of future applications in environmental decontamination processes.

RESULTS AND DISCUSSION

Different types of microorganisms (*Bacillus methylotrophicus, Bacillus cereus and Pseudomonas cepacia*) were cultivated in different substrates and compared with regard to biosurfactant production. The cultivation was carried out in mineral medium supplemented with 2,0 % (glucose, sucrose, cane molasses, frying residual oil) individually, during 96 hours, 200 rpm, 28 ºC and 37 ºC. Initially the surface tension was used as a preliminary screening standard for potential biosurfactant producer bacteria

Figure 1: Surface tension for microorganisms at different carbon sources

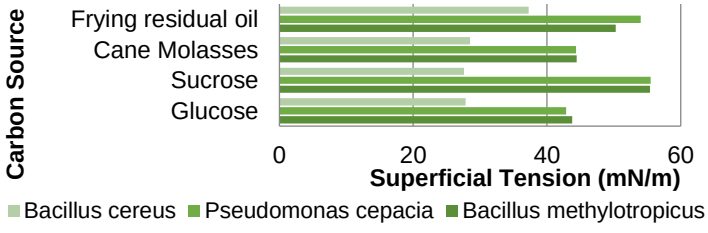
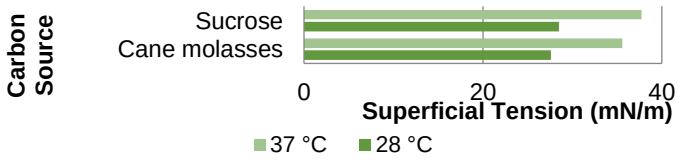


Figure 2: Surface tension for temperatures at selected carbon sources for the cultivation of B. cereus



The microbial tensoactive was submitted to emulsification tests in different hydrophobic compounds (motor oil, motor residual oil and soybean oil) and applied as petroderivative dispersing agent in wather.

Figure 3: Emulsification rates for selected carbon sources in the cultivation of B. cereus at temperatures of 28 °C and 37 °C

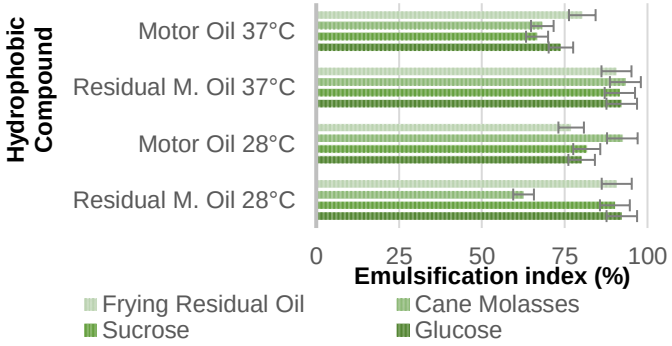


Table 1: Application of the Bacillus cereus biosurfactant obtained at 28 °C and 37 °C in the petroderivative dispersion in the proportions of 1:2, 1:8 and 1:25 (v/v)

Carbon source	Temperature					
	28 °C			37 °C		
	1:2	1:8	1:25	1:2	1:8	1:25
Glucose	14.8	28.8	14.2	36.0	39.6	18.0
Sucrose	39.6	18.0	18.0	18.0	34.5	21.6
Cane Molasses	32.4	32.4	10.8	39.6	32.4	25.2
Frying Residual Oil	43.2	43.2	18.0	46.8	28.8	13.2

CONCLUSION

In the present work, B.cereus microorganism presented promising properties regarding surface tension and emulsification activity and potential for application in industries. The new biosurfactant produced has potential for industrial and environmental applications, especially in the oil industry, where it can be used for oil recovery.

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(PO34) Production of Levan by *Bacillus subtilis var. Natto* in Bioreactor

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Keywords: polysaccharide, bacteria, fermentation.

INTRODUCTION

Levan is an exopolysaccharide of fructose, bound by ß-(2→6) glycosidic bonds and possible ß-2(→1) branching, produced by microorganisms and plants (1). Levan produced by microorganism is extracellularis and its production is faster. Several microorganisms produce levan, among them: *Actinomyces viscosus* (2), *Bacillus subtilis* (3), *Bacillus subtilis var. natto* (4), *Zymomonas mobilis* (5). The Levan has many applications in several industries.

RESULTS AND DISCUSSION

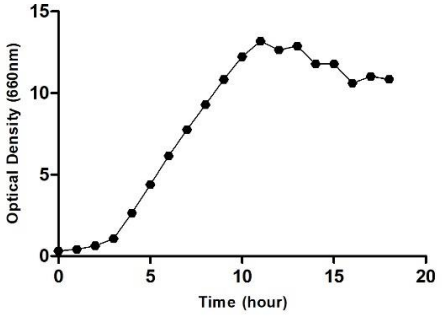
Levan was produced by *Bacillus subtilis var. Natto* in bioreactor. During the production were monitored the growth of Bacillus by spectrophotometry and the consumption of sucrose and quantified the fructose and glucose by chromatography of high efficiency.

Activation Medium	Production Medium
1.5g of meet extract, 1.5g of yeast extract, 5g of NaCl, 5g of peptone	250g of sucrose, 2g of urea, 5g of yeast extract, 1g of KH_2PO_4, 8g of K_2HPO_4, 1g of $MgSO_4.7H_2O$, 0.10g of $FeSO_4.7H_2O$, 0.0088g of $CuSO_4.5H_2O$, 0.0076g of $MnSO_4.H_2O$, 0.01g of $ZnSO_4.7H_2O$

Table 1. Medium used on the production of Levan

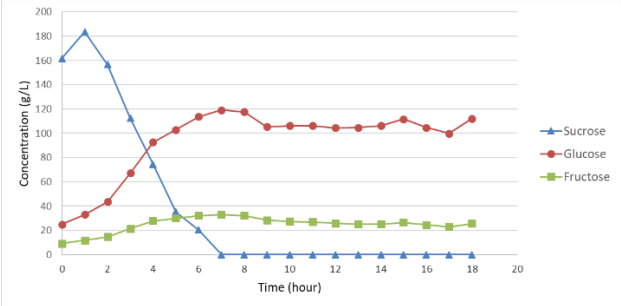
Bacillus subtilis var. natto produced 41 g/L of levan in 18h fermentation.

Figure 1. Growth of Bacillus subtilis during the production of Levan.



The figure 1 shows that the microorganism remained in the lag phase in the first 4 hours, was in linear growth for 8 hours and declined from the eleventh hour of the levan production. In production of Levan using the same microorganism, the authors used 20% (v/v) of sucrose in the medium and obtained an average of 40-50 g/L of levan in 21h of fermentation (6).

Figure 2. Quantification of sucrose, glucose and fructose during the production of Levan.



Freire dos Santos (7), studying optimal conditions for *Bacillus subtilis var natto*, found that the best condition to produce levana would be using 400g/L of sucrose, in a 16h fermentation lasting, would have 111.6g/L of the polysaccharide.

CONCLUSION

Bacillus subtilis var. Natto is able to produce Levan in bioreactor and produced 41g/L with 250g of Sucrose in the medium.

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(PO35) Valorization of cotton waste using deep eutectic solvents

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Keywords: Biomass; Enzymatic hydrolysis; Cotton residues; High pressure; Deep Eutectic Solvents.

INTRODUCTION

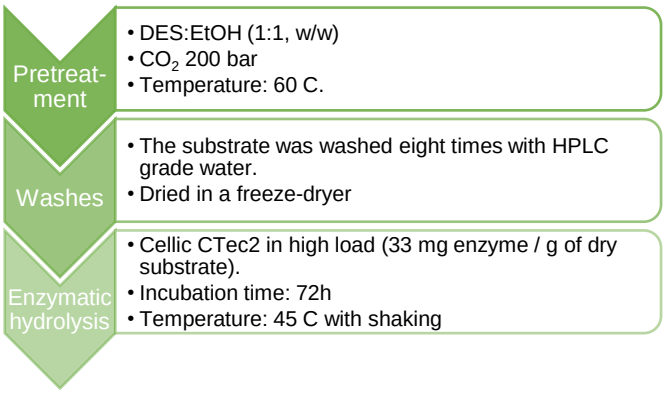
The global energy supply is currently based on fossil fuels, whose reserves are limited and will end in the near future. Biomass has a big potential to replace petroleum to produce biofuels and chemicals. Every year industry produces tons of organic residues and most of it is discarded or incinerated.

Cotton is a natural fiber that produces one of the highest amount of residues in the agro-industry, being able to generate waste almost five times the weight of the produced fiber. Brazil is the fifth largest world producer of refined cotton fibers. In 2014, the country produced 1.5 million tons of cotton fiber. The conversion of the resulting residues in value added products has been studied to decrease accumulation in environment and produce important sub-products, like bioethanol. [1,2]

To produce bioethanol from lignocellulose, it is necessary to pretreat biomass adequately to make the cellulose fraction, present in the complex matrix, more accessible to enzyme hydrolysis. In this work, cotton residues from spinning factories were soaked in different deep eutectic solvents (DES) together with ethanol and subjected to CO2 at high pressure in a continuous flow extractor and evalutated with respect to their hydrolyzability

RESULTS AND DISCUSSION

In the pretreatment we utilized three different DES, Choline:Urea (1:1), Choline:Oxalic acid (1:2), Potassium carbonate:Glycerol (1:100). Experiments were realized with biomass *in natura* (IN) and extractable free (EF). The process of these experiments is shown in the following scheme:



After the enzymatic hydrolysis, an aliquot of each sample was collected and analyzed with HPLC. The amount of glucose produced is show in Table 1.

Table 1. Amount of glucose produced in the enzymatic hydrolysis

DES	(%) Glucose	
	Biomass IN	Biomass EF
Ch:Ur	17,967	20,484
Ch:Oac	8,867	13,772
Pc:Gl	17,882	22,426
Native	8,282	9,601

Ch:Ur = Choline:Urea; Ch:Oac = Choline:Oxalic acid; Pc:Gl = Potassium carbonate:Glycerol; Biomass IN = Biomass *in natura*; Biomass EF = Biomass extract free.

On the basis of the enzymatic hydrolysis performance, the best cellulosic substrate was produced with extractable free biomass and the DES Potassium carbonate:Glycerol. For this sample a 233,6 % increase in glucose yield compared to the native material was observed.

CONCLUSION

The pretreatment with DESs was efficient to improve the glucose yield after enzymatic hydrolysis when compared with the untreated biomass. The samples extractable free presented better result than the biomass *in natura* for all experiments, and the most efficient DES was found to be Potassium carbonate:Glycerol.

New studies with the pretreated materials will be carried out to further increase the glucose yield utilizing different enzyme concentrations.

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UFPR FURB FAPESC CNPq

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(PO36) Influence of deep eutectic solvents on the enzymatic hydrolysis of cellulose

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Keywords: Deep Eutectic Solvent, Enzymatic hydrolysis, Cellulose

INTRODUCTION

Among the greatest current challenges is the need to meet society's demands for energy, liquid fuels, products and materials in a sustainable manner following the principles of Green Chemistry. Renewable resources such as lignocellulosic biomass, urban organic waste and industrial effluents can serve as feedstock for biorefinery and the production of biofuels. Brazil generates a large quantity of these lignocellulosic residues, being the largest one of the cultivation of the sugar cane, whose production was 768,090,444 tons in 2013, of which two thirds are residues.

The enzymatic hydrolysis of biomass is used for the depolymerization of cellulose and hemicellulose to produce fermentable sugars. The next step, the fermentation of these sugars results in second generation ethanol production, that can be used as liquid fuel in cars.

Deep Eutectic Solvent (DESs), which have the characteristics of both ionic liquids and organic solvents, have emerged as a new generation of green solvents with the characteristics of good atom efficiency and low cost. DESs have shown good potential for biomass pretreatment. After pretreatment the cellulosic fraction is usually enzymatically hydrolyzed and DESs may interfere in this process. In this preliminary study we have used DESs as reaction media or ingredient in order to evaluate their their effect on the efficiency of the enzymatic hydrolysis of cellulose.[1, 2]

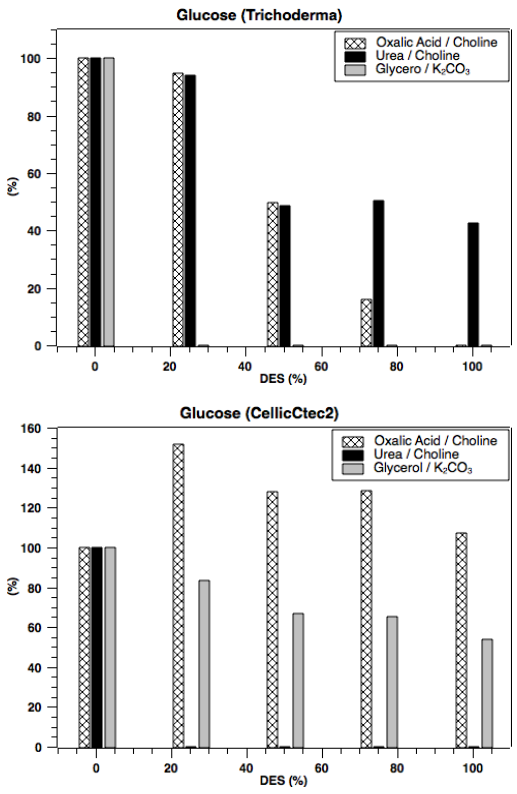
RESULTS AND DISCUSSION

DESs were evaluated as solventes or cosolvents in the enzymatic hydrolysis of cellulose in the form of filter paper.

We investigated the effects of two enzymes preparations during hydrolysis, using DESs and buffer mixtures. The DES/Buffer proportions and correlation between products were evaluate during enzymatic process of cellulose hydrolysis of filter paper Whatman Nr 1.

The activity of Enzyme preparations Cellic CTec2 (Novozymes A/S) and *Trichoderma reesei* (TC crude; Rhoem Enzyme) were studied in the following DESs proportions in buffer: 0, 25, 50, 75 and 100%. The Figure 1 and 2 show the amount of glucose produced at different concentrations of DESs with the enzymes *Trichoderma reesei* and CellicCtec2.

Figure 1. Amount of glucose produced with *Trichoderma reesei and Celic Ctec2*



CONCLUSION

Results obtained from enzymatic hydrolysis of filter paper with *T. reesei* indicate inhibition of enzymatic hydrolysis with increasing DESs proportion. For CelicCtec2 oxalic acid/choline seems to have a positive effect on activity, while urea/choline does not release any sugar at all. Further investigations are necessary for a better understanding of these results.

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CAPES, CNPq, FURB.

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(PO37) Bioconversion of syngas into biodegradable plastics using *Rhodospirillum rubrum*

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Keywords: Syngas fermentation, carbon monoxyde, polyhydroxyalkanoate, bioprocess, process analytical technology

INTRODUCTION

With the recent reports on plastic pollution, both the society and political authorities have now become aware of the importance of moving from a fossil-based to a bio-economy and of minimizing the environmental impact of plastic disposal. In this context, the bio-based and biodegradable poly(3-hydroxyalkanoates) (PHA) appear as promising alternatives to conventional polymers. Nevertheless, their implementation still requires process optimization in terms of sustainability and cost efficiency.

The bioprocess we present in this work relies on syngas fermentations using pyrolyzed organic wastes (CO, CO_2, H_2 and N_2) as main substrate and *Rhodospirillum rubrum* as CO-metabolizing and PHA-producing strain. Depending on the organic material and the pyrolysis condition different gas compositions can be detected. In order to identify the optimal growth conditions, a cutting-edge process analytical technology platform including measurements of dissolved oxygen and redox potential, gas concentrations by mass spectrometry, as well as cell concentration and PHA content by flow cytometry was set up to monitor the bioprocess and cell physiology (1-3).

Figure 1. A modern fermentation platform has been designed for the safe usage of syngas.

RESULTS AND DISCUSSION

First experiments revealed difficulties in culturing *R. rubrum* under anaerobic conditions directly on syngas as single carbon source. Therefore, a set of experiments had been designed to enhance the biomass concentration that could be used as starter culture for subsequent syngas fermentation. Aerobic growth on malate, succinate, and fructose, as well as mixtures of them were investigated revealing a maximal specific growth on malate (μ_{max} = 0.09 h^{-1}) and a growth yield of $Y_{X/S}$ = 0.24 g g^{-1}. The addition of 0.5 g L^{-1} yeast extract revealed not only a further improvement of growth rate and biomass yield but also a significantly better adaptation during the switch to syngas under anaerobic conditions. Alternatively, the performance of anaerobic starter culture was tested. Malate was found to be not a suitable carbon source, however, fructose showed good performances and could also be used as a transitionary substrate for the switch from aerobic to anaerobic, syngas conditions.

Interestingly, in all conditions there was practically no PHA formation without addition of acetate.

CONCLUSION

A strategy was developed for enhancing the productivity of semi-continuous processes by adding a heterotrophic, aerobic growth phase on fructose prior PHA production from syngas and acetate. This approach proved to be feasible but only if an anaerobic adaptation phase on fructose was included in-between.

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