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**PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS  
(BIOLOGIA CELULAR, MOLECULAR E MICROBIOLOGIA)**

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**SUGARCANE BAGASSE XYLAN ACETYLATION AND ITS EFFECT ON  
BIOPLASTIC FORMULATION, PROPERTIES AND BIODEGRADATION**

**JÚLIA RIBEIRO MARTINS**

**RIO CLARO - SP  
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**JÚLIA RIBEIRO MARTINS**

Dissertação apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Mestre em Biologia Celular, Molecular e Microbiologia.

Orientador: Prof. Dr. Michel Brienzo

**RIO CLARO - SP  
2022**

M386s      Martins, Júlia Ribeiro  
Sugarcane bagasse xylan acetylation and its effect on  
bioplastic formulation, properties and biodegradation /  
Júlia Ribeiro Martins. -- Rio Claro, 2022  
143 p. : il., tabs.

Dissertação (mestrado) - Universidade Estadual Paulista  
(Unesp), Instituto de Biociências, Rio Claro  
Orientador: Michel Brienzo

1. Bioplástico. 2. Bagaço de Cana-de-Açúcar. 3. Xilana.  
4. Acetilação. 5. Modificação Química. I. Título.

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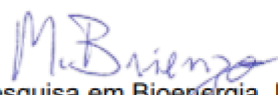
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Aprovada como parte das exigências para obtenção do Título de Mestra em CIÊNCIAS BIOLÓGICAS (BIOLOGIA CELULAR, MOLECULAR E MICROBIOLOGIA), área: Estrutura, Função e Produção de Biomoléculas pela Comissão Examinadora:

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Rio Claro, 25 de agosto de 2022

## ACKNOWLEDGMENT

I thank Professor Michel Brienzo, for being an inspiration and for awakening in me a love for research and bioenergy. Thank you for your teachings, partnership and patience. I thank my laboratory colleagues for the journey, for the exchange of experiences and knowledge, for the moments of relief and company. Special appreciation to João and Mateus for their direct contributions to this project, both with technical and personal support. To all the employees of IPBEN for their services and personal help. To Maria, for her kindness, care and affection when my energy had long disappeared.

I thank my boyfriend, Vitor, for the constant effort to be by my side and support me. For the endless love and affection in every moment of despair and exhaustion. I thank my friends for being a point of refuge. Livia, Verônica and Gabi, don't you ever forget how much impact you all had in my life.

I thank my family for their constant support. For offering me a foundation based on love and understanding, which gives me the courage to go further. I thank my brother Gabriel for his inspiring calm. To my brother Pedro for the advices, so remarkable and decisive in this trajectory. I thank Luiza for being such an inspiring researcher. To Morena for being a small, but bright and constant, ray of sunshine in my life. I thank my parents, Dirlene and Paulo, for being my biggest supporters and for always believing in me, sometimes even more than I would believe in myself. I dedicate every and each one of my achievements to my family.

I thank the SUS for the vaccine, CNPq, CAPES and FAPESP for all the financial support in so many years of research and Unesp for being like a second home in the last 9 years.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

## RESUMO

A aplicação de materiais biodegradáveis renováveis em substituição ao plástico é urgente. Devido ao crescimento da população mundial e ao aumento de seus hábitos de consumo, até 23 milhões de toneladas de resíduos plásticos estão sendo descartados em ambientes naturais, como ecossistemas aquáticos, trazendo danos irreversíveis à vida selvagem. Um biopolímero com potencial para a produção de bioembalagens são as hemiceluloses. No entanto, a molécula de hemicelulose apresenta algumas desvantagens, como baixa resistência térmica, e o bioplástico formado pode apresentar fragilidade e baixa resistência mecânica. Assim, modificações químicas de hemiceluloses podem ser aplicadas para superar tais problemas. A acetilação é um tipo de modificação química comumente aplicada às hemiceluloses e pode melhorar algumas das características do bioplástico. No presente estudo, xilana de cana-de-açúcar foi acetilada aplicando ácido sulfúrico como catalisador, e diferentes condições de reação como teor de ácido sulfúrico e tempo de reação foram testadas. Gras de acetilação baixo (0.45) e médio (0.9) foram alcançados, o que resultou em xilana de cana solúvel em água (como vantagem). A reação de acetilação foi responsável pela hidrólise da xilana devido à presença de ácido sulfúrico, que causou grande perda de massa. Isso pode ser observado principalmente em amostras com alto teor de ácido sulfúrico ou com tempo de reação aumentado. Os bioplásticos foram formados utilizando xilana acetilada com baixo e médio DS e xilana não acetilada, aplicando-se amido como copolímero e glicerol como plastificante. Xilana acetilada em blenda com amido na formação de bioplásticos é aqui relatado pela primeira vez. A água foi utilizada como solvente para a formação do bioplástico e o bioplástico à base de amido, sem adição de xilana, foi utilizado para comparação. A acetilação em graus baixos e médios causou um aumento na resistência mecânica e térmica. A acetilação DS de 0,45 aparentemente não afetou a taxa de desintegração do bioplástico, enquanto o DS médio de 0,9 pode ter sido responsável pela diminuição da atividade microbiana, o que foi atestado em análise de ecotoxicidade. Todas as amostras estudadas apresentam propriedade de barreira ao óleo até 30 dias, uma vantagem para aplicação em embalagens. Os resultados indicam que a acetilação em grau moderado trouxe a vantagem de criar xilana solúvel em água, e foi suficiente para melhorar as propriedades dos bioplásticos.

**Palavras-chave:** Bioplástico, Bagaço de Cana-de-açúcar, Xilana, Acetilação, Solúvel em Água, Modificação Química

## ABSTRACT

The application of renewable biodegradable materials replacing plastic is urgent. Due to world population growth and an increase in its consuming habits, up to 23 million metric tons of plastic wastes are being disposed of in natural environments, such as aquatic ecosystems, bringing irreversible damage to wildlife. A potential biopolymer for biopackaging production is hemicelluloses. However, hemicelluloses molecule presents some disadvantages such as low thermal resistance, and the bioplastic formed may present brittleness and low mechanical strength. Therefore, chemical modifications of hemicelluloses can overcome such issues. Acetylation is a common chemical modification applied to hemicelluloses, and can enhance some of the bioplastic features. In the present study, sugarcane xylan was acetylated applying sulfuric acid as catalyst, and different reaction conditions such as sulfuric acid content and reaction time were tested. Low (0.45) and medium (0.9) acetylation degrees substitution (DS) were achieved, which resulted in water-soluble sugarcane xylan (as an advantage). Acetylation reaction was responsible for xylan hydrolysis due to the presence of sulfuric acid, which caused for high mass loss. This could be seen specially in samples with high sulfuric acid content or with increased reaction time. Bioplastics were formed using acetylated xylan with low and medium DS and non-acetylated xylan, applying starch as copolymer and glycerol as a plasticizer. Acetylated xylan blended with starch in bioplastics formation is here reported for the first time. Water was used as a solvent for bioplastic formation, and starch-based bioplastic, without the addition of xylan was used for comparison. Acetylation in both low and medium degrees caused an increase in mechanical and thermal resistance. Acetylation DS of 0.45 apparently didn't affect the disintegration rate of the bioplastic, while medium DS of 0.9 may have been responsible for decreasing microbial activity, which was attested in ecotoxicity analysis. All of the samples studied present oil barrier property up to 30 days, an advantage for package application. The results indicate that acetylation in a moderate degree brought the advantage of creating water-soluble xylan, and it was sufficient for enhancing bioplastics properties.

**Keywords:** Bioplastic, Sugarcane Bagasse, Xylan, Acetylation, Water-soluble, Chemical Modification

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## CHAPTER I: INTRODUCTION AND OBJECTIVES

### 1. Introduction

The world population is growing significantly, and the current average population increase is estimated at 81 million people per year (WORLDMETER, 2022). That, associated with its increasing consumption habits, resulted in an increase in the production of plastic materials. It is estimated that 19 to 23 million metric tons of plastic waste entered aquatic ecosystems in 2016, which represents 11 % of plastic waste generated globally in that year (BORRELLE et al., 2020). Synthetic polymers degradation is a slow process, as they are not directly consumed by microorganisms. They are initially degraded by environmental factors, such as light, heat, moisture, and chemical conditions, which rupture the bonds of macromolecules (SINGH et al., 2021; WILKES et al., 2017). In this process, micro and nanoplastics are created, resulting in radical sites, which are essential for the action of microorganisms.

With increasing worldwide concerns over such problematics, much effort has been directed to the application of biopolymers in the production of bioplastics, such as cellulose and hemicelluloses. Hemicelluloses are a group of polysaccharides with highly diversified structures incorporating various sugars, usually presenting an amorphous structure. It accounts for 20 ~ 35 percent of plant biomass and is the second most abundant biopolymer after cellulose (LING et al., 2020). In sugarcane bagasse, xylose content represents up to 80 % of hemicelluloses (ALVES et al., 2021).

Acetyl group is a common substituent found on hemicelluloses in their natural state, usually connected to its backbone or ramification through ester linkages (GILLE; PAULY, 2012). It is thought that hemicelluloses acetylation was obtained through an evolutionary process, adjusting the plant biological functions, such as flexural properties of wood, polysaccharide interaction, plant growth, and stress resistance (GAO et al., 2017; GRANTHAM et al., 2017; JOHNSON et al., 2017; PAULY; RAMÍREZ, 2018; VOGEL et al., 2004; YAN et al., 2018; ZHANG et al., 2017; ZHU et al., 2014). However, for industrial processing, hemicelluloses are deacetylated during separation from other biomass polymers, due to alkaline solubilization frequently applied (PENG et al., 2012). Therefore, there is a great amount of free hydroxyl groups on hemicellulosic backbones, specifically two per xylose unit. The free hydroxyl groups present an opportunity for chemical modifications, which may be used to hinder the molecule drawbacks such as poor solubility in common

organic solvents and low thermal stability during sample processing (HUDA et al., 2005; LÖNNBERG et al., 2006). Among the modification strategies are esterification, including acetylation, oleoylation, lauroylation, fluorination, crosslinking, and etherification, including methylation, carboxymethylation, and benzylation (ALEKHINA et al., 2014; MARTINS; ABE; BRIENZO, 2022; PENG; REN; SUN, 2010; REN; SUN; PENG, 2008).

Chemical acetylation is an esterification reaction that substitutes hydroxyl groups in the hemicelluloses backbone with relatively hydrophobic acetyl groups (compared to the hydroxyl groups). Such modification influences the molecule properties, such as hydrophobicity, solubilization in different solvents, and thermal degradation. However, such properties will depend on the degree of acetylation obtained, as acetyl groups will be responsible for impeding hydroxyl groups to create hydrogen bonds with water and spontaneous intra-molecular hydrogen bonding, keeping the xylan chains from aggregating (STEPAN et al., 2013; ZHU et al., 2020). Also, relatively hydrophobic acetyl groups present good affinity with some organic solvents such as chloroform, dimethylsulfoxide, and tetrahydrofuran (SUN; HUGHES, 1999).

Numerous methods for hemicelluloses acetylation have been reported to achieve varying degrees of substitution and controlled hemicelluloses properties (AKKUS; OZKAN; BAKIR, 2018; MORAIS DE CARVALHO et al., 2019; REN; SUN; LIU, 2007; SUN; HUGHES, 1999; YILMAZ-TURAN et al., 2020; ZHU et al., 2020). Hemicelluloses may be acetylated by varying reaction conditions such as the solvents and catalysts applied, as well as reaction time and temperature, which will also influence the molecule degradation in the process. Therefore, this study evaluated the sugarcane bagasse xylan acetylation reaction conditions, applying acetic anhydride as an acetylation agent, and sulfuric acid as a reaction catalyst. The conditions were applied to produce xylan with different acetylation degrees, which were employed in starch/xylan-based bioplastics formulation. Bioplastic properties such as mechanical resistance, thermal degradation, water-solubility, oil barrier, and disintegration were evaluated.

## CHAPTER V: GENERAL CONCLUSIONS

The present study aimed to evaluate the influence of reaction conditions on the acetylation degree obtained, as well as the influence of the acetylation degree in the bioplastic formation and properties. It was possible to control xylan degree of acetylation by varying the reaction parameters such as catalyst content and reaction time. Increasing reaction time was responsible for increasing the DS obtained, while the higher sulfuric acid content caused hemicelluloses hydrolysis. Sugarcane bagasse xylan acetylation with DS ranging from 0.42 to 0.91 was achieved and confirmed by FTIR analysis. Acetylated xylan with such DS was water-soluble, which allowed for the application of water as a solvent, and starch and glycerol as copolymer and plasticizer in the bioplastic formation. Both low (0.45) and medium (0.9) acetylation DS showed influence on xylan-based bioplastic properties when compared to non-acetylated xylan and starch-based bioplastics.

In general, the low DS of 0.45 caused an increase in the bioplastics water affinity, while the medium DS of 0.9 has the opposite effect. For instance, low DS bioplastic presented a higher moisture content and water permeability rate when compared to the non-acetylated bioplastic, whereas medium DS bioplastic presented a reduction in both of those properties. This indicates an increase in the hydrophilicity of the low DS bioplastic due to the hindrance of aggregation among xylan chains. However, the low DS applied was not enough to cause an increase in hydrophobicity, which was observed in the bioplastic with medium DS of 0.9, which was indicated by water contact angle analysis.

Acetylation in both low and medium degrees caused an increase in the bioplastics Young's modulus up to 100 times higher when compared to non-acetylated xylan bioplastic. All of the bioplastics studied present oil barrier property, an advantage for package application. Although acetylation is known to hinder polymer biodegradation, the low DS applied in the present study of 0.45 apparently was sufficient to impede the bioplastics disintegration in soil. However, the medium DS of 0.9 may have hindered microbial activity, which caused lower alterations in the soil and, therefore, presented reduced ecotoxicity when compared to other bioplastics in the present work.

The results indicated that acetylation shows potential for enhancing bioplastics properties for packaging material, especially by the increase in mechanical and thermal resistance, and water barrier properties. Polymers chemical modifications have shown to be a great tool in order to enhance biomaterials properties, while still applying renewable sources as

feedstocks. Such researches are detrimental to gradually reducing the application of traditional petroleum-based plastics in society. Therefore, reducing environmental damage, such as threatening wildlife, spreading toxins, and contributing to global warming. Some examples of renewable polymers developed through research and produced and distributed on an industrial scale are cellulose acetate, polylactic acid, starch thermoplastics, and, more recently, ethylene derived from sugarcane, among others. Therefore, the investigation of increasing the application of renewable materials in a wider variety of fields is one of the main tools for a more sustainable society.

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