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PROGRAMA INTEGRADO (UNESP, USP E UNICAMP) DE PÓS-GRADUAÇÃO
EM BIOENERGIA

**PILOT-SCALE PRETREATMENT OF SUGARCANE STRAW USING A
COMBINED SULFURIC ACID- AND BISULFITE-CATALYZED STEAM
EXPLOSION PROCESS FOR CELLULOSIC ETHANOL PRODUCTION**

MARCELO BRANT WURTHMANN SAAD

Rio Claro - SP

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Tese apresentada ao Instituto de Pesquisa em Bioenergia de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências.

Orientador(a): Adilson Roberto Gonçalves

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To my beloved Thalys and Laura

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“Life is like riding a bicycle. To keep your balance you must keep moving”

Albert Einstein

RESUMO

A palha da cana-de-açúcar representa uma matéria-prima potencial para biorrefinarias celulósicas no Brasil, uma vez que a colheita mecanizada sem queima foi adotada. Diferentes estratégias têm sido propostas para obter sólidos pré-tratados altamente digeríveis por enzimas, visto que o consumo enzimático representa um fator crítico na viabilidade econômica da produção de etanol celulósico. O pré-tratamento por explosão a vapor catalisado por ácido tem sido considerado um método promissor para reduzir a recalcitrância da biomassa à hidrólise enzimática. No entanto, a formação de pseudo-lignina (humina) durante o pré-tratamento ácido pode reduzir significativamente o desempenho da hidrólise enzimática. O catalisador bissulfito pode reagir com precursores da pseudo-lignina, mitigando a formação de estruturas de humina altamente inibitórias. Este trabalho teve como objetivo avaliar o pré-tratamento da palha da cana-de-açúcar utilizando um processo combinado de explosão a vapor catalisado por ácido sulfúrico e bissulfito. A palha da cana-de-açúcar foi pré-tratada em um sistema de explosão a vapor em escala piloto, utilizando um procedimento de imersão e desaguamento para impregnar os catalisadores e preparar a palha para a reação de pré-tratamento. A influência de diferentes condições de embebição e pré-tratamento foi avaliada considerando os rendimentos de glicose e xilose obtidos durante o pré-tratamento e após a hidrólise enzimática com a enzima Cellic® CTec3 HS. Os materiais pré-tratados foram caracterizados por meio de diferentes técnicas para elucidar sua composição e estrutura, incluindo difração de raios X, espectroscopia FTIR, RMN de estado sólido de ^{13}C , microscopia confocal de varredura a laser e medições de relaxometria por RMN de ^1H . Um conjunto de experimentos iniciais catalisados por ácido indicou uma condição ideal de pré-tratamento com severidade próxima de 1,90. Os experimentos catalisados por ácido e bissulfito revelaram um efeito positivo significativo da combinação de pHs de embebição mais elevados e da adição de bissulfito na acessibilidade dos sólidos pré-tratados à enzima. A palha pré-tratada obtida com pH de embebição de 1,45, adição de 1,65% m/m de metabissulfito de sódio (com base na biomassa seca) e severidade de pré-tratamento em torno de 1,90 resultou em 100% de acessibilidade e atingiu os maiores rendimentos de hidrólise com as menores dosagens enzimáticas. No entanto, os resultados evidenciaram que em pHs de embebição mais baixos (~1,20) maiores dosagens de bissulfito são necessárias para mitigar a formação de pseudo-lignina e beneficiar a performance da hidrólise. Por fim, este trabalho mostrou a possibilidade de alcançar cerca de 80% de conversão dos polissacarídeos da palha em glicose e xilose sob condições realistas de processo de pré-tratamento e hidrólise, utilizando 10 mg de proteína (ensaio BCA) por grama de celulose na dosagem enzimática.

Palavras-chave

Palha de cana-de-açúcar. Explosão a vapor. Ácido diluído. Bissulfito. Etanol celulósico.

ABSTRACT

Sugarcane straw represents a potential feedstock for cellulosic biorefineries in Brazil since green mechanized harvesting without burning has been adopted. Different strategies have been proposed to obtain pretreated solids highly digestible to enzymes because enzyme consumption represents a critical factor in the economic feasibility of cellulosic ethanol production. Acid-catalyzed steam explosion pretreatment has been considered a promising method to reduce biomass recalcitrance for enzymatic hydrolysis. However, pseudo-lignin (humin) formation during acid pretreatment can significantly reduce enzymatic hydrolysis performance. Bisulfite catalyst may react with pseudo-lignin precursors, mitigating the formation of high inhibitory humin-like structures. This work aimed to evaluate the pretreatment of sugarcane straw using a combined sulfuric acid- and bisulfite-catalyzed steam explosion process. Sugarcane straw was pretreated in a pilot-scale steam explosion system, using a soaking and dewatering procedure to impregnate catalysts and prepare straw for the pretreatment reaction. The influence of different soaking and pretreatment conditions was evaluated considering the glucose and xylose yields obtained during pretreatment and after the enzymatic hydrolysis with Cellic® CTec3 HS enzyme. The pretreated materials were characterized through different techniques to elucidate their composition and structure, including X-ray diffraction, FTIR spectroscopy, ^{13}C solid-state NMR, confocal laser scanning microscopy, and ^1H NMR relaxometry measurements. A set of acid-catalyzed initial experiments indicated an optimum pretreatment condition at nearly 1.90 severity. The acid- and bisulfite-catalyzed experiments revealed a significant positive effect of the combined higher soaking pHs and bisulfite addition on pretreated solids' accessibility to the enzyme. The pretreated straw obtained with 1.45 soaking pH, 1.65% w/w sodium metabisulfite addition (dry biomass basis), and about 1.90 pretreatment severity resulted in 100% accessibility and achieved the highest hydrolysis yields under the lowest enzyme dosages. Nevertheless, the results evidenced that at lower soaking pHs (~ 1.20) higher bisulfite dosages are required to mitigate pseudo-lignin formation and benefit hydrolysis performance. Finally, this work showed the possibility of reaching about 80% conversion of the straw polysaccharides to glucose and xylose through realistic process conditions of pretreatment and hydrolysis using 10 mg protein (BCA assay) per gram of cellulose of enzyme dosage.

Keywords

Sugarcane straw. Steam explosion. Diluted acid. Bisulfite. Cellulosic ethanol.

TABLE OF CONTENTS

GENERAL INTRODUCTION	13
CHAPTER 1 – INDUSTRIAL PRETREATMENT OF LIGNOCELLULOSIC BIOMASS: A REVIEW OF THE EARLY AND RECENT EFFORTS TO SCALE-UP PRETREATMENT SYSTEMS AND THE CURRENT CHALLENGES	14
ABSTRACT	14
1 INTRODUCTION	15
2 EARLY EFFORT	18
2.1 Hydrolysis of Lignocellulosic Biomass.....	18
2.2 Pretreatment Overview	22
2.3 Thermochemical Systems.....	26
2.3.1 Vertical Digesters	26
2.3.2 Horizontal Digesters	28
2.3.3 Hybrid Systems.....	31
2.4 Thermomechanical Systems	32
3 RECENT EFFORT	35
3.1 POET-DSM and Andritz	35
3.2 Iogen and Raízen	40
3.3 Beta Renewables.....	45
3.4 GranBio	50
3.5 Abengoa.....	55
3.6 DuPont.....	60
3.7 Clariant and Valmet.....	68
3.8 Longlive Bio-tech	76
4 CURRENT CHALLENGES.....	81
4.1 Feedstock Quality Challenge.....	82
4.1.1 Biomass Collection and Storage	82
4.1.2 Biomass Cleaning	85
4.2 Operational Performance Challenge.....	88
4.2.1 Pretreatment Uptime	89
4.2.2 Digester Residence Time	90

5 FINAL CONSIDERATIONS	92
CHAPTER 2 - PILOT-SCALE PRETREATMENT OF SUGARCANE STRAW USING A COMBINED SULFURIC ACID- AND BISULFITE-CATALYZED STEAM EXPLOSION PROCESS FOR CELLULOSIC ETHANOL PRODUCTION	94
ABSTRACT	94
1 INTRODUCTION	95
2 MATERIALS AND METHODS	104
2.1 Sugarcane Straw	104
2.2 Pretreatment.....	104
2.2.1 Acid-catalyzed Initial Experiments	106
2.2.2 Combined Acid- and Bisulfite-catalyzed Experiments	107
2.2.3 Performance Calculations	108
2.3 Enzymatic Hydrolysis	109
2.3.1 Standard Protocol.....	109
2.3.2 Hydrolysis Optimization.....	110
2.3.3 Performance Calculations	110
2.4 Analytical Methods	112
2.4.1 Chemical Composition	112
2.4.2 Pretreated Solids Particle-size	112
2.4.3 X-ray Diffraction (XRD)	113
2.4.4 FTIR Spectroscopy	113
2.4.5 ¹³ C Solid-state NMR Spectroscopy	113
2.4.6 Confocal Laser Scanning Microscopy (CLSM)	114
2.4.7 ¹ H NMR Relaxometry Measurements	114
3 RESULTS AND DISCUSSION	115
3.1 Acid-catalyzed Initial Experiments	115
3.2 Combined Acid- and Bisulfite-catalyzed Experiments	120
3.2.1 S1-S4 Trials	120
3.2.2 S5-S6 Trials	136
3.3 Hydrolysis Optimization	138
4 CONCLUSIONS.....	140
REFERENCES	143

APPENDIX A – SUGARCANE STRAW COLLECTION	180
APPENDIX B – RAW STRAW USED DURING S1-S6 TRIALS.....	181
APPENDIX C – RAW STRAW PARTICLE-SIZE DISTRIBUTION.....	182
APPENDIX D – ACID SOAKING SYSTEM.....	183
APPENDIX E – PRETREATMENT SYSTEM.....	184
APPENDIX F – STRAW AND SOAKING DATA	185
APPENDIX G – ACID-CATALYZED INITIAL EXPERIMENTS DATA	186
APPENDIX H – S1-S4 COMBINED ACID- AND BISULFITE-CATALYZED EXPERIMENTS DATA	187
APPENDIX I – S5-S6 COMBINED ACID- AND BISULFITE-CATALYZED EXPERIMENTS DATA	188
APPENDIX J – HYDROLYSIS OPTIMIZATION DATA.....	189

GENERAL INTRODUCTION

This work is divided into two chapters. Chapter 1 provides a historical perspective on biomass pretreatment developments, describing the methods and systems that have reached the industrial scale. The information in Chapter 1 served as the foundation for developing this PhD thesis, particularly in understanding pretreatment technologies. Initially, Chapter 1 was intended to be concise; however, my curiosity about recent cellulosic ethanol industrial developments, combined with the time available during the 2020-2021 period (due to COVID-19 my experimental work could only begin in 2022), led to a more in-depth exploration. While writing this chapter, several points particularly caught my attention, including 1) the geniality of the early biomass conversion systems, which enabled continuous processes and served as inspiration for recent pretreatment developments; 2) the significant challenge of processing non-wood biomass at an industrial scale, even after demonstration-scale operations; 3) the largely overlooked success story of the non-wood pulping industry; and 4) the critical role of biomass quality in ensuring the viability of non-wood processing. Ultimately, Chapter 1 became a valuable discussion on lessons learned from industrial pretreatment operations, contributing to the literature on biomass pretreatment.

Chapter 2 describes the PhD project concept, the experimental approach, and the results obtained from the pretreatment experiments. Three main driving forces guided the development of this work: 1) conducting a pilot-scale study simulating Iogen's successful industrial pretreatment technology; 2) employing sugarcane straw as a feedstock, an underexplored material compared to sugarcane bagasse; and 3) proposing a solution to mitigate pseudo-lignin formation during pretreatment, as it is detrimental to the enzymatic hydrolysis of the pretreated biomass. Iogen's process is a dilute-acid steam explosion pretreatment successfully employed industrially to produce cellulosic ethanol from sugarcane bagasse. Sugarcane straw is considered a potential feedstock for fuel and chemical production in Brazil, given recent advancements in sugarcane harvesting that have enabled the efficient collection and transport of straw. Since enzyme consumption is a major contributor to cellulosic ethanol production costs, sodium metabisulfite was selected as a potential pretreatment additive to reduce pseudo-lignin formation. Finally, this project elucidates sugarcane straw behavior under a dilute-acid steam explosion process and provides a promising pretreatment process using a combined acid- and bisulfite-catalyzed condition.

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