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UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
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INTEGRATED PROCESS FOR PRODUCTION OF BRIQUETTES AND SECOND-GENERATION ETHANOL FROM AGRO-INDUSTRIAL AND URBAN WASTES

RODRIGO PAGANO MARTINS

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Tese apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Biologia Celular, Molecular e Microbiologia).

Orientador: Prof. Dr. Michel Brienzo

Rio Claro – SP
2026

M386i

Martins, Rodrigo Pagano
Integrated process for production of briquettes and second-generation ethanol from agro-industrial and urban wastes / Rodrigo Pagano Martins. -- Rio Claro, 2026
201 p.

Tese (doutorado) - Universidade Estadual Paulista (UNESP), Instituto de Biociências, Rio Claro
Orientador: Michel Brienzo

1. Biocombustíveis. 2. Gerenciamento de resíduos. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Dados fornecidos pelo autor(a).

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Rio Claro

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: INTEGRATED PROCESS FOR PRODUCTION OF BRIQUETTES AND SECOND-GENERATION ETHANOL FROM AGRO-INDUSTRIAL AND URBAN WASTES

AUTOR: RODRIGO PAGANO MARTINS
ORIENTADOR: MICHEL BRIENZO

Aprovado como parte das exigências para obtenção do Título de Doutor em Ciências Biológicas (Biologia Celular, Molecular e Microbiologia), área: Estrutura, Função e Produção de Biomoléculas pela Comissão Examinadora:

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DEDICATION

I dedicate this work to my wife Camila and my children Artur and Cecília.

AGRADECIMENTOS

Agradeço a Deus pela força da vida que me mantém firme, mesmo nos momentos difíceis.

À minha esposa Camila, por sua companhia e dedicação à nossa família e aos meus filhos Artur e Cecília, por serem as alegrias da minha vida e fontes de energia.

Aos meus pais Ambrósio e Izilda (*in memoriam*), pelo incentivo e ajuda nos meus estudos. Sinto muita saudade, mãe.

Ao meu orientador, Prof. Dr. Michel Brienzo, pela oportunidade, apoio e atenção.

À Prof. Dr. Andrea de Conti e ao seu aluno de doutorado Marcos Paulo Granada, da UNESP - Rosana, pelo apoio com a briquetagem.

Aos meus amigos do IPBEN – Jefferson, Rogério, Carol Forsan, Carol Freitas, Beatriz, Manuel, João Vitor, Alisson, Danieli, Mateus, Renato, Josiel, todos contribuíram com trabalho de equipe e ajuda.

Agradeço também à UNESP pela oportunidade concedida através da parceria com o Centro Paula Souza, que me permitiu ingressar no programa de mestrado e, posteriormente, no doutorado.

O presente trabalho foi realizado com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001,

O presente trabalho foi realizado com o apoio do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, processo nº 305078/2025-9, 403481/2025-2)

O presente trabalho foi realizado com apoio da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brasil. Processo nº 2017/22401-8. As opiniões, hipóteses e conclusões ou recomendações expressas neste material são de responsabilidade do(s) autor(es) e não necessariamente refletem a visão da FAPESP.

EPIGRAPH

"Some men see things as they are and say, 'Why?' I dream of things that never were and say, 'Why not?'

George Bernard Shaw

ABSTRACT

This work proposed the development of an Integrated Biorefinery process for the simultaneous production of second-generation (2G) ethanol and densified solid biofuels (briquettes) from lignocellulosic residues of agro-industrial and urban origin: Banana Pseudostem (BPS), Guava Seed Cake (GSC), and Urban Tree Pruning (UTP). The study was divided into four main stages: pretreatment optimization, scale-up, briquetting, and fermentation. A 2^3 experimental design was carried out to optimize the pretreatment conditions with diluted sulfuric acid, defining the best conditions of 40 min, 140 °C, and 3.5% H_2SO_4 (w/v). Statistical analysis confirmed that the solubilization of xylose (acid hydrolysis) and glucose (enzymatic hydrolysis) were respectively: 5.66 $g.L^{-1}$ and 8.60 $g.L^{-1}$ for BPS; 9.24 $g.L^{-1}$ and 2.33 $g.L^{-1}$ for GSC; and 8.13 $g.L^{-1}$ and 1.06 $g.L^{-1}$ for UTP. In a bench up-scale diluted acid pretreatment the final glucose concentration using BPS, GSC, and UTP was 8.10 $g.L^{-1}$, 0.61 $g.L^{-1}$, and 5.07 $g.L^{-1}$, and the xylose concentration was 4.51 $g.L^{-1}$, 4.0 $g.L^{-1}$, and 6.22 $g.L^{-1}$, respectively. These results validated the fractionation process for generating a liquor rich in fermentable sugars. There was also the production of xylooligosaccharides (XOS) during bench up-scale in the pretreatment, with values of 41.51% (xylobiose – X2) and 8.33% (xylopentaose – X5) for BPS, 43.1% (X2) for GSC, and 20.57% (X2) and 7.75% (X5) for UTP. The solid residue, enriched in cellulose and lignin, was subjected to briquetting under conditions of 90 °C, 10 MPa, 3 min residence time, and 7 min cooling time. All briquettes demonstrated exceptional durability, exceeding 98.7%, with negligible volumetric expansion. The pre-treated GSC residues exhibited the most advantageous characteristics, including lower moisture content, higher apparent density, superior heating value (HHV), and improved composition in terms of fixed carbon. The comparison between raw and pre-treated residues revealed that the pre-treated materials presented improved energy density and HHV, confirming the benefit of fractionation. Although the raw GSC showed the best performance in the combustion test (reaching and sustaining the highest temperature), the densification results consolidate the logistical and energy potential of the pre-treated materials. The fermentation stage utilized the thermophilic and xylose-utilizing bacterium, *Parageobacillus caldoxylosilyticus*, applied to the hydrolysate generated in the pretreatment. The evaluation demonstrated that the condition of 10 $g.L^{-1}$ of sugars was ideal, with UTP and BPS showing the greatest potential. However, increasing the sugar concentration led to a drop in efficiency, indicating the microorganism's high sensitivity to inhibitors and a tendency to divert the metabolic flow towards the production of byproducts (organic acids) at the expense of ethanol. The low tolerance and the marked reduction in post-peak production were identified as crucial biological bottlenecks for large-scale application. The results of the study highlight the potential of the residues and techniques used for the integrated production of solid and liquid biofuels, validating the concept of an Integrated Biorefinery. The main breakthrough relates in identifying process bottlenecks in fermentation, highlighting the need for metabolic engineering for the industrial use of thermophilic bacteria and validating a commercial solution for briquettes.

Keywords: banana pseudostem, guava seed cake, urban tree pruning, pretreatment, fruit waste, biofuels.

RESUMO

O presente trabalho propôs a elaboração de um processo de Biorrefinaria Integrada para a produção simultânea de etanol de segunda geração (2G) e biocombustíveis sólidos densificados (briquetes) a partir de resíduos lignocelulósicos complexos de origem agroindustrial e urbana: Pseudocaule de Banana (BPS), Torta de Semente de Goiaba (GSC) e Poda de Árvores Urbanas (UTP). O estudo foi dividido em quatro etapas principais: otimização do pré-tratamento, aumento de escala, briquetagem e fermentação. Foi realizado um planejamento experimental 2^3 para otimizar as condições do pré-tratamento com ácido sulfúrico diluído, definindo as melhores condições em 40 min, 140 °C e 3,5% H_2SO_4 (m/v). A análise estatística confirmou que a solubilização de xilose (hidrólise ácida) e glicose (hidrólise enzimática) foram, respectivamente: 5,66 $g.L^{-1}$ e 8,60 $g.L^{-1}$ para BPS; 9,24 $g.L^{-1}$ e 2,33 $g.L^{-1}$ para GSC; e 8,13 $g.L^{-1}$ e 1,06 $g.L^{-1}$ para UTP. Em uma etapa de aumento da escala em laboratório (bench *up-scale*), onde a hidrólise enzimática foi eliminada, a concentração final de glicose em BPS, GSC e UTP foi de 8,10 $g.L^{-1}$, 0,61 $g.L^{-1}$, e 5,07 $g.L^{-1}$, e a de xilose foi de 4,51 $g.L^{-1}$, 4,0 $g.L^{-1}$, e 6,22 $g.L^{-1}$, respectivamente. Esses resultados validaram o processo de fracionamento para gerar um licor rico em açúcares fermentáveis. Houve também a produção de xilooligosacarídeos (XOS) durante o aumento de escala no pré-tratamento, com valores de 41,51% (xilobiose – X2) e 8,33% (xilopentaose – X5) para BPS, de 43,1% (X2) para GSC e de 20,57% (X2) e 7,75% (X5) para UTP. O resíduo sólido, enriquecido em celulose e lignina, foi submetido à briquetagem nas condições de 90 °C, 10 Mpa, 3 min de residência e 7 min de resfriamento. Todos os briquetes demonstraram durabilidade excepcional, ultrapassando 98,7%, com expansão volumétrica insignificante. Os resíduos de GSC pré-tratados exibiram as características mais vantajosas, incluindo menor teor de umidade, maior densidade aparente, valor de aquecimento superior (HHV) e composição aprimorada em termos de carbono fixo. A comparação entre resíduos brutos e pré-tratados revelou que os materiais pré-tratados apresentaram densidade de energia e HHV aprimorados, confirmando o benefício do fracionamento. Embora o GSC bruto tenha apresentado o melhor desempenho no teste de combustão (atingindo e sustentando a temperatura mais alta), os resultados de densificação consolidam o potencial logístico e energético dos materiais pré-tratados. Paralelamente, a etapa de fermentação utilizou a bactéria termofílica e xilose-utilizadora, *Parageobacillus caldoxylosilyticus*, aplicada ao hidrolisado gerado no pré-tratamento. A avaliação demonstrou que a condição de 10 $g.L^{-1}$ de açúcares foi a ideal, com UTP e BPS apresentando o maior potencial. Contudo, o aumento da concentração de açúcares levou a uma queda drástica de eficiência, indicando a alta sensibilidade do microrganismo aos inibidores e uma tendência a desviar o fluxo metabólico para a produção de subprodutos (ácidos orgânicos) em detrimento do etanol. A baixa tolerância e a acentuada redução da produção pós-pico foram identificadas como gargalos biológicos cruciais para a aplicação em escala. Os resultados do estudo evidenciam o potencial dos resíduos e das técnicas utilizadas para a produção integrada de biocombustíveis sólidos e líquidos, validando o conceito de Biorrefinaria Integral. O principal avanço reside na identificação dos gargalos de processo na fermentação, direcionando a necessidade de engenharia metabólica para o uso industrial da bactéria termofílica e na validação de uma solução comercial para os briquetes.

Palavras-chave: pseudocaule de banana, torta de semente de goiaba, poda de árvore urbana, pré-tratamento, resíduo de frutas, biocombustíveis.

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THESIS INTRODUCTION

The growing concern about climate change and the search for sustainable energy sources have led to the exploration of alternatives to fossil fuels (Cherwoo et al., 2023). In this context, biofuels, especially those derived from agro-industrial and urban waste have gained prominence as a viable solution for mitigating environmental impacts and promoting a more circular economy (Mujtaba et al., 2023). Agro-industrial waste, generated in agriculture and the food industry, such as straw, bagasse, peels, and crop wastes and urban waste, which includes tree brunches, papers, woods and textile fibers, represents a vast source of biomass that can be converted into fuels (Gonçalves; Dos Santos; De Macedo, 2015), such as liquid biofuel like second-generation ethanol and solid fuel like briquettes (Wu et al., 2021).

Second-generation ethanol can be produced from non-food biomass using lignocellulosic waste and this approach has the advantage of not competing directly with food production while adding value to materials that might otherwise be discarded (Turner; Pansino; De Lemos, 2023). The conversion processes of this biomass involve pretreatments, enzymatic hydrolysis, and fermentation steps aimed at maximizing the release of fermentable sugars, enabling efficient ethanol production. The use of advanced technologies, such as enzymes and genetically modified microorganisms contributed to improve the efficiency of this process, making it more competitive with fossil fuels (Aditiya et al., 2016; Dos Santos et al., 2016).

In addition to ethanol, agro-industrial and urban waste can also be used to produce briquettes, a form of solid biofuel. Briquettes are made with compacted biomass that has a high energy density and can be used as an alternative to coal and other fossil fuels in heating and energy generation processes (Dinesha; Kumar, Rosen, 2019). The production of briquettes from lignocellulosic waste is an effective way to add value to materials that would otherwise be considered waste. Moreover, the use of briquettes contributes to reducing carbon emissions, as the combustion of biomass is considered a carbon-neutral energy source, since the CO_2 released during combustion is reabsorbed by plants during their growth (De Conti et al., 2022).

The integration of liquid and solid biofuel production from agro-industrial and urban waste offers several advantages. First, the utilization of waste reduces pressure on landfills (Turner; Pansino; De Lemos, 2023) and minimizes the environmental impacts associated with the decomposition of organic waste, which generates methane emissions, a potent greenhouse gas. Second, integrated production allows for the optimization of available resources, increasing the efficiency and sustainability of conversion processes (Souza; Seabra, 2013). For example, the by-products of ethanol production can be used as raw materials in briquette manufacturing, creating a closed-loop system that maximizes the recovery of energy. Additionally, biofuel production from waste contributes to energy security by diversifying energy sources and reducing dependence of fossil fuels (Perez et al., 2020). However, despite the advantages, the implementation of integrated biofuel production processes from waste has significant challenges. The variability in the chemical composition of waste, the efficiency of pretreatment processes, and the need for investments in technology are factors that need to be considered. The development of more efficient conversion technologies, as well as the creation of suitable logistics chains for the collection and processing of waste, are essential to ensure the economic viability of these processes (Perez et al., 2020).

Continuous research and innovation are crucial to overcoming these challenges. Collaborative projects between universities, research institutions, and industry can facilitate knowledge exchange and the development of new technologies that make biofuel production from waste more accessible and competitive (Rodionova et al., 2017). Furthermore, public policies that encourage the use of biofuels, such as subsidies and favorable regulations, can play an important role in promoting the adoption of these energy alternatives (Ebadian et al.,2020).

In conclusion, the use of agro-industrial and urban waste in the production of biofuels, such as second-generation ethanol and briquettes, represents an innovative and sustainable approach to energy generation. The integrated production of these fuels not only adds value to materials that would otherwise be discarded but also contributes to the reduction of greenhouse gas emissions, promotes energy security, and provides economic opportunities for urban and rural communities. The future of biofuel production depends on the ability to address the challenges associated with the variability of waste and process efficiency, as well as on the ongoing commitment to research, innovation, and public awareness.

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THESIS GENERAL CONCLUSION AND FUTURE PERSPECTIVE

The work carried out demonstrated the technical feasibility and robustness of the Integrated Biorefinery concept applied to the valorization of non-conventional agro-industrial and urban waste (BPS, GSC, and UTP). The objective of producing solid and liquid biofuels in an integrated and optimized way was fully achieved. The first fundamental conclusion is that the chemical pretreatment strategy was highly effective in biomass fractionation, confirming the upscale's ability to simultaneously generate two high-value-added raw materials: a hydrolysate rich in fermentable sugars for second-generation ethanol and a lignin-enriched pretreated for briquettes. The successful optimization of the process ensured the conversion of hemicellulose with minimization of the formation of inhibitory compounds.

The second essential conclusion lies in the energy valorization of the solid residue. Briquetting conferred significantly improved logistical and energy properties to the pretreated materials compared to the raw biomass. Densified solid biofuels showed superior characteristics of durability, energy density, and combustion profile, highlighting GSC as a promising material, and UTP as a sustainable solution for urban waste that is difficult to dispose of.

The fermentation stage marked a crucial advance in the research, as it utilized the thermophilic and xylose-utilizing bacterium, *Parageobacillus caldoxylosilyticus*, in complex lignocellulosic hydrolysates from non-traditional biomass. This approach represents one of the first studies to investigate the application of this microorganism in substrates generated from residues such as BPS, GSC, and UTP. The evaluation confirmed the bacterium's potential for sugar consumption, essential for second-generation ethanol. However, the study also identified the main biological bottleneck: the bacterium demonstrated extreme sensitivity to high sugar concentrations in the hydrolysate, resulting in a probable metabolic shift towards byproducts. Although thermophilia promises faster and more contamination-resistant processes, genetic engineering work will be necessary to improve ethanol production by the bacterium. In summary, the research validates a circular and waste-free biorefinery model.