SCALE-UP OF ENZYMATIC PRE-TREATMENTS FOR LIGNOCELLULOSIC BIOMASS AIMING AT THE PRODUCTION OF HIGH-VALUE-ADDED BIOPRODUCTS: A REVIEW

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ABSTRACT

Reducing waste generation is essential to mitigate the effects of pollution already observed today. The industrial sector is one of the largest waste producers globally, requiring a specific approach to dealing with it. For example, fruit and vegetable waste (FVW) can undergo fermentation processes that generate various bioproducts, such as fuels, food additives, pharmaceuticals, and chemical products. However, lignocellulosic biomasses, due to their complex structure, need to be subjected to single or combined pre-treatments, whether physical, chemical, and/or enzymatic, to release sugars, mainly glucose and xylose, to be used in bioprocesses. Several pretreatments have been investigated over the years. However, most have been conducted on a laboratory scale, representing an obstacle to practical industrial application. Enzymatic hydrolysis is highly specific, occurring under mild pressure and temperature conditions, generating less waste than other methods, resulting in lower environmental impacts. This review discussed the potential for scaling up enzymatic pretreatment to obtain hydrolysates, which can be used as raw material in industrial bioprocesses.

Keywords: Enzymatic hydrolysis. Biorefinery. Biofuels. Cellulose.

1. INTRODUCTION

The growing and inevitable global waste production makes it necessary to establish practical approaches for its reuse or treatment. In 2019, the global population generated 931 million metric tons of food waste, corresponding to around 17% of the total food available. This waste contributes approximately 9% of global greenhouse gas emissions from the food system, totaling 17.9 billion metric tons of carbon dioxide[1]. Agro-industrial waste has a complex composition, mostly cellulose, hemicellulose, and lignin. In turn, these biomass fractions can be pre-treated to release fermentable sugars, mainly glucose and xylose, which could be a carbon source in processes via microbial fermentation to produce renewable fuels, food ingredients, pharmaceuticals, and various chemical commodities.

Although this conversion of lignocellulosic fractions into sugars is possible, some barriers make the process difficult, such as, for example, the high organization and crystallinity of cellulose, the recalcitrance of hemicellulose and the presence of lignin, which acts as a physical and chemical barrier[2]. These factors make it difficult for enzymes or microorganisms to convert sugars. Several pretreatment methods, whether physical, chemical, or enzymatic, have been explored to overcome these challenges, highlighting enzymatic hydrolysis as a promising approach. Thus, enzymatic methods offer advantages, such as high specificity, the requirement for mild conditions to occur, such as low temperatures, and the non-generation of toxic products, the production of enzymes is still an expensive process, which often limits studies to laboratory scales[3]. In this context, scaling up becomes essential to enable the industrial application of these processes.

The present work proposes to review the literature on scaling up enzymatic processes. Enzymes are used for the hydrolysis of vegetable biomass, aiming to obtain fermentable sugars for conducting bioprocesses to produce value-added bioproducts sustainably.

2. MATERIAL & METHODS

This review was conducted using the Scopus database in February and March 2024. Search terms included "enzymatic hydrolysis," "scale-up," and "lignocellulosic biomass". The focus was on articles, reviews, and case studies discussing the scale-up of enzymatic pre-treatments for lignocellulosic biomass to produce high-value-added bioproducts, mainly biofuels. Articles were selected based on relevance and quality to provide a concise analysis on the topic.

3. RESULTS & DISCUSSION

In Brazil, diverse fruit production, such as orange, banana, and mango, generates significant agro-industrial waste like straw and peels. Around 10% of fruit is lost during harvesting and up to 50% during processing, resulting in approximately 140 gigatons of annual waste[4]. This waste, influenced by plant species and soil properties, presents environmental and social challenges. Lignocellulosic biomass, a key biomass type, is vital for sustainable biofuel production, especially second-generation ethanol, which can reduce CO_2 emissions by up to 90% compared to fossil fuels. Biotechnological processes can convert this waste into biofuels like hydrogen and alcohol without increasing CO_2 emissions. Lignocellulosic biomass consists of cellulose, hemicellulose, and lignin, with carbohydrates making up 55±76% of the dry weight, including glucose and xylose polymers. Before microbial fermentation into ethanol or other products, these polymers require enzymatic breakdown into fermentable sugars, a crucial step due to its impact on the total production cost of lignocellulosic biofuels[2].

Hydrolysis can be achieved through either acid or enzymatic methods, with a growing emphasis on environmentally sustainable and economically viable technologies. Acid hydrolysis of cellulose typically requires high temperatures, leading to glucose degradation and reduced yields. In contrast, enzymatic approaches offer higher conversion efficiency, reduced byproduct formation, milder operating conditions, and lower energy requirements[5]. Enzymatic hydrolysis is crucial in lignocellulosic biorefinery processes using feedstocks from fruit and vegetable residues, generating fermentable sugars like glucose and xylose. These sugars can then be converted into high-value products, including biofuels, organic acids, and bioplastics, through microbial action.

Despite its promise, enzymatic hydrolysis of lignocellulosic biomass for large-scale applications faces challenges. Increased degradation products and sugar concentrations can inhibit enzyme activity, while higher solids loading can lead to water constraints and viscosity issues, affecting mass transfer efficiency. Strategies to mitigate these challenges include reducing particle size and using fed-batch methods. Scaling up high-solids enzymatic hydrolysis is challenging due to the non-Newtonian nature of lignocellulosic slurries, necessitating specialized reactors like free-fall horizontal configurations and helical impellers. While promising, the peg mixer configuration remains underexplored. The stability of enzymes across different reactor designs needs evaluation. Given the relationship between biomass slurry rheology, biomass type, and pretreatment, a systematic approach can guide effective pretreatment strategies. However, measuring these rheological properties is still challenging due to equipment limitations, highlighting the need for online measurements[6].

It was discovered that smaller initial particle sizes, ranging from 33 µm to 850 µm, in sawdust slurries led to higher enzymatic reaction rates, increased cellulose conversion to glucose, and lower viscosity[7]. For instance, at 10% w/w solids loading, viscosity decreased significantly. This reduction in particle size can shorten enzymatic hydrolysis time, allow for higher solids loading, and potentially reduce reactor sizes in large-scale processing. Roller bottle reactors were assessed for the Simultaneous Saccharification and Fermentation (SSF) of lignocellulosic biomass (LCB) on a laboratory scale and, in this research, five different biomass pretreatments and two enzyme preparations were involving, demonstrated the system's efficiency[8]. While SSF has inherent mass transfer limitations, these can be effectively managed with limited mixing. The reactor system's scalability, ranging from 125 mL benchtop to 2 L pilot scale at 20% initial insoluble solids, proves valuable for evaluating new biomass pretreatments and enzyme systems at elevated solids loading.

Fed-batch operations effectively maintain a low viscosity in high total solids enzymatic hydrolysis. The choice between batch and fed-batch operations is influenced by key parameters, including initial solids, and feeding frequency, which are linked to biomass characteristics and pretreatments. While fed-batch operations can reduce energy costs, they don't necessarily lead to higher sugar concentrations[6]. An 8 L scraped surface bioreactor was employed for enzymatic hydrolysis, improving heat transfer and preventing particle settling more effectively than conventional stirred tanks. Their optimal efficiency was found at 20% initial solids loading, highlighting the bioreactor's superiority over shake flasks[9]. In a related development, an integrated industrial system for non-isothermal simultaneous solid-state hydrolysis, fermentation, and separation was designed[10]. This system, operating at approximately 50°C for hydrolysis and 37°C for fermentation with 25% solids loading, addresses challenges such as low solids content and sugars feedback inhibition. It enables yeast to consume glucose promptly and facilitates online ethanol separation. Successfully scaled up from a 300 L pilot reactor to a 110 m^3 industrial facility, this innovative approach marks a significant advancement in the field.

In addition to their use in enzymatic hydrolysis, some studies have reported the use of enzymes as a biological pretreatment of lignocellulosic biomass, which can be compared to classical methods such as acid and alkaline hydrolysis. This pretreatment is one of the latest and most efficient biological strategies for lignocellulosic biomass application[11]. Several studies have focused on evaluating the action of lignin-degrading enzyme systems, such as Lignin peroxidase, Manganese peroxidase, and laccase, along with other enzymes indirectly involved, like aryl-alcohol oxidase, glyoxal oxidase, and glucose oxidase[12,13]. These enzymes catalyze various chemical transformations in lignin, ultimately breaking it down into monomers. In the case of wheat straw, pretreatment with laccase using 1-hydroxybenzotriazole led to a significant 48% lignin removal and a 60% increase in glucose yield[14].

Similarly, pretreating corn straw with crude enzyme solutions from lignin-degrading enzymes for two days resulted in a 50.2% increase in sugar hydrolysis rate (323 mg/g) and lower levels of microbial inhibitors compared to acid pretreatment[15]. In modern biorefining approaches, enzymes that degrade lignin play a crucial role as potent tools in biotechnology[11]. However, the high cost required to produce the enzyme remains a significant barrier to the large-scale application of lignin-degrading enzymes. However, the scale of biofuel plants is not solely limited by technology. Factors such as the high costs of enzyme production and market costs, the required volume of biomass, and logistical challenges in collecting and transporting feedstock within a reasonable radius and cost also significantly influence the scale-up process (SILVA et al., 2020)[6]. As highlighted in this article, the main obstacles to industrial biorefining processes encompass high capital and operational expenses, technical immaturity, and challenges in scaling up[16].

Figure 1 schematically represents the potential of lignocellulosic waste biorefineries, including fruit and vegetable waste as raw material, the wide range of value-added bioproducts that can be obtained, and the main limitations and challenges in scaling up these processes, from laboratory-scale flasks to industrial production.

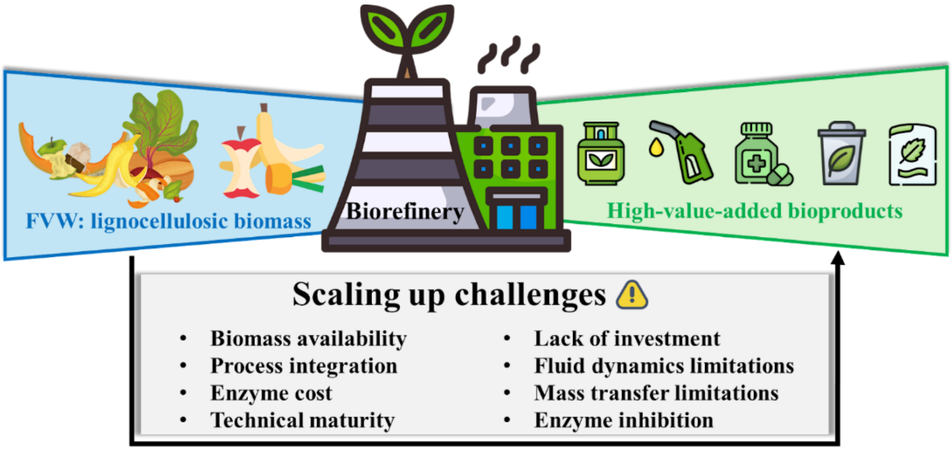

Figure 1 - The potential for utilizing lignocellulosic biomass waste to produce high-value-added bioproducts, and the challenges faced in scaling up and large-scale production.

The integration of artificial intelligence (AI) into biorefineries stands out as an innovative and efficient approach to address some of these operational challenges. This technological incorporation not only optimizes biorefineries' potential but also facilitates their market penetration, promoting circular and sustainable production models. AI-driven "smart biorefineries" exemplify enhanced sustainability, efficiency, and environmental compatibility by swiftly analyzing comprehensive datasets, discerning operational patterns, and bolstering the productivity of bio-based processes[17].

4. CONCLUSION

In Brazil, the diverse fruit production results in substantial agro-industrial waste, presenting both environmental and logistical challenges. This lignocellulosic biomass holds potential for sustainable biofuels and high value-added products. However, high enzymatic production costs and operational challenges hinder its efficient utilization. Promising solutions like enzymatic pretreatments and advanced reactors are on the horizon, with artificial intelligence emerging as a promising tool to optimize these processes. Despite these advancements, scaling up remains limited by technological barriers and logistical constraints. Addressing these challenges is essential to unlock the full potential of agricultural waste for biofuels and high value-added products.

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