JCEC
THE JOURNAL OF ENGINEERING AND EXACT SCIENCES

OPEN ACCESS

The Journal of Engineering and Exact Sciences – jCEC, Vol. 08 N. 11 (2022)
journal homepage: https://periodicos.ufv.br/jcec
eISSN: 2527-1075
ISSN: 2446-9416

Factors that influence the enzymatic hydrolysis of agricultural wastes for ethanol production: a review

Fatores que influenciam a hidrólise enzimática de resíduos agrícolas para produção de etanol: uma revisão

Factores que influyen en la hidrólisis enzimática de residuos agrícolas para la producción de etanol: una revisión

Article Info:
Article history: Received 2022-08-01/ Accepted 2022-12-20 / Available online 2022-12-22
doi: 10.18540/jcecvl8iss11pp15137-01e

Natasha Kevellyn dos Santos
ORCID: https://orcid.org/0000-0003-3099-1936
Federal University of Uberlândia (UFU), Brazil
E-mail: natasha.santos@ufu.br
Daniel Pasquini
ORCID: https://orcid.org/0000-0001-7441-5143
Federal University of Uberlândia (UFU), Brazil
E-mail: daniel.pasquini@ufu.br
Milla Alves Baffi
ORCID: https://orcid.org/0000-0003-0983-889X
Federal University of Uberlândia (UFU), Brazil
E-mail: milla.baffi@ufu.br

Abstract
Lignocellulosic biomass, such as agricultural and forestry residues, can be reused and serve as sources of sugars for the production of second-generation ethanol (2G) and other bioproducts. However, these wastes are composed by molecules of difficult degradation, which require steps of pretreatment and enzymatic hydrolysis for their bioconversion into fermentable sugars. At the same time, chemical substances with a potential inhibitory effect on the microbial metabolism can also be produced after the pretreatments and hinder the overall yield of the hydrolytic process. For an efficient and low-cost hydrolysis, homemade enzymes produced from agroindustrial residues, such as sugarcane bagasse, can be employed. However, a set of parameters might be adjusted, such as: kind of pretreatment, enzyme load, solids load, hydrolysis time and use of additives, to improve the yields in free sugars using these onsite enzymatic preparations. In this sense, studies involving the optimization of the conditions of pretreatment and saccharification are essential to increase the bioconversion rate of lignocellulose. These strategies are important for the production of value-added products from these wastes and, consequently, offer a correct and profitable destination to them. Hence, this study presents a review of the main features that influence the enzymatic hydrolysis of agricultural wastes and the yield in reducing sugars for ethanol production.
Keywords: Agricultural Wastes. Lignocellulose. Pretreatment. Hydrolysis. Homemade Enzymes.

Resumo
A biomassa lignocelulósica, como resíduos agrícolas e florestais, pode ser reaproveitada e servir como fonte de açúcares para a produção de etanol de segunda geração (2G) e outros bioprodutos. No entanto, esses resíduos são compostos por moléculas de difícil degradação, que requerem etapas de pré-tratamento e hidrólise enzimática para sua bioconversão em açúcares fermentáveis. Ao mesmo tempo, substâncias químicas com potencial efeito inibitório no metabolismo microbiano

também podem ser produzidas após os pré-tratamentos e prejudicar o rendimento geral do processo hidrolítico. Para uma hidrólise eficiente e de baixo custo, podem ser empregadas enzimas caseiras produzidas a partir de resíduos agroindustriais, como o bagaço da cana-de-açúcar. No entanto, um conjunto de parâmetros pode ser ajustado, tais como: tipo de pré-tratamento, carga enzimática, carga sólida, tempo de hidrólise e uso de aditivos, para melhorar os rendimentos em açúcares livres usando essas preparações enzimáticas in situ. Nesse sentido, estudos envolvendo a otimização das condições de pré-tratamento e sacarificação são essenciais para aumentar a taxa de bioconversão da lignocelulose. Essas estratégias são importantes para a produção de produtos com valor agregado a partir desses resíduos e, consequentemente, oferecer uma destinação correta e rentável a eles. Portanto, este estudo apresenta uma revisão das principais características que influenciam a hidrólise enzimática de resíduos agrícolas e o rendimento em açúcares redutores para a produção de etanol.
Palavras-chave: Resíduos Agrícolas. Lignocelulose. Pré-tratamento. Hidrólise. Enzimas Caseiras.

Resumen
La biomasa lignocelulósica, como los residuos agrícolas y forestales, puede reutilizarse y servir como fuente de azúcares para la producción de etanol de segunda generación (2G) y otros bioproductos. Sin embargo, estos residuos están compuestos por moléculas de difícil degradación, que requieren etapas de pretratamiento e hidrólisis enzimática para su bioconversión en azúcares fermentables. Al mismo tiempo, las sustancias químicas con un efecto inhibidor potencial sobre el metabolismo microbiano también se pueden producir después de los pretratamientos y perjudicar el rendimiento general del proceso hidrolítico. Para una hidrólisis eficiente y de bajo costo se pueden utilizar enzimas caseras producidas a partir de residuos agroindustriales, como el bagazo de caña de azúcar. Sin embargo, se puede ajustar un conjunto de parámetros, tales como: tipo de pretratamiento, carga enzimática, carga sólida, tiempo de hidrólisis y uso de aditivos, para mejorar los rendimientos de azúcares libres utilizando estos preparados enzimáticos in situ. En este sentido, los estudios que involucran la optimización de las condiciones de pretratamiento y sacarificación son esenciales para aumentar la tasa de bioconversión de la lignocelulosa. Estas estrategias son importantes para la producción de productos con valor agregado a partir de estos residuos y, en consecuencia, ofrecer un destino correcto y rentable para los mismos. Por lo tanto, este estudio presenta una revisión de las principales características que influyen en la hidrólisis enzimática de los residuos agrícolas y el rendimiento en azúcares reductores para la producción de etanol.
Palabras clave: Residuos Agrícolas. Lignocelulosa. Pretratamiento. Hidrólisis. Enzimas Caseras.

1. Introduction

Brazil has an economy greatly based on the agricultural activity and the generation of agroindustrial wastes is quite considerable, especially sugarcane bagasse, which is largely available throughout the country. Thus, methods that add value to these byproducts indirectly contribute for the reduction of the environmental impacts caused by their improper disposal (Nunes et al., 2014; Florencio et al., 2017). Such residues can be reused for the sustainable production of biofuels and chemicals of industrial interest (Banerjee et al., 2017; Cordeiro et al., 2020). In this scenario, lignocellulosic biorefineries have been identified as substitutes for the traditional oil refineries, being the second-generation ethanol (2G) as one of their main products (Julio et al., 2017).

However, the degradation of the lignocellulosic biomass presents some bottlenecks due to its recalcitrant nature and the structural complexity of its components. For the release of sugars from the polysaccharides of the plant cell wall, saccharifications with enzymatic cocktails of high synergistic power are necessary (Fernandes et al., 2017; Rodrigues et al., 2021). Nevertheless, the commercial cocktails currently used are expensive, being one of the main limitations to the production of 2G ethanol (Rodrigues et al., 2021). Thus, the search for more economical processes in the production of enzymes is of paramount importance, since the high value of this input represents a barrier to the large-scale production of ethanol (Johnson, 2016; Florencio et al., 2017).

Among these processes, the production of enzymes by solid state fermentation (SSF) through the cultivation of filamentous fungi in agroindustrial residues is a strategy that can aid in the reduction of the costs and add value to environmental liabilities (Santos et al., 2018; Rodrigues et al., 2018; Rodrigues et al., al. 2020). For that, studies looking for new microbial producer species of hydrolytic enzymes have been carried out, with filamentous fungi cultured in monocultures or in consortia (Rodrigues et al., 2017; Delabona et al., 2019; de Souza et al., 2021). For example, Rodrigues et al. (2020) produced enzymatic cocktails by SSF, using sugarcane bagasse and wheat bran as substrates for the fungal growth, and the action of such cocktails was investigated in sugarcane bagasse saccharifications in order to replace commercial cocktails (Rodrigues et al., 2021). This on-site integrated approach for onsite enzyme production using lignocellulosic wastes was also investigated by de Souza et al. (2021), in which used steam-pretreated sugarcane bagasse (SPSB) as carbon source for the production of cellulases by *Trichoderma reesei* Rut C30 and observed a glucose yield of 80% after the hydrolysis of SPSB with this laboratory-made mixture. In this sense, the use of homemade enzymes that act synergistically has been considered a potential strategy to become the 2G ethanol economically viable (Florencio et al., 2017). In this scenario, this work presents a review of literature on the feasibility of the use of homemade enzymes in the hydrolysis of lignocellulosic residues, based on recent studies. The study also aimed to point out the factors that can influence the enzymatic performance in saccharifications of residual biomass and illustrate the future perspectives of their use in relation to the commercial cocktails.

2. Influence of the enzymatic synergism

For an efficient hydrolysis, the action of different enzymes, which cooperate with each other to improve the performance of the process, is needed (Malgas et al., 2017). However, a single microorganism does not produce all the necessary enzymes in adequate concentrations. In addition, some enzymes are more tolerant to the environmental conditions, such as pH, temperature and inhibitors, than other ones. Thus, to accelerate the rate of hydrolysis, an enzymatic cocktail with high synergistic power is desirable (Adsul et al., 2020).

The cellulases are the main enzymes responsible for the degradation of lignocellulosic residues, acting on the cellulose polymer and releasing oligosaccharides and glucose monomers (Florencio, 2017). These enzymes act in synergism, with endoglucanases performing the hydrolysis of the internal β-1.4-glycosidic bonds, exoglucanases act on the terminal units of the chain, removing the cellobiose dimers and β-glucosidases convert cellobiose into glucose (Kont et al., 2016). Therefore, an ideal enzymatic cocktail might have satisfactory concentrations of all these enzymes. Thus, the search for new microbial species producing these enzymes is constant (Zang et al., 2018; Nwamba et al., 2021). A simple and rapid screening method is the evaluation of the fungal growth on petri plates containing microcrystalline cellulose (Avicel) as the sole carbon source and staining with Congo red, which reveals halos of hydrolysis. For example, Delabona et al. (2019) screened 156 isolates of the genus *Scytalidium* and selected an enzyme cocktail obtained from the MIBA247 strain, using sugarcane bagasse as substrate for fungal growth. This enzymatic extract was able to hydrolyze 62% of bagasse, demonstrating the high potential of this strain for the production of enzymes by SSF and for saccharifications of lignocellulosic biomass.

Previous studies have demonstrated that the investigation of enzymes production and their characteristics can aid to replace the high cost commercial cocktails and also reduce environmental impacts (de Souza et al., 2021). For example, Mahmood et al. (2021) used sugarcane bagasse for enzyme production by *Phaeolus spadiceus* and demonstrated that this species had the potential to produce efficient and low-cost endoglucanases. In another study, the fungus *Curvularia affinis* showed considerable production of endoglucanases and exoglucanases when it was cultured on bean straw (Alawlaqi and Alharbi, 2020). However, the lignocellulosic biomass is a complex and heterogeneous material, composed not only of cellulose, but also of hemicellulose and lignin. Thus, in addition to the cellulases, accessory enzymes are necessary to potentiate the degradation of the

raw material, such as hemicellulases. Among the hemicellulases, xylanases act in the depolymerization of hemicelluloses and help in the conversion of biomass, contributing to the opening of the fibers and to the access of cellulases to cellulose (Amorim et al., 2019). Agrawal et al. (2021), for example, produced D-feruloyl esterase and β-xylosidase by the fungus *Scytalidium thermophilum* and observed that the use of these homemade cocktails improved the hydrolysis of rice straw and sugarcane bagasse, demonstrating potential to reduce the load of commercial enzymes, and consequently, the cost of the sugars production from agricultural wastes. The action of accessory enzymes was also evaluated by Nwamba et al. (2021), who employed β-glucosidase (Novozymes) and endoxylanase (Qingdao Vland Biotech) in association with cellulolytic cocktail LT4, in the hydrolysis of sugarcane bagasse and released high concentration of glucose (131 g/L). These results showed that the use of accessory enzymes can be an important tool to favor the saccharification and make it economically viable (Nwamba et al., 2021).

Other important accessory enzymes are the lytic polysaccharide monooxygenases (LPMOs), which perform the oxidative cleavage of cellulose and hemicelluloses (Zhang et al., 2020). In commercial preparations with a high concentration of cellulases and hemicellulases, LPMOs are considered important components (Agrawal et al., 2020). Some studies have demonstrated that the cellulase load required in saccharification can be considerably reduced with the addition of LPMOs (AA9 protein), and there is still a significant increase in the glucose yield (Agrawal et al., 2019; Zhang et al., 2020). Agrawal et al. (2020) evaluated the pre-incubation of biomass with AA9 proteins before the addition of the hydrolytic cocktail and observed a positive effect, suggesting that this strategy could also promote savings in saccharifications. Mukasekuru et al. (2020) also obtained significant results in hydrolysis of sugarcane bagasse using endo-xylanase and AA9 enzymes (125 g/L glucose and 56 g/L xylose).

3. Influence of time

During the biomass conversion, the enzymatic synergism is influenced by several factors: reaction time, enzyme and substrate characteristics, among others (Malgas et al., 2017). When considering the reaction time, it is observed that the highest degree of synergy is observed at the initial stages of hydrolysis and decreases as it approaches to the final stages (Andersen et al., 2008; Lucarini et al., 2017; Malgas et al., 2017). Such behavior is justified by the fact that the enzymes cooperate more in the initial stages of degradation and, consequently, over the time, the binding sites become more available, requiring a lower degree of synergism (Malgas et al., 2017). In this sense, an efficient hydrolysis in a shorter time can be a potential alternative (Adsul et al., 2020). On the other hand, a high degree of synergism can also be achieved in the later stages, as the amorphous regions of the cellulose are limited for the enzymes at the beginning of the process, a scenario that changes over time, since when a component is hydrolyzed, the other becomes more exposed (Malgas et al., 2017). Previous studies have demonstrated the importance of the reaction time in the enzymatic hydrolysis and indicated that the longer the time, the greater are the yields in monosaccharides, with a tendency towards to the fixation at 72 hours of reaction. The Table 1 summarizes the effect of the time in the sugar concentration after the enzymatic hydrolysis in some studies.

Table 1 – Effect of the reaction time in the enzymatic hydrolysis.

Biomass	Enzyme source	Time (h)	Sugar concentration (g/L)	References
Sugarcane bagasse	*Lichtheimia ramosa* homemade extract	48	1.45 glucose	Garcia et al., (2018)
Sugarcane bagasse	Onsite cocktails of *Botryosphaeria sp.* AM01 and *Saccharicola sp*. EJC04	20	3.56 glucose / 1.66 xylose	Marques et al., (2018)
Sugarcane bagasse	*Scytalidium sp*. MIBA247 extract	72	43.57 glucose	Delabona et al., (2019)
Oak	Commercial Cellic CTec2	120	120.2 glucose / 37.3 xylose	Kim et al., (2019)
Sugarcane bagasse	Cellic CTec2	72	146 glucose	Gomes et al., (2018)
Corn husk	Cellic CTec2	72	54 glucose / 23.8 xylose	Gong et al., (2020)
Sugarcane bagasse	Cellic CTec2	72	125 glucose / 56 xylose	Mukasekuru et al., (2020)
Sorghum	Commercial NS22257 and NS22244 (Novozymes)	60	147 glucose	Cheng et al., (2020)
Sugarcane bagasse and wheat bran	*Aspergillus niger* onsite extract	72	10.8 glucose / 3.1 xylose	Rodrigues et al., (2021)
Sugarcane bagasse	Commercial LT4 (cellulase)	72	158 total sugars	Nwamba et al (2021)

4. Influence of pretreatment

The lignocellulose is evolutionarily adapted to be resistant to the biodegradation, and among the resistance mechanisms stands out the presence of lignin, a complex macromolecule that prevents the enzymes to access the polysaccharides (Santiago et al., 2017). In order to break this barrier and reach the cellulose and hemicellulose fractions, a pretreatment step is carried out before the enzymatic hydrolysis. In addition, the pretreatment changes the porosity and crystallinity of the material, with the substrate characteristics influencing its performance (Gunes et al., 2019; Silva et al., 2020). In turn, the substrate will be altered in a way that can facilitate or hinder the saccharification (Santiago et al., 2017).

The pretreatments are based on the application of chemical, physical and/or biological techniques. However, some methods have advantages and disadvantages related to the selectivity of the components and the generation of inhibitors (Santiago et al., 2017; Nwamba et al., 2021). Thus, the yield and efficiency of the hydrolytic and fermentative processes can be affected by substances generated after the pretreatment, which inhibit the enzymatic and microbial activities (Colombi et al., 2017; Santiago et al., 2017). Therefore, the formation of inhibitory compounds, as well as the degradation of carbohydrates, must be avoided (Santiago et al., 2017). In addition, the availability of fermentable sugars should be enhanced in order to consolidate the ethanol production. Finally, the method used must be economically viable and ecologically correct (Rodrigues et al., 2022). Table 2 presents the advantages and disadvantages of the pretreatments commonly used in previous studies.

In order to compensate the weaknesses of an individual pretreatment, such as environmental impacts, energy expenditure and cost, as well as highlight its positive points, a combination of methods can be performed, with the most assertive set depending on the substrate characteristics

(Vergara et al., 2021). For example, Andrade et al. (2018) subjected sugarcane, elephant grass and angelim red sawdust to physicochemical pretreatments, using an alkaline process with sodium hydroxide (NaOH), followed by an acid method with phosphoric acid (H_3PO_4). These authors observed a significant delignification rate (62.16% of reduction for elephant grass, 46.53% for sugarcane and 28.45% for sawdust), demonstrating the positive effect of the combined pretreatment. Tallarico et al. (2019) also observed a positive effect of the combined pretreatment of microwaves and ultrasound on the conversion of cellulose into lactic acid.

Table 2 - Comparison of lignocellulose pretreatment methods.

Pretreatment		Advantages	Disadvantages
Physical	Milling	Low risk of recalcitrant compounds production	High demand of electricity
	Ultrasound	Particle size reduction and scalability	High demand of electricity
	Microwave	Particle size reduction and low risk of formation of recalcitrant compounds	High demand of electricity and scalability
Chemical	Acid Alkaline	Low energy demand, hemicellulose hydrolysis Delignification, alteration of the cellulose structure	Cost, chemical contamination and risk of inhibitor formation
Physico-chemical	Steam explosion	Scalability	High energy demand and risk of formation of recalcitrant compounds
	Hydrothermal	Scalability	High energy demand and risk of formation of recalcitrant compounds
Biological	Fungal	Scalability and low energy demand	High exposure time, consumption of sugars generated by microorganisms
	Enzymatic	Scalability and low energy demand	Specificity and cost of enzymes

However, it is a challenge to assertively choose the kind of pretreatment and the strategy to remove possible inhibitors. The cellulose can be degraded to hexoses which, after dehydrated, which can be converted into 5-hydroxymethyl furfural (HMF). This substance, in turn, will potentially give rise to formic acid and levulinic acid. On the other hand, hemicelluloses can be degraded into aliphatic acids, furan aldehydes and other inhibitors, being furfural (FF) the most abundant. Finally, in the degradation of lignin, the formation of 4-hydroxybenzoic acid, vanillin and other phenols can occur (Kumar et al., 2018). Colombi et al. (2017) analyzed the performance of the yeast *Saccharomyces cerevisiae* in alcoholic fermentation in the presence of inhibitors (acetic acid, vanillin, vanillic acid and 4-hydroxybenzoic acid). The results indicated that these substances affected the fermentative metabolism of the yeast and inhibited its growth. In addition, when the acetic acid concentration was equal to or greater than 3.5 g/L, there was no ethanol production. Therefore, since some pretreatment methods can lead to the formation of these inhibitor compounds, some alternatives to separate inhibitors and concentrated sugars have been evaluated, such as detoxification with oxidative enzymes (laccases for the reduction of phenolic compounds) and the use of genetic engineering to increase the enzymatic tolerance (Kumar et al., 2020).

The pretreatment can also influence the enzyme production. Alawlaqi and Alharbi (2020) evaluated the production of enzymes in bean biomass submitted to acid, alkaline or hydrogen peroxide pretreatments and observed that the production of cellulases by the fungus *Curvularia affinis* was higher in the pretreated samples, with emphasis on the alkaline method, which increased the surface area of the biomass. However, the pretreatment with hydrogen peroxide showed lower

activities compared to the others, probably due to the generation of inhibitory substances. On the other hand, the pretreatment can also decrease the enzyme production depending on the kind of biomass. Rodrigues et al. (2017), for example, observed that raw sugarcane bagasse showed higher β-glucosidase and xylanase activities when compared to the bagasse submitted to alkaline, organosolv and hydrogen peroxide pretreatments. The lower production of these enzymes in the pretreated samples may also be due to the presence of inhibitors generated after the pretreatments.

The Table 3 presents studies of kinds of pretreatments and the yields of enzymatic hydrolysis. Considering only the pretreatment effect, the combined methods have positively influenced the saccharifications. In addition, the chemical pretreatments obtained the most satisfactory results for sugar release. However, the sugar yields varied even when the same pretreatments were used, depending on the kind of biomass and other factors.

Table 3 - Influence of the kind of pretreatment in the enzymatic hydrolysis.

Pretreatment	Conditions	Sugar concentration (g/L)	References
Organosolv	10 g of sugarcane bagasse : 30 mL glicerol 100%	1.45 glucose	Garcia et al., (2018)
Hydrothermal	sugarcane bagasse autoclaving in 5% NaOH solution	3.56 glucose / 1.66 xylose	Marques et al., (2018)
Alkaline	sugarcane bagasse in NaOH and anthraquinone (liquid-solid ratio 15:1) at 90 ºC	43.57 glucose	Delabona et al., (2019)
Acid	30% of oak in 1% maleic acid at 190 °C	120.2 glucose / 37.3 xylose	Kim et al., (2019)
Acid / Alkaline	sugarcane bagasse in 1% sulfuric acid (liquid-solid ratio 1:3) at 121 °C and after in 3% NaOH (w/v) at 100 °C	146 glucose	Gomes et al., (2018)
Organosolv / Alkaline	Corn straw in methanol/NaOH with 50% solids	54 glucose / 23.8 xylose	Gong et al., (2020)
Organosolv / Alkaline	sugarcane bagasse in atmosferic glycerol/NaOH	125 glucose / 56 xylose	Mukasekuru et al., (2020)
Hydrothermal	Sorghum in reactor at 190°C for 10 minutes	147 glucose	Cheng et al., (2020)
Hydrothermal	sugarcane bagasse in reator at 180°C for 44 minutes	10.8 glucose / 3.1 xylose	Rodrigues et al., (2021)
Organosolv / Alkaline	Glicerol/NaOH at 240 ºC for 30 minutes	158 total sugars	Nwamba et al., (2021)

4. Influence of the residual lignin after the pretreatment

After the pretreatment, the lignin repolymerization may occur and significantly influence the enzymatic hydrolysis (Pielhop et al., 2015; Santos et al., 2019; Zhou et al., 2021). The repolymerized lignin can partially cover the cellulose fibers, preventing the access of the enzymes, or even deactivate them by irreversible binding (Pielhop et al., 2015; Nwamba et al., 2021). One strategy to overcome this obstacle is the use of hydrophobic additives, which interact with the lignin, being adsorbed and preventing the enzyme adsorption by the lignin (Pielhop et al., 2015; Zhou et al., 2021). Santos et al. (2019) suggested the use of low-cost additives, such as bovine serum albumin, as a strategy to avoid the enzymatic inhibition. The bovine serum albumin has hydrophobic sites that easily bind to nonpolar surfaces and can be used to increase the biomass digestibility (Pielhop et al., 2015; Santos et al., 2019; Zhou et al., 2021). Other substances, such as surfactants and saponins, can also be employed to decrease the adsorption of enzymes by lignin and thus,

increase the glucose yields (Mukasekuru et al., 2020; Vergara et al., 2021). For example, Vergara et al. (2021) investigated the effect of different additives on the enzymatic hydrolysis of pre-treated wheat straw, such as Tween 80 (poly(oxyethylene)20-sorbitan-monooleate) and polyethylene glycol-6000, and proteins, as bovine serum albumin, casein and skimmed milk powder. They observed that the most favorable condition combined high delignification with the use of the low cost casein protein, with a glucose yield of 88.6% after the saccharification.

Pielhop et al. (2015) observed that the surface area of lignin increases substantially after the repolymerization of the biomass, resulting in increased adsorption and inactivation of the enzymes, due to unproductive binding. The biomass used was spruce wood pretreated by autohydrolysis with 2-naphthol, which is considered efficient in preventing the lignin repolymerization. A directly proportional relationship was found between the concentration of 2-naphthol in the pretreatment and the yields of sugars in the enzymatic hydrolysis. According to Zanchetta et al. (2018), the temperature of the saccharification of pretreated sugarcane bagasse may have mitigating effects against the impacts of lignin. In the study, it was reported that the adsorption of the enzymes was directly proportional to the incubation temperature and that the processes carried out at 30 °C revealed minimal adsorption and lower thermal denaturation of the enzymes. However, at this temperature, the time required for hydrolysis and fermentation was extended.

In order to minimize the problem of enzyme inactivation, the use of surfactants such as polysorbate (Tween) and polyethylene glycol (PEG), which prevent the unproductive adsorption, has also been investigated (Sun et al., 2020). These authors compared the effect of commercial surfactants on the hydrolysis of Miscanthus straw (Silwet L-77, Tween-80 and PEG-4000) and observed that the highest rates of conversion to sugars after the saccharification were observed with the use of Silwet L -77. Madadi et al. (2021) evaluated the performance of proteins extracted from amaranth leaf as a biosurfactant and compared to soy proteins and the commercial surfactant Tween-80 in the hydrolysis of different biomass (rice, wheat, rapeseed, miscanthus, corn, poplar, eucalyptus and poplar). These authors observed that the yields of sugars and ethanol were significant in the samples wherein the amaranth proteins were used as biosurfactant, effectively preventing the entrapment of the cellulases by the lignin.

5. Influence of solids load

Low solids loads can be used in hydrolysis to avoid unsatisfactory mixing between the enzymes and biomass (Godoy et al., 2019). However, this procedure can result in low concentrations of sugars and also increase the costs of the steps of separation and purification. On the other hand, saccharifications with high solid loads can generate higher concentrations of sugars, and also reduce the consumption of water, resources and energy (Gong et al., 2020; Nwamba et al., 2021). Nonetheless, the high solids content can hinder the mixing process, and consequently affect the mass and heat transfer, reducing the sugar yields due to the high viscosity (Godoy et al., 2019; Gong et al., 2020). In addition, the high solids concentration may reflect in an increase in the production of inhibitors, such as lignin, phenols and furans, becoming the enzymes more susceptible to the unproductive adsorption (Silva et al., 2020).

To overcome these obstacles, the fed mode is used, which consists of the continuous addition of biomass to the mixture, contributing to reduce the viscosity of the medium and, consequently, promote greater mass and heat transfer during the process (Cheng et al., 2020; Nwamba et al., 2021). According to Nwamba et al. (2021), this fed-batch approach is ideal for hydrolysis processes with high solids and low enzyme concentrations. Some studies have demonstrated the importance of this method in saccharifications. For example, Cheng et al. (2020) used solids loading greater than 30% in the hydrolysis of sorghum, supplemented with the surfactant PEG 4000, and obtained high fermentable sugar concentrations (232 g/L). These authors concluded that the fed-batch biomass was efficient to enhance the glucose yield. Gong et al., (2020) also released high concentrations of sugars in the saccharification of corn husk pretreated by

alkaline/organosolv method using high solids loading in fed mode. Mukasekuru et al. (2020) evaluated the enzymatic hydrolysis in fed batch mode with pretreated sugarcane bagasse by the alkaline/organosolv method with different solids loads (8, 10, 12 and 14%), feed dosages (5, 6, 7 and 8 %) and reaction times (6, 12 and 24 hours). The authors reported that the addition of sugarcane bagasse in a fed batch mode prevented the high viscosity in the system. Besides, the highest sugar release (180 g/L) was obtained with 30% of solids, in combination to a set of additives (Tween 80, bovine serum albumin, tea saponin) and accessory enzymes (endo-xylanase and AA9), contributing to the low enzymatic load of 3.0 filter paper units (FPU) of CellicCTec2 cocktail per gram of substrate. Table 4 exhibits studies of enzymatic hydrolysis as a function of solids content and shows that, as this factor increases, the yield of sugars proportionally increases. In general, at approximately 30% of biomass, the best sugar yields are usually obtained.

On the other hand, Mukasekuru et al. (2020) used the fed-batch mode in the saccharification of sugarcane bagasse and observed that glucose yields increased in the first 24 hours and then decreased at 48 hours. These results suggested that the increase of the concentration of solids in the mixture decreased the amount of free water in the system, and consequently, restricted the mass transfer. According to Silva et al. (2020), the water restriction is apparently one of the main elements that can negatively reflect on the enzymatic saccharification with high solids content. However, this constraint is not limited to the amount of water present, but can also be related to its mobility, which may have its free flow impeded by the increase of soluble species. The Table 4 presents a summary of influence of solids content in the enzymatic hydrolysis in previous studies.

Table 4 - Influence of solids content in the enzymatic hydrolysis.

Enzime source	Pretreatment	Solids load (%)	Sugar concentration (g/L)	References
Lichtheimia ramosa extract	Organosolv (sugarcane bagasse)	3	1.45 glucose	Garcia et al., (2018)
Botryosphaeria sp. AM01 and *Saccharicola sp*. EJC04 cocktails	Hydrothermal (sugarcane bagasse)	5	3.56 glucose / 1.66 xylose	Marques et al., (2018)
Scytalidium sp. MIBA247 extract	Alkaline (sugarcane bagasse)	10	43.57 glucose	Delabona et al., (2019)
Cellic CTec2	Acid (oak)	30	120.2 glucose / 37.3 xylose	Kim et al., (2019)
Cellic CTec2	Acid (sugarcane bagasse)	27	146 glucose	Gomes et al., (2018)
Cellic CTec2	Alkaline (corn husk)	25	54 glucose / 23.8 xylose	Gong et al., (2020)
Cellic CTec2	Organosolv/Alkaline (sugarcane bagasse)	30	125 glucose / 56 xylose	Mukasekuru et al., (2020)
NS22257 e NS22244 (Novozymes)	Organosolv/Alkaline (sorghum)	50	147 de glucose	Cheng et al., (2020)
LT4 (cellulase)	Alkaline (sugarcane bagasse)	20	158 total sugars	Nwamba et al., (2021)

6. Influence of the enzymatic load

Technical hitches arising from hydrolysis with high solids content (> 15%), such as high viscosity and difficulty for the mass transfer, can be mitigated with high dosages of enzymes. However, the cost of enzymes is one of the main contributors to the increase of the final price of ethanol (Larnaudie et al., 2019). Bhagia et al. (2018) demonstrated that in the hydrolysis of biomasses with low lignin content, the deactivation of cellulases at the air-liquid interface can occur

when low enzyme loads are employed, being the main reason of incomplete cellulose conversion. The scientific literature considers that the hydrolysis of lignocelluloses with solids content greater than 20% requires at least 10 FPU of cellulase load per gram of biomass (Jung et al., 2017; Lucarini et al., 2017; Mukasekuru et al., 2020). Studies using homemade enzymes often apply the same enzyme dosage (10 FPU/g) used for commercial enzymes (Garcia et al., 2018; Delabona et al., 2019; Rodrigues et al., 2021). Nevertheless, in general, the sugar yields tend to be higher when commercial enzymes are used (Gomes et al., 2018; Mukasekuru et al., 2020). Conversely, the search for low-cost homemade cocktails with high activities of cellulases and accessory enzymes is essential to optimize and economically viable the saccharification processes. For example, Delabona et al., (2019) compared the performance of an onsite extract produced by *Scytalidium* sp. MIBA247 with the commercial Celluclast® cocktail using both in the concentration of 10 FPU/g of sugarcane bagasse and observed a superior efficiency of the *Scytalidium* extract in the glucose yield. In 72 h of hydrolysis, the *Scytalidium* extract released 43.57 g/L of glucose, while the commercial preparation released 33.52 g/L. This superior efficiency of the homemade solution can probably be due to the action of the accessory enzymes present in the crude preparation, which contributed to the opening of the cellulose fibers and improve the access of the cellulases to the biomass.

On the other hand, it is important to emphasize that the efficiency of the accessory enzymes is highly dependent on the kind of pretreatment and its effects on the biomass structure. The Table 5 presents studies of hydrolysis and sugar yield, highlighting the enzymatic load. Observing only this factor, there was no linear relationship, as a higher enzymatic dosage does not always result in a more significant yield, as the sugar release can vary depending on other factors, such as the kind of biomass, pretreatment, solids loads, among others (Nwamba et al., 2021).

Table 5 - Influence of enzymatic load in saccharifications of biomass.

Biomass	Enzyme source	Enzyme load (FPU/g)	Sugar concentration (g/L)	References
Sugarcane bagasse	*Lichtheimia ramosa* extract	5	145 glucose	Garcia et al., (2018)
Sugarcane bagasse	*Botryosphaeria sp.* AM01 and *Saccharicola sp*. EJC04 cocktails	150	3.56 glucose / 1.66 xylose	Marques et al., (2018)
Sugarcane bagasse	*Scytalidium sp*. MIBA247 extract	10	43.57 glucose	Delabona et al., (2019)
Oak	Cellic CTec2	30	120.2 glucose / 37.3 xylose	Kim et al., (2019)
Sugarcane bagasse	Cellic CTec2	26	146 glucose	Gomes et al., (2018)
Corn husk	Cellic CTec2	5	54 glucose / 23.8 xylose	Gong et al., (2020)
sugarcane bagasse	Cellic CTec2	3	125 glucose / 56 xylose	Mukasekuru et al., (2020)
Sorghum	NS22257 and NS22244 (Novozymes)	50	147 glucose	Cheng et al., (2020)
Sugarcane bagasse	*Aspergillus niger* extract	10	10.8 glucose / 3.10 xylose	Rodrigues et al., (2021)
Sugarcane bagasse	LT4 (cellulase)	2	158 total sugars	Nwamba et al., (2021)

7. Effect of the combination of factors

There are several features that can influence the enzymatic conversion of lignocellulosic wastes, and they must be examined in association in order to achieve effective results (Santiago, 2017). The Table 6 exhibits the studies previously discussed, but with all the factors included. In order to reach the cellulose and hemicellulose fractions and then produce fermentable sugars, a step of pretreatment is typically carried out, resulting in modifications in the porosity and crystallinity of the biomass (Nwamba et al., 2021). There is a wide variety of pretreatments, with the chemical methods generally performing better than the other methods. In addition, combined methods can also be advantageous. In general, the main disadvantage of the pretreatments is the risk of formation of inhibitor compounds of the hydrolytic and fermentative processes (Nwamba et al., 2021).

According to Larnaudie et al. (2019), another noteworthy feature is the enzymatic load, which can directly affects the efficiency of the hydrolysis and the cost of the process. For an efficient saccharification, when low enzymatic loads are used, it was observed that it is necessary to use high solids and/or additives such as surfactants, in addition to the accessory enzymes. Consequently, the enzymatic saccharifications with high solids loads can generate higher concentrations of free sugars and also origin less environmental impacts (Silva et al., 2020; Nwamba et al., 2021). In order to make the process more profitable, the homemade enzymes, produced by solid state fermentation using agroindustrial wastes as substrates for microbial growth, have a lower cost in comparison to the commercial ones. However, in general, they present a lower performance, probably due to the concentration of the cocktail used in the enzymatic hydrolysis (Rodrigues et al., 2022). Regarding the enzymatic synergy, the factor time also seems to have a positive effect on the bioconversion of residues into fermentable sugars (Malgas et al., 2017; Mukasekuru et al., 2020).

8. Conclusions

The study concluded that an efficient biomass hydrolysis requires the action of several enzymes in synergism and the supplementation with accessory enzymes can increase the sugars yield and reduce the enzymatic loading. The enzymatic load, in turn, must be chosen considering the concentration of cellulases present in the employed cocktail. Additionally, the assertive choice of the pretreatment can result in desired changes in the biomass, with a reduction in the lignin content and low formation of inhibitors. The repolymerization of lignin can also have its effects minimized with the use of surfactants and some protein additives. Another important strategy is to carry out the hydrolysis with high solids loads in fed-batch mode. Finally, the choice of reaction time depends on its effect on the enzymatic synergy. Therefore, that to obtain satisfactory and economically viable sugar yields from residual biomass saccharifications, it is necessary to focus on the optimization of several factors that are closely linked to each other.

Acknowledgements

The authors appreciate the financial and technical support from Minas Gerais State Research Foundation (FAPEMIG—Grant number APQ-01360-14) and Federal University of Uberlandia (UFU), respectively. N.K.S. is grateful to the Tutorial Education Program in Environmental and Sanitary Engineering (PET-EAS) for her undergraduate studentship.

References

Adsul, M., Sandhu, S.K., Singhania, R.R., Gupta, R., Puri, S.K., & Mathur, A. (2020). Designing a cellulolytic enzyme cocktail for the efficient and economical conversion of lignocellulosic biomass to biofuels. *Enzyme Microbial Technology*, 133, 109442-109492. http://dx.doi.org/10.1016/j.enzmictec.2019.109442

Agrawal, D., Basotra, N., Balan, V., Tsang, A., & Chadha, B.S. (2019). Discovery and Expression of Thermostable LPMOs from Thermophilic Fungi for Producing Efficient Lignocellulolytic Enzyme Cocktails. *Applied Biochemistry and Biotechnology*, 191(2), 463-481. https://doi.org/10.1007/s12010-019-03198-5

Agrawal, D., Kaur, B., Brar, K.K., & Chadha, B.S. (2020). An innovative approach of priming lignocellulosics with lytic polysaccharide mono-oxygenases prior to saccharification with glycosyl hydrolases can economize second generation ethanol process. *Bioresource Technology*, 308, 123257. http://dx.doi.org/10.1016/j.biortech.2020.123257

Agrawal, D., Tsang, A., & Chadha, B.S. (2021). Economizing the lignocellulosic hydrolysis process using heterologously expressed auxiliary enzymes feruloyl esterase D (CE1) and β-xylosidase (GH43) derived from thermophilic fungi *Scytalidium thermophilum*. *Bioresource Technology*, 339, 125603. http://dx.doi.org/10.1016/j.biortech.2021.125603

Alawlaqi, M.M., & Alharbi, A.A. (2020). Exo- and endoglucanase production by *Curvularia affinis* using bean (*Phaseolus vulgaris* L.) waste biomass. *Bioresources and Bioprocessing*, **7**(1), 1-2. http://dx.doi.org/10.1186/s40643-020-0296-y

Amorim, C.C., Farinas, C.S., & Miranda, E.A. (2019). Liquefied wheat bran as carbon source and inducer in high-solids submerged cultivation of *Aspergillus niger* for xylanase production. *Biocatalysis and Agricultural Biotechnology*, 21, 101346. http://dx.doi.org/10.1016/j.bcab.2019.101346

Andrade, T.C.C., Siqueira, L.N., Souza, D.A., Silva, F.V., Guarda, P.M., & Guarda, E.A. (2018). Análise da influência do pré-tratamento químico e físico sob a caracterização das frações celulose e lignina de diferentes biomassas e seu potencial para produção de etanol de segunda geração. *Revista em Agronegócio e Meio Ambiente – RAMA*, 11(3), 955-975. http://dx.doi.org/10.17765/2176-9168.2018v11n3p955-974

Banerjee, J., Singh, R., Vijayaraghavan, R., MacFarlane, D, Patti, A.F., & Arora, A. (2017). Bioactives from fruit processing wastes: green approaches to valuable chemicals. *Food Chemistry*, 225(1), 10-22. http://dx.doi.org/10.1016/j.foodchem.2016.12.093

Bhagia, S., Dhir, R., Kumar, R., & Wyman, C.E. (2018). Deactivation of Cellulase at the Air-Liquid Interface Is the Main Cause of Incomplete Cellulose Conversion at Low Enzyme Loadings. *Scientific Reports*, 8, 1350. http://dx.doi.org/10.1038/s41598-018-19848-3

Cheng, M.H., Kadhum, H.J., Murthy, G.S., Dien, B.S., & Singh, V. (2020). High solids loading biorefinery for the production of cellulosic sugars from bioenergy sorghum. *Bioresource Technology*, 318, 124051. http://dx.doi.org/10.1016/j.biortech.2020.124051

Colombi, B.L., Almeida, O.M., Silva, Z.P., Magalhães, W.L.E., & Tavares, L.B.B. (2017). Efeito de compostos inibidores na bioconversão de glucose em etanol por levedura *Saccharomyces cerevisiae*. *Engevista*, 19(2), 339-352. http://dx.doi.org/10.22409/engevista.v19i2.838

Cordeiro, N.K., Cardoso, K.P.S., da Mata, T.C., Barbosa, J.A., & Gonçalves Jr, A.C. (2020). Gestão de resíduos agrícolas como forma de redução dos impactos ambientais. *Revista de Ciências Ambientais*, 14(2), 23-34. https://revistas.unilasalle.edu.br/index.php/Rbca/article/view/5593/pdf

Delabona, P.S., Silva, M.R., Paixão, A.A.D., Lima, D.J., Rodrigues, G.N., Lee, M.S., Souza, M.G.S., Bussamra, B.C., Santos, A.S., & Pradella, J.G.C. (2019). A novel *Scytalidium* species: understand the cellulolytic system for biomass saccharification. *Brazilian Journal of Chemical Engineering*, 36(1), 85-97. http://dx.doi.org/10.1590/0104-6632.20190361s20170495

Fernandes, F.S., Farias, A.V., Dos Santos, R.A., De Souza, E.S., & De Souza, J.V.B. (2017). Determinação da quantidade de açúcares redutores dos hidrolisados enzimáticos de resíduos amazônicos para obtenção de bioetanol. . *The Journal of Engineering and Exact Sciences,* 3(4), 0608-0613. doi: https://doi.org/10.18540/24469416030420170608

Florencio, C., Badino, A.C., & Farinas, C.S. (2017). Current challenges on the production and use of cellulolytic enzymes in the hydrolysis of lignocellulosic biomass. *Química Nova*, 40(9), 1082-1093. http://dx.doi.org/10.21577/0100-4042.20170104

Garcia, N.F.L., Santos, F.R.S., Bocchini, D.A., da Paz, M.F., Fonseca, G.G., & Leite, R.S.R. (2018). Catalytic properties of cellulases and hemicellulases produced by *Lichtheimia ramosa*: potential for sugarcane bagasse saccharification. *Industrial Crops and Products,* 122, 49-56. http://dx.doi.org/10.1016/j.indcrop.2018.05.049

Gomes, A.C., Moysés, D.N., Santa Anna, L.M.M., & de Castro, A.M. (2018). Fed-batch strategies for saccharification of pilot-scale mild-acid and alkali pretreated sugarcane bagasse: effects of solid loading and surfactant addition. *Industrial Crops and Products,* 119, 283-289. http://dx.doi.org/10.1016/j.indcrop.2018.04.026

Gong, Z., Wang, X., Yuan, W., Wang, Y., Zhou, W., Wang, G., & Liu, Y. (2020). Fed-batch enzymatic hydrolysis of alkaline organosolv-pretreated corn stover facilitating high concentrations and yields of fermentable sugars for microbial lipid production. *Biotechnology for Biofuels,* 13, 13. http://dx.doi.org/10.1186/s13068-019-1639-9

Gunes, B., Stokes, J., Davis, P., Connolly, C., & Lawler, J. (2019). Pre-treatments to enhance biogas yield and quality from anaerobic digestion of whiskey distillery and brewery wastes: a review. *Renewable and Sustainable Energy Reviews,* 113, 109281. http://dx.doi.org/10.1016/j.rser.2019.109281

Johnson, E. (2016). Integrated enzyme production lowers the cost of cellulosic ethanol. *Biofuels, Bioproducts and Biorefining,* 10(2), 164-174. http://dx.doi.org/10.1002/bbb.1634

Julio, R., Albet, J.,Vialle, C., Vaca-Garcia, C., & Sablayrolles, C. (2017). Sustainable design of biorefinery processes: existing practices and new methodology. *Biofuels, Bioproducts and Biorefining*, 11(2), 373-395. https://doi.org/10.1002/bbb.1749

Jung, Y.H., Park, H.M., Kim, D.H., Yang, J., & Kim, K.H. (2017). Fed-Batch Enzymatic Saccharification of High Solids Pretreated Lignocellulose for Obtaining High Titers and High Yields of Glucose. *Applied Biochemistry and Biotechnology,* 182(3), 1108-1120. http://dx.doi.org/10.1007/s12010-016-2385-0

Kim, D.H., Park, H.M., Jung, Y.H., Sukyai, P., & Kim, K.H. (2019). Pretreatment and enzymatic saccharification of oak at high solids loadings to obtain high titers and high yields of sugars. *Bioresource Technology,* 284, 391-397. http://dx.doi.org/10.1016/j.biortech.2019.03.134

Kont, R., Kari, J., Borch, K., Westh, P., & Valjamae, P. (2016). Inter-domain Synergism Is Required for Efficient Feeding of Cellulose Chain into Active Site of Cellobiohydrolase Cel7A. *Journal of Biological Chemistry,* 291(50), 26013-26023. http://dx.doi.org/10.1074/jbc.m116.756007

Kumar, S., Ahluwalia, V., Kundu, P., Sangwan R.S., Kansal, S.K., Runge, T.M., & Elumalai, S. (2018). Improved levulinic acid production from agri-residue biomass in biphasic solvent system through synergistic catalytic effect of acid and products. *Bioresource Technology*, 251, 143-150. http://dx.doi.org/10.1016/j.biortech.2017.12.033

Kumar, V., Yadav, S.K., Kumar, J., & Ahluwalia, V. (2020). A critical review on current strategies and trends employed for removal of inhibitors and toxic materials generated during biomass pretreatment. *Bioresource Technology,* 299, 122633. http://dx.doi.org/10.1016/j.biortech.2019.122633

Larnaudie, V., Ferrari, M.D., Lareo, C. *(*2019). Techno-economic analysis of a liquid hot water pretreated switchgrass biorefinery: effect of solids loading and enzyme dosage on enzymatic hydrolysis. *Biomass and Bioenergy*, 130, 105394. http://dx.doi.org/10.1016/j.biombioe.2019.105394

Lucarini, A.C., Delquiaro, A.C.T., Vidoca, L.C.P.T., Braz, R., Martins, R.M., & Alves, T.P. (2017). Estudo da hidrólise enzimática da palha da cana de açúcar para produção de etanol de segunda

geração. *The Journal of Engineering and Exact Sciences*, 3(2), 242–253. https://doi.org/10.18540/jcecvl3iss2pp242-253

Madadi, M., Wang, W., Chu, C., Liu, P., Wang, Y. , Xia, T., Tu, Y., Lin, X., Song, B., Yang, X., Zhu, W., Duanmu, D., Tang, S., & Peng, L. (2021). Using *Amaranthus* green proteins as universal biosurfactant and biosorbent for effective enzymatic degradation of diverse lignocellulose residues and efficient multiple trace metals remediation of farming lands. *Journal of Hazardous Materials,* 406, 124727. http://dx.doi.org/10.1016/j.jhazmat.2020.124727

Mahmood, R.T., Masood, M., Zia, N., Safder, A., Asad, M.J., Nasreen, S., Ahmed, D., & Iqbal, R. (2021). Production and Characterization of Endoglucanase from *Phaeolus spadiceus*. Pakistan *Journal of Biochemistry and Biotechnology*, 2(1), 27-37. https://doi.org/10.52700/pjbb.v2i1.31

Malgas, S., Thoresen, M., Van Dyk, J., & Pletschke, B.I. (2017). Time dependence of enzyme synergism during the degradation of model and natural lignocellulosic substrates. Enzyme and Microbial Technology, 103, 1-11. http://dx.doi.org/10.1016/j.enzmictec.2017.04.007

Marques, N.P., Pereira, J.C., Gomes, E., da Silva, R., Araújo, A.R., Ferreira, H., Rodrigues, A., Dussán, K.J., & Bocchini, D.A. (2018). Cellulases and xylanases production by endophytic fungi by solid state fermentation using lignocellulosic substrates and enzymatic saccharification of pretreated sugarcane bagasse. *Industrial Crops and Products,* 122, 66-75. http://dx.doi.org/10.1016/j.indcrop.2018.05.022

Mukasekuru, M.R., Kaneza, P., Sun, H., Sun, F.F., He, J., & Zheng, P. (2020). Fed-batch high-solids enzymatic saccharification of lignocellulosic substrates with a combination of additives and accessory enzymes. *Industrial Crops and Products,* 146, 112156. http://dx.doi.org/10.1016/j.indcrop.2020.112156

Nwamba, M.C., Song, G., Sun, F., Mukasekuru, M.R., Ren, H., Zhang, K., Cao, T., Wang, H., Sun, H., & Hong, J. (2021). Efficiency enhancement of a new cellulase cocktail at low enzyme loading for high solid digestion of alkali catalyzed atmospheric glycerol organosolvent pre-treated sugarcane bagasse. *Bioresource Technology,* 338, 125505. http://dx.doi.org/10.1016/j.biortech.2021.125505

Nunes, R.M., Araújo, R.F., Guarda, E.A. (2014). Avaliação do potencial de produção de etanol a partir do efluente da produção de papel. *Revista de Ciências Ambientais,* 8(1), 29-35. http://dx.doi.org/10.18316/1415

Pielhop, T., Larrazábal, G.O., Studer, M.H., Brethauer, S., Seidel, C.M., & von Rohr, P.R. (2015). Lignin repolymerisation in spruce autohydrolysis pretreatment increases cellulase deactivation. *Green Chemistry* 17(6), 3521-3532. http://dx.doi.org/10.1039/c4gc02381a

Rodrigues, P.O., Pereira, J.C., Santos, D.Q., Gurgel, L.V.A., Pasquini, D., & Baffi, M.A. (2017). Synergistic action of an *Aspergillus* (hemi-)cellulolytic consortium on sugarcane bagasse saccharification. *Industrial Crops and Products,* 109, 173-181. http://dx.doi.org/10.1016/j.indcrop.2017.08.031

Rodrigues, P.O., Gurgel, L.V.A., Pasquini, D., Badotti, F., Goes-Neto, A., & Baffi, M.A. (2020). Lignocellulose-degrading enzymes production by solid-state fermentation through fungal consortium among Ascomycetes and Basidiomycetes. *Renewable Energy,* 145, 2683-2693. http://dx.doi.org/10.1016/j.renene.2019.08.041

Rodrigues, P.O., Barreto, E.S., Brandão, R.L., Gurgel, L.V.A., Pasquini, D., & Baffi, M.A. (2021). On-site produced enzyme cocktails for saccharification and ethanol production from sugarcane bagasse fractionated by hydrothermal and alkaline pretreatments. *Waste and Biomass Valorization,* 13, 95–106. https://doi.org/10.1007/s12649-021-01499-7

Rodrigues, P.O., Moreira, F.S., Cardoso, V.C., Santos, L.D., Gurgel, L.V.A., Pasquini, D., & Baffi, M.A. (2022). Combination of High Solid Load, On-site Enzyme Cocktails and Surfactant in the Hydrolysis of Hydrothermally Pretreated Sugarcane Bagasse and Ethanol Production. *Waste and Biomass Valorization,* 13, 3085-3094. https://doi.org/10.1007/s12649-022-01685-1

Santiago, B.L.S., & Rodrigues, F.A. (2017). Processamento de biomassa lignocelulósica para produção de etanol: uma revisão. *The Journal of Engineering and Exact Sciences,* 3(7), 1011-1022. http://dx.doi.org/10.18540/jcecvl3iss7pp1011-1022

Santos, A.C., Ximenes, E., Kim, Y., & Ladisch, M.R. (2019). Lignin–Enzyme Interactions in the Hydrolysis of Lignocellulosic Biomass. *Trends in Biotechnology,* 37(5), 518-531. http://dx.doi.org/10.1016/j.tibtech.2018.10.010

Santos, P.S., Solidade, L.S., Souza, J.G.B., Lima, G.S., Braga Junior, A.C.R., Assis, F.G.V., & Leal, P.L. (2018). Fermentação em estado sólido em resíduos agroindustriais para a produção de enzimas: uma revisão sistemática. *The Journal of Engineering and Exact Sciences,* 4(2), 0181-0188. http://dx.doi.org/10.18540/jcecvl4iss2pp0181-0188

Silva, A.S., Espinheira, R.P., Teixeira, R.S.S., Souza, M.F., Leitão, V.F., & Bon, E.P.S. (2020). Constraints and advances in high-solids enzymatic hydrolysis of lignocellulosic biomass: a critical review. *Biotechnology for Biofuels,* 13, 58. http://dx.doi.org/10.1186/s13068-020-01697-w

Souza, M.F., Bon, E.P.S., Silva, A.A. (2021). Production of cellulases and β-glucosidases by *Trichoderma reesei* Rut C30 using steam-pretreated sugarcane bagasse: an integrated approach for onsite enzyme production. *Brazilian Journal of Chemical Engineering,* 38, 435–442. https://doi.org/10.1007/s43153-021-00114-5

Sun, D., Yang, Q., Wang, Y., Gao, H., He, M., Lin, X., Lu, J., Wang, Y., Kang, H., Alam, A., Tu, Y., Xia, T., & Peng, L. (2020). Distinct mechanisms of enzymatic saccharification and bioethanol conversion enhancement by three surfactants under steam explosion and mild chemical pretreatments in bioenergy *Miscanthus*. *Industrial Crops and Products,* 153, 112559. http://dx.doi.org/10.1016/j.indcrop.2020.112559

Tallarico, S., Costanzo, P., Bonacci. S., Macario, A., Di Gioia, M.L., Nardi, M., Procopio, A., & Oliverio, M. (2019). Combined Ultrasound/Microwave Chemocatalytic Method for Selective Conversion of Cellulose into Lactic Acid. *Scientific Reports,* 9, 18858. https://doi.org/10.1038/s41598-019-55487-y

Vergara, P., Ladero, M., Carbajo, J.M., Garcia-Ochoa, F., & Villaer, J.C. (2021). Effect of additives on the enzymatic hydrolysis of pre-treated wheat straw. *Brazilian Journal of Chemical Engineering*, 38, 241–9. https://doi.org/10.1007/s43153-021-00092-8

Zanchetta, A., Santos, A.C.F., Ximenes, E., Nunes, C.C.C., Boscolo, M., Gomes, E., & Ladish, M.R. (2018). Temperature dependent cellulase adsorption on lignin from sugarcane bagasse. *Bioresource Technology*, 252, 143-149. http://dx.doi.org/10.1016/j.biortech.2017.12.061

Zang, X., Liu, M., Fan, Y., Xu, J., Xu, X., & Li, H. (2018). The structural and functional contributions of β-glucosidase-producing microbial communities to cellulose degradation in composting. *Biotechnology for Biofuels,* 11(51). http://dx.doi.org/10.1186/s13068-018-1045-8

Zhang, R. (2020). Functional characterization of cellulose-degrading AA9 lytic polysaccharide monooxygenases and their potential exploitation. *Applied Microbiology and Biotechnology,* 104, 3229-3243. http://dx.doi.org/10.1007/s00253-020-10467-5

Zhou, Z., Ju, X., Chen, J., Wang, R., Zhong, Y., & Li, L. (2021). Charge-oriented strategies of tunable substrate affinity based on cellulase and biomass for improving in situ saccharification: a review. *Bioresource Technology,* 319, 124159. http://dx.doi.org/10.1016/j.biortech.2020.124159