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Soybean protein as a cost-effective lignin-blocking additive for the saccharification of sugarcane bagasse

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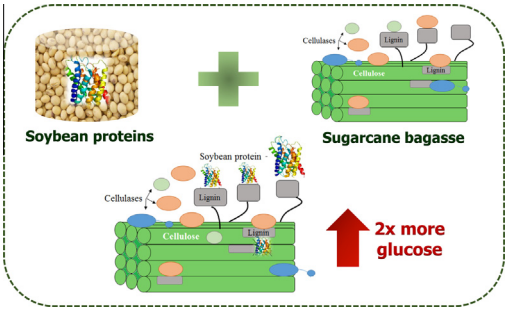
HIGHLIGHTS

- Soybean protein is a potential cost-effective lignin-blocking additive.
- Addition of soybean protein led to 2-fold increase in sugarcane bagasse hydrolysis.
- The positive effect of low-cost soybean protein was comparable to that of BSA.
- On-site enzyme cocktails with soybean protein resulted in 2× higher glucose release.

GRAPHICAL ABSTRACT



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ABSTRACT

Addition of surfactants, polymers, and non-catalytic proteins can improve the enzymatic hydrolysis of lignocellulosic materials by blocking the exposed lignin surfaces, but involves extra expense. Here, soybean protein, one of the cheapest proteins available, was evaluated as an alternative additive for the enzymatic hydrolysis of pretreated sugarcane bagasse. The effect of the enzyme source was investigated using enzymatic cocktails from *A. niger* and *T. reesei* cultivated under solid-state, submerged, and sequential fermentation. The use of soybean protein led to approximately 2-fold increases in hydrolysis, relative to the control, for both *A. niger* and *T. reesei* enzymatic cocktails from solid-state fermentation. The effect was comparable to that of BSA. Moreover, the use of soybean protein and a 1:1 combination of *A. niger* and *T. reesei* enzymatic cocktails resulted in 54% higher glucose release, compared to the control. Soybean protein is a potential cost-effective additive for use in the biomass conversion process.

1. Introduction

The first industrial-scale cellulosic ethanol plants have already started operating worldwide. However, several technological challenges still need to be addressed in order to obtain a commercially competitive product. One such challenge is the need to use high loadings of solids in the hydrolysis process. In addition to the associated difficulties from the process engineering perspective, such as the problems of pumping and agitation at high solids loading, the presence of inhibitors of the biochemical reactions can negatively affect the efficiency of both the enzymatic hydrolysis and the alcoholic fermentation (Jonsson and Martin, 2016; Ko et al., 2015b; Michelin et al., 2016; Ximenes et al., 2011, 2010). These inhibitors, together with the residual lignin, have impact on a critical aspect that has a major influence on the final cost of the cellulosic ethanol: the quantity of enzyme required to convert cellulose into glucose. Despite significant progress already made in this

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regard, studies indicate that the cost of the enzymes is much more significant than has been commonly assumed (Johnson, 2016; Klein-Marcuschamer et al., 2012).

A potential strategy to address these issues and contribute to improving the efficiency of the enzymatic hydrolysis step would be the development of cost-effective technologies to reduce the amount of enzyme lost in the process due to unproductive adsorption onto lignin. One approach that has been described for this purpose is the addition of lignin-blocking agents to the hydrolysis medium. The use of additives such as Tween 20 or 80 surfactants, polyethylene glycol (PEG), and bovine serum albumin (BSA) has been shown to increase the yield and rate of enzymatic hydrolysis (Brethauer et al., 2011; Cannella and Jorgensen, 2014; Eriksson et al., 2002; Jin et al., 2016; Kim et al., 2015; Ko et al., 2015a,c; Kristensen et al., 2007; Kumar and Wyman, 2009; Yang and Wyman, 2006).

Although the use of an additive increases the cost of the cellulosic ethanol production process, there are clear benefits in terms of improving the saccharification reaction. Reduction of unproductive binding to lignin enables more effective use of the added enzymes and, most importantly, can help to decrease the enzyme loading required (Kim et al., 2015). Studies have found that addition of PEG improved the rate of wheat straw hydrolysis by up to 45% (Cannella and Jorgensen, 2014; Hsieh et al., 2015), while the presence of Tween 80 during the hydrolysis of spruce increased the conversion rate by up to 58% (Kristensen et al., 2007). The mechanism proposed for the positive effect of additives on lignocellulosic hydrolysis may vary according to the biomass, enzyme preparation, pretreatment, experimental conditions, among others. For instance, a recent study demonstrated that the mechanism for Tween 80 enhancing steam-exploded biomass hydrolysis is either blocking lignin adsorption of enzymes or dissociating hemicellulose from lignin (Jin et al., 2016). The latter effect is related to the fact that hemicelluloses are central linkers between cellulose and lignin, and the disruption of their interactions should make cellulose more accessible to enzymatic attack (Wang et al., 2016). However, there is a clear need to find more cost-effective additives for use in enzymatic biomass hydrolysis processes. Recently, Hui et al. (2015) studied the effect of non-enzymatic protein additives (corn steep liquor, peptone, and yeast extract) in the enzymatic hydrolysis and simultaneous saccharification and fermentation of rice straw, showing that glucose release increased by up to 13.7% with the use of such additives. The authors showed that the positive effect exerted by the non-enzymatic proteins tested was clearly better than that provided by a model protein, BSA. These findings further highlight that the use of inexpensive non-enzymatic proteins as lignin-blocking agents could be very promising for future applications in glucose and ethanol production from lignocellulosic materials.

Another approach to address the limitation related to loss of enzymes due to unproductive adsorption could be to tailor enzymatic preparations that are less liable to adsorption onto lignin. It has been reported previously that enzymes from *Trichoderma reesei* and *Aspergillus niger* respond differently, in terms of lignin adsorption, during the hydrolysis of hardwoods pretreated using liquid hot water (Ko et al., 2015c). Therefore, the on-site production of enzymes using different fungal strains and cultivation methods could be a promising strategy to obtain enzymatic cocktails with different lignin adsorption characteristics. Among the cultivation methods employed to produce enzymes, traditional cultivation systems such as solid-state fermentation (SSF) and submerged fermentation (SmF) have been widely described for obtaining cellulases and hemicellulases (Farinas, 2015). More recently, a sequential fermentation (SF) method was developed with the aim of combining the advantages of the two conventional methods (SSF and SmF) for cellulase production (Cunha et al., 2015, 2012b; Florencio et al., 2015). The use of sequential fermentation was shown to result in enzymatic cocktails that exhibited different characteristics, compared to those from SSF and SmF (Vasconcellos et al., 2015), and that provided more efficient saccharification, relative to the SmF cocktail (Florencio et al., 2016). Therefore, it is of interest to investigate how the performance of different enzymatic cocktails in the saccharification of lignocellulosic materials is likely to be affected by the presence of lignin-blocking additives. Among the possible options, soybean protein stands out as a promising cost-effective candidate, since it is one of the cheapest proteins available on the market (Klein-Marcuschamer et al., 2012). However, to the best of our knowledge, there have been no studies focusing on the evaluation of soybean protein as an additive to improve the saccharification of lignocellulosic biomass.

This paper reports a systematic study of the effects of using soybean protein as a cost-effective lignin-blocking additive in the enzymatic hydrolysis of pretreated sugarcane bagasse. The effect of adding soybean protein in biomass saccharification was evaluated in comparison to other established additives (Tween, PEG, and BSA). Assessment was made of the performance of enzymatic cocktails produced in-house by *T. reesei* and *A. niger*, under different cultivation systems (SSF, SmF, and SF), and used for sugarcane bagasse hydrolysis in the presence of soybean protein, with the aim of reducing the unproductive adsorption of enzymes onto lignin.

2. Materials and methods

2.1. Additives

The concentration of soybean protein (isolated protein with 90% protein content, from Bremil, Rio Grande do Sul, Brazil) was initially tested at 1, 4 and 7% (w/w). Then, for all of the subsequent experiments, soybean protein was used at 4% (w/w) concentration. Other additives tested were polyethylene glycol (PEG) with molecular weights of 1500, 6000, and 8000 g/mol (Sigma Aldrich, USA), at a concentration of 4% (w/w) (Cannella and Jorgensen, 2014), and Tween 20 and 80 (polyoxyethylene sorbitan monooleate, Dinamica, Brazil), at 0.1% (w/v) (Okino et al., 2013). BSA (Sigma, USA) was used at a concentration of 4% (w/w), for comparison with the effects of the other additives.

2.2. Fungal strains

The *T. reesei* Rut-C30 strain was purchased from the Centre for Agricultural Bioscience International (CABI) culture collection in the United Kingdom (IMI number: 345108). The *A. niger* wild-type A12 strain was obtained from the Embrapa Food Technology collection (Rio de Janeiro, Brazil). The strains were maintained at −18 °C in a 20% w/w glycerol/water solution and were activated in potato dextrose agar (PDA) for 5 days at 30 °C (*T. reesei*) or for 3 days at 32 °C (*A. niger*) (Florencio et al., 2016).

2.3. Substrate

The inducer substrate used for cultivation of *T. reesei* and *A. niger* was washed steam-exploded sugarcane bagasse (SEB_w), donated by a local sugarcane mill (Usina Nardini, Vista Alegre do Alto, São Paulo, Brazil). The washing procedure was carried out as described by Vasconcellos et al. (2015). The sugarcane bagasse samples were subsequently milled and sieved, and the particle size selected was $1.0 \leqslant dp \leqslant 2.0$ mm (particle diameter). Chemical characterization was performed as described previously (Gouveia et al., 2009). The composition of the pretreated bagasse was (w/