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# Intensification of corn fiber saccharification using a tailor made enzymatic cocktail

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ABSTRACT

The transition from an economic model based on resource extraction to a more sustainable and circular economy requires the development of innovative methods to unlock the potential of raw materials such as lignocellulosic biomasses. Corn fiber differs from more traditional lignocellulosic biomasses due to its high starch content, which provides additional carbohydrates for fermentation-based biomanufacturing processes. Due to its unique chemical composition, this study focused on the development of a tailor made enzymatic cocktail for corn fiber saccharification into monosaccharides. Three commercially available hydrolytic enzymes (Cellic® CTec2, Pentopan® Mono BG, and Termamyl® 300 L) were combined to hydrolyze the polysaccharide structure of the three main carbohydrate fractions of corn fiber (cellulose, hemicellulose and starch, respectively). Prior to saccharification, corn fiber was submitted to a mild hydrothermal pretreatment (30 min at 100 °C). Then, two experimental designs were used to render an enzymatic cocktail capable of providing efficient release of monosaccharides. Using 60 FPU/g DM of Cellic® CTec2 and 4.62 U/g DM of Termamyl® 300 L, without addition of Pentopan® Mono BG, resulted in the highest efficiencies for glucose and xylose release (66% and 30%, respectively). While higher enzyme dosages could enhance the saccharification efficiency, adding more enzymes would have a more pronounced effect on the overall process costs rather than in increasing the efficiency for monosaccharides release. The results revealed that the recalcitrance of corn fiber poses a problem for its full enzymatic degradation. This fact combined with the unique chemical composition of this material, justify the need for developing a tailor made enzymatic cocktail for its degradation. However, attention should also be given to the pretreatment step to reduce even more the recalcitrance of corn fiber and improve the performance of the tailored cocktail, as a consequence.

## 1. Introduction

The decoupling of industrial production methods from petrochemical resources is a necessary process for the sustainable development of modern societies [1]. In this transition from oil-based economies to bio-based ones, lignocellulosic materials have gained attention as low-cost materials for the production of biofuels and biochemical compounds [1–5]. Among the different types of lignocellulosic biomasses, agro-industrial wastes stand out as promising sources of carbon for the production of biomolecules. Not only are they cheap and readily available but being residual compounds from the food and feed industries, they do not represent a threat to food security or additional land use [2,3].

To unlock the carbohydrate potential of lignocellulosic biomasses, pretreatment and enzymatic hydrolysis steps are typically needed to reduce their natural recalcitrance [4]. Enzymatic hydrolysis has been deemed as one of the most appropriate methods for saccharification of biomass polysaccharides into monomers [6]. However, for an efficient enzymatic hydrolysis of lignocellulosic biomass, different enzymes are needed to respond to the heterogeneous structure of these materials. In addition, different lignocellulosic materials require a different combination of enzymes to result in maximum saccharification. Therefore, numerous studies have been published in recent years focusing on either the optimization of the enzymes themselves [7,8] or on the optimization of enzymatic cocktails [9–12].

Corn fiber, a by-product from the corn wet-milling process, is a

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lignocellulosic material consisting mainly of the pericarp layer of the corn kernels [13]. Currently, it is mainly used as an ingredient in animal feed [14], but it has steadily been gaining attention as a potential carbon source for biotechnological uses, mostly related to the production of bioethanol [15,16]. Corn fiber is an uncommon lignocellulosic material, as it contains a relatively low lignin amount, which is contrasted by its high residual starch content, which serves as an additional source of glucose for fermentation processes [13]. Moreover, its hemicellulosic fraction is a heavily branched glucuronoarabinoxylan structure, with numerous substitutions of phenolic compounds [14,17], which makes it more resistant to enzymatic degradation compared to the hemicellulose of other lignocellulosic biomasses [17,18]. Thus, carefully designed enzymatic cocktails are needed for its efficient saccharification.

Based on the above, this study focused on the tailoring of an enzymatic cocktail for saccharification of corn fiber into monosaccharides. Utilizing statistical experimental design approaches, various combinations of commercial enzymes were tested to create an optimized enzymatic cocktail able to efficiently yield fermentable sugars from mildly hydrothermally pretreated corn fiber.

## 2. Materials and methods

### 2.1. Lignocellulosic biomass composition and pretreatment

Corn fiber (Refinazil®) was kindly provided by Ingredion Incorporated (Brazil). Compositional analysis was carried out by following the standard analytical protocol developed by the National Renewable Energy Laboratory (NREL) [19]. The composition of the (extractive free) corn fiber was (in wt%) 24.87 ± 1.96 cellulose, 42.41 ± 3.03 hemicellulose, 23.37 ± 0.85 starch, and 9.34 ± 0.28 lignin. All values denote the percentages in dry weight and their correspondent standard deviations.

Prior to saccharification, corn fiber was submitted to a mild hydrothermal pretreatment, which consisted in heating a 10% (w/v) mixture of the biomass in distilled water for 30 min at 100 °C. This pretreatment step was performed in an autoclave (MultiControl 2, CertoClav, Austria). After pretreatment, the biomass was cooled in an ice bath and the solid fraction was separated and dried in a circulating oven (VENTI-Line®, VWR, Denmark) at 60 °C until it reached a moisture level of around 10%, being then kept frozen until further use. The liquid fraction's total carbohydrate content was analyzed by means of the Anthrone-Sulfuric Acid assay [20]. As the total carbohydrate amount released during the pretreatment corresponded to approximately 5% of the solid's total carbohydrate content, it was decided to discard the liquid fraction.

### 2.2. Enzymes

Different combinations of three commercial enzymes were evaluated to tailor the enzymatic cocktail. Each of the enzymes aimed for the hydrolysis of one of the three carbohydrate fractions of the corn fiber: the cellulolytic enzyme mixture Cellic® CTec2 (CCT2) was used for cellulose degradation; the xylanase Pentopan® Mono BG (PMBG) (Novozymes) aimed at hemicellulose saccharification, aided by CCT2′s complementary activities, while the alpha-amylase Termamyl® 300 L (TM300) (Novozymes) was used for hydrolyzing the starch fraction. CCT2 was kindly provided by Novozymes (Bagsværd, Denmark), while PMBG and TM300 were purchased from Sigma-Aldrich.

CCT2′s general cellulase activity was determined by the method developed by Adney and Baker [21] and PMBG's xylanase activity by the method described by Bailey, Biely and Poutanen [22]. The activities were determined to be 165 FPU/mL and 17554 XU/g respectively. To evaluate the amylase activity of TM300, 0.1 mL of enzyme was incubated in a 1% starch solution made in 50 mM Na-citrate buffer (pH 5). The incubation step was performed for 30 min at 45 °C. The glucose release was determined colorimetrically by a glucose oxidase/peroxidase kit (GOPOD kit, Megazyme, Ireland) [23]. The amylase activity of TM300 was determined to be 2 U/mL. One amylase unit was defined as the release of 1 μmol of glucose per minute under reaction conditions.

### 2.3. Design of experiments for enzymatic cocktail optimization

To optimize the composition of the enzymatic cocktail for monosaccharide release from corn fiber, two sequential experimental designs were utilized. The saccharification experiments were carried out in horizontal mode in a roller system (Thermo Scientific, USA) using 50-mL tubes with a working volume of 25 mL of 50 mM Na-citrate (pH 5) and an agitation speed of 80 rpm. A 10% (w/v) solid load of corn fiber (on a dry weight basis) was used. The hydrolysis was performed for 72 h at a constant temperature of 45 °C.

#### 2.3.1. Box-Behnken experimental design

For the optimization of the combination of the three enzymes, a three variable, three level Box-Behnken experimental design with three replicates at the central point was used. The studied variables included different dosages (in enzymatic activity per gram of dry biomass) of CCT2 (10–30 FPU/g DM), PMBG (500–1000 XU/g DM), and TM300 (1–8 U/g DM). The saccharification efficiencies of glucose and xylose (in grams of released monosaccharide per theoretical maximum amount of monosaccharide, Equation 1) at 72 h of hydrolysis were used as response variables. Glucose and xylose maximum achievable monosaccharide release was calculated based on the biomass characterization performed in Section 2.1. The experimental design with the efficiencies achieved for each monosaccharide is shown in Table 1. A hydrolysis assay under the optimal conditions was carried out to confirm the results predicted by the analysis.

$$Efficiency\ (\%) = \frac{Monosaccharide\ release\ at\ 72h}{Maximum\ achievable\ release\ of\ monosaccharide} \times 100$$

#### 2.3.2. Central composite experimental design

To increase the xylose release and obtain a more complete hydrolysis of the biomass, a $2^2$ central composite experimental design with a triplicate at the central point was performed following the Box-Behnken experimental design. In these assays, the TM300 dosage was fixed at 4.62 U/g dry matter (optimal value obtained in the previous design); while the other two enzymes, CCT2 and PMBG were studied in a different range of values (20–60 FPU/g DM and 100–900 XU/g DM, respectively) (Table 2). Following the trends observed in Fig. 1, higher CCT2 dosages were tested, while in the case of PMBG, lower enzymatic loads were used. As in the previous design, an additional hydrolysis assay was performed under the optimum conditions suggested by the

**Table 1**
Variables tested and responses obtained according to the Box-Behnken experimental design used for corn fiber saccharification. DM: dry matter.

| Assay | Cellic® CTec2 (FPU/g DM) | Pentopan® Mono BG (XU/g DM) | Termamyl® 300 L (U/g DM) | Glucose release efficiency (%) | Xylose release efficiency (%) |
|---|---|---|---|---|---|
| **1** | 10 | 500 | 4.5 | 47.09 | 18.24 |
| **2** | 30 | 500 | 4.5 | 43.27 | 22.54 |
| **3** | 10 | 1000 | 4.5 | 45.20 | 14.51 |
| **4** | 30 | 1000 | 4.5 | 44.88 | 23.42 |
| **5** | 10 | 750 | 1 | 43.00 | 13.65 |
| **6** | 30 | 750 | 1 | 50.70 | 20.15 |
| **7** | 10 | 750 | 8 | 41.02 | 13.92 |
| **8** | 30 | 750 | 8 | 57.52 | 24.43 |
| **9** | 20 | 500 | 1 | 47.27 | 19.29 |
| **10** | 20 | 1000 | 1 | 50.52 | 18.28 |
| **11** | 20 | 500 | 8 | 45.41 | 20.27 |
| **12** | 20 | 1000 | 8 | 53.72 | 19.94 |
| **13** | 20 | 750 | 4.5 | 50.27 | 20.50 |
| **14** | 20 | 750 | 4.5 | 58.90 | 22.24 |
| **15** | 20 | 750 | 4,5 | 60.41 | 22.99 |

**Table 2**
Variables tested and responses obtained according to the central composite experimental design used for corn fiber saccharification. DM: dry matter.

| Assay | Cellic® CTec2 (FPU/g DM) | Pentopan® Mono BG (XU/g DM) | Glucose release efficiency (%) | Xylose release efficiency (%) |
|---|---|---|---|---|
| **1** | 20 | 100 | 43.90 | 18.38 |
| **2** | 20 | 500 | 42.54 | 16.85 |
| **3** | 20 | 900 | 46.16 | 14.98 |
| **4** | 40 | 100 | 65.09 | 28.37 |
| **5** | 40 | 900 | 58.86 | 24.44 |
| **6** | 60 | 100 | 72.78 | 30.99 |
| **7** | 60 | 500 | 78.15 | 31.49 |
| **8** | 60 | 900 | 60.04 | 27.68 |
| **9** | 40 | 500 | 63.33 | 24.54 |
| **10** | 40 | 500 | 63.77 | 24.68 |
| **11** | 40 | 500 | 61.15 | 26.36 |

model to validate it.

Finally, an assay was also carried out removing PMBG, TM300 or both enzymes from the optimized enzymatic cocktail and the monosaccharide release efficiencies at 72 h of hydrolysis were determined.

### *2.4. Analytical methods*

Glucose and xylose concentrations were measured by HPLC. The chromatographic separation was performed using an Ultimate 3000 Basic Automated System from Thermo Scientific, with an Aminex HPX-87 H column (dimensions: 300 ×7.8 mm), under the following conditions: 5 mM $H_2SO_4$ as mobile phase in a flow rate of 0.4 mL/min, at 40 °C and injection volume of 20 μL. StatisticaTM 14.0.1 (TIBCO Software Inc., Palo Alto, California, USA) was the software used for statistical analysis of the results.

## 3. Results

### *3.1. First round of cocktail improvement using a Box-Behnken experimental design*

As a first step for tailoring the enzymatic cocktail, a Box-Behnken experimental design was used where different concentrations of three commercial hydrolytic enzymes were tested. The experimental design, as well as the results obtained for each individual experiment, are shown in Table 1. As can be seen, a significant variation in the responses occurred according to the conditions used for saccharification, from 41.02% to 60.41% for glucose release efficiency, and from 13.65% to 24.43% for xylose release efficiency. Statistical analysis of these results revealed that the dosage of the cellulolytic cocktail CCT2 was the main factor contributing to a high monosaccharide release (Fig. 1). Its effect was significant at 95% confidence level in a linear form for both glucose and xylose release efficiency. For glucose release efficiency, CCT2 was the only enzyme with significant effect, while for xylose release efficiency, TM300 and PMBG also presented significant effects at 95% and 90% confidence level, respectively.

Interestingly, although both enzymes CCT2 and PMBG presented linear effects on xylose release efficiency, CCT2 showed a strong positive effect, while PMBG had a weaker and negative effect (Fig. 1b), meaning that the results were improved when the amount of PMBG was reduced. For TM300, a quadratic effect was observed only for xylose release efficiency, which was significant at 95% confidence level. Overall, the statistical model adjusted for these responses suggests an improved cocktail composition consisting of 30 FPU/g DM of CCT2, 678 XU/g DM of PMBG and 4.62 U/g DM of TM300. This cocktail would render release efficiencies of 52.5% and 18.6% for glucose and xylose, respectively.

To test the accuracy of the model and verify the development of the hydrolysis using the enzyme combination, a validation experiment was carried out in triplicate (Fig. 2). The kinetics of glucose release show that most of this monosaccharide was released during the first 24 h hydrolysis. Afterward, the efficiency exhibited a less pronounced increase until the end of the experiment. In the case of xylose, the release occurred in an almost linear manner during the whole experiment.

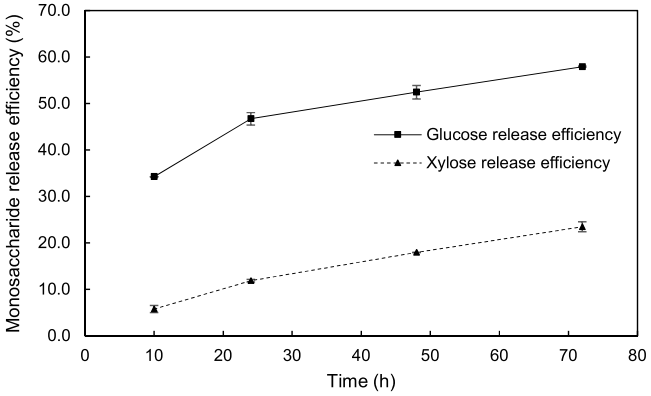


**Fig. 2.** Evolution of corn fiber saccharification using the enzymatic cocktail suggested by the model obtained through the Box-Behnken experimental design. Error bars depict standard deviation of triplicates. The saccharification was carried out at 45 °C, with a 10% (w/v) solid load.

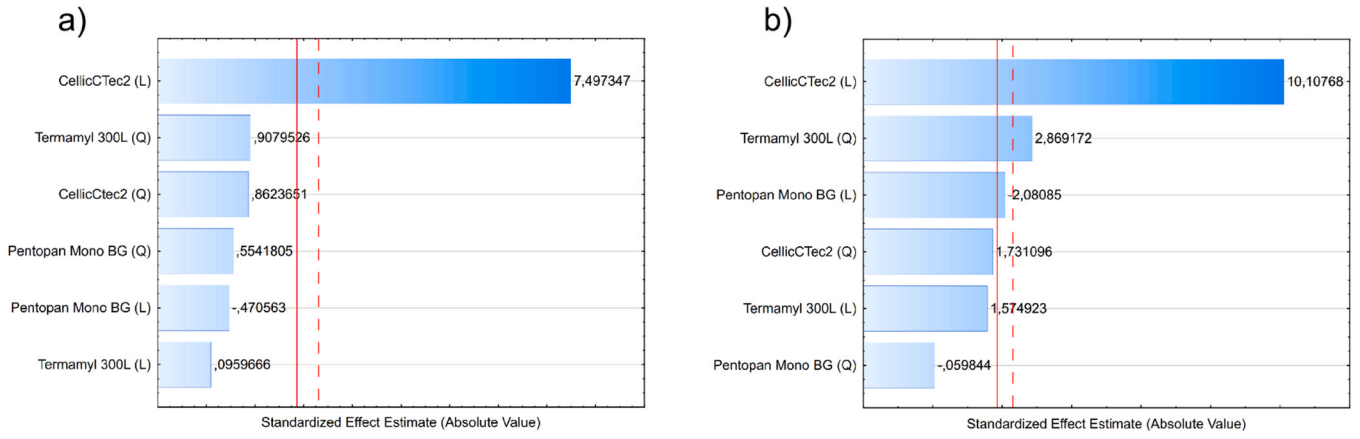


**Fig. 1.** Pareto charts describing the effects of the three tested enzymes in the Box-Behnken experimental design on the release of monosaccharides from corn fiber. A: enzyme effects on glucose release efficiency; B: enzyme effects on xylose release efficiency. The continuous vertical line denotes a significance level for $p = 0.1$ and the dashed line for $p = 0.05$.

Glucose release efficiency reached a maximum value of 57.9%, which fits into the 95% confidence interval predicted by the statistical analysis. Xylose release was also higher than the one predicted by the model, reaching a value of 23.5% at 72 h, however, this value exceeded the model's prediction by 5%.

### 3.2. Second round of cocktail improvement using a central composite experimental design

As the results depicted in Fig. 2 showed room for improvement, a second round of enzymatic cocktail optimization was performed. The linear and positive effect observed for CCT2 (Fig. 1, S1 and S2) suggests that an increase in the enzyme dosage would result in improved monosaccharide release efficiencies. The effect of PMBG on xylose release was rather flat (Fig. 1 and S2), with a small increase in its effect towards lower dosages. As a flat response could indicate that the selected range in the experimental design was too narrow, a wider range of enzyme levels was tested (100–900 XU/g DM), focusing on lower dosages. Finally, TM300 appeared to have reached the peak of its effect at a level of 4.62 U/g DM (Figs. S1 and S2), so, this level was fixed for the next round of optimization. Having only two variables to test, it was decided to continue the design of experiments using a central composite design where the dosages of CCT2 and PMBG were varied (Table 2).

The statistical analysis of the central composite experimental design (Fig. 3) showed that similarly to Fig. 1, CCT2 showed a highly significant linear effect for both responses, glucose and xylose release. In this case, its contribution was statistically significant ($p < 0.05$) even in a quadratic form for xylose release efficiency. Again, PMBG showed a significant ($p < 0.05$) linear and negative effect for xylose release only.

The statistical model adjusted for these responses suggested an improved enzyme cocktail composition consisting of 60 FPU/g DM of CCT2 and 177 XU/g DM of PMBG, with the aforementioned fixed value of 4.62 U/g DM of TM300. With this cocktail, the expected monosaccharide release efficiencies would be 72.6% for glucose and 31.5% for xylose. The fitness of the model reached $R^2$ values of 0.89 and 0.98 for glucose and xylose release efficiencies, respectively.

To confirm the results predicted by the model, a validation experiment was carried out. As can be seen in Fig. 4, the release of glucose and xylose using the enzyme loads suggested by the model followed a very similar pattern, being relatively faster during the first 48 h and becoming more stagnant after that. At the end of the experiment, release efficiencies of 62.5% and 30.3% were obtained for glucose and xylose, respectively. Both values fall into the model's 95% prediction interval, which indicates good accuracy.

Finally, additional experiments were carried out to evaluate the contribution of each enzyme present in the tailored cocktail on the saccharification of corn fiber. In these experiments, PMBG and/or TM300 were removed from the cocktail optimized according to the central composite design. The rest of the experimental conditions were maintained as explained previously. The results of glucose and xylose release efficiencies obtained at 72 h of hydrolysis were submitted to Tukey's test ($\alpha = 0.05$) to verify their similarities and differences. The analysis revealed no statistically significant differences on glucose release when PMBG or TM300 were removed from the cocktail. On the other hand, removing TM300 from the optimized cocktail reduced the xylose release efficiency by 3.3%. When CCT2 was used alone, glucose release efficiency was increased by almost 15% (compared to the efficiency obtained in the central composite design), but xylose release efficiency decreased by 3.4%.

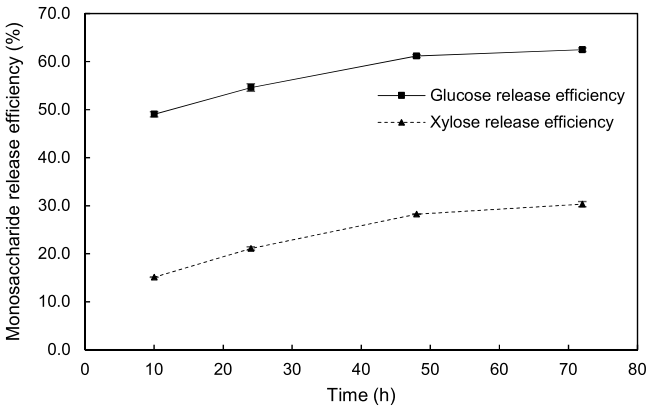


**Fig. 4.** Evolution of corn fiber saccharification using the enzymatic cocktail suggested by the model obtained through the central composite experimental design. Error bars depict standard deviation of the triplicates. The saccharification was carried out at 45 °C, with a 10% (w/v) solid load.

## 4. Discussion

The enzymatic saccharification experiments carried out according to the Box-Behnken experimental design showed less than 60% release efficiency for glucose and less than 25% for xylose. Overall, the biggest factor contributing to monosaccharide release was the dosage of CCT2 (Fig. 1), a well-known cellulolytic cocktail with side activities. Even with a high starch content in corn fiber (23.37%), TM300's effect was not statistically significant for glucose release (Fig. 1). The Termamyl® product range are thermostable alpha-amylases that have been reported

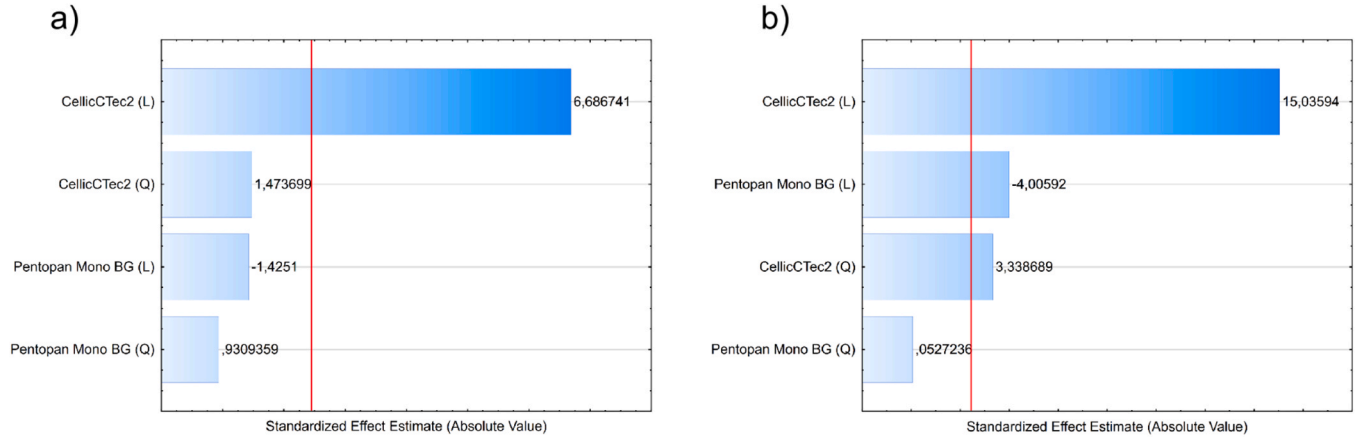


**Fig. 3.** Pareto charts describing the effects of the two tested enzymes in the central composite experimental design assay on the release of monosaccharides from corn fiber. A: enzyme effects on glucose release efficiency; B: enzyme effects on xylose release efficiency. The continuous vertical line denotes a significance level for $p = 0.05$.

to withstand high temperatures (90–120 °C) for hydrolysis [13]. The need for a higher temperature could explain both the relatively low activity (2 U/mL) at 45 °C and the low significance for the glucose release.

In the Box-Behnken experiments, PMBG did not significantly contribute to xylose release (Fig. 1), and its response was surprisingly a flat surface for this monosaccharide (Fig. S2), which could be an indicator of a too narrow range selected for the experimental design. In fact, other studies conducting enzymatic saccharification of lignocellulosic biomass vary wildly in the xylanase dosage utilized [24,25], even if they utilize the same method for determining the xylanase activity [22]. This, combined with PMBG's flat response and corn fiber's heavily branched hemicellulosic structure [17], suggest that the range tested in the Box-Behnken design was too narrow in order to see a response. Consequently, the optimization procedure was continued by testing higher CCT2 and lower PMBG dosages.

The enzymatic cocktail predicted by the central composite design achieved 4.5% and 6.8% higher monosaccharide release efficiencies for glucose and xylose, respectively (Fig. 4). The obtained statistical model's accuracy also presented high $R^2$ values, and the validation experiments confirmed a satisfactory prediction capability of the model. Similar to the Box-Behnken design, CCT2 was the enzyme with the highest effect on hydrolysis also in the experiments of the central composite design (Fig. 3). In fact, the highest dosage tested, 60 FPU/g DM, was the one recommended by the model as the optimal one. Even if the observed trend in the pareto chart (Fig. 3) and the response surfaces (Fig. S3) suggests that higher CCT2 dosages would increase the saccharification efficiency, the cost of the enzymes poses an important burden to the overall process. Higher enzyme dosages would increase the overall process costs at a faster rate than they would increase the efficiency, as evidenced by the 4.5% and 6.8% efficiency increases for glucose and xylose when the CCT2 dosage was doubled. Furthermore, it can be observed in Fig. 4 how the increase in monosaccharide release was much lower between 48 h and 72 h, while this is not the case in Fig. 2. This suggests that higher enzyme levels would accelerate the saccharification process but will not necessarily increase the maximum efficiency achieved.

Several articles have described the recalcitrance of the corn fiber hemicellulosic fraction [14,17,18], which poses a problem for the enzymatic degradation of the biomass. It is known that for an efficient saccharification of the hemicellulose, a combination of different enzymes acting in synergy is needed [17,26,27]. CCT2′s accessory enzymes' activities have been previously reported [28], and it was expected that the increase in endoxylanase activity from the addition of PMBG would help take full advantage of CCT2′s side activities. However, results suggest that this was not the case and that the hemicellulosic fraction was not completely hydrolyzed. This could explain the slower release towards the end of the process in Fig. 4 and why different levels of PMBG, an endoxylanase, had a low impact on the release of monosaccharides, especially xylose. Pretreatments stronger than the one performed in this study could help overcome this issue [29] by providing a better disruption of the lignocellulosic structure, which would give better access for the enzymes to the fibers and hence, increase the saccharification efficiency. Increasing the operating temperature would also lead to higher enzymatic activity, as CCT2′s optimal temperature is close to 50 °C [30]. A de-starching process at 90 °C prior to the lignocellulosic hydrolysis process [13] could also unlock the full potential of TM300. However, all these approaches come with increased operating costs. As a matter of fact, the operating temperature used in this study would allow for a simultaneous saccharification and fermentation scenario using thermotolerant microorganisms [30], which could render the process economically profitable if the fermentation end product has a high value. However, a combination of different approaches, like more potent pretreatments and better use of the enzymes, would most probably be the solution for a more efficient corn fiber saccharification.

When evaluating the effect of each individual enzyme present in the optimized enzymatic cocktail, it was observed that the removal of TM300 negatively affected the release of xylose. This outcome was predicted in Fig. 1, where TM300 was statistically significant only for xylose release. This enzyme's alpha-amylase activity could remove starch and grant better access for the rest of the enzymes to contribute to hemicellulose degradation. On the other hand, the removal of PMBG did not yield any statistically significant difference when compared to the tailored cocktail's result. In fact, in both Figs. 1 and 3 it can be observed how, compared to the other two enzymes, PMBG's significance is lower. In the response surfaces (Figs. S1, S2 and S3), PMBG also showed a quite flat response.

As mentioned before, higher enzyme loads would inevitably result in higher costs for any hydrolysis process. In the present study, all the experiments showed a clear and important contribution of CCT2 for an efficient hydrolysis of corn fiber. PMBG showed a statistically significant contribution to xylose release efficiency. However, its effect on the release was negative, as depicted in the Pareto charts (Figs. 1 and 3). Removing PMBG from the optimized cocktail also did not change the results in a statistically significant manner. For this reason, the removal of this enzyme would be recommended, as it would result in lower operational costs without a significant loss in monosaccharide release efficiency. For the case of TM300, removing it from the cocktail would decrease the cost of the hydrolysis and increase glucose release, but would result in a reduction of xylose release. In this sense, a techno-economic assessment considering the end product of a given process (high or low value end product) would be necessary to determine the necessity of utilizing this enzyme for hydrolysis. Finally, a process consisting of a more potent pretreatment than the one used in this study combined with an enzymatic cocktail consisting of 60 FPU/g DM of CCT2, and 4.62 U/g DM of TM300 could result in higher monosaccharide release efficiencies.

## 5. Conclusions

This study focused on the development of an enzymatic cocktail for an efficient saccharification of corn fiber into monosaccharides. An enzymatic cocktail with a composition of 60 FPU/g DM of Cellic® CTec2 and 4.62 U/g DM of Termamyl® 300 L was found as the best one resulting in glucose and xylose release efficiencies of 66% and 30%, respectively. Although this enzyme load can be considered higher compared to other values reported in the literature, it is worth noting that the corn fiber used in the present study has not been previously milled and/or de-starched, and was pretreated under mild hydrothermal conditions (30 min at 100 °C), resulting in a simpler process, with less steps, less energy requirement, avoiding the formation of inhibitors during pretreatment and their negative effect during the saccharification step. So, the process described in the present study has potential to be a more sustainable approach for corn fiber saccharification.

The results of this study also revealed that the recalcitrance of corn fiber poses a problem for its full enzymatic degradation. In this sense, future challenges include testing pretreatment methods/conditions able to reduce more significantly the biomass recalcitrance and increase hydrolysis efficiency as a consequence. Attention can also be given to the use of more advanced hemicellulolytic enzymes, capable of debranching the complex hemicellulose structure of corn fiber. Finally, a techno-economic assessment is recommended to assess the feasibility of utilizing Termamyl® 300 L in the tailored cocktail based on the final desired application.

## CRediT authorship contribution statement

**Julen Ordeñana Manso**: Methodology, Validation, Formal analysis, Investigation, Writing-original draft. **Martin B. Nielsen**: Methodology, Validation, Investigation. **Eva Balaguer Moya**: Methodology, Investigation. **Juliana P. Sandri**: Methodology, Investigation. **Celina K. Yamakawa**: Methodology, Supervision, Writing-original draft. **Solange**

**I. Mussatto**: Conceptualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Writing- review and editing.

## Data availability

Data will be made available on request.

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*Author agreement*

We certify that all authors have seen and approved the final version of the manuscript being submitted. We also confirm that this is an original work that has not been published previously and that is not under consideration for publication elsewhere. All the authors agreed that this manuscript should be submitted to Enzyme and Microbial Technology. If accepted for publication, it will not be published elsewhere in the same form, in English or any other language, including electronically, without the consent of the copyright holder.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.enzmictec.2023.110347.

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