α-Galactosidase-assisted Hydrolysis of Spent Coffee Grounds Reveals a Trade-off Between Manno-oligosaccharide Production and Glucose Release

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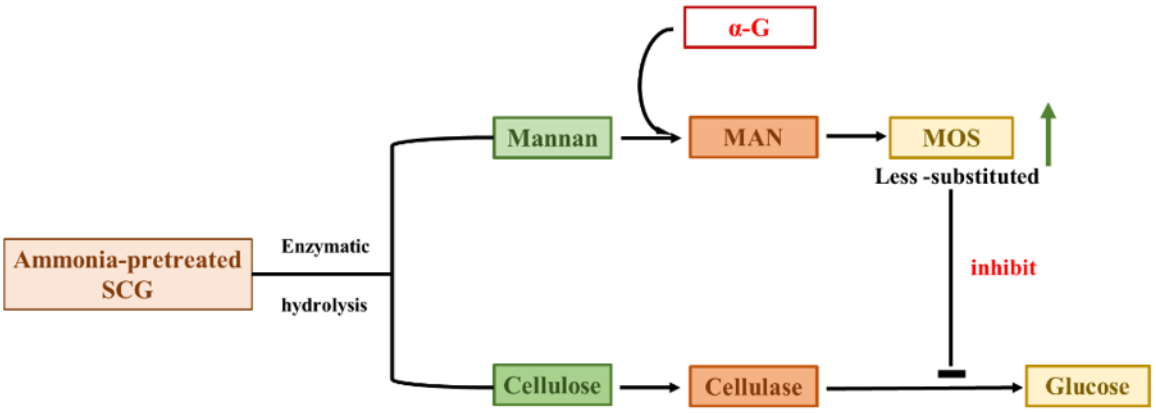
GRAPHICAL ABSTRACT



α-Galactosidase-assisted Hydrolysis of Spent Coffee Grounds Reveals a Trade-off Between Manno-oligosaccharide Production and Glucose Release

Hong Yin,[a] Zhenni Jiao,[b] and Donglin Xin [a,*]

Spent coffee grounds (SCG) are an abundant lignocellulosic biomass rich in galactomannan and cellulose, rendering them a promising feedstock for biorefinery, especially for the production of manno-oligosaccharides (MOS) as high-value prebiotics. This study investigated the role of α-galactosidase (α-G) in the enzymatic hydrolysis of ammonia-pretreated SCG, with particular emphasis on MOS production and cellulose hydrolysis. Enzymatic hydrolysis was conducted at 50 °C and pH 5.0, and the resulting sugars and MOS were quantified by HPLC. Supplementation with α-G effectively removed α-1,6-linked galactose side groups from galactomannan and increased MOS yield. Notably, the addition of α-G enabled a 50% reduction in mannanase dosage without compromising MOS production, indicating its potential to improve galactomannan conversion efficiency. However, α-G treatment altered the properties of the resulting MOS and was associated with stronger inhibition in MOS-containing cellulase systems. Under the tested conditions, α-G supplementation in MOS-containing systems was associated with additional numerical reductions in glucose yield of 15.8% for CBHI and 8.1% for EGII, whereas little numerical change was observed in the βG system. These results reveal a trade-off associated with α-G supplementation: although it promotes MOS generation, stronger inhibition was observed in MOS-containing cellulase systems, which may impair downstream cellulose hydrolysis.

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Keywords: Ammonia-pretreatment; Spent coffee grounds; α-Galactosidase; Manno-oligosaccharides; Cellulose

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INTRODUCTION

With the continuous growth of global coffee consumption, the issue of spent coffee grounds (SCG) disposal has become increasingly prominent. About 6 million tons of coffee grounds are produced globally each year, most of which are directly landfilled or incinerated, not only causing a waste of resources but also potentially leading to environmental problems (Mussatto *et al*. 2011). The SCG are composed of considerable proportions of cellulose and galactomannan embedded in the cell wall matrix, highlighting their potential as a renewable source for carbohydrate valorization (Ballesteros *et al*. 2014). Particularly, galactomannan can be converted into mannan oligosaccharides (MOS) with

prebiotic properties, which have significant application value in functional foods and the pharmaceutical industry (Gibson *et al*. 2017). Therefore, developing efficient biological conversion technologies for coffee grounds has significant environmental and economic significance (Karmee 2017).

At present, the hydrolysis methods of coffee grounds primarily include physical, chemical, and enzymatic methods. Although physical and chemical methods can effectively destroy the fiber structure, they have disadvantages such as high energy consumption and large pollution (Mekonnen *et al*. 2025). In contrast, enzymatic hydrolysis has advantages, such as mild conditions, lesser need for chemical reagents, and good selectivity, but the existing enzymatic hydrolysis system still has the problem of low efficiency (Brummer *et al*. 2014; Kuila *et al*. 2017). Previous studies have used cellulase, xylanase, and β-mannanase (MAN) to degrade coffee grounds to increase the yield of reducing sugars (Widjaja *et al*. 2019; Tsafrakidou *et al*. 2023; Xin *et al*. 2024a). However, when cellulase is used alone, the cellulose conversion percentage of coffee grounds remains considerably low; the degradation efficiency of MAN toward galactomannan is also markedly limited. This suboptimal performance can be attributed to the structurally recalcitrant nature of coffee grounds: a large number of α-1,6-galactose side chains in galactomannan molecules form spatial hindrance, seriously hindering the action of MAN on the main chain. Therefore, the removal of galactose substituents is crucial for enhancing galactomannan degradation and potentially improving overall substrate accessibility for subsequent enzymatic hydrolysis.

α-galactosidase (α-G) is a glycoside hydrolase that can specifically hydrolyze non-reducing terminal α-1,6-galactoside bonds; it plays an important role in the degradation of galactomannan (Bhatia *et al*. 2020). The addition of α-G may affect cellulose hydrolysis in two ways. On the one hand, removal of galactose side chains may disrupt the compact hemicellulose network, thereby increasing cellulose accessibility. On the other hand, α-G treatment may generate MOS with altered structures, and how α-G-mediated debranching influences MOS-induced cellulase inhibition under SCG-oriented hydrolysis conditions remains insufficiently characterized.

In this study, the authors investigated how α-galactosidase supplementation influences MOS generation and cellulose hydrolysis during the enzymatic conversion of ammonia-pretreated SCG. Particular attention was paid to whether α-G-mediated debranching improves galactomannan conversion while simultaneously generating MOS with stronger inhibitory effects on cellulase components. Through clarifying this dual effect, this work aimed to provide a better understanding of the trade-off between MOS production and cellulose digestibility, and to offer guidance for enzyme formulation in integrated SCG biorefinery systems.

EXPERIMENTAL

Materials

The SCG were collected from a coffee shop in Xi'an, Shaanxi Province, China. The SCG were subjected to aqueous ammonia pretreatment (AA-SCG) according to the method described by Xin *et al*. (2024a). The main composition of AA-SCG was: galactomannan, 38.5%; cellulose, 21.3%; and lignin, 21.5%. Celluclast 1.5 L and microcrystalline cellulose (Avicel PH-101) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade and acquired from the same supplier.

Enzymes

Endo-1,4-β-mannanase (MAN) derived from *Aspergillus niger* (Megazyme, Ireland), with a specific activity of 650 U/mg protein (10 mg protein/mL) was obtained from Megazyme. α-Galactosidase (α-G) was acquired from Sigma with an activity of 620 U/mg protein. Cellobiohydrolase I (CBHI) sourced from *Trichoderma reesei* (Novozymes, 120 U/mg protein) was used for exo-glucanase activity. Endoglucanase II (EGII) with a specific activity of 85 U/mg protein was purchased from Megazyme and used for endo-glucanase activity assays. β-glucosidase (βG) derived from *Aspergillus fumigatus* (Sigma-Aldrich, 250 U/mg protein) was used to complete the saccharification process by converting cellobiose into glucose. All enzymes were equilibrated with 50 mM sodium citrate buffer (pH 5.0) *via* dialysis before application, and their protein concentrations were confirmed by Bradford assay using bovine serum albumin as the standard.

Synthesis of Phosphoric Acid-swollen Cellulose (PASC)

The PASC was synthesized according to an adapted method described by Zhang *et al*. (2006). Microcrystalline cellulose (Avicel® PH-101, 0.4 g) was first prehydrated with 1 mL deionized water to prevent aggregation, followed by dropwise addition of 100 mL ice-cold 85% (w/v) phosphoric acid under continuous vortex mixing (2000 rpm). The hydrolysis was conducted on ice (0 to 4 °C) for 60 min with intermittent shaking every 15 min, then quenched by slow addition of 40 mL ice-cold DI water (1 mL/min). The white precipitate obtained was recovered *via* centrifugation (4000 × g, 10 min, 4 °C), followed by successive washes with deionized water and a 2% (w/v) Na_2CO_3 to achieve neutralization. The resulting PASC preparation was then resuspended in 50 mM citrate buffer at pH 5.0.

Preparation of MOS

The MOS production was achieved through citric acid pretreatment of guar gum followed by neutralization and MAN hydrolysis. The hydrolysate was subjected to yeast fermentation to remove monosaccharides, ion-exchange resin desalination, and spray-drying to obtain solid MOS, as described in Xin *et al*. (2024b). The resulting MOS contained a galactose substitution degree of 18.4%.

Enzymatic Hydrolysis

Enzymatic hydrolysis of Avicel, PASC, AA-SCG, and MOS was performed using various combinations of cellulase components and/or mannan-degrading enzymes. The enzyme set included EGII (2 mg/g dry matter, DM), CBHI (8 mg/g DM), βG (0.2 mg/g DM), MAN at 1 and 2 mg/g DM, and α-G at 0.05, 0.1, 0.5, 1, and 2 mg/g DM. The loadings of MAN, CBHI, EGII, and βG were adopted from the authors' previous published studies on related hydrolysis systems and were used here as established enzyme conditions to enable direct comparison of the effects of α-G supplementation (Xin *et al.* 2022, 2025). All reactions were performed in 10-mL centrifuge tubes with a final volume of 1 mL in 50 mM sodium citrate buffer (pH 5.0). The mixtures were incubated at 50 °C under continuous agitation (200 rpm) for 48 h. Unless stated otherwise, substrate loading was maintained at 2% (w/v) based on dry matter. Enzymatic activity was terminated by heating the reaction mixtures in a boiling water bath for 10 min. After cooling, the samples were centrifuged at 10,000 × g for 10 min to obtain the supernatants. The resulting hydrolysates were analyzed using high-performance liquid chromatography (HPLC) to determine the concentrations of galactose, mannose (M1), MOS, and glucose.

All enzymatic hydrolysis experiments were conducted in two independent replicates. Given the limited number of independent replicates (n=2), no inferential statistical analyses were performed, and the data are presented descriptively as means with standard errors.

Carbohydrate Analysis

Monosaccharide and oligosaccharide concentrations were determined by high-performance liquid chromatography (HPLC) using an Agilent 1260 Infinity II system (Agilent Technologies) equipped with a refractive index detector. Separation was carried out on a Waters Sugar Pak-1 column maintained at 80 °C. The mobile phase consisted of ultrapure water containing 50 mg/L Ca-EDTA, delivered isocratically at a flow rate of 0.4 mL/min. Analytes were identified and quantified by comparing their peak retention times with those of authenticated reference standards.

The glucose yield represents the percentage obtained by dividing the mass of released glucose (after applying the 0.9 conversion factor) by the theoretical mass of cellulose in the substrates.

The MOS yield represents the percentage ratio of released MOS to the theoretical galactomannan present in the substrates.

The total hydrolysis yield of mannan (%) is constituted by the combined contributions from MOS and monosaccharides.

RESULTS AND DISCUSSION

Effects of α-Galactosidase Dosage on Mannan Hydrolysis

Before investigating the role of α-G in the preparation of fermentable sugars and MOS during enzymatic hydrolysis of AA-SCG, this experiment first evaluated its hydrolytic efficiency on MOS to select a suitable dosage for the subsequent experiments.

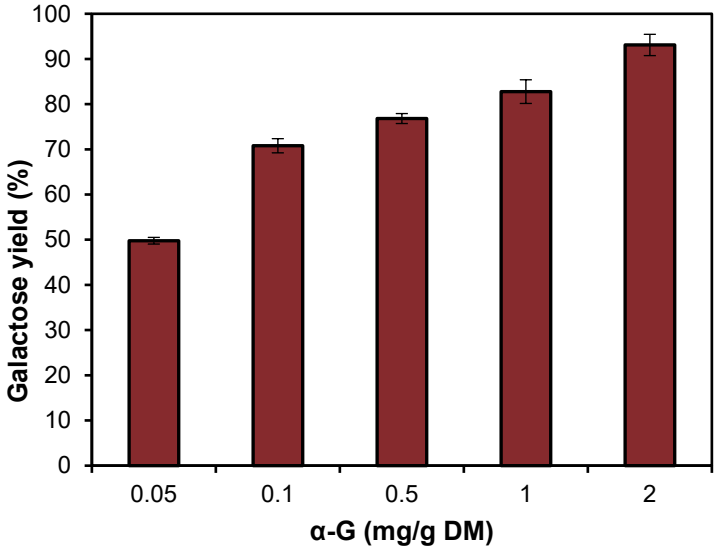

Fig. 1. Hydrolysis of manno-oligosaccharides (MOS, 5%, w/v) by α-galactosidase (α-G) at different loadings (0.05, 0.1, 0.5, 1, and 2 mg/g dry matter, DM) at 50 °C and pH 5.0 for 48 h; Error bars represent the standard errors of two independent experiments.

This evaluation was conducted using the MOS as the substrate under conditions of 50 °C and pH 5.0 (adjusted with sodium citrate buffer). Different concentrations of α-G (0.05, 0.1, 0.5, 1, and 2 mg/g) were added, and the enzymatic reaction proceeded for 48 h. As shown in Fig. 1, the hydrolytic efficiency of α-G toward MOS increased with increasing α-G loading. When the α-G dosage reached 2 mg/g DM, the galactose yield was as high as 93.1%. This result indicates that most galactose units were effectively hydrolyzed by α-G. The process involves the specific action of α-G on the α-1, 6-glycosidic bonds in MOS, which removes the α-1, 6-D-galactopyranosyl substituents from the main chain and facilitates further degradation of the mannose backbone. Based on these results, 2 mg/g DM was selected as the α-G loading for the subsequent enzymatic systems, because galactose release had already reached 93.1% at this dosage, suggesting that most accessible galactosyl side chains had been hydrolyzed under the tested conditions. Therefore, higher α-G loadings were not further evaluated in the present study, as only limited additional improvement was expected.

Effect of α-Galactosidase on the Hydrolysis of Mannan in AA-SCG

Galactomannan in SCG is composed of a β-1,4-linked mannose backbone and galactose substituents attached *via* α-1,6 glycosidic bonds, which are randomly distributed along the mannose backbone, forming a complex branched structure (Mary *et al*. 2019). These side chains hinder the effective binding of MAN to the backbone, thereby reducing the hydrolysis efficiency. α-G is specifically responsible for hydrolyzing the α-1,6 glycosidic bonds between galactose side chains and the mannose backbone, removing the galactose substituents (Bhatia *et al*. 2020).

Therefore, in this experiment, α-G was introduced into the MAN hydrolysis system to examine its influence on the hydrolysis of galactomannan present in AA-SCG. As illustrated in Fig. 2, the addition of MAN at 1 mg/g DM resulted in M1 and MOS yields of 12.8% and 36.0%, respectively.

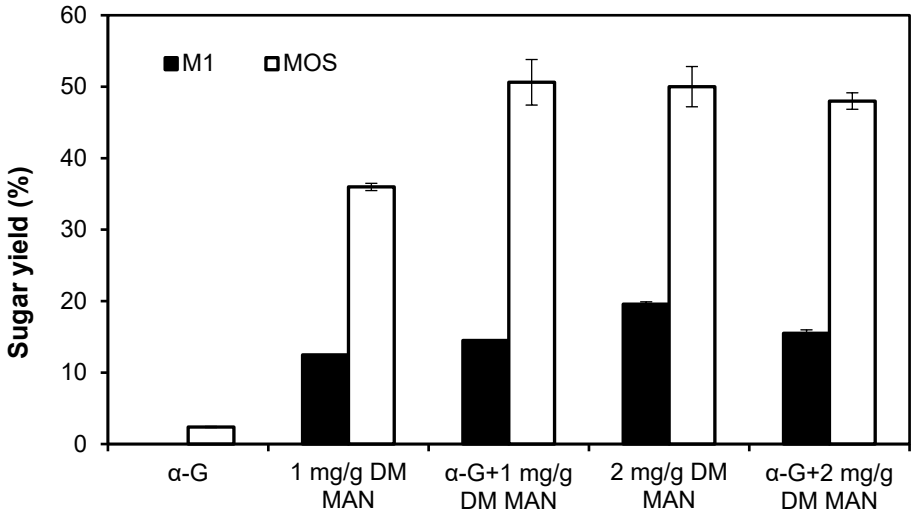

Fig. 2. Hydrolysis of ammonia-pretreated spent coffee grounds (AA-SCG, 2%, w/v) by endo-1,4-β-mannanase (MAN; 1 and 2 mg/g DM) with or without α-galactosidase (α-G; 2 mg/g DM) at 50 °C and pH 5.0 for 48 h; Error bars represent the standard errors of two independent experiments.

After supplementation with α-G, the yields of M1 and MOS increased to 14.5% and 50.6%, respectively, suggesting that α-G-mediated removal of galactose side chains improved the accessibility of MAN to the mannose backbone (Sørensen *et al.* 2007). When the MAN dosage was increased to 2 mg/g DM without α-G supplementation, the M1 and MOS yields reached 19.6% and 50.0%, respectively. Notably, the MOS yield obtained with 1 mg/g DM MAN plus α-G (50.6%) was comparable to that achieved with 2 mg/g DM MAN alone (50.0%), indicating that α-G supplementation can reduce the required MAN dosage by half while maintaining MOS production under the tested conditions. However, the corresponding M1 yield remained lower (14.5% *vs.* 19.6%), indicating that this benefit is specific to MOS production and may not apply when monosaccharide recovery is the primary objective.

Effect of α-Galactosidase on the Hydrolysis of Cellulose in AA-SCG

In galactomannan-rich biomass such as SCG, galactomannan may be associated with or partially cover cellulose surfaces. Its α-1,6-linked galactose side chains may create steric hindrance that restricts cellulase access to cellulose, thereby potentially reducing hydrolysis efficiency (Wang *et al*. 2017). Theoretically, α-G can specifically cleave these galactose side chains, loosening the galactomannan, reducing its physical shielding effect on cellulose, and improving cellulase accessibility. Therefore, this study introduced α-G into the cellulase hydrolysis system of AA-SCG to evaluate its potential in promoting cellulose degradation.

As shown in Fig. 3A, when only pure cellulase was added, the hydrolysis yields of cellulose and mannan were 5.9% and 8.5%, respectively. Upon supplementation with α-G, the corresponding yields were 6.2% and 9.4%, respectively, remaining numerically similar to those obtained with cellulase alone. These results suggest that α-G supplementation alone did not substantially enhance cellulose or mannan hydrolysis under the tested conditions. In contrast, the addition of MAN alongside cellulase enhanced the hydrolysis efficiency of both cellulose and mannan, reaching 49.1% and 57.7%, respectively. We hypothesize that MAN-mediated depolymerization of galactomannan may reduce its association with or coverage of cellulose surfaces, thereby potentially increasing cellulose accessibility to cellulases and contributing to the observed improvement in cellulose hydrolysis. However, this proposed mechanism was not directly examined in the present study and requires further validation using structural or accessibility measurements.

Notably, when α-G was further added to the cellulose and MAN system, the yield of MOS increased 13.2% (Fig. 3B). However, cellulose hydrolysis did not increase; instead, it showed a slight decrease, declining from 49.1% to 45.7%. This result is somewhat unexpected, because the enhanced degradation of galactomannan would be expected to expose more cellulose and thereby favor cellulose hydrolysis. However, the present results suggest that improved galactomannan conversion did not directly translate into a higher glucose yield in this system. A similar phenomenon has also been observed in other accessory-enzyme-assisted lignocellulose hydrolysis systems, where improved hemicellulose deconstruction did not necessarily lead to a proportional enhancement of cellulose saccharification (Xin *et al*. 2019). This implies that, besides substrate accessibility, other factors generated during α-G-assisted hydrolysis may also influence the final cellulose conversion.

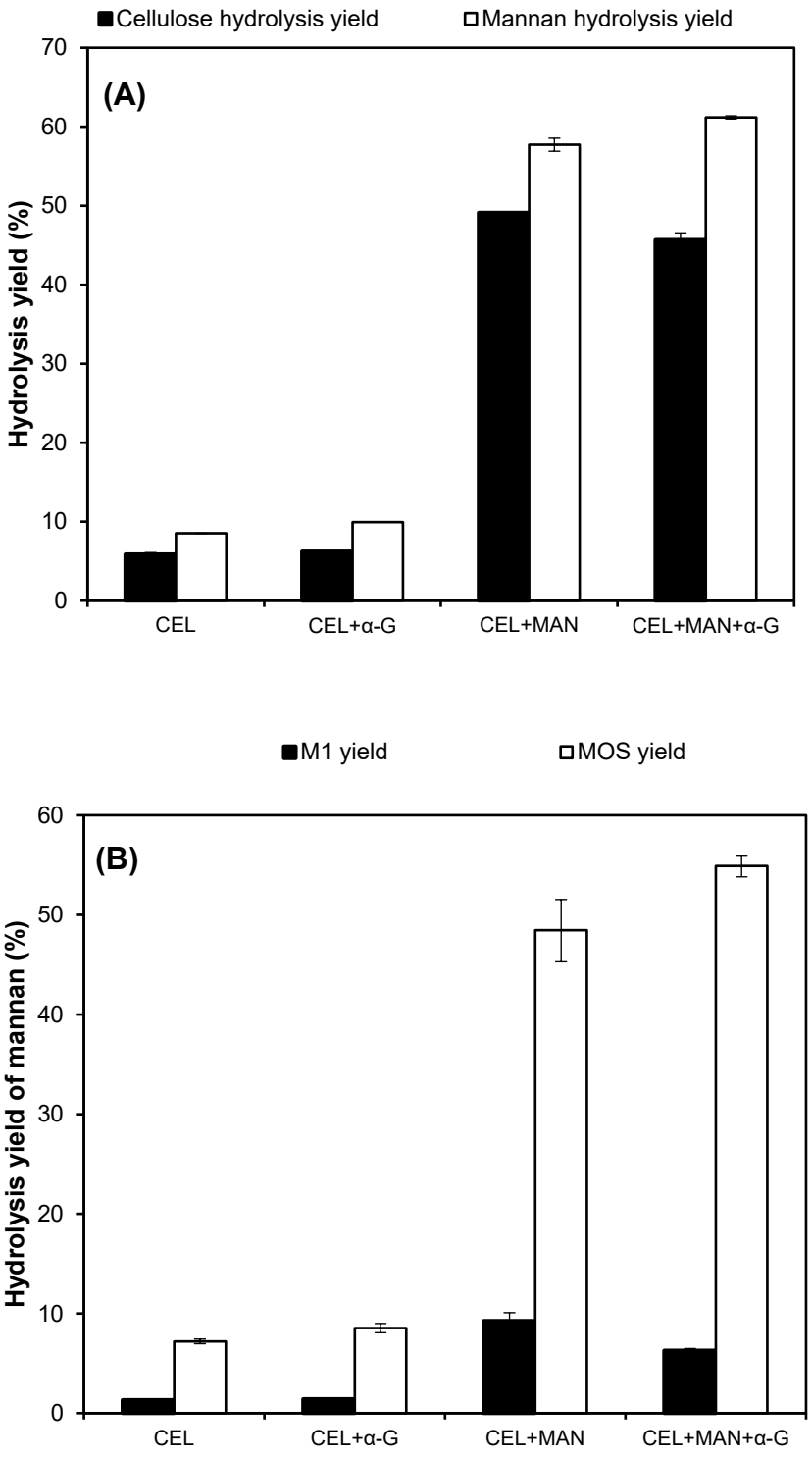

Fig. 3. Hydrolysis of ammonia-pretreated spent coffee grounds (AA-SCG, 2%, w/v) by a cellulase mixture (CEL; 8 mg/g DM cellobiohydrolase I, CBHI; 2 mg/g DM endoglucanase II, EGII; and 0.2 mg/g DM β-glucosidase, βG), with or without endo-1,4-β-mannanase (MAN; 1 mg/g DM) and α-galactosidase (α-G; 2 mg/g DM), at 50 °C and pH 5.0 for 48 h; Error bars represent the standard errors of two independent experiments.

The Effect of α-G Addition on MOS-mediated Inhibition

Based on the above observations, the authors further examined whether α-G treatment altered the inhibitory effect of MOS on cellulase hydrolysis. For this purpose, cellulose was used as the substrate, and α-G was introduced into a cellulase system containing MOS to evaluate whether debranching of MOS would further affect cellulase inhibition. The MOS used in this experiment was prepared from guar gum by citric acid pretreatment followed by MAN hydrolysis, and its initial galactose substitution degree was 18.4% (Xin *et al*. 2024b). Guar gum-derived MOS were used as a controlled model substrate to evaluate the effect of α-G-mediated debranching on MOS-induced cellulase inhibition. The purified MOS preparation had a defined initial galactose substitution degree of 18.4%, allowing the effect of changes in galactose substitution to be examined under controlled conditions while minimizing interference from other soluble components present in AA-SCG hydrolysates. However, the structure, substitution pattern, and oligosaccharide distribution of guar gum-derived MOS may differ from those of MOS generated directly from AA-SCG. Therefore, the quantitative inhibitory effects observed using this model system should not be directly extrapolated to AA-SCG hydrolysates, although the observed trends provide useful insight into the relationship between MOS debranching and cellulase inhibition.

As shown in Fig. 4A, glucose yield in the cellulase-only system gradually increased with reaction time and reached 22.9% after 48 h. In contrast, the addition of MOS reduced the glucose yield to 9.3% at 48 h, corresponding to a 59.4% decrease. This observation is consistent with previous studies showing that mannan-rich polysaccharides and their hydrolysis products can strongly inhibit cellulase-mediated cellulose conversion. For example, Kumar and Wyman (2014) reported that guar gum galactomannan markedly reduced Avicel hydrolysis by fungal cellulases. These previous observations support the general view that mannan-derived soluble carbohydrates can act as potent inhibitors of cellulase systems during lignocellulose conversion.

Consistent with doctoral observations in a model Avicel system (Xin 2019), α-G treatment was associated with stronger MOS-mediated inhibition in the present system. The current study further related this response to a quantified decrease in the galactose substitution degree of MOS from 18.4% to 5.2% and evaluated its implications in the context of SCG valorization. After α-G supplementation, the glucose yield decreased further by 18.3%, indicating that the effect of MOS on cellulase hydrolysis depended not only on their presence, but also on their structural state after enzymatic modification. Figure 4B shows that α-G treatment increased the concentration of M1 to 1.2 mg/mL while reducing the MOS concentration to 2.9 mg/mL. At the same time, the galactose substitution degree of MOS decreased from 18.4% to 5.2%, indicating substantial removal of galactose side chains. Moreover, previous work has shown that galactose inhibits cellulase hydrolysis only at relatively high concentrations (Hsieh *et al*. 2014), whereas the galactose concentration in the present system was below that range, suggesting that the strengthened inhibition after α-G treatment was unlikely to be caused simply by released galactose.

Taken together, these results indicate that the strengthened inhibition observed after α-G treatment was associated with the reduced galactose substitution degree of MOS. Previous studies have shown that MOS can competitively inhibit CBHI by interfering with productive substrate binding (Xin *et al.* 2015). Based on this evidence, it is hypothesized that α-G-mediated debranching may reduce steric hindrance from galactose side chains and thereby facilitate interactions between less-substituted MOS and cellulases, including CBHI. However, the present results demonstrate only an association between reduced

galactose substitution and increased cellulase inhibition, rather than a direct causal relationship. Because direct binding or adsorption measurements were not performed in this study, this proposed mechanism remains hypothetical and requires further validation using approaches such as surface plasmon resonance (SPR) or isothermal titration calorimetry (ITC).

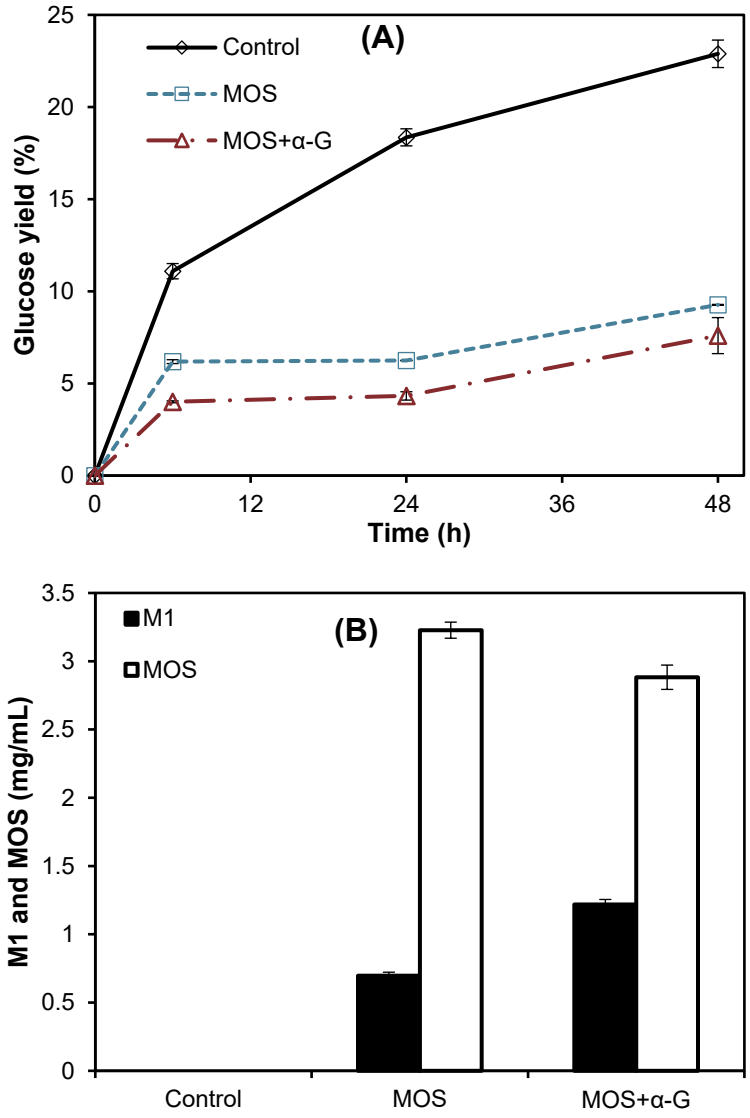

Fig. 4. Hydrolysis of microcrystalline cellulose (1%, w/v) by a cellulase mixture (CEL; 8 mg/g DM cellobiohydrolase I, CBHI; 2 mg/g DM endoglucanase II, EGII; and 0.2 mg/g DM β-glucosidase, βG) under control conditions, with MOS (4 mg/mL), or with MOS (4 mg/mL) plus α-G (2 mg/g DM), at 50 °C and pH 5.0 for 6, 24, and 48 h (A). Concentrations of mannose (M1) and MOS after 48 h hydrolysis (B). Error bars represent the standard errors of two independent experiments.

The Effect of α-G on the Inhibitory Effect of MOS on Individual Cellulases

To further evaluate whether the response observed above was reproduced across the individual cellulase components used in the present study, the effects of MOS with or without α-G treatment were examined separately in systems containing CBHI, EGII, and βG.

As shown in Fig. 5A, in the βG hydrolysis system, the glucose yield was 69.6% in the control. The corresponding yields in the presence of MOS and MOS plus α-G remained numerically similar to the control, suggesting that these treatments had only minor effects on βG activity under the tested conditions.

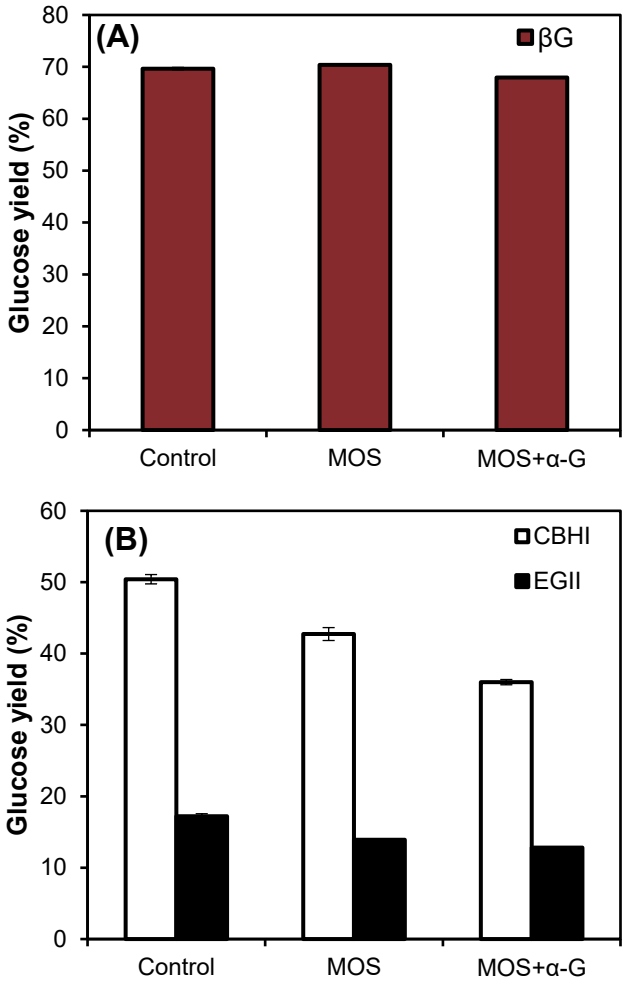

Fig. 5. Hydrolysis of cellobiose (2%, w/v) by β-glucosidase (βG; 0.2 mg/g DM) (A), or hydrolysis of phosphoric acid-swollen cellulose (PASC, 0.5%, w/v) by cellobiohydrolase I (CBHI; 8 mg/g DM), endoglucanase II (EGII; 2 mg/g DM), and β-glucosidase (βG; 0.2 mg/g DM) under control conditions, with MOS (2 mg/mL), or with MOS (2 mg/mL) plus α-G (2 mg/g DM) (B), at 50 °C and pH 5.0 for 3 or 24 h. Error bars represent the standard errors of two independent experiments.

Because only minor numerical changes in βG activity were observed in the presence of MOS under the tested conditions, βG was further added in the system inhibiting CBHI and EGII by MOS, converting the PASC hydrolysis products into more easily detectable glucose. As shown in Fig. 5B, in the hydrolysis of PASC by CBHI and EGII, MOS reduced glucose yield 15.2% (from 50.4% to 42.7%) and 19.0% (from 17.2% to 13.9%), respectively, indicating that MOS inhibited the hydrolytic activity of these enzymes. With the addition of α-G, the glucose yield from cellulose hydrolyzed by CBHI further decreased to 36.0%, corresponding to a numerical reduction of 15.8%, whereas that of EGII decreased to 12.8%, corresponding to a numerical reduction of 8.1%. Thus, the

additional reduction in glucose yield associated with α-G supplementation was numerically larger in the CBHI system than in the EGII system under the tested conditions.

The magnitude of the numerical changes differed among the cellulase components tested. Under the tested conditions, the additional reduction in glucose yield observed in the MOS + α-G treatment was larger for CBHI than for EGII, whereas little numerical change was observed in the βG system. These differences are interpreted descriptively because no formal statistical comparisons were performed. This distinction is particularly important because CBHI is a major component of *Trichoderma reesei* cellulase systems and plays a central role in cellulose depolymerization. Therefore, even a moderate increase in CBHI inhibition may have a disproportionate negative effect on the overall hydrolytic performance of commercial cellulase mixtures.

Related control experiments were performed in the cited doctoral work using purified cellulase components under model hydrolysis conditions (Xin 2019). In those experiments, α-G alone was included as a control in systems containing Cel5A, Cel6A, or Cel7A and did not indicate a substantial direct effect on cellulose hydrolysis. In a separate Cel3A system, cellobiose hydrolysis remained essentially unchanged when galacto-MOS was treated with α-G. However, because the enzyme preparations, substrates, α-G loadings, and reaction conditions used in those experiments differed from those of the present study, these previous observations cannot substitute for matched α-G-only controls in the current system. Therefore, a direct effect of α-G on the cellulase components under the present conditions cannot be completely excluded.

Taken together, these results support the interpretation that α-G supplementation in AA-SCG hydrolysis has a dual effect. On the one hand, it promotes galactomannan conversion, increases MOS yield, and allows a reduction in mannanase dosage. On the other hand, it was associated with stronger inhibition in the MOS-containing cellulase systems, with the largest numerical reduction observed for CBHI under the tested conditions. This dual effect may help explain why α-G supplementation enhanced MOS production but did not improve the overall glucose yield from cellulose hydrolysis in the AA-SCG system.

From a biorefinery perspective, α-G supplementation may offer a practical advantage by reducing the required mannanase dosage, which could lower enzyme input for MOS production. However, this potential benefit should be balanced against the strengthened MOS-mediated inhibition of cellulases observed in the present study, because this may reduce downstream glucose production efficiency. Therefore, the practical process value of α-G supplementation will depend on the targeted product distribution and will require further optimization of enzyme balance, hydrolysis sequence, and product valorization pathway.

CONCLUSIONS

1. α-Galactosidase (α-G) plays a dual role in the enzymatic valorization of spent coffee grounds (SCG). On the one hand, it promotes galactomannan hydrolysis, enhances manno-oligosaccharides (MOS) production, and allows a reduction in the required mannanase dosage. On the other hand, α-G supplementation was associated with stronger inhibition in the MOS-containing cellulase systems, with the largest numerical reduction observed for a cellobiohydrolase enzyme (CBHI) under the tested conditions, thereby limiting the improvement of overall cellulose hydrolysis. These findings

highlight a trade-off between efficient MOS generation and downstream cellulose digestibility in SCG hydrolysis systems.

2. From an application perspective, the potential reduction in enzyme input associated with reduced mannanase usage should be evaluated together with the negative impact of stronger cellulase inhibition on glucose production. Further process-level and techno-economic analyses are required to determine the practical feasibility of this strategy and to optimize enzyme formulation, hydrolysis sequence, and process integration in SCG biorefinery systems.

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Use of Generative AI

During manuscript preparation, DeepSeek was used only for language polishing and improving clarity. The authors reviewed and edited all AI-assisted text and take full responsibility for the final content. No generative AI was used to prepare images, figures, graphs, or diagrams.

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