Optimization of enzymes production by *Aspergillus niger* in solid state fermentation of agro-industrial by-products

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Introduction

It is estimated that the annual world production of agro-industrial by-products is around 1 billion tones [1]. Those by-products are often discarded or used in low-value applications. Solid by-products of lignocellulolytic structure have been proven to be suitable substrates in solid-state fermentation (**SSF**) to produce carbohydrases, that have many industrial applications [2]. In this work, the by-products rice husk (**RH**), brewer's spent grain (**BSG**), and vine shoot trimmings (**VST**) were used to carry out SSF experiments using *Aspergillus niger* CECT 2088 and to produce enzymes of relevance in the textile industry, namely as cellulases, xylanases and amylases, that are used for desizing and/or scouring [3, 4]. Several factors were studied, specifically supplementation, solid particle size, substrate mixtures and, finally, kinetics.

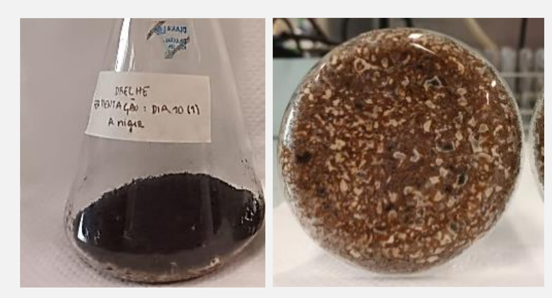
Methods

By-products

Granulometry
1, 4 and 10 mm

Supplementation
K_2HPO_4 and/or $(NH_4)_2SO_4$

Solid-state fermentation

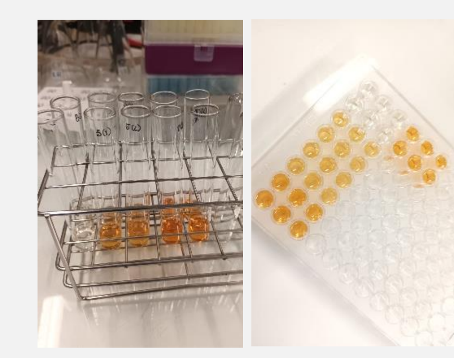


- 5 g of dry solid
- 25 °C
- 75 % (w/w) of humidity
- *A. niger* CECT 2088 (10^6 spores/mL)

Aqueous extraction

- 1:10 (w/v)
- 20 °C
- 30 min

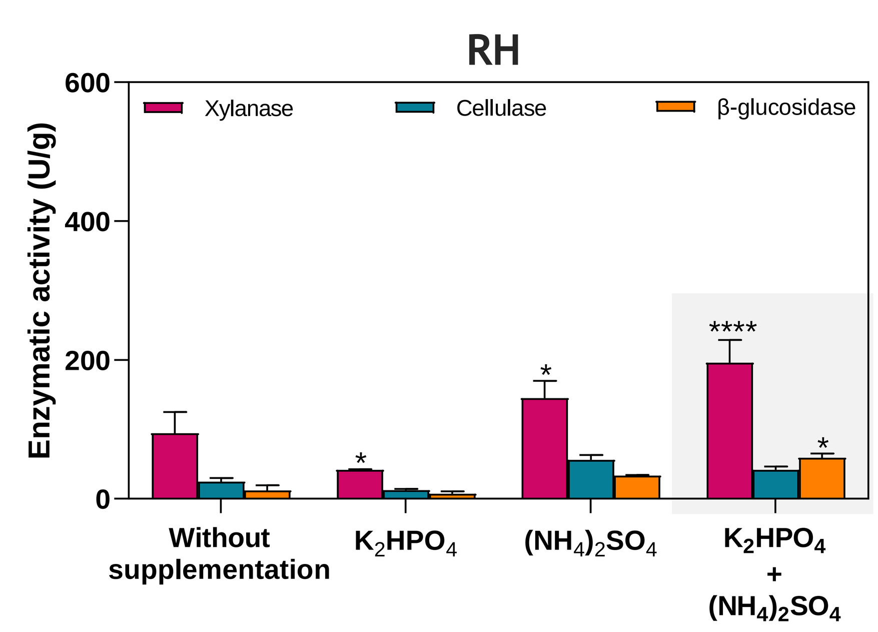
Enzymatic analysis

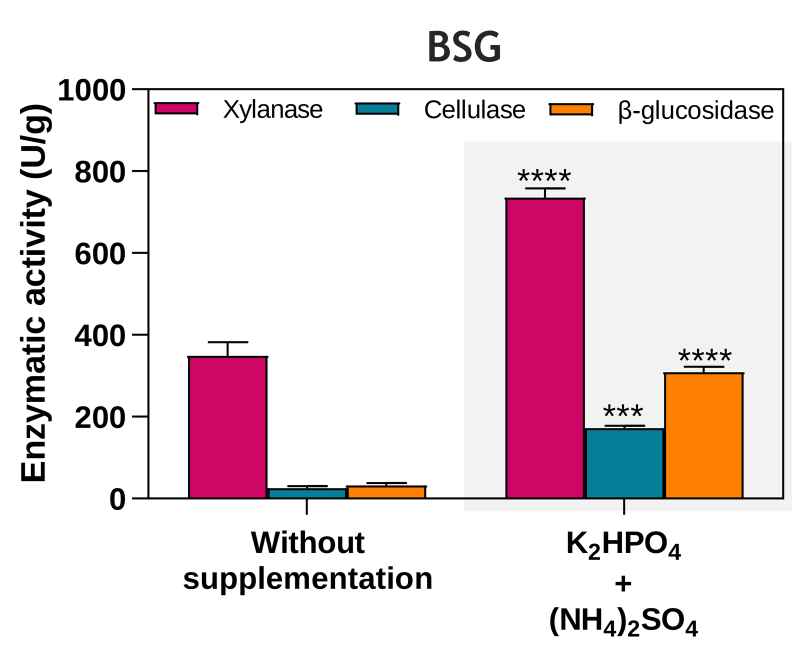


- Xylanase
- Cellulase
- β-glucosidase
- Amylase

Results

1. Effect of potassium and nitrogen supplementation on enzyme production

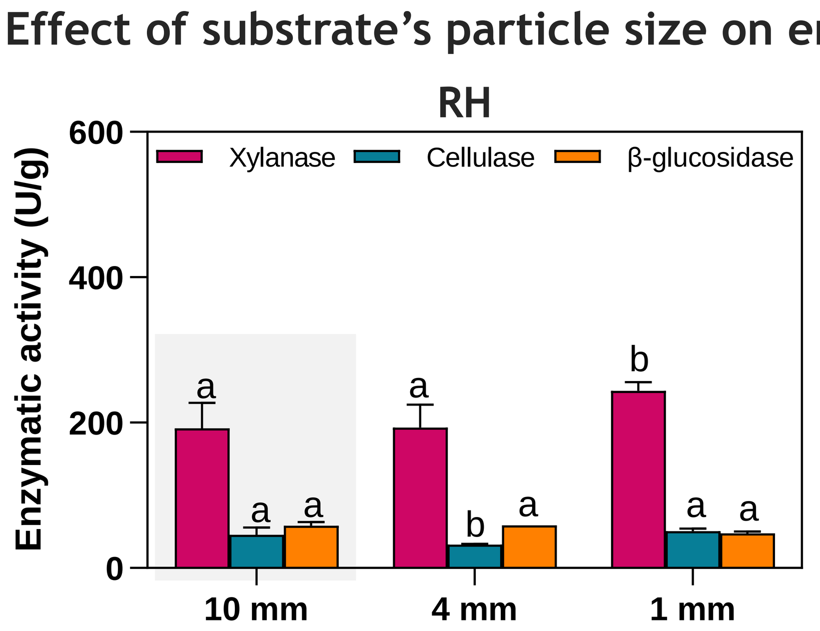


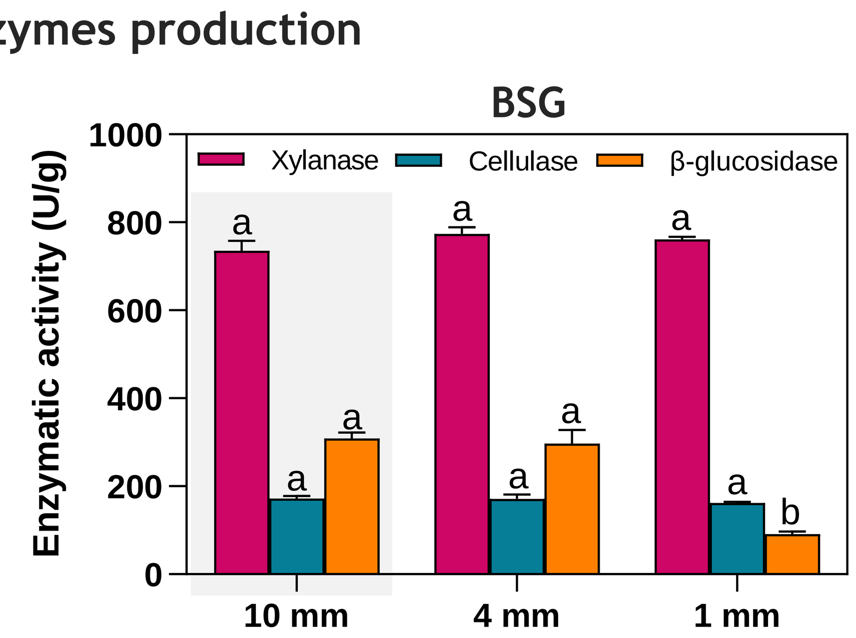


2 way ANOVA; Dunnett's test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$)

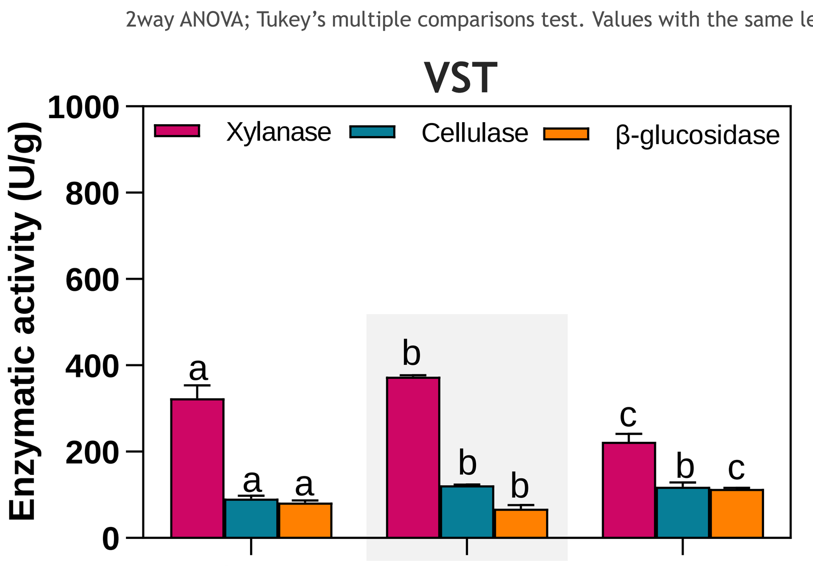
✓ Supplementation with both 1 % (w/w) K_2HPO_4 and 2 % (w/w) $(NH_4)_2SO_4$ increased xylanase and β-glucosidase activities in SSF of RH, as well as all the enzymes in SSF of BSG.

2. Effect of substrate's particle size on enzymes production





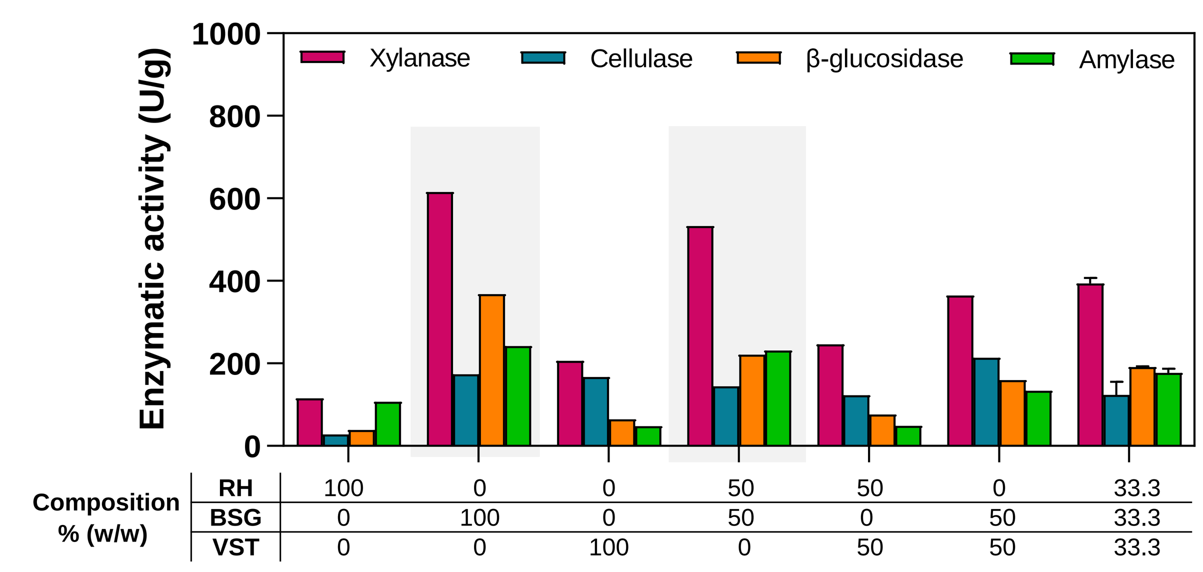
2way ANOVA; Tukey's multiple comparisons test. Values with the same letters for each enzyme are not significantly different (p<0.05).



✓ Particle size had no significant effect on enzymes production in RH (slight increase of xylanase at 1mm) and BSG.

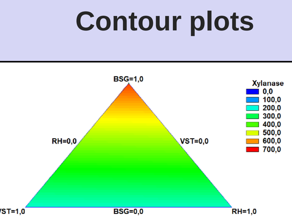
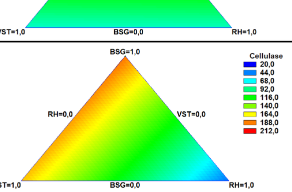
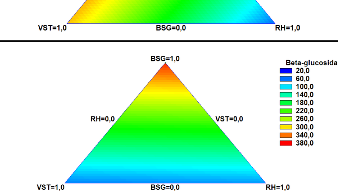
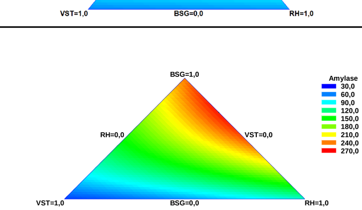
✓ The decrease of VST granulometry from 10 to 4 mm increased all enzymes production.

3. Optimization of substrate mixtures in enzyme production

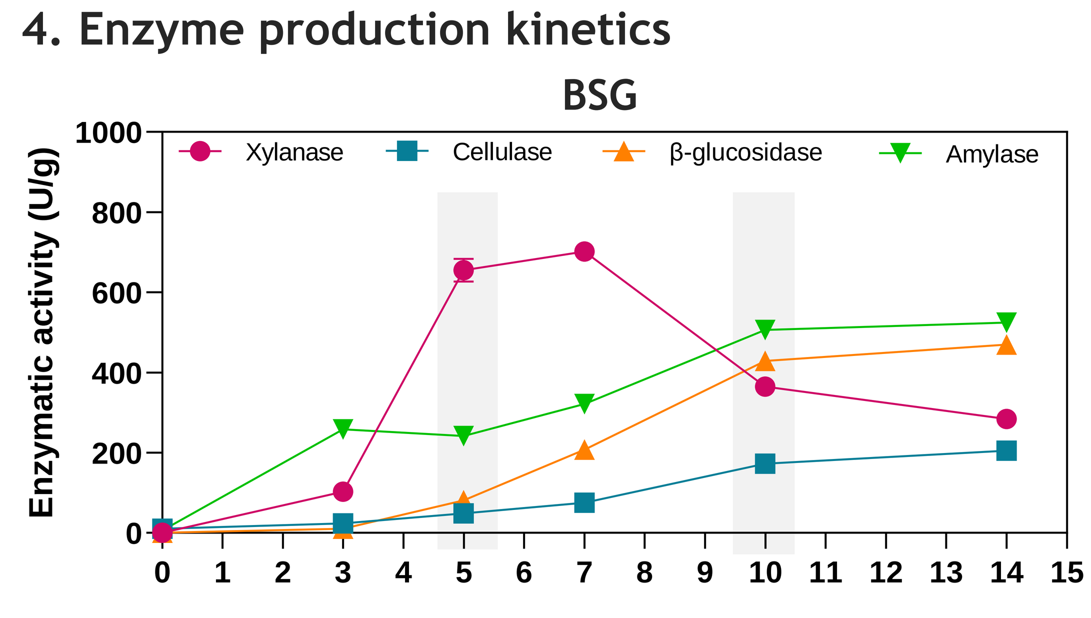


✓ Overall, 100 % (w/w) of BSG resulted in highest enzymes activity, followed by 50/50 mixtures of RH/BSG.

The simplex centroid mixture design optimization

Enzyme	Contour plots	Model	Optimum predicted (U/g)
Xylanase		Xylanase = 651.1*BSG + 209.1*VST + 216.8*RH $p \leq 0.05$ $R^2 = 0.82$	651
Cellulase		Cellulase = 189.1*BSG + 175.0*VST + 40.0*RH $p \leq 0.05$ $R^2 = 0.7$	189
β-glucosidase		β-glucosidase = 362.8*BSG + 62.7*VST + 71.16*RH $p \leq 0.05$ $R^2 = 0.91$	363
Amylase		Amylase = 237.7*BSG + 43.7*VST + 102.7*RH + 41.9*BSG*VST + 311.9*BSG*RH - 32.1*VST*RH $p \leq 0.05$ $R^2 = 0.96$	263

4. Enzyme production kinetics



✓ Xylanase activity peaked on the 5th day.

✓ Cellulase, amylase, and β-glucosidase reached their maximum activity on the 10th day.

References

[1] Moayedi, H., Aghel, B., Nguyen, H., & Rashid, A. S. A. (2019). Applications of rice husk ash as green and sustainable biomass. Journal of Cleaner Production, 237, 117851.
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Conclusions

1. SSF with *A. niger* CECT 2088 is a suitable biotechnological process to produce lignocellulosic enzymes from RH, BSG and VST.
2. Supplementation with nitrogen and phosphorous sources and particle sizes are important factors to enzymes production by SSF.
3. BSG was the best substrate for enzyme production, however mixtures of BSG with RH or VST can be used to achieve similar results as BSG alone.
4. Time is an important parameter to select the target enzyme to be produced by SSF of BSG with *A. niger*.

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