FOODCONF fouth international conference on food science and technology

Enhanced biological biotreatment of wheat bran using two consortiums of fungal strains

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Introduction

Lignocellulosic biomass is one of the most abundant renewable resources on earth, it provides an attractive feedstock for ethanol production due to its rich carbohydrates compound (55-75% dry basis). Thermal/ chemical pre-treatment methods have been regarded are the most used pre-treatment technologies; however, they usually need expensive corrosion resistant reactors, processing large volumes of the waste stream, extensive washing of treated solids, and detoxification of compounds inhibitory to ethanol-fermenting microorganisms. **[1]**

From both economic and environmental perspectives, fungal pre-treatment with lignin-degrading microorganisms, preliminary white rot fungi, has received renewed interest as an alternative to thermal/chemical pre-treatment for cellulosic ethanol production. **[2]**

Materials and methodology

Materials

- strains were received from National Collection of Agricultural and Industrial Microorganisms (NCAIM, Hungary) *A. awamori, P. granulatum T. viride and Thermothelomyces thermophila*
- Commercial wheat bran was used.
- In addition to single strains, many combinations were tested. (Consortium A : *A. awamori, P. granulatum T. viride,* Consortium B: *A. awamori, P. granulatum T. viride Thermothelomyces thermophila*).

Biological pretreatment

- Liquid: solid ratio = 4:1 5:1 7:1, culture medium pH 4.25-pH6.25 , initial cell concentration 1.10^5 -2.10^5 conidia/gram substrate . The flasks were maintained at 30C°-45C° for 6 days under static condition

Evaluation

- **Soluble carbohydrates:** HPLC (Thermo Fisher Scientific Corporation, USA).

Experimental design and statistical analysis

To determine the optimum concentration of sugar produced in this study, a statistical tool, Response Surface Methodology (RSM) was used. To perform RSM MODDE v5 software was used. It was conducted through the central composite design, which is one of the most common design used for analysis. In central composite design four variables were added i.e. Temperature, moisture content, pH and initial conidia spores added. The analysis of variance (ANOVA) and regression analysis were performed to evaluate the effectiveness of the model as a whole, i.e., to determine whether at least one of the term is significant or not. To check whether the terms in model are fitted correctly, a test for the lack of fit and p-value was performed. Model and all the statistical analysis was performed using software Modde v5.

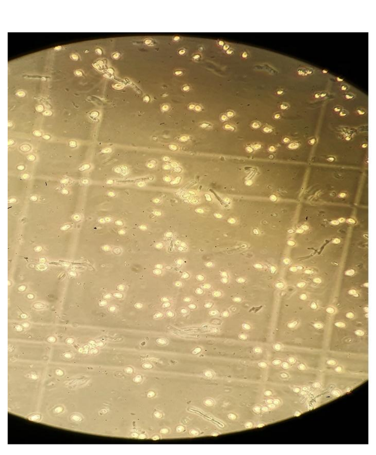
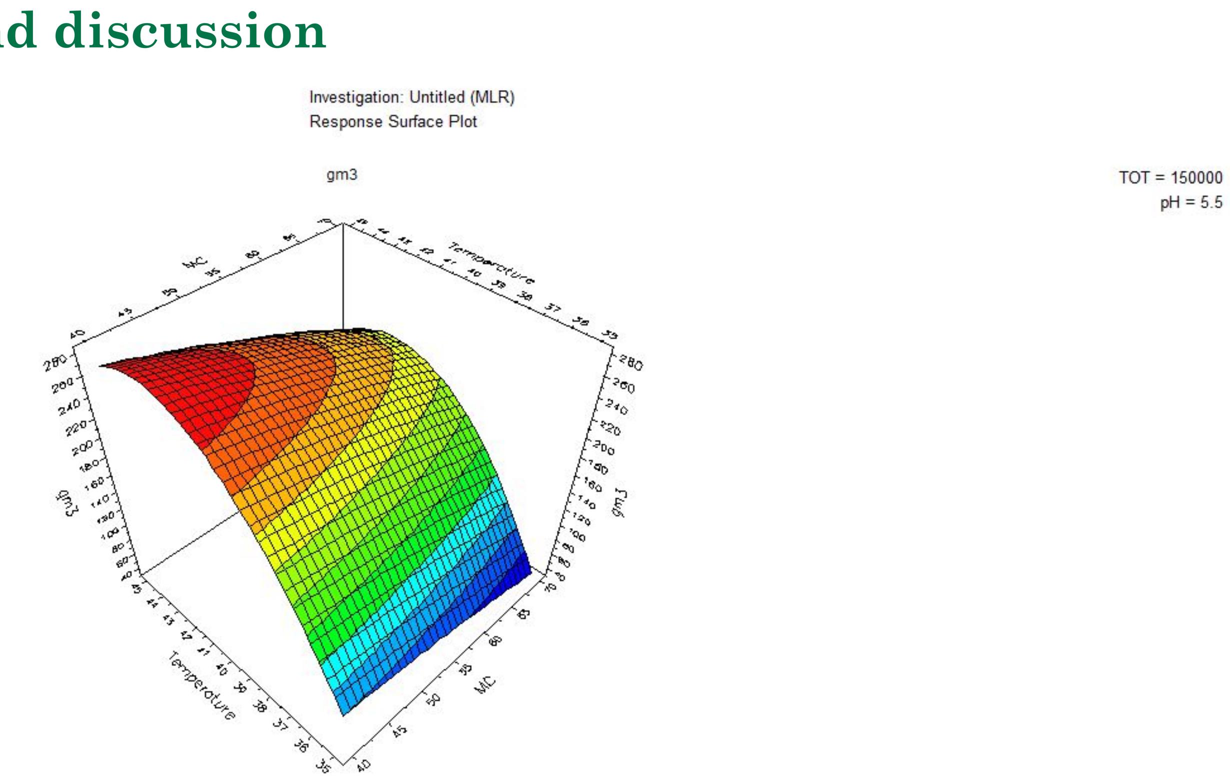


Fig. 1: Fungal conidia counted with Burker chamber with Olympus Plan 40x/0.65 Ph2 microscope objective

Fig. 2: Consortium growth after 3 days of pretreatment

Results and discussion



Graph. 1: Response surface plot of total soluble sugar yield, under moisture content and temperature effects of Consortium A

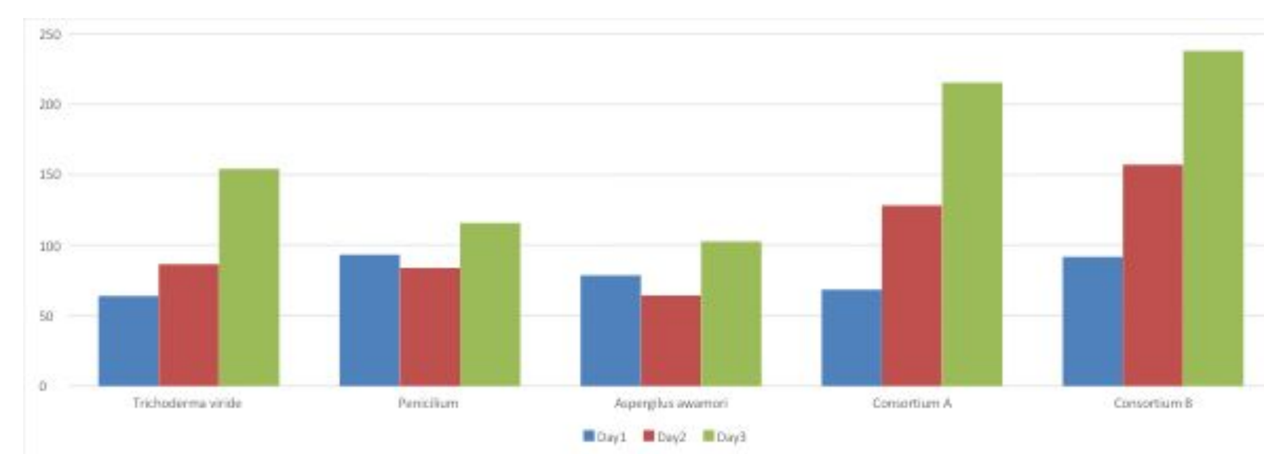


Fig. 3: Amounts of soluble carbohydrates Sugar yield after 3days of pretreatment of multiple single strains and two consortiums mg/gds

The influence of variables on the sugar yield was further investigated by performing ANOVA to determine the significance of model. The model for the response was only significant for only two factors as noticeable from p-value which is <0.05. The lower p value indicates that the model is more significant.

Moisture content and temperature are significant and give best sugar yield at 4:1 moisture to solid ratio, and the temperature at 44C°.

Initial conidia didn't have a significant effect from the range of 1.10^5 spores/gds to 2.10^5, as well as pH range from 4.75 to 6.25.

Furthermore, using the optimizer function on Modde software indicates that the optimal parameters for a better sugar yield after 3 days of pretreatment are:

- pH: 6.25 Moisture content: 4:1 Temperature: 44C° Initial conidia added is 2.10^5 spore/gds to get 350 mg/gds

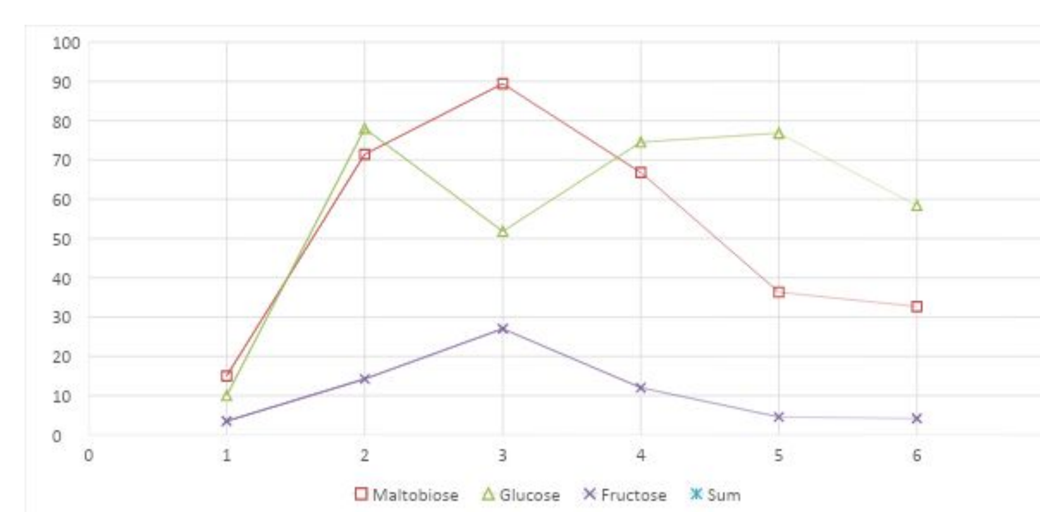


Fig. 4: Amounts of soluble carbohydrates mg/gds (Consortium A)

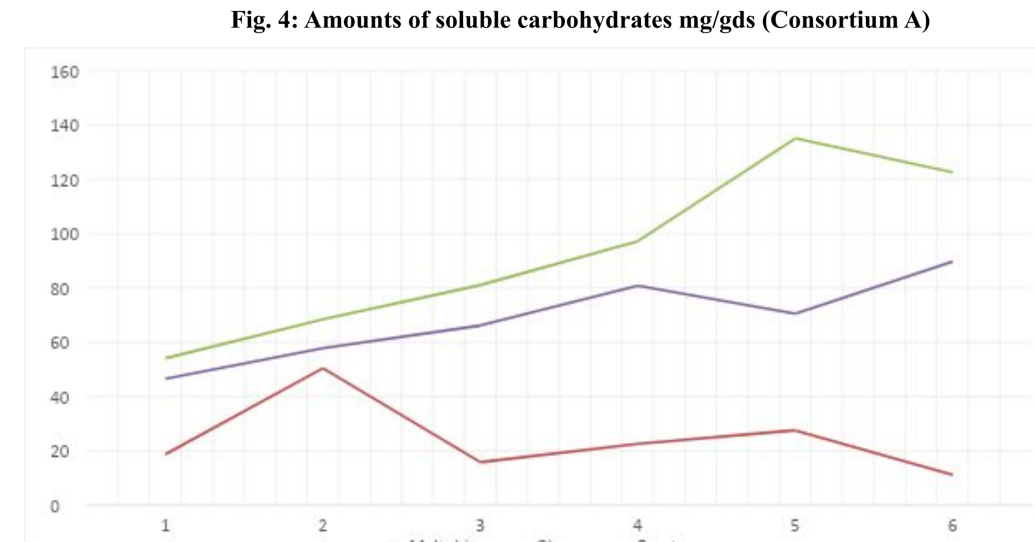


Fig. 5: Amounts of soluble carbohydrates mg/gds (Consortium B)

The optimal time of Consortium A and B for the highest reducing sugars (215 mg/gds) and (250 mg/gds) was discovered in the flask having the optimal parameters after 72h of pretreatment, With Glucose yield peak after 2 days of pretreatment (68 mg/gds) and (75 mg/gds) and Maltobiose and Fructose yield peaking after 3 days of pretreatment at (90 mg/gds and 28 mg/gds) for consortium A and (50 mg/gds and 58 mg/gds) for consortium B respectively.

Conclusion

- Moisture content and temperature has a significant effect on the hydrolysis by A. awamori, P. granulatum and T. viride in the SSF pretreatment.
- Initial conidia and pH didn't have a significant effect with the range used.
- Our results are preliminary, but it may serve very good base for development of biological pretreatment technology for processing lignocellulose biomass

References

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[2] Sanches, C., Lignocellulosic residues: Biodegradation and bioconversion by fungi. Biotechnol. Adv. 2009

Acknowledgement

This work is supported by the New Széchényi Plant Project and carried out at the department of Brewery and Distilling, Faculty of food science, The Hungarian university of agriculture and life sciences.