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Production and characterization of Talf1 yeast invertases and further application to biodesulfurization

B.F. Arez, L. Alves, S.M. Paixão*

LNEG – U. Bioenergia, Estrada do Paço do Lumiar, 22, 1649-038 Lisboa, Portugal

**Corresponding author:* susana.alves@lneg.pt

Introduction – Combustion of fossil fuels generates emissions of numerous toxic gases which in later years have become a major concern internationally. One of the most concerning problems is sulfur and sulfur dioxide, and maximum levels have been established through the years. Biodesulfurization (BDS) could be a complementary technology to the commonly used physico-chemical process. BDS is based on the use of microorganisms for the removal of sulfur even from the most recalcitrant compounds at atmospheric pressure and temperature, making it cheaper and more eco-friendly. However, this bioprocess has a few limitations, such as the high costs of the culture medium, which makes the process very expensive. Thus, in order to reduce its costs, it is important to search for cheaper carbon sources which can contribute to produce the microbial biomass. The goal of this work was the production and characterization of novel *Z. bailii* strain Talf1 invertases for further application to BDS, in order to expand the usable alternative carbon sources to high sucrose level feedstock, comparing two different approaches: Separated Hydrolysis and Fermentation (SHF) and Simultaneous Saccharification and Fermentation (SSF) processes.

Experimental – The invertases of *Z. bailii* strain Talf1 [1] were produced using different inducers. For determination of the invertase activity in each crude enzymatic crude extract, the amount of reducing sugars released from 1% sucrose hydrolysis in each reaction mixture containing 25 µL enzyme extract diluted was assayed using DNS method [2]. One unit of invertase activity (U) was defined as the amount of enzyme responsible for the production of 1 µmol of reducing sugar per minute at 50°C. The best enzyme crude extract was used for further characterization and BDS tests. The optimal temperature and pH for sucrose hydrolysis conditions were determined at temperatures ranging from 20°C to 70°C, and pH's ranging from 3.0 to 8.5. Enzyme stability assays for temperature and pH were also carried out as well as the influence of several cations on invertase activity. The applicability of invertases to BDS process through SHF and SSF was evaluated in dibenzothiophene (DBT) desulfurization assays with *Gordonia alkanivorans* strain 1B, using 10 g/l sucrose as sole carbon source and 250 µM DBT as sole sulfur source. Growth and sugar consumption profiles, as well as the DBT consumption and/or 2-hydroxybiphenyl (2-HBP) production were determined.

Results and Discussion – Induction assays showed Jerusalem artichoke as the best substrate for invertase production by *Z. bailii* strain Talf1 (134U/ml) and the characterization of this enzyme extract showed that 50°C and pH = 5.5 are the optimal conditions for Talf1 invertase activity, but the higher stability from the range tested was achieved at 30°C, the optimal temperature for BDS processes. The addition of invertases within the DBT desulfurization process by strain 1B, using sucrose as carbon source, contributed to a significant improvement in relation to the control assay without invertases, both in maximum growth rate (μ_{max} = 0,072 h^{-1} in SHF and μ_{max} = 0,070 h^{-1} in SSF *vs.* μ_{max} = 0,037 h^{-1} in control) and 2-HBP production (186.28 µM in SHF and 196.57 µM in SSF *vs.* 149.93 µM in control). Both bioprocesses with invertases reduced growth and desulfurization time from 7 to about 3 days, and achieved similar results, but for SHF approach an extra 24 h are required for the prior saccharification step.

Conclusion – The application of Talf1 invertases to BDS, either in SHF or SSF approach, can open a new focus of research, allowing the utilization of high sucrose feedstock as alternative cheaper carbon sources.

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References

[1] Paixão S.M., et al., *New Biotechnol* (2013). (doi.org/10.1016/j.nbt.2013.02.002)

[2] Miller G.L., *Analyt Chem* 31:426-428 (1959).