

Acid sulfite pretreatment in the enzymatic hydrolysis of *Cytisus striatus*: Optimization strategy

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Abstract

Ethanol production from lignocellulosic material includes three major steps: biomass pretreatment, which fragments the lignocellulosic matrix to facilitate the enzymes access to the substrate; hydrolysis, where the polysaccharides are converted into fermentable sugars (e.g. glucose and xylose) [1]; and finally, fermentation that produces ethanol or other biologically based chemicals (e.g. lactic acid, succinic acid) [2]. The aim of the present work was to study the effect of some operative variables of the pretreatment stage, namely sodium hydrogen sulfite and sulfuric acid loadings, temperature and time, on the release of sugars in the enzymatic hydrolysis of *Cytisus striatus*, performed applying a Novozymes® cocktail, with fixed charges and operating conditions. Wood branches were chipped and submitted to different reaction conditions, with a central composite experimental design 2⁴+star, exploring the following variables: sulfuric acid charge (0-3%, on wood), sodium bisulfite charge (0-4 %, on wood), maximum temperature (150-190°C) and time at maximum temperature (0-30 minutes). After pretreatment, the acid hydrolysates were recovered, the solid residues were mechanically disintegrated and thereafter subjected to enzymatic hydrolysis with an enzymatic cocktail from Novozymes®. Sugars and by-products released in the sulphite pretreatment and enzymatic treatment hydrolysates were analyzed by HPLC.

The different factors effect (NaHSO₃ load [A], H₂SO₄ load [B], temperature [C] and time at Tmax [D]) on different response variables was mathematically established. As an example, the equation corresponding to the XMG (Xylose + Mannose + Galatose) and to the released sugars:

$$XMG = 13,987 + 0,394 A + 3,060 B + 1,543 C + 0,545 D - 0,124 A^2 - 0,957 AB - 0,652 AC - 0,081 AD - 0,037 B^2 - 5,377 BC - 2,403 BD - 1,802 C^2 - 2,295 CD - 0,741 D^2 \quad (\text{Eq. 1})$$

$$\text{Sugars} = 32,51 + 7,166 A + 13,022 B + 26,535 C + 8,940 D + 4,729 A^2 + 0,665 AB + 1,955 AC + 0,783 AD + 2,539 B^2 - 1,743 BC - 0,55 BD + 1,619 C^2 + 1,575 CD - 0,231 D^2 \quad (\text{Eq. 2})$$

Standard effects and yield surface response were also obtained and analysed, as seen in Fig. 1 and Fig. 2:

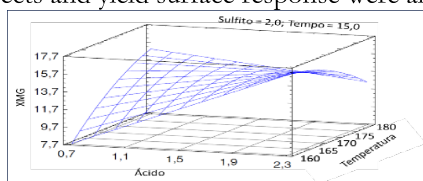


Fig. 1. XMG surface response.

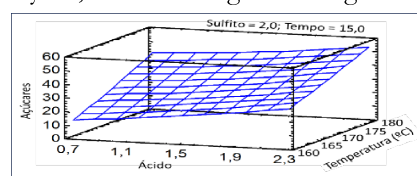


Fig. 2. Sugar surface response.

Pretreatment of biomass with sodium hydrogen sulfite in acid environment alters the feedstock structure and composition, making it more suitable for enzymatic treatment. The pretreatment conditions affect the amount of total recovered sugars and the generated degradation products. For a given sulfite load, more acidic conditions lead to higher sugar release and further material fragmentation, but also to an increase in degradation products. Moderate loads of sodium bisulfite (1%) and sulfuric acid (2.25%), release practically all raw material hemicelluloses. The enzymatic treatment showed to be very sensitive to the pretreatment conditions. The released sugars percentage in the enzymatic hydrolysis ranged from 6.0% to 68.9%. More acidic conditions increase the rate and extent of the enzymatic hydrolysis of the polysaccharides.

References

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- [2] Gil N, Domingues FC, Amaral ME, Duarte AP. Optimization of dilute acid pretreatment of *Cytisus striatus* and *Cistus ladanifer* for bioethanol production, 2012. *J. of Biob. Mat. and Bioen.*, 6 (3), 292-298.