

ENHANCING ETHANOL PRODUCTION: THE ROLE OF ANTHRAQUINONE IN SODA PRETREATMENT OF SUGARCANE BAGASSE

Elisa da S. Barreto^{1*}, André L. R. Ferreira¹, Valéria M. Guimarães² & Leandro A. Gurgel¹

¹ Organic Physical Chemistry Group, Federal University of Ouro Preto/ICEB, Ouro Preto, Brazil.

² Laboratory of Biochemical Analyses/ Federal University of Viçosa/BIOAGRO, Viçosa, Brazil.

* Corresponding author's email address: elisa.barreto@ufop.edu.br

ABSTRACT

This study investigated the soda-anthraquinone pretreatment of raw sugarcane bagasse, focusing on its effects on yield, cellulose and hemicellulose preservation, and enzymatic conversion. Anthraquinone, a redox mediator, promotes the oxidation of aldehyde groups to aldonic acids at the reducing ends of carbohydrate chains, stabilizing them and preventing further degradation, which commonly occurs during soda delignification. Anthrahydroquinone (AHQ) simultaneously reduces lignin, speeding up delignification. In the experiments, 30 g of sugarcane bagasse (dry-weight basis) were placed in a 475 mL reactor with 15% (w/w) sodium hydroxide, 9.6 mL of water per gram of bagasse, and optionally 0.493% (w/w) anthraquinone. The reactor was heated to 150°C for 60 minutes. The solid fractions were washed to neutral pH and subjected to enzymatic hydrolysis using 10 FPU/g of CellicCtec + CellicHtec (85:15, v/v). Results showed that anthraquinone pretreatment increased hemicellulose preservation by 8% compared to experiments without the mediator. Additionally, 87.87% of the cellulose fraction and 78.47% of the hemicellulose fraction were enzymatically converted to monosaccharides. While the soda-anthraquinone pretreatment improved polysaccharide preservation, further optimization of pretreatment conditions is needed to enhance delignification and cellulose preservation.

Keywords: Biomass. Delignification. Keyword 3. Keyword 4. Keyword 5.

1 INTRODUCTION

The application of anthraquinone (AQ) as a catalyst in alkaline pulping has been well documented in scientific studies for paper and pulping applications to accelerate pulp delignification and obtain higher yields. The anthraquinone is a redox mediator that promotes cycles of oxidation and reduction in the lignocellulosic fiber. During the redox cycle, anthraquinone promotes oxidation of the aldehyde groups to aldonic acids at the reducing end of the carbohydrate chains, stabilizing them and avoiding further degradation, as occurs in alkaline delignification processes. At the same time, anthrahydroquinone (AHQ) reduces lignin, accelerating the delignification (Figure 1). In addition to the electron transfer ability, the anthraquinone is stable in alkaline medium and at high temperatures (DIMMEL & DONALD, 1985).

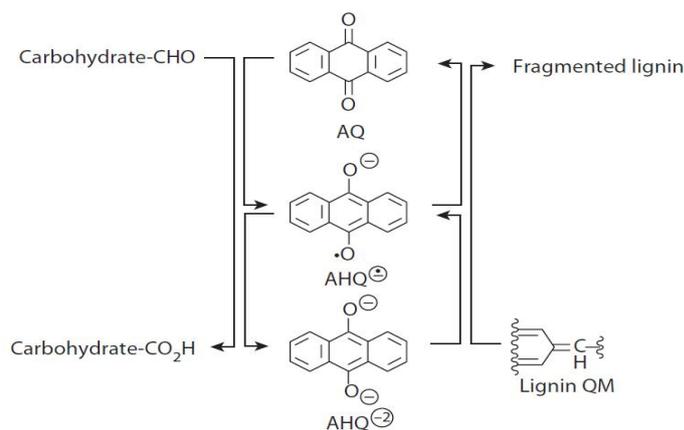


Figure 1. Redox chemistry for AQ in alkaline; two electron transfers (AQ directly to AHQ⁻ and back) and/or one electron transfers involving AHQ⁻ ion radicals. (DIMMEL & DONALD, 1985)

A large amount of studies has been devoted to the chemistry of alkaline pulping, particularly kraft pulping of woods; however, few studies regarding the chemistry of the soda-anthraquinone (AQ) pulping applied to non-woody lignocellulosic materials have been done, especially for second-generation ethanol production.

This study investigated the soda-anthraquinone pretreatment to evaluate the influence of the redox mediator on yield, preservation of cellulose and hemicelluloses and enzymatic conversion of polysaccharides to monosaccharides for second-generation ethanol production.

2 MATERIAL & METHODS

The pretreatment experiments was carried out in a tubular reactor made stainless steel (190mm of height x 65mm of internal diameter x 75mm of external diameter) with a volume capacity of 475mL. In each pretreatment experiment, 30 g of dry sugarcane bagasse was placed into the reactor with 15% (w/w) of sodium hydroxide, 9.6 mL of water per gram of sugarcane bagasse and with or without 0.493% (w/w) of anthraquinone (AQ) at 150o C for 60 minutes. After the end of the soda-AQ pretreatment, the vessel reactor was immediately cooled in an ice bath, opened, discharged and the solid fraction was thoroughly washed until neutral pH. After pretreatment the solid fractions were weighted to determine mass yield (according to Nascimento et. al. 2016), submitted to physical- chemical characterization (according to Baeta et. al. 2016).

The enzymatic hydrolysis assays was performed with 1g of biomass pretreated and 10 mL of solution containing citrate buffer (0.05 mol/ L), 10 FPU/ g of enzymes mixture (85% CellicCtec + 15% CellicHtec) (Novozymes) with pH adjusted to 4.8. Hydrolysis experiments were carried in an orbital shaker agitated at 150 rpm at 50 °C for 72h. After the reaction time, the solid and liquid fractions were separated by centrifugation at 2780 x g and the hydrolysates were heated in boiling water bath for 5 min to prevent further hydrolysis. Sugars were quantified by high performance liquid chromatography (HPLC), according to Barreto et al. (2020). To evaluate the anthraquinone redox potential, the electrochemical measurements were carried out with a potentiostat (AUTOLAB Potentiostat-Galvanostat 128N) according to Santos et al. (2016).

3 RESULTS & DISCUSSION

The chemical composition of sugarcane bagasse used in this study was 48.7% cellulose, 21.4% hemicelluloses, 21.81% lignin, 2.31% extractives and 0.41% ash (inorganic). This composition is in agreement with the literature (LIMA et al., 2018). The main effect of anthraquinone was preservation of 30.16% of the hemicellulose, 8% more than without the mediator (Table 1). Nascimento et al. (2016) observed solubilization of 47.71% hemicelluloses after pretreatment with 0.15% AQ and 15% (w / w) NaOH at 170 °C for 30 min.

However, the effect NaOH-AQ pretreatment on preservation of cellulose fraction was 4.91%, which was lower than the pretreatment performed without the redox mediator. Electrochemical measurements assays showed the anthraquinone redox potential in a pH range of 12-13, as shown in Figure 2. Thus, the low cellulose preservation may be related to low diffusion of the anthraquinone into fiber structure. Therefore, the redox mediator probably acted better on the outermost polysaccharide fraction, such as hemicelluloses. According to Nascimento et al. (2016), experimental conditions such as temperature, heating ramp, reaction time and agitation system (type and speed) may contribute to the diffusion of AQ into fiber structure.

Table 1. Pretreatment yield, chemical composition pretreated sugarcane bagasse in terms of cellulose, hemicelluloses and lignin, and enzymatic conversion.

Assay	Y (%)	Lig.(%)	Cel. (%)	Hemi.(%)	EC _{cel} (%)	EC _{hemi} (%)	Sol _{Cel} (%)	Sol _{Hemi} (%)	Sol _{Lig} (%)
NaOH	56,5	7,87 ± 2,4	70,83 ± 0,4	22,70 ± 0,1	91,80 ± 0,4	96,08 ± 1,5	17,80	38,16	82,07
AQ	57,5	10,35 ± 3,2	65,69 ± 1,5	25,70 ± 0,1	87,87 ± 0,9	78,57 ± 2,1	22,51	30,16	76,03

Cel: cellulose; Hemi: hemicelluloses; Lig: lignin; EC: enzymatic conversion; Y: pretreatment yield yield; Sol: solubilization of a biomass component

The anthraquinone showed a slight negative effect on the enzymatic hydrolysis, presenting 3.93% and 17.51% less enzymatic conversion of cellulose and hemicellulose, respectively (table 1). These results may be due to the presence of complex lignin carbohydrates (CLCs). Yang et al. (2016) observed 16.3% less in the enzymatic conversion of the cellulose in the soda-AQ pretreatment, compared to the kraft process. The group observed that the material delignified by soda-AQ presented presence of lignin-carbohydrate complexes, unlike the kraft process. According to Min et al. (2014), the CLCs play a role like hemicelluloses in protecting the cell wall from enzyme attack.

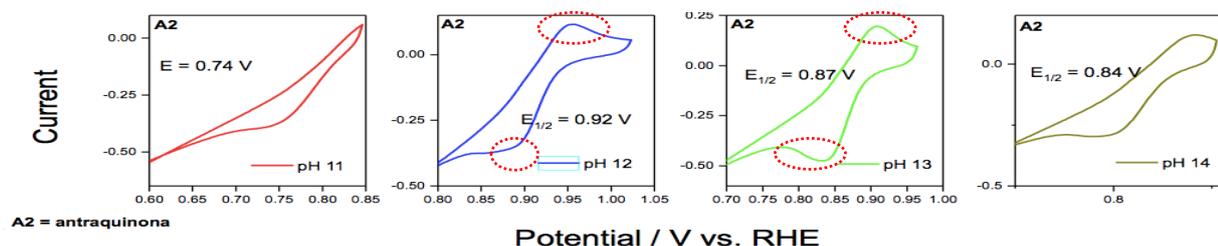


Figure 2. electrochemical measurements of anthraquinone at different pHs. The circles show that anthraquinone is capable of donating and receiving electrons between pHs 12 and 13.

4 CONCLUSION

The results obtained in this study showed that the use of anthraquinone as redox mediator makes possible the preservation of the hemicelluloses fraction, which can contribute to increase ethanol production in a co-fermentation process or to produce added-value byproducts if they are isolated from cellulose fraction. Although, the presence of redox mediator had a slight negative effect on the enzymatic conversion of carbohydrates, the NaOH-AQ pretreatment showed a good potential to be applied to non-woody materials. However, more experiments are needed to increase the diffusion of the redox mediator into fiber structure to improve the cellulose preservation.

REFERENCES

- ¹ BARRETO, E.D.S., BAÊTA, B.E.L., PEREIRA, M.C., PASQUINI, D., GUIMARÃES, V.M., GURGEL, L.V.A. 2-Hydroxy-1,4-naphthoquinone (Lawson) as a Redox Catalyst for the Improvement of the Alkaline Pretreatment of Sugarcane Bagasse. *Energy & Fuels*, 34(12), 16228-16239, 2020.
- ² BAÊTA, B. E. L. et al. Evaluation of hydrogen and methane production from sugarcane bagasse hemicellulose hydrolysates by two-stage anaerobic digestion process. *Bio. Tech.*, v. 218, p. 436–446, 2016.
- ³ DIMMEL, Donald R. Electron transfer reactions in pulping systems (i): Theory and applicability to anthraquinone pulping. *Jour. of Wood Chem.and Tech.y*, v. 5, n. 1, p. 1–14, 1985
- ⁴ LIMA, D. R. S. et al. Influence of different thermal pretreatments and inoculum selection on the biomethanation of sugarcane bagasse by solid-state anaerobic digestion: A kinetic analysis *Industrial Crops and Prod.*, v. 111, n. May 2017, p. 684–693, 2018.
- ⁵ MIN, D. Y. et al. The influence of lignin-carbohydrate complexes on the cellulase-mediated saccharification I: Transgenic black cottonwood (western balsam poplar, California poplar) *P. trichocarpa* including the xylan down-regulated and the lignin down-regulated lines. *Fuel*, v. 119, p. 207–213, 2014.
- ⁶ NASCIMENTO, V. M. et al. Effect of anthraquinone on alkaline pretreatment and enzymatic kinetics of sugarcane bagasse saccharification: Laboratory and pilot scale approach. *ACS Sust. Chem. and Eng.*, v. 4, n. 7, p. 3609–3617, 2016.
- ⁷ SANTOS, W. S. et al. A hole inversion layer at the BiVO₄ / Bi₄V₂O₁₁ interface produces a high tunable photovoltage for water splitting. *Sci. Rep.*, v. 6, p. 1–9, 2016.

ACKNOWLEDGEMENTS

Capes, CNPq, Fapemig, BioAgro UFV and Federal University of Ouro Preto.