Valorization of corn stover from phytoremediation of heavy metal contaminated soils through bioethanol production

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This work aimed to assess the possibility of valorization of corn stover (CS) from heavy metals contaminated soil phytoremediation through the production of bioethanol. Thus, CS was submitted to an acid pre-treatment with 3% (v/v) H_2SO_4, HCl, HNO_3 or CH_3COOH at 85 °C for 48 hours. An enzymatic hydrolysis step with *Accellerase* or *Ultraflo* was then applied at 50 °C for 13 hours. *Saccharomyces cerevisiae* was used to ferment the released sugars at 37 °C for 11 days, followed by broth distillation to recover ethanol The average yield in ethanol obtained for the CS produced in the two contaminated soils was 0.51 and 0.32 $g_{ethanol}/g_{stover}$ for the CS treated with HCl and *Accellerase* and 0.39 and 0.27 $g_{ethanol}/g_{stover}$ for the CS treated with HNO_3 and *Ultraflo*, respectively. For the CS produced in the control soil, the yield in ethanol was 0.44 and 0.37 $g_{ethanol}/g_{stover}$ for the treatment with HCl and *Accellerase* and HNO_3 and *Ultraflo*, respectively. This research demonstrated the feasibility of valorization of CS from heavy metals contaminated soil phytoremediation through the production of a valuable biofuel, that contributes to a more sustainable process of soil phytoremediation.

Keywords: Bioethanol; Cd; Corn stover valorization; Soil phytoremediation; Zn

1. Introduction

Increasing soil contamination due to intensive agriculture and to industrial activities such as mining, which introduces considerable quantities of heavy metals into agricultural soil, has been a growing concern, as heavy metals make soil unusable for food agriculture (Silva et al., 2019). This has motivated the need to develop sustainable techniques for the extraction of its contaminants. Currently, phytoremediation is one of the most commonly used processes since it has the advantage of being a treatment performed at the contaminated site (*in situ*). Therefore, phytoremediation plays an important role in soil remediation, making them suitable again for cultivation by removing contaminants (Coutinho and Barbosa, 2007). However, biomass resulting from phytoremediation, that may contain high levels of contaminants such as heavy metals, is usually composted, landfilled or sometimes submitted to thermal processes, with a significant loss of valuable resources in such process (Marques et al., 2020).

To produce second generation bioethanol, it is necessary to convert polysaccharides from lignocellulosic material. Lignocellulose is a material belonging to the wall constitution of plant cells and refers to the material composed of lignin, cellulose and hemicelluloses. Its composition varies with the type of plant, age and environment where it grows. Generally, plants have in their constitution 45-25% of leach, 40-50% cellulose and 25-30% of hemicellulose (Brethauer *et al*, 2020; Carpita and McCann, 2020; Da Costa *et al*, 2019).

Corn has been reported as potentially effective in cadmium, zinc, chromium, copper and lead removal from contaminated soil (Hong *et al*, 2009; Jiang *et al*, 2010; Soudek *et al*, 2010). Thus, in the present work corn was used to perform the Cd and Zn contaminated soil phytoremediation. In order to further valorize this organic matter of lignocellulosic nature that would otherwise be discarded, it was proposed to convert the CS resulting from phytoremediation of a soil contaminated with heavy metals (cadmium and zinc) into bioethanol.

2. Materials and methods

The main objective of pre-treatment is to break down the cellulose and hemicellulose polymers present in lignin, thus increasing the accessibility to enzymes in the enzymatic hydrolysis process. In the present research an acid pre-treatment was applied. Thus, about 20 g of CS were weighed into a 500 ml glass bottle. Subsequently, 150 ml of one of each of the acids was added to each of the flasks used - H_2SO_4, HNO_3, HCl or CH_3COOH, at 3% (v/v) concentration. The pre-treatment was performed for 48 hours in a thermostatic water bath with shaking at 100 rpm (Julabo, SW22) at 85 °C. Each treatment was performed in duplicates.

To achieve the maximum efficiency of the enzymatic hydrolysis process, after the end of pre-treatment, the glass bottles were cooled and the pH value was measured, and adjusted to 5, through small additions of NaOH at 40% (w/v). Commercial *Saccharomyces cerevisiae* was used for the fermentation process. 40 g of yeast was diluted in 200 ml of deionized water and after homogenization, 25 ml of inoculum was added to each of the bottles. The

mixture was stirred and the bottles were covered with porous stopper, placed in the thermostatic water bath with agitation at 60 rpm at 37 °C for 11 days. After fermentation, the samples were filtered under vacuum, to separate the liquid phase from the sluge. Ethanol was recovered from the filtered fermentation broth in a rotaty evaporator (Buchi, R-210) at 120 mbar and 60 °C. The reducing sugars content in the samples collected during the different stages of ethanol production was evaluated according to the methodology described by Miller (1959), reading the absorbance at $\lambda = 500$ nm using a UV-Vis spectrophotometer (*Shimadzu, UV-1700 Pharma Spec; Shimadzu, V260*) and a calibration curve previously prepared.

3. Results and discussion

Through the analysis of the sugar concentration along the process, it was concluded that at all stages, the treatment with HNO_3 and the enzyme *Ultraflo* provided a rather constant amount of sugars. For the treatment with HCl and *Accellerase*, it was concluded that there was a high consumption of the sugars present in the liquor, which may indicate that this combination contributes to higher consumption by the yeasts in the fermentation process, probably due to the presence of lower amount of inhibitors.

It was also concluded that the concentration of sugars for the biomass from control soil is higher. Possibly, the contaminants present in biomass may have a positive effect on the conversion of sugars. To verify the effects they have on fermentation, readings of the concentration of contaminants in the obtained ethanol should be performed. Table 1 sumarizes the results of the sugar and ethanol concentration values obtained for the biomass from contaminated soil. The values of ethanol obtained for biomass from non-contaminated soil under the same experimental conditions are also shown.

Table 1. Summary of sugar and ethanol concentration for biomass from contaminated and control soil.

Soil	Pre-treatment + enzyme	Glucose concentration after hydrolysis (g/l)	Glucose concentration after fermentation (g/l)	Volume of ethanol solution recovered (ml)	Volume of ethanol (ml)
Estarreja	HCl + *Accellerase*	9,74	2,47	338,7	8,05
	HNO_3 + *Ultraflo*	9,47	5,40	288,2	5,13
Panasqueira	HCl + *Accellerase*	10,61	0,94	330,5	6,22
	HNO_3 + *Ultraflo*	8,15	5,30	253,0	4,25
Control	HCl + *Accellerase*	13,38	6,17	355,2	7,02
	HNO_3 + *Ultraflo*	12,91	5,20	293,0	5,79

4. Conclusions

By comparing the amounts of ethanol obtained in the first phase, with a volume of ethanol recovered of 1.23 ml and 0.67 ml for HCl and HNO_3, respectively, with the corresponding enzymes, and the second experimental phase, with a volume of ethanol recovered of 7.02 ml and 5.79 ml, it is perceived that the quantities differ significantly. It is believed that this is a consequence of the quality of the yeast used in the first phase being lower than that used in the second phase, thus limiting the extent of the fermentation process. With the results obtained in the experimental work, it was concluded that the production of ethanol from CS from contaminated soil has a high potential. Based on the results obtained, the alcohol yield for CS produced in both contaminated soils was 0.51, 0.39, 0.32 and 0.27 $g_{ethanol}/g_{stover}$ and for the CS produced in non-contaminated soil it was 0.44 and 0.37 $g_{ethanol}/g_{stover}$ for HCl and HNO_3, respectively. Thus, it can be concluded that there is feasibility of ethanol production from biomass or corn plants from phytoremediation of heavy metal contaminated soils.

References

Marques, A.P.G.C., Caetano, N.S.C., Castro, P.M.L., 2020. Chapter 12:Strategies for Enhancing Soil Phytoremediation and Biomass Valorization. Handb. Environ. Remediat. 331–356.

Silva, T.J., Hansted, F.A.S., Tonello, P.S., Goveia, D., 2019. Fitorremediação de Solos Contaminados com Metais : Panorama Atual e Perspectivas de uso de Espécies Florestais. Rev. Virtual Quim. 11, 1–17.

Brethauer, S., Shahab, R.L., Studer, M.H., 2020. Impacts of biofilms on the conversion of cellulose. Appl. Microbiol. Biotechnol. 104, 5201-5212.

Carpita, N.C., McCann, M.C., 2020. Redesigning plant cell walls for the biomass-based bioeconomy. J. Biol. Chem. 295, 15144–15157.

Coutinho, H.D., Barbosa, A.R., 2007. Fitorremediação: Considerações gerais e características de utilização. Silva Lusit. 15, 103–117.

Da Costa, R.M.F., Pattathil, S., Avci, U., Winters, A., Hahn, M.G., Bosch, M., 2019. Desirable plant cell wall traits for higher-quality miscanthus lignocellulosic biomass. Biotechnol. Biofuels 12, 1–18.

Hong, C.O., Gutierrez, J., Yun, S.W., Lee, Y.B., Yu, C., Kim, P.J., 2009. Heavy metal contamination of arable soil and corn plant in the cicinity of a zinc smelting factory and stabilization by liming. Arch. Environ. Contam. Toxicol. 56, 190–200.

Jiang, C. ai, Wu, Q.T., Sterckeman, T., Schwartz, C., Sirguey, C., Ouvrard, S., Perriguey, J., Morel, J.L., 2010. Co-planting can phytoextract similar amounts of cadmium and zinc to mono-cropping from contaminated soils. Ecol. Eng. 36, 391–395.

Soudek, P., Petrová, Š., Benešová, D., Vaněk, T., 2010. Phytoextraction of toxic metals by sunflower and corn plants. J. Food, Agric. Environ. 8, 383–390.