The effect of acid pretreatment on bio-ethanol and bio-hydrogen production from sunflower straws

G. Antonopoulou[1,*], G. Dimitrellos[1], D. Vayenas[1,2] and G. Lyberatos[1,3]

[1] Institute of Chemical Engineering Sciences, Stadiou 1, Platani, Patras,26504, Greece

[2] Department of Environmental and Natural Resources Management, University of Patras,. 2 G. Seferi st, Agrinio, GR30100, Greece

[3]School of Chemical Engineering, National Technical University of Athens, GR 15780 Athens, Greece

*Corresponding author: E-mail: geogant@chemeng.upatras.gr, Tel +302610965318, Fax: +302610965318

Abstract

Lignocellulosic biomass including agricultural residues such as straws, leaves, or pruning could be used as feedstocks for second generation bio-ethanol or bio-hydrogen production. Although being abundant with an annual world production exceeding 220 billion tons, the main obstacles of lignocellulosic biomass use, are the low yields attained, due to natural resistance, often called ‘‘recalcitrance” of its lignocellulosic content, to microbial and enzymatic deconstruction (Zhu et al., 2009). Therefore, biomass pretreatment is necessary to enhance the hydrolysis of hemicellulose and make the cellulose fraction more accessible for enzymatic attack, prior to fermentation. A variety of pretreatment including mechanical, thermochemical or thermal processes, have been developed for changing the chemical and structural composition of biomass and improving the enzymatic conversion efficiency (Hendriks and Zeeman, 2009). Acid pretreatment, has been proposed as an efficient pretreatment method for hydrogen and ethanol production (Schell et al., 2003; Sanchez et al., 2004; Garcia et al., 2014). During acid pretreatment, hemicellulosic fraction of biomass is hydrolyzed to soluble sugars and compounds such as furfural and/or hydroxylmethylfurfural (HMF), formic and acetic acids can also be observed (Ramos, 2003). These compounds might have an inhibitory or toxic effect on bacteria or yeasts that are used in subsequent bioconversions to hydrogen/ ethanol.

In this study, sunflower straws were pretreated with different inorganic acids such as H_2SO_4, H_3PO_4 and HCl at different concentrations (2-20 g /100g TS) at mild conditions (1h at 120^{o}C) and the effect of type and acid concentration on carbohydrates’ solubilization and fractionation of the lignocellulossic content (cellulose, hemicellulose, lignin), was evaluated. The possible release of compounds such as acetic acid, ethanol, furfural and HMF during the pretreatment, was also determined while a detailed characterization of the pretreated feedstocks was carried out, through techniques such as scanning electron microscopy (SEM) and IR spectroscopy.

In the sequel, bioethanol production from acid pretreated sunflower straws was studied in batch experiments at 30^{o}C, using the xylose - fermenting yeast of *P. stipitis* via a Simultaneous Saccharification and Fermentation (SSF) concept. The initial pH was 5. For enzymatic hydrolysis, a mixture of commercial enzymes such as Celluclast 1.5L (Cellulase from *Trichoderma reesei*, ATCC 26921) at a concentration of 40 FPU of Celluclast /g TS sunflower straws and Novozyme 188 (Cellobiase from *Aspergillus niger*) at a ratio (v/v) of (3:1) was used. Kinetic characterization of *P. stipitis* in terms of maximum ethanol production rates and yields was carried out by using synthetic substrates as glucose (10g/L), xylose (10g/L) and mixtures of them (glucose 5g/L and xylose 5g/L as well as glucose 2g/L and xylose 8g/L) and was correlated to the ethanol yields obtained from the acid pretreated feedstocks. The experiments showed that all treatment methods enhanced ethanol production from sunflower straws and the treatment with HCl at a concentration of 10 and 20g/100gTS, led to higher ethanol yield.

In addition, fermentative hydrogen production from acid pretreated sunflower straws was studied in batch experiments at 35ºC, using heat treated mixed anaerobic sludge, via a SSF concept. For enzymatic hydrolysis, a mixture of Celluclast 1.5L (40 FPU/g TS sunflower straws) and Novozyme 188 at a ratio (v/v) of (3:1), was used. The metabolic products generated (acetic, porpionic and butyric acid, ethanol and butanol) during fermentation were determined and correlated to the produced hydrogen productivities and yields.
For all experiments, it was resulted that the optimum, for hydrogen production, pretreatment method, was not the optimum for ethanol production, implying that the same pretreatment method is not appropriate for all subsequent bio-conversion processes.

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References

Cheng, K.-K., Cai, B.-Y., Zhang, J.-A. Ling, H.-Z., Zhou, Y.-J., Ge, J.-P., Xu, J.-M. 2008. Sugarcane bagasse hemicellulose hydrolysate for ethanol production by acid recovery process, Biochem. Eng. J. 38: 105–109.

Garcia, A., Cara, C., Moya, M., Rapado, J., Puls, J., Castro, E., Martin, C., 2014. Dilute sulphuric acid pretreatment and enzymatic hydrolysis of *Jatropha curcas* fruit shells for ethanol production. Industrial Crops Products 53: 148– 153

Hendriks, A.T.W.M., Zeeman, G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass *Bioresour. Technol*. 100: 10–18

Jeffries, T.W., 2006. Engineering yeasts for xylose metabolism. Curr. Opin. Biotechnol. 17, 320–326.

Ramos, L.P., 2003. The chemistry involved in the steam treatment of lignocellulosic materials. Quim. Nova. 26 (6), 863–871

Sanchez, G., Pilcher, L., Roslander, C., Modig, T., Galbe, M., Liden, G., 2004. Dilute-acid hydrolysis for fermentation of the Bolivian straw material Paja Brava. Bioresour. Technol. 93: 249–256

Sanchez, S., Bravo, V., Castro, E., Moya, A.J., Camacho, F., 2002. The fermentation of mixtures of D-glucose and D-xylose by *Candida shehatae, Pichia stipitis* or *Pachysolen tannophilus* to produce ethanol J Chem Technol Biotechnol. 77: 641-648

Schell, D.J., Farmer,J., Newman, M., Mc Millan, J.D., 2003. Dilute–sulfuric acid pretreatment of corn stover in pilot-scale reactor: Investigation of yields, kinetics and enzymatic Ddigestibilities of solids. Appl. Biochem. Biotechnol. 105–108

Zhu, J.Y., Pan, X.J., Wang, G.S., Gleisner, R., 2009. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresour. Technol.* 100(8): 2411–2418