

# Assessing straw digestate from anaerobic digestion as feedstock for sugars production

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## Introduction

Anaerobic digestion (AD) is a biological conversion process that has been widely used to convert organic residues into renewable energy, while alleviating environmental concerns associated with the waste. There are three output streams of AD: biogas, liquid effluent, and solid digestate (AD fiber). As for the AD fiber, cellulose and lignin are the major components, which undergo relatively little changes during conventional AD processes. It has been widely accepted by the scientific community that AD fiber is not suitable to be further converted to other useful energy/chemical products due to its “recalcitrant” structure and low nutrient value (Tambone et al., 2009). Thus, it is currently used by the agricultural industry as soil amendment or animal bedding (Johnson et al., 2006).

The aim of this paper was to assess the potential of straw digestate as sugar source. Therefore, the application of chemical pretreatment (dilute acid or alkaline) and cellulase saccharification as pretreatment options was studied.

## Materials and Methods

**Fiber samples:** AD fiber sample was the solid digestate after liquid/solid separation from a pilot scale CSTR anaerobic digester that deals with wheat straw in the premises of NTUA. The digester was operated at 37°C with a hydraulic retention time of 20 days. Wheat straw (WS) was obtained from Aspropyrgos province, Greece. It mainly composed of 33.8% cellulose, 45.1% hemicellulose, 16.4% lignin (15.4% Klason lignin and 1.0% acid-soluble lignin), and 4.7% ash.

**Chemical pretreatment:** The CSTR AD fiber was pretreated in autoclave with/ without dilute NaOH or H<sub>2</sub>SO<sub>4</sub> at 120°C.

**Enzymatic hydrolysis:** Enzymatic saccharification of untreated and pretreated solid samples was executed at 50 °C containing 10% w/w dry solids and the cellulolytic formulation, Cellic CTec2 (Novozymes, Denmark for 72h. Buffer solutions were employed in order to adjust the pH to 5.0.

**Factorial experimental procedure:** The aim of the experimental procedure was to determine the influence of some basic process parameters on the saccharification efficiency SG (optimization parameter). The latter is estimated as the glucose production compared to the maximum theoretical glucose production from the total conversion of carbohydrates. The parameters that generally influence the performance of the pretreatment scheme are chemicals' concentration, autoclave retention time and enzyme loading during enzymatic hydrolysis (controlling parameters). The effect of the controlling parameters on each optimization parameter was estimated by performing a 2<sup>3</sup> factorial experiment. In general, by using a 2<sup>n</sup> factorial design, n controlling parameters interrelate to an optimization parameter through an appropriate linear model. Their significance can also be estimated and assessed [23, 24]. The levels of the controlling parameters are given in Tables 1 and 2. The experimental area of the factorial design was pre-determined in preliminary trials.

Table 1. Controlling parameters and their levels at the factorial experiment (alkaline pretreatment prior to enzymatic hydrolysis)

Controlling Parameter	Variation Intervals		
	Low level (-)	High Level (+)	Center
Time autoclave, $t_{\text{auto}}$ (h)	1	1,5	1,25
NaOH (%)	2	4	3
CellicCTec2, $C_{\text{enz}}$ ( $\mu\text{L}/\text{g}$ cellulose)	100	400	250

Table 2. Controlling parameters and their levels at the factorial experiment (acidic pretreatment prior to enzymatic hydrolysis)

Controlling Parameter	Variation Intervals		
	Low level (-)	High Level (+)	Center
Time autoclave, $t_{\text{auto}}$ (h)	1	1,5	1,25
H <sub>2</sub> SO <sub>4</sub> (%)	1	3	2
CellicCTec2, $C_{\text{enz}}$ (μL/ g cellulose)	100	400	250

In the 2<sup>3</sup> factorial designs, 16 experiments were carried out in triplicate. Five extra experiments in the centre of the designs were also conducted for statistical purposes. From these data, mathematical models were constructed whose adequacy was checked by the Fisher criterion.

### Results and discussion

According to the results of the factorial experiment for alkaline pretreatment and by following a specific analytical procedure (Alder et al., 1995; Cochran and G.M. Cox, 1957), the following linear model was estimated, interrelating the saccharification yield with the statistically important controlling parameters of the system:

$$SG_{\text{NaOH}} = 3.975 + 32 * t_{\text{auto}} + 0.0525 * C_{\text{enz}}$$

The adequacy of the mathematical model derived from the factorial design was checked by the Fisher criterion and it proved to be adequate.

The plus (+) in the above equation indicates that an increase of the autoclaving time and/or enzyme loading leads to a higher saccharification yield and consequently to a more attractive feedstock for ethanol production. It was shown through statistical analysis that the NaOH concentration within the range of 2 to 4%, as well as the interactions between two or more parameters were negligible.

In the experimental range studied, the higher saccharification yield achieved was 76% in the experimental point 1,5 h autoclaving time, 2% NaOH and 400 μL CellicCTec2/ g cellulose.

The acidic pretreatment combined with the enzymatic hydrolysis presented saccharification yields in the range of 2-39%, much lower than the respective yields achieved by the application of alkaline pretreatment.

In the experimental range studied, the higher saccharification yield achieved was 39% in the experimental point 1,5 h autoclaving time, 3% H<sub>2</sub>SO<sub>4</sub> and 400 μL CellicCTec2/ g cellulose, which represents the harshest pretreatment conditions examined. Nevertheless, the same analytical procedure was also adopted and the following model derived:

$$SG_{\text{H}_2\text{SO}_4} = 11.35 + 0.03 * C_{\text{enz}}$$

The model proved to be inadequate poor experimental reproducibility and low efficiencies.

### Conclusions

Anaerobically treated agricultural wastes, such as straw digestate, still contain important components of remaining carbohydrates and lignin that can be used as feedstock for sugars production and alcoholic fermentation. Chemical pretreatment combined with enzymatic hydrolysis was investigated as an alternative valorisation route for digestate. Acid pretreatment along with enzymatic hydrolysis was found to yield low sugars recoveries (2-39%), casting doubt on its suitability for ethanol production. In contrary, alkaline pretreatment and enzymatic hydrolysis is a better approach with elevated saccharification yields reaching up to 76%. Conclusively, according to the experimental results the perspective of a new integrated system is enforced. This system combines ethanol production with anaerobic digestion simultaneously producing energy in the form of methane and ethanol and improving the overall energy balance.

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