



# Enzymatic hydrolysis of steam exploded corncob residues after pretreatment in a twin-screw extruder



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## ABSTRACT

A modified twin-screw extruder incorporated with a filtration device was used as a liquid/solid separator for xylose removal from steam exploded corncobs. A face centered central composite design was used to study the combined effects of various enzymatic hydrolysis process variables (enzyme loading, surfactant addition, and hydrolysis time) with two differently extruded corncobs (7% xylose removal, 80% xylose removal) on glucose conversion. The results showed that the extrusion process led to an increase in cellulose crystallinity, while structural changes could also be observed via SEM. A quadratic polynomial model was developed for predicting the glucose conversion and the fitted model provided an adequate approximation of the true response as verified by the analysis of variance (ANOVA).

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## 1. Introduction

Bioconversion of lignocellulosic biomass to ethanol is considered to be one of the most important alternatives to petroleum based liquid fuels [14,15,17,29,35]. Lignocellulosic biomass are highly abundant, have high energy potential and are low cost materials for ethanol production. Typical sources are forest products, agricultural residues, municipal solid waste, and dedicated energy crops [18,31].

Corncoobs, a byproduct of corn grain production, were once used for heat, animal feed and manure for agricultural production in some parts of Europe, while in the United States, corncoobs are currently being used as a potential feedstock for cellulosic ethanol production due to its low lignin and high carbohydrate contents. Moreover, corncoobs have a high heating value (HHV) producing approximately 8000 Btu/lb. The average corncob yield is about 14% of grain yield, which represents about 16% of the total corn stover in a field [32,22,4].

Among the different technologies [25,33] available for the conversion of lignocellulosic biomass to suitable fermentation substrates, the enzymatic conversion of cellulose seems to be the most promising approach to get a high yield of fermentable sugars [8] because it is highly specific and does not produce substantial amounts of unwanted byproducts [38]. The enzymatic hydrolysis

process is usually catalyzed by cellulase enzymes and the process is affected by many factors including cellulose fibre protection by hemicelluloses and lignin, cellulose crystallinity, degree of polymerization, degree of acetylation of hemicelluloses and the accessible surface area of the biomass [28]. The presence of hemicelluloses and lignin makes the cellulase enzymes' access to cellulose difficult, which will reduce hydrolysis efficiency. Therefore, the structure of cellulosic biomass must be pretreated prior to enzymatic hydrolysis to make cellulose more accessible to enzymatic conversion [29,11]. Various physical, chemical, physico-chemical and biological pretreatment methods have been well-investigated for ethanol production from lignocellulosic biomass [36,16,35]. The purpose of the pretreatment is mainly to increase the accessibility of the enzymes to cellulose the by solubilisation of hemicelluloses or/and lignin, and by decreasing the degree of polymerization and cellulose fibre crystallinity [12]. Moreover, adding surfactants has also improved the effectiveness of the cellulose hydrolysis [3,10].

To improve the rate of enzymatic hydrolysis, researchers have focused on the study of multiple enzymatic hydrolysis process parameters, including substrate concentration, and reaction conditions such as hydrolysis time, pH, temperature and addition of surfactants [35]. Optimal parameters are highly dependant on the physico-chemical structure of the digested biomass, and different pretreatment methods will produce substantially different biomass. Pretreatment in a twin-screw extruder can be used (among other things) to hydrolyze and remove the hemicellulose fraction [23,24,7]. However, the effect of xylose removal via

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extrusion pretreatment, along with other process parameters on the enzymatic hydrolysis of corncobs, has not yet been systematically characterized. In the present study, two differently extruded corncobs with 7% xylose removal and 80% xylose removal, respectively, were used as a source of enzymatic hydrolysis. The characteristics of these two materials were examined by SEM and XRD. A face-centered central composite design was used to study the combined effects of various enzymatic hydrolysis process variables (enzyme loading, surfactant addition, and hydrolysis time) with these two extruded corncobs (7% xylose removal, 80% xylose removal).

## 2. Materials and methods

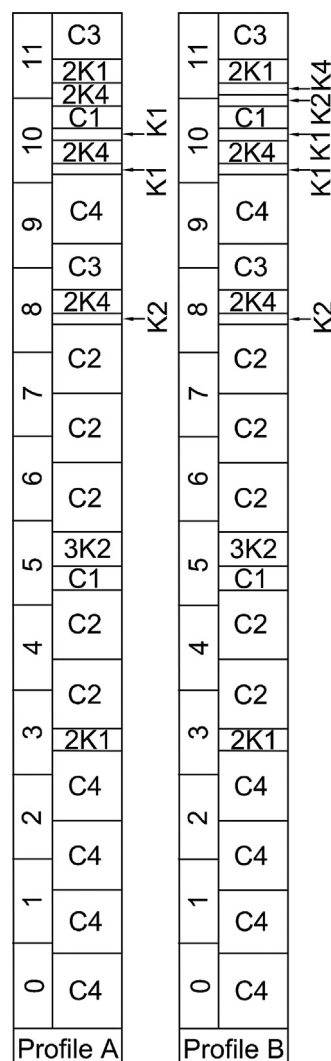
### Materials

Corncoobs were obtained from local farmers in Chatham, ON, Canada. Corncoobs were cleaned and ground to the particle size of 0.5–1 cm<sup>3</sup> and moisture was adjusted to 50% dry matter. Corncoobs were then fed into a continuous steam explosion pretreatment reactor (GreenField Ethanol, Chatham). The reactor was set at a temperature of 205 °C with pH 4.8 in a system pressurized with saturated steam. The overall retention time of the corncoobs during pretreatment was 5 min. Hemicellulose was hydrolyzed to xylose or xylo-oligosaccharides under these conditions. The pressure of the reactor was rapidly released to atmospheric pressure, thus the pressurized corncoobs were flashed into a cyclone separator, which increased the accessible surface area of the fibres for the enzymes. Pretreated corncoobs with 80% moisture content were collected and adjusted to 60% by air drying for further xylose removal during the extrusion process.

All other chemicals (e.g., acetic acid, sodium sulfate anhydrous, tetracycline, cycloheximide, glucose and xylose) were of analytical grade and purchased from Sigma–Aldrich (USA). The Cellic CTec 2 cellulose enzyme was obtained from Novozyme (Canada).

### 2.2. Xylose removal during extrusion process

Experiments were conducted with a Leistritz co-rotating twin screw extruder (American Leistritz Extruder Corp, USA). The extruder was composed of twelve modular barrels that were each 200 mm long. The barrels were electrically heated using thermal induction and cooled by water circulation. Barrel temperature, water flow rate, feed flow rate and pressure were monitored from a control panel. The material was fed into the extruder inlet port (Barrel 0, Fig. 1) at 4 kg/h by a gravimetric feeder (Brabender Technology, Canada). Water was injected into Barrel 8 by a positive displacement pump (Milton Roy USA). A solid/liquid separator was positioned in Barrel 9 to collect the filtrate mainly containing dissolved xylose. Two pressure sensors were positioned in Barrels 8 and 10, respectively, to detect the pressure on both sides of the filter. Two screw configuration profiles (Fig. 1A and B) were used to produce the extruded corncoobs with 7% and 80% xylose removals, respectively. These two screw configuration profiles were built by placing conveying, kneading and reverse screw elements at different positions and intervals. The conveying screw elements



**Fig. 1.** Screw configuration profiles used to achieve 7% (a) and 80% (b) xylose removal. Conveying element: C1: GFA-2-30-30, C2: GFA-2-30-90, C3: GFA-2-40-60, C4: GFA-2-40-90. Kneading elements: K1: KB2-15-30°, K2: KB2-15-60°, K3: KB2-15-90°, K4: KB2-15-30°-Li. GFA-2-XX-XX: G = co-rotating, F = conveying, A = free-meshing, 2 = number of threads, the first XX = pitch, the second XX = length of screw element. KB5-2-30-XX-Li: KB = kneading block, 5 = number of kneading segments, 2 = number of threads, 30 = length of kneading block, XX = twisting angle of the individual kneading segments, RE = conveying element, Li = reverse element, X2 = two same elements.

were used for material transportation and their smaller pitch could compress the products and achieve a high degree of filling within each barrel. Kneading screw elements oriented at different angles were used to break down large solids and to mix biomass and water to achieve a homogeneous distribution. In addition, reverse screw elements carrying the materials in the opposite direction were placed immediately before and after the filter to increase

**Table 1**

Carbohydrate composition of corncoob samples after different treatment conditions (average of triplicates ± standard error).

	Ground corncoobs (%)	Steam exploded corncoobs (%)	Extruded corncoobs with 80% xylose removal	Extruded corncoobs with 7% xylose removal
Glucose	41.3 ± 0.75	55.4 ± 1.61	65.5 ± 1.50	58.1 ± 1.52
Xylose	29.0 ± 1.25	22.8 ± 1.29	7.4 ± 0.10	19.4 ± 1.00
Arabinose	4.2 ± 0.18	2.4 ± 0.07	1.5 ± 0.07	2.3 ± 0.06
Galactose	1.5 ± 0.10	0.6 ± 0.05	0.2 ± 0.03	0.6 ± 0.03
Mannose	0.4 ± 0.03	0.3 ± 0.03	0.3 ± 0.03	0.2 ± 0.02

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