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### Research paper

# Optimization of biological pretreatment to enhance the quality of wheat straw pellets



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#### ABSTRACT

Pelleting increases the efficiency of handling and transportation of biomass for conversion into biofuels either through biochemical or thermochemical process. Pretreatment of biomass makes lignin fragments accessible by disrupting the lignocellulosic structure, and ensures the production of high-quality pellets. In this study, biological pretreatment using solid-state fermentation (SSF) was investigated as a means to improve the quality of pellets produced from wheat straw. SSF of wheat straw using Trametes versicolor 52] (TV52]), T. versicolor m4D (TVm4D) and Phanerochaete chrysosporium (PC) were conducted. Response surface methodology was employed by using a four-factor, three-level Box-Behnken design with moisture content (% mass fraction of water), hammer mill screen size used for chopping wheat straw (mm), fermentation time (days) and fermentation temperature (°C) as process parameters. Pellet density, dimensional stability and tensile strength were the response variables. The fermentation temperature significantly affected all the responses. The optimization options of moisture content (70% mass fraction of water), hammer mill screen size (50-52.8 mm) and fermentation temperature (22-22.1 °C) of wheat straw with pretreatment using the three fungal strains were very similar. The optimized fermentation time of wheat straw pretreated with PC was the longest at 35 days; the shortest at 21 days was for wheat straw pretreated with TV52]. The microscopic structural changes induced by microbial pretreatment were examined by scanning electron microscopy (SEM). Results showed that the combination between single fibers became relatively loose, and the connection was never tight which was advantageous to improve the physical quality of the compressed pellets.

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#### 1. Introduction

Depleting resources of fossil-based fuels along with greenhouse gas emissions have triggered the search for alternative sources of energy to ensure energy access and security [1,2]. Lignocellulosic biomass such as agricultural residue is considered an important renewable source for bioenergy production due to its abundance and inexpensiveness. Among all the agricultural residues generated, cereal straw is the largest quantity of feedstock available, with a global annual production of about 1.5 Gt [3,4]. Crop residues are the non-edible portion of crops, so they do not interfere with food supplies [5]. Some of the main issues regarding the utilization of agricultural straw are its irregular shape and size and relatively low bulk density in its original or baled form compared to wood or coal. For example, the bulk density of loose straw, standard baled straw, and wood residue is approximately 40, 100 and 250 kg m<sup>-3</sup>, respectively [3,6]. This results in high transportation and storage costs, which constitute 35% or more of the production expenditures of cellulosic biofuels and comprise more than 50% of the feedstock price [7,8]. Consequently, the new logistics strategies should be designed so that they minimize biomass handling and transportation costs and facilitate the establishment of large-scale processes [9].

Pelleting, which is a form of biomass densification, aims to increase the bulk density of agricultural residues more than what is achieved by baling [10,11]. Pellets provide biomass with sufficient caloric density for efficient transportation, and could be an option

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for higher utilization of (mass) payload capacities, which in turn would result in cost reduction [9,11]. Pellets are the most energy intensive densified products, having higher density than other densified forms such as briquettes or cubes [4]. However, without an adequate pretreatment, agricultural biomass is difficult to densify. It yields weak and powdery pellets, which not only are costly to produce but also cannot withstand the physical rigors of transportation [11].

Agricultural residues are composed of a complex network of cellulose microfibrils embedded in a matrix of cross-linked hemicellulose and lignin [12]. These three major components are organized in such highly recalcitrant structures in plant cell walls, that overcoming this recalcitrance has been considered as the most important unresolved issue of plant-based green technologies [13].

Conventional physicochemical methods are the leading pretreatment technologies, although they require large inputs of energy, and also generate pollution. Typically, they need expensive corrosion resistant reactors, processing of large volumes of waste stream, extensive washing of treated solids, etc. [14]. The pretreatment step is regarded as a crucial and costly unit process in converting lignocellulosic materials into fuels, and it is the major contributor to the cost of producing energy from biomass [15,16]. In contrast to physicochemical methods, biological pretreatment of lignocellulosic biomass is a safe, environmentally friendly, less energy intensive and low-cost alternative [6,17].

Biological pretreatment employs certain microorganisms, including white-rot, brown-rot, or soft-rot fungi, or bacteria, to deconstruct recalcitrant biomass [18,19]. White-rot fungi can degrade cellulose, hemicellulose and lignin approximately equally, and they are widely considered to be the most effective type of microorganism for this purpose [15,17]. Their unique enzymatic machineries, consisting of lignin peroxidase (LiP), manganese peroxidase (MnP), laccase (Lac), and versatile peroxidase (VP), are aimed at breaking down lignin and altering lignocellulose structures [20,21].

Solid-state fermentation (SSF) as a strategy for fungal pretreatment offers advantages over liquid-state cultivations, which present many limitations such as low substrate loading (<5%) [14]. In SSF, microorganisms are grown on moist, solid supports like insoluble substrates which they use as a carbon and energy source. As the fermentation occurs in conditions of lower moisture compared to liquid fermentations, it imitates the natural environment to which many higher filamentous fungi are adapted [22,23]. SSF offers important advantages over submerged fermentation; for instance, non-aseptic conditions, lower capital costs, low energy expenditure, less water usage, high volumetric productivity and reproducibility [24,25].

Fungal pretreatment has been previously proposed for biopulping applications [26,27]. Currently, many studies have been focused on biological pretreatment of lignocellulosic biomass for further enzymatic hydrolysis or saccharification [28,29]. However, the possibility of using fungi to precondition biomass for densification processes has been barely examined. In this study, the option of using SSF as biological pretreatment to improve the quality of pellets produced from wheat straw was explored. The influence of the fungal strain, fermentation time, moisture content, particle size, and temperature of fermentations on the biomass, and their effect on pellet density, dimensional stability and tensile strength were determined.

#### 2. Materials and methods

#### 2.1. Experimental design

In this study, the real values and the coded factor levels of the

independent variables are given in Table 1. The regression equations show the effect of fermentation time, moisture content, particle size and fermentation temperature on the density 0 (pellet density immediately after pelleting), density 1 (pellet density 14 days after pelleting), dimensional stability, and tensile strength of wheat straw pellet samples that was determined using analysis of variance (ANOVA) and response surface methodology (RSM) using the Design Expert software version 7.0 [30]. The main advantage of RSM is that it can evaluate multiple parameters and their interactions with a small number of experiments. Hence, RSM was used to optimize the process parameters to maximize the response variables (density 0, density 1, tensile strength and minimize dimensional stability of pellets). The parameters studied were fermentation time, moisture content, particle size and fermentation temperature. It was assumed that the independent variables affected the response variables. The response variables which are density 0, density 1, dimensional stability and tensile strength were calculated after the experiments and can be defined as:

$$y_n = \beta_0 + \sum_{i=1}^4 \beta_i x_i + \sum_{i=1}^4 \beta_{ii} x_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^4 \beta_{ij} x_i x_j \ (n = 1, 2, 3, 4)$$
(1)

 $y_1$  = density 0 (kg m<sup>-3</sup>);  $y_2$  = density 1 (kg m<sup>-3</sup>);  $y_3$  = dimensional stability (%);  $y_4$  = tensile strength (MPa);  $x_1$  = fermentation time (days);  $x_2$  = moisture content (% mass fraction of water);  $x_3$  = particle size (mm);  $x_4$  = fermentation temperature (°C);  $\beta_0$ ,  $\beta_i$ ,  $\beta_{ij}$ ,  $\beta_{ij}$  = the regression coefficients of intercept terms, linear terms, quadratic terms, and interaction terms in the equation, respectively.

#### 2.2. Wheat samples

In the experiment, the wheat straw of variety 'AC Utmost' (Canadian Western Red Spring) was obtained from a field in Mayfield No. 406, Saskatchewan, Canada ( $52.66^{\circ}$  N,  $107.78^{\circ}$  W) in October 2013. The wheat straw samples were collected shortly after the kernel was harvested and transferred in plastic bags to the University of Saskatchewan. The moisture content of the wheat straw samples was 5.99% (mass fraction of water) after it was stored for 12 months in the laboratory, was measured using ASAE S358 [31], where 25 g of material was oven-dried at  $103 \pm 2 \,^{\circ}$ C for 24 h. All of the moisture content tests were performed in triplicate. The wheat straw samples were chopped into 50, 100 and 150 mm segments (particle size) using a crop straw crusher (Model CTR, Belfast Mini-Mills Ltd., Belfast, PE, Canada).

#### 2.3. Fungal strains

The white-rot fungi used in this study were the wild-types of Trametes versicolor 52J (TV52J) (ATCC 96186) and Phanerochaete

Table 1	
Coded levels for independent variables used in the experiment.	

$\frac{\text{Code}}{z_j}$	Factor				
	$\begin{pmatrix} x_1 \\ (d) \end{pmatrix}$	x <sub>2</sub> (%)	<i>x</i> <sub>3</sub> (mm)	<i>x</i> ₄ (°C)	
1	35	70	150	34	
0	28	65	100	28	
-1	21	60	50	22	
$\varDelta j$	7	5	50	6	

 $x_1$  = fermentation time (days);  $x_2$  = moisture content (% mass fraction);  $x_3$  = hammer mill screen size (mm);  $x_4$  = fermentation temperature (°C).

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