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Simultaneous saccharification and co-fermentation of whole wheat in integrated ethanol production



Borbála Erdei*, Mats Galbe, Guido Zacchi

Lund University, Department of Chemical Engineering, P.O. Box 124, SE-221 00 Lund, Sweden

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ABSTRACT

Two of the most important ways of reducing the production cost of lignocellulosic ethanol are to increase the ethanol yield and the concentration in the fermentation broth. This can be facilitated by co-fermentation of glucose and xylose from agricultural residues such as wheat straw, due to the high amount of xylose in the hemicelluloses in these materials.

Simultaneous saccharification and co-fermentation (SSCF) of steam-pretreated wheat straw (SPWS) with and without the addition of liquefied wheat meal (LWM) was performed using the pentose-fermenting yeast, TMB3400. The highest overall ethanol yield in batch operation, of around 70%, equivalent to an ethanol concentration of 43.7 g L^{-1} , was achieved using SPWS with 7.5% water-insoluble solids (WIS) and addition of LWM with 1% WIS. Using SPWS with a higher WIS (10%) resulted in a decreased yield, 60%, although the concentration of ethanol increased to 53.0 g L^{-1} . SSCF of 7.5% straw was also performed with a single (after 20 h) or fed-batch addition of 1% WIS LWM (after 20, 24 and 28 h) resulting in an increase in both ethanol yield and concentration compared to the reference, without wheat meal addition, but no significant difference compared to the batch experiments.

The addition of wheat meal to SSCF did not improve xylose utilization significantly, probably due to the instant release of glucose from the liquefied meal, which hampers the uptake of xylose. The instant release of glucose was shown to be caused by the high amylase activity of the β -glucosidase enzyme preparation.

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1. Introduction

First-generation ethanol based on sugar- and starch-rich raw materials is already being produced on a commercial scale in the US, Brazil and several European countries. The leading producer of ethanol today is the United States, which produced over 40 hm^3 ethanol from corn in 2009 [1], followed by Brazil (24 hm^3 ethanol) based on sucrose from sugarcane. The total ethanol production in the EU countries is about 3.7 hm^3 , and is based primarily on wheat grain [1].

Second-generation ethanol based on lignocellulosic material, such as softwood, bagasse or wheat straw, has the potential to supply transportation fuel in the near future. However, the commercial production of second-generation ethanol has been hampered by problems such as the cost of raw material, high projected capital cost [2,3] and processing costs [4]. One of the most energy-demanding process steps is distillation, i.e. the recovery of ethanol from the fermentation broth [5,6]. The energy required for distillation is lower when the ethanol concentration in the broth is higher; it is therefore important to reach ethanol concentrations of at least a mass

* Corresponding author. Tel.: +46 46 222 8296; fax: +46 46 222 4526.

E-mail addresses: Borbala.Erdei@chemeng.lth.se, borbala.erdei@gmail.com (B. Erdei), Mats.Galbe@chemeng.lth.se (M. Galbe), Guido.Zacchi@chemeng.lth.se (G. Zacchi).

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fraction of 4–5% to make ethanol production from lignocellulosic substrates economically feasible [5,6].

A higher ethanol concentration in the distillation step can be achieved by increasing the concentration of available sugars and/or improving the efficiency of fermentation. The sugar concentration can be increased by increasing the water-insoluble solids (WIS) content. However, this can also lead to a higher concentration of inhibitors when the whole slurry from pretreatment is used, and may lead to a decreased yield due to increased inhibition and poorer mass transfer [7–10]. An alternative is to dilute the inhibitors by the addition of another process stream, which must be rich in fermentable sugars. The effect of glucose-rich streams, such as the hydrolysate from wheat or corn kernels, has previously been studied in the simultaneous saccharification and fermentation (SSF) of wheat straw [11] and lignocellulosic residue from furfural production [12]. Erdei et al. demonstrated that the integrated process not only resulted in a higher ethanol concentration, but also improved ethanol yield compared with SSF, when mixing pre-hydrolysed wheat meal and steam-pretreated wheat straw (SPWS). Other studies have also shown that using the hydrolysate from wheat [13] or corn [12] is also beneficial due to their rich nutrient content.

The ethanol yield can also be improved by co-fermentation of hexose and pentose sugars due to the high amount of xylose in the hemicellulose in the wheat straw. The wild-type *Saccharomyces cerevisiae*, which is the yeast most commonly used for industrial ethanol production, does not ferment xylose. A genetically modified strain, TMB3400 [14], has been developed by introducing genes encoding xylose reductase (XR) and xylitol dehydrogenase (XDH) from fungi [15,16]. To obtain efficient xylose uptake, the glucose concentration must be low, since glucose and xylose compete for the same transport system [17,18] and the affinity for xylose is 200-fold lower [19]; thus xylose transport into the cell is inhibited by glucose. However, it has also been shown that glucose can enhance xylose utilization at low concentrations [20,21]. This has been attributed to the induction of transport systems [20–22], the induction of glycolytic enzymes [23], and improved co-factor generation [21]. The positive effect of a low glucose concentration on xylose uptake makes SSF an interesting process for co-fermentation, since glucose is released from the WIS by the hydrolytic effect of cellulases. However, when the cellulose is fully degraded no glucose is available to induce xylose uptake. Adding wheat meal increases the amount of glucose, ensuring a sufficiently high level of glucose for the xylose to be taken up by the cells.

In the current work, simultaneous saccharification and co-fermentation (SSCF) of SPWS was investigated using *S. cerevisiae* TMB3400 with and without the addition of liquefied wheat meal (LWM). The main focus was on ethanol yield and concentration, but xylose utilization was also investigated when LWM was added to the integrated process.

2. Materials and methods

2.1. Wheat straw

The wheat straw (from SW Gnejs winter wheat) used was provided by Johan Håkansson Lantbruksprodukter, collected from

fields of Lunnarp (Lat: 55° 39' North, Long: 13° 22' East, Sweden) with a Claas Dominator 86 harvester, air dried and then pressed with a Claas Quadrant press into bales in September 2010. The straw had a 9% water mass fraction and on a dry basis the mass fractions of glucan, xylan, galactan, arabinan, mannan lignin and ash consisted of respectively 38.8%, 22.2%, 2.7%, 1.4%, 1.7%, 18.5% and 2.4%, determined according to the standard methods of the National Renewable Energy Laboratory (NREL) [24]. It was ground using a knife mill (Retsch GmbH, Haan, Germany), sieved to obtain particles in the range 2–10 mm, and then stored in plastic bags at room temperature prior to pretreatment.

2.1.1. Pretreatment

The wheat straw was pretreated in two batches (SPWS 1 and 2). It was soaked in excess dilute (mass fraction of 0.2%) sulphuric acid solution (20 g liquid g⁻¹ dry straw) for 1 h and then pressed to a mass fraction of about 50% using a 5-L filter press (Fischer Maschinenfabrik GmbH, Germany). The pressed material was stored in plastic buckets at room temperature before steam pretreatment. Steam pretreatment was performed at 190 °C for 10 min [25] using saturated steam, in a unit described previously [26]. The carbohydrate and the lignin contents of the solid fraction and the total sugars in the liquid fraction were analysed according to NREL standard methods [24,27]. The results are presented in Table 1. The WIS of SPWS 1 was adjusted to 10% by pressing and to 7.5% by the addition of water for the SSCF experiments.

2.2. Wheat meal

Wheat meal (ground wheat kernels) intended for fuel alcohol production was kindly provided by Sileco (Laholm, Sweden). It had an average particle size of 2.5–3 mm, and was stored in a plastic bucket at 5 °C until used. It contained a mass fraction of 72.7% starch and 24.3% starch-free residue on a dry basis. The

Table 1 – Composition of the two steam-pretreated wheat straw batches, SPWS 1 and SPWS 2.

	SPWS 1	SPWS 2
WIS (%)	8.7	9.5
Solids (% of WIS)		
Glucan	62.5	63.4
Xylan	4.0	6.4
Arabinan	1.1	–
Galactan	–	1.5
Mannan	1.4	3.4
Lignin	25.9	28.4
Liquid (g L ⁻¹)		
Glucose	4.2	5.2
Xylose	33.2	37.3
Arabinose	4.7	4.6
Galactose	0.3	4.2
Mannose	–	1.8
Furfural	1.6	2.0
HMF	0.2	0.1
Acetic acid	2.3	2.9

Percentage (%) represents mass fraction. Concentration (g L⁻¹) of total sugars contains both monomer and oligomer sugars.

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