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Enzymatic hydrolysis at high dry matter content: The influence of the substrates' physical properties and of loading strategies on mixing and energetic consumption

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ABSTRACT

The present work investigates the impact of the physical properties and loading strategies of wheat straw and miscanthus on enzymatic hydrolysis at high DM concentration. Three parameters have been chosen to evaluate the enzymatic hydrolysis performance: (i) the mixing time, (ii) the energetic mixing consumption and (iii) the glucose concentration. It was demonstrated that the hydrolysis of miscanthus is easy to perform and has low viscosity. On the contrary, the higher porosity grade of wheat straw than miscanthus (73% against 52%) contributed to have a very high viscosity at 20% *w/w* DM. The development of a fed-batch strategy allowed the reduction of viscosity inducing the energetic consumption lowering from 30 kJ to 10 kJ. It has been also proven that the miscanthus addition in wheat straw achieved to decrease mixing energy consumption at 5–8 kJ, when it represented more than 30% of the total mass of the reaction medium.

1. Introduction

The global warming, due to the increase of the greenhouse gas (GHG) emissions and the simultaneous depletion of fossil fuels, have encouraged the research of alternative and clean energy sources for the anthropic activities. In particular, the bioethanol industry boomed in the last decades (Battista et al., 2016a). Bioethanol production usually starts from simple sugars derived from cane and corn (first generation biofuels), whose fermentation has very good efficiency. Nevertheless, this production is expensive and non-sustainable because of the competitive use of these substrates with food industry (Clomburg and Gonzalez, 2013). Agro-food residues (second generation biofuels) are becoming important substrates for bioethanol production, limiting the use of fields for non-food production. Wheat straw and miscanthus are common second generation substrates for the bioethanol production. Wheat straw is a waste material from agricultural production and miscanthus is a grass family crop with a high energetic yield by its beneficial chemical composition (low content of lignin) (Lewandowska et al., 2016).

Bioethanol production involves four steps: (i) the pretreatments of the substrates, (ii) the hydrolysis to convert ligno-cellulosic material into glucose, (iii) the fermentation of glucose in ethanol and (iv) the distillation. The pretreatments of straw and miscanthus are necessary to optimize the glucose concentration during the hydrolysis and to reduce the viscosity of the reaction medium (Battista et al., 2016b). The pretreatment stage is followed by the hydrolysis, often conducted by purified enzymes able to degrade hemicellulose and cellulose into soluble sugars (Zhou et al., 2008). The enzymatic hydrolysis has currently high yields (75–85%) and improvements are still projected (85–95%) (Balat, 2011).

The last phase of the bioethanol production is represented by the distillation. It has been evaluated that to be economically advantageous the distillation requires a minimum ethanol concentration of 4% *w/w*, which means a minimum glucose concentration of 8% *w/w* and an associate ligno-cellulose loading of at least 15% *w/w* DM content during the enzymatic hydrolysis (McIntosh et al., 2016). Working at high DM concentration also permits to reduce the volume of the reactor and consequentially to have lower economic and energetic costs of the process (Larsen et al., 2008).

Typical enzymatic hydrolysis of lignocellulosic materials is conducted at low DM concentration (maximum 5% *w/w*) to ensure a good contact between enzymes and substrates (Boussaid and Saddler, 1999; Xue et al., 2012). There are few studies regarding the enzymatic hydrolysis at high DM concentration. Kristensen et al. (2009) and Jorgensen et al. (2007) have demonstrated that the conversions of cellulose into glucose decreases by the increasing of DM concentration.

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In addition, Cara et al. (2007) and Battista et al. (2016c) have underlined that at high DM content, the complexity of the lignocellulosic polymers, causes an increase of the reaction medium viscosity and consequentially bad mixing within the bioreactor. The mechanism by which cellulases permit the hydrolysis of cellulose follows three steps: (i) external mass transfer of enzyme, (ii) diffusion/adsorption of the enzyme on the substrate surface and (iii) cellulase catalytic action. The overall reaction rate is determined by the rates of these three events occurring in sequence. If the external mass transfer is neglected (at low DM content), the overall reaction rate will be controlled by the second step (internal diffusion). At high DM content, the mixing is not efficient: the external mass transfer controls the overall reaction rate (Corre et al., 2016) and the hydrolysis efficiency is 20% lower than observing at 5% *w/w* DM concentration (Xue et al., 2012).

The aim of this work is the improvement of the enzymatic hydrolysis of wheat straw and miscanthus at high DM concentration (20% *w/w*), reducing the reaction medium viscosity. The physical properties influence on viscosity has been observed and different loading strategies of batch and fed-batch have been tested on straw, on miscanthus and on a combination of both substrates. The performances of the tests have been evaluated taking into account the most important factors affecting the bioprocesses: (i) mixing time, (ii) mixing energetic consumption and (iii) the glucose concentration contained in the reaction medium at the beginning and at the end of the hydrolysis phase.

2. Materials and methods

2.1. Substrates, enzymatic cocktail characteristic and description of the tests

The substrates used for the tests were wheat straw and miscanthus, pretreated at appropriate operative conditions (data not shown). Table 1 summarises the physical and chemical features of the pretreated wheat straw and the pretreated miscanthus. The features of raw substrates were not available. Zhang et al. (2012) founded that soil and climate conditions influence the raw substrates porosity, which can vary in a very range: 45–85%. This demonstrates that the results obtained by this work are not dependent on the substrates conditions (raw or pretreated). The content of lignin, hemicellulose and cellulose of both substrates have been determined by an external company which supplied the substrates (Table 1).

Cellic CTec-2 (Novozymes) cellulase blend was used for all enzymatic hydrolysis tests and loadings were quoted as FPU (Filter Paper Units)/g glucan. The amount of the enzymatic cocktail has been determined following the methods by McIntosh et al. (2016).

Batch and fed-batch tests have been realised using wheat straw, miscanthus and wheat straw-miscanthus mixture as substrates in order to see the rheological behavior and the conversion of the substrates into glucose. All tests, described in Table 2, have been prepared in order to reach the DM concentration of 20% *w/w* and have conducted in triplicate to ensure their repeatability. The duration of each test has been established at 5 h, when was proved that a stable torque trend was reached. This time was not sufficient to guarantee a complete cellulose conversion in glucose. But this aspect was not relevant in this work which had the aim to investigate the correlation between the substrates feature and the apparent viscosity within the reactor.

S-B test has been prepared loading the reactor with 2.4 kg of wheat straw-water mixture (0.9 kg of wheat straw), while M-B test loading 2.4 kg miscanthus-water mixture (1 kg of miscanthus). Fed batch tests (S-FB65, S-FB50, S-FB35, M-FB65, M-FB50 and M-FB35, Table 2) consisted a first loading of the 65%, the 50% and 35% of the 2.4 kg reaction medium at the beginning of the tests. The rest of the loading has been gradually added in equal parts after 10, 30, 60, 105 and 120 min after the beginning of each test. These fed batch tests had the aim to improve the rheological performance into the reactor and to reduce the power consumption without decreasing the yield from cellulose to glucose. Batch tests have been also conducted on wheat straw-miscanthus-water mixtures (SM-80:20, SM-70:30, SM-50:50, SM-30:70) according the ratios reported in Table 2.

The enzymatic hydrolysis of all the tests have been conducted at optimal operative conditions that are at 50 °C, 50 rpm and a pH range of 5.0–5.5.

Table 1
Chemical and physical characteristics of wheat straw and miscanthus.

	Miscanthus	Wheat Straw
DM content (% *w/w*)	73.35 ± 1.01	70.79 ± 1.29
Cellulose content (%*w/w*)	45.30 ± 2.35	49.20 ± 2.07
Hemicellulose content (% *w/w*)	27.10 ± 1.23	12.20 ± 1.91
Lignin content (% *w/w*)	9.80 ± 0.14	14.90 ± 1.41
Apparent density (kg/m^3)	516.10 ± 8.67	433.90 ± 13.40
Density a 0.212 MPa (g/mL)	0.93 ± 0.05	0.70 ± 0.04
Porosity (%)	52.00 ± 2.60	73.00 ± 3.65
Volume of macroporosity (mL/g)	0.34 ± 0.03	0.65 ± 0.07
Volume of mesoporosity (mL/g)	0.02 ± 0.00	0.02 ± 0.00
Volume of microporosity (mL/g)	0.00 ± 0.00	0.00 ± 0.00
Average diameter of the pores (nm^3)	16461.10 ± 823.06	43128.40 ± 2156.42
d (0.1) µm	158	175
d (0.5) µm	516	551
d (0.9) µm	1210	1340

2.2. The equipment

The bioethanol production from wheat straw and miscanthus have been conducted in a 3 L reactor (Fig. 1) equipped with a torque meter Kistler 4503A measuring torque till a value of 2 Nm and with a data detection frequency variable from 1 to 10 Hz. Data were recollected by LEIRI software reporting them in an Excel file. The reactor was also equipped with a water-heater and with temperature and pH control sensors. The mixing system was an helicoidal impeller properly designed to deal with high DM concentration and high viscosity medium. The helicoidal impeller (Fig. 1) had a diameter of 130 mm and is located at 30 mm from the bottom of the reactor.

2.3. Analytical methods

DM of the wheat straw and miscanthus have been determined according to standard methods described in literature (APHA/AWWA/WEF, 1998). DM represented the content of solids present in the substrates, including the inert materials and the degradable ones (Battista et al., 2016b). The apparent density was determined by the use of Archimedes' principle (Zhao et al., 2016).

The apparent viscosity of the wheat straw-water and of miscanthus–water mixtures have been determined at 10 and 20% DM *w/w* before the beginning of the enzymatic hydrolysis. The equipment used was the viscometer DV-II-PRO by Brookfield provided with a cross rotating spindle working at 50 rpm.

The glucose concentration has been quantified by an enzymatic reaction using the GLUCOSTAT YSI2700 at the beginning and at the end of the tests.

Porosity of the substrates was a very important parameter that was directly linked to the absorption capacity of water: obviously, a major grade of porosity favored the absorption of water molecules by substrates. By this way, the amount of water available for the dispersion of the substrates particles decrease, affecting the viscosity of the reaction medium. The grade of porosity and the average volume pore for macroporosity, mesoporosity and microporosity have been evaluated by N_2 adsorption isotherms method. Initially the sample was degassed at 60 °C for 48 h. The average pore volume was obtained using the Horvath-Kawazoe approximation (Horvath and Kawazoe, 1983). The absorption capacity of wheat straw and miscanthus has been evaluated in