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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.

KEYWORDS: Bioethanol; High Dry Matter; Mixing; Enzymatic hydrolysis; Energy consumption; Lignocellulosic materials

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. 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).

<Table 1>

93 Cellic CTec-2 (Novozymes) cellulase blend was used for all enzymatic hydrolysis tests
94 and loadings were quoted as FPU (Filter Paper Units)/g glucan. The amount of the
95 enzymatic cocktail has been determined following the methods by McIntosh et al, 2016.
96 Batch and fed-batch tests have been realised using wheat straw, miscanthus and wheat
97 straw-miscanthus mixture as substrates in order to see the rheological behavior and the
98 conversion of the substrates into glucose. All tests, described in Table 2, have been
99 prepared in order to reach the DM concentration of 20% *w/w* and have conducted in
100 triplicate to ensure their repeatability. The duration of each test has been established at 5
101 hours, when was proved that a stable torque trend was reached. This time was not
102 sufficient to guarantee a complete cellulose conversion in glucose. But this aspect was
103 not relevant in this work which had the aim to investigate the correlation between the
104 substrates feature and the apparent viscosity within the reactor.
105 <Table 2>
106 S-B test has been prepared loading the reactor with 2.4 kg of wheat straw-water mixture
107 (0.9 kg of wheat straw), while M-B test loading 2.4 kg miscanthus-water mixture (1 kg
108 of miscanthus). Fed batch tests (S-FB65, S-FB50, S-FB35, M-FB65, M-FB50 and M-
109 FB35, Table 2) consisted a first loading of the 65%, the 50% and 35% of the 2.4 kg
110 reaction medium at the beginning of the tests. The rest of the loading has been gradually
111 added in equal parts after 10, 30, 60, 105 and 120 minutes after the beginning of each
112 test. These fed batch tests had the aim to improve the rheological performance into the
113 reactor and to reduce the power consumption without decreasing the yield from
114 cellulose to glucose. Batch tests have been also conducted on wheat straw-miscanthus-
115 water mixtures (SM-80:20, SM-70:30, SM-50:50, SM-30:70) according the ratios
116 reported in Table 2.

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

119 **2.2 The equipment**

120 The bioethanol production from wheat straw and miscanthus have been conducted in a
121 3L reactor (Figure 1) equipped with a torque meter Kistler 4503A measuring torque till
122 a value of 2 Nm and with a data detection frequency variable from 1 to 10 Hz. Data
123 were recollected by LEIRI software reporting them in an Excel file. The reactor was
124 also equipped with a water-heater and with temperature and pH control sensors. The
125 mixing system was an helicoidal impeller properly designed to deal with high DM
126 concentration and high viscosity medium. The helicoidal impeller (Figure 1) had a
127 diameter of 130 mm and is located at 30 mm from the bottom of the reactor.

128 **2.3 Analytical methods**

129 DM of the wheat straw and miscanthus have been determined according to standard
130 methods described in literature (APHA/AWWA/WEB, 1998). DM represented the
131 content of solids present in the substrates, including the inert materials and the
132 degradable ones (Battista et al., 2016b). The apparent density was determined by the use
133 of Archimedes' principle (Zhao et al., 2016).
134 The apparent viscosity of the wheat straw-water and of miscanthus–water mixtures have
135 been determined at 10 and 20% DM *w/w* before the beginning of the enzymatic
136 hydrolysis. The equipment used was the viscometer DV-II-PRO by Brookfield provided
137 with a cross rotating spindle working at 50 rpm.

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

140 Porosity of the substrates was a very important parameter that was directly linked to the
141 absorption capacity of water: obviously, a major grade of porosity favored the
142 absorption of water molecules by substrates. By this way, the amount of water available
143 for the dispersion of the substrates particles decrease, affecting the viscosity of the
144 reaction medium. The grade of porosity and the average volume pore for macroporosity,
145 mesoporosity and microporosity have been evaluated by N_2 adsorption isotherms
146 method. Initially the sample was degassed at 60 °C for 48 h. The average pore volume
147 was obtained using the Horvath-Kawazoe approximation (Horvath and Kawazoe, 1983).
148 The absorption capacity of wheat straw and miscanthus has been evaluated in
149 qualitative way by a simple experiment: 50 g of dry straw and 50 g dry of miscanthus
150 have been located in two different separatory funnels. 100 mL of distilled water have
151 been poured into the funnels at t = 0. The time of the beginning of water percolation
152 from the outlet of the separatory funnels has been measured in order to have qualitative
153 evaluation of the different grade of porosity of both substrates. Also, at the end of
154 percolation, the amount of the recollected water from the funnels has been weighted in
155 order to have an estimation of the adsorption capacity of straw and miscanthus. The
156 average equivalent diameter of the particles of both substrates has been determined by
157 light scattering with a particles size analyzer in a 10 nm–2 mm range.

158 **2.4 Definition of the parameters used for the evaluation of the tests**

159 The evaluation of the performances of the different tests has been realised by three
160 different parameters taking into account the factors affecting all the bio-technological
161 processes. First, a good fluid-dynamic within the reactor was important to ensure
162 sufficient mass and heat transfer and a good contact between the substrates and the
163 enzymes, avoiding inhibition with too high mechanical shear stress of the impeller. The

second factor was mixing energy consumption calculated from torque measurement. Finally, the cellulose conversion was the last factor to evaluate the bioprocess performances. The three parameters used to express these three factors are respectively the mixing time, the energy consumption and the glucose concentration at the beginning and at the end of the enzymatic hydrolysis.

2.4.1 The Mixing Time

Mixing time (tm) is the characteristic parameter used to investigate the performance of stirred tank reactors. Mixing time is defined as the period of time necessary to achieve the desired level of homogeneity in a given vessel (Jafari et al., 2005) and it is often used as an indication of impeller effectiveness. The shorter the mixing time the more effective the blending (Gumienna et al., 2011). The mixing time was determined by the pH pulse method (Tan et al., 2011), (Correa et al., 2016). 10 mL of NaOH (2 N) solution will be put in the reaction medium. Mixing time was estimated as the time required for the pH to reach 95 % of its final value. The determination of the mixing time, has been conducted at the beginning of the tests, when the adjustment of the acid reaction medium is necessary to reach the operative pH value of 5.5, required by the enzymatic hydrolysis. Mixing time has been also evaluated at the end of the hydrolysis, before the discharging of the reactor.

2.4.2 Power input required by the mixing system

The power consumption was determined by means of a torque meter mounted on the shaft of each impeller. Due to the friction factor, the torque generated by the motor (Mm) is not fully transmitted by the impeller to the reaction medium (Wang et al., 2012). The corrected torque value Mc was calculated by subtracting the residual torque from each measurement:

188 $Mc\ (Nm) = Mm - Mr$ /1/

189 where Mm is the measured torque and Mr is the residual torque, in Nm, respectively.
190 Mr is determined by measuring the torque at 50 rpm. The values of Mm were recorded
191 each second by the torque-meter for all the duration of the test (5 h). An average value
192 of Mc has been calculated each 15 minutes (Δt) and used for the following calculation
193 of the power (P) and mixing energy consumptions (E):

194 $P\ (W) = Mc\ 2\pi\ N$ /2/

195 Where N is the rotational speed of the helicoidal impeller, fixed to 50 rpm for all the
196 tests. Finally, the mixing energy consumption is given by the equation:

197 $E\ (J) = \Sigma\ P_i\ \Delta t_i$ /3/

198 Where P_i is the power consumption for the th-esim time range Δt_i of 15 minutes (900 s).

199 ### 2.4.3 Conversion of ligno-cellulosic materials

200 In order to evaluate if the best operative conditions, obtained by the tests, are adequate
201 for the degradation activity of the enzymes, the conversion of the ligno-cellulosic
202 compounds into glucose has been also considered. Thus, the glucose concentration in
203 the reaction medium has been also measured at the beginning and at end of the tests.

204 ## 3. RESULTS AND DISCUSSIONS

205 ### 3.1 Different viscosity between wheat straw and miscanthus

206 <Figure 2>

207 <Figure 3>

208 The tests demonstrated that the enzymatic hydrolysis depends on the substrates used to
209 feed the reactor (Figure 2 and Figure 3): the torque (Mc) was higher for wheat straw
210 than for miscanthus. In addition, the wheat straw has recorded a considerable decreasing
211 of the torque values between the beginning and the end of the tests. On the one hand,
212 the required torque for the reaction medium mixing at 50 rpm dropped from 0.64 Nm to
213 0.22 Nm for the S-B test and to 0.09 Nm for the S-FB50 and S-FB35 tests (Figure 2).
214 On the other hand, the torque in the case of miscanthus tests had a lower reduction: it
215 passed from 0.09 to 0.045 Nm (Figure 3). These different values can be explained by
216 the viscosity. The reaction medium composed by 20% *w/w* DM of wheat straw particles
217 was very viscous, having an apparent viscosity of 200.1 ± 1.7 cP at 10% *w/w* DM and of
218 420.1 ± 3.1 cP at 20% *w/w* DM. The miscanthus presented a gentle fluid-dynamic
219 behavior with low viscosity values of 43.81 ± 4.49 cP and 79.40 ± 4.05 cP at
220 respectively 10% and 20% *w/w* DM. The torque values for miscanthus were only 5-10
221 times higher than torque required by water (about 0.01 Nm) at 50 rpm (Figure 3). The
222 difference in viscosity had an immediate effect on mixing time (Table 3). Mixing time
223 was higher than 50s for the wheat straw and only of 17s for the miscanthus. The high
224 values of DM and the complex polymerization of the lignocellulosic substrates allowed
225 to define the reaction medium as pseudoplastic fluids, whose behavior can be described
226 by models such as power law, Bingham, Cason and Herschel-Bulkley models
227 (Sotaniemi et al., 2016). The remarkable difference between wheat straw and
228 miscanthus viscosity could be due to a different physical structure of the two substrates.
229 Table 1 shows that wheat straw had a higher degree of porosity than miscanthus (73%
230 and 52% respectively) and a higher average diameter value of pores (about 43,100 nm
231 and 16,400 nm respectively). This porosity determined also a different water absorption,

higher for wheat straw than for miscanthus particles. This theory has been confirmed by the experimental results obtained from the measurement of the beginning time of percolation and the amount of the water adsorption: 25 s and 45 mL for the wheat straw and 49 s and 29 mL for the miscanthus. The higher water absorption of straw caused a lower amount of liquid in the reaction medium and consequentially an increase of the viscosity. Therefore, there was a reduction of the dispersion and of the homogenization of wheat straw particles within the reactor. Mondebach and Nokes (2013) explained that water reduces the viscosity of the slurry by increasing the lubricity of the particles. It permits to decrease the required shear stress and consequentially the power input for mixing. The water absorption was mainly due to macroporosity which is twice bigger for wheat straw than for miscanthus (Table 1) (Ros et al., 2013). It is also interesting to observe that the total porosity of the substrates was not given by microporosity and mesoporosity (which was almost 0 mL/g). The different porosity degree also permits to explain the apparent density (Table 1), which was about 430 kg/m^3 for wheat straw and 520 kg/m^3 for miscanthus. It was coherent with the density measured at 0.2 MPa: 0.7 and 0.93 g/mL for wheat straw and miscanthus, respectively.

The analysis for the determination of the average diameter value of the particle, reported in Table 1, permitted to verify that the wheat straw particles are slightly bigger than miscanthus, contributing to the further increasing of the reaction medium's viscosity for the wheat straw.

Finally, the higher viscosity of straw was also due by a different distribution of the three major lignocellulosic components (lignin, hemicellulose and cellulose) (Table 1). In fact, Lewandowska et al., 2016 have reported that miscanthus has a higher content of hemicellulose, the most easily degradable and flexible compounds between ligno-

256 cellulosic materials, and a lower concentration of lignin which gives the property of
257 rigidity to ligno-cellulosic polymers (Table 1).

258 **3.2 Batch and fed-batch tests**

259 The effects of the enzymatic hydrolysis have been summarized in Table 3. All the tests
260 have recorded the reduction of the mixing time. It moved from about 50 s to 40 s for the
261 wheat straw, which means a reduction of about 20%. While, the mixing time values
262 declined from 17 s to 10.5 s for miscanthus, with a reduction of more than 35%.

263 <Table 3>

264 The mixing time reduction was clearly imputable to the decreasing of the reaction
265 medium by the effect of the enzymatic hydrolysis. The enzymes attacked the cellulose
266 permitting a significant structural change in terms of the crystallinity and
267 polymerization degrees (Adani et al., 2011). Consequentially, the mixing inside the
268 reactor was improved with a reduction of the torque transmitted by the motor to the
269 impeller.

270 Figure 3 reports the torque values during time for batch and fed-batch tests with
271 miscanthus substrate. All the tests have followed almost the same decreasing evolution
272 of torque during time, recovering almost the same values, from 0.07 Nm at the
273 beginning of the test (average value for all the miscanthus tests) to 0.045 Nm at the end
274 of the hydrolysis. On the contrary, strong difference in the torque values have been
275 recorded for the wheat straw, where the loading reactor strategy had a great impact
276 (Figure 2). The higher values of torque belonged to the S-B test with an initial value of
277 0.65 Nm. The tests S-FB65, S-FB50 and S-FB35 had an initial torque more and more
278 inferior (0.45, 0.19 and 0.12 Nm respectively, Figure 2) due to the minor mass loaded at

279 the beginning of the test. During the first additions, the benefic effects of hydrolysis
280 were not enough efficient and the viscosity remained high, so that a considerable
281 increasing of the torque has been recorded. By the time, the action of the enzymes
282 allowed a sufficient denaturation of the ligno-cellulosic materials (Adani et al., 2011)
283 and the new substrates additions have been quickly homogenized in the reaction
284 medium. Figure 2 also shows that the reduction of the torque was more efficient for the
285 S-FB50 and S-FB35, whose final values of the torque were around 0.09 Nm, inferior
286 than the final values of torque of about 0.23 Nm for the S-FB65. It suggests that a
287 gradual addition of the substrates had a beneficial effect on the denaturation of the
288 ligno-cellulosic materials, probably due to the high concentration of enzymes (which are
289 fed at the beginning of the test) for mass of substrates initially charged. In general, it has
290 been demonstrated that fed-batch offers advantages in the enzymatic hydrolysis over the
291 batch mode: the initial substrates quantity fed into the reactor is lower, so diffusion and
292 mixing limitations can be minimized. In addition, fed-batch strategy permitted to the
293 enzymes to better liquefy the recalcitrant lignocellulosic materials before additional
294 solids addition (Mondebach and Nokes, 2013).

295 As reported in the previous part, torque is strictly linked to the power demand of the
296 mixing system and to the energy consumption for the reaction medium agitation. For
297 this reason, the lower torque of S-FB50 and S-FB35 implied also energetic and
298 economic advantages. Table 3 reported the energy consumption during the tests. It was
299 evident that a gradual addition of the feeding permitted a considerable saving of
300 electrical energy: the S-B test required over 32.5 kJ, while S-FB35 test required only 9.5
301 kJ, which means an energy reduction of about 60%. Also, all the tests having
302 miscanthus as substrate required between 7 and almost 5 kJ, with a slightly energetic

303 reduction for M-FB50 and M-FB35 tests which required 4.8 kJ against the 7 kJ of the
304 M-B and M-FB65. This little difference for the miscanthus tests can be easily explained
305 by considering the lower viscosity of this substrate for the reasons previously discussed.

306 Regarding the cellulose conversion into glucose, Table 3 reported glucose concentration
307 in the reaction medium at the beginning and at the end of the tests. There is no
308 difference between the tests conducted with the wheat straw and the miscanthus. About
309 19.00 - 19.50 g/L of glucose have been found in wheat straw tests after the hydrolysis
310 stage. A slightly higher concentration of 20.00 – 21.00 g/L has been obtained from
311 miscanthus. It is probably due to the different distribution of the three ligno-cellulosic
312 (lignin, cellulose and hemicellulose) between wheat straw and miscanthus, reported in
313 Table 1. Wheat straw had a major concentration of cellulose, which is the main
314 precursor of glucose production. Miscanthus had a minor lignin which limits the access
315 to cellulose to the enzymes, by its more external position between all the lignocellulosic
316 polymers and by its strong covalent bonds (Elgharbawy et al., 2016). The work of
317 Adani et al. (2011) confirmed that the access of cellulase enzymes to crystalline
318 cellulose was greatly reduced by the presence of some compounds, in particular lignin
319 and other structural proteins. Anyway, as previously reported, a short hydrolysis time of
320 5 h allowed to achieve only a partial conversion of the substrates, and longer times are
321 necessary to complete the cellulose conversion into glucose. Finally, if the fed-batch
322 permitted to have benefic effects on the mixing and consequentially a strong reduction
323 in the energetic consumption, it was irrelevant for the glucose concentration. This result
324 was coherent with the review work of Modenbach and Nokel (2013) which has
325 concluded their analysis asserting that the fed-batch mode has given unclear results in
326 the cellulose conversion into glucose.

3.3 Batch tests with straw-miscanthus mixture

Batch tests (Table 2) have been conducted using a mixture of wheat straw and miscanthus at different concentrations in order to observe when the effect of wheat straw on the viscosity begins to decline. Figure 4 reports the torque values of these batch tests and, for comparisons, the ones of S-B and M-B tests. It has been confirmed that mixing time, torque and energetic consumption of SM-80:20 were still very influenced by the presence of wheat straw, like SB Test. However, when the ratio of miscanthus in the reaction medium reached the 30% *w/w* (SM-70:30), the viscosity dropped down and its behavior was almost the same of M-B test. It means that the content of water absorbed into the internal porosity of the wheat straw is not sufficient to compromise an adequate lubrication of the particles, allowing a good mixing of the reaction medium.

4. CONCLUSIONS

The physical properties influence on the reaction medium viscosity at high DM concentration has been studied. Batch test with straw was characterized by bad mixing and high energy consumption. It was due to the high porosity and to high water absorption of straw which caused an increase of the reaction medium viscosity. The problem can be solved or by the fed-batch mode, which permitted to reduce diffusion and mixing limitations, or preparing a mixture of wheat straw-miscanthus with a minimal ratio of miscanthus of 30% *w/w*. By these ways, it was possible to reduce the mixing time and the energy consumption from 30 to 5 kJ.

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CAPTIONS

Figures

Figure 1. The reactor and the helicoidal impeller used for the enzymatic hydrolysis of wheat straw and miscanthus.

Figure 2. Torque values *vs* time for batch and fed-batch tests having wheat straw as substrate.

Figure 3. Torque values *vs* time for batch and fed-batch tests having miscanthus as substrate.

Figure 4. Torque values *vs* time for batch tests having wheat straw and miscanthus as substrates.

Tables

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

Table 2. Abbreviation and description of the tests.

Table 3. Mixing time, energy consumption and glucose concentration of the tests.

Figure 1

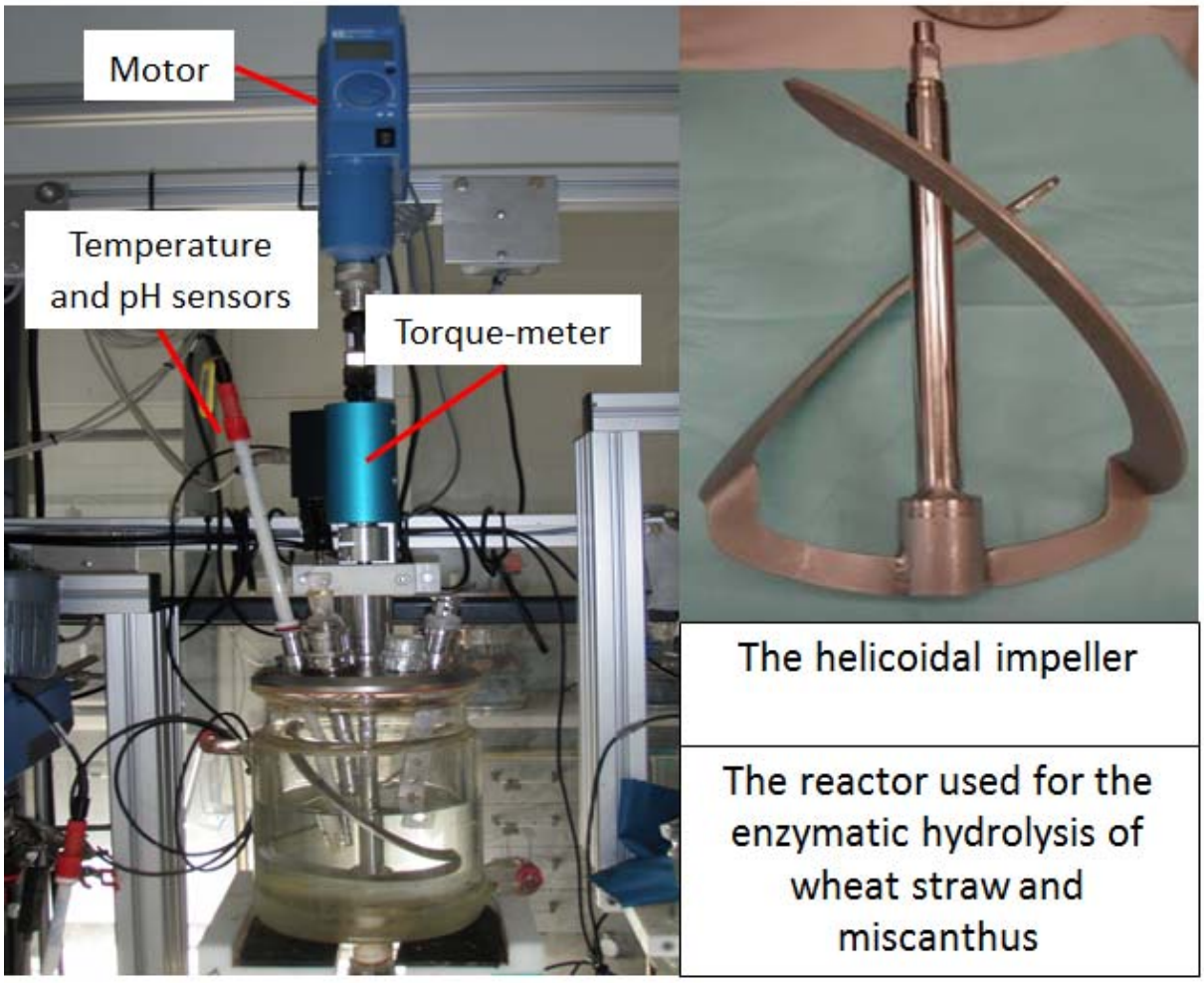
Motor
Temperature and pH sensors
Torque-meter
The helicoidal impeller
The reactor used for the enzymatic hydrolysis of wheat straw and miscanthus

Figure 2

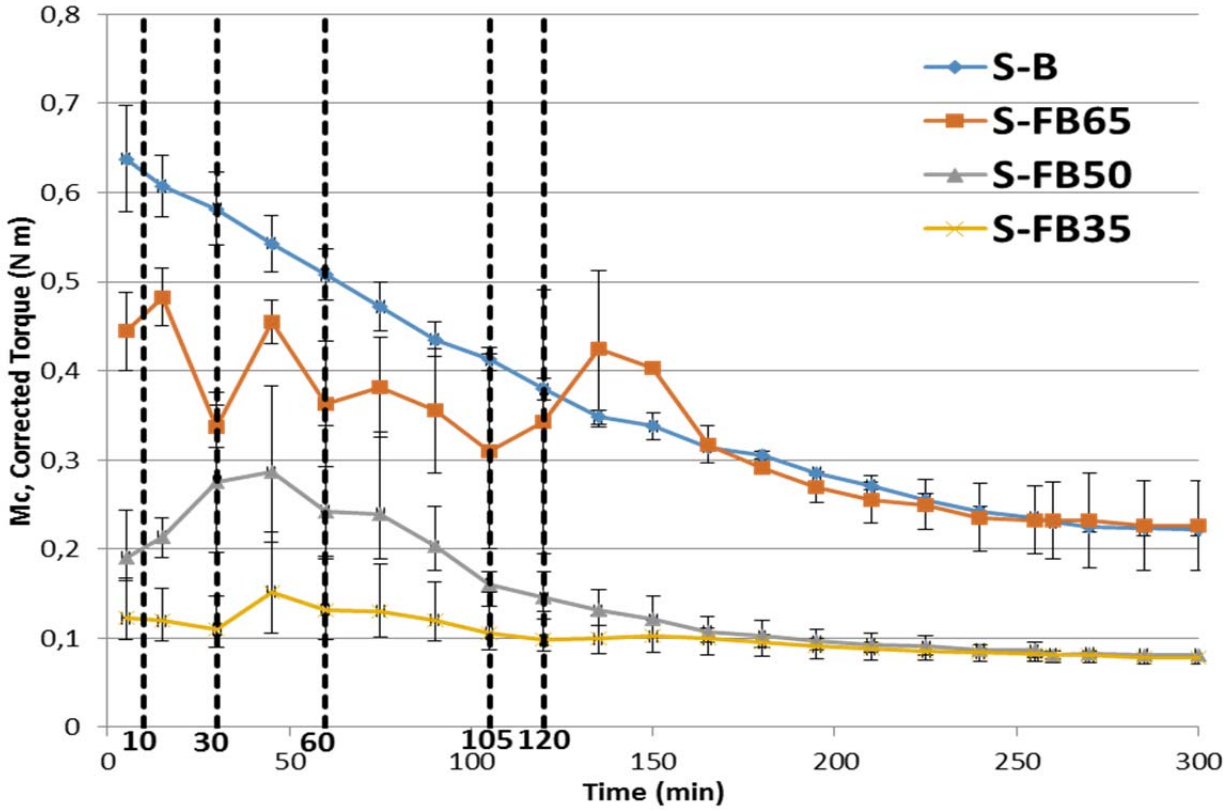
S-B
S-FB65
S-FB50
S-FB35
Mc, Corrected Torque (N m)
Time (min)
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0
50
100
150
200
250
300
10
30
60
105
120

Figure 3

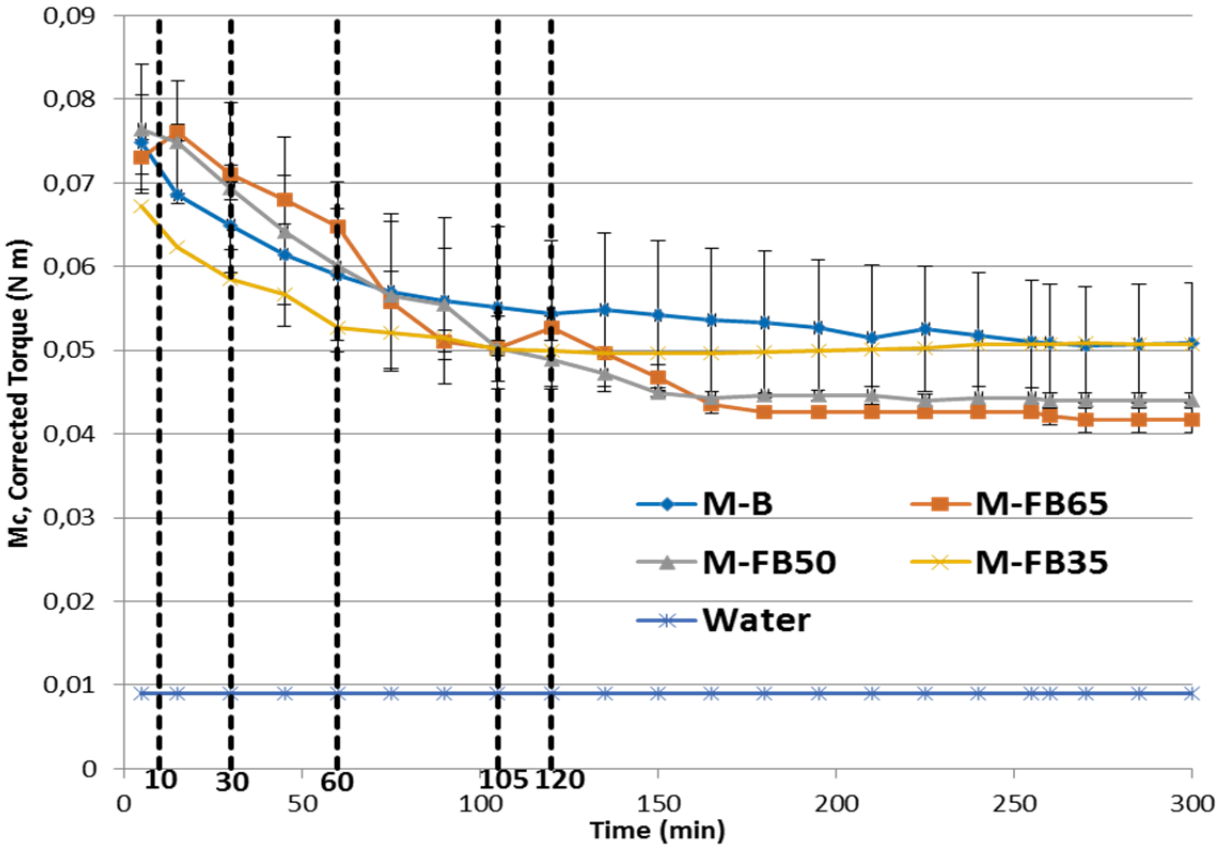
0,09
0,08
0,07
0,06
0,05
0,04
0,03
0,02
0,01
0
Mc, Corrected Torque (N m)
10
30
60
105
120
0
50
100
150
200
250
300
Time (min)
M-B
M-FB65
M-FB50
M-FB35
Water

Figure 4

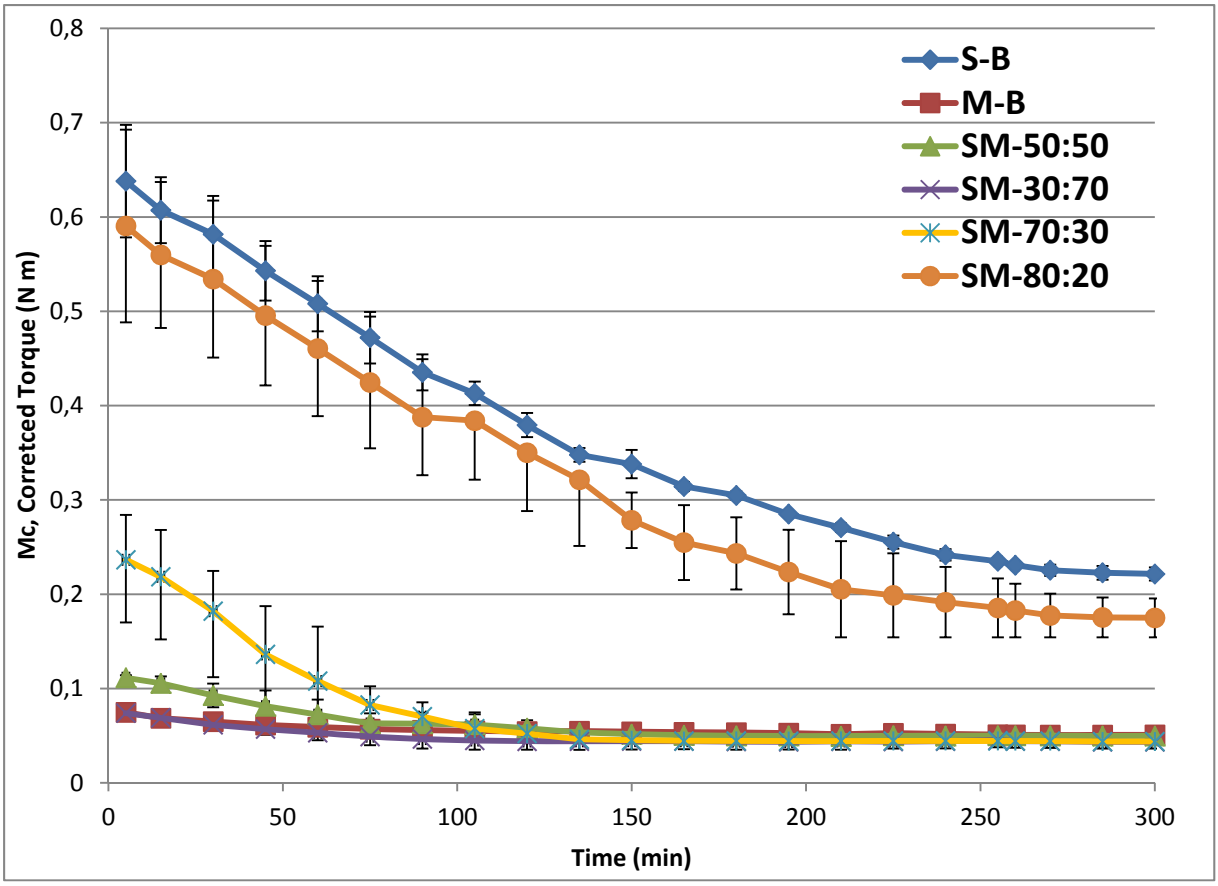
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
Mc, Corretced Torque (N m)
0
50
100
150
200
250
300
Time (min)
S-B
M-B
SM-50:50
SM-30:70
SM-70:30
SM-80:20

Table 1

	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

Table 2

Labels	Description of the tests
S-B	Batch test with wheat straw
S-FB65	Fed batch test with 65% of the total mass of wheat straw loaded at the beginning of the experiment
S-FB50	Fed batch test with 50% of the total mass of wheat straw loaded at the beginning of the experiment
S-FB35	Fed batch test with 35% of the total mass of wheat straw loaded at the beginning of the experiment
M-B	Batch test with miscanthus
M-FB65	Fed batch test with 65% of the total mass of miscanthus loaded at the beginning of the experiment
M-FB50	Fed batch test with 50% of the total mass of miscanthus loaded at the beginning of the experiment
M-FB35	Fed batch test with 35% of the total mass of miscanthus loaded at the beginning of the experiment
SM-80:20	Batch test with a mixture composed by 80% of wheat straw and 20% of miscanthus
SM-70:30	Batch test with a mixture composed by 70% of wheat straw and 30% of miscanthus
SM-50:50	Batch test with a mixture composed by 50% of wheat straw and 50% of miscanthus
SM-30:70	Batch test with a mixture composed by 30% of wheat straw and 70% of miscanthus

Table 3

	Mixing Time (s)	**Energy Consumption (J)**	**Glucose concentration (g/L)**
Water	3.33 ± 0.44	847.12 ± 9.42	xxxxxxxxxx
Wheat Straw at t = 0	53.27 ± 2.10	xxxxxxxxxxxx	4.11 ± 0.15
S-B	41.28 ± 1.81	32,623.21 ± 272.94	18.42 ± 1.23
S-FB65	42.57 ± 1.50	34,105.87 ± 797.05	19.96 ± 0.83
S-FB50	41.20 ± 1.64	13,028.88 ± 1010.21	19.54 ± 1.26
S-FB35	39.20 ± 1.68	9,567.57 ± 1442.52	19.67 ± 1.21
Miscanthus at t = 0	17.20 ± 1.26	xxxxxxxxxxx	3.51 ± 0.18
M-B	11.26 ± 0.84	6,814.98 ± 635.90	21.85 ± 0.25
M-FB65	10.00 ± 2.17	7,287.02 ± 150.41	20.10 ± 1.15
M-FB50	10.42 ± 0.85	4,788.05 ± 263.05	20.11 ± 0.74
M-FB35	10.16 ± 0.57	4,888.74 ± 128.19	20.25 ± 0.85
SM-80:20	38.57 ± 2.37	30,548 ± 542.34	20.21 ± 0.85
SM-70:30	36.50 ± 1.42	7,946.21 ± 124.91	19.81 ± 0.63
SM-50:50	24.50 ± 3.53	5,661.82 ± 476.88	20.87 ± 1.35
SM-30:70	15.50 ± 2.12	4,492.21 ± 85.43	20.57 ± 1.01