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### ► To cite this version:

Federico Battista, Mélanie Gomez Almendros, Romain Rousset, Serge Boivineau, Pierre-Antoine Bouillon. Enzymatic hydrolysis at high dry matter content: The influence of the substrates' physical properties and of loading strategies on mixing and energetic consumption. *Bioresource Technology*, 2018, 250, pp.191 - 196. 10.1016/j.biortech.2017.11.049 . hal-01931421

**HAL Id: hal-01931421**

**<https://ifp.hal.science/hal-01931421>**

Submitted on 22 Nov 2018

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1        **ENZYMATIC HYDROLYSIS AT HIGH DRY MATTER CONTENT: THE**  
2        **INFLUENCE OF THE SUBSTRATES' PHYSICAL PROPERTIES AND OF**  
3        **LOADING STRATEGIES ON MIXING AND ENERGETIC CONSUMPTION**

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9    **ABSTRACT**

10    The present work investigates the impact of the physical properties and loading  
11    strategies of wheat straw and miscanthus on enzymatic hydrolysis at high DM  
12    concentration. Three parameters have been chosen to evaluate the enzymatic hydrolysis  
13    performance: (i) the mixing time, (ii) the energetic mixing consumption and (iii) the  
14    glucose concentration. It was demonstrated that the hydrolysis of miscanthus is easy to  
15    perform and has low viscosity. On the contrary, the higher porosity grade of wheat  
16    straw than miscanthus (73% against 52%) contributed to have a very high viscosity at  
17    20% w/w DM. The development of a fed-batch strategy allowed the reduction of  
18    viscosity inducing the energetic consumption lowering from 30 kJ to 10 kJ. It has been  
19    also proven that the miscanthus addition in wheat straw achieved to decrease mixing  
20    energy consumption at 5-8 kJ, when it represented more than 30% of the total mass of  
21    the reaction medium.

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

## 24 **1. INTRODUCTION**

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

39 Bioethanol production involves four steps: (i) the pretreatments of the substrates, (ii) the  
40 hydrolysis to convert ligno-cellulosic material into glucose, (iii) the fermentation of  
41 glucose in ethanol and (iv) the distillation. The pretreatments of straw and miscanthus  
42 are necessary to optimize the glucose concentration during the hydrolysis and to reduce  
43 the viscosity of the reaction medium (Battista et al., 2016b). The pretreatment stage is  
44 followed by the hydrolysis, often conducted by purified enzymes able to degrade  
45 hemicellulose and cellulose into soluble sugars (Zhou et al., 2008). The enzymatic

46 hydrolysis has currently high yields (75-85%) and improvements are still projected  
47 (85-95%) (Balat, 2011).

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

55 Typical enzymatic hydrolysis of lignocellulosic materials is conducted at low DM  
56 concentration (maximum 5% *w/w*) to ensure a good contact between enzymes and  
57 substrates (Boussaid and Saddler, 1999), (Xue et al., 2012). There are few studies  
58 regarding the enzymatic hydrolysis at high DM concentration. Kristensen et al. (2009)  
59 and Jorgensen et al. (2007) have demonstrated that the conversions of cellulose into  
60 glucose decreases by the increasing of DM concentration. In addition, Cara et al. (2007)  
61 and Battista et al. (2016c) have underlined that at high DM content, the complexity of  
62 the lignocellulosic polymers, causes an increase of the reaction medium viscosity and  
63 consequentially bad mixing within the bioreactor. The mechanism by which cellulases  
64 permit the hydrolysis of cellulose follows three steps: (i) external mass transfer of  
65 enzyme, (ii) diffusion/adsorption of the enzyme on the substrate surface and (iii)  
66 cellulase catalytic action. The overall reaction rate is determined by the rates of these  
67 three events occurring in sequence. If the external mass transfer is neglected (at low DM  
68 content), the overall reaction rate will be controlled by the second step (internal  
69 diffusion). At high DM content, the mixing is not efficient: the external mass transfer

70 controls the overall reaction rate (Corre et al., 2016) and the hydrolysis efficiency is  
71 20% lower than observing at 5% *w/w* DM concentration (Xue et al., 2012).  
72 The aim of this work is the improvement of the enzymatic hydrolysis of wheat straw  
73 and miscanthus at high DM concentration (20% *w/w*), reducing the reaction medium  
74 viscosity. The physical properties influence on viscosity has been observed and  
75 different loading strategies of batch and fed-batch have been tested on straw, on  
76 miscanthus and on a combination of both substrates. The performances of the tests have  
77 been evaluated taking into account the most important factors affecting the  
78 bioprocesses: (i) mixing time, (ii) mixing energetic consumption and (iii) the glucose  
79 concentration contained in the reaction medium at the beginning and at the end of the  
80 hydrolysis phase.

## 81 **2. MATERIALS AND METHODS**

### 82 **2.1 Substrates, enzymatic cocktail characteristic and description of the tests**

83 The substrates used for the tests were wheat straw and miscanthus, pretreated at  
84 appropriate operative conditions (data not shown). Table 1 summarises the physical and  
85 chemical features of the pretreated wheat straw and the pretreated miscanthus. The  
86 features of raw substrates were not available. Zhang et al. (2012) founded that soil and  
87 climate conditions influence the raw substrates porosity, which can vary in a very range:  
88 45-85%. This demonstrates that the results obtained by this work are not dependent on  
89 the substrates conditions (raw or pretreated). The content of lignin, hemicellulose and  
90 cellulose of both substrates have been determined by an external company which  
91 supplied the substrates (Table 1).

92 <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<sub>2</sub> 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



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

#### 169 **2.4.1 The Mixing Time**

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

#### 182 **2.4.2 Power input required by the mixing system**

183 The power consumption was determined by means of a torque meter mounted on the  
184 shaft of each impeller. Due to the friction factor, the torque generated by the motor  
185 ( $M_m$ ) is not fully transmitted by the impeller to the reaction medium (Wang et al.,  
186 2012). The corrected torque value  $M_c$  was calculated by subtracting the residual torque  
187 from each measurement:

188  $M_c \text{ (Nm)} = M_m - M_r$  /1/

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

194  $P \text{ (W)} = M_c 2\pi N$  /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  $E \text{ (J)} = \sum P_i \Delta t_i$  /3/

198 Where  $P_i$  is the power consumption for the  $i$ -th time range  $\Delta t_i$  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  $200.1 \pm 1.7$  cP at 10% w/w DM and of  
218  $420.1 \pm 3.1$  cP at 20% w/w DM. The miscanthus presented a gentle fluid-dynamic  
219 behavior with low viscosity values of  $43.81 \pm 4.49$  cP and  $79.40 \pm 4.05$  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,

232 higher for wheat straw than for miscanthus particles. This theory has been confirmed by  
233 the experimental results obtained from the measurement of the beginning time of  
234 percolation and the amount of the water adsorption: 25 s and 45 mL for the wheat straw  
235 and 49 s and 29 mL for the miscanthus. The higher water absorption of straw caused a  
236 lower amount of liquid in the reaction medium and consequentially an increase of the  
237 viscosity. Therefore, there was a reduction of the dispersion and of the homogenization  
238 of wheat straw particles within the reactor. Mondebach and Nokes (2013) explained that  
239 water reduces the viscosity of the slurry by increasing the lubricity of the particles. It  
240 permits to decrease the required shear stress and consequentially the power input for  
241 mixing. The water absorption was mainly due to macroporosity which is twice bigger  
242 for wheat straw than for miscanthus (Table 1) (Ros et al., 2013). It is also interesting to  
243 observe that the total porosity of the substrates was not given by microporosity and  
244 mesoporosity (which was almost 0 mL/g). The different porosity degree also permits to  
245 explain the apparent density (Table 1), which was about 430 kg/m<sup>3</sup> for wheat straw and  
246 520 kg/m<sup>3</sup> for miscanthus. It was coherent with the density measured at 0.2 MPa: 0.7  
247 and 0.93 g/mL for wheat straw and miscanthus, respectively.

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

252 Finally, the higher viscosity of straw was also due by a different distribution of the three  
253 major lignocellulosic components (lignin, hemicellulose and cellulose) (Table 1). In  
254 fact, Lewandowska et al., 2016 have reported that miscanthus has a higher content of  
255 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 beneficial 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.

### 327 **3.3 Batch tests with straw-miscanthus mixture**

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

### 339 **4. CONCLUSIONS**

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

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438

439 **CAPTIONS**

440 **Figures**

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

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

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

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

449 **Tables**

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

451 Table 2. Abbreviation and description of the tests.

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

**Figure 1**

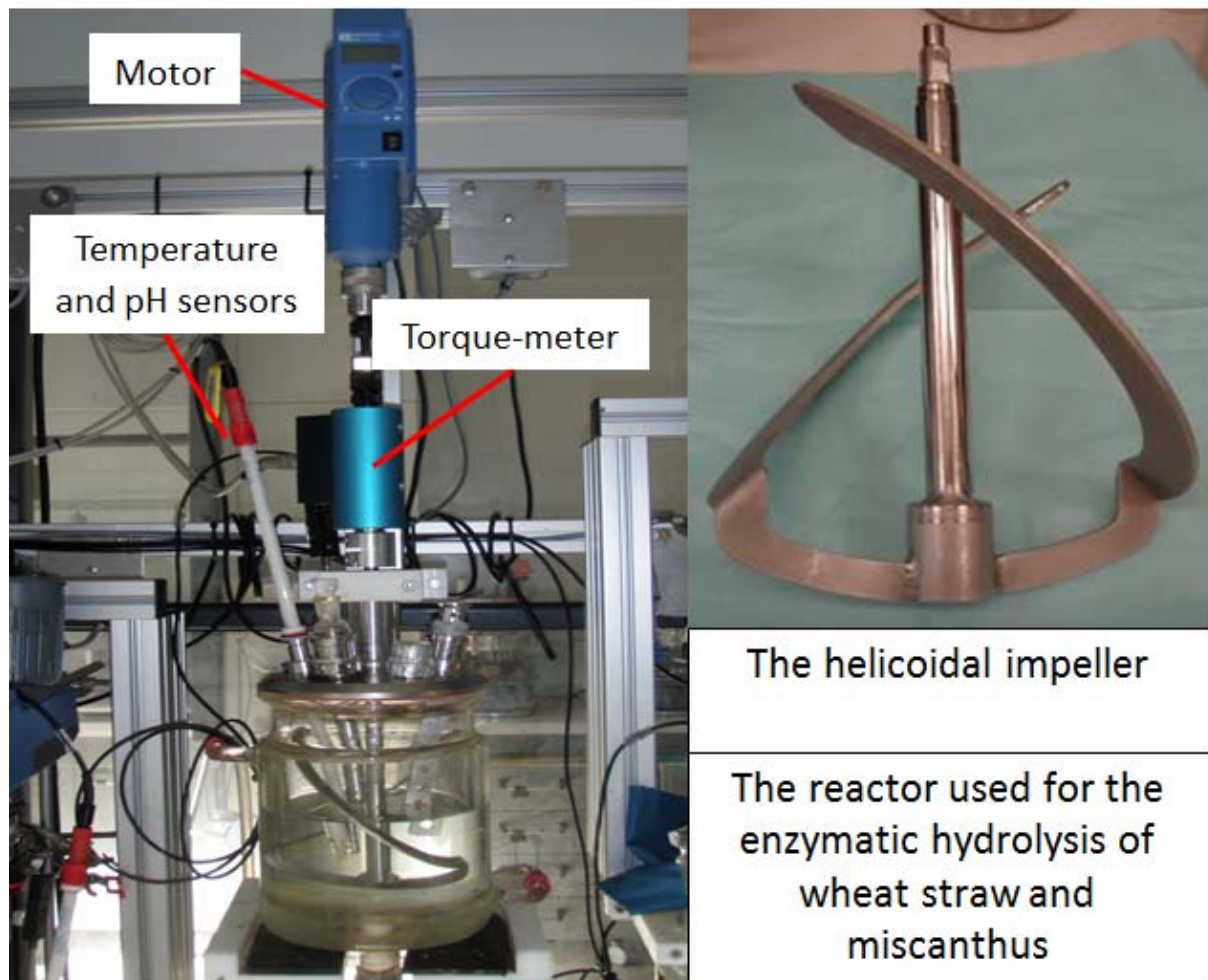


Figure 2

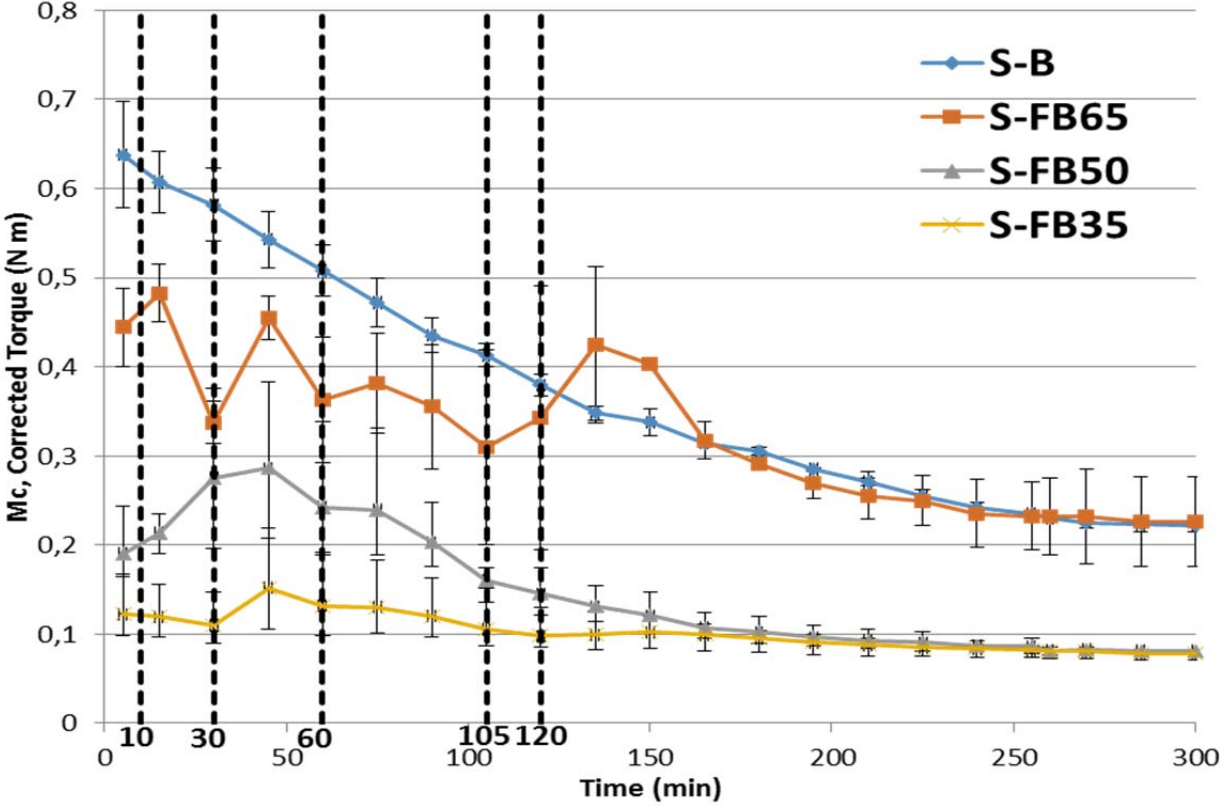


Figure 3

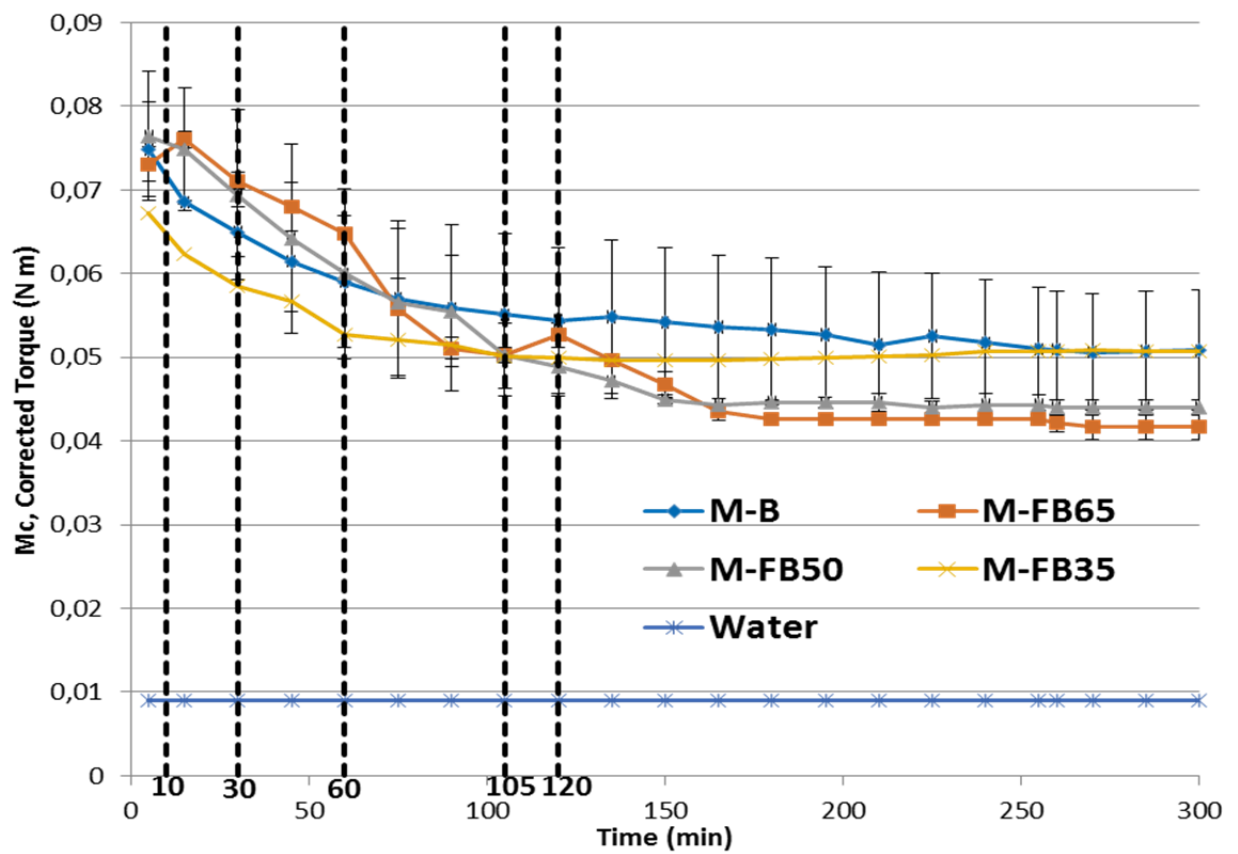
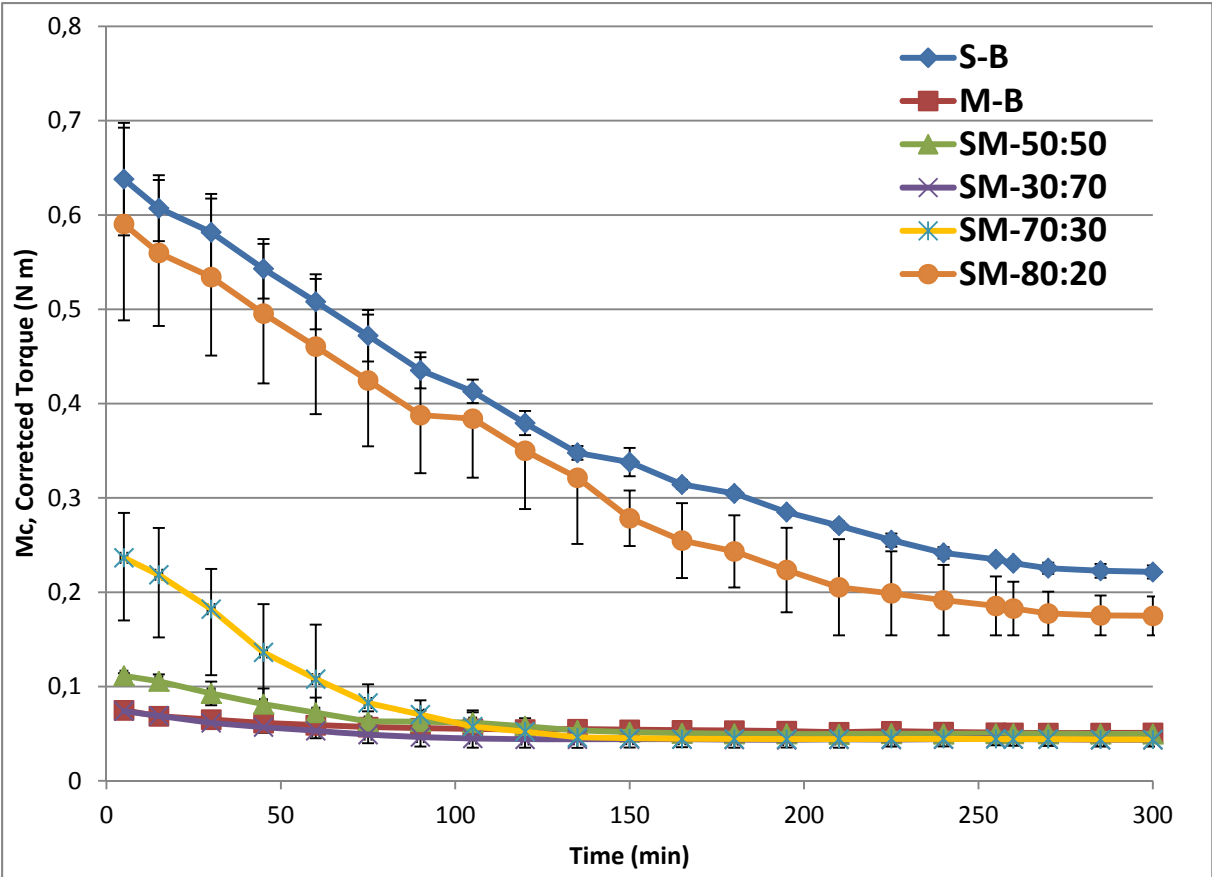




Figure 4



**Table 1**

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

**Table 2**

<b>Labels</b>	<b>Description of the tests</b>
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**

	<b>Mixing Time (s)</b>	<b>Energy Consumption (J)</b>	<b>Glucose concentration (g/L)</b>
Water	3.33 ± 0.44	847.12 ± 9.42	xxxxxxxxxxx
Wheat Straw at t = 0	53.27 ± 2.10	xxxxxxxxxxxxx	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	xxxxxxxxxxxxx	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