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de Assis Castro, Rafael Cunha; Mussatto, Solange I.; Conceicao Roberto, Inês

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1 **A vertical ball mill as a new reactor design for biomass**
2 **hydrolysis and fermentation process**

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4 **Rafael Cunha de Assis Castro¹, Solange I. Mussatto², Inês Conceição Roberto^{1,*}**

5
6 *¹ Departamento de Biotecnologia, Escola de Engenharia de Lorena, Universidade de*
7 *São Paulo, CEP:12602-810, Lorena, São Paulo, Brazil.*

8 *² Novo Nordisk Foundation Center for Biosustainability, Technical University of*
9 *Denmark, Kemitorvet, Building 220, 2800, Kongens Lyngby, Denmark.*

10
11
12 ***Corresponding author:** Inês Conceição Roberto, Departamento de Biotecnologia,
13 Escola de Engenharia de Lorena, Universidade de São Paulo, Estrada Municipal do
14 Campinho, s/nº, CEP: 12.602-810, Lorena - São Paulo, Brazil. Tel: (+55) 12-3159-
15 5026. E-mail: ines@debiq.eel.usp.br

24 **Abstract**

25

26 A vertical ball mill (VBM) reactor was evaluated for use in biomass conversion
27 processes. The effects of agitation speed (100-200 rpm), number of glass spheres (0-30
28 units) and temperature (40-46 °C) on enzymatic hydrolysis of rice straw and on glucose
29 fermentation by a thermotolerant *Kluyveromyces marxianus* strain were separately
30 studied. The results revealed an important role of the spheres during biomass' fiber
31 liquefaction and yeast's fermentative performance. For hydrolysis, the spheres were the
32 only variable with significant positive impact on cellulose conversion, while for
33 fermentation all the variables have influenced the ethanol volumetric productivity (Q_P).
34 For Q_P , the spheres showed an interactive effect with temperature, being obtained a
35 maximum of 2.16 g/L.h when both variables were used in the lowest level. By applying
36 the needed adjustments on the levels of the variables for each process (hydrolysis and
37 fermentation), the VBM reactor could be efficiently used for biomass conversion into
38 ethanol.

39

40 **Keywords:** Non-conventional reactor; Rice straw; Enzymatic hydrolysis; Ethanol;
41 *Kluyveromyces marxianus*

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48 **1. Introduction**

49

50 The use of lignocellulosic materials as feedstocks to produce fuels, power,
51 materials and chemicals is a promising and sustainable alternative to petroleum-based
52 platform. Lignocellulose is the fibrous part of plant materials, mainly composed of
53 cellulose, hemicellulose and lignin in a highly organized structure that makes the plant
54 biomass recalcitrant to physical, chemical and microbial attack [1]. Among the
55 lignocellulosic raw materials, rice straw (the stalk of the plant that is left over on the
56 field upon harvesting of the rice grain) is one of the main agricultural residues
57 worldwide, with an estimated availability of 685 million tons per year [2].

58 The conversion of polysaccharides from lignocellulosic materials into ethanol by
59 the biochemical route is performed in three steps: 1) biomass pretreatment to make
60 polysaccharides more accessible to further hydrolysis; 2) hydrolysis of polysaccharides
61 into monosaccharides by hydrolytic enzymes, and 3) fermentation of the obtained
62 sugars into ethanol [3]. However, there are still some aspects to be enhanced in order to
63 reach a more economically competitive technology, such as the slow rate of cellulose
64 enzymatic hydrolysis, low fermentation yield and productivity, the costs of the enzymes
65 and high-energy requirements [4]. To solve these issues, several efforts have been
66 carried out considering crops management, pretreatment methods, hydrolytic enzymes,
67 microorganisms, and bioreactor systems [5].

68 Regarding the pretreatment step, a variety of methods has been reported in the
69 literature with different specificities on altering the physical and chemical structure of
70 the lignocellulosic materials [6]. Considering the biorefinery approach, the pretreatment
71 technology must focus on biomass fractionation, not only improving the subsequent

72 hydrolysis of cellulose, but also providing separation of the main constituents of
73 lignocellulosic biomass. In this way, each individual main component of biomass may
74 be handled toward different categories of products [7]. Nevertheless, depending on the
75 type of pretreatment and conditions employed, some biomass components can be lost
76 during this process [8]. The loss of these components, especially the polysaccharide
77 ones, must be avoided in order to increase the process efficiency. Recently, we have
78 proposed a two-steps pretreatment that improved the ethanol production from both
79 cellulose and hemicellulose fractions of rice straw [9]. This two-steps process consists
80 in applying a mild alkaline pretreatment to remove acetyl groups from the biomass
81 structure, prior to dilute acid hydrolysis to produce a hemicellulosic hydrolysate with
82 lower toxicity degree, thus obtaining a pretreated cellulose-rich solid (cellulignin) with
83 minimal loss of polysaccharide fractions.

84 In order to obtain soluble glucose from the cellulose fraction, the pretreated solid
85 must be submitted to an enzymatic hydrolysis step by the action of cellulases. However,
86 the heterogeneous nature of the biomass fibers creates rheological complexities,
87 hindering the mass transfer rate in the substrate matrix and limiting the cellulose
88 conversion [10]. In addition, as only a limited amount of free water is present when the
89 process is performed at high solids content, a much longer time is required to liquefy
90 the matrix to attain an effective hydrolysis [11]. Therefore, the reactor design plays an
91 important role to achieve an effective bioconversion process. In this sense, non-
92 conventional reactors with novel design and stirring modes have been suggested as a
93 possibility to overcome some of the mixing problems related to insoluble solids
94 liquefaction, as recently reviewed by Liguori et al. [5].

95 Within this context, this work aimed to evaluate the enzymatic hydrolysis of rice
96 straw in a non-conventional reactor, named a Vertical Ball Mill (VBM) reactor, as well
97 as to study the use of this reactor for ethanol production by fermentation using semi-
98 defined glucose medium. The effects of operational conditions including agitation
99 speed, number of glass spheres and temperature were investigated on each process. The
100 novelty of this research lies in the use of this new reactor design, regarding a conceptual
101 impeller type in combination with a grinding element. This study represents an initial
102 approach to estimate the efficiency of the VBM reactor for use in future SSF processes.

104 **2. Materials and methods**

106 **2.1. Feedstock and pretreatment**

107 Rice straw was collected from fields in the region of Canas, São Paulo state,
108 Brazil. The material was dried until approximately 10% moisture content, hammer-
109 milled to attain particles of about 1 cm in length and 1 mm in thickness, and stored until
110 treatment. Milled rice straw was submitted to a two-steps pretreatment as previously
111 defined by Castro et al. [9]. Firstly, the material was deacetylated employing NaOH
112 solution with a loading of 80 mg NaOH/g of biomass, using a solid:liquid ratio of 1:10,
113 at 70 °C for 45 min. After washing, the deacetylated solid material was pretreated by
114 dilute acid hydrolysis using 100 mg H₂SO₄/g deacetylated rice straw, a solid:liquid ratio
115 of 1:10, at 121 °C for 85 min. The resulting solid (referred as deacetylated cellulignin)
116 was washed and dried until 10% moisture content. The composition of the raw and
117 pretreated material was determined according to NREL-LAP standard protocol [12], as
118 shown in **Table 1**.

119

Table 1

120

121 2.2. *Enzymes and microorganism*

122 Cellulase from *Trichoderma reesei* (Cellubrix, Novozymes Corp.) with an activity
123 of 30 FPU/mL was used for enzymatic hydrolysis. Additional β -glucosidase produced
124 from *Aspergillus niger* (Novozyme 188, Novozymes Corp.) with an activity of 920
125 IU/mL was also added to the experiments to enhance the cellulose conversion to
126 glucose.

127 The thermotolerant yeast *Kluyveromyces marxianus* NRRL Y-6860 was used for
128 fermentation. For inoculum preparation, cells from malt extract agar slants were
129 cultivated in Erlenmeyer flasks containing semi-defined medium with the following
130 composition (g/L): 30.0 glucose, 1.5 KH_2PO_4 , 1.0 $(\text{NH}_4)_2\text{SO}_4$, 0.1 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and
131 3.0 yeast extract. The inoculum was cultivated in an orbital shaker at 40 °C, 200 rpm for
132 16 h. After this time, the cells were recovered by centrifugation (2500 \times g, 10 min),
133 washed twice in sterile distilled water, and resuspended in the fermentation medium to
134 obtain the desired initial cell concentration (1.0 g/L).

135

136 2.3. *Vertical Ball Mill (VBM) reactor set-up*

137 A 1.5-L VBM reactor (120 mm inner diameter) made of 316 stainless steel and
138 jacketed for temperature control by water recirculation using an external thermostatic
139 water bath was used for the experiments. This reactor was equipped with three flat
140 round plates impellers of 94 mm diameter, which were positioned at a distance of 28
141 mm each other, as shown in **Fig. 1**. Above each plate, glass spheres (23 mm diameter

142 and 8.16 ± 0.35 g each) were placed as grinding elements. The impeller was rotated by
143 an electric motor (IKA RW 20) able to operate from 60 to 2000 rpm.

144 **Figure 1**

145

146 ***2.4. Enzymatic hydrolysis of pretreated rice straw in the VBM reactor***

147 A 2^3 full-factorial experimental design, composed by 11 independent assays, was
148 used to evaluate the effects of the following operational variables on enzymatic
149 hydrolysis of pretreated rice straw in the VBM reactor: agitation (100 to 200 rpm),
150 number of glass spheres (0 to 30) and temperature (40 to 46 °C). The experimental error
151 was estimated by the three central points to give important information on the
152 reproducibility of the experiments, which was considered into statistical analysis of
153 significance. All the experiments were conducted using 8% (w/v) solid loading (40 g
154 dry mass in 0.5 L final volume), 50 mM sodium citrate buffer (pH 4.8), 21.5 FPU
155 cellulase/g cellulose and final β -glucosidase loading of 64.5 IU/g cellulose. The enzyme
156 solution was added after reaching the desired temperature, from which the reaction has
157 begun. Samples were taken at appropriate times to estimate glucose in order to assess
158 the kinetics of enzymatic hydrolysis in each evaluated condition, until 24 h process.
159 Cellulose conversion (CC) was the response considered for these experiments. CC was
160 calculated according to Eq. 1 where [G] is the glucose concentration in the supernatant
161 of the slurry (in grams per liter), F_C is the fraction of cellulose in the substrate (in gram
162 per gram), and T_S is the initial solids content (in grams per liter).

163

$$164 \quad CC(\%) = \frac{[G] \times 0.9}{F_C \times T_S} \times 100 \quad (\text{Eq.1})$$

165

166 **2.5. Ethanol production from semi-defined medium in the VBM reactor**

167 The same 2^3 full-factorial experimental design, composed by 11 independent
168 assays, was also used to evaluate the effects of the same operational variables levels on
169 ethanol production in the VBM reactor. In the same way, the experimental error was
170 estimated by three central point replicates. All assays were carried out using a semi-
171 defined medium composed of (g/L): 50.0 glucose, 1.5 KH_2PO_4 , 1.0 $(\text{NH}_4)_2\text{SO}_4$, 0.1
172 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 3.0 yeast extract, in 0.5 L total final volume and employing 1 g/L
173 initial cell concentration of *K. marxianus* NRRL Y-6860, added after reaching the
174 desired temperature, from which the reaction has begun. Samples were periodically
175 taken until 24 h to measure biomass, glucose and ethanol concentrations. The ethanol
176 yield ($Y_{P/S}$, g/g), determined by ratio between ethanol produced and glucose consumed,
177 and ethanol volumetric productivity (Q_P , g/L.h), calculated by the ratio between the
178 maximum ethanol concentration and the respective fermentation time, were the
179 responses considered for these experiments.

180

181 **2.6. Analyses**

182 Biomass concentration was determined from the cell optical density (OD) at 600
183 nm measured in a UV-Vis Spectrophotometer (Genesys 10S, Thermo Fischer Scientific)
184 and converted to cell concentration using a suitable calibration curve $\text{OD} \times \text{dry weight}$.
185 Glucose and ethanol concentrations were quantified by HPLC using a refractive index
186 detector (Agilent Technologies 1260 Infinity), a Bio-Rad Aminex HPX-87H column
187 (Bio-Rad, Hercules, CA, USA) at 45 °C, and sulfuric acid (0.005 M) as the mobile
188 phase in a flow rate of 0.6 mL/min.

189

190 3. Results and discussion

191

192 *3.1. Effect of operational conditions on enzymatic hydrolysis and fermentation* 193 *process*

194 The results obtained for enzymatic hydrolysis of pretreated rice straw and glucose
195 fermentation from semi-defined medium using the VBM reactor and the different
196 operational conditions are shown in **Table 2**.

197

Table 2

198 *3.1.1. Enzymatic hydrolysis*

199 As can be seen in **Table 2**, the cellulose conversion of pretreated rice straw after
200 24 h varied from 68 to 87%. The highest results (84-87%) were obtained in the assays 7
201 and 8, both using 30 spheres at 46 °C. On the other hand, the lowest values were
202 observed in the assays 1 and 2, which were conducted without spheres and under the
203 lowest temperature (40 °C), regardless of agitation speed. These results suggest that the
204 agitation speed has a minor impact on enzymatic hydrolysis of rice straw in VBM
205 reactor, being 100 rpm enough to perform this process; whereas increasing the number
206 of spheres and temperature have favored the cellulose conversion. This behavior
207 supports the hypothesis that the spheres can act as effective grinding agents, by causing
208 mechanical stress on biomass's fiber, which in turn improved the superficial contact
209 between enzyme and substrate. In general, mass and heat transfer problems stand up as
210 an important drawback in the bioconversion process, especially on the enzymatic
211 hydrolysis step [13]. Samaniuk et al. [14] verified a synergistic relationship between
212 mixing and enzyme activity during enzymatic hydrolysis. According to the authors, in
213 mixed systems, the enzyme distribution is improved and the particle surface area rapidly
214 decreases during the hydrolysis that in turn reduces the mass transfer limitations.

215 Some authors have described new reactor configurations and agitation systems in
216 order to improve the enzymatic hydrolysis of lignocellulosic materials. For example,
217 Kadić et al. [15] investigated the effect of agitation rate on enzymatic hydrolysis of
218 steam pretreated *Arundo donax* and spruce in reactor equipped with a pitched-blade
219 impeller with three blades at an angle of 45°. The authors observed an improvement in
220 the hydrolysis rate from 20 to 37% using spruce (13% w/w) when the impeller speed
221 was increased from 100 to 600 rpm. Such improvement was related to the reduction of
222 particle size, which increased the hydrolysable surface area. Another example was
223 reported by Du et al. [16] who compared the enzymatic hydrolysis of sulfuric
224 acid/steam pretreated corn stover employing two different reactor systems, the
225 horizontal rotating bioreactor (HRR) and the vertical stirred-tank reactor (VSTR),
226 equipped with a double helical ribbon impeller. The authors reported that HRR's
227 performance on biomass saccharification at 25% (w/w) was about 18% higher than that
228 of VSTR.

229 A combined strategy of simultaneous ball milling and enzymatic hydrolysis was
230 evaluated by Mais et al. [17] using a 1.1-L ball-mill reactor and small porcelain beads as
231 grinding elements. According to these authors, increasing the numbers of beads present
232 in the reaction vessel improved the efficacy of hydrolysis conversion of α -cellulose at 5
233 % (w/v). The conversion yields after 48 h were 67, 66, and 73% with 0, 50, and 100
234 beads, respectively.

235 The results of the present study on enzymatic hydrolysis of deacetylated rice straw
236 cellulignin in the VBM reactor are also promising and represent a significant
237 improvement in process efficiency. For example, under the conditions of assay 7 (100
238 rpm, 30 spheres, 46 °C) a cellulose conversion of 87% was achieved at 24 h, while

239 under the same process conditions but in the absence of spheres (assay 5), the cellulose
240 conversion was reduced to 73.4%. These results are also better when compared to a
241 previous study performed in shake flasks [18], which resulted in 79.2% conversion at 48
242 h, employing the same solids content and enzyme loading. **Fig. 2** shows the kinetic
243 profile of enzymatic hydrolysis performed in both experiments. As can be seen, the
244 cellulose conversion rate was mainly enhanced in the first 24 h of process, reaching
245 10% improvement when using the VBM reactor and the conditions of assay 7 (100 rpm,
246 30 spheres and 46 °C). Besides reducing the hydrolysis time in 24 h, improving the
247 cellulose conversion in approx. 10% is relevant from the economical point of view,
248 since the literature has reported a great impact of this step on second-generation ethanol
249 production costs [19].

250 **Figure 2**

251 The present results demonstrate that the new VBM reactor used in this study can
252 be efficiently employed for saccharification of lignocellulosic raw materials.

253 254 **3.1.2. Fermentation process**

255 The effects of the same operational conditions previously studied in the VBM
256 reactor for enzymatic hydrolysis were also evaluated on ethanol production from
257 glucose using the yeast *K. marxianus*. As can be seen in **Table 2**, in the studied range of
258 values, $Y_{P/S}$ showed a little variation (from 0.38 to 0.44 g/g), whereas Q_P showed a more
259 significant variation (from 0.74 to 2.16 g/L.h). *K. marxianus* was able to convert
260 glucose into ethanol with high efficiency (80% in average) even at the highest
261 temperature (assays 5-8), regardless of the agitation speed and number of spheres, thus
262 confirming its thermotolerant characteristic. Glucose consumption was also higher than
263 81% for all the experiments. On the other hand, cultivation at the highest temperature

264 resulted in the lowest cell growth (< 1.5 g/L) and ethanol volumetric productivity (< 0.9
265 g/L.h).

266 It is interesting to note in **Table 2** that the conditions of the assay 7, which
267 provided the highest cellulose conversion by enzymatic hydrolysis (87%), resulted in
268 the lowest value of Q_p (0.74 g/L.h). Such results indicate that the conditions that
269 enhanced enzymatic hydrolysis were different from those that benefited the
270 fermentative process in the VBM reactor. In order to better understand the effects of the
271 process variables on both processes (hydrolysis and fermentation), a statistical analysis
272 of the data was performed, as follows.

273

274 **3.2. Statistical analysis**

275 Pareto's charts (a graphical representation of Student's t -test) representing the
276 estimated effects and interaction of the independent variables on the evaluated
277 responses are shown in **Fig. 3**. In these charts, the bars beyond the vertical line
278 correspond to effects significant at $p < 0.05$.

279

Figure 3

280 For cellulose conversion (**Fig. 3A**), the number of spheres was the only variable
281 with a significant individual effect, which was positive suggesting that increasing the
282 number of spheres improved the efficiency of hydrolysis. Regarding the fermentation
283 process, the three studied variables (agitation, number of spheres and temperature)
284 presented effects significant at 95% confidence level on Q_p (**Fig. 3B**), while none of
285 them had a significant effect on $Y_{P/S}$ (**Fig. 3C**). These results suggest that the ability of
286 the yeast to convert glucose into ethanol was not affected by varying the process

287 conditions, but the conversion rate was strongly dependent on the level of the variables
288 employed for fermentation.

289 Besides the individual effects, two interactions were also significant for the
290 response Q_P (**Fig. 3C**). The interaction between agitation speed and temperature had a
291 negative effect ($X_1 \cdot X_3 = -4.22$) on this response, suggesting that Q_P is positively
292 impacted by decreasing the temperature and increasing the agitation speed. In addition,
293 the temperature showed an interaction effect with the number of spheres, but with a
294 positive signal ($X_2 \cdot X_3 = +3.37$), indicating that Q_P increases in the conditions of lower
295 temperature and number of spheres. It is worth mentioning that Q_P was increased in
296 about 3-fold when the agitation speed was increased from 100 to 200 rpm and the
297 temperature was reduced from 46 to 40 °C, in the absence of glass spheres.

298 A multiple regression analysis of the results was performed in order to obtain
299 mathematical models explaining the variation of both responses as a function of the
300 operational variables. Linear models were adjusted with R^2 equal to 0.84 for cellulose
301 conversion and 0.98 for Q_P , which explain 84 and 98% of the total variation in the
302 responses, respectively (**Table 3**).

303 **Table 3**

304 Contour surfaces plotted for the evaluated responses according to the previous
305 established models (**Fig. 4**) clearly show that the enzymatic hydrolysis and fermentation
306 processes are maximized in different regions. The effect of the spheres was the most
307 important influencing in such opposite behaviors. The use of glass spheres in the VBM
308 reactor improved the cellulose conversion during the enzymatic hydrolysis of pretreated
309 rice straw. However, the presence of spheres decreased the ethanol productivity during
310 the fermentation step. The positive effect of the spheres on hydrolysis could be

311 attributed to two types of phenomena: 1) shear stress due to impacts of the spheres on
312 lignocellulosic fibers and/or 2) increased mass transfer due to the generation of a more
313 homogeneous mixture during the hydrolysis. On the other hand, the negative effect of
314 the spheres on fermentation performance could be explained by possible viability losses
315 of the cells because of the shear stress generated.

316 **Figure 4**

317 **Fig. 5** shows the kinetic profile of fermentation process performed in the VBM
318 reactor compared with that observed in the shake flasks experiments previously reported
319 by Ref. [18]. As can be seen, experiments in the VBM reactor under the conditions of
320 assay 2 showed ethanol concentration similar to that obtained in shake flasks (20.9 and
321 20.1 g/L, respectively). However, a longer time was required to obtain this ethanol titer
322 in the VBM reactor, thus leading to a lower ethanol volumetric productivity.

323 **Figure 5**

324 **4. Conclusions**

325

326 The results of the present study indicate that the VBM reactor significantly
327 improved the saccharification of alkali-acid-pretreated rice straw. The glass beads added
328 to the VBM reactor was the main factor affecting both processes, enzymatic hydrolysis
329 and fermentation, with a positive effect on cellulose conversion and a negative effect on
330 ethanol volumetric productivity. Therefore, by applying the needed adjustments on the
331 levels of the variables for each process (hydrolysis and fermentation), the VBM reactor
332 could be efficiently used for biomass conversion in ethanol, presenting also potential for
333 use in SSF process, for example. Future studies would be useful in order to better
334 understand the fluid dynamics involved in VBM reactor, especially when operating with

335 high solids content. Such information, together with the results of the present work, will
336 represent a step forward towards the development of the market in this sector.

337

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344

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Figure Captions

Figure 1. Image of the Vertical Ball Mill (VBM) reactor (A). Illustration of inside details (B): inlet (1) and outlet (2) water for temperature control; sampling duct (3); port for addition of reaction components (4); gases outlet port (5); agitation rotor (6); flat round impellers with spheres (7). Image (C) and illustration (D) of impellers.

Figure 2. Kinetic profile of cellulose conversion from pretreated rice straw biomass using the VBM reactor (present study) and shake flasks experiments from Castro and Roberto (2014).

Figure 3. Pareto's charts for the effects of agitation (X_1), number of spheres (X_2), temperature (X_3) and their interactions on cellulose conversion by enzymatic hydrolysis, CC (A), ethanol volumetric productivity, Q_p (B) and ethanol yield, $Y_{p/S}$ (C) during fermentation using the VBM reactor.

Figure 4. Contour surfaces plotted according to the models representing (A) cellulose conversion and (B) ethanol volumetric productivity. The agitation speed was set at 150 rpm for both responses.

Figure 5. Ethanol production from glucose fermentation using the VBM reactor (present study) and shake flasks experiments from Castro and Roberto (2014).

Table 1. Chemical characterization of raw material before (rice straw) and after pretreatment (deacetylated cellulignin).

Components	Composition (wt%)	
	Rice straw	Deacetylated cellulignin
Cellulose	35.3 ± 0.2	61.8 ± 0.7
Hemicellulose	23.8 ± 0.4	11.1 ± 0.1
Acetyl groups	2.6 ± 0.4	0.06 ± 0.01
Lignin	17.5 ± 0.5	17.1 ± 0.3
<i>Acid soluble lignin</i>	4.4 ± 0.2	0.9 ± 0.1
<i>Acid insoluble lignin</i>	13.1 ± 0.7	16.2 ± 0.6
Ash	11.3 ± 0.1	6.0 ± 0.1
Extractives	14.0 ± 0.2	nd

nd: non- determined

Table 2. Experimental design and results obtained for enzymatic hydrolysis of pretreated rice straw and glucose fermentation using the VBM reactor.

Experimental runs	Independent Variables*			Responses						
	X_1	X_2	X_3	Enzymatic hydrolysis**		Fermentation process			Biomass*** (g/L)	
				CC (%)		Glucose consumption (%)	Ethanol (g/L)	$Y_{P/S}$ (g/g)		Q_P (g/L.h)
1	100	0	40	71.29		100.0	19.97	0.38	1.48	2.37
2	200	0	40	68.25		100.0	19.29	0.40	2.16	2.34
3	100	30	40	80.85		100.0	19.83	0.39	1.13	2.54
4	200	30	40	77.56		100.0	21.52	0.40	1.50	2.12
5	100	0	46	73.43		93.5	20.50	0.41	0.85	1.30
6	200	0	46	71.48		88.3	19.05	0.41	0.79	1.42
7	100	30	46	87.00		81.4	17.74	0.38	0.74	1.18
8	200	30	46	84.85		84.8	18.37	0.42	0.76	1.48
9	150	15	43	82.87		100.0	21.65	0.43	1.22	1.84
10	150	15	43	81.95		100.0	20.43	0.40	1.07	1.74
11	150	15	43	80.01		100.0	20.78	0.44	1.21	1.87

* X_1 = agitation speed (rpm); X_2 = number of glass spheres (units) and X_3 = temperature (°C); **Results for 24 h process; *** An initial biomass concentration of 1 g/L was used for fermentation.

Table 3. Model equations for the responses cellulose conversion, CC in % (\hat{y}_1) and ethanol volumetric productivity, Q_P in g/L.h (\hat{y}_2) during the processes of enzymatic hydrolysis and fermentation, respectively, in the VBM reactor.

Model equation	R ²
$\hat{y}_1 = 42.62 - 0.03X_1 + 0.38X_2 + 0.78X_3$	0.84
$\hat{y}_2 = 2.25 + 0.04X_1 - 0.11X_2 - 0.03X_3 - 0.001X_1X_3 + 0.002X_2X_3$	0.98

X_1 , X_2 , X_3 represent the coded levels of agitation speed, number of spheres and temperature, respectively.

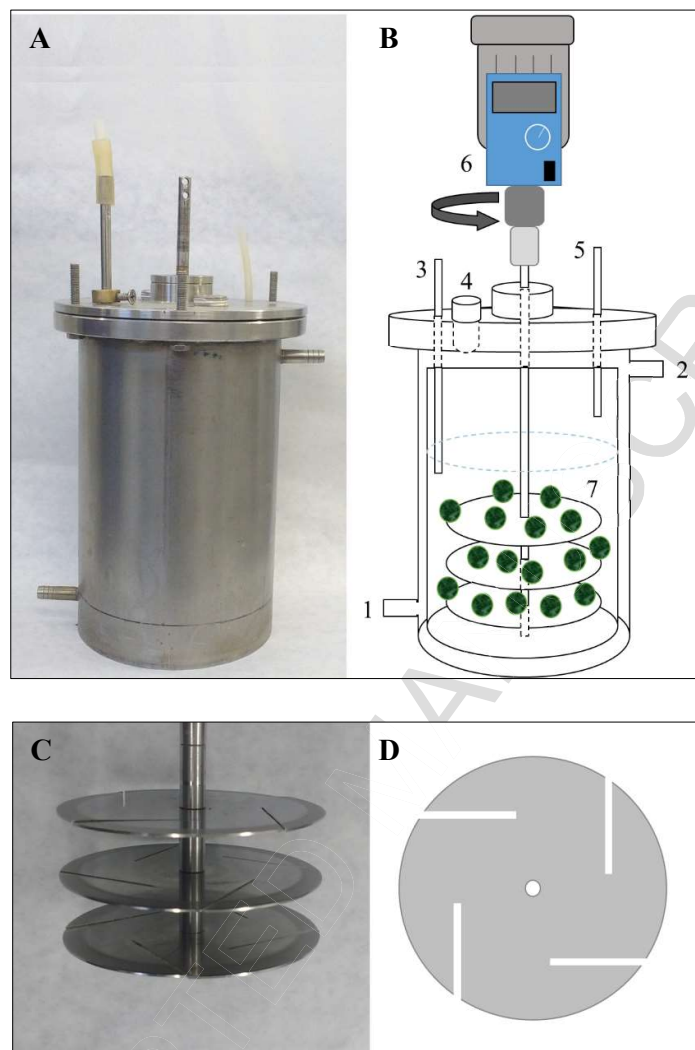
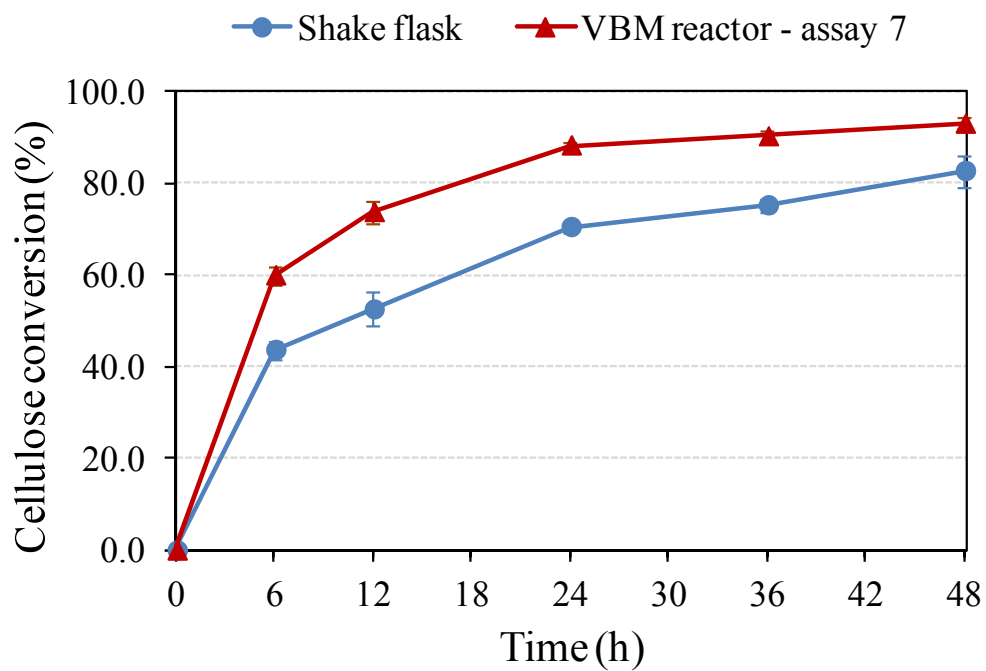


Figure 1

**Figure 2**

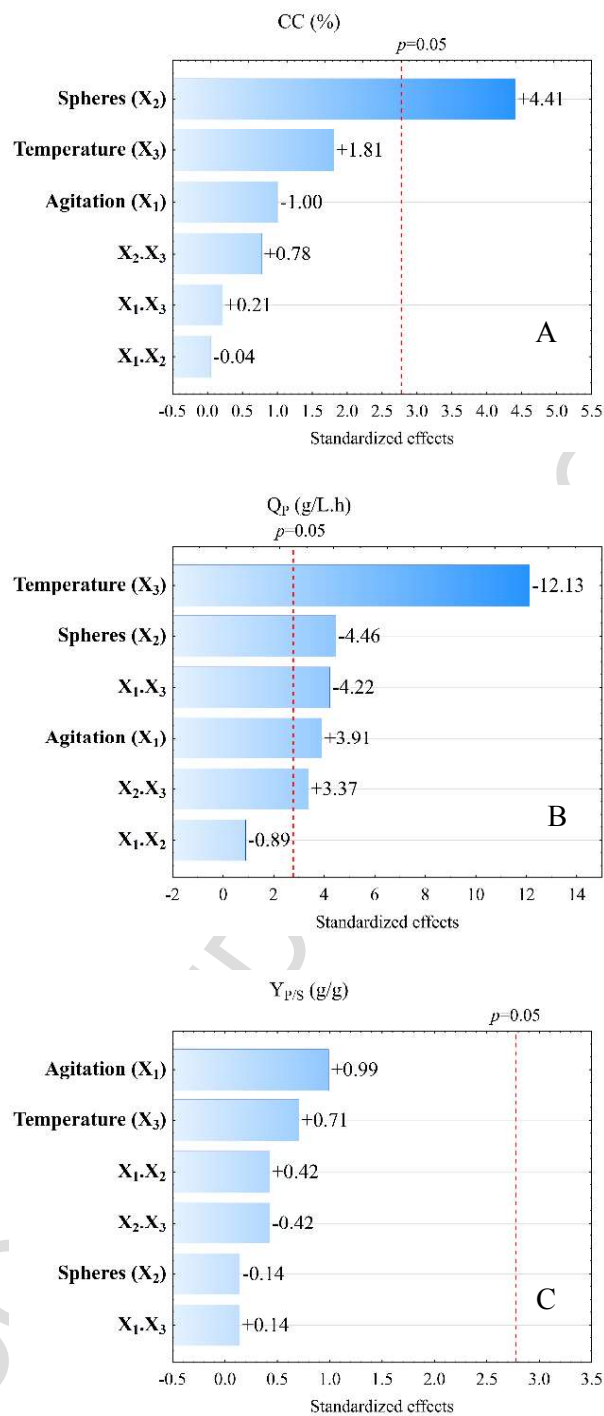


Figure 3

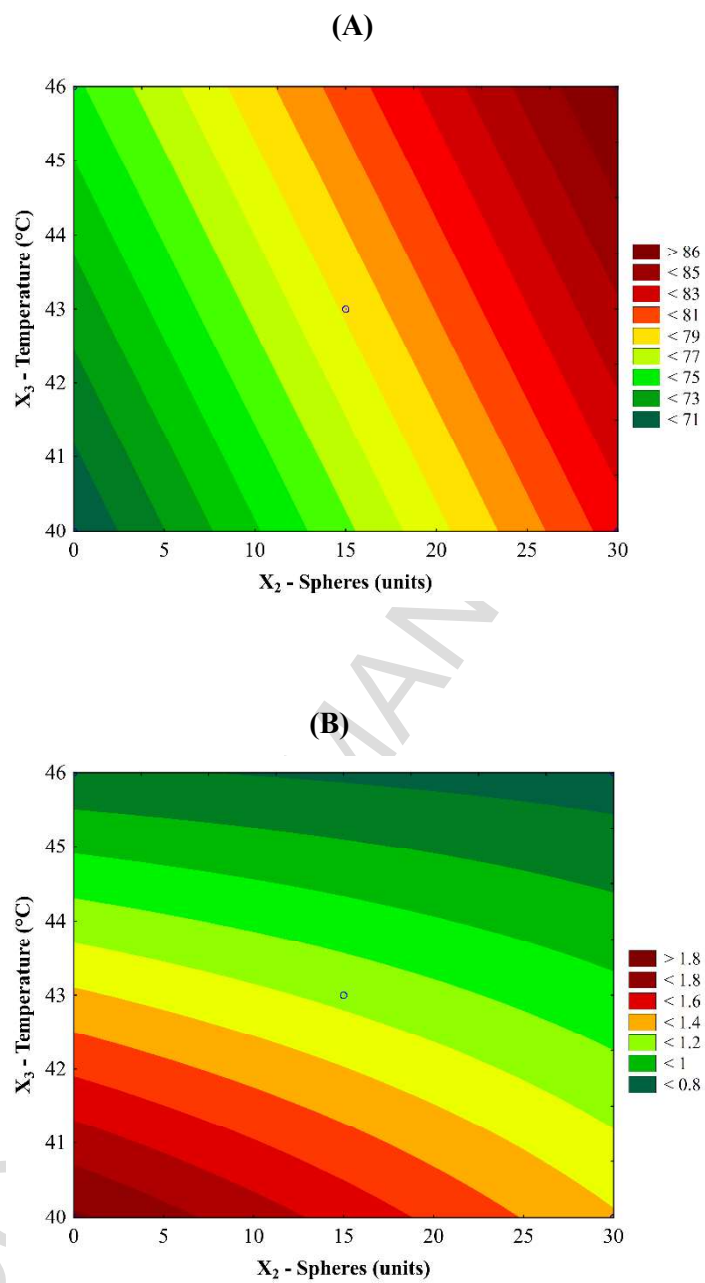
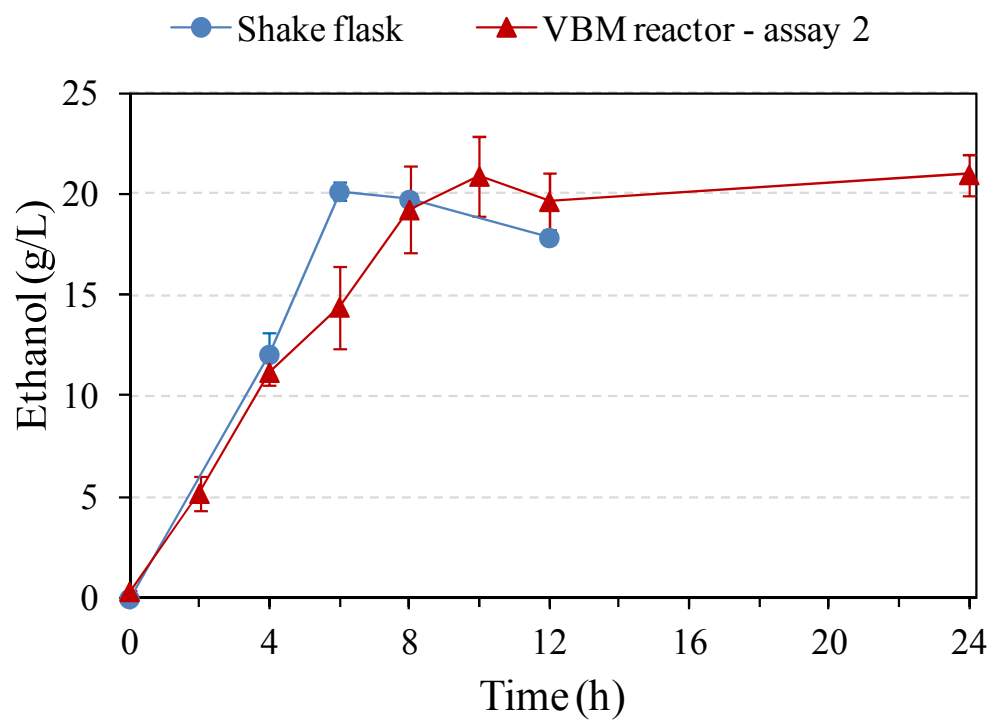


Figure 4

**Figure 5**

Highlights

- A vertical ball mill (VBM) reactor was proposed for biomass conversion
- Enzymatic hydrolysis of rice straw and glucose fermentation were studied
- VBM significantly improved the enzymatic hydrolysis of pretreated rice straw
- *Kluyveromyces marxianus* showed high ethanol efficiency in the VBM reactor
- Operational conditions for each process in the VBM reactor were established