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

Rafael Cunha de Assis Castro[1], Solange I. Mussatto[2], Inês Conceição Roberto[1,*]

[1] Departamento de Biotecnologia, Escola de Engenharia de Lorena, Universidade de São Paulo, CEP:12602-810, Lorena, São Paulo, Brazil.

[2] Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kemitorvet, Building 220, 2800, Kongens Lyngby, Denmark.

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

Abstract

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

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

1. Introduction

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

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

Regarding the pretreatment step, a variety of methods has been reported in the literature with different specificities on altering the physical and chemical structure of the lignocellulosic materials [6]. Considering the biorefinery approach, the pretreatment technology must focus on biomass fractionation, not only improving the subsequent hydrolysis of cellulose, but also providing separation of the main constituents of lignocellulosic biomass. In this way, each individual main component of biomass may be handled toward different categories of products [7]. Nevertheless, depending on the type of pretreatment and conditions employed, some biomass components can be lost during this process [8]. The loss of these components, especially the polysaccharide ones, must be avoided in order to increase the process efficiency. Recently, we have proposed a two-steps pretreatment that improved the ethanol production from both cellulose and hemicellulose fractions of rice straw [9]. This two-steps process consists in applying a mild alkaline pretreatment to remove acetyl groups from the biomass structure, prior to dilute acid hydrolysis to produce a hemicellulosic hydrolysate with lower toxicity degree, thus obtaining a pretreated cellulose-rich solid (cellulignin) with minimal loss of polysaccharide fractions.

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

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

2. Materials and methods

2.1. Feedstock and pretreatment

Rice straw was collected from fields in the region of Canas, São Paulo state, Brazil. The material was dried until approximately 10% moisture content, hammer-milled to attain particles of about 1 cm in length and 1 mm in thickness, and stored until treatment. Milled rice straw was submitted to a two-steps pretreatment as previously defined by Castro et al. [9]. Firstly, the material was deacetylated employing NaOH solution with a loading of 80 mg NaOH/g of biomass, using a solid:liquid ratio of 1:10, at 70 °C for 45 min. After washing, the deacetylated solid material was pretreated by dilute acid hydrolysis using 100 mg H_2SO_4/g deacetylated rice straw, a solid:liquid ratio of 1:10, at 121 °C for 85 min. The resulting solid (referred as deacetylated cellulignin) was washed and dried until 10% moisture content. The composition of the raw and pretreated material was determined according to NREL-LAP standard protocol [12], as shown in **Table 1**.

Table 1

2.2. Enzymes and microorganism

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

The thermotolerant yeast *Kluyveromyces marxianus* NRRL Y-6860 was used for fermentation. For inoculum preparation, cells from malt extract agar slants were cultivated in Erlenmeyer flasks containing semi-defined medium with the following composition (g/L): 30.0 glucose, 1.5 KH_2PO_4, 1.0 $(NH_4)_2SO_4$, 0.1 $MgSO_4 \cdot 7H_2O$, and 3.0 yeast extract. The inoculum was cultivated in an orbital shaker at 40 °C, 200 rpm for 16 h. After this time, the cells were recovered by centrifugation (2500×*g*, 10 min), washed twice in sterile distilled water, and resuspended in the fermentation medium to obtain the desired initial cell concentration (1.0 g/L).

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

A 1.5-L VBM reactor (120 mm inner diameter) made of 316 stainless steel and jacketed for temperature control by water recirculation using an external thermostatic water bath was used for the experiments. This reactor was equipped with three flat round plates impellers of 94 mm diameter, which were positioned at a distance of 28 mm each other, as shown in **Fig. 1**. Above each plate, glass spheres (23 mm diameter and 8.16 ± 0.35 g each) were placed as grinding elements. The impeller was rotated by an electric motor (IKA RW 20) able to operate from 60 to 2000 rpm.

Figure 1

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

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

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

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

The same 2^3 full-factorial experimental design, composed by 11 independent assays, was also used to evaluate the effects of the same operational variables levels on ethanol production in the VBM reactor. In the same way, the experimental error was estimated by three central point replicates. All assays were carried out using a semi-defined medium composed of (g/L): 50.0 glucose, 1.5 KH_2PO_4, 1.0 $(NH_4)_2SO_4$, 0.1 $MgSO_4.7H_2O$, and 3.0 yeast extract, in 0.5 L total final volume and employing 1 g/L initial cell concentration of *K. marxianus* NRRL Y-6860, added after reaching the desired temperature, from which the reaction has begun. Samples were periodically taken until 24 h to measure biomass, glucose and ethanol concentrations. The ethanol yield ($Y_{P/S}$, g/g), determined by ratio between ethanol produced and glucose consumed, and ethanol volumetric productivity (Q_P, g/L.h), calculated by the ratio between the maximum ethanol concentration and the respective fermentation time, were the responses considered for these experiments.

2.6. Analyses

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

3. Results and discussion

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

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

Table 2

3.1.1. Enzymatic hydrolysis

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

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

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

The results of the present study on enzymatic hydrolysis of deacetylated rice straw cellulignin in the VBM reactor are also promising and represent a significant improvement in process efficiency. For example, under the conditions of assay 7 (100 rpm, 30 spheres, 46 °C) a cellulose conversion of 87% was achieved at 24 h, while under the same process conditions but in the absence of spheres (assay 5), the cellulose conversion was reduced to 73.4%. These results are also better when compared to a previous study performed in shake flasks [18], which resulted in 79.2% conversion at 48 h, employing the same solids content and enzyme loading. **Fig. 2** shows the kinetic profile of enzymatic hydrolysis performed in both experiments. As can be seen, the cellulose conversion rate was mainly enhanced in the first 24 h of process, reaching 10% improvement when using the VBM reactor and the conditions of assay 7 (100 rpm, 30 spheres and 46 °C). Besides reducing the hydrolysis time in 24 h, improving the cellulose conversion in approx. 10% is relevant from the economical point of view, since the literature has reported a great impact of this step on second-generation ethanol production costs [19].

Figure 2

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

3.1.2. Fermentation process

The effects of the same operational conditions previously studied in the VBM reactor for enzymatic hydrolysis were also evaluated on ethanol production from glucose using the yeast *K. marxianus*. As can be seen in **Table 2**, in the studied range of values, $Y_{P/S}$ showed a little variation (from 0.38 to 0.44 g/g), whereas Q_P showed a more significant variation (from 0.74 to 2.16 g/L.h). *K. marxianus* was able to convert glucose into ethanol with high efficiency (80% in average) even at the highest temperature (assays 5-8), regardless of the agitation speed and number of spheres, thus confirming its thermotolerant characteristic. Glucose consumption was also higher than 81% for all the experiments. On the other hand, cultivation at the highest temperature resulted in the lowest cell growth (< 1.5 g/L) and ethanol volumetric productivity (< 0.9 g/L.h).

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

3.2. Statistical analysis

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

Figure 3

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

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

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

Table 3

Contour surfaces plotted for the evaluated responses according to the previous established models (**Fig. 4**) clearly show that the enzymatic hydrolysis and fermentation processes are maximized in different regions. The effect of the spheres was the most important influencing in such opposite behaviors. The use of glass spheres in the VBM reactor improved the cellulose conversion during the enzymatic hydrolysis of pretreated rice straw. However, the presence of spheres decreased the ethanol productivity during the fermentation step. The positive effect of the spheres on hydrolysis could be attributed to two types of phenomena: 1) shear stress due to impacts of the spheres on lignocellulosic fibers and/or 2) increased mass transfer due to the generation of a more homogeneous mixture during the hydrolysis. On the other hand, the negative effect of the spheres on fermentation performance could be explained by possible viability losses of the cells because of the shear stress generated.

Figure 4

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

Figure 5

4. Conclusions

The results of the present study indicate that the VBM reactor significantly improved the saccharification of alkali-acid-pretreated rice straw. The glass beads added to the VBM reactor was the main factor affecting both processes, enzymatic hydrolysis and fermentation, with a positive effect on cellulose conversion and a negative effect on ethanol volumetric productivity. Therefore, by applying the needed adjustments on the levels of the variables for each process (hydrolysis and fermentation), the VBM reactor could be efficiently used for biomass conversion in ethanol, presenting also potential for use in SSF process, for example. Future studies would be useful in order to better understand the fluid dynamics involved in VBM reactor, especially when operating with high solids content. Such information, together with the results of the present work, will represent a step forward towards the development of the market in this sector.

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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
Acid soluble lignin	4.4 ± 0.2	0.9 ±0.1
Acid insoluble lignin	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					
				Enzymatic hydrolysis**	Fermentation process				
	X_1	X_2	X_3	CC (%)	Glucose consumption (%)	Ethanol (g/L)	$Y_{P/S}$ (g/g)	Q_P (g/L.h)	Biomass*** (g/L)
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.

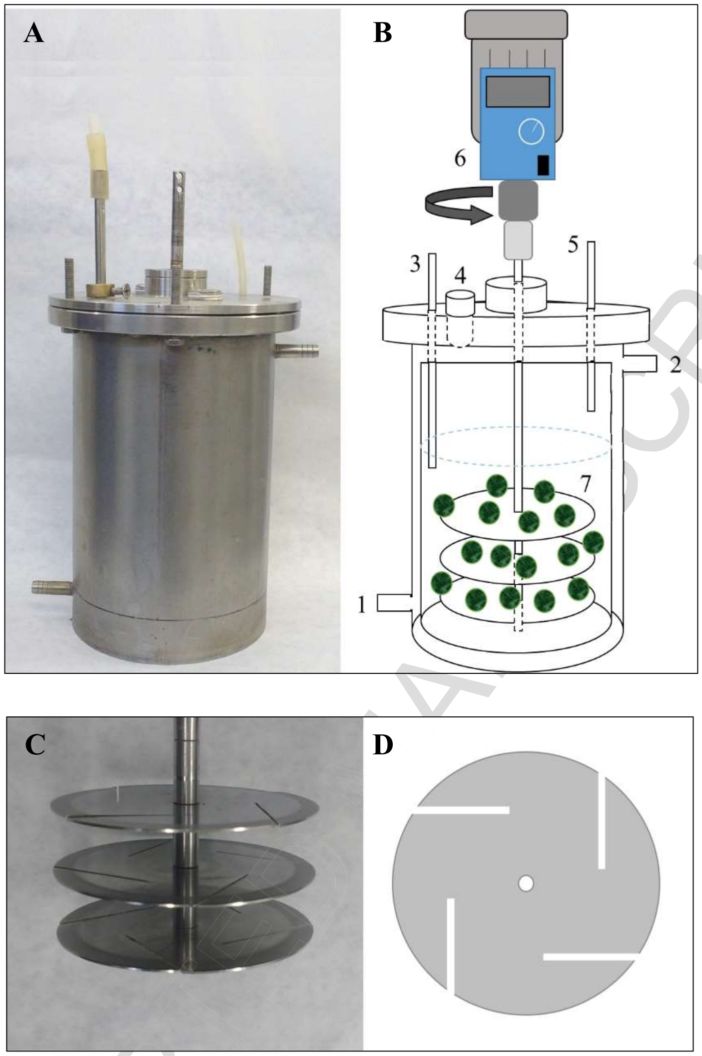


Figure 1

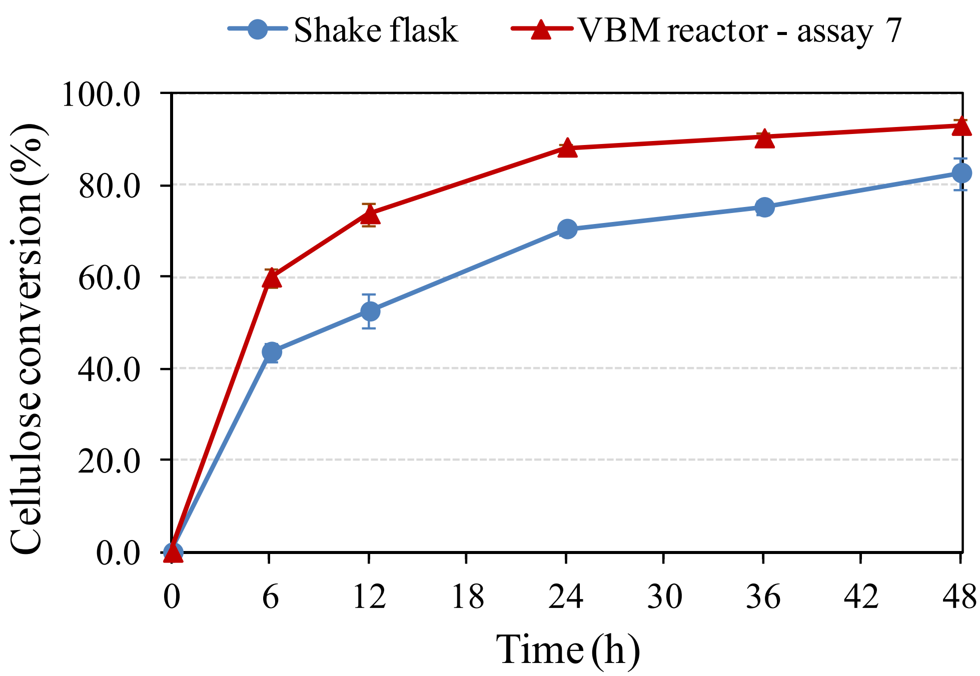
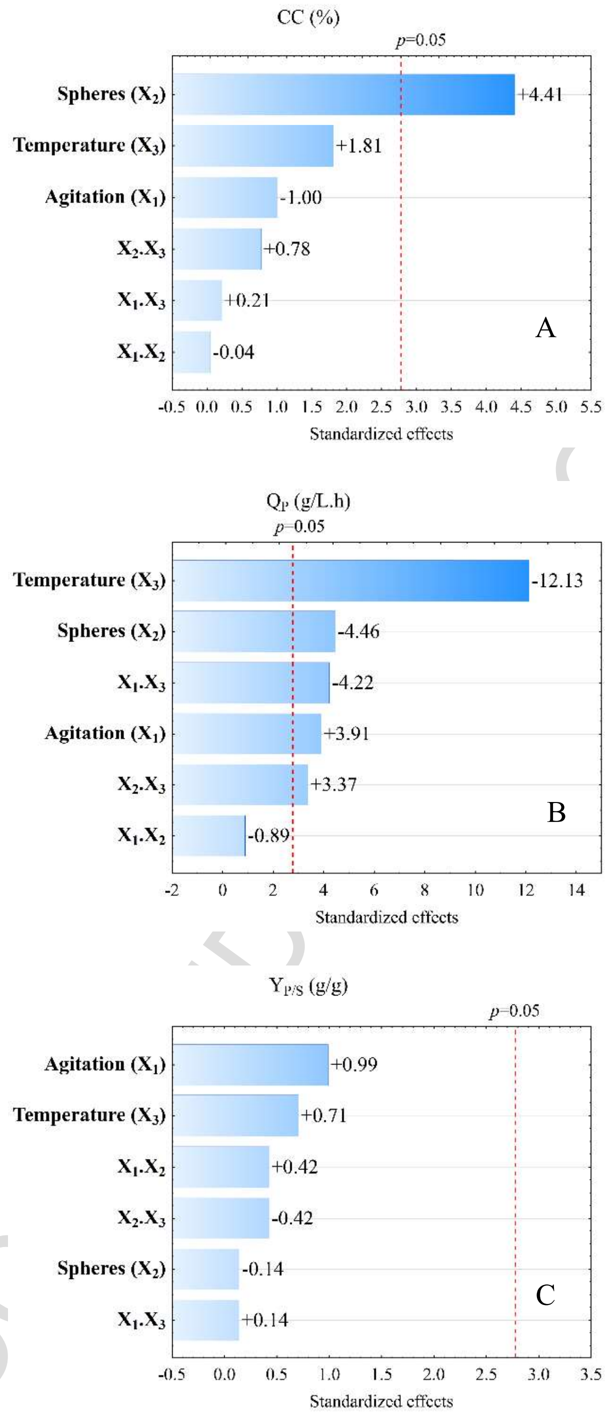

Shake flask
VBM reactor - assay 7
Cellulose conversion (%)
100.0
80.0
60.0
40.0
20.0
0.0
0
6
12
18
24
30
36
42
48
Time (h)

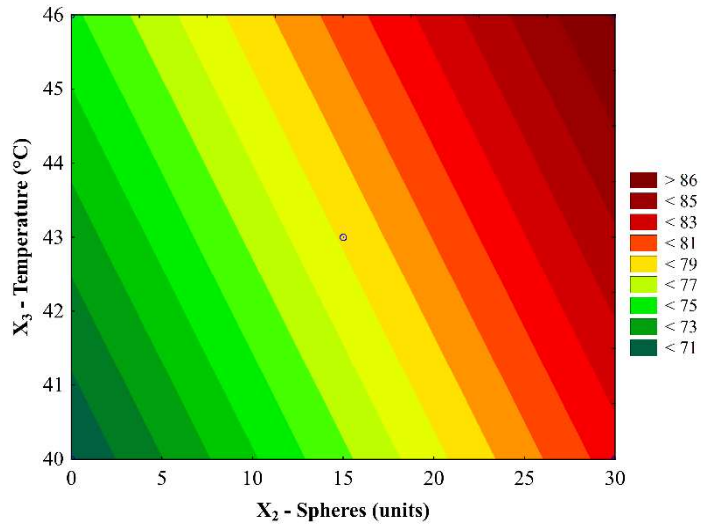
Figure 2



CC (%)
p=0.05
Spheres (X2)
+4.41
Temperature (X3)
+1.81
Agitation (X1)
-1.00
X2.X3
+0.78
X1.X3
+0.21
X1.X2
-0.04
A
-0.5 0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5
Standardized effects
QP (g/L.h)
p=0.05
Temperature (X3)
-12.13
Spheres (X2)
-4.46
X1.X3
-4.22
Agitation (X1)
+3.91
X2.X3
+3.37
X1.X2
-0.89
B
-2 0 2 4 6 8 10 12 14
Standardized effects
YP/S (g/g)
p=0.05
Agitation (X1)
+0.99
Temperature (X3)
+0.71
X1.X2
+0.42
X2.X3
-0.42
Spheres (X2)
-0.14
X1.X3
+0.14
C
-0.5 0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
Standardized effects

Figure 3

(A)


46
45
44
43
42
41
40
0
5
10
15
20
25
30
X3 - Temperature (°C)
X2 - Spheres (units)
> 86
< 85
< 83
< 81
< 79
< 77
< 75
< 73
< 71

(B)

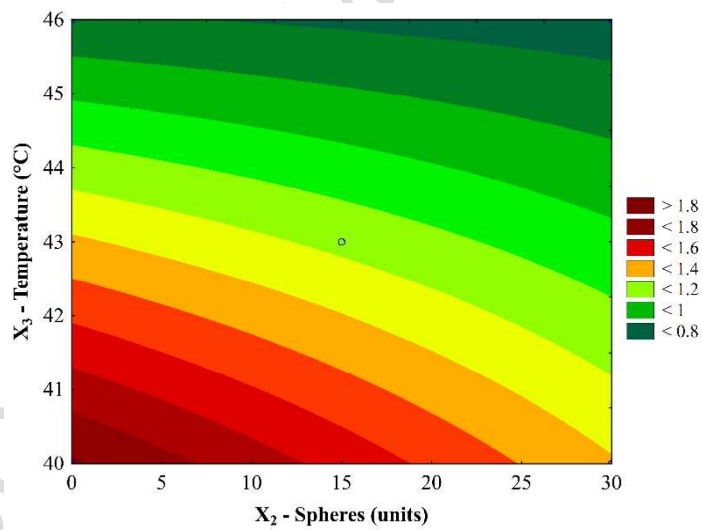

46
45
44
43
42
41
40
0
5
10
15
20
25
30
X3 - Temperature (°C)
X2 - Spheres (units)
> 1.8
< 1.8
< 1.6
< 1.4
< 1.2
< 1
< 0.8

Figure 4

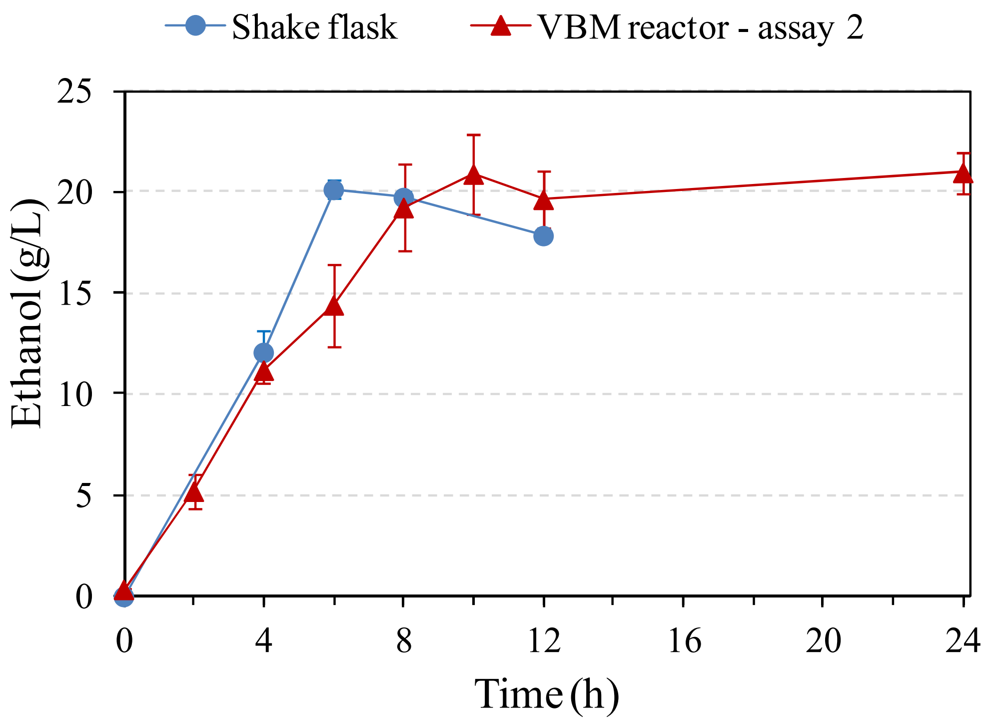

Shake flask
VBM reactor - assay 2
25
20
15
10
5
0
Ethanol (g/L)
0
4
8
12
16
20
24
Time (h)

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