Comparison of different lignocellulosic biomass for Solvent production by Clostridium beijerinckii strains

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Research Article

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Abstract

The aim of the present study was to compare the effect of lignocellulosic sugar hydrolysate, obtained after acid pretreatment of different lignocellulosic biomass on production of solvents by two different *Clostridium beijerinckii* strains. *C. beijerinckii* C-01 produced solvents *via* the isopropanol-butanol-ethanol (IBE) pathway and *C. beijerinckii* B-17 via the acetone-butanol-ethanol (ABE). The hydrolysate was obtained after acid pretreatment of rice-husk, rice-straw and sugarcane-bagasse. Solvent production was observed from all the three substrates by both the strains. However, the highest solvent production of 9.2 gL^{-1} similar to medium supplemented with commercial sugars was observed in sugarcane-bagasse hydrolysate by *C. beijerinckii* B-17. Degradation products *i.e.* 5-Hydroxy-methyl-furfural, levulinic acid, furfural and formic acid were found in different concentrations during acid pretreatments. The highest amount of formic acid (3.5 gL^{-1}), along with significant amounts of levulinic acid and furfural, was found to be present in rice-husk hydrolysate. The study shows that rice-straw and sugarcane-bagasse obtained after acid pretreatment are better substrates in comparison to rice-husk for butanol production by *Clostridium beijerinckii* strains.

1. Introduction

Keeping in view the increasing awareness towards environmental safeguards, stringent government policies, and volatile crude oil markets with simultaneous increase in energy demands due to increasing population, the focus of global economies has been directed in shifting towards sustainable and greener energy dependency. In developing nations like India, this increasing energy demand is currently met largely by non-renewable energy sources which put a strain on the finite fossil fuel resources [1]. India is largely dependent on fossil fuel imports to meet its energy demands and is one of the world's fastest growing energy markets accounting for 18% of the rise in global energy consumption. To bring about a transitional change from a fossil fuel based economy to a more sustainable carbon–neutral economy and to mitigate the environmental damages and climatic changes, maximum efforts have to be carried out on the reduction of the dependency of fossil fuels and alarming levels of greenhouse gases (CHGs).

Given India's growing energy demands and limited domestic oil and gas reserves, the country has proposed several plans and mandates to explore for its renewable energy sources. India being the second largest agro-based economy generates a large amount of agricultural waste, of which a major part is unutilized and generally subjected to open field burning causing serious environmental problems [2]. The use of these unutilized agro-residues can serve as enormous resource for energy generation. Now-a-days, with several bio-refineries being established in the country due to the mandate shift towards cleaner bio-energy systems, the utilization of these agro lignocellulosic substrates can not only provide a sustainable solution for alternate energy resource but also help in better agricultural waste management [3].

The lignocellulosic residues can be utilized for bio-fuel production only after a pre-treatment process which weakens the naturally recalcitrant structure to unlock the available fermentable sugars [4] in the form of monomeric sugars (C_5 andC_6).

Pre-treatment strategies include chemical, biological and physical processes or a combination of all these three [5, 6]. Among these the chemical pre-treatment method, through the use of dilute sulphuric acid is very effective in disrupting the recalcitrant structure of lignocellulosic biomass [7, 8]. During dilute acid pretreatment process, a variety of inhibitory compounds are generated, along with the hydrolysed sugars, which generally inhibit cell growth and fermentation [9, 10]. These include different salts, acetic, furfural, ferulic, hydroxymethyl furfural (HMF), phenolic compounds, glucuronic and r-coumaric acids [9, 10]. Therefore, their generation and removal becomes an important parameter that needs to be optimized for any lignocellulosic biomass in bio-fuel production [11].

In recent years, large amount of research has been carried out to produce bio-butanol [12, 13]. Bio-butanol is a superior fuel than bio-ethanol because of higher energy content and better air-to-fuel ratio. It is also less volatile and explosive than bio-ethanol and has a higher flash point and lower vapor pressure [14, 15]. Commercially butanol is generally synthesized chemically from fossil fuels [16]. However, with the depletion of fossil fuels and the environmental constrains resulting thereof, the bio-butanol production through fermention is becoming lucrative.

Clostridium species [12] in nature produce bio-butanol through two pathways *i.e.* Acetone-Butanol-Ethanol (ABE) or Isopropanol-Butanol-Ethanol (IBE) fermentation pathways [17]. The cost of the substrate, substrate inhibition, microbial strain performance, fermentation process mode, productivity and the product recovery process are some of the several factors which directly influence the economics of bio-butanol production [18]. Among this the cost of the substrate utilized, roughly contributes to 60% of the total processing cost [19]. Hence, exploring low cost substrates for ABE fermentation is vital for making the process economically feasible., Lignocellulosic biomass is one such substrate, which is abundant and sustainable, offering an attractive and alternative for economical bio-butanol production. Among the different biomass's available, rice straw (RS) and sugarcane bagasse (SB) have high cellulose and hemicellulose content that is hydrolyzed into hexoses and pentoses [20, 21]. In contrast to bio-ethanol production by yeasts, a mixture of hexose and pentose sugars obtained as a result of pre-treatment and hydrolysis of biomass can be fermented by solventogenic *Clostridium* spp. [22]. Bio-butanol production has been reported utilizing different lignocellulosic biomass like wheat straw [23], corn stover [24, 25], barley straw [11], corn fiber [26], switchgrass [27], distillers dry grains and solubles [28, 29], corncob [30], sugarcane bagasse and rice straw (RS) (31, 32, 33). However, most of these results have reported an additional detoxification step in order to make the hydrolysate suitable for the growth of bacteria. Additionally the yields obtained were not comparable to processes with commercial sugars.

Though a number of studies have been carried out to understand solvent production by *C. beijerinckii* from lignocellulosic biomass, this study aims for a direct comparison of solvent production from sugar hydrolysates of rice straw, rice husk and sugarcane bagasse biomass. The main parameters assessed in this study are: (a) cellulose & hemicellulose hydrolysis and sugar yields from substrates; (b) amount of inhibitor formation and (c) production of different solvents.

2. Materials And Methods

2.1. Screening and isolation of solvent producing bacterial strains

The isolation method was similar to that described by Weizmann (1935) [13]. Briefly 2% of crushed grains (wheat, gram, barley and millet) were added separately to autoclaved; hot (90–100°C, to counter select against non-spore formers) reinforced clostridial media (RCM) in a 120 mL Wheaton bottle and incubated anaerobically at 35°C for 5 days. The bottles showing most gas production were tested for solvent production using gas chromatography (GC). The bottles showing the presence of solvents were used to inoculate sterile acid pretreated RS hydrolysate. The cultures growing in RS hydrolyzate were plated by pipetting 500 µL on reinforced clostridial agar (RCA) for isolation of solvent producing bacterial strains. Distinct single colonies were picked up and stored as spore suspension in sterile double distilled water at 4°C. The pure cultures obtained were sent for 16s-rRNA gene sequencing which were outsourced to Macrogen, Korea. The sequences obtained were searched for 16s-rRNA gene sequence homology using BLAST algorithm with NCBI and deposited in the GenBank database with accession numbers KC851824 and KC915012.

2.2. Organism, culture maintenance and inoculum preparation

Clostridium beijerinckii was maintained as spore suspensions in sterile double distilled water at 4°C. *C. beijerinckii* spores were heat shocked for 10 min at 80°C followed by cooling in ice cold water. Heat shocked spores (10 % *v/v*) were inoculated in 30 mL reinforced clostridial (RCM, Himedia, India) medium and incubated anaerobically for 15–16 h at 35°C to generate pre-inoculum. Actively growing cells from pre-inoculum were inoculated into fresh RCM to generate inoculum.

2.3. Dilute sulphuric acid pre-treatment of biomass

Rice straw, rice husk and sugarcane bagasse obtained locally were air dried, milled and sieved to obtain a maximum particle size of + 850 µm/-425 µm. The compositional analysis was carried out for the raw and treated biomass to estimate cellulose, hemicellulose, lignin and other components according to National Renewable Energy Laboratory [34] protocols. Biomass was dried at 105°C overnight to ensure low moisture content prior to treatment. Dry biomass was pretreated with 1.5% (*w/w*) H_2SO_4 at a solid loading of 10% (*w/v*) and at 135°C for 18 min. The hydrolysate obtained was separated from the un-hydrolysed biomass by filtration. The inhibitor content of the hemicellulose hydrolysate was determined according to standard methods of the NREL. The hydrolysate was neutralized with calcium carbonate prior to fermentation. The C_5 hydrolysate was supplemented with 2 gL^{-1} yeast extract and 3 gL^{-1} sodium acetate.

2.4. Solvent production from hydrolysate

All experiments were carried out in 125 mL Wheaton bottles at pH 6.5 and temperature 35°C. Batch fermentation was done with the hydrolysate obtained after acid pre-treatment. Sodium acetate and yeast

extract was also added to the hydrolysate for fermentation [13]. The hydrolysate was inoculated with 10% (*v/v*) of actively growing cells. An initial sample (0 h) was taken after inoculation for analysis. Experiments were run for 72 h. At the end of fermentation final samples were taken for determination of solvent production and sugar utilization. ABE/IBE yield were calculated as the amount of solvents produced per gram of sugar consumed (gg^{-1}).

2.5. Analyses

The culture supernatant was analyzed for sugar, organic acids, inhibitors and solvents, after centrifugation at 5000 × g (5 min) and filtration with 0.22 µ filter (Millpore, USA).

The sugar concentration was analyzed using an Agilent 1260 Series (Agilent Technologies, Inc., Palo Alto, CA) high pressure liquid chromatography (HPLC) system. A Bio-Rad HPX-87P column (Bio-Rad Laboratories, Inc., Hercules, CA; 300 × 7.8 mm) equipped with a guard column (30 × 4.6 mm) was used at a flow rate of 0.6 mL min^{-1} (Millipore water was used as the mobile phase) for separation of sugars. The temperature of the column was kept at 60°C. A refractive index (RI) detector was used at 50°C for signal detection.

Acids (acetic and butyric) and inhibitors (Furfural, 5-Hydroxy Methyl Furfural and formic acid) in the acid treated hydrolysate were analyzed using an Agilent 1290 Ultra Series (Agilent Technologies, Inc., Palo Alto, CA) high pressure liquid chromatography (HPLC) system. An Aminex HPX-87H column (Bio-Rad Laboratories, Inc., Hercules, CA; 300 × 7.8 mm) equipped with a guard column (30 × 4.6 mm) was used at a flow rate of 0.6 mL min^{-1} (0.008N H_2SO_4 was used as the mobile phase) for separation of organic acids and inhibitors. The temperature of the column was kept at 35°C. A Diode Array (DA) detector was used for signal detection. The wavelength was read between 250 to 360 nm.

Solvent (acetone, butanol, isopropanol and ethanol) concentrations were quantified using a Clarus 580 gas chromatograph (Perkin Elmer Inc., USA), equipped with a headspace analyzer Turbomatrix 16 (Perkin Elmer Inc., USA), a flame ionization detector (FID) and a PE Elite M1 column; 30 × 0.28 × 3 mm (Perkin Elmer Inc., USA). The injector and detector temperature were 250 and 240°C respectively and the oven temperature was programmed from 50 to 120°C at the rate of 5°C min^{-1}, held for 2 min and increased to 150°C at the rate of 15°C min^{-1}.

Scanning electron microscope (SEM) was used to study the morphological changes of the fiber structure on raw untreated & acid treated biomass. Samples were coated with gold according to the method of Ibbett et al., (2013) and observed under scanning electron microscope (Vega II LSU, Tescan) at a voltage of 15KV & 100x magnification.

3. Results And Discussion

3.1. Composition and dilute acid pretreatment of biomass

Lignocellulosic biomasses originating from different types of plants have typical properties in terms of hemicellulose structures and composition, lignin content, cellulose crystallinity and the degree of polymerization [35]. In the untreated rice straw, rice husk and sugarcane bagasse the main carbohydrates were xylan and glucan, originating from xylan in hemicellulose and glucan from cellulose (Table 1). Cellulose-derived glucose was the most abundant sugar (39.7% in rice straw, 36.0% in rice husk and 39.9% in sugarcane bagasse) followed by hemicellulosic xylose (19.6% in rice straw, 17.2% in rice husk and 24.5% in sugarcane bagasse) [36]. The lignin composition was found to be higher in rice husk (23.5% *w/w*) compared to rice straw (19.3% *w/w*) and sugarcane bagasse (22.3% *w/w*) which are in accordance with the values referred by Lee et al. (2014) and Biswas et al., (2015). As a result of higher hemicellulose content in sugarcane bagasse, in comparison to rice straw and rice husk, it can serve as a better feedstock for biochemical butanol production.

Table 1
Compositional (% *w/w*, dry basis) analysis of the lignocellulosic substrates before (raw) and after dilute acid pretreatment

	Rice husk		**Sugarcane bagasse**		**Rice straw**	
	Raw	Treated	Raw	Treated	Raw	Treated
Acid Soluble Lignin (%)	1.1	0.7	1.6	1.1	2.1	0.1
Acid Insoluble Lignin (%)	22.4	35.4	20.7	22.1	17.2	25.4
Cellulose (%)	36.0	43.4	39.9	56.9	39.7	56.9
Hemicellulose (%)	17.2	1.9	24.5	1.0	19.6	2.9
Ash (%)	12.4	10.4	2.8	2.2	10.1	5.3
Extractives (%)	10.2	-	10.9	-	9.5	-
Results presented are an average of three replications performed under the same conditions and have an error margin of ± 5%.						

During the dilute acid pretreatment of lignocellulosic biomass, both physical and chemical properties of lignocellulosic biomass are modified. The effects of the same pretreatment conditions may vary on different lignocellulosic biomass based on the biomass type, lignin composition and its cell wall structure [37]. In this study, different lignocellulosic biomass (rice straw, rice husk and sugarcane bagasse) were treated with dilute sulfuric acid to release the monomeric C_5 sugar (xylose) from the hemicellulose, the percentage hydrolysis of which can be inferred from the residual composition as summarized in Table 1. From the results, it can be seen that sugarcane bagasse and rice straw have higher capacity for sugar release than rice husk due to higher cellulosic and hemicellulosic content directly leading to higher yield of fermentable sugars. The cellulose content proportionately increases during pretreatment which results due to the hydrolysis of hemicellulose and lignin. After pretreatment, the cellulose content of sugarcane bagasse, rice straw and rice husk increased from 39.9 to 56.8%, 39.7 to 56.9% and 36 to 43.4% respectively. In contrast the hemicellulose percentage decreased to 2.9%, 1.0% and 1.9% for rice straw,

sugarcane bagasse and rice husk respectively, indicating that the pretreatment was effective in removing the xylan from biomass. However, it was seen that the percentage recovery of xylose in the hydrolysate was different for the three biomasses (Fig. 1). Rice straw hydrolysate showed maximum xylose recovery of 64%, followed by sugarcane bagasse (61%) and rice husk (60%). [38] also reported that on subjecting different lignocellulosic biomass to similar hydrolytic conditions resulted in varying xylose sugar recovery. The recovery of xylose from rice husk was observed to be the least which could be attributed to the high percentage of lignin in the biomass. This increases the compactness and therefore recalcitrance of biomass to pretreatment [39]. The acid insoluble lignin content increased in all the cases after acid pretreatment (Table 1). The acid insoluble lignin percentage of rice husk increased to 35.4% in the pretreated biomass, which was much higher than sugarcane bagasse (22.1%) and rice straw (25.4%). The dilute acid pre-treatment also resulted in the release of lower concentration of glucose in the hydrolysate in all the three biomasses indicating only slight hydrolysis of the cellulose content.

3.2. Scanning electron microscope (SEM) images of biomass after dilute acid pretreatment

Scanning electron microscopy (SEM) is an effective technique widely used to observe lignocelluloses surfaces to analyze the effect of acid pre-treatments based on the surface morphology of the biomasses [40]. The SEM images of untreated and acid pretreated biomass are presented in Fig. 2 which shows the changes as indicated by the disrupted and ruptured structure in the pretreated biomass in comparison to the compact and ordered structure in the untreated raw biomass. Amongst the three biomasses, the cellulose bundles were found to be more evident with less cohesion between them in the case of rice straw and sugarcane bagasse in comparison to rice husk as shown in Fig. 2b and 2f. This can be attributed to higher level of hemicellulose and lignin removal. Results also confirm the removal of the outer protective layer, mainly lignin (as determined by chemical composition), with consequent disruption of the original biomass's structure and separation of fiber bundles in the case of rice straw and sugarcane bagasse. Similar disrupted structures of sugarcane bagasse were reported by [39, 41] after dilute acid pretreatment. However, lignin precipitation on the surface of fibers was not observed, which has been generally reported in literature after acid pretreatment [42].

3.3. Isolation and identification of solvent producing bacterial strains

Species of genus Clostridium were first isolated from grains [43] and were found to metabolize lignocellulosic sugars, both hexoses (mannose, galactose, glucose) and pentoses (xylose, arabinose) [44]. In this study, bacteria were screened from gram, barley, millet and wheat grains for the production of butanol. Eleven isolates of spore forming, anaerobic bacteria, producing solvents were purified by serial dilutions in C_5 sugar hydrolysate. On RCM agar plates under anaerobic conditions, the newly isolated bacteria formed circular to irregular, raised to flat, white to lavender colonies at 35°C. The cells of all isolates were straight rods, stained Gram-positive and formed endospores.

The solvent production profile of isolates was assayed by gas chromatography in small-scale batch fermentation with the model-substrate glucose. The bacterial strains produced between 2 gL^{-1} to 14 gL^{-1} total solvents, showing a wide variation in the amount of solvents formed.

Two distinct bacterial strains were taken up for further studies. *Clostridium beijerinckii* C-01 (Dalal et al., 2019), isolated from gram grain, produced isopropanol-butanol-ethanol (IBE) and *Clostridium beijerinkcii* B17, isolated from barley grain, produced acetone-butanol-ethanol (ABE) as the major solvents. Both the microbial strains produced ethanol in a negligible amount (0.1 gL^{-1}). This is in favour of the industrial process as butanol produces more energy than ethanol on burning (Cheng et al., 2012). *C. beijerinckii* C-01 did not produce acetone as one of the by-products of fermentation, which is also highly desirable [45, 31, 46] as the mixture of isopropanol, butanol and ethanol can be directly used as a fuel [47].

3.4. Solvent production by *C. beijerinckii* C-01 on different biomass

In order to compare results obtained in hydrolysate fermentations, control batch experiments were run using 60 gL^{-1} of glucose and xylose as model substrates with *C. beijerinckii* C-01 and are given in Table 2. The control experiments resulted in the production of 12.7gL^{-1} IB from 40 gL^{-1} of glucose and 10.5 gL^{-1} from 37.5 gL^{-1} of xylose after 72 h of fermentation. The resulted IB yields after fermentation were 0.32 and 0.28 with glucose and xylose respectively. Of the two products, 3.0 gL^{-1} of isopropanol and 9.7 gL^{-1} of butanol were formed from glucose. When xylose was used the concentrations of isopropanol and butanol were 2.2 gL^{-1} and 8.5 gL^{-1} respectively. There are only a few wild IBE producing strains which have been reported to date, including *Clostridium beijerinckii* NRRL B-593 [48], *Clostridium beijerinckii optinoi* [49], *Clostridium* sp. A1424 [50] and *Clostridium* spp. strain NJP7 [51] which either show low production of butanol or do not utilize xylose efficiently for conversion of sugars from lignocellulosic biomass to solvents [50, 51].

Table 2
Comparative analysis of sugar, solvents and yield of solvents in different hydrolysates and control(Glucose or Xylose) after 72 h of fermentation

		Sugar utilized (gL^{-1})	Total Solvents (gL^{-1})	Yield (gg^{-1})
Rice straw	C-01	23.0	6.60	0.29
	B17	23.0	8.00	0.35
Rice husk	C-01	03.0	0.70	-
	B-17	08.0	2.80	-
Sugarcane bagasse	C-01	27.8	7.80	0.28
	B17	29.5	9.20	0.31
Glucose (Control)	C-01	40.0	12.70	0.32
	B17	36.5	13.70	0.38
Xylose (Control)	C-01	37.0	10.50	0.28
	B17	36.0	10.10	0.28

Results are an average of three replications performed under the same conditions and have an error margin of ± 5%.

Hydrolysates of sugarcane bagasse, rice straw and rice husk were prepared and subjected to fermentation with *C. beijerinckii* C-01 for 72 h. Figure 3 presents a comparative analysis of concentration of solvent production in all the three lignocellulosic hydrolysates. From Fig. 3 we can see that after 72 h of growth *C. beijerinckii* C-01 on sugarcane bagasse hydrolysate, maximum solvent production of 7.8 $gL^{-1}$was obtained from 27.8 gL^{-1} of sugar. The IB production on rice straw hydrolysate was 7.5 gL^{-1} from 25 gL^{-1} of sugars. However the solvent production on rice husk hydrolysate was only 0.7 gL^{-1}.

3.5. Solvent production by *C. beijerinckii* B17 on different biomass

Control batch experiments were also run using glucose and xylose as model substrates with *C. beijerinckii* B17 containing 60 gL^{-1} sugar at 0 hr. The results show (Table 2) a production of 13.7 and 10.1 gL^{-1} of AB from glucose and xylose respectively. During the fermentation, 36.5 gL^{-1} of glucose was utilized giving an AB yield of 0.38. In case of xylose, 36 gL^{-1} was utilized giving an AB yield of 0.28. The result also shows, 2.8 gL^{-1} of acetone and 10.9 gL^{-1} of butanol were produced from glucose and 1.6gL^{-1} of acetone and 8.5 gL^{-1} of butanol were produced from xylose.

Hydrolysates of sugarcane bagasse, rice straw and rice husk were prepared and subjected to fermentation with *C. beijerinckii* B17 for 72 h. Figure 3 presents a comparative analysis of concentration of solvent production in all the three lignocellulosic hydrolysates. From the Figure we can see that after 72 h of growth *C. beijerinckii* B17 produced maximum solvents on sugarcane bagasse hydrolysate. It produced 8.0gL^{-1}, 2.8 gL^{-1} and 9.2 gL^{-1} Acetone-Butanol from rice straw, rice husk and sugarcane bagasse respectively. The concentration of 9.2 gL^{-1}on sugarcane bagasse is comparable to the results reported by [26] which were 9.3 gL^{-1} of ABE production from sulfuric acid treated corn fiber hydrolysate.

3.6. Comparison of solvent production by *C. beijerinckii* on different lignocellulosic hydrolysates

Butanol can be produced by bacteria from hydrolysates of lignocellulosic biomass after pretreatment. Hydrolysates obtained after dilute acid pretreatment of wheat straw [52], [11], corn stover and switchgrass [25] have been used for ABE fermentation in which a detoxification process was necessary prior to the fermentation especially for barley straw and corn stover. In the present study three different hydrolysates obtained after dilute sulfuric acid pretreatment were fermented with two bacterial strains without any detoxification. The hydrolysates were not supplemented with minerals because of the natural presence of minerals like potassium, phosphorous, iron, calcium, magnesium, sulfur and sodium [53]. In a previous study, higher ABE was formed in experiments done without mineral supplementation than with supplementation [31]. However, the hydrolysates were supplemented with yeast extract and sodium acetate.

Table 2 compares the production and yield of total solvents obtained from the 3 hydrolysates and commercial sugars by both the strains. Among the hydrolysates, a maximum yield of 0.31 g/g AB was obtained for C. *beijerinckii* B17 from sugarcane bagasse. A maximum solvent yield of 0.38 g/g from glucose and 0.28 g/g from xylose was obtained for this strain. Qureshi et al., (2010) reported a maximum butanol production of 4.75 gL^{-1} from *C. beijerinckii* P260 utilizing un-detoxified barley straw hydrolysate containing 60 gL^{-1} reducing sugar. Another AB producing *Clostridium* spp. strain BOH3 was reported to accumulate 4.38 gL^{-1} butanol and 1.13 gL^{-1} acetone from sulfuric acid pretreated horticultural waste hydrolysate [54]. Compared to these previous studies in literature, the *Clostridium* strain C-01 showed at par butanol production from un-detoxified hydrolysate without generating any acetone and strain B17 showing higher production of butanol.

From the fermentation results it can be inferred that both the strains showed optimum production of solvents in sugarcane bagasse hydrolysate, Rice husk hydrolysate was found to be an inferior substrate in comparison to rice straw and sugarcane bagasse hydrolysates in spite of the same pretreatment processes. The fermentation results are consistent with the result of the compositional analysis and SEM analysis which showed less disruption in rice husk biomass on pretreatment in comparison to rice straw and sugarcane bagasse. These results suggest that sugarcane bagasse and rice straw are superior fermentation lignocellulosic substrates than rice husk and were found to be comparable to the results reported by [55, 56].

3.7. Study of inhibitors in the different biomass

The presence of inhibitors or sugar degradation products, resulting from pretreatment process, is toxic to fermentation [11]. Therefore, to understand if the poor fermentabilty of rice husk was due to the presence of inhibitors, analysis of all the three hydrolysates was done. Some of the inhibitors that were present in the hydrolysates are presented in Fig. 4. The different inhibitors that were identified were formic acid, acetic acid, furfural, 5-HMF and levulinic acid. All the hydrolysates showed the presence of acetic acid, with sugarcane bagasse hydrolysate showing a significant amount at 6.68 gL^{-1}, which was below the inhibitory concentration of 9.7 gL^{-1} for all the hydrolysates [57]. All the three hydrolysates showed the presence of formic acid which was higher than the inhibitory concentration of 0.4 gL^{-1} [10, 57]. The concentration of formic acid was 3.06 gL^{-1} and 3.46 gL^{-1} in rice straw and rice husk hydrolysates respectively. It should be noted that solvent production of 8 gL^{-1} was achieved in rice straw hydrolysate in spite of the presence of high formic acid. The concentration of levulinic acid was 2.73 gL^{-1} for rice husk and 2.64 gL^{-1} for sugarcane bagasse. However further studies are required to be carried out in order to identify the inhibitory limits of levulinic acid for butanol fermentation.

Other inhibitors, 5-HMF and furfural are degradation products generated from cellulose and hemicellulose respectively. The concentration of 5-HMF (Fig. 4) was within the inhibitory limit of 2.0 gL^{-1} for all the 3 substrates [10]. In sugarcane bagasse hydrolysate high concentration of 5-HMF was present (0.89 gL^{-1}), which may have had a stimulatory effect on solvent production. 5-HMF has been found to enhance solvent production in studies reported by [10, 58] from *C. beijerinckii*. Qureshi et al. (2012) reported that furfural concentration is stimulatory for *C. beijerinckii* P260 below 1.0 gL^{-1} and inhibitory above 2.0 gL^{-1}. In sugarcane bagasse hydrolysate and rice husk hydrolysate, the concentration of furfural was 1.69 and 1.72 gL^{-1} respectively (Fig. 4). This concentration is much higher than 0.64 gL^{-1}, which have been reported in previous studies [11]. However this high concentration did not seem to be inhibitory for AB production in sugarcane bagasse hydrolysate. The results obtained in this study support the results obtained by [58] who reported that the presence of 5-HMF and furfural in wheat straw hydrolysate enhanced the ABE productivity by 300%. The high concentration of furfural and 5-HMF were reported to be inhibitory only when present together [58]. From this study it can be inferred that the presence of inhibitory concentrations of formic acid and significant amounts of levulinic acid and furfural together could be responsible for poor fermentation yield in rice husk hydrolysate. As per the reports in literature, the presence of higher levels of phenolic inhibitors in rice husk hydrolysate in comparison to rice straw may have also played a role in making it less amenable for fermentation [59].

Rice straw and rice husk are important sources of lignocellulosic agro residues globally and have almost similar chemical compositions. However, when subjected to identical dilute acid thermal pre-treatments, rice husk was found to be poorly saccharifable, generating higher levels of fermentation inhibitors and lower yields of butanol during batch fermentation. This higher recalcitrance and lower pretreatment efficiency observed in rice husk may be attributed to several factors such as, the presence of considerably higher levels of lignin. Though not assessed in this study, rice straw and rice husk contain considerably

higher levels of silica in their cell walls compared with other cereal lignocellulose, being comparatively much higher in rice husk than in rice straw [60].

4. Conclusion

Globally, rice straw, sugarcane bagasse and rice husk are important lignocellulosic biomass with similar composition. In this study, fermentation of three different lignocellulosic biomass hydrolysates obtained after dilute sulphuric acid pretreatment was carried out with an IBE producing *C. beijerinckii* C-01 strain and an ABE producing *C. beijerinckii* B17 strain. It was found that, on subjecting to identical dilute acid pre-treatment, sugarcane bagasse and rice straw hydrolysates were superior substrates for solvent production in comparison to rice husk hydrolysate. Rice husk was found not only to be poorly saccharifable, but also generated considerable amounts of fermentation inhibitors namely formic acid, levulinic acid and furfural, which synergistically may have been toxic to the organisms.

Declarations

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Credit authorship contribution statement

Jyotsana Dalal, Sam Joy & Mriganko Das: Investigation, Conceptualization, Data curation, Formal analysis & Writing - original draft; **Jaya Rawat:** Project administration, funding acquisition.

Declaration of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

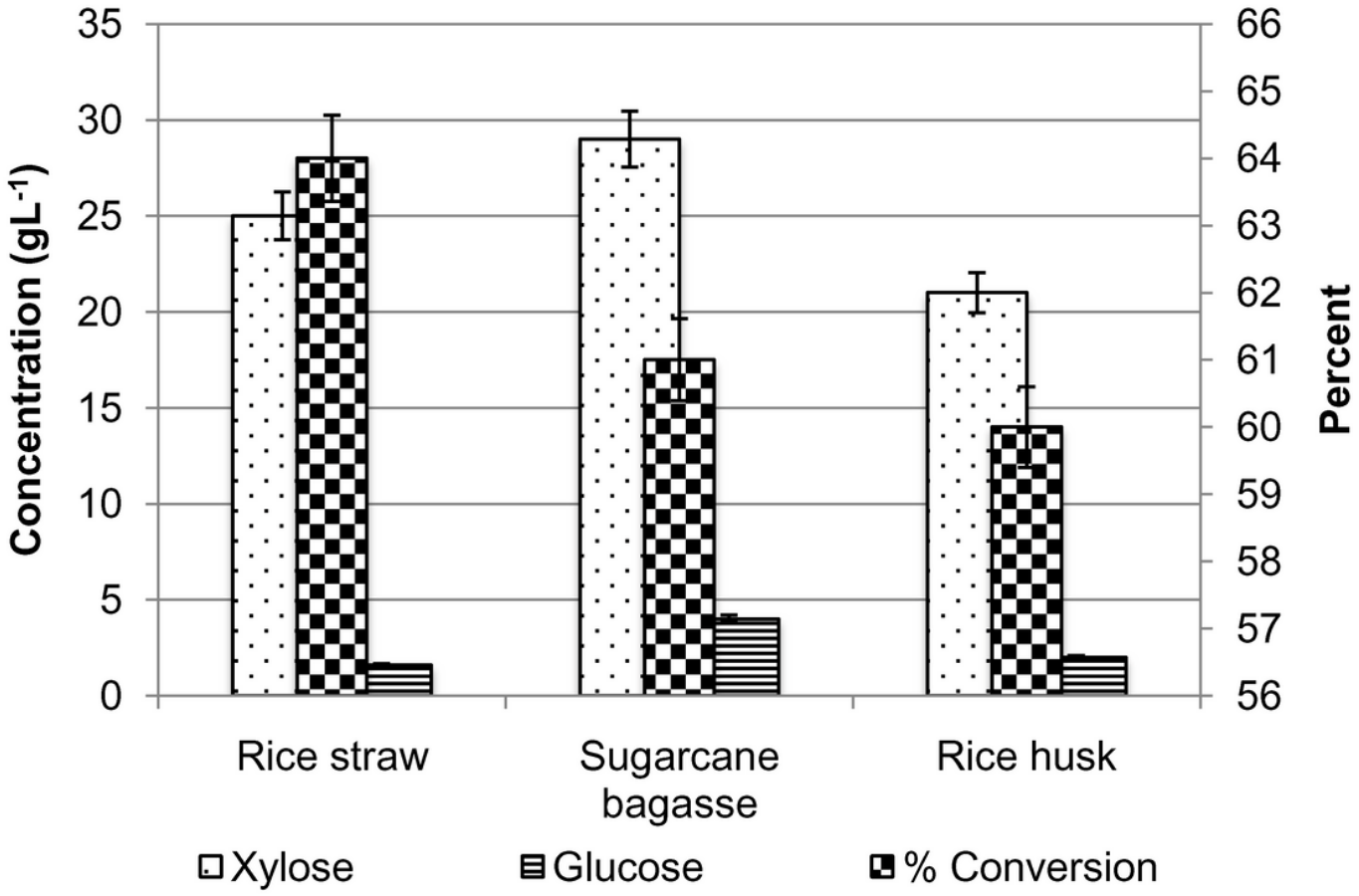

Figure 1

Sugar recovery after dilute acid pretreatment

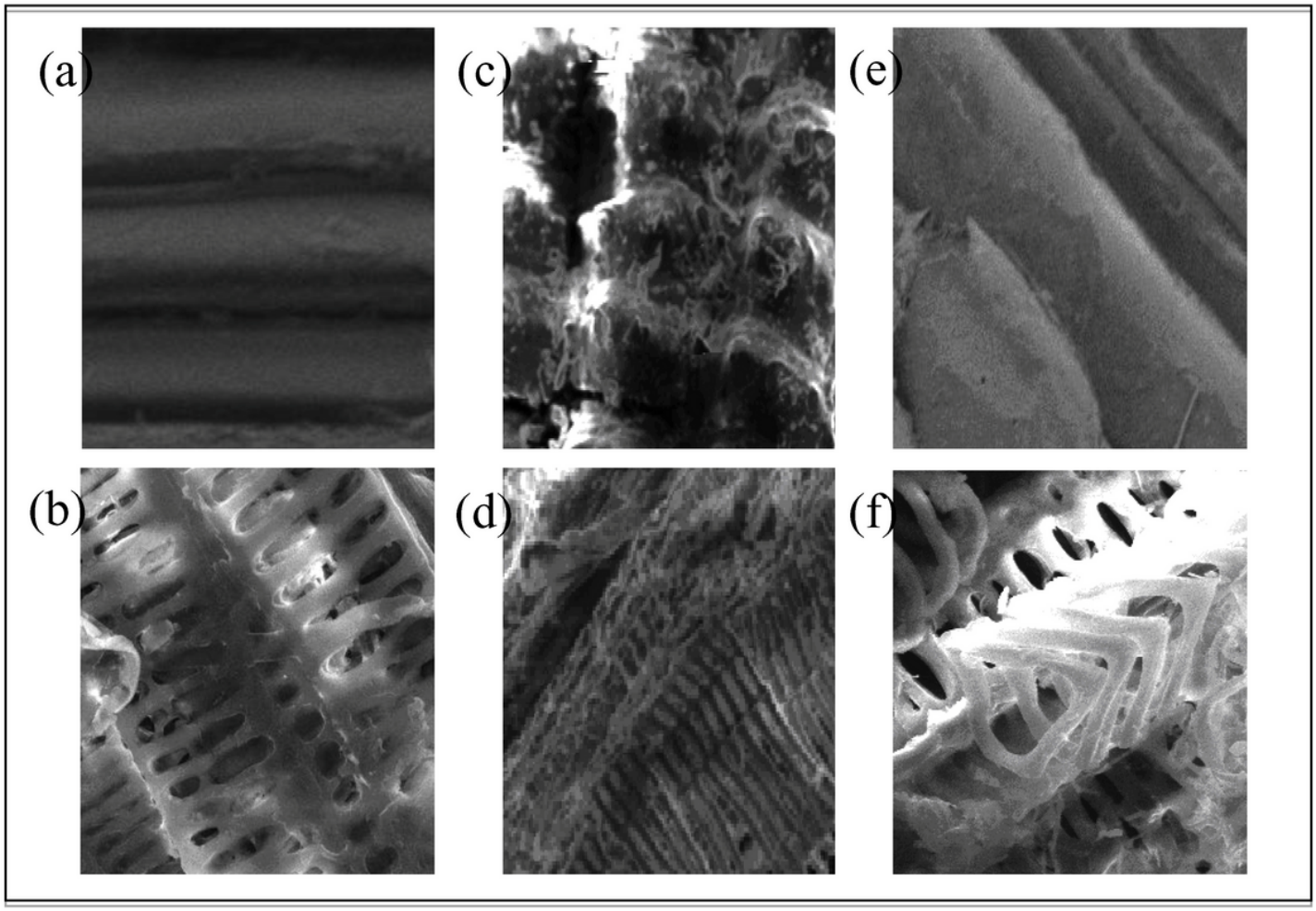

Figure 2

SEM micrograph of biomass before and after pretreatment with dilute sulfuric acid; (a) untreated rice straw, (b) treated rice straw, (c) untreated rice husk, (d) treated rice husk, (e) untreated sugarcane bagasse, (f) treated sugarcane bagasse; Magnification 100X.

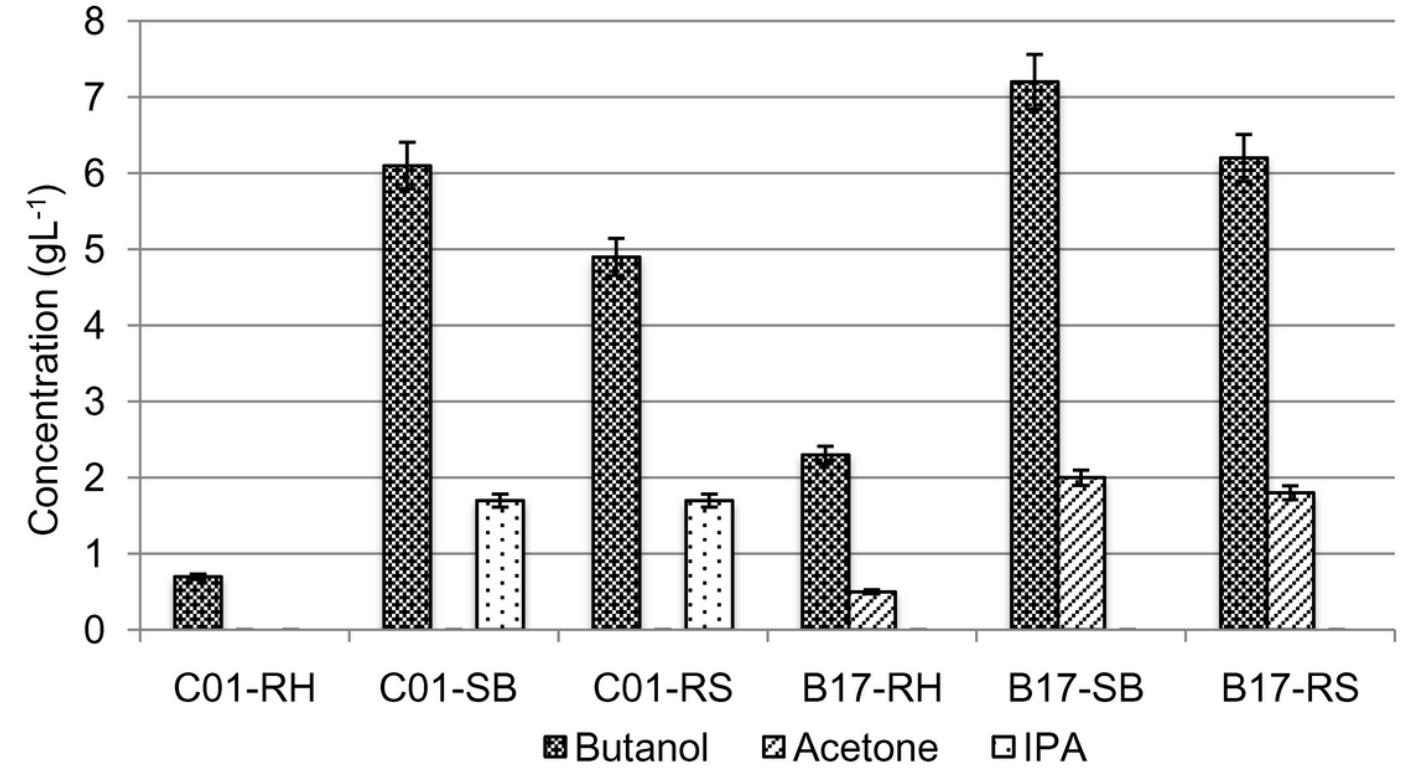

Figure 3

Comparative analysis of solvent production by C. beijerinckii C-01 and C. beijerinckii B-17 on various lignocellulosic hydrolysates.

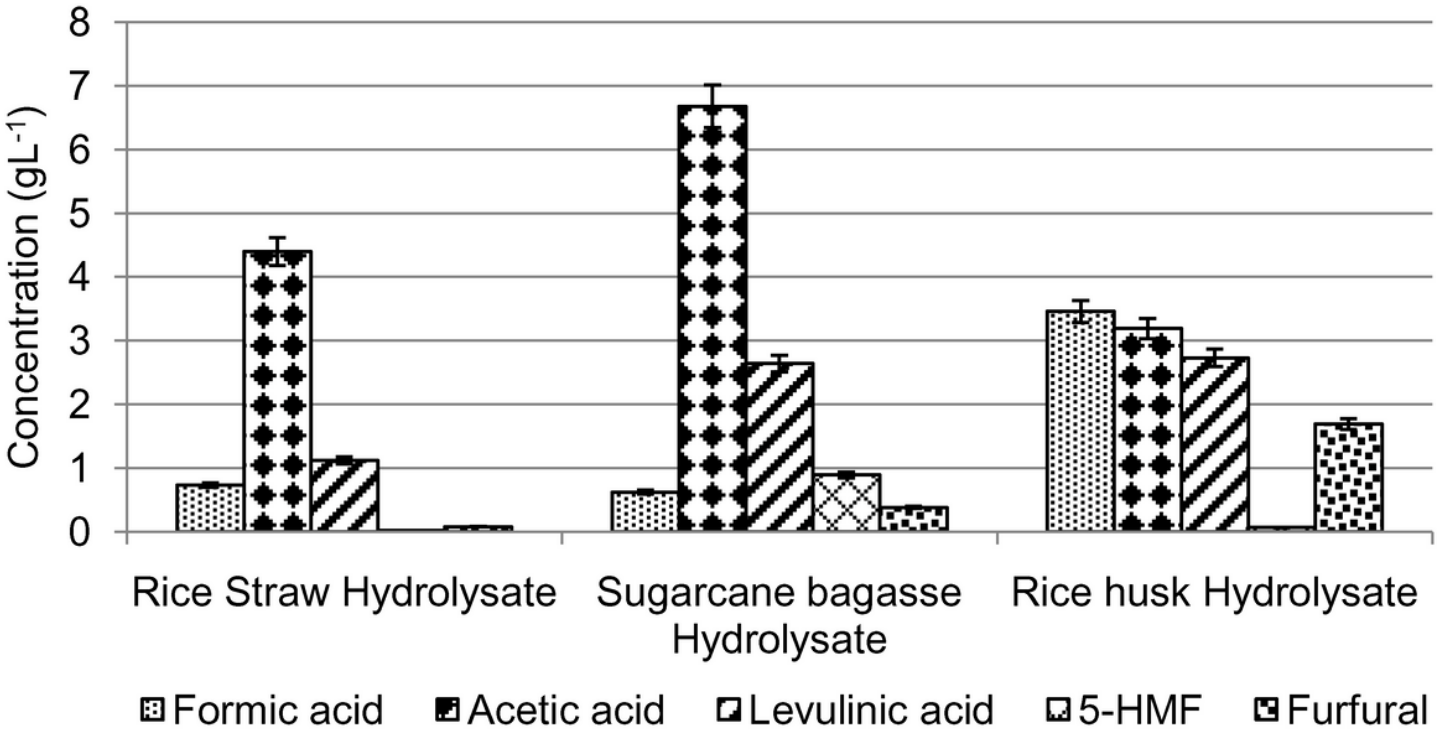

Figure 4

Presence of sugar inhibitors that were measured in different lignocellulosic hydrolysates obtained after dilute acid pretreatment.