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EFFECTS OF PRETREATMENT ON BIOBUTANOL YIELDS FROM RICE-BRAN AND DEOILED RICE-BRAN PROCESSED WITH *Clostridium saccharoperbutylacetonicum N1-4*

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ABSTRACT

Acid and enzymatic pre-treatment of selected lignocellulosic materials [Rice Bran (RB) and Deoiled Rice Bran (DRB)] were conducted to facilitate the release of simple sugars for fermentation process leading to biofuel production. In this study, an innovative approach involving the use of acids to breakdown the complex lingo-cellulosic structure of RB and DRB was utilised. In addition, RB and DRB samples were subjected to physical (heat treatment) and enzymatic treatments, sequentially; in order to improve the amount of simple sugar available as substrate in a microorganism assisted fermentation process. The released sugar from the various treatment regimes were subjected to fermentation process. The highest total fermentable sugar obtained from the Trichloroacetic Acid (TCA), HCl and H_2SO_4 treated hydrolysate were 33.07 g L^{-1}, 27.14 g L^{-1} and 31.93 g L^{-1} for treated hydrolysates, respectively. DRB samples showed a higher total sugar yields and fermentation products than RB samples. The highest butanol yield obtained from the TCA, HCl and H_2SO_4 treated hydrolysate were 8.66 g L^{-1}, 5.32 g L^{-1} and 7.86 g L^{-1}, respectively, after fermentation. The Acetone-Butanol-Ethanol (ABE) yield and productivity of the TCA, HCl and H_2SO_4 treated DRB hydrolysates were 0.47 g/g and 0.1g L^{-1} h^{-1}, 0.32g/g, and 0.07 g L^{-1} $h^{-}1$ as well as 0.42 g/g, and 0.08 g L^{-1} h^{-1}, respectively. (This is consistent with Table 1) The high ABE yield and productivity values suggest that TCA is a good novel pre-treatment agent for biomass fermentation.

Keywords: Acidic hydrolysis, Clostridia, Enzymatic hydrolysis, Ligno-cellulosic materials

INTRODUCTION

In the recent times, there has been an increased interest in the usage of biofuel as an alternative to fossil fuel in the global energy market due to its non-toxic, biodegradable and renewable nature (Negro *et al.,* 2017). The use of biomass for biofuel production on a large scale, though relatively new technology, is attracting an expanding interest and subsequently, special focus through researches is being directed towards exploring series of feedstock and processes that can give the optimum biomass to biofuel conversion ratio (Paulova *et al.*, 2015).

RB, a lignocellulosic material and a by-product of the rice-milling industry has a great potential as a substrate for ABE production because, in addition to being a non-edible commodity most especially in the rural areas, it also contains a mixture of carbohydrates that can be utilized by a solvent-producing microorganism (clostridia) for the production of biofuel. DRB is obtained when the lipid content of RB has been removed. DRB contains mixtures of carbohydrates as well as lignocellulosic polysaccharides (Saunders, 1985). The usage of RB and DRB further assists in solving an environmental problem it creates as wastes considering the fact that it is produced in large quantities mostly in the rice growing areas of Nigeria. The use of DRB in this study is to investigate the effect of the presence of the lipid content of RB on the yield of fermentation products.

RB and DRB as typical examples of lignocellulosic materials undergo either thermochemical or biological processes in the presence of microorganisms before they are converted to ABE. Limited study on this exists in the literature. *Clostridia spp.* is one of the most prominent and productive groups of microorganism associated with the optimum industrial production of ABE by the fermentation process. Microbial fermentation is currently attracting a lot of attention and has a great potential of attaining economic viability in fermentation technology (Dada *et al* , 2013). A major aspect of this conversion process is the pretreatment of the lignocellulosic material (Yang *et al.,* 2017). The lignin-hemicellulose-cellulose complex structure of lignocellulosic materials ensures that the monomers (fermentable sugars) of the carbohydrate polymers (cellulose and

hemicellulose) are bound together with crystalline fibers held by a broad network of hydrogen bonds (in the case of cellulose) and then integrated into the matrix of hemicellulose and lignin. Lignocellulosic materials are thus pretreated in order to break and distort this complex bonding structure and subsequently liberate the monomers for further hydrolysis and fermentation (Baral *et al*, 2015), as it has been shown in some previous works that certain physical, chemical and biological pretreatment mechanisms increase accessibility of enzymes (pretreatment agents) to the embedded carbohydrate polymers (Thompson *et al.*, 1992; Kootstra *et al.*, 2009).

The use of organic acids such as TCA, has been suggested as an alternative to these mineral acids because not only do these organic acids promote the hydrolysis of polysaccharides, they also do not degrade the free sugars (Kootstra, *et al.*, 2009).

MATERIALS AND METHODS

Feedstock

Freshly milled RB sample was obtained from a local rice mill in Ogbomoso Nigeria. The sample was sieved using a 10-mesh particle size sieve to ensure a uniformly sized sample. The RB was kept dry and away from light in a dark air-tight dry container and stored for subsequent usage in a cold room. The temperature of the cold room was kept between 8 and 12 °C. Exposure of oil containing materials to light makes the materials to become rancid.

Preparation of inoculum and fermentation media

The PG medium consisted of 150 g potato, 10 g glucose, 0.5 g $(NH_4)_2SO_4$ and 3 g $CaCO_3$ in 1 L of distilled water, while the TYA medium consisted of 20 g glucose, 6 g tryptone, 2 g yeast extract, 3 g CH_3COONH_4, 0.5 g KH_2PO_4, 0.3 g $MgSO_4.7H_2O$ and 0.01 g $FeSO_4.7H_2O$ in 1 L of distilled water. Similarly, the Fermentation medium consisted of 6 g Tryptone, 2 g Yeast extract, 3 g CH_3COONH_4, 0.5 g KH_2PO_4, 0.3 g $MgSO_4.7H_2O$ and 0.01 g $FeSO_4.7H_2O$ in 1 L of distilled water. In this work, 10% lingo-cellulosic material (treated Rice bran and DRB) was used as the carbon source for the fermentation medium.

Extraction of oil

Oil from the RB was extracted through a solvent extraction process using soxhlet extractor with hexane as the solvent at 60 °C for 6 h (Kar and Mittel, 1981; AOAC, 1984). The soxhlet extraction method was used because of its simplicity, greater yield and purity (Kar and Mittel, 1981).

Physical pretreatment and acidic hydrolysis

Sample of RB or DRB (100 g) was suspended in 1 L distilled water acidified with 1 % (w/v) of TCA to generate TCA-treated RB hydrolysate (TRB) and TCA-treated DRB hydrolysate (TDRB) respectively. Control experiments for both RB and DRB pretreatment were also set up using distilled water only. The mixture was left for 1 h at room temperature after which it was then subjected to boiling at 100 °C for 3 h to generate boiled TRB hydrolysate (BTRB) and boiled TDRB (BTDRB) hydrolysates respectively. The physically treated (boiled) sample was then allowed to cool to room temperature and the pH value was recorded. The sample was then neutralized with 10 M NaOH to pH 7 and then subjected to enzymatic hydrolysis process . The above procedures were repeated with 1% (v/v) of both HCl and H_2SO_4 in separate experiments.

Enzymatic hydrolysis

The enzymes used for enzymatic hydrolysis in this study were cellulose (Celluclast 1.5 L, Sigma Chemicals) and β-glucosidase (Novozyme 188, Sigma Chemicals). Prior to the commencement of enzymatic hydrolysis, the pH of the samples obtained from the acid and physical pretreatments described above was adjusted to pH 4.5 with 0.1M dilute HCl. The pH value was not controlled during the experiment. Enzyme mixture (6 mL/100 g DRB or RB of each enzyme) of cellulase and β-glucosidase was then added to the sample to generate enzyme-treated acid-treated boiled samples (EBTRB and EBTDRB respectively for RB and DRB), and incubated for 72 h at 45 °C in an orbit shaker, the speed of rotation was 170 rpm. A control experiment (without the addition of a mixture of enzymes) was also set up. The above procedures were repeated with HCl and H_2SO_4 in order to determine the efficiency and appropriateness of the use of TCA as a pretreating agent for acidic hydrolysis. The pH of the treated samples was then adjusted to pH7 with 10 M NaOH and samples were taken for total sugar analysis.

Kommentar [U1]:

Kommentar [U2]:

Microorganisms and inoculum preparation

C. saccharoperbutylacetonicum N1-4 which was used in all experiments in this work. It was obtained from the stock culture of the microorganism managed in the Biotechnology Laboratory of the Department of Chemical Engineering of Ladoke Akintola University of Technology (LAUTECH) Ogbomoso. It was maintained as a suspension of

spores in a potato glucose medium (PG medium) as a stock culture and kept at 4 °C. The spores in suspension were transferred into a PG medium at ratio 1:10 ml (v/v). The fresh culture was then transferred into a freshly prepared sterilized Tryptone–yeast extract–acetate medium (TYA medium) and was incubated for 15-18 h at 30 °C and used as the inoculum.

Batch fermentation process

All fermentation experiments were conducted using a 250 mL Duran bottle with the working volume of 100 mL. The hydrolysates that have been subjected to physical, acidic and enzymatic pretreatment were used as the sole source of carbon in the fermentation medium. The pH of the medium was adjusted to 6.0 ± 0.2 after which the medium was then sterilized by autoclaving at 121 °C, 15 psi for 15 min. Anaerobic condition was attained by allowing nitrogen gas to pass through the medium for about 90 sec. in a sterilized environment and the medium was thereafter inoculated with the freshly prepared *C. saccharoperbutylacetonicum N-14*. All the experiments were conducted in duplicate and the average of the values were reported.

Analytical methods

The samples, taken at designated intervals, were centrifuged at 7,500x *g* for 10 min and the supernatant were taken and filtered using 0.2µm cellulose acetate filter. The composition of fermentable sugars prior to and during fermentation and their concentrations were determined using high-performance liquid chromatography (HPLC Agilent 12000 series, Agilent Technologies, Palo Alto, CA, USA) as described by Khamaiseh *et al.* (2012). The equipment is equipped with a refractive index detector (RID) and a 300×7.80 mm Rezex RCM-Monosaccharide Ca^{+2} (8%) column. The mobile phase which was 100% water was run at a flow rate of 0.6 mL/ min. The temperature of the column was maintained at 70 °C. The total run time was 18 min for each sample.

Concentration of the solvents (ABE) and acids were determined using Gas Chromatography (7890A GC-System, Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionisation detector and a 30m capillary column (Equity1; 30 m × 0.32 mm× 1.0 µm film thickness; Supelco Co, Bellefonte, PA, USA). The oven temperature was programmed to increase from 40 °C to 130 °C at a rate of 8 °C/min. The injector and detector temperatures were set at 250 °C and 280 °C, respectively. Helium, as the carrier gas, was set at a flow rate of 1.5 mL/min . Inoculum cell density was determined using a Hach DR2800 spectrophotometer (Hach, Colorado, USA) at 660 nm. The ABE productivity, defined as the ABE concentration divided by the total fermentation time given by g/L h, as well as the ABE yield, defined as the total ABE divided by the total carbohydrate consumed and given by g/g were determined as described by Dada *et al.* (2013).

RESULTS AND DISCUSSION

Effect of different pre-treatment methods on the concentration of sugars

The composition, initial and final concentrations of the different sugars as well as the concentration of the fermentation products with their yields and productivities present in the treated RB hydrolysates are shown in Table 1. The highest amount of total sugar from RB, 29.51 g/L was obtained from the sample with combined pretreatment of boiling for 3 h, TCA hydrolysis and enzymatic hydrolysis (EBTRB) while the highest amount of total sugar from DRB, 33.07 g/L (Table 1) was also obtained from the DRB sample treated with combined pretreatment methods (EBTDRB).

For the effect of heat treatment (boiling), there was an increment of 43 % from the total sugar obtained for RB, 11.38 g/L to 16.28 g/L (BRB) (Table 1) while BDRB increased by 29.1 % from 16.16 g/L (DRB) to 20.82 g/L (Table 1). The total ABE for BRB hydrolysate, 6.47 g/L (Table 1) represents an increase of 65 % on the ABE of RB while for BDRB, there was an increment of 13% over the unboiled DRB hydrolysate (Table 1). The total ABE for the boiled DRB hydrolysate BDRB was 8.13 g/L, this was also higher than the total ABE for the untreated hydrolysate. The implication of this is that the physical treatment (boiling) significantly affects the release of sugars and subsequently, the amount of solvents produced. This is in agreement with an observation from a previous study (Hipolito *et al.*, 2008).

Samples of RB and DRB that were not subjected to any treatment were used after sterilization as the control experiments. Interestingly, a small amount of sugar was found in these samples, 11.38 g/L and 16.16 g/L respectively. The presence of sugar after sterilization of RB and DRB was suspected to be due to the effect of heat on the lignocellulosic structure of the biomass which must have caused a slight degradation of the polysaccharides thus releasing some sugar during the sterilization process. This effect contributes to the overall pretreatment effect on the samples. However, it was observed that there was a significant increase in the amount of sugars released after the various pretreatment mechanisms according to the applied treatments. The hydrolysates from RB showed the lowest sugar content compared to DRB. This may

be due to the presence of oil in RB which has been removed in DRB.

The data for ABE and total acid production as well as the productivity of the untreated RB are shown in Tables 1. The low solvent produced may be attributed to the small amount of sugar released due to untreated sample. The productivity of ABE using this hydrolysate is the smallest of all the productivities recorded in this work. However, the total ABE production (7.17 g/L) of the untreated DRB, is higher than that of the untreated RB, so also was the productivity (0.05 g/L h). This may be due to the availability of the higher amount of fermentable sugar which was due to the removal of lipid structure through extraction. In addition, there is the possibility that the presence of oil adversely affects the sugar uptake aspect of the fermentation reaction thus inhibiting the formation of solvents. This agreed with the observations from previous work (Lin and Tanaka, 2008). The data for ABE and total acid production as well as the productivity of the untreated RB are shown in Tables 1. The low solvent produced may be attributed to the small amount of sugar released due to untreated sample. The productivity of ABE using this hydrolysate is the smallest of all the productivities recorded in this work. However, the total ABE production (7.17 g/L) of the untreated DRB, is higher than that of the untreated RB, so also was the productivity (0.05 g/L h).

Table 1: Fermentation of RB Hydrolysates and DRB Hydrolysates

	Medium	Glucose conc (g/L)		Fructose conc (g/L)		Sucrose conc (g/L)		Xylose Conc (g/L)		Products (g/L)					ABE Productivity (g/L h)	Yield (g/g)
		Initial	Final	Initial	Final	initial	Final	Initial	Final	Acetone	Butanol	Ethanol	Acetic Acid	Butyric Acid		
RB Hydrolysates	RB	6.81	0.02	1.59	0.33	1.59	0.02	1.39	0.05	1.37	2.02	0.52	1.28	0.52	0.03	0.38
	BRB	12.09	0.02	2.09	0.02	0.02	0.02	2.06	0.05	1.98	3.7	0.79	1.83	0.8	0.05	0.39
	TRB	13.37	0.02	3.55	0.02	0.02	0.02	0.05	0.05	1.02	4.07	0.71	0.84	0.59	0.05	0.34
	BTRB	16.73	3.22	3.35	0.02	0.02	0.02	3.57	0.05	3.06	4.73	0.57	1.71	1.08	0.06	0.41
	EBTRB	14.46	0.02	1.02	0.02	13.97	2.01	0.05	0.05	2.26	5.32	1.1	0.73	0.89	0.07	0.32
DRB Hydrolysates	DRB	16.1	0.02	0.02	0.02	0.02	0.02	0.05	0.05	3.21	3.07	0.89	2.25	0.77	0.06	0.45
	BDRB	20.77	0.02	0.02	0.02	0.02	0.02	0.05	0.05	3.74	3.5	0.89	3.47	1.27	0.06	0.39
	TDRB	20.73	0.02	3.45	0.02	0.02	0.02	0.05	0.05	5.02	4.43	0.95	2.74	0.79	0.08	0.43
	BTDRB	14.74	5.93	1.05	0.02	14.28	0.02	0.81	0.08	3.04	5.03	1.24	2.67	2.05	0.08	0.37
	EBTDRB	28.82	8.42	4.17	0.02	0.02	0.02	0.05	0.05	2.73	8.66	0.25	3.81	1.3	0.1	0.47

This may be due to the availability of the higher amount of fermentable sugar which was due to the removal of lipid structure through extraction. In addition, there is the possibility that the presence of oil adversely affects the sugar uptake aspect of the fermentation reaction thus inhibiting the formation of solvents. This agreed with the observations from previous work (Lin, and Tanaka, 2008).

Fermentation using EBTRB hydrolysate

In the first 12 h of the fermentation of EBTRB hydrolysate, 3.97 g/L of sugar was consumed (Figure 1). The product profiles for this process which demonstrated a typical biphasic growth pattern of solventogenic microorganism are shown below (Figures 2a and 2b). This is understandable as it had earlier been established that for solventogenic microorganisms such as *C. saccharoperbutylacetonicum N1-4*, acids are mainly produced at the early stage of the exponential phase which normally falls within the first 12 h, while the production of solvents takes place during the late exponential phase (referred to as solventogenesis stage) (Mitchell *et al.*, 1991).

It has been reported that in the early stage of fermentation if the amount of sugar consumed is less than 10 g/L, the process is most likely to produce more of acids than solvents (Fond *et al.*, 1985). At 120 h, the total ABE produced was 8.69 g/L while the total concentration of acids produced was 1.62 g/L (0.73 g/L acetic acid and 0.89 g/L butyric acid) (Figure2b). The small value of the total acid after 120 h compared to the total ABE produced also suggested that there had been a reaction shift from acidogenesis to solventogenesis which was responsible for the higher amount of solvent obtained. The ABE productivity was 0.07 g/L h while the ABE yield was 0.32 g/g (Table 2). In addition, the pH profile (Figure 3) showed compliance with the established pattern reported elsewhere (Hatzinikolaou and Wang, 1991).

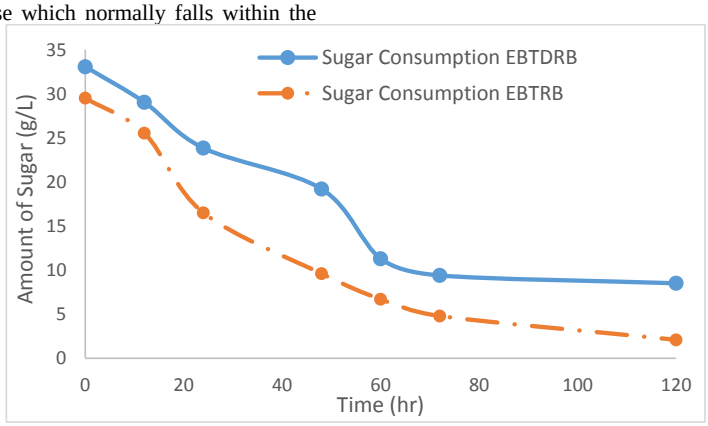

Fig. 1. Sugar consumption of EBTRB and EBTDRB

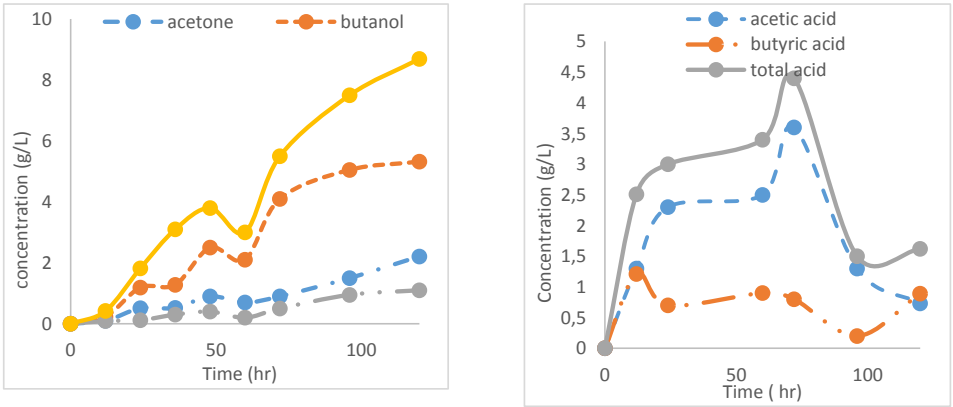

Fig. 2. ABE fermentation using EBTRB (a) Total ABE, acetone, butanol, and ethanol. (b) Acetic acid, butyric acid and total acid

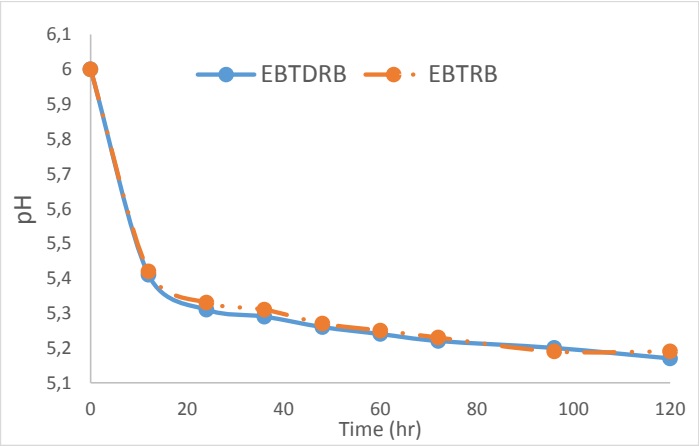

Fig. 3. pH profiles of EBTDRB and EBTRB

The pH profile of EBTDRB hydrolysate (Figure 3) agreed with the pH profile for inorganic acids such as HCl and H_2SO_4 that are commonly used as pretreatment agents (Hatzinikolaou and Wang, 1991). The ABE productivity was 0.097 g/L h while the ABE yield was 0.47 g/g.

Fermentation using EBTDRB hydrolysate

The sugar consumed in the first 24 h of the fermentation of EBTDRB hydrolysate is 9.17 g/L (Figure 1). The total ABE produced during this time was 1.95 g/L while the total acid was 1.83 g/L (Figures 4a and 4b). The total ABE produced, after 120 h, was 11.65 g/L while the total acid was 5.1 g/L. The product profiles for this process (Figures. 4a and 4b), indicates the characteristic growth behavior of biphasic solventogenic microorganisms is exhibited. Though the transition from acidogenesis to solventogenesis takes place in the early stages of fermentation as explained earlier, however, this shift may undergo delay due to some factors. It is possible that the activities of the microorganism are inhibited because of the absence of appropriate nutritional enhancement. This may delay or alter the mechanism of the acidogenesis stage which will obviously affect the rate and extent of solvent formation in the solventogenesis stage. There is still an appreciable production of butyrates and acetates (Figure 4b), during solventogenesis and this may be due to incomplete phase transition; this unusual occurrence may be attributed to the biphasic nature of the microorganism. It is possible that the microorganism could not attain complete growth and consequently, degeneration- a situation where microorganisms find it difficult to survive and eventually sporulate may have set in, this may be responsible for the continuous production of the acids. In addition, as observed by Lee *et al.*, (2009), the formation of growth inhibitors during acid hydrolysis may also influence the carbon flux from the metabolized sugars to favour both acid and solvent production simultaneously. However, the absence of oil from the DRB hydrolysates might have been responsible for the higher yield of fermentation since this would have ensured the absence of the several inhibitors attributable to the presence of oil during acid hydrolysis.

The pH profile of EBTDRB hydrolysate (Figure 3) agreed with the pH profile for inorganic acids such as HCl and H_2SO_4 that are commonly used as pretreatment agents (Hatzinikolaou and Wang, 1991). The ABE productivity was 0.097 g/L h while the ABE yield was 0.47 g/g (Table 2).

Fermentation using hydrolysates of HCl and H_2SO_4

The result of the HCl treated and H_2SO_4 treated hydrolysates are shown in Table 4. After 120 h incubation, the HCl treated hydrolysate gave a total ABE of 8.69 g/L while the total sugar consumed during the same time was 21.62 g/L implying that the yield and productivity of the process were 0.4 g/g and 0.07 g/L h respectively. For H_2SO_4 treated hydrolysate, the amount of the total sugar consumed in 120 h was 31.85 g/L and the total ABE produced was 13.67. The yield and productivity of this hydrolysate were 0.42 g/g and 0.1 g/L h respectively. The presence of the inhibitors formed during acid hydrolysis of HCl and H_2SO_4 hydrolysates might have hindered the activities of the microorganisms during the fermentation process and this may have contributed

to the lower yield and productivity of the hydrolysates.

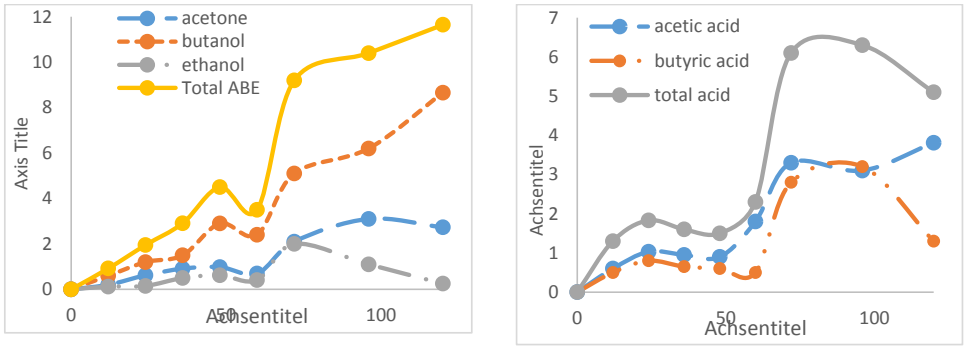

Fig. 4. Product profiles for EBTDRB (a) Total ABE, acetone, butanol, and ethanol (b) Acetic acid, butyric acid and total acid.

Table 4: Comparison of Fermentation of Lignocellulosic Materials

Substrate	Hydrolysis Method	Culture	Butanol Produced (g/L)	Total ABE (g/L)	Yield (g/g)	Productivity (g/L h)	Reference
Bagasse	Alkali pre-treatment and enzyme hydrolysis	*Clostridium saccharobutylicum ATCC 27022*	11	14	0.3	0.12	Ezeji and Blaschek
Rice straw	Alkali pre-treatment and enzyme hydrolysis	*Clostridium saccharobutylicum ATCC 27022*	10	13	0.28	0.11	Ezeji and Blaschek
Wheat straw	Alkali pre-treatment and enzyme hydrolysis	*Clostridium acetobutylicum IFP 921*	10.3	17.1	0.22	0.14	Ezeji and Blaschek
Corn stover	Steam explosion	*Clostridium acetobutylicum NCIB 644*	8	12.8	0.27	0.11	Ezejiand Blaschek
Corn fibre	Dilute H_2SO_4	*Clostridium beijerinckii BA101*	6.4	9.3	0.39	0.07	Ezeji and Blaschek
DDGS	Dilute H_2SO_4	*Clostridium saccharobutylicum 262*	7.2	12.1	0.35	0.1	Ezeji and Blaschek
RB	TCA+Boiling + Enzymatic hydrolysis	*Clostridium saccharoperbutylacetonicum N1-4*	5.32	8.68	0.32	0.07	This study
DRB	Dilute H_2SO_4 +Boiling + Enzymatic hydrolysis	*Clostridium saccharoperbutylacetonicum N1-4*	7.86	13.67	0.42	0.1	This study
DRB	Dilute HCl + Boiling +Enzyme hydrolysis	*Clostridium saccharoperbutylacetonicum N1-4*	5.33	8.69	0.32	0.07	This study
DRB	TCA+Boiling + Enzymatic hydrolysis	*Clostridium saccharoperbutylacetonicum N1-4*	8.66	11.65	0.47	0.1	This study

Table 5. Standard Deviation of Total Sugar Consumption in Two Experiments

Samples	Average Sugar Consumed (g/L)	Standard Deviation
RB	10.96	0.033941
BRB	16.15	0.067882
TRB	16.88	0.039598
BTRB	20.36	0.050912
EBTRB	27.31	0.206475
DRB	16.08	0.002828
BDRB	20.75	0.028284
TDRB	24.14	0.042426
BTDRB	24.83	0.042426
EBTDRB	24.55	0.056569

Inorganic acids such as HCl and H_2SO_4 have been reported to degrade pentose sugar monomers (e.g. xylose) and hexose sugar monomers (e.g. glucose) into furfural and hydroxymethyl-furfural, respectively, during acid hydrolysis (Zheng *et al.*, 2009). These two are very toxic inhibitors that adversely affect the yield and productivity of fermentation products. Despite the high amount of sugar contained in the inorganic acid hydrolysates, the yield and productivity of biobutanol from the organic acid hydrolysate are higher (Table 4)

The productivity and yield of butanol and total ABE for different biomass in comparison with the biomass used in this study is presented in Table 4. The result shows the efficiency of the current pretreatment method compared with the existing methods as reported by Ezeji and Blaschek (2010). As stated earlier, the average of values from duplicated experiments was used for the final analysis. The small values of the standard deviation show that the average data set used did not deviate so much from the original values obtained in the actual experiments (Table 5)

CONCLUSION

In order to get maximum yield of fermentation products from RB, it must first be deoiled as it has been shown from this study that the yield of DRB hydrolysates is higher than those of the corresponding RB hydrolysates. The oil is seen as a natural inhibitor for the production of biofuels from RB. The oil removed can be further treated to generate additional biofuel product (biodiesel). It has also been shown here that DRB should be hydrolysed in order to increase the total fermentable sugar that will be available for the fermentation process. The use of TCA for acid hydrolysis in DRB hydrolysates generated the highest amount of total sugar available for fermentation when compared with the existing methods. The DRB hydrolysate that was physically pretreated (by boiling), and later subjected to acid (TCA) as well as enzymatic hydrolysis; EBTDRB, produced the highest amount of butanol out of all the hydrolysates considered in this study. Furthermore, the ABE productivity (0.1 g/L h) and yield (0.47 g/g) of this approach have shown that TCA is a good pretreatment agent for acid hydrolysis of RB and DRB. Above all, the strategic use of TCA as a pre-treatment agent in this work is considered a novel idea because the high yield and productivity obtained had disproved the notion that TCA as a phytotoxic acid, will inhibit the growth of microorganism in the fermentation medium and hence not suitable as a pre-treatment agent for lignocellulosic materials. The successful usage of TCA for pre-treatment of biomass for fermentation is yet to be reported.

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