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Pretreatment and hydrolysis methods for recovery of fermentable sugars from de-oiled Jatropha waste

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HIGHLIGHTS

- Release of reducing sugars from the de-oiled Jatropha waste was evaluated.
- Enzymatic hydrolysis resulted in the maximal sugar concentration release.
- Ultrasonication and heat pretreatments could not significantly enhance sugar recovery.
- Higher sugar recovery was efficiently achieved via combined hydrolysis.
- H_2 fermentation from the released sugars was evaluated.

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ABSTRACT

The release of reducing sugars (RS) upon various pretreatments and hydrolysis methods from de-oiled Jatropha waste (DJW) was studied. The highest RS concentration of 12.9 g/L was observed at 10% enzyme hydrolysis. The next highest RS of 8.0 g/L and 7.8 g/L were obtained with 10% HCl and 2.5% H_2SO_4, respectively. The NaOH (2.5%), ultrasonication and heat (90 °C for 60 min) treatments showed the RS concentration of 2.5 g/L, 1.1 g/L and 2.0 g/L, respectively. Autoclave treatment slightly enhanced the sugar release (0.9 g/L) compared to no treatment (0.7 g/L). Glucose release (11.4 g/L) peaked in enzyme hydrolysis. Enzyme treated acid unhydrolysed biomass showed 11.1 g/L RS. HCl and H_2SO_4 pretreatment gave maximal xylose (6.89 g/L and 6.16 g/L, respectively). Combined (acid and enzyme) hydrolysis employed was efficient and its subsequent batch hydrogen fermentation showed a production 3.1 L H_2/L reactor.

1. Introduction

Lignocellulosic materials are more reliable and promising feedstock for the fermentative biofuels production once the sugars such as cellulose are recovered from its complex structure. However, extraction of soluble sugars from these recalcitrant materials is a tedious process. Biofuels fermentation with the raw waste is more energy intensive and low yield process. Thus pretreatment must be done to make the process viable and sustainable (Fan et al., 2005). Pretreatment using acid or alkali promises more sugar recovery. In addition, the formation of inhibitors also is not avoidable due to the harshness of these agents. These inhibitors are not suitable for the hydrogen fermentation since they show negative effects on the hydrogen-producing organisms (Cao et al., 2009; Klinke et al., 2004).

The hydrolysis methods to recover the fermentable sugars vary with different biomass. For example, Ren et al. (2010) reported that alkali treatment of corn stover could recover more sugars whereas Datar et al. (2007) found acid hydrolysis could recover high quantity of RS. The possible reason for this discrepancy is the varying composition (cellulose, hemicellulose, and lignin content) based on the chemical nature of the biomass. Suitable hydrolysis steps and methods or inhibitor production control could reduce the cost and simultaneously improve the fermentation process (Cao et al., 2009).

The generation of de-oiled Jatropha waste (DJW) increased tremendously in recent years due to the high demand of biodiesel as an alternative biofuel. A Jatropha based biodiesel plant produces 2.5–3.0 tons of solid waste (DJW) per one ton of biodiesel (Srividhya et al., 2010). This DJW could not be used as animal fodder due to its toxic nature. Besides, the disposal or management of

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this solid waste is expensive and hazardous to environment if improperly treated (Sricharoenchaikul and Atong, 2009). Previous studies reported that the reducing sugars recovered from this lignocellulosic waste could be a feasible source for growing marine organism and solublising protein as a valoralized product (Liang et al., 2010; Kootstra et al., 2011). In addition it has been used in the co-composting and enzyme production such as xylanase (Das et al., 2011; Joshi and Khare, 2011). However, in a recent study, this cellulose-based material was shown to be a feasible feedstock in hydrogen fermentation (Kumar et al., 2012). Pretreatment of this waste enhances the availability of sugars which could be a substrate for anaerobic H_2 fermentation. In this study we demonstrated different pretreatment and hydrolysis methods for recovering sugars from DJW. Additionally, the possibility of hydrogen fermentation using the released reducing sugars was evaluated.

2. Methods

2.1. DJW characterization

DJW used in this study was collected from a Jatropha biomass-based biodiesel producing industry in central Taiwan. The cellulosic content was analyzed as 42.3% of fermentable sugars (14.1% cellulose and 28.2% of hemicellulose) using FIBERTEC™ 1020 (M6) analyzer as mentioned elsewhere (Kumar et al., 2012).

2.2. Sugar recovery and hydrogen fermentation

Heat treatment was done at various temperatures (80, 90 and 100 °C) and different time intervals (30, 45 and 60 min) in a boiling water bath. Ultrasonication was performed for various time (30, 45 and 60 min) in a sonicator operated at ultrasound wave 95 Hz and sonic temperature of 45 °C. Acid (HCl and H_2SO_4), enzyme (Viscozyme L, purchased from Sigma, product no.: V2010) and alkaline treatments were done in the range of 0.5–10% as a mixture of 1:20 ratio (w/v) (i.e. 5 g of dried and powdered substrate was mixed with 100 mL aqueous solution of the above mentioned chemicals). Acid and alkaline samples were further kept in an autoclave (121 °C for 30 min), whereas enzyme hydrolysis was operated at 50 °C in a water bath with a contact time of 3 h. Autoclave pretreatment was performed as mentioned before, but without any acid or alkali addition. After pretreatment the hydrolysate pH was neutralized (7.0) using 6 N HCl or 6 N NaOH. Enzymatic hydrolysis after acid pretreatment was carried out with the unhydrolysed biomass that remained after 10% HCl pretreatment. All reagents used were analytical grade.

Batch hydrogen fermentation experiments were carried out in 60 mL bottles, which contained 40 mL of hydrolysate, 12 mL of sewage sludge, 1–3 mL for pH adjusting solution as either 1 N HCl or NaOH and 5 mL of micronutrient solution. The micronurtrient solution components were (mg/L): K_2HPO_4, 125; $MgCl_2{\cdot}6H_2O$, 100; $MnSO_4{\cdot}6H_2O$, 15; $FeSO_4{\cdot}7H_2O$, 25; $CuSO_4{\cdot}5H_2O$, 5; and $CoCl_2{\cdot}5H_2O$, 0.12. The batch reactors were kept in an incubator operating at 37 °C and 150 rpm. The biogas volume was measured using a glass syringe at 1 atm and room temperature.

2.3. Analytical methods

The hydrolysates were filtered through a 0.22 μm filter and analyzed by HPLC equipped with RID (Shimadzu LC-10AT) for the sugars, sugar derivatives and inhibitors. Reducing sugar (RS) concentration was measured by DNS method (Miller, 1959). Sugar recovery was calculated based on the total solids (TS) and cellulosic contents (cellulose + hemicellulose) of DJW. The gas composition was analyzed using a GC equipped with a thermal conductivity detector. The yield of unhydrolysed biomass (YUHB) was calculated based on the formula given below:

$$\text{YUHB } (\%) = \frac{\text{Amount of unhydrolysed biomass remained}}{\text{Amount of initial biomass added (5g)}} * 100$$

3. Results and discussions

3.1. Selection of efficient pretreatment method for DJW

A previous study has revealed that a two step (lime followed by enzyme) pretreatment is necessary to release maximal fermentable sugars (Liang et al., 2010). In the first step, acid or alkali acts on the hemicellulose structure to release the pentose sugars like xylose and arabinose. In the second step, enzyme hydrolyses the residual waste (rich in cellulose) to recover the hexose sugar as glucose (Liang et al., 2010). In the present study both single and two step pretreatment of DJW was evaluated and the amount of released sugars is summarized in Table 1. Single step enzymatic hydrolysis resulted in the release of 12.9 ± 0.22 g/L reducing sugars (RS), and was the highest among the single step pretreatments.

The main role of pretreatment is to retrieve the monomeric sugars such as glucose, xylose, arabinose and cellobiose from the cellulosic and hemicellulose part of the lignocellulosic compartment (Cui et al., 2009). Pretreatment of DJW with enzyme solution at a concentration of 10% (v/v) (DJW (1 g): 10% enzyme solution (2 mL)) showed 60.6 ± 1.0% and 25.7 ± 0.4% sugar recovery based on total cellulosic and total solids contents, respectively.

The next higher recovery was attained via acid treatments. HCl pretreatment showed 37.7 ± 0.3% and 16.0 ± 0.1% whereas H_2SO_4 showed 37.0 ± 0.45 and 15.7 ± 0.2% based on total cellulosic and total solids contents, respectively. Autoclave treatment slightly enhanced RS (0.9 ± 0.03 g/L) as compared to no treatment (0.7 ± 0.08 g/L). Ultrasonication and heat treatment also improved the recovery but not significantly.

A positive correlation (correlation coefficient >0.9) was observed between the sugar release and the concentration of acid during the HCl (0.5–10%) and H_2SO_4 (0.5–5%) pretreatment. The total sugar released ranged from 1.4–7.9 g/L and 1.4–7.8 g/L by using HCl and H_2SO_4 pretreatments, respectively. Similar results were found in all other pretreatment methods except heat-treatment and ultrasonication. In heat treatment it was found that incubation time of more than 45 min reduced the sugar release (Fig 1). In case of ultrasonication 45 min of incubation was found to be suitable for maximal sugar release. The sugars released from ultrasonication,

Table 1
Maximal sugar release and recovery from various pretreatment methods.

Pretreatment	RS (g/L)	Sugar recovery (%)		Yield of unhydrolysed biomass (YUHB-%) (g/100 g TS)
		(g RS/ 100 g TS)	(g RS/100 g cellulosic content)	
NT	0.7 ± 0.08	1.3 ± 0.2	3.2 ± 0.4	96.7 ± 1.2
AC	0.9 ± 0.03	1.8 ± 0.1	4.2 ± 0.2	78.0 ± 2.0
US	1.1 ± 0.06	2.2 ± 0.1	5.1 ± 0.3	81.3 ± 1.2
HT	2.0 ± 0.07	4.1 ± 0.3	9.6 ± 0.3	77.3 ± 1.2
HCl (10%)	8.0 ± 0.05	16.0 ± 0.1	37.7 ± 0.3	46.0 ± 2.0
H_2SO_4 (2.5%)	7.8 ± 0.08	15.7 ± 0.2	37.0 ± 0.4	45.3 ± 3.1
NaOH (2.5%)	2.5 ± 0.19	5.0 ± 0.5	11.8 ± 0.9	68.0 ± 0.0
Viscozyme (10%)	12.9 ± 0.22	25.7 ± 0.4	60.6 ± 1.0	69.3 ± 1.3
A–E	11.1 ± 0.10	22.3 ± 0.2	52.5 ± 0.4	60.7 ± 3.1
CH	NC	38.3 ± 0.3	90.2 ± 0.7	NC

NT – no treatment, HT – heat treatment (90 °C, 60 min), AC – autoclave, US – ultrasonication (95 Hz 45 min), A–E – acid enzyme hydrolysis (10% HCl + 5% enzyme), CH – combined hydrolysis (10% HCl + A–E), NC – not calculated.