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Effect of Rice-Bran Pretreatment in Biohydrogen Production

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Authors' contributions

This work was carried out in collaboration between all the authors. Author EOD designed the study. Author AOA wrote the protocol while author ATA wrote the first draft of the manuscript. All authors managed the analyses of the study. Author TJA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the effect of different pretreatment methods on sugar liberation from Rice bran (RB) and Deoiled Rice bran (DRB). An amount of 100 g of RB or DRB were soaked in 1% (w/v) trichloroacetic acid (99.0 % stock concentration) separately and each mixture was made up to 1L with distilled water to generate the Acid treated RB hydrolysate (ARB) and acid treated DRB hydrolysate. RB and DRB were also subjected to a combined treatment of acid, hydrothermal (boiling) and enzyme treatments to investigate the effects of the combination of the three different methods. Sugar compositions of pretreated samples were determined using high-performance liquid chromatography. It was that there was an increment of more than 400 % and 300 % respectively in the concentration of sugar obtained from acid treated RB and DRB hydrolysates over the untreated RB and DRB hydrolysates. In combined treatment, RB hydrolysates showed an increment of 584%

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in the amount of total sugar released as well as 500 % increment in DRB hydrolysates. The amount of sugar liberated from DRB hydrolysates is slightly more than the respective RB hydrolysates subjected to same treatment. The increase in the amount of rice bran hydrolysates produced demonstrated that pretreatment of Rice bran with acid, boiling and via enzymatic reactions can increase the amount of biohydrogen produced.

Keywords: Rice bran; De-oiled Rice bran; Boiling; Acid; Enzymes.

1. INTRODUCTION

The role of fossil fuel in meeting the ever-increasing demand of power generation in the world today cannot be overemphasized as it has contributed approximately 80 % of the total power generation [1]. However, the burning of fossil fuels has led to the increase in the amount of greenhouse gas (majorly CO_2 and NOx) in the atmosphere. Also, these gases have a negative consequence in the public, environmental and ecological health [2]. Reduction in the volume of these dangerous gases to the atmosphere calls for a clean and sustainable energy. Hydrogen fuel provides a green and sustainable energy as it has no trace of Nitrous oxides when burnt in air. The product of hydrogen fuel combustion is only water vapour with a low energy density (12 MJ/kg) when compared to that of methane (53 MJ/kg) and gasoline (46 MJ/kg) [3], placed it above other fossil fuel alternatives. Hydrogen fuel supply to industrial users has become a major business around the globe. The demand for hydrogen fuel has risen to threefold since 1975 [4]. However, the present production of hydrogen (120 million tonnes per year) is insufficient to meet the huge demand. In fact, green hydrogen demand is expected to have an annual increase of 5.48 % [5] and according to Goldmansach's 2020 equity research report, green hydrogen provides close to one-fourth of the global energy demand by 2050 with a potential market of around US$10 trillion [6]. Although, hydrogen production from biomass via biotransformation methods involving either microbial fermentation or thermochemical activities have been reported, however, biohydrogen production from cellulosic materials especially lignocellulosic have not been extensively researched.

Lignocellulosic are complex materials that is comprised of cellulose, hemicellulose and lignin at it is regarded as a good feed for biohydrogen production as it is almost ubiquitous and low cost. Some past studies have demonstrated the potential of lignocellulosic materials in biohydrogen production.

Rice bran is an example of a lignocellulosic material that is a byproduct obtained from rice milling industry. Rice bran is a good substrate for biohydrogen production due to its non-food nature. It is a mixture of celluloses, hemicellulose and starch which are useful materials in hydrogen production. It also consists of natural nutrients like Ca^{2+}, Zn^{2+}, and Mg^{2+} that are useful for biohydrogen production [7], [8]. However, the complex nature of Rice bran restricts the access to its cellulosic compounds useful for hydrogen production [9]. Hence, disintegrating such biomass before hydrogen production is the possible solution to this challenge. Disintegration of biomass has the capability to modify the chemical and structural arrangement of hemicellulose, cellulose and increases the polymerization extent and surface area [9], [10]. A lot of disintegration processes have been reported such as the use of chemicals (acid, alkali), physical (thermal) mechanical (ball mill, ultrasonication) to improve the biomass biodegradability and biohydrogen production [1]. The different disintegration methods have their own advantages and shortcomings. Dispersion is one of the finest disintegration techniques in biomass liquefaction [11], however, reported studies have shown that it is energy intensive [12]. Thermal process is also an effective disintegration method for enriched biohydrogen production, however, an adverse operating condition for example at high temperature above 160^0C, hemicellulose and lignin solubilizes which thus limiting thermal pretreatment potential of biomass [1], [11]. In addition, the use of alkalis has proved to be an effective pretreatment method as it offers improved biomass porosity and internal surface area, and increases biohydrogen production, however, some alkalis may get trapped in the waste biomass which could result in environmental challenges especially in disposal and recycling of biomass and chemicals [12]. With all this in mind, the objectives of this study were to investigate the effect of various pretreatment methods on sugar liberation from rice bran and de-oiled rice bran hydrolysates as well as comparison of the initial

rice bran concentration with sugar amount liberated for fermentation.

2. MATERIALS AND METHODS

2.1 Preparation of Samples

2.1.1 Milled ricebran

Milled RB sample (Fig. 1) was sourced from a local rice milling industry in Kilang BERNAS, Selangor, Malaysia. The sample was strained by No. 45 mesh particle size sieve to obtain uniform particle size (Sigma-Aldrich 2012). The sample particle size ranged between 0.18 – 0.39 mm as determined by (Schmidt and Furlong). The RB was preserved and kept away from light in a dark, air-tight, dry container and stored in a cold room for further use, this is because oil-containing materials under storage undergo rancidity when exposed to light over a long period of time [13]. The temperature of the cold room was maintained at between 8 and 12^{o}C.

2.1.2 Deoiled ricebran

Oil from the RB was extracted through solvent extraction process using a Soxhlet extractor (Fig. 2) with hexane as the solvent at 60^{o}C for 6 hr. Soxhlet extraction method was used because of its simplicity, greater yield, and purity [14]. The de-oiled samples were kept dried in an airtight container and stored for subsequent usage in the cold room with the temperature maintained between 8- 12^{o}C.

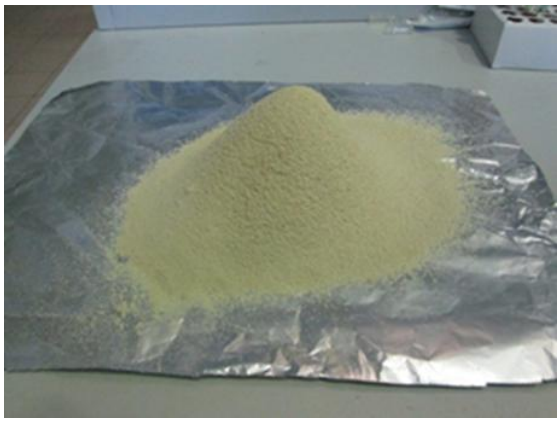

Fig. 1. Rice bran

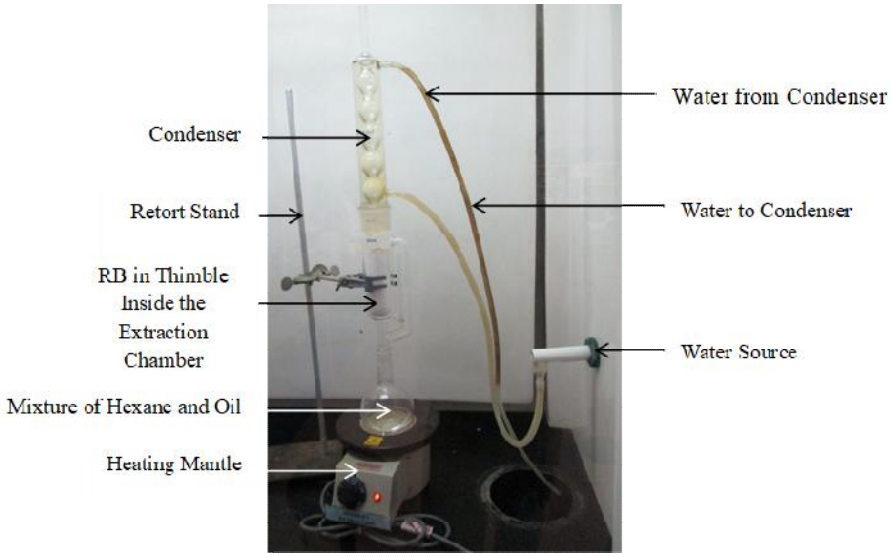

Fig. 2. Soxhlet extractor used for oil extraction from rice bran

2.2 Pretreatment Methods

2.2.1 Acid pretreatment

The effects of trichloroacetic acid (TCA) on pretreatment of RB and DRB for the purpose of fermentable sugar liberation were investigated in this work. An amount of 100 g of RB or DRB were soaked in 1% (w/v) TCA (99.0 % stock concentration) separately and each mixture was made up to 1L with distilled water to generate the Acid treated RB hydrolysate (ARB) and acid treated DRB hydrolysate (ADRB) (Fig. 3). A control of was set up consisting of 20 g of either RB or DRB in 1 L of distilled of W but not subjected to acid pretreatment. The acid hydrolysis took place at 85 °C for 3h at constant stirring of 100 rpm. To arrive at these acid hydrolysis conditions, based on the reports of Lee et al. [15] and Al-Shorgani et al. [16] on studies carried out on RB, RB hydrolysates were subjected to different acid hydrolysis conditions using different temperatures and hydrolysis time. Acid hydrolysis was carried out differently at 75°C, 80°C, and 85°C temperatures for 1 hr., 2 hr., and 3 hr. Samples of the hydrolysates were then taken for sugar analysis. Subsequently, the best acid hydrolysis condition, which gave the highest amount of sugar was then used for the acid hydrolysis process in this study. Control experiments for acid pretreatment for both RB and DRB hydrolysates were also set up by soaking 100 g of RB and DRB in 1L distilled water only. The hydrolysates were left for 3 hr. at ambient temperature with constant stirring at 100 rpm. Samples were taken for sugar analysis at the end of the 3 h duration.

2.2.2 Combined (acid, boiling and enzyme) pretreatment

RB and DRB were also subjected to a combined treatment of acid, hydrothermal (boiling) and enzyme treatments to investigate the effects of the combination of the three different methods on the ability of the hydrolysates to release fermentable sugars. The samples were initially subjected to acid treatment as described extensively under section 2.3 using TCA and after acid hydrolysis, the pH of the mixture was adjusted to pH 7 with drops of 5M NaOH and then subjected to boiling at 100 ^{o}C for 3 hours with constant stirring at 100 rpm using a commercial grade Digital Precise Shaking Water-bath (WSB-18 Water Bath). After 3 hours of boiling, the pH of the hydrolysates was adjusted to pH 4.5 and subjected to enzyme treatment as described by Dada. et al. (2013) to generate combined acid-hydrothermal-enzyme treated RB hydrolysate (ABERB) and combined acid-hydrothermal-enzyme treated DRB hydrolysate (ABEDRB) respectively [3]. The pH of the treated samples was then adjusted to pH 7 with 5M NaOH and samples were taken for sugar analysis.

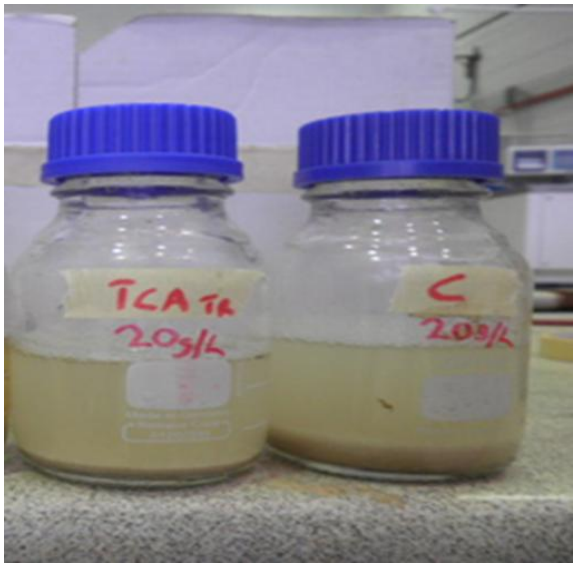

Fig. 3. Acid-treated Hydrolysates A: TCA treated hydrolysate B : Control Sample (Without Acid)

2.3 Sugar Analysis

Sugar compositions of pretreated samples were determined using high-performance liquid chromatography (HPLC Agilent 12000 series). It has a 300 × 7.80 mm Rezex RCM-Monosaccharide Ca^{2+} (8%) column and a refractive index detector (RID). The mobile phase contained 100% water which was ran at 0.6 mL/minute. The temperature of the column was kept constant at 70 ^{o}C. In order to prevent wastage of the hydrolysate, 3 mL of the fermented sample was taken at designated intervals and centrifuged at 7,500 x g (g is relative centrifugal force- RCF) for 10 minutes using a micro-centrifuge (Gyrospin GS111004, Gyrozen centrifuge). The resulting supernatant was filtered using a 0.2 µm cellulose acetate filter and 1 mL of the filtered supernatant was put in a 2 mL HPLC vial and used for the sugar analysis via the HPLC with total run time of 18 min.

All equipment used were of commercial grade and purchased locally.

3. RESULTS AND DISCUSSION

3.1 Effect of Acid Pretreatment

It is observed (Tables 1&2) that there was an increment of more than 400 % and 300 % respectively in the concentration of sugar obtained from acid treated RB and DRB hydrolysates over the untreated RB and DRB hydrolysates. It is suspected that acid treatment disrupted the structure of cellulose, increased its pore size in the process and thus reduced its crystallinity, and subsequently hydrolyzing the glycosidic bonds present in the polysaccharides (which led to the release of more sugars). This observation agreed with the general trend that acid hydrolysis liberates more sugar in lignocelluloses [15,17]. It can also be seen from the two tables that acid treatment has significant effect on the liberation of fructose from the hydrolysates [18].

3.2 Effect of Combined (Acid, Boiling and Enzyme) Pretreatment

Combined pretreatment method comprising of acid, boiling and enzyme treatments gave the highest number of fermentable sugars both for RB and DRB hydrolysates (Tables 1 and 2). In RB hydrolysates, there was an increment of 584 % between ABERB and RB and 29 % between ABERB and ARB in the amount of total sugar released. Also, in DRB hydrolysates, there was an increment of 500 % between ABEDRB and DRB and 36 % between ABEDRB and ADRB and in the amount of sugar released. It is observed that the acid treatment led to the release of most sugar after the combined treatment in both RB and DRB hydrolysates, this was followed by boiling treatment and enzyme treatment have the least effect on the release of fermentable sugars of all the treatments methods used. These differences may be attributed to distinctive effect of each treatment method. According to an earlier report by Mosier et al. [19], the reasons for subjecting lignocellulosic materials to pretreatment methods are to alter or remove structural and compositional blockage to hydrolysis such that enzymes can have access to cellulose and hemicellulose for easier conversion into fermentable sugars [19]. The boiling and acid treatments were believed to have impacted on the degree of polymerization and the crystallinity index of cellulose and thus disrupted its structure resulting in the release of more sugar as earlier observed by Chua and Wayman [20]. Although, hemicellulose has a much lower degree of polymerization (< 200), it is believed that the acid treatment was able to solubilize it and liberated more fermentable sugars while the de-polymerization of lignin, which provides mechanical strength to the cell wall structure was believed to have been achieved by the disruption of the carbon-carbon bonds as well as the ether bonds existing between lignin precursors by combination of prolonged high temperature (100 ^{o}C for 3 hours) and acid treatment. These observations agree with results observed from a previous study [19]. It should however be noted that the ultimate determinant for sugar liberated is the initial substrate concentration. The amount of sugar liberated is linearly proportional to the initial substrate concentration. This applies to both RB and DRB hydrolysates. This corroborated the earlier works [15], [16], [21] & [22].

3.3 Effect of Oil Content

Presence of oil in the hydrolysates showed a slight difference in the amount of sugar released. The data displayed in Tables 1 and 2 shows that the amount of sugar liberated from DRB hydrolysates is slightly more than the respective RB hydrolysates subjected to same treatment. The fact that the respective hydrolysates (whose only difference is either presence or absence of oil) were subjected to the same treatment method while the amount of sugar released were

Table 1. Composition and Concentrations of Sugar from Differently Treated RB Hydrolysates

Sample	Glucose Conc. (g/L)	Fructose Conc. (g/L)	Sucrose Conc. (g/L)	Xylose Conc. (g/L)	Total Sugar Conc. (g/L)	Standard Deviation of Total Sugar
RB	3.15	0.58	0.029	0.55	4.31	0.07
ARB	12.31	3.6	3.4	3.61	22.92	0.891123
ABERB	14.46	1.02	13.97	0.05	29.51	0.537029

Table 2. Composition and Concentrations of Sugar from Differently Treated DRB Hydrolysates

Sample	Glucose Conc. (g/L)	Fructose Conc. (g/L)	Sucrose Conc. (g/L)	Xylose Conc. (g/L)	Total Fermentable Sugar Conc. (g/L)	Standard Deviation of Total Sugar
DRB	4.24	0.69	0.08	0.47	5.47	0.423202
ADRB	20.73	3.45	0.03	0.05	24.26	0.457056
ABEDRB	28.82	4.17	0.03	0.05	33.07	0.03

sparingly different is enough to suspect that the difference in the observed amount of sugar liberated is because of the oil content. Acid hydrolysis of rice bran oil was suspected to have resulted in the formation of polyunsaturated fatty acids (PUFA) and glycerol which further encouraged non-degradation of cellulose into monomers [12] & [23]. hence the reduction in amount of liberated sugars in RB hydrolysates. This observation agreed with submission of Abdul-Hamid et al. [24] that Rice bran oil, containing between 80 to 85% unsaturated fatty acids, is known as a rich source of polyunsaturated fatty acids (PUFA) [24]. In addition, the presence of tocopherol, tocotrienol and oryzanol (derivatives of phenols) as natural antioxidants in Rice bran oil [23] might have further strengthened the lignocellulosic structure of RB hydrolysates by their capability to hinder oxidation thus making their degradation more difficult. However, in the DRB hydrolysates, the absence of oil was suspected to be responsible for the almost direct liberation of more sugar since less resistance was encountered by the pretreatment agents. The was an increment of 12 % in the amount of sugar released from DRB to that released from RB, however, the effectiveness and viability of the extraction process cannot yet be determined since it is the fermentation that can effectively determine the better option between RB and DRB.

4. CONCLUSION

The effect of various pretreatment methods has been investigated. A combined acid, boiling and enzymatic hydrolysis method produced the highest rice bran hydrolysates in terms of total sugar. Also, de-oiled rice bran has shown to produce more rice bran hydrolysates than ordinary rice bran. Pretreated deoiled rice bran produced the highest amount of rice bran hydrolysates than other pretreated rice bran. In sum, the increase in the amount of rice bran hydrolysates produced have demonstrated that pretreatment of Rice bran with acid hydrolysis, boiling and /or via enzymatic reactions is beneficial towards increasing the amount of biohydrogen produced and reducing production cost. The result contained in this study is outstanding in that this study, to the best knowledge of the authors, is one of the very few, where data on the release of fermentable sugar from pretreated RB and DRB were presented. Data on the quantity of fermentable sugar obtainable from ricebran (pretreated and otherwise) is scanty in literature as this area has not been extensively explored by previous researchers. In a previous study by Tiwari et al (2015), emphasis was on amount of bioethanol obtainable from rice bran, the study was silent on the quantity of fermentable sugar gotten from the treated ricebran [25]. The result of this work also agreed with the submission of Phwan et al (2019) on the use of weak acid for pretreating biomass feedstock during fermentation process [17].

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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