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Recycling Rice Straw into Biofuel "Ethanol" by *Saccharomyces cerevisiae* and *Pichia guilliermondii*

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ABSTRACT

This study suggests a new effective chemical pretreatment to hydrolyze rice straw for efficient ethanol production. It introduces a new yeast strain that ferments rice straw hydrolyzate more efficiently than *Saccharomyces cerevisiae*. The results proved the effectiveness of alkali application before HCl to delignify rice straw and to make it more appropriate for hydrolysis. The application of the hydrolyzing enzymes (cellulase and pectinase) resulted in hydrolysis of pretreated rice straw up to 94.3%. The total sugars released due to pretreatment-enzyme system was about 624 mg g^{-1} dry mass and the glucose fraction was 198 mg g^{-1}. The results indicated that *Pichia guilliermondii* is more effective to ferment rice straw hydrolyzate than *S. cerevisiae*. *P. guilliermondii* produced larger amounts of bioethanol (7.72 g L^{-1}) than *S. cerevisiae* (6.13 g L^{-1}) under the same conditions. Our results suggest an appropriate pretreatment system (the cold dilute alkali-acid) and a new effective yeast strain to ferment the rice straw hydrolyzate to produce large amounts of bioethanol.

Keywords: Bioethanol, Hydrolysis, Pretreatment, *Pichia guilliermondii,* Rice straw, *Saccharomyces cerevisiae*.

INTRODUCTION

Energy consumption has been increasing steadily with population growth and industrial development. Conventional energy sources have difficulty in matching the increasing demands. Over the last two decades, a great interest in exploring alternative energy sources has been developed (Qiu *et al*., 2010). Biofuel, a clean and renewable energy source, which can be produced through fermentation of sugars, has drawn much attention (Taleghani *et al*., 2010). A worldwide interest in the utilization of bioethanol as an energy source has its concern on the cost and efficiency of industrial processes for bioethanol production. Intensive studies (Prasad *et al*., 2007) suggested efficient fermentative organisms, low cost fermentation substrates, and optimal environmental conditions for fermentation to occur. Even though the fermentative process of bioethanol production is well known, the production costs are still the key impediment for the wide use of bioethanol as a fuel. Therefore, development of fermentation processes using cheap carbon sources is important for commercial scale production (Cazetta *et al*., 2007).

The use of lignocellulosic materials from agricultural wastes provides a low-cost fermentative substrate (Dipardo, 2002; Anwar *et al*., 2012). Preliminary studies using residual waste as lignocellulosic feedstocks for bioethanol production was greatly promising. Further research warranted the development of an innovative waste management approach that uses

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agricultural, municipal and industrial residues as cheap substrates (Zhang *et al*., 2009). On the other hand, accumulation and disposing of agricultural residues like rice straw represent a worldwide challenge (Zayed and Abdel-Motaal, 2005).

The global production of rice straw amounts to 600–900 million tons per year (Karimi *et al*., 2006). Egypt's share is 6% that is 7 million tons year^{-1}. Rice straw predominantly contains cellulose (32–47%), hemicellulose (19–27%) and lignin (5–24%) (Garrote *et al*., 2002; Saha, 2003). The carbohydrate content of typically estimated to be about 60% involves glucose (41–43.4%), xylose (14.8–20.2%), arabinose (2.7–4.5%), mannose (1.8%) and galactose (0.4%) (Vlasenko *et al*., 1997; Ma *et al*., 2009; Singh et al., 2011). Several biotechnological techniques were studied to make use of carbohydrates fraction in rice straw. For example, in high pressure steaming followed by rapid decomposition (steam explosion or autohydrolysis), lignin is partially depolymerized, hemicellulose is solubilized and depolymerized improving the accessibility of the cellulose component to the action of hydrolytic enzymes (Cazetta *et al*., 2007).

The production of bioethanol by yeast based sugar fermentation has already been commercially established but innovative studies could bring improvements to reactors and separation systems. Screening a wide spectrum of fermentative organisms to utilize the different components of rice straw is of great interest to reduce the costs of bioethanol production process. It is essential to hydrolyze lignocellulosic materials before fermentation. Enzymatic hydrolysis is still at an early stage, requiring intensive research for increased yields (Kucuk and Demirbas, 1997). Efforts are underway to improve different steps to reduce the overall cost of ethanol production (Qiu *et al*., 2010; Taleghani *et al*., 2010).

The objective of this work was to develop an effective method for pretreatment and enzymatic saccharification of cellulose and hemicelluloses components of rice straw into fermentable sugars, as well as fermentation of the hydrolyzate to bioethanol by new yeast strains.

MATERIALS AND METHODS

Rice Straw

Rice straw (*Oryza sativa* L.) was obtained from local farms, Egypt. It was cut to 1–2 cm lengths and washed thoroughly with tap water until the washing became clear and colorless and then air-dried. The chemical characterization of cellulose, hemicellulose and lignin from rice straw was made according to Galbriath and Shields (1981) and Selvendran and O'Neill (1987).

Pretreatment of Rice Straw

Different diluted (1.0%) solutions of NaOH, NH_4OH, HCl, Oxalic acid and EDTA were used in the pretreatment stage of straw. The application of these pretreatments was carried out in two ways. In the first, each solution was applied singly. In the second, after the application of each of the above solutions, the cocktail was filtered and the residues were pretreated again with 1.0% HCl. The pretreatment was carried out by soaking 5 g of rice straw in 50 mL of any solution for 24 hours and shaking (150 rpm) at room temperature (30±1°C). The mixture was filtered through filter paper Whatman No. 1 and the filtrate was collected. Reducing sugars in the collected fractions were estimated using Nelson's method (Somogyi, 1952). The hydrolyzed percent of cellulose, hemicelluloses and lignin were determined.

Determination of the Hydrolyzed Cellulose, Hemicelluloses and Lignin of Rice Straw

To determine the hydrolyzed cellulose, hemicelluloses and lignin contents of the

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pretreated rice straw, the method described by Baour-Hoch *et al.* (1990) was followed. Briefly, the lipid-free (ethanol washed) rice straw was extracted twice in boiling distilled water and twice in 80% (v/v) ethanol (10 mL each time) by stirring the mixture for 30 minutes at 60°C. Extracts of water and ethanol were combined and completed to a volume in which soluble sugars were estimated. The residue was resuspended and extracted three times in 0.5N NaOH (100 mL each time) by stirring the mixture for 30 minutes at 60°C (Wagner et al., 1983). According to Baour-Hoch *et al.* (1990), starch was completely extracted by this method; only very trivial cell wall components were included in the extract. The residue of the "0.5N NaOH" extract was considered as cell wall preparation (Baour-Hoch *et al*., 1990). The soluble sugars of the starch fractions were estimated by the anthrone-sulfuric acid method**,** as well as hemicelluloses, cellulose and lignin fractions (Southgate, 1976). The rice straw contains a significant amount of pectin, so we used a step to remove pectin. In this step, the cell wall preparation was extracted twice with 0.5% ammonium oxalate-oxalic acid (pH 4) at 90°C for 24 hours. Ammonium oxalate solution precipitates calcium ions, which connect the glycosidic bonds in pectin molecules and thus becomes water soluble. The residue was next extracted three times with 17.5% NaOH, each for 18 hours. Following the removal of insoluble residue by centrifugation, the alkali-soluble extracts "the hemicelluloses fraction" were neutralized with HCl (Galbriath and Shields, 1981). The residue after hemicelluloses extraction was dried and resuspended in 72% H_2SO_4 and kept at 0–4°C for 48 hours with occasional stirring. Then, the suspension was diluted with distilled water to a final concentration of 1% H_2SO_4 and autoclaved for 2 hours. The supernatant contained the acid hydrolyzed cellulose (Dever *et al*., 1968; Selvendran and O'Neill, 1987).

Enzymatic Hydrolysis of Rice Straw

The cellulase and pectinase enzymes used in this study were commercial products of *Aspergillus niger* (Sigma). The cellulase's caboxymethyl cellulose (CMC) saccharification activity was 15 $IUmg^{-1}$, measured as the initial rate of reducing sugars formation during hydrolysis of 0.5% caboxymethyl cellulose at pH 5.0 and 50°C (Mandels *et al.,* 1969). Its filter-paper activity was 0.53 FPU mg^{-1}, using the standard procedure (Ghose, 1987).

The straw was pretreated with 1.0% NaOH for 24 hours at room temperature (30±5°C), followed by shaking in 1.0% of HCl for another 24 hours. We used 0.1% of NaOH and HCl based on preliminary test (data not shown). After filtration, the residue of the straw was enzymatically hydrolyzed by adding 50 mL of 1.0% of cellulose or cellulose+pectinase enzymes. Enzymes were prepared in 0.05M sodium acetate buffer at pH 5.0. The enzyme mixture (enzyme and residue of pretreated rice straw) was shaken in 250-mL glass flasks (150 rpm at room temperature for 24 hours). The mixture was then filtered and the sugars were determined as explained above.

Determination of Reducing Sugars and Carbohydrates

After chemical pretreatments and enzymatic hydrolysis, the Nelson-Somogyi method (Somogyi, 1952) was used to determine the soluble reducing sugars (RS), using glucose as standard control. Carbohydrate contents were determined by anthrone-sulfuric acid method (Fales, 1851; Karimi *et al*., 2006)

Yeast Strains

Saccharomyces cerevisiae y-1646 was obtained from South Africa (Department of Microbiological, Biochemical and Food Biotechnology, Faculty of Natural and

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Agricultural Sciences, University of the Free State) and *Pichia guilliermondii* Moh10 was isolated and identified in a previous work (Hashem, 2005). Yeast strains were propagated and stored on yeast extract-malt extract agar (YMA) slants at 4°C. Active cultures for inoculation were prepared by growing the yeast in YM broth on a rotary shaker at 150 rpm for 16 hours at 25°C (initial pH 3.8 - 4.5).

Biomass and Fermentations of Yeast under Semi-anaerobic Conditions

Repeated batch cultures of yeast were carried out in triplicates using a medium containing rice straw hydrolyzate obtained from the above mentioned hydrolysis systems. The medium was used in biomass and bioethanol production by both yeast strains under semi-anaerobic conditions. The prepared medium was sterilized at 121°C for 20 minutes. Experiments were initiated by transferring the prepared cell suspension with 10 mL (1.2×10^6 cell mL^{-1}) into 150 mL of the medium in 250 mL bottles fitted with rubber plugs, and shaken in the incubator at 150 rpm at 30°C for 72 hours. The experiments were monitored by taking 5 mL samples under sterilized conditions after 72 hours for biomass determination by the absorbance method at 500 nm. The solution was centrifuged at 1000×*g* for 10 minutes and the supernatant was used in bioethanol analysis.

Bioethanol Determination

Bioethanol content was estimated by dichromate method as follows; 5 mL of sample to be analyzed and 20 mL of 0.5N NaOH was transferred to an evaporator and distilled until approximately 10 mL evaporated from the mixture. The evaporated gas was collected in the collection flask containing 30 mL of 0.2N potassium dichromate solution and 10 mL of concentrated H_2SO_4. The content of the collecting flask was quantitatively transferred into a 250 mL Erlenmeyer flask with additional 100 mL distilled water, 10 mL of 0.2N potassium iodide and 3-5 drops of 5% starch solution and then titrated against 0.1 N sodium thiosulfate solution. Alcohol content (% volume) in the sample= [(Bichromate volume×2-thiosulfate volume)×0.0289]. This method was adapted from IMECA Co. (Zohri and Moustafa, 2000). All experiments were repeated three times and the standard deviation was estimated.

RESULTS AND DISCUSSION

Chemical Composition of the Investigated Rice Straw

Analysis of rice straw used in the present study revealed that it contains 40.84% cellulose, 28.06% hemicellulose, 7.86% lignin and 23.24% ash and other components (Table 1). Jeya *et al*. (2009) mentioned that the main composition of rice straw was 12% moisture, 36.8% cellulose, 25.8% hemicellulose, 15.8% lignin. The carbohydrate content of rice straw typically involves glucose (41–43.4%), xylose (14.8–20.2%), arabinose (2.7–4.5%), mannose (1.8%) and galactose (0.4%) (Karimi *et al*., 2006).

Table 1. Average chemical composition of used rice straw.

Constituent	% of dry weight	Standard deviation
Cellulose	40.84	±1.85
Hemicellulose	28.06	±0.96
Lignin	7.86	±0.52
Ash and others	23.24	±0.67

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Pretreatment and Hydrolysis of Rice Straw Cell Wall

Figure 1 shows that among the nine different hydrolyzing treatments, application of HCl after NaOH was the most effective pretreatment. This treatment resulted in a reduction of solid yield by 40.0% followed by NaOH pretreatment that reduced the solid yield by 37.1%. Application of HCl only or after NH_4OH and oxalic acid produced similar values indicating unfeasibility of these combinations. Application of ammonia or oxalic acid resulted in hydrolysis of 16.6 and 19.4% of the straw weight whereas EDTA showed the lowest hydrolysis of straw. We assume that the considerable reduction in solid yield following chemical pretreatment of the straw was due to lignin removal and the depolymerization of hemicellulose. This finding is greatly supported by similar results by Wyman (1996) and Dawson and Boopathy (2007)

Table 2 presents the data of the cellulose, hemicelluloses and lignin hydrolysis as a result of the application of different chemical pretreatments. Cellulose was almost hydrolyzed by the application of HCl after NaOH (80.12%). Ammonia, oxalic acid with or without HCl in addition to EDTA produced close values of cellulose hydrolysis (64.11-79.52%). Hydrolysis of hemicellulose was also enhanced by the application of HCl after NaOH (36.25%) or oxalic acid (36.07%), as well as the application of ammonia (37.31%). Lignin was nearly hydrolyzed when treated with HCl after EDTA or HCl+NaOH (84.19 and 83.82%, respectively). The hypothesis behind applying EDTA or oxalic acid is to chelate calcium ion that is legated in the rich cell wall carboxyl group and pectates, thus, losing the tightness of the complex cell wall structure and finally facilitating its hydrolysis. Sodium hydroxide and ammonia were also involved in the hydrolysis of 77.75% and 73.05% of the lignin content, respectively. The action of ammonia or NaOH is the lignin fraction. He *et al*. (2008) reported that the ester bond of lignin-carbohydrate complexes was destroyed by NaOH pretreatment. As a result, the large molecular weight of lignin became smaller and the three dimensional network structure became linear after NaOH pretreatment while cellulose crystallinity was increased (Anwar *et al*., 2012).

The obtained results indicated that acid hydrolysis of rice straw is effective especially after the application of alkali such as NaOH or NH_4OH (Table 2). These results are in agreement with those obtained by Jeya *et al*. (2009) who pretreated rice straw and found that it consists of 52.2% cellulose, 18% hemicellulose, 9.8% lignin and 20% ash with 2% NaOH prior to hydrolysis. They concluded that, alkali pretreatment increased the proportion of cellulose by 41.8% and

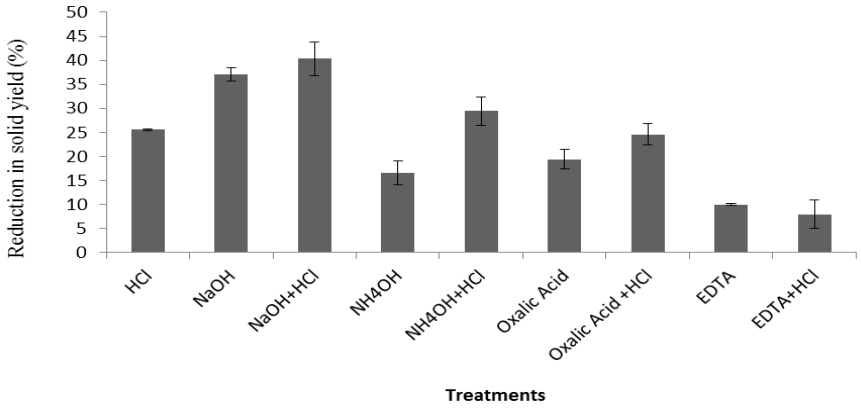

Figure 1. Effect of different pretreatments on the hydrolysis of rice straw (expressed as reduction in solid yield).

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Table 2. Degradation percentage of the main components of rice straw (cellulose, hemicellulose and lignin) after the application of different pretreatments.

Treatmentnts	Cellulose		Hemicellulose		Lignin	
	Hydrolyzed (%)	SD [a]	Hydrolyzed (%)	SD	Hydrolyzed (%)	SD
HCl	69.29	±2.08	16.38	±0.57	65.07	±2.32
NaOH	60.43	±3.21	31.77	±1.53	77.75	±2.58
NaOH+HCl	80.12	±4.31	36.25	±0.31	83.82	±4.23
NH_4OH	76.99	±1.67	37.31	±0.71	73.05	±2.53
NH_4OH+HCl	76.80	±6.19	34.61	±0.16	51.50	±2.34
Oxalic Acid	79.52	±0.69	35.04	±0.38	33.40	±1.22
Oxalic Acid +HCl	75.17	±0.90	36.07	±0.21	58.84	±2.04
EDTA	76.72	±2.92	27.90	±1.12	40.00	±2.30
EDTA+HCl	64.11	±1.64	36.00	±0.37	84.19	±5.23

[a] Standard deviation.

decreased that of hemicellulose and lignin by 45.7 and 38.0%, respectively. The high cellulose content and decreased hemicellulose and lignin contents would allow for the enhancement of enzymatic saccharification (Jeya *et al*., 2009).

Figure 2 shows that availability of glucose as the end-product of the hydrolysis of carbohydrates was greatly affected by the application of HCl either as a single pretreatment or after the application of other chemicals. The highest amount of glucose was obtained when HCl was applied after NaOH (26.88 mg mL^{-1}) followed by the application of the acid after oxalic acid (23.4 mg mL^{-1}). The application of HCl alone or after ammonia resulted in close values of glucose (21.1 and 20.13, respectively). However, the application of HCl after EDTA produced a low value of glucose (15.6 mg mL^{-1}). Glucose could originate from either the hemicellulose or cellulose fractions. The glucose liberated at mild hydrolysis conditions most likely originated from hemicelluloses (Sanchez *et al*., 2004), while the main part of glucose originated from the cellulose fraction (Taherzadeh *et al*., 1997).

It is obvious that the application of NaOH, NH_4OH, oxalic acid and EDTA alone did not release any considerable amounts of glucose (0.67 – 2.75 mg mL^{-1}). This finding confirms that HCl is an important factor for the hydrolysis of cellulose and hemicelluloses that comprise the main constituents of the cell wall of rice straw. Our results are greatly supported by the findings of many authors who applied

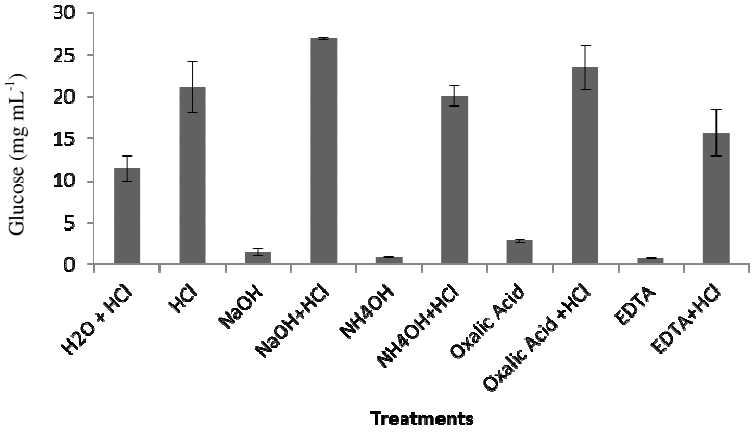

Figure 2. Glucose (mg mL^{-1}) produced from the application of different pretreatments.

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different chemicals for the hydrolysis and pretreatment of crop residues. Karimi *et al*. (2006) reported that dilute-acid hydrolysis is a suitable process to produce sugars from rice straw for further processing into bioethanol production.

Enzymatic Hydrolysis of Rice Straw

Figure (3-a) shows the effect of cellulase and pectinase enzymes on the hydrolysis of pretreated and untreated rice straw. The application of the cellulase alone or in combination with pectinase to untreated rice straw resulted in the hydrolysis of 43.7 and 60.7% of the straw, respectively. The percentage of hydrolysis was greatly enhanced up to 94.3% when rice straw was pretreated by NaOH and HCl before the application of the enzymes. Our results prove that alkali-acid pretreatment is a very effective method to facilitate and enhance the enzymatic hydrolysis of rice straw. Our results are supported by recent work of Singh and Bishnoi (2012), who used microwave and alkali-pretreatment of rice straw to enhance its enzymatic hydrolysis. Also, Anwar *et al*. (2012) proved that the hydrolysis of cellulose in pretreated samples is a key step for the production of ethanol.

It was proved that lignocellulosic biomass could not be enzymatically saccharified to high yields without a pretreatment, mainly because the lignin in plant cell walls forms a barrier against enzymatic attack (Sewalt *et al*., 1997). An ideal pretreatment would reduce the lignin content and crystallinity of the cellulose

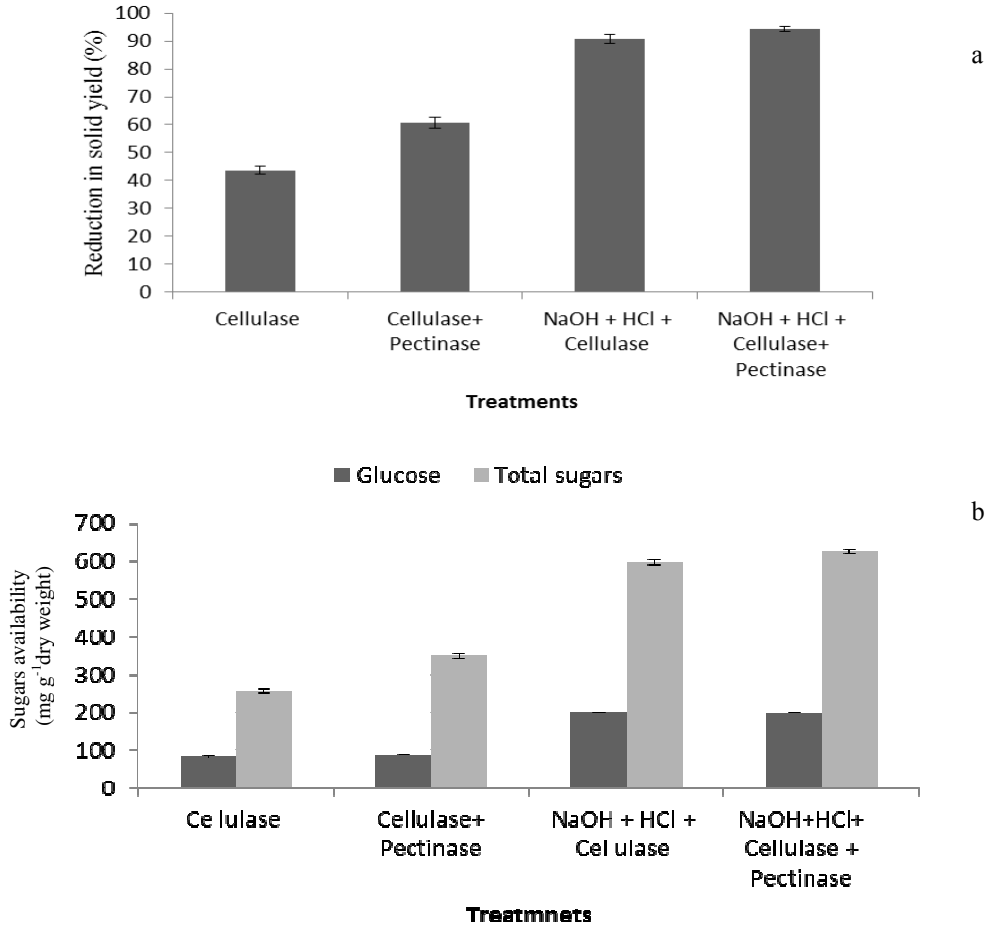

Figure 3. Effect of enzymatic degradation of pretreated rice straw (a) on the reduction of solid yield (%) (b) on glucose and total sugars availability (mg g^{-1}dry weight).

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and increase the surface area (Krishna and Chowdary, 2000). This result is supported by the findings of Wyman (1996), who concluded that dilute-acid hydrolysis can significantly improve the enzymatic hydrolysis since the presence of hemicellulose hinders the enzymatic hydrolysis. Our results are comparable with the results of Yu *et al.* (2007) and Anwar *et al*. (2012). The enzymatic hydrolysis is a more effective way to obtain reducing sugars leading to the production of bioethanol (Wayman *et al*., 1992).

Figure (3-b) shows the effect of enzymatic hydrolysis on the availability of glucose and total sugars. The application of cellulase alone released 256.6 mg sugars g^{-1} dry weight, of which 83.6 mg were glucose. However, these quantities increased up to 2 fold of glucose and up to 1.5 fold of total sugars when rice straw was pretreated by NaOH-HCl and then subjected to enzymatic hydrolysis. It is obvious that the application of both enzymes was able to hydrolyze 90% of the pretreated rice straw and released most of the sugar content (623.9 mg sugars g^{-1} dry weight) and 198.14 mg glucose g^{-1} dry weight. This finding indicates that the hydrolysis of straw wall components containing polysaccharides and lignin is largely possible by combining both chemical and enzymatic treatments. Production of enzymes and their application at a large scale is a challenge because of industrial and economic drawbacks. Our study utilized the cumulative findings of several researchers on the hydrolysis of agricultural residues using alkali and acid hydrolysis as a pretreatment facilitating enzyme action. Alkali treatment of lignocellulosic substances such as cereal straw disrupts the cell wall by dissolving hemicelluloses, lignin, and silica, by hydrolyzing uronic and acetic esters, and by swelling and decreasing the crystallinity of cellulose (Jackson, 1977). Alkali treatment, in addition, cleaves the α-ether linkages between lignin and hemicelluloses and the ester bonds between lignin and/or hemicelluloses and hydroxycinnamic acids, such as *p*-coumaric and ferulic acids (Sun and Cheng, *et al*., 2002). Xiao *et al*. (2001) reported that the treatment of the dewaxed maize stems, rye straw, and rice straw with 1 M NaOH at 30°C for 18 hours resulted in the dissolution of 78.0, 68.8, and 82.1% of lignin, and 72.1, 72.6, and 84.6% of hemicelluloses, respectively. This cleavage of the ester bonds undoubtedly resulted in high solubility of lignin and hemicelluloses from the cell walls of maize stems, rye straw, and rice straw by alkali treatment. It was also found that this treatment removed 89.2, 92.6, and 88.9% of the original silica from maize stems, rye straw, and rice straw, respectively.

Utilization and Fermentation of Rice Straw Hydrolyzate by *P. guilliermondii* and *S. cerevisiae*

Figure 4 shows the ability of both *P. guilliermondii* and *S. cerevisiae* to grow on and utilize the hydrolyzate of different pretreatments throughout the conventional semi anaerobic conditions. Growth of *P. guilliermondii* was markedly higher than *S. cerevisiae* in all treatments. The highest growth rate of both yeasts in the hydrolyzate resulted from the application of HCl after oxalic acid treatment (27.6×10^6 and 19.8×10^6 cells mL^{-1}, respectively). The hydrolyzate of EDTA provided the second best medium for yeasts growth where 24.8×10^6 cell mL^{-1} were detected for *P. guilliermondii* and 17.2×10^6 cell mL^{-1} were determined for *S. cerevisiae*. The results support the hypothesis that removal of calcium facilitates hydrolysis of straw and further the most appropriate hydrolyzate for yeast growth. It was shown that the hydrolyzate produced by NaOH treatment was the most suppressive one for both yeasts, and the total count did not exceed 15.6×10^6 and 11.2×10^6 cell mL^{-1} for *P. guilliermondii* and *S. cerevisiae*, respectively.

Data of semi anaerobic fermentation of different hydrolyzates produced from different pretreatments are given in Figure 5. Bioethanol production by *P. guilliermondii* was obviously higher than that by *S. cerevisiae* in all cases. The highest production values were achieved when *P. guilliermondii* was grown on hydrolyzates of EDTA+HCl (2.93 g

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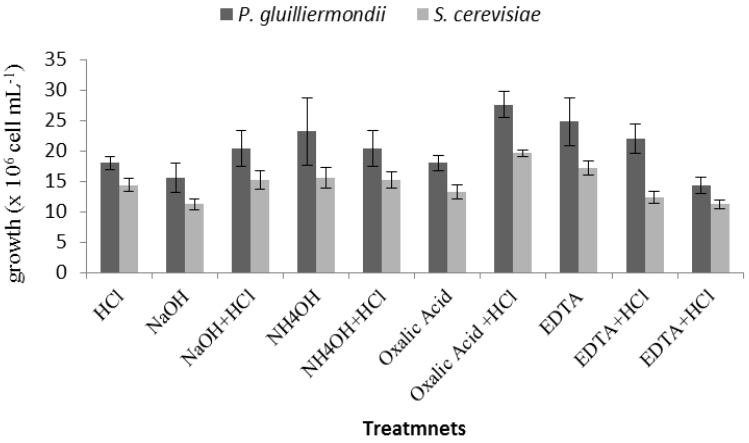

Figure 4. Growth of *P. guilliermondii* and *S. cerevisiae* on hydrolyzates of different pretreatments at 25°C after 72 hours under semi aerobic conditions.

l^{-1}) followed by NaOH+HCl or HCl only (2.67 g l^{-1}). The lowest value was obtained from fermentation of hydrolyzate using oxalic acid (1.45 g l^{-1}). The highest bioethanol concentration produced by *S. cerevisiae* was detected when yeasts were grown on hydrolyzate of ammonia+HCl (1.82 g l^{-1}). However, the lowest concentration was obtained when either ammonia or EDTA was applied as a hydrolyzing solution (0.3 g l^{-1}). Collectively, the results indicate that relevant hydrolyzate for growth does not necessarily coincide with its fermentative ability.

When *P. guilliermondii* and *S. cerevisiae* were grown on hydrolyzates of cellulase and pectinase enzymes, the production of bioethanol was dramatically increased (Figure 6). *P. guilliermondii* seems to be the most appropriate yeast to utilize and ferment the hydrolyzate. It produced 7.72 g l^{-1} bioethanol when grown on the hydrolyzate of combined cellulase and pectinase after pretreatment with alkali-acid system. The production was slightly lower when the cellulase enzyme was applied (7.39 g l^{-1}) indicating the unnecessity of pectinase addition in the hydrolysis cocktail. However, bioethanol production was reduced by 50% when cellulase and pectinase were applied without pretreatment or by 42% when cellulase alone was applied without

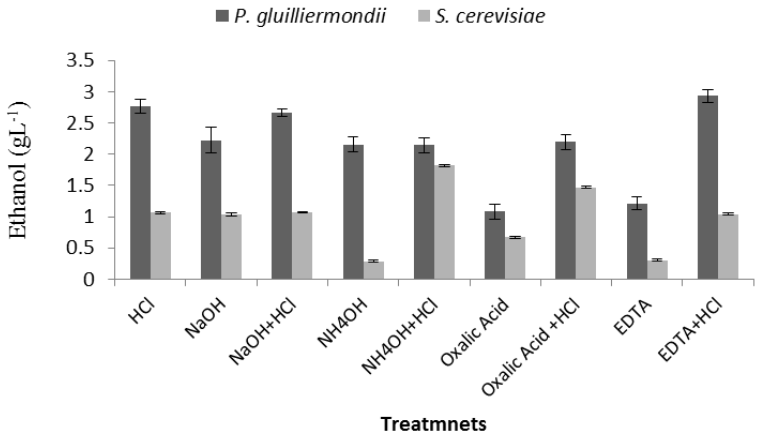

Figure 5. Fermentability of hydrolyzates of different pretreatments into ethanol by *P. guilliermondii* and *S. cerevisiae* at 25°C after 72 hours under semi anaerobic conditions.

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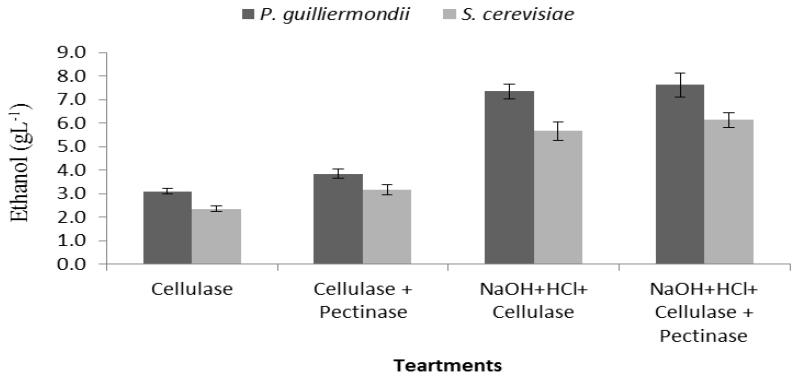

Figure 6. Fermentability of enzymatic hydrolyzates of rice straw into ethanol production by *P. guilliermondii* and *S. cerevisiae* at 25°C after 72 hours under semi aerobic conditions.

pretreatment. *S. cerevisiae* fermented all hydrolyzates at a relatively similar efficiency. It ultimately produced 6.13 g l^{-1} bioethanol from the hydrolyzate of cellulase and pectinase on pretreated rice straw. Also, the lowest productivity was achieved with the application of cellulase only.

The results prove the ability of *P. guilliermondii* to utilize and ferment the hydrolyzate of alkali-acid pretreated and enzymatic hydrolyzed rice straw. Thus, we could assume the ability of this yeast strain to utilize all types of sugars (particularly pentoses) produced from hydrolysis. However, *S. cerevisiae* utilized only hexoses (glucose). Our findings are in close agreement with Slininger *et al*. (1985) who found that *Pichia stipitis* NRRL strain Y-7124 utilized over 95% xylose based on 150 g l^{-1} initial concentration and produced 52 g l^{-1} of bioethanol with a yield of 0.39 g per g xylose. The crude acid hydrolyzate was inhibitory to all strains of yeast, even at low concentrations. Therefore, the other substitute for bioethanol production can be the formation of xylitol from lignocellulosic hydrolyzates by yeasts such as *Candida tropicalis* and *Candida guilliermondii* (Horitsu *et al*., 1992).

CONCLUSIONS

The study suggested cheap pretreatments to enhance the enzymatic hydrolysis and fermentability of rice straw. Application of cold diluted (1.0%) HCl after NaOH (1.0%) resulted in complete delignification of the straw. This pretreatment greatly improved the enzymatic hydrolysis up to 94.3%. *P. guilliermondii* fermented the hydrolyzate of rice straw more efficiently than *Saccharomyces cerevisiae*. It produced 7.72 g l^{-1} bioethanol when it was grown on the hydrolyzate of pretreated and enzymatically hydrolyzed straw. Our study recommends the application of the designed system at an industrial scale to reduce the overall cost of bioethanol production from the lignocellulosic residues. In addition, we recommend investigating other yeasts, which could be more effective than conventional ones.

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بازیافت کاه برنج به سوخت زیستی اتانول توسط *Saccharomyces cerevisiae* و *Pichia guilliermondii*

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چکیده

این مطالعه پیش تیمار جدیدی را برای آبکافت موثر کاه برنج جهت تولید بایو اتانول پیشنهاد می‌نماید. همچنین در این مقاله یک سویه جدید از مخمر که می‌تواند آبکافته کاه برنج را با کارایی بیشتری نسبت به *Saccharomyces cerevisiae* آبکافت کند معرفی می‌شود. نتایج تاثیر استفاده از قلیا قبل از تیمار اسیدی با HCL جهت لیگنین زدایی از کاه برنج به منظور آماده سازی آن برای آبکافت را اثبات کردند. استفاده از آنزیم‌های آبکافت کننده (سلولاز و پکتیناز) موجب آبکافت کاه برنج تا ۳/.۹۴ شد. کل قندهای آزاد شده بر اثر سیستم پیش تیمار-آنزیم حدودا ۶۲۴ میلی‌گرم بر گرم وزن خشک و کسر گلوکز ۱۹۸ میلی-گرم بر گرم بود. نتایج نشان دادند که *Pichia guilliermondii* نسبت به *S. cerevisiae* کارایی بیشتری برای تخمیر آبکافته کاه برنج دارد. تحت شرایط مشابه، *P. guilliermondii* اتانول بیشتری (۷۲/۷ گرم بر لیتر) از *S. cerevisiae* (۱۳/۶ گرم بر لیتر) تولید نمود. نتایج ما معرف یک سیستم پیش تیمار جدید (اسید-قلیای رقیق سرد) و یک سویه جدید مخمر برای تولید بایو اتانول در حجم زیاد می‌باشند.

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