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Acid hydrolysis and fungal biodegradation of pretreated sugarcane bagasse for bioethanol production

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ABSTRACT

This study examines the prospect of biofuel production from high carbohydrate containing lignocellulosic material, e.g. sugarcane bagasse through chemical and biological means. The chemical composition of raw and pretreated SCB was determined. Hydrolysis of TSCB chemically by acids or enzymatically by fungi was performed to produce hydrolyzates for the fermentation process. Conversion of SCB to free sugars by acid hydrolysis varied from one treatment to another. Acid catalysis and fractionation of sugarcane bagasse to RS occurred at high temperature within short reaction times. High temperature in lower acid concentrations is favorable for TSCB hydrolysis, however, at lower temperatures RS production enhanced by increasing acid concentration. This treatment when neutralized, amended with some nutrients and inoculated with 2 % of *Saccharomyces cerevisiae* yeast, achieved the highest ethanol concentration (1.145 % v/v) using H₂SO₄ as catalyst. The highest bioconversion of 5 % waste (63.425 % w/w) was recorded on TSCB by *A. niger*. Some relevant features limiting the overall treatment effectiveness were identified, paving way for future studies to significantly improve this process.

KEYWORDS

Sugarcane bagasse; *Aspergillus niger*; *Trichoderma harzianum*; Chemical hydrolysis; Enzymatic hydrolysis; Fermentation; Bioethanol.



INTRODUCTION

Unlike fossil fuels, ethanol is a renewable energy source produced through fermentation of sugars. Ethanol is widely used as a partial gasoline replacement in the US. Fuel ethanol that is produced from corn has been used in gasohol or oxygenated fuels since the 1980s. Ethanol is also a safer alternative to methyl tertiary butyl ether (MTBE), the most common additive to gasoline^[1]. The US Environmental Protection Agency recently announced the beginning of regulatory action to eliminate MTBE in gasoline^[2]. However, the cost of ethanol as an energy source is relatively high compared to fossil fuels.

A dramatic increase in bioethanol production using the current cornstarch-based technology may not be practical because corn production for ethanol will compete for the limited agricultural land needed for food and feed production. In the last few decades, bioethanol has assumed a very important place among renewable fuel resources and its market is continuously expanding. Lignocellulosic biomass (LB) is mainly composed of two polymeric carbohydrates: cellulose and hemicellulose. Lignin, another constituent of LB, acts as a “skin” and prevents easy access to cellulose. A potential source for low-cost ethanol production is to utilize lignocellulosic materials such as crop residues, grasses, sawdust, sugarcane bagasse, wood chips, and solid animal waste^[3].

Sugarcane bagasse (SCB), a waste in the process of sugarcane industries, is an abundant and low-cost lignocellulosic source^[4], which can be hydrolyzed to fermentable sugars for the production of value added bio-products, consequently increasing the economy of the process. On addition, it gives a solution for the removal of this abundant waste solving a problem of the sugar industry. Therefore, a double effect is obtained economic and ecologic. Rapid growth of sugarcane plants, climate and soil property making the material of sugarcane bagasse easily available and annually renewable. So, it can act as a cheap substrate with constant supply as a substrate for bioconversion to fuel ethanol. There is an increased interest in producing bioethanol as an octane booster or as liquid fuel. Lignocellulosic materials from different crop residues have been used for conversion to ethanol^[5,6].

Ethanol production from LB is done through four main steps: pretreatment, hydrolysis, fermentation and recovery and purification. The aim of the pretreatment is breaking down the LB structure and preparing it for enzymatic hydrolysis. In hydrolysis, fermentable monosaccharides are produced from hydrolysis of cellulose and hemicelluloses. Hydrolysis and fermentation can perform separately or simultaneously. In Separate Hydrolysis and Fermentation (SHF), hydrolysis and fermentation is done sequentially and in Simultaneous Saccharification and Fermentation (SSF), cellulose hydrolysis and hexose fermentation is simultaneously performed^[7].

Conversion of SCB into fermentable sugars is possible through thermal, chemical, or enzymatic hydrolysis^[8-12]. Treating lignocellulosic agricultural residues with mineral acids can be used to release fermentable sugars. The level and composition of the sugars released depends on the type of acid and its concentration in the hydrolysis mixture^[13]. The acids most used were H_2SO_4 ^[3,9,14-22], HCl ^[3,20,23-26], and HNO_3 ^[27]. The role of acids in SCB hydrolysis is the breakdown of the heterocyclic ether bonds between sugar monomers in the polymeric chains, which are formed by hemicellulose and cellulose^[14]. Disruption of lignocellulosic structure of biomass by means of dilute sulfuric acid pretreatment with microwave-assisted heating plays a key role in producing bioethanol, as a substitute fuel to gasoline, from lignocelluloses^[17].

The interest in using H_3PO_4 as acid in acid hydrolysis is that after neutralization of hydrolysates with $NaOH$, the salt formed is sodium phosphate which remains in the hydrolysates and could be used as nutrient by microorganisms in the fermentation process, therefore, its removal is not required^[28].

In the operation of neutralization, it is usually to add chemicals that neutralize the acids of the hydrolysates forming salts^[16]. These salts have low solubility and are normally removed by filtration. For example, hydrolysates containing sulphuric acid are neutralized with calcium carbonate forming calcium sulphate. Finally the processed hydrolysates are supplemented with several nutrients to be a favorable fermentation medium. These nutrients contribute the nitrogen and micronutrients needed for the growth of the microorganisms. A great number of microorganisms are capable of bioethanol formation among which *Saccharomyces cerevisiae* (baker's yeast) is the most frequently and traditionally used organism^[29].

Previously we reported enzymatic degradation of pretreated SCB using fungal microorganisms in liquid culture medium^[30]. El-katatny et al.^[30] have been investigated and discussed the optimum

conditions for CMC-ase production by *Aspergillus niger* and *Trichoderma harzianum* using the pretreated SCB as a carbon source. Therefore, the aim of this study to investigate acid hydrolysis or fungal biodegradation of the pretreated SCB to obtain the hydrolysate containing high fermentable sugar for use as a substrate for bioethanol production by fermentation.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade. Media and chemicals used in this study were purchased from Sigma (USA), Merck (Germany), and SD Fine Chemicals (India).

Organisms and culture conditions

The experimental organisms *Aspergillus niger* and *Trichoderma harzianum* were isolated from soil of sugarcane bagasse store in Gerga sugarcane factory, Sohag Governorate, Egypt, using CMC agar plate. The culture was maintained on PDA slant at 4°C and sub-cultured on fresh sterile PDA slant and incubated for 120 hrs.

Sugarcane bagasse pretreatment

SCB was milled down to 40-mesh on a Willy mill, and treated with 2% NaOH at [1:10, (solid : liquid)], and autoclaved for 1 hour at 15 lb/in² pressure and 121°C and the residue was freed of alkali by washing several times with distilled water and dried at 50±2°C for two days before use. SCB and delignified SCB were analysed for their contents of lignin, cellulose, and total carbohydrates using standard methods that will be shown in the next.

Estimation of total carbohydrate

Estimation of the total carbohydrates was performed using an anthrone reagent^[31]. The anthrone reagent consists of 0.2 g anthrone (Merck), 8 ml absolute ethyl alcohol, 30 ml distilled water, and 8 ml concentrated H₂SO₄ (D = 1.84 Merck). These were successively mixed in a conical flask under continuous cooling. This reagent should be always freshly prepared.

A certain amount of residual waste material after being dried and weighed was hydrolyzed by 4 N HCl for 2 hours in a boiling water bath. After cooling, the hydrolysate was filtered and the filtrate was completed to a definite volume. One ml of filtrate (containing carbohydrate solution) was introduced into a clean Pyrex test tube and was mixed with 9 ml anthrone reagent. The mixture was then heated in a boiling water bath for exactly 7 minutes, after which it was directly cooled under tap water. The developed blue-green colour was read at the wavelength of 620 nm against a blank containing only water and anthrone reagent. Carbohydrate estimation is calculated according to^[31].

Determination of cellulose, lignin and ash

To one gram of dried sample was added, 15 ml of 80% acetic acid and 1.5 ml concentrated nitric acid and refluxed for 20 min. It was filtered, the residue was washed with ethanol, dried in an oven at 100-105 °C and weighed (A). Then it was incinerated at 540°C (B). Cellulose content was determined in accordance with^[32].

$$\% \text{ Cellulose} = [(\text{material A}) - (\text{material B}) / \text{Weight of sample taken}] \times 100$$

Lignin was estimated by Adams method³³. Ash was determined according to Chopra and Kanwar^[34].

Acid hydrolysis

The dried treated sugarcane bagasse (TSCB), was hydrolysed by H₃PO₄, H₂SO₄, or HOClO₃ with concentrations 0.5%, 1%, 2%, 4% and 6% (v/v). Concentration values were selected according to the literature^[35]. The temperature of the hydrolysis was controlled at 90, 100, and 120°C, and the reaction time was varied at 1, 2, and 3 hrs. All conditins were carried out using a liquid solid ratio (LSR) of 15 ml liquor/g dry weight of TSCB. The hydrolysis was performed in triplicate at each condition, modified from^[14,24,27]. The effect of amount of acid solution (ml) per gram TSCB was also investigated. After hydrolysis the mixtures were diluted to a definite volume, nutralized and centrifuged. The superntants

were determined for reducing sugars (RS) using dinitrosalicylic acid method^[36]. Yields for sugars were calculated using the formula referred by Neureiter et al.⁹ as described below.

$$Y = (CV/W) \times 100$$

Where Y is the yield of sugars expressed as percent of reducing sugars in dry weight. C is the concentration of reducing sugars (g/L), V is total volume of the liquid phase (L) and W is dry weight of the corresponding lignocellulosic material (g). The hydrolysates produced through the maximal values of chemical (hydrolysates of H₂SO₄) hydrolysis were chosen for fermentation by an isolate of *Saccharomyces cerevisiae*.

Enzymatic hydrolysis

Biodegradation of sugarcane bagasse by growing of fungal species on Czapek's Dox medium in which sucrose was replaced by TSCB and the optimum conditions of El-Katatny et al.^[30] were performed. Culture medium is containing (g/l): NaNO₃, 2; K₂HPO₄, 1; MgSO₄.7H₂O, 0.5; KCl, 0.5; FeSO₄.7H₂O, 0.001 and TSCB, 50 g. Fifty ml aliquots of the medium were dispensed into 250 ml Erlenmeyer flasks and sterilized by autoclaving for 15 min at 15 lb/in² pressure and 121°C then inoculated mycelium disc of the *A. niger* or *T. harzianum* individually. After incubation period flasks were filtered and reducing sugars were assayed, using dinitrosalicylic acid method^[36]. The hydrolysates were tested in the process of fermentation by an isolate of *Saccharomyces cerevisiae*.

Fermentation

Strain of *Saccharomyces cerevisiae* was obtained from Faculty of Agriculture, Microbiology Department, Minia University, for fermentation of hydrolysate. The yeast culture was maintained on medium containing 20.0 g glucose, 20.0 g peptone and 10.0 g yeast extract per liter, after subculturing at regular time intervals and stored in a refrigerator. The biomass of yeast after growth for 1 day at 30°C in medium containing 100.0 g sucrose, 5.0 g yeast extract and 5.0 g peptone per liter was centrifuged at 5000 rpm for 5 min and inoculated into the hydrolysate at a concentration of 2 % (w/v). The fermentation was carried out in 250 ml conical flasks containing 200 ml hydrolysates supplemented with 0.3 % urea, 0.15% potassium dihydrogen phosphate, 0.5 % yeast extract and 0.5 % peptone and the pH was adjusted to 5 by ammonium hydroxide solution. The flasks were incubated at 30°C, under stationary conditions for two days. Samples were analysed for ethanol content according to the methods of Kumnuanta et al.^[37], and Caputi et al.^[38]. The amount of ethanol in blank containing the same nutrients dissolved in distilled water was also estimated.

Estimation of ethanol

Ethanol produced in the fermentation medium was estimated by titration with potassium dichromate oxidation method^[37]. Also ethanol was estimated colorimetrically, using uv-spectrophotometer model UVD-2950, in Analytical Chemistry Unit (ACAL), Department of Chemistry, Annex (B), Faculty of Science, Assiut University, Assiut 71516, Egypt^[38].

Statistical analysis

Means and standard errors of 3 replicates for each experiment were undertaken using the SPSS for Windows (Release 10.0.1) computer package.

RESULTS

Pretreatment and chemical composition of sugarcane bagasse

In this study, the composition of SCB raw material, and TSCB were calculated and reported in TABLE 1. Bagasse composition in our results consists of approximately 42.78% and 22.32% of cellulose and lignin, respectively. Ash percentage in our results was 2.68% and 2.25 of bagasse before and after SCB pretreatment, respectively.

In our results the alkaline pretreatment with 2% NaOH, resulted in 74.22% delignification (TABLE 1). 2% NaOH we tested in pretreatment was used for lignin extraction from the sugarcane bagasse in all subsequent experiments.

TABLE 1: Chemical composition of sugarcane (SCB) and treated sugarcane bagasse (TSCB)

Substrate	Chemical Composition (% w/w)*				
	Cellulose	Hemicellulose	Total Carbohydrate	Lignin	Ash
SCB (0% NaOH)	42.780±1.10	22.500 ± 0.50	65.280±1.60	22.320 ± 0.86	02.680±0.20
TSCB (2% NaOH)	73.002±1.061	16.285±1.215	89.287±2.242	05.754±1.055	02.258±0.470

* % w/w: Percentage based on dry weight

Chemical hydrolysis

(a) Hydrolysis by H₃PO₄

Figure 1a-c shows the concentrations of the reducing sugars (RS) percentage (%) determined in the hydrolysates for different hydrolysis times and different acid concentration of H₃PO₄ in chemical hydrolysis of TSCB by phosphoric acid. At 90–120°C, it was observed that the longer the reaction time was, the higher RS concentration was obtained (Figure 1a–c). The highest percentage of RS was 34.564 in the experiment carried out at 4% H₃PO₄, at 100°C, and after incubation period of 3 hrs (Figure 1b). However, at the highest temperature (120°C), a slight decrease in RS concentration occurred compared with the other produced at 90 and 100°C temperatures of hydrolysis. RS produced by phosphoric acid hydrolysis of TSCB proportionally increased with increasing the acid concentration from (0.5 - 6%), and the optimum values were shown at the range of 4% - 6% of acid concentrations (Figure 1a, b, c). However, at 120°C the produced reducing sugars at 1% of acid concentration were mostly similar to the average of RS produced at higher percentage of acid concentrations which showed sometime approximately a slightly increase (Figure 1c).

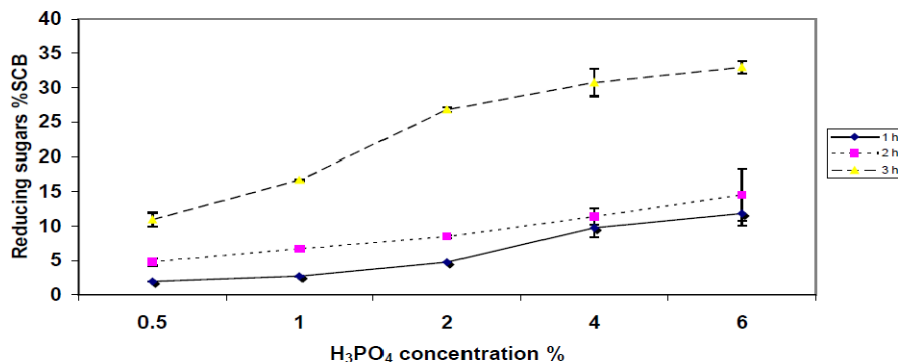


Figure 1 a: Effect of H₃PO₄ concentration on the hydrolysis of SCB, at 90°C

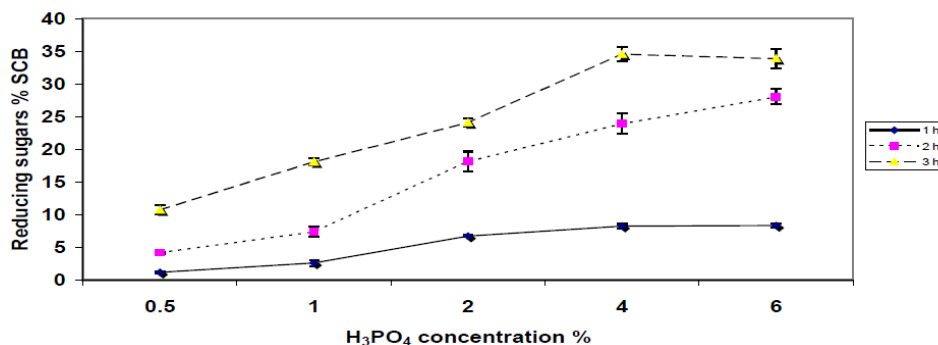


Figure 1 b: Effect of H₃PO₄ concentration on the hydrolysis of SCB, at 100°C

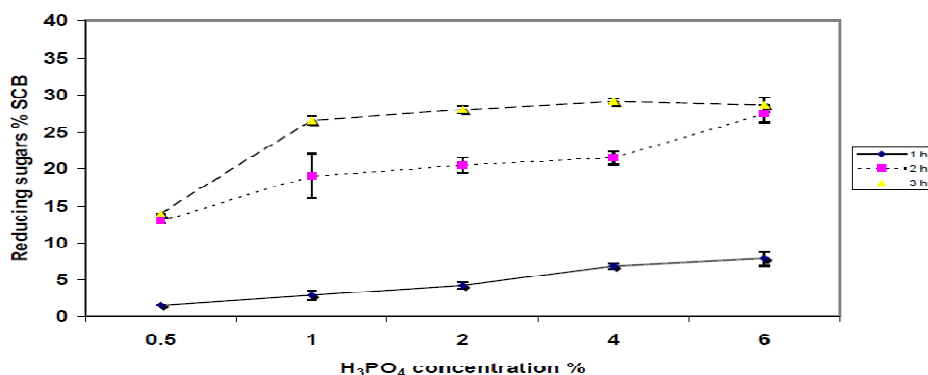


Figure 1 c: Effect of H₃PO₄ concentration on the hydrolysis of SCB, at 120°C

(b) Hydrolysis by H₂SO₄

The profiles of RS produced from sugarcane bagasse hydrolysed by 0.5% -6% of H₂SO₄ at different temperatures were similar to those of H₃PO₄ treatment (Figure 2a-c) after incubation period of 1, 2 and 3 hours, except for the highest percentage of RS (63.5 %) was shown at 90°C (Figure 2a). Values of RS at 90°C, reached to 63.5 % comparing with 42 % reducing sugars produced at 100°C, or 120°C. Mostly, production of RS increased with acid concentration increasing as well as hydrolysis by H₃PO₄, except for incubation temperature of 120°C, whereas, RS greatly decreased with increasing of acid concentration starting at the point of 1% of H₂SO₄ concentration at incubation periods 2 hrs and 3 hrs.

Interestingly, values of reducing sugars produced after 3 hrs incubation period were shown to be the highest compared with 1 hr or 2 hrs incubation periods, either at 90°C or at 100°C, and ranged between 41.752- 63.512 % reducing sugars of TSCB. On the other hand, at 120°C incubation temperature, values of RS after 3 hrs showed the lowest values and gave 15.733 % of TSCB (Figure 2c). The highest percentage of RS was 63.5% in the experiment carried out at 4% H₂SO₄, at 90°C, and after incubation period of 3 hrs (Figure 2a).

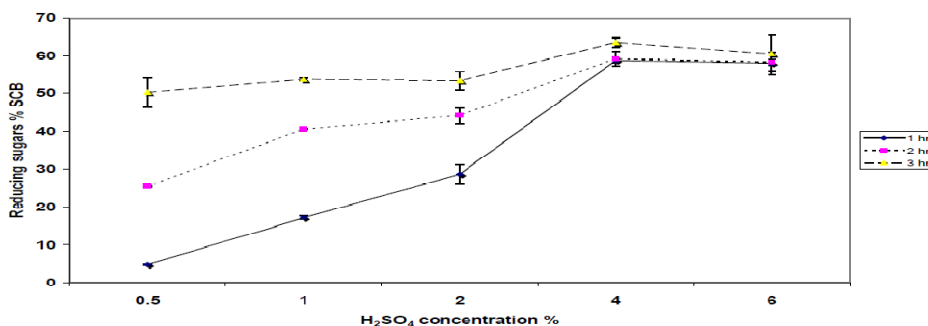


Figure 2 a: Effect of H₂SO₄ concentration on the hydrolysis of SCB, at 90°C

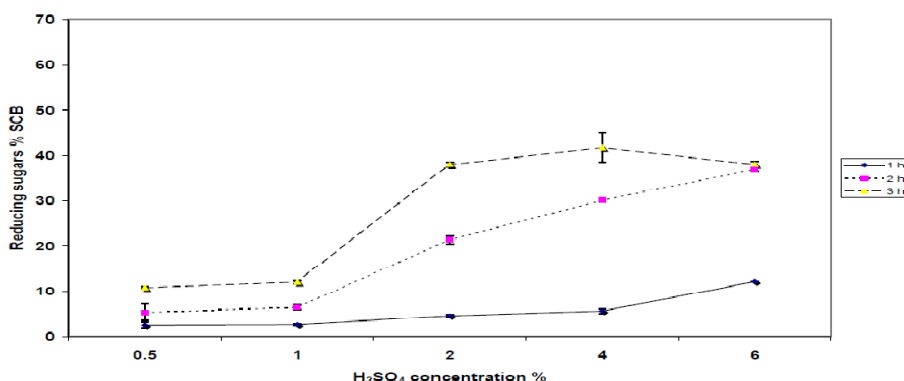


Figure 2 b: Effect of H₂SO₄ concentration on the hydrolysis of SCB, at 100°C

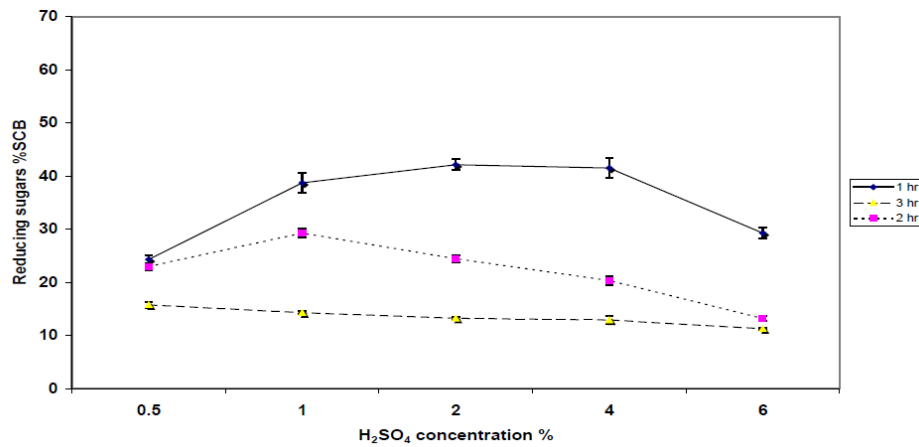


Figure 2 c: Effect of H₂SO₄ concentration on the hydrolysis of SCB, at 120°C

(c) Hydrolysis by HOClO₃

Hydrolysis of treated SCB by HOClO₃ for RS production are presented in Figure 3a-c. Result of RS concentration was always increasing with acid concentration except in the range of 4-6% HOClO₃ of high concentrations at all incubation temperatures and with most incubation periods.

Prolongation time increased TSCB hydrolysis by HOClO₃ at 90°C and decreased at 120°C. In this case RS concentration decreased from 49.563% at 90°C to 35.333 % at 120°C after 3 hours of incubation period. The highest percentage of RS was 49.5% in the experiment carried out at 4% HOClO₃, at 90°C, and after incubation period of 3 hrs (Figure 3a).

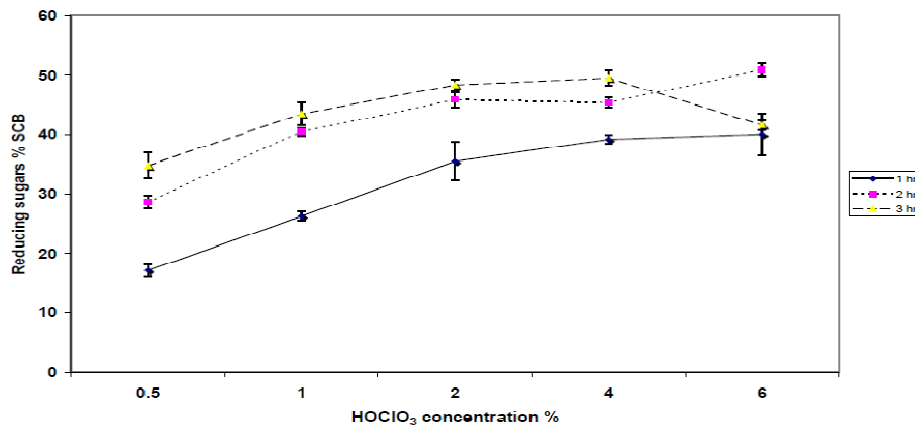


Figure 3 a: Effect of HOClO₃ concentration on the hydrolysis of SCB, at 90°C

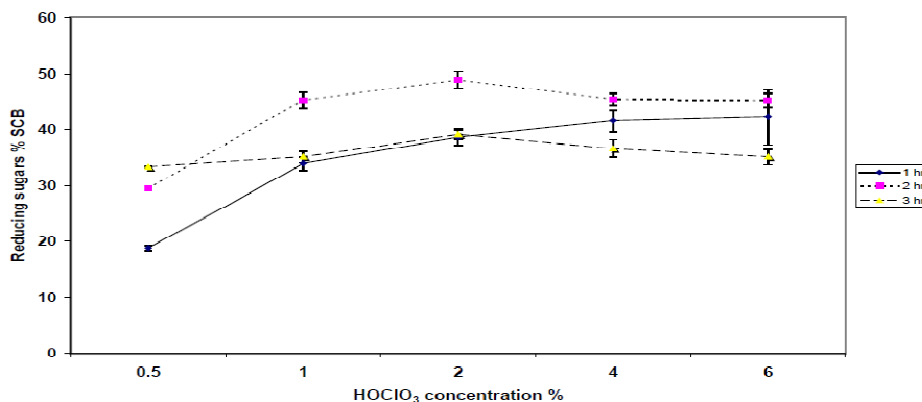


Figure 3 b: Effect of HOClO₃ concentration on the hydrolysis of SCB, at 100°C

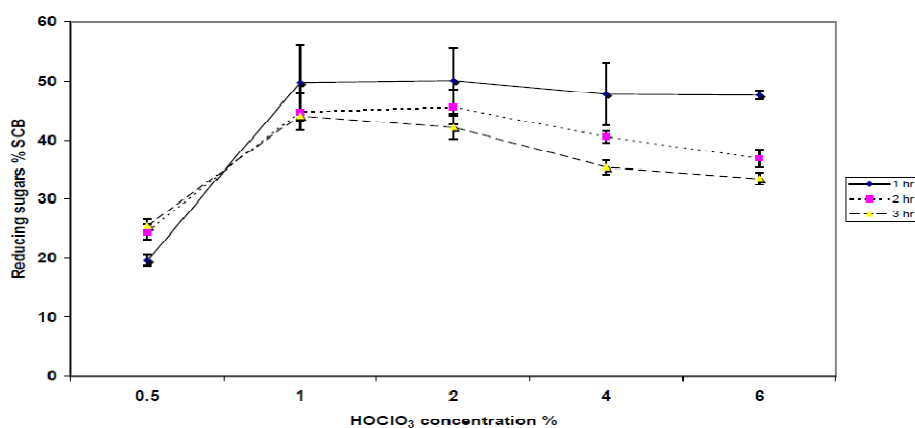


Figure 3 c: Effect of HOCIO₃ concentration on the hydrolysis of SCB, at 120°C

The profile of sugarcane bagasse hydrolysis by 0.5%-6% of HOCIO₃ at different incubation temperatures (90, 100, 120°C) were similar to those of H₂SO₄ although the sum of RS produced by HOCIO₃ was individually higher. The maximum value of H₂SO₄ hydrolysis (63.5%) was obtained under the concentration of 4% H₂SO₄, at 90°C, and after incubation period of 3 hrs (Figure 2a), whereas, that of HOCIO₃ hydrolysis (49%) was obtained under the same condition (Figure 3a), implying that H₂SO₄ could break sugarcane bagasse better than HOCIO₃. So, H₂SO₄ gave higher hydrolysis efficiency for sugarcane bagasse at lower temperature, therefore, treated sugarcane bagasse hydrolysis using 4% of H₂SO₄ at 90°C for 3 hrs was the optimum conditions performed in producing of RS for fermentation experiment.

The effects of using different volumes (5, 10, 15 ml/g TSCB) of the three tested acids (4% concentration) at 90°C for 3 hrs were investigated on the production of reducing sugars. Results in TABLE 2 show a great increase in RS with increasing acid volume (ml/g TSCB) to hydrolyze TSCB substrate.

TABLE 2: Effect of different amounts of acids solutions (ml/g TSCB) on the hydrolysis of treated sugarcane bagasse at (4%, 90°C, 3hrs.)

Catalyst	Amount of acid solution (ml/g TSCB)	Conditions (Acid conc., Temp., Incubation time)	RS % (g/g TSCB) ± SD
H ₃ PO ₄	5	(4%, 90°C, 3hrs.)	16.561 ± 1.286
	10		28.376 ± 1.247
	15		30.727 ± 1.988
H ₂ SO ₄	5	(4%, 90°C, 3hrs.)	40.471 ± 1.096
	10		61.248 ± 2.084
	15		63.512 ± 1.223
HOCIO ₃	5	(4%, 90°C, 3hrs.)	37.948 ± 2.724
	10		45.433 ± 0.945
	15		49.563 ± 1.202

Enzymatic hydrolysis

The results for fungal enzymatic hydrolysis of TSCB by *A. niger* gave more RS than hydrolysis by *T. harzianum*, and the values of RS were 0.634 g/g TSCB (63.4 % of reducing sugars), and 0.406 g/g TSCB (40.6 % of reducing sugars), respectively.

Fermentation

The results of reducing sugars, ethanol and calculated ethanol yield g/g TSCB for the different hydrolysates produced chemically by H₂SO₄ or obtained from enzymatic hydrolysis by *A. niger* or *T. harzianum* are given in TABLE 3.

TABLE 3: Production of ethanol (%) by *Saccharomyces cerevisiae* using reducing sugars produced from chemical and enzymatic hydrolysis of alkali treated SCB*

Process of TSCB hydrolysis	% of Reducing sugars (g g ⁻¹ TSCB) before fermentation	Percentage of ethanol production (%)		
		Blank (v/v)	Hydrolysate (v/v)	g g ⁻¹ TSCB
Chemically : -H ₂ SO ₄	60.513	0.258	1.403	17.9765
Enzymatically: - <i>A. niger</i>	63.425	0.258	1.840	24.8374
- <i>T. harzianum</i>	40.640	0.258	1.081	12.9211

*Alkali treated SCB was used at 5% concentration

Ethanol yield in different hydrolysates was calculated as the amount of ethanol in the hydrolysate sample subtracted from the amount of ethanol in blank sample according to the following equation.

$$\text{Ethanol \% (g/g TSCB)} = [\text{Ethanol \% hydrolysate (v/v)} - \text{Ethanol \% Blank (v/v)}] \times d/w \times 100$$

Where:

d = density of ethanol at 25 °C = 0.785 g/cm³

w = weight of TSCB

Results show that ethanol yield by fermentation was maximum in case of *A. niger* hydrolysates (0.2484g g⁻¹), followed by hydrolysates of sulphuric acid (0.1798 g g⁻¹), and in the last the hydrolysates of *T. harzianum* (0.1292 g g⁻¹) using 5% (w/v) as initial substrate of TSCB.

DISCUSSION

This study examines the prospect of biofuel production from high carbohydrate containing lignocellulosic material, e.g. sugarcane bagasse through chemical and biological means. The purpose of alkaline pretreatment for SCB was delignification. The removal of lignin is necessary for cellulose to become readily available for hydrolysis chemically or by enzymes, which permit the yeast to convert the glucose into ethanol^[39].

Lignocellulosic biomass cannot be saccharified by enzymes to high yield without a pretreatment, mainly because the lignin in plant cell walls forms a barrier against enzymatic attack^[40]. An ideal pretreatment would reduce the lignin content and crystallinity of the cellulose and increase the surface area^[41]. Lignin prevents the degradation of cellulose mainly by acting as a physical barrier between the cellulolytic enzyme and its substrate, consequently, the rate and extent of enzymatic cellulose degradation in lignocellulosic materials is inversely related to the lignin content^[42] with maximum cellulose degradation occurring only after 50 % or more of the lignin has been removed. Therefore, 2% NaOH we tested in pretreatment for all subsequent experiments.

NaOH is thought to have some saponification activity on bagasse^[43]. During alkaline pretreatment lignocelluloses undergo two reactions, namely solvation and saponification, causing the lignocelluloses structure to swell leading to an increase in internal surface area, decreasing the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates and making the lignocellulosic components more accessible to enzymatic or chemical hydrolysis^[44].

The composition obtained for the SCB in this study was in range values are found for this kind of materials, and one of these reported that SCB consists of cellulose 43.6%, hemicellulose 33.8%, lignin 18.1%, ash 2.3% and wax 0.8% on a dry weight basis^[45]. The percentages of cellulose and lignin in the chemical composition of SCB raw material used in this study are also consistent with different previously reports^[12,46,47]. The high cellulose and hemicellulose content measured for sugarcane bagasse indicates a high potential for production of a great variety of products, as ethanol, which may be then used as a fuel or raw material for synthesis of different chemicals.

Ash percentage in our results is 2.68% of bagasse offer numerous advantages in comparison to other crop residues because of its low ash content in comparison with rice straw and wheat straw, which have 17.5% and 11.0%, ash contents, respectively. This recommended its usage in bioconversion processes using microbial cultures^[48].

Hydrolysis of TSCB chemically by acids or enzymatically by fungi was performed to produce hydrolyzates for the fermentation process. During the enzymatic hydrolysis, cellulose is degraded by the cellulases to reducing sugars that can be fermented by yeasts or other organisms like bacteria to ethanol. A neutralization of pH is necessary for the downstream enzymatic hydrolysis or fermentation processes.

In acid hydrolysis of TSCB by H_3PO_4 and H_2SO_4 at lower or moderate temperature (90 or $100^\circ C$), values of RS proportionally improved with increasing of acid concentration from 0.5-4% and lowed mostly at 6%. However, at the higher temperature ($120^\circ C$), the maximum of RS was shown at the H_2SO_4 acid concentration of 2%. Gupta et al.^[49] reported that the release in sugar from *Prosopis juliflora* a wood substrate increased with increase in acid concentration and it declined thereafter because of the increase in release of some toxic compounds or inhibitors. On the contrary, increasing of hydrolyzing acids concentration from 1 to 5% (v/v) for treated rice straw, corn stalks, sawdust, sugar beet waste and sugarcane bagasse did not enhance the sugars availability^[50].

High temperature in lower acid concentrations is favorable for TSCB hydrolysis, however, at lower temperatures RS production enhanced by increasing acid concentration as well as prolongation of incubation period from 1 h to 3 hrs. This result is consistent with McMillan^[51], and reviewed by Sun and Cheng,³.

The highest percentage of RS in this result was carried out mostly at 4% of acid concentration, at the range of $90-100^\circ C$, and after incubation period of 3 hrs. Our results show a good agreement with works of Jonglertjunya et al.^[52]. These authors suggested that acid hydrolysis can be enhanced with increasing acid concentration and reaction temperature, and the optimum glucose and xylose concentrations occurred at $121^\circ C$ for 1 hour hydrolysis time in 10% sulphuric acid solution. Moreover, acid catalysis and fractionation of sugarcane bagasse to RS occurred at high temperature within short reaction times. This investigation is consistent with previously report^[53].

In this study, cellulolytic enzymes were produced in filtrate of *T. harzianum* or *A. niger* grown on pre-treated sugarcane bagasse. CMCase enzyme activities under optimum conditions of El-Katatny et al.³⁰ were used for production hydrolysate which was used in fermentation process by an indigenous isolate of *S. cerevisiae* as well as hydrolysate of chemical hydrolysis. *A. niger* exhibited higher enzyme activity on pretreated biomass of sugarcane compared with *T. harzianum* when performed for production of RS. This result is similar to some previously reports, i.e. Sharada et al.^[54] studied production of cellulase using *Trichoderma reesei* and *A. niger* using different cellulose substrates and reported maximum cellulase production in solid state fermentation by *A. niger*. Moreover, Bokhary, et al.^[55] reported that, *Aspergillus* was a predominant genus, among 61 fungal species isolated as cellulose degraders.

The calculated ethanol production after the process of fermentation ranged from 0.129 to 0.248 $g\ g^{-1}$ TSCB. These results around the amount of ethanol produced previously which was 0.1415 $g\ g^{-1}$ by *A. fumigatus* and 0.1915 $g\ g^{-1}$ in case of *Cladosporium cladosporioides*^[56]. Romero, et al.^[57] reported on the fermentation of hydrolysates obtained from olive tree pretreated with different sulphuric acid concentrations, and the maximum ethanol yield was 0.38 $g\ g^{-1}$ which reached with the hydrolyzate obtained with 0.75 N sulphuric acid.

The highest bioconversion of 5 % wastes gave 37.8 % w/w of hydrolysates by *Trichoderma viride* EMCC 107. This treatment when followed by baker's yeast fermentation, 0.41 % (v/v) ethanol and 8.2 % (v/v) conversion coefficient were obtained^[50].

Of course, the yield of ethanol was dependent on initial reducing sugars concentration. To raise reducing sugars content in the hydrolysate to the desired level, concentrating the hydrolysate by evaporation prior to use it for fermentation process is recommended. The hydrolysates obtained after chemical or fungal bio-treatment need to be processed if they are going to be used as fermentation media. In general the operations are needed (in this sequence): concentration, detoxification, neutralization and supplementation with nutrients^[28].

The formation of furfural and hydroxymethylfurfural HMF as toxic compounds gradually increases when the acids concentration or hydrolysis residence time are increased, regardless of the type of feedstock⁵⁰. The two-stage dilute acid hydrolysis increased the conversion percentage of all agro-industrial wastes to total sugars and reduced the formation of furfural and HMF comparing with single-

stage acid hydrolysis^[50]. The sugars potential of *Paulownia tomentosa* is estimated by dilute acid pretreatment and cellulase hydrolysis, whereas, dilute (1%) sulfuric acid hydrolysis was followed by cellulase complex NS 50013 and β -glucosidase NS 50010, for bio-ethanol production process^[58]. Single-stage acid hydrolysis and two-stage acids hydrolysis were assayed on agro-industrial wastes for obtainment of free sugars for bioethanol production^[50]. The fungal biotreatment of agro-industrial wastes as an alternative to the acid hydrolysis attained less released sugars in some cases; nevertheless it could be a promising choice on the economic basis.

CONCLUSION

This study examines the prospect of biofuel production from high carbohydrate containing lignocellulosic material, e.g. sugarcane bagasse through chemical and biological means. Pretreatment of SCB with NaOH followed by enzymatic hydrolysis with *A. niger* or *T.harzianum* provides a good substrate for ethanol production by *S.cerevisiae*. Acid catalysis and fractionation of sugarcane bagasse to RS occurred at high temperature within short reaction times. High temperature in lower acid concentrations is favorable for TSCB hydrolysis, however, at lower temperatures RS production enhanced by increasing acid concentration. Chemical hydrolysis of treated SCB with 4% H₂SO₄ followed by fermentation with *S. cerevisiae* gave 0.1798 g ethanol per gram treated SCB. The maximum ethanol yield was 0.2484 g g⁻¹ TSCB by *S. cerevisiae* from enzymatic hydrolysis hydrolysate produced by *A. niger*.

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