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Research Article

A Study on the Valorization of Rice Straw into Different Value-Added Products and Biofuels

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This work depicts that rice straw (RS), which is one of the major lignocellulosic wastes all over the world and causing many environmental problems, has considerable amounts of protein, ash, macronutrients, and micronutrients of approximately 11.38%, 16.77%, 2.27 mg/kg, and 771.9 mg/kg, respectively; besides, a C/N ratio of 15.18, a total N, P_2O5, and K_2O content of 1.85%, and a considerably low concentration of undesirable heavy metals and silica of approximately 77.69 mg/kg and 109 mg/kg are also present, which recommends its applicability as a precursor feedstock for the production of organic fertilizer and animal fodder. The batch solid-state fermentation (SSF) of RS by *Trichoderma longibrachiatum* DSMZ 16517 produced considerable amount of total reducing sugars (TRS) of approximately 339.2 mg TRS/g RS under the optimum operatic conditions of 20% (w:v) substrate concentration, pH 7, 1% inoculum size, a 9-day incubation period, and 30°C incubation temperature. The readily available and cost-effective agroindustrial waste, sugarcane molasses, proved to enhance the fungal biomass growth and (hemi) cellulolytic enzymes activities. The inoculated RS-SSF batch process with *T. longibrachiatum* precultured on 10% molasses enhanced the (hemi) cellulolytic enzymatic activities and TRS production rate by approximately 5.82 and 3.8 folds, respectively, relative to that inoculated by *T. longibrachiatum* precultured in the conventional potato dextrose broth medium. The separate hydrolysis and fermentation processes by different yeast strains *Candida tropicalis* DSM 70156, *C. shehatae* ATCC 58779, and *Saccharomyces cerevisiae* ATCC 64712 revealed an efficient bioethanol yield and productivity that ranged between 0.36 and 0.38 g/g sugars and 0.22 and 0.23 g/L/h, respectively, with concomitant competent fermentation efficiencies that ranged between 48.35% and 51.25%. The proximate analysis of rice straw before and after fungal hydrolysis proved calorific values of approximately 15.8 MJ/kg and 16.05 MJ/kg, respectively, recommending their applicability as primary and secondary solid biofuels. Thus, this study proved the waste prosperity of RS for environmental opulence and sustainability.

1. Introduction

The worldwide increase in population and rising demand for energy for boosted industrial activities coincide concomitantly with the depletion of nonrenewable sources of energy and the increased price of fossil fuels [1]. Transportation fuels are considered as one of the main causes of air pollution and acid rains, which negatively impact life on land and underneath water [2]. According to the UN environment program (UNEP), approximately 25% of all energy-related greenhouse gas (GHG) emissions come from the transportation sector [3]. Bioethanol as an alternative and/or complementary to gasoline would overcome the energy crisis and environmental issues [4–7].

Rice, or *Oryza sativa*, is considered the third most important grain crop worldwide, after wheat and corn [8].

It represents approximately 2% of the worldwide cultivated region [9]. Egypt is one of the main rice producers [10, 11]. The majority of the lignocellulosic waste produced by rice farming, which makes up between 40 and 60 percent of the paddy plant, is rice straw [12]. The worldwide annual production of rice straw can reach more than 731 million tons, representing approximately 2–9 tons/ha of rice cultivation [11, 13, 14]. Egypt alone contributes for about 4-5 million tons/year [11, 15]. This leads to governmental waste management concerns, air pollution, and the phenomenon of "black cloud" due to the open burning of about 3.1 million tons per year [11, 15]. Conversely, farmers continue to openly burn rice straw in fields around the world, which damages soil and increases greenhouse gas (GHG) emissions, carbon footprint, air pollution, and climate change issues, causing a detrimental effect on ecosystems and human health [16, 17]. As a solution to such issues, a portion of this waste is utilized as mulch for other crops, for the growth of mushrooms, and for the development of biofuels, biogas, biofertilizers, charcoal, and medium-density fiberboard (MDF), as well as in the creation of wood products and building materials [18].

The price of feedstock used for bioethanol production is reported to account for more than 1/3 of the total production process cost [2]. Rice straw is considered a good candidate for lignocellulosic waste that can be successfully used to produce second-generation bioethanol [6, 16, 19]. It is also considered a win-win solution for sustainable and clean energy with lower emissions and carbon footprints, waste management, and economy [1]. Although the bioethanol has a high oxygen content, which causes an increase in the fuel volumetric consumption, however, it has an excellent resistance to knock, high heat of vaporization, and it permits low-temperature combustions with fewer PM and exhaust gas emissions. Moreover, the high octane rating of bioethanol (108) allows for higher engine compression ratios and advancing the spark, which improves engine performance and efficiency [2]. Thus, bioethanol is seen as having a substantial market value in many industrial processes as well as a fuel. In addition to the emerging need for the depletion of GHG emissions, air pollution, and mitigation of climate change problems, it is also being considered a global mandate for economy and energy security as well as to resolve the food versus fuel problem [20, 21], especially after the Renewable Energy Directive (RED II), which sets a target for the percentage of biofuels used in transport at 14% by 2030 and excludes first-generation biofuels from the definition of renewable energy sources [21]. However, the cost of rice straw pretreatment is reported to represent approximately 33% of the overall bioethanol production cost [22]. The conventional dilute acid hydrolysis of lignocellulosic wastes, especially hemicellulose, is a corrosive, nonecofriendly, and costly process. Besides, it produces many acidic inhibitors, lowering the sugars and bioethanol yields [7]. Likewise, the alkali pretreatment for delignification, followed by the enzymatic saccharification, is also costly [20]. The cost of rice straw shredding, baling, collection, and transportation to the place of its processing for bioethanol is also one of the big obstacles to bioethanol production from rice straw [23]. Furthermore, the price of rice straw varies based on its excess availability for industrial purposes other than as a feedstock for the manufacturing of bioethanol [5].

The fungal saccharification of lignocellulosic wastes, followed by bioethanol fermentation, is a promising technology [24]. Yet, to be a feasible process, applying fungal isolates with sufficient delignification and saccharification efficiencies, besides high capabilities of generating (hemi) cellulolytic enzymes, is mandatory for producing a high yield of fermentable sugars within a short-time solid-state fermentation (SSF) process [25, 26]. The *Trichoderma* genus strains have been reported to play a crucial role in these processes [21, 27]. The main species involved in that criteria are *T. harzianum*, *T. viride*, and *T. reseei* [28]. Furthermore, SSF is preferable to submerged fermentation (SmF) for lower water consumption, cost-effectiveness, and higher production of (hemi) cellulolytic enzymes and fermentable sugars [29, 30]. The simplicity of the fermentation media and the absence of complicated machines, equipment, and control systems are two other benefits [31]. Moreover, applying yeast or bacterial isolate capable of fermenting hexoses and pentoses is also essential to maximize the bioethanol yield [32–34]. In addition, from an environmental and economic point of view, the bioprocessing of rice straw into bioethanol should be a zero-waste process, with the beneficial utilization of any waste or byproducts engendered during the bioprocessing platforms [11].

This manuscript aims to valorize the abundant and readily available rice straw into different value-added products, namely, organic fertilizer, animal fodder, (hemi) cellulolytic enzymes, bioethanol, and primary and secondary solid biofuels (Figure 1). Parametric optimization of rice straw SSF into a sugary solution for bioethanol production using *Trichoderma longibrachiatum* DSMZ 16517 will be performed in this study. Different species of *Trichoderma* have been used for the pretreatment of rice straw or for (hemi) cellulolytic enzymes production to be utilized for the hydrolysis of lignocellulosic wastes [16, 21, 34–37]. However, to our best knowledge, this is the first time that whole fungal cells of *T. longibrachiatum* DSMZ 16517 have been used simultaneously for (hemi) cellulolytic enzymes production besides the hydrolysis and saccharification of rice straw. In an attempt to decrease the cost of fungal preparation as an inoculum in SSF bioprocesses, the enhancement of fungal biomass yield and enzymatic activities will also be adventurously investigated using some readily available and low-cost agroindustrial wastes.

2. Materials and Methods

2.1. Microbial Strains. *Trichoderma longibrachiatum* DSMZ 16517 was used for fungal hydrolysis and saccharification of rice straw. *Candida tropicalis* DSM 70156, *Candida shehatae* ATCC 58779, and *Saccharomyces cerevisiae* ATCC 64712 were used for bioethanol fermentation.

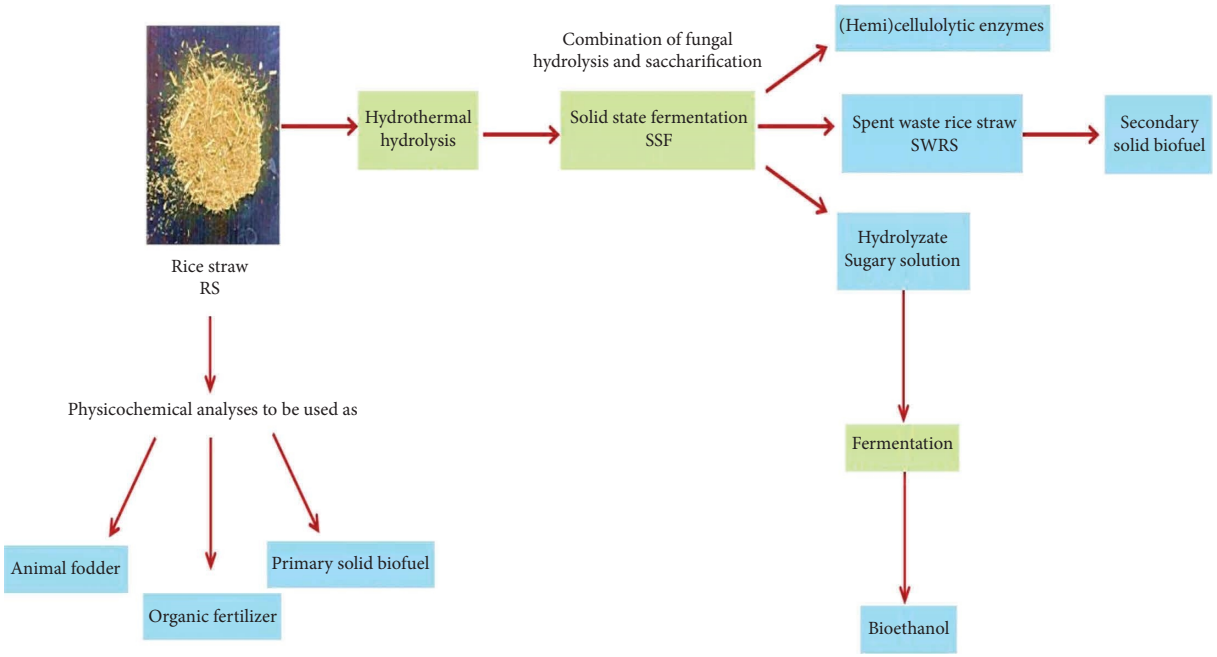


Figure 1: Schematic representation for valorization of rice straw into different value-added products.

2.2. Media. Potato dextrose (PD) agar medium (PDA) [16] and Wickerham (WH) agar medium (WHA) [38] were used for maintenance and cultivation of fungal and yeast strains, respectively.

2.3. The Agroindustrial Wastes Used in This Study. Rice straw was collected from local farmers in Kafr El-Sheikh governorates, Egypt. The collected rice straw was chipped and grinded, then dried in sunlight, and finally, sieved to a constant size (≈0.5–1 cm). The obtained dried biomass was then stored in plastic bags at room temperature until analysis and treatment were performed.

To enhance the biomass yield and enzymatic activity of *T. longibrachiatum* DSMZ 16517, the following two readily available, sustainable, and cheap sources of essential nutrients were used:

(1) Sugarcane molasses (SCM) which is one of the main waste products of Egyptian sugarcane industries. It was obtained from Sugars and Integrated Industries Egyptian Distillation Plants, El-Hawmdia City, Giza, Egypt. It costs 0.36 $/kg. Its physicochemical characteristics and minerals composition are listed in Tables 1 and 2.

(2) Corn steep liquor (CSL) which is one of the main waste products of starch industries. It was obtained from Corn Refining Company, Mostorod, Cairo, Egypt. It costs 0.65 $/kg. Its physicochemical characteristics and minerals composition are listed in Tables 1 and 2.

2.4. Methods of Analysis. The Walkley and Black method [39] was used to determine the concentrations of organic carbon and organic matter. The Jackson [40] method was used for measuring the pH and electrical conductivity. The Kjeldahl digestion method, as reported by Chapman and Pratt [41], was utilized to quantify total N, and the crude protein concentration was computed using the conversion factor of 6.25. The analysis of total phosphorus was conducted using the methodology expounded by Watanabe and Olsen [42]. The inductively coupled plasma-mass spectrometry (ICP-MS, Spectro Ciros CCD ICP-OES, Spectro Analytical Instruments, Kleve, Germany) was used to quantify minerals and heavy metals in accordance with the methodology outlined by Benton [43]. All the aforementioned physicochemical analyses have been performed at Soil and Water Unit, Central Lab, Faculty of Agricultural, Ain Shams University. Hemicellulose, cellulose, and lignin percentages in rice straw before and after fungal hydrolysis were determined according to Moubasher et al. [44], at the Egyptian General Organization for Export and Import Control (GOEIC). The proximate analysis of rice straw before and after fungal hydrolysis has been also performed at

Table 1: Physicochemical analysis of the purchased CSL and molasses.

Parameters	CSL	Molasses
Electrical conductivity (dS/m)	1.27	1.15
Total dissolve solids (mg/L)	813	736
Organic C (%)	19.93	20.99
Organic matter (%)	34.28	36.10
Total nitrogen (%)	1.71	1.81
Protein content (%)	10.71	11.31
P_2O_5 (%)	0.22	0.25
K_2O (%)	0.31	0.88
$N + P_2O_5 + K_2O$ (%)	2.24	2.94
C/N	11.65	11.60
pH	4.3	4.8

Table 2: Mineral composition of the purchased CSL and molasses.

	Macronutrients				Micronutrients				Undesired heavy metals						
	P	K	Ca	Mg	Fe	Zn	Mn	Cu	Ni	Cd	Cr	Pb	As	Hg	Co
	mg/kg				mg/kg				mg/kg						
CSL	9.56	25.79	28.91	1.55	11.56	8.12	14.40	1.22	0.34	0.13	0.01	0.02	0.0	0.0	0.37
Molasses	10.91	71.37	27.08	1.51	16.44	15.36	2.10	1.38	0.34	0.12	0.01	0.02	0.0	0.0	0.36

GOEIC, and the moisture content, volatile matter, ash content, and calorific value were determined according to the standard methods (EN15414-3/2011[45], EN15402/2011[46], EN15403/2011[47], and ASTM D5865-19 [48], respectively).

The 3,5-dinitro salicylic acid (DNSA) method [48] was used to determine the total reducing sugar (TRS) concentration in the hydrolyzates before and after bioethanol fermentation. The TRS concentrations were calculated as the equivalent to the glucose standard curve (10–2000 mg/L) at $\lambda_{540\ nm}$, using an ultraviolet/visible/near infrared (UV/Vis/NIR) double beam spectrophotometer (model V-570, JASCO International Co., Ltd., Tokyo, Japan).

The identification of sugars profile and their quantification in the hydolyzates before and after bioethanol fermentation were performed using high performance liquid chromatography (HPLC) model Agilent 1200 series USA equipped with a refractive index RI detector model (Agilent 1260 infinity, Santa Clara, CA, USA) and Suplcosil™ LC-NH_2 (25 cm × 4.6 mm length and internal diameter, respectively, and 5 μm particle size, Sigma-Aldrich Co. LLC) column. The injection volume was 10 µL. Isocratic separation mode was used, with a flow rate of 1.0 mL/min and analytical mobile phase of water/acetonitrile 25 : 75 (v/v). The column temperature was 35°C. A mixture of standard sugars was used, which constituted of rhmanose, xylose, arabinose, fructose, mannose, glucose, galactose, sucrose, maltose, and lactose (Figure 2).

Gas chromatography (model 6890 (G1530A), Agilent, Santa Clara, CA, USA) with a flame ionization detector and nominal capillary column (HP-5, 5% phenyl-95% methylsiloxane 30 m × 250 m I.D., 5.00 μm film, Santa Clara, CA, USA) was used to measure the amount of ethanol in g/L. The carrier gas, nitrogen, flowed at a rate of 25 mL/min, and the oven and detector temperatures were 300°C [38]. The samples were stored in a fridge at −18°C until analysis to prevent spoilage by microbes and loss of ethanol. All analyses were performed in triplicates, and the average mean values were taken into account and presented as average mean values ± standard deviation (StD), where the StD value is within the 95% confidence interval's bound.

2.5. Optimization of Rice Straw Solid State Fermentation. One-factor-at-a-time (OFAT) has been applied for the optimization of rice straw solid-state fermentation (SSF). The effect of pH, incubation temperature, incubation period, salinity, and inoculum size on TRS yield was studied. Table 3 describes the conditions of each performed experiment.

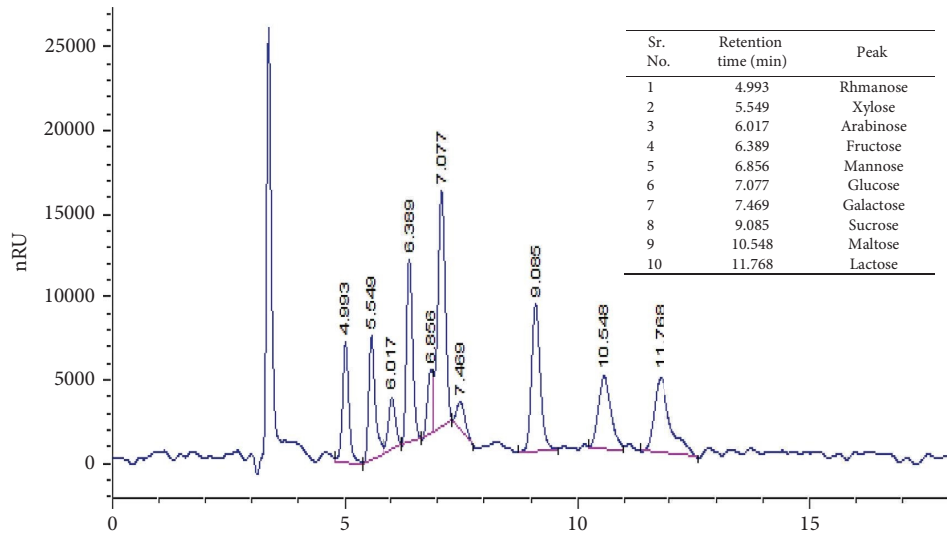
Ten grams of rice straw were put into 250 mL Erlenmeyer flasks containing 50 mL distilled water. The flasks were hydrothermally pretreated by autoclaving at 121°C and 1.2 bar for 20 min [49]. Then, flasks were inoculated with the required inoculum size of *T. longibrachiatum* DSMZ 16517, after cooling to room temperature. The flasks were then incubated statically at the set temperature. After the prescribed incubation period, the flask content was extracted by mixing vigorously with 100 mL distilled water and then filtered within a cloth cheese to separate the content into liquid and solid parts. The liquid filtrate (i.e., the hydrolyzate) was then centrifuged at 10,000 rpm for 10 min to remove any rice straw or fungal biomass debris. Finally, the TRS concentration was determined in the decanted hydrolyzate by the DNSA method. The saccharification percentage was calculated according to Nawaz et al. as follows [50]:

$$\text{Sacchrification \%} = \frac{\text{TRS}\,(g/L) \times 0.9 \times \text{reaction volume}\ (L) \times 100}{\text{Rice straw weight}\ (g)}. \quad (1)$$

2.6. Fungal Biomass and Enzymes Enhancement. The enhancement of fungal biomass and enzymatic activities was also investigated using different readily available, sustainable, and cheap sources of essential nutrients (SM and CSL) in comparison to the conventional PD broth medium (PDM).

2.6.1. Fungal Biomass Enhancement. Approximately, 0.1 g of *T. longibrachiatum* DSMZ 16517 were inoculated into three batch groups of 250 mL Erlenmeyer flasks containing 50 mL of sterilized PDM and different concentrations of SCM or CSL dissolved in distilled water (5–25% w/v) of pH 7. The inoculated flasks were then incubated for 15 d in an orbital shaking incubator set at 30°C and 50 rpm. The fungal biomass was determined as dry weight at prescribed time intervals.

2.6.2. Screening of Enzymatic Activities of T. longibrachiatum DSMZ 16517 Precultured in Different Enriching Media on Rice Straw. Three groups of SSF 250 mL bioreactor batches



Sr. No.	Retention time (min)	Peak
1	4.993	Rhmanose
2	5.549	Xylose
3	6.017	Arabinose
4	6.389	Fructose
5	6.856	Mannose
6	7.077	Glucose
7	7.469	Galactose
8	9.085	Sucrose
9	10.548	Maltose
10	11.768	Lactose

Figure 2: HPLC separation of sugars.

Table 3: Rice straw solid state-fermentation experimental conditions.

Studied parameters	Experimental conditions
Effect of pH	Incubation temperature 30°C, substrate concentration 20% (w:w), inoculum size 1% (w:w), 7 d incubation period, and pH ranged between pH 4.5 and pH 7.5
Effect of incubation temperature	Substrate concentration 20% (w:w), inoculum size 1% (w:w), 7 d incubation period, pH 7, and incubation temperature ranged between 20°C and 40°C
Effect of incubation period	Incubation temperature 30°C, substrate concentration 20% (w:w), inoculum size 1% (w:w), pH 7, and incubation period ranged between 3d and 11 d
Effect of salinity	Incubation temperature 30°C, substrate concentration 20% (w:w), 9 d incubation period, inoculum size 1% (w:w), pH 7, and salinity as NaCl ranged between 0 g/L and 40 g/L
Effect of inoculum size	Incubation temperature 30°C, substrate concentration 20% (w:w), 9 d incubation period, pH 7, and inoculum size ranged between 0.5% and 2.5% (w:w)

containing sterilized 20% (w/v) rice straw, and adjusted at pH 7, were inoculated with 1% (w/w) *T. longibrachiatum* DSMZ 16517 precultured in different enriching media and harvested at the late exponential phase (approximately 9 d). The inoculated batches were statically incubated for 9 d at 30°C. After the prescribed incubation period, the flasks contents were extracted by 100 mL distilled water and the hydrolyzates were obtained as previously described. Finally, the TRS concentration was determined in the decanted hydrolyzates by the DNSA method, the saccharification percentages were calculated according to Nawaz et al. [50], and the (hemi) cellulolytic enzymes concentrations were also measured.

2.6.3. Enhancement of (Hemi) Cellulolytic Enzymatic Activities. Two groups of SSF 250 mL bioreactor batches containing sterilized 20% (w/v) rice straw, and adjusted at pH 7, were inoculated with 1% (w/w) *T. longibrachiatum* DSMZ 16517 precultured in PD-broth or 10% SCM media and harvested at the late exponential phase (approximately 9 d). The inoculated batches were statically incubated for 11 d at 30°C. After a set of prescribed time intervals, the flasks' contents were extracted by 100 mL distilled water and the hydrolyzates were obtained as previously described. Finally, the TRS concentrations in the decanted hydrolyzates, the saccharification percentages, and the (hemi) cellulolytic enzymatic activities were determined with time.

2.6.4. (Hemi) Cellulolytic Enzymes Assays

(1) Endoglucanase Activity. Endoglucanase activity as carboxymethyl cellulase (CMCase) activity in the hydrolyzate was determined according to the method described by Li et al. [51] as follows: 1% (w/v) solution of carboxymethyl cellulose (CMC) in sodium acetate buffer pH 5.0 was preprepared. In a clean dry tube, 1 mL of the hydrolyzate (i.e., the cocktail enzymes) was then mixed with 1 mL of the prepared 1% CMC solution in acetate buffer. The tube was then incubated at 63°C for 30 min. Finally, the liberated TRSs were measured by the DNSA method as described before, at $\lambda_{540\ nm}$. The blank was 1 mL of distilled water instead of 1 mL of supernatant (enzyme). The concentration of the resulted reducing sugars was determined using the glucose standard curve. One unit of CMCase is the micromole of glucose liberated per mL of hydrolyzate filtrate per minute.

(2) Exoglucanase Activity. Exoglucanase activity as filter paperase (FPase) activity in the hydrolyzate was determined according to the method described by Chandra and Reddy [52] as follows: 1 mL of the hydrolyzate (i.e., the cocktail enzymes) was mixed with 2 mL of 0.1 M citrate buffer pH 4.8 containing 0.05 g of filter paper (Whatman no.1). The tube was incubated at 50°C for 1 h. The liberated TRSs were measured by the DNSA method, as described before, at $\lambda_{540\ nm}$. The blank was 1 mL of distilled water instead of enzyme. The concentration of the resulted TRS was determined using the glucose standard curve. One unit of FPase is the micromole of glucose liberated per mL of hydolyzate filtrate per minute.

(3) Cellobiase Activity. Cellobiase activity as β-1,4-glucosidase activity in the hydrolyzate was determined according to the method described by Tomaz et al. [53] as follows: 0.5 mL of hydrolyzate was incubated with 0.5% cellobiose in 0.05 M acetate buffer pH 5.0 for 60 min at 30°C. The reaction was stopped by heating in a boiling water bath for 10 min. The enzyme activity was determined by measuring the concentration of the glucose released in the medium using the DNSA method, as described before, at $\lambda_{540\ nm}$. The blank was 1 mL of distilled water instead of enzyme. The concentration of the resulted TRS was determined using the glucose standard curve. One unit of β-1,4-glucosidase is the micromole of glucose liberated per mL of hydolyzate filtrate per minute.

(4) Xylanase Activity. Hemicellulase activity was measured as xylanase activity in the hydrolyzate was determined according to the method described by Meng et al. [54] as follows: 1% (w/v) solution of Brich wood xylan in sodium acetate buffer pH 5.0 was prepared. In a clean dry tube, 1 mL of the hydrolyzate (i.e., the cocktail enzymes) was mixed with 1 mL of the preprepared 1% xylan solution in acetate buffer. The tube was then incubated at 63°C for 30 min. Finally, the liberated TRS were measured by the DNSA method as described before, at $\lambda_{540\ nm}$. The blank was 1 mL of distilled water instead of 1 mL of supernatant (enzyme). The concentration of the resulted TRS was determined using the xylose standard curve. One unit of xylanase is the micromole of xylose liberated per mL of hydolyzate filtrate per minute.

For the (hemi) cellulolytic enzymatic activity in solid-state fermentation (SSF), CMCase U/mL, FPase U/mL, cellobiose U/mL, and xylanase U/mL are converted to U/g lignocellulosic weight, applying the following equation [55]:

$$\text{Enzyme activity U/g} = \frac{\text{Enzyme activty } U/mL \times \text{hydrolyzate volume } mL}{\text{Lignocellulosic weight } g}. \quad (2)$$

2.7. Bioethanol Fermentation. This was performed anaerobically, in 250 mL bioreactors with a working volume of 100 mL, at 30°C, 48 h, and a mixing rate of 100 rpm [34]. 10.0 g/L peptone, 2.0 g/L KH_2PO_4, and 1.0 g/L $MgSO_4.7\ H_2O$ were supplemented to the obtained rice straw hydrolyzate and the pH was adjusted to 5.5 using 1M HCl, before sterilization by autoclaving at 121°C, 1.2 bar for 20 min. The sterilized hydrolyzate was then individually inoculated by 10% (v/v) of the preselected yeast isolates, namely, *Candida tropicalis* DSM 70156, *Candida shehatae* ATCC 58779, and *Saccharomyces cerevisiae* ATCC 64712. The produced ethanol was then quantified using GC-FID. The residual TRS was determined by the DNSA method [48] and the residual total sugars profile was quantified by HPLC.

The ethanol yield was calculated according to Ma et al. [20] as follows:

$$\text{Bioethanol yield} = \frac{\text{Produced bioethanol } g/L}{\text{Utilized sugars } g/L}. \quad (3)$$

The theoretical ethanol yield was calculated according to Legodi et al. [56] as follows:

$$\text{Theoretical yield \%} = \frac{\text{Produced bioethanol } g/l}{\text{cellulose content } g/g \times \text{rice straw } g \times 1.111 \times 0.51} \times 100, \quad (4)$$

where 0.51 is the conversion factor for glucose to ethanol based on stoichiometric biochemistry of yeast, while 1.111 is the conversion factor of cellulose to equivalent glucose.

The fermentation efficiency is also calculated according to El-Metwally et al. [57] as follows:

$$\text{The fermentation effeciency} = \frac{\text{Produced ethanol } g/L}{\text{Theoretical recovery } g/L} \times 100, \quad (5)$$

$$\text{Theoretical recovery} = \text{Total sugars g/L} \times 0.64. \quad (6)$$

2.8. Physicochemical Characterization of Rice Straw before and after Fungal Hydrolysis. The surface morphology of rice straw before and after fungal hydrolysis was examined by a field emission scanning electron microscope implanted with smart energy dispersive X-ray spectroscopy (FESEM-EDX, model Sigma 300VP, Carl Zeiss, Jena, Germany). The proximate and biochemical analyses' rice straw before and after fungal hydrolysis were also performed; besides, the calorific value was also measured according to the ASTM D5865-19 [48], using the calorific value tester (Parr 6200, Parr Instrument Company, IL, USA), to be used as a solid biofuel.

3. Results and Discussion

3.1. The Physiochemical Characteristics and Mineral Composition of the Collected Rice Straw. The listed chemical constituents (Table 4) and mineral content (Table 5) of the collected rice straw were found to be within the permissible limits of the Egyptian standard for solid organic fertilizer [59]. As such, this recommends the application of rice straw as a precursor feedstock in the preparation of an ecofriendly organic fertilizer. The protein content in the collected rice straw was recorded to be $11.38 \pm 0.2\%$, and ICP analysis proved considerably low Si-content of 109 ± 2 mg/kg. Sherief et al. [60] reported that the protein content in rice straw can range between 3.6% and 7.2%. The chemical composition of rice straw varies according the regional area of cultivation, climate, cultivation practices, and time between harvesting and baling [61]. However, rice straw is known to be mainly composed of carbohydrates, including cellulose and hemicellulose biopolymers crusted by lignin, with a relatively high C/N ratio [62], but low content of protein. It can be mixed with vital nutritional elements to be used as animal fodder.

3.2. Hydrothermal Pretreatment of Rice Straw. The hydrothermal pretreatment of rice straw by autoclaving at 121°C and 1.2 bar for 20 min produced 2.18 g/L TRS with a saccharification percentage of approximately 2.85%. The hydrothermal pretreatment of lignocellulosic wastes deteriorate the lignin layer and hemicellulose linkages, which would make cellulose more accessible for fungal hydrolyzes and scarification [34].

3.3. Optimization of Rice Straw Fungal Hydrolysis Using the Standard Strain T. longibrachiatum DSMZ 16517. SSF process is reported to be more advantageous than the conventional SmF as it consumes less water with lower probabilities of contamination and small bioreactors' size [30]. It enriches enzymes activities with higher stabilities over wide ranges of temperatures and pH and produces higher products' yields at relatively high temperature and pH, with lower disposed waste effluents [9]. It consumes lower amounts of solvents for products' recovery and energy as there is usually no need for agitation [30]. Besides, it is reported to be approximately 10-fold more cost-effective than SmF in the production of cellulases [9].

The pH and incubation temperature are crucial parameters for fungal growth and metabolism besides enzymatic activity and production [19]. Figure 3(a) denotes that the maximum fabrication of TRS and saccharification efficiency occur at pH 7. Raghuwanshi et al. [63] reported that the production of hydrolytic enzymes by *T. asperellum* RCK2011 and its mutant strain SR1-7 retained more than 90% of their optimal activity at pH ranging from 4.0 to 10.0.

Figure 3(b) reveals that the maximum TRS production and saccharification efficiency occur at 30°C. Similar observation was reported for *Trichoderma* sp. RCK65 [64]. Temperature is known to unswervingly impact the microbial growth and its protein dynamics [65]. Legodi et al. [55] reported that the increase in temperature over 30°C would affect the fungal cell membrane, growth, and cellulases production of *T. longibrachiatum* LMLSAUL 14-1. According to Aggarwal et al. [19], the increase in temperature over 28°C may lead to denaturation of the produced enzymes and consequently decrease the saccharification efficiency.

The optimum incubation period was found to be within 9 d; thereafter, a significant decrease in the TRS yield and saccharification efficacy occurred (Figure 3(c)). This might be due to the occurrence of feedback inhibition because of the accumulation of some inhibitory compounds, for example, cellobiose on CMCase, besides the changes in pH and decline in moisture content that would happen with time [19, 66]. The optimum incubation period differs with substrate, inoculated fungi, initial pH, and fermentation medium [19].

The inhibitory effect of NaCl as an example of inorganic salts is so obvious (Figure 3(d)). Similar observation was reported by Mutrakulcharoen et al. [67].

Results illustrated in Figure 3(e) show the importance of inoculum size on the efficiency of RS-SSF. Similar observation was reported by Aggarwal et al. [19] and attributed that to the fungal sporulation and metabolic activities.

Table 4: Physicochemical analysis of the collected rice straw.

Parameters	Rice straw	Egyptian organic fertilizer standard (8079/2017)
Electrical conductivity (dS/m)	1.14 ± 0.2	6–10
Total dissolve solids (mg/L)	729.6 ± 0.2	3840–6400
Organic C (%)	27.63 ± 0.5	Min 9
Organic matter (%)	47.52 ± 0.5	Min 16
Moisture content (%)	7.20 ± 0.6	Max 30
Total nitrogen (%)	1.82 ± 0.2	Min 0.5
P_2O_5 (%)	0.025 ± 0.01	Min 0.6
K_2O (%)	0.004 ± 0.001	Min 0.4
$N + P_2O_5 + K_2O$ (%)	1.85 ± 0.07	Min 1.5
C/N	15.18 ± 0.2	18–22 : 1
pH	6.82 ± 0.05	6–8

At relatively low inoculum size, longer time is required for the fungal proliferation, colonization on the substrate, enrichment of enzymes system, and substrate consumption [19]. However, at relatively high inoculum size, the spore density increases, burdening oxygen diffusion, which consequently inhibits the fungal growth, enzyme production, and substrate consumption [68]. According to Chang and Web [69], the increased inoculum size is not favorable as it utilizes readily available fermentable sugars in hydrolyzate for growth.

Approximately 339.2 mg TRS/g RS was obtained at optimum operatic conditions of the SSF batch process of 20% (w:w) substrate concentration, pH 7, 1% inoculum size, 9 d incubation period, and 30°C incubation temperature.

3.4. Enhancement of Fungal Biomass and (Hemi) Cellulolytic Enzymes Using Agroindustrial Wastes

3.4.1. Screening for a Cost-Effective Fungal Precultivation and Enrichment Medium

(1) Biomass Enrichment. According to the data illustrated in Figures 4(a) and 4(b), it can be depicted that the highest biomass yield obtained after 9 days of incubation recording approximately 17.89 g/L, 12.99 g/L, and 15.21 g/L for 10% molasses, 10% CSL, and PD broth media, respectively. Based on the physicochemical analysis (Table 1) and minerals composition (Table 2) of the purchased SCM and CSL, they can act as sources of C, N, P, K, and other essential elements required for microbial growth and enrichment. Molasses as a cheap and readily available feedstock has been previously reported for fungal biomass production [70]. The presence of nitrogen source in the cultural medium is essential, as it is important for protein synthesis and it is also one of the major components of cellular proteins [19]. Phosphorus is another important element that is mandatory for fungal growth and metabolism. It is an essential component of phospholipids, and it aids in the development of cell membranes. In addition to joining the nucleotides that make up nucleic acid strands, it plays a crucial part in the synthesis of several intermediates, enzymes, and coenzymes that are needed for intracellular activities, other oxidative reactions, and the metabolism of carbohydrates [71]. The SCM was found to possess lower Si-content than that of CSL, recording approximately 0.63 and 0.73 mg/kg, respectively. Moreover, the SCM attested to have relatively higher N, P, K, Fe, and protein contents than CSL (Tables 1 and 2). Besides, the SCM ascertained to have relatively lower undesirable heavy metals than CSL (Tables 1 and 2). Thus, that would explain its relatively higher efficiency for promoting the fungal growth. Similar observation was reported by Zabidi et al. [72], whereas molasses showed better promotion to microbial growth than the agroindustrial waste soybean pulp, which was attributed to the molasses' higher sugars and protein contents [72].

(2) Enhancement of (Hemi) Cellulolytic Enzymatic Activity. This experiment was done to study the effect of the inoculation of rice straw-SSF bioreactors by *T. longibrachiatum* DSMZ 16517 precultivated and enriched on 10% molasses, 10% CSL, or the conventional PD-broth medium on TRS yields and (hemi) cellulolytic enzymatic activity. The applied SSF of RS using precultured *T. longibrachiatum* DSMZ 16517 on 10% (w/v) SCM medium proved to have the most sufficient hydrolysis and saccharification efficiencies (Table 6 and Figure 5) and (hemi) cellulolytic enzymatic activity (Table 6 and Figure 6).

T. longibrachiatum DSMZ 16517 precultivated on 10% SCM performed a saccharification efficiency of approximately 33.14% and TRS yield of 24.55 g/L (Table 6 and Figure 5). This was comparable to *T. longibrachiatum* DSMZ 16517 precultivated on the conventional PD-broth medium, which recorded 31.12% and 23.05 g/L, respectively (Table 6 and Figure 5). But it was approximately 1.7-fold higher than those occurred in RS-SSF bioreactors inoculated by *T. longibrachiatum* DSMZ 16517 precultivated on 10% CSL, which recorded 19.83% and 23.05 g/L, respectively (Table 6 and Figure 5). This recommends the application of SCM for enrichment of fungal biomass in biofuels and enzymes production processes using lignocellulosic wastes. Molasses has been reported as a favorable medium for microbial growth and production of valuable metabolites and products [4, 38, 73, 74].

Trichoderma species are known to have a cellulolytic enzymes system composed of exoglucanases, endoglucanases, and β-glucosidase that synergistically hydrolyzes and scarifies cellulose into fermentable sugars, e.g., glucose [28, 75, 76]. Data listed in Table 6 and illustrated in Figure 6 depicted that SCM enhanced the (hemi) cellulolytic enzymatic activities of *T. longibrachiatum* DSMZ 16517. The cellulolytic and xylanase activities of *T. longibrachiatum* DSMZ 16517 precultured on 10% SCM were approximately 3.65-fold and 2-fold of that precultured on 10% CSL, respectively.

Nevertheless, the CMCase, FPase, β-glucosidase, and xylanase activities of *T. longibrachiatum* DSMZ 16517 precultured on 10% SCM were approximately 1.39, 1.44, and 1.73 folds of that precultured in conventional PD-broth medium, while the xylanase activities were comparable, recording 946.5 U/g and 933.33 U/g, respectively.

Table 5: Mineral composition of the collected lignocellulosic wastes.

	Macronutrients				Micronutrients			Undesired heavy metals							
	P	K	Ca	Mg	Fe	Zn	Mn	Cu	Ni	Cd	Cr	Pb	As	Hg	Co
	mg/kg					mg/kg						mg/kg			
Rice straw	1.08 ± 0.02	0.28 ± 0.02	0.41 ± 0.02	0.50 ± 0.02	320.3 ± 0.5	72.3 ± 0.5	327 ± 0.5	52.3 ± 0.5	38.4 ± 0.5	1.10 ± 0.2	0.35 ± 0.05	1.64 ± 0.05	0.0002 ± 0.0	0.001 ± 0.0	36.2 ± 0.05
Egyptian organic fertilizer standard 8079/2017	—	—	—	—	—	Max 300	—	Max 100	Max 180	Max 5	Max 300	Max 300	—	Max 4	Max 100

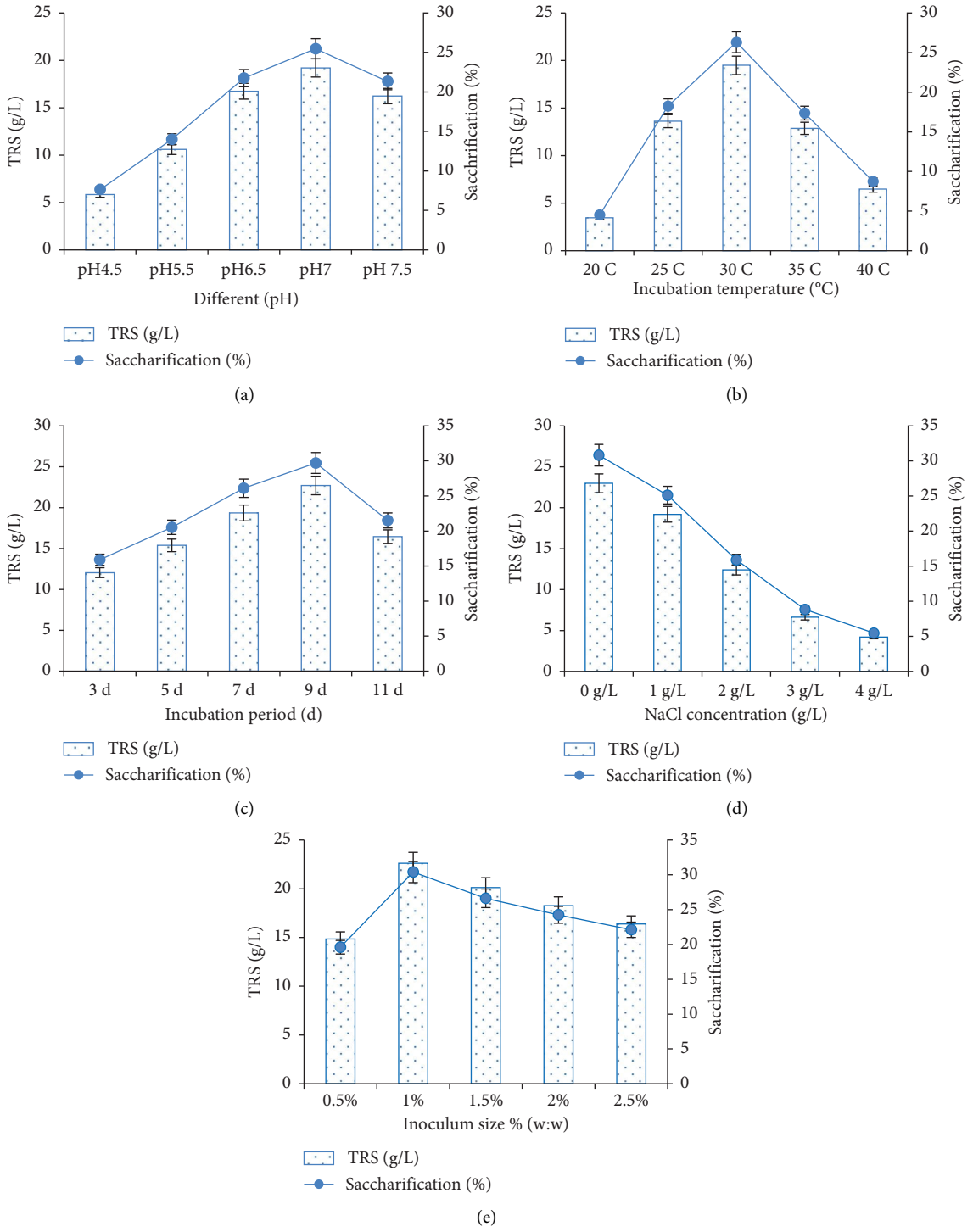


Figure 3: Optimization of fungal hydrolysis of rice straw.

Although the growth of *T. longibrachiatum* DSMZ 16517 on CSL was enhanced, the enzymatic activities and hydrolysis and saccharification efficiencies were reduced. The CMCase and FPase activities decreased by approximately 38%, while the β-glucosidase and xylanase activities decreased by approximately 50%, relative to those of *T. longibrachiatum* DSMZ 16517 precultured in the conventional PD-broth medium. This consequently decreased the hydrolysis and saccharification efficacies by approximately 64%. According to Zabidi et al. [72], molasses is regarded as a cellulosic feedstock that stimulates the synthesis of cellulolytic enzymes in cellulolytic microbes. It also serves as cost-effective conveniently available source of fermentable sugars to produce extracellular (hemi) cellulolytic enzymes [73, 74]. Assorted sources of carbon were reported to induce the fungal (hemi) cellulolytic enzymes

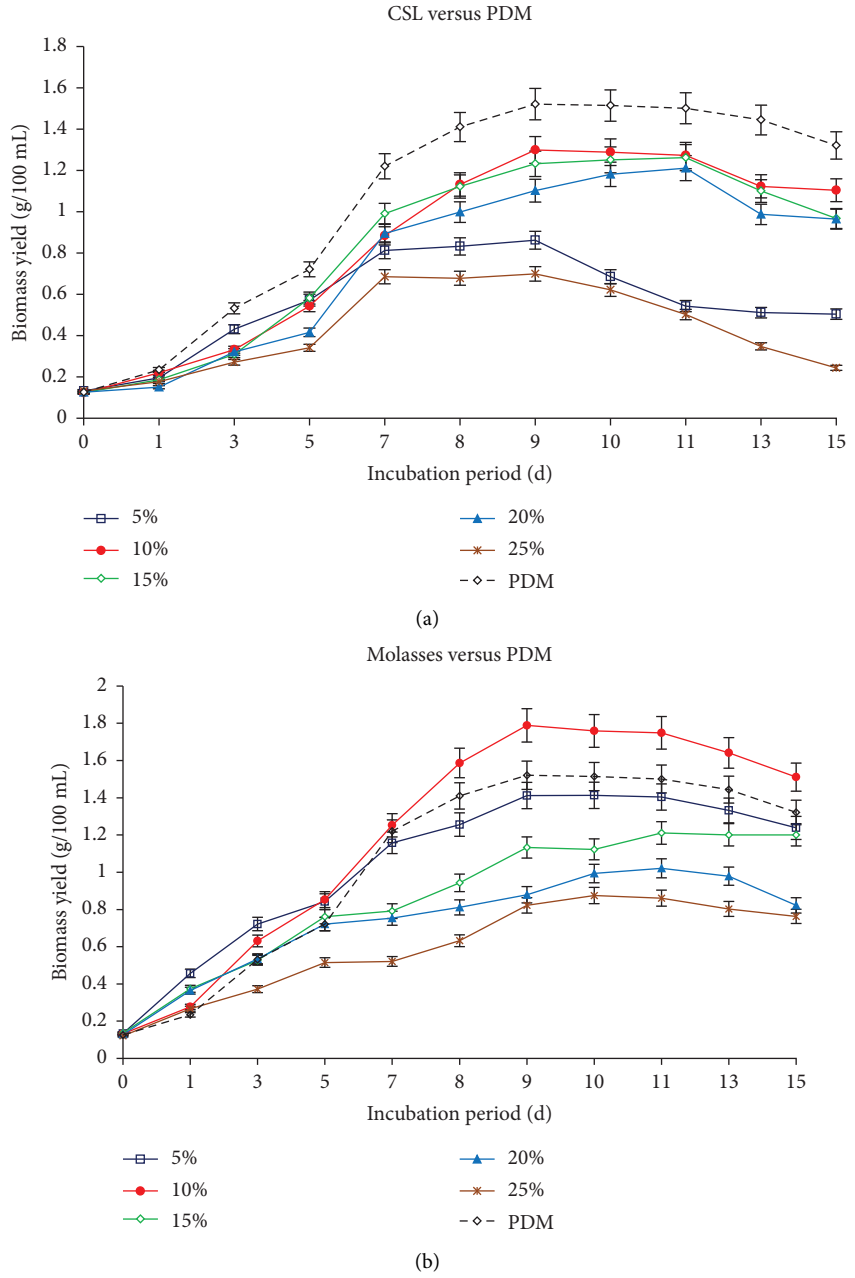


Figure 4: Effect of media components on the biomass yield.

system [19, 77, 78]. Moreover, organic nitrogen sources are known to have higher positive cellulase activity responses than inorganic ones [79]. However, that varies with fungal strains, whereas the presence of readily available C- and N-sources may enhance the fungal growth but may not be inductive for (hemi) cellulolytic enzymes [80]. *T. longibrachiatum* DSMZ 16517 precultured on 10% SCM were chosen for further studies.

3.4.2. Rice Straw-SSF by T. longibrachiatum DSMZ 16517 Precultivated in Molasses. The cultivation medium and its components reported to affect the time reaching maximum cellulolytic activities [81]. Thus, it was important to investigate the time profile of saccharification and (hemi) cellulolytic enzymatic activities of *T. longibrachiatum* DSMZ 16517 precultivated and enriched on 10% SCM relative to the conventional PD-broth medium. The rate of rice straw hydrolysis and saccharification in bioreactors inoculated with *T. longibrachiatum* DSMZ 16517 precultivated and enriched on 10% SCM was approximately four times higher than those inoculated by *T. longibrachiatum* DSMZ 16517 precultivated and enriched on the conventional PD-broth medium (Figure 7). Upon the application of *T. longibrachiatum* DSMZ 16517 precultivated and enriched on SCM, the maximum TRS yield occurred after 3 d of incubation period at 30°C and recorded 27.71 g/L with a corresponding saccharification % of approximately 37.41%

Table 6: Screening of *T. longibrachiatum* (DSMZ 16517) enzymatic activities precultured in different enriching media on rice straw.

Different media	Enzymatic activities of *T. longibrachiatum* DSMZ 16517 on rice straw				TRS (g/L)	Saccharification (%)
	CMCase (U/g)	FPase (U/g)	Glucosidase (U/g)	Xylanase (U/g)		
Molasses	1774.5 ± 5	244.5 ±.5	1167 ± 5	946.5 ± 5	24.55 ± 2	33.14 ± 2
Corn steep liquor	483.33 ± 5	63.78 ± 5	337.5 ± 5	471.66 ± 5	14.69 ± 2	19.83 ± 2
Potato dextrose media	1272.21 ± 5	170.08 ± 5	674.88 ± 5	933.33 ± 5	23.05 ± 2	31.12 ± 2

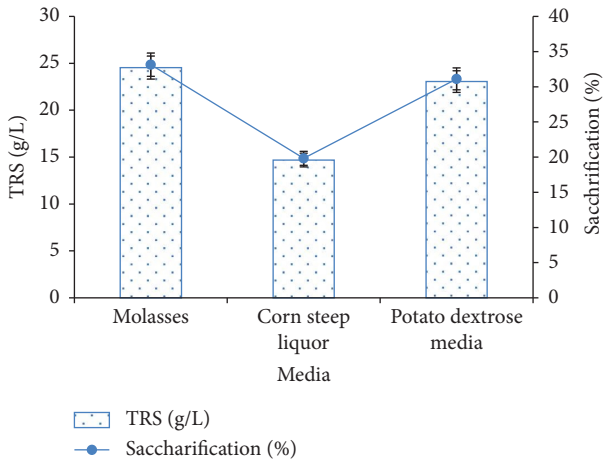


Figure 5: Screening of *T. longibrachiatum* (DSMZ 16517) hydrolytic and saccharifying activities precultured in different enriching media on rice straw.

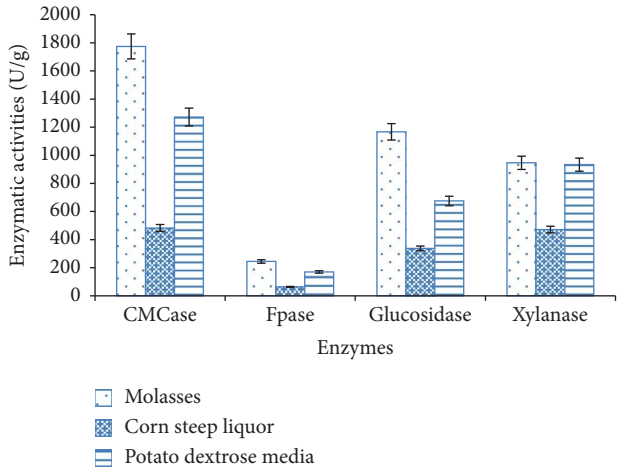


Figure 6: Screening of *T. longibrachiatum* DSMZ 16517 enzymatic activities precultured in different enriching media on rice straw.

and production rate of 8.51 g TRS/L/d (Figure 7). On the other hand, upon utilizing *T. longibrachiatum* DSMZ 16517 precultivated and enriched on the conventional PD-broth medium, the maximum TRS yield occurred after 9 d of incubation period at 30°C and recorded 22.33 g/L with a corresponding saccharification % of approximately 31.50% and production rate of 2.24 g TRS/L/d (Figure 7).

The enhancement of TRS production yield and rate (Figure 7) is very beneficial on the industrial scale. As from the economic point of view, it is feasible to produce the largest amount of fermentable sugars at lowest time to save energy and time. It can be depicted from data illustrated in Figures 7 and 8 that the fungal hydrolysis and saccharification efficacies were concomitant with the (hemi) cellulolytic enzymatic activities. Similar observation was reported by Madian et al. [16].

It is obvious from Figure 8 that the precultivation and enrichment of *T. longibrachiatum* DSMZ 16517 on SCM enhanced its (hemi) cellulolytic enzymatic activities. It yielded approximately 2859.99 U/g CMCase, 539.85 U/g FPase, 1474.07 U/g β-glucosidase, and 1918.89 U/g xylanase after 3 d of incubation on rice straw, with a total cellulases and xylanase activities of approximately 1182.30 U/gds and 639.63 U/gds, respectively. Upon the precultivation and enrichment of *T. longibrachiatum* DSMZ 16517 on the conventional PD-broth medium, the (hemi) cellulolytic enzymes expressed their maximum activities on rice straw after 9 d of incubation, recording 1550 U/g CMCase, 200.19 U/g FPase, 700 U/g β-glucosidase, and 1050.5 U/g xylanase, with a total cellulases and xylanase activities of approximately 272.24 U/gds and 116.72 U/gds, respectively. The cellulases and xylanase activities were enhanced by approximately 5.97 and 5.48 folds, respectively, with an enhanced TRS production rate of approximately 3.8 folds. Thus, from an economic point of view, it is beneficial for large-scale production since the cost of 1 kg of sugarcane molasses is 0.36 $/kg, relative to the cost of PD-broth medium of approximately 25.89 $/100 g.

The decrease in TRS yields after 3 d and 9 d in SSF bioreactors inoculated by *T. longibrachiatum* DSMZ 16517 precultured on SCM and PD-broth media, respectively, might be attributed to the decrease in fungal enzymatic activities and/or the utilization of the produced readily available fermentable sugars as C-source. Similar observation has been reported by Prasad et al. [1]. It has been reported that the optimum time for (hemi) cellulolytic enzymes production during SSF of different lignocellulosic wastes ranged between 3 and 8 d [19].

The production and activities of (hemi) cellulolytic enzymes by different *Trichoderma* sp. has been widely reported (Table 7), but it is a pioneer step to investigate the (hemi) cellulolytic enzymatic capabilities of *T. longibrachiatum* DSMZ 1651 on rice straw.

One of the bottlenecks in bioethanol production is the cost of (hemi) cellulolytic enzymes [16]. Thus, the recorded enhancement of (hemi) cellulolytic enzymes using *T. longibrachiatum* DSMZ 1651 precultured on a cheap and readily available molasses is very advantageous. The three main constituents of cellulase enzyme are the endoglucanase CMCase, the exoglucanase FPase, and the cellobiase β-glucosidase [86]. The recorded (hemi) cellulolytic enzymes' production and activities (Table 7 and Figure 8)

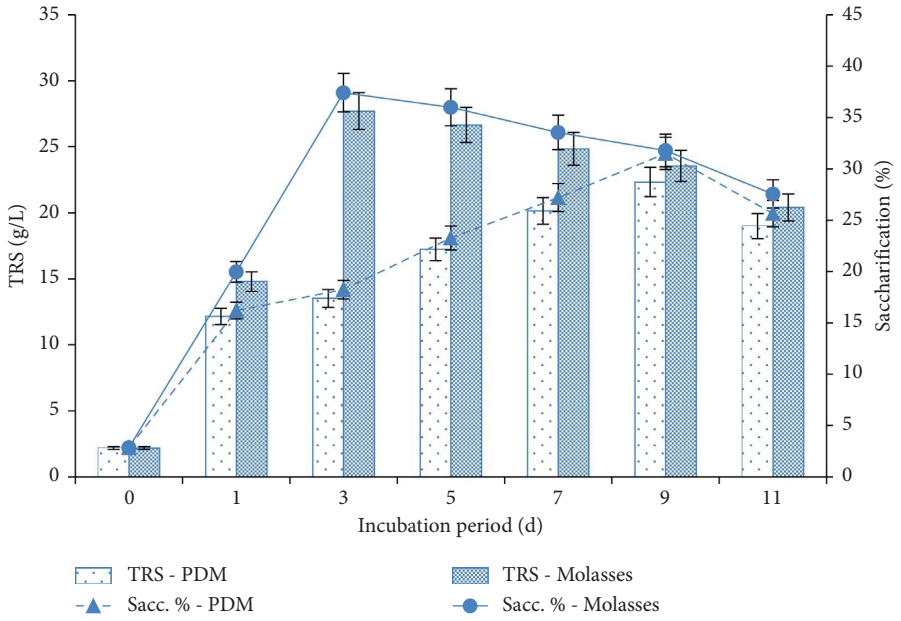


Figure 7: Rice straw fungal hydrolysis and saccharification time profile using *T. longibrachiatum* DSMZ 16517 precultured and enriched on molasses relative to the conventional potato dextrose broth medium.

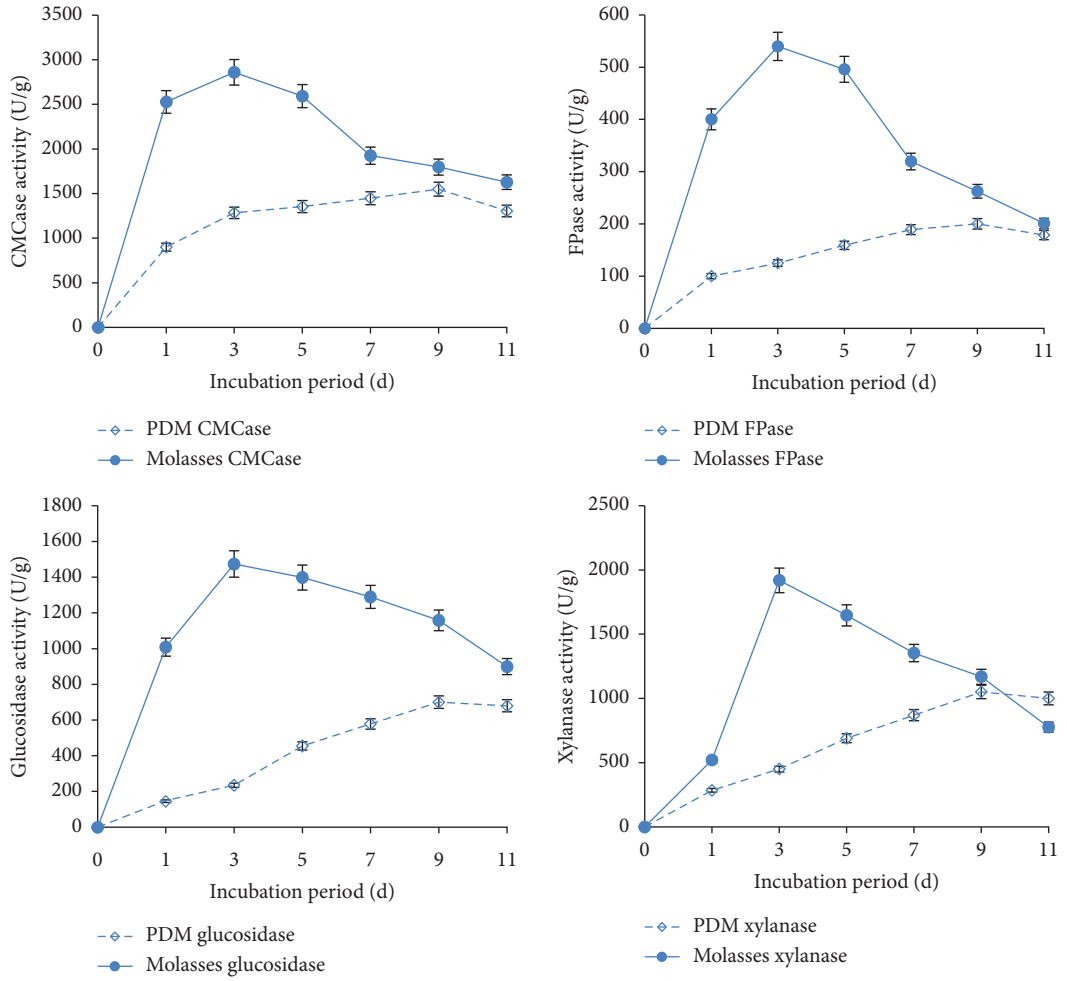


Figure 8: (Hemi) cellulolytic enzymatic activities of *T. longibrachiatum* DSMZ 16517 precultured and enriched on molasses relative to the conventional potato dextrose broth medium on rice straw.

Table 7: (Hemi) cellulolytic enzymatic activities of different *Trichoderma* sp. in SSF of different lignocellulosic wastes.

Trichoderma sp.	Bioprocess conditions	(Hemi) cellulolytic enzymes	References
T. aurantiacus	Technique: solid-state fermentation (SSF) Substrate: wheat straw (WS)	CMCase: 1709 U/g FPase: 5.5 U/g β-glucosidase: 79 U/g Xylanase: 4490 U/g	Kalogeris et al. [82]
T. longibrachiatum	Technique: solid-state fermentation (SSF) Substrate: wheat straw (WS)/wheat bran (WB)	Xylanase: 592.7 U/g–4 d	Azin et al. [83]
T. harzianum SNRS3	Technique: solid-state fermentation (SSF) Substrate: rice straw (RS)	CMCase: 111.31 U/g–6 d FPase: 6.25 U/g–6 d β-glucosidase: 173.71 U/g–8 d Xylanase: 433.75 U/g–7 d	Rahnama et al. [9]
T. longibrachiatum MDU-6	Technique: solid-state fermentation (SSF) Substrate: wheat bran	CMCase: 9.9 U/g–10 d Xylanase: 3811 U/g–10 d	Chutani and Sharma [84]
T. longibrachiatum	Technique: solid-state fermentation (SSF) Substrate: wheat bran	CMCase: 1087.3 U/gds–3 d FPase: 180 U/gds–3 d	Leghlimi et al. [85]
T. longibrachiatum LMLSAUL 14-1	Technique: solid-state fermentation (SSF) Substrate: banana pseudostem (BPS)	CMCase: 11.35 U/gds–8 d FPase: 75 U/gds–7 d β-glucosidase: 235.83 U/gds–8 d	Legodi et al. [55]
T. longibrachiatum DSMZ 1651	Technique: solid-state fermentation (SSF) Substrate: rice straw (RS)	CMCase: 2859.99 U/g–3 d FPase: 539.85 U/g–3 d β-glucosidase: 1474.07 U/g–3 d Xylanase: 1918.89 U/g–3 d	This study

recommend its application in many industrial applications, for example, biofuels, food, animal fodders, paper, textiles, detergents, biofertilizers, and alcoholic beverages. It is known that *Trichoderma* sp. insufficiently produce β-glucosidase [9, 87]; however, the recorded β-glucosidase activity of 1474.07 U/g within 3 days (Table 7 and Figure 8) is very beneficial.

The β-glucosidase is considered as the rate-limiting factor in the cellulose hydrolysis and saccharification. It is responsible for the hydrolysis of cellobiose into two moieties of glucose, thus overcoming the inhibition effect of cellobiose onto cellobiohydrolase and endoglucanase enzymes. As much as the β-glucosidase activity is high, as less as is the need for adding external sources of β-glucosidase in the SSF bioprocesses [9]. Moreover, the recorded sufficient xylanase production and activities (Table 7 and Figure 8) are also very beneficial as it mainly hydrolyzes and saccharifies hemicellulose [9]. Thus, the collaborative action of both cellulase and xylanase is very crucial for a successful bioprocess.

The relatively high yield of FPase recorded in this study (Table 7 and Figure 8) is considered also as a breakthrough for (hemi) cellulolytic enzymes production using SSF of untreated lignocellulosic wastes. *T. reesei* NCIM 992 recorded FPase production of approximately 30.7 U/g in a SSF process of an alkali-treated rice straw [88].

3.5. Full Characterization of Rice Straw before and after Fungal Hydrolysis. Table 8 showed that cellulose and hemicellulose contents collectively contribute to approximately 67.62% of the rice straw total weight. This is comparable to what is reported by Prasad et al. [1]. Rice straw is reported to constitute 10–27% hemicellulose, 30–56% cellulose, and 3–30% lignin [1].

T. longibrachiatum DSMZ 16517 showed efficient hydrolysis and saccharification efficiency on rice straw, approximately 37%, with a total weight loss of approximately 55.34%. It also expressed an efficient delignification proficiency which reached to approximately 70.34% after 3 d of incubation, with a decrease in cellulose, hemicellulose, and ash contents by approximately 77.12%, 69.52%, and 61.22%, respectively. The recorded fungal hydrolysis of hemicellulose (Table 8) is very advantageous, as according to Madian et al. [16], it would lower the bioethanol production cost by 25%. *Trichoderma* mutant AA1 has been reported before for its delignification capabilities [89]. *Trichoderma viride* reported a rice straw-delignification efficiency of approximately 74% in the presence of Tween-80 and under optimum operating conditions [36].

The proximate analysis of rice straw before and after hydrolysis depicted considerable calorific values (CV) of approximately 15.8 MJ/kg and 16.05 MJ/kg, respectively (Table 9). This is comparable to those derived from lignocellulosic straw [90], thus it can be used as a solid biofuel. It was also noticed that the CV slightly increased after the fungal hydrolysis (Table 9). This might be related to the recorded increase in the fixed carbon content with the concomitant decrease in the ash content after the fungal hydrolysis process [90]. It is well recognized that a sample's ash content indicates its mineral richness [91]. The recorded decrease in the ash content after fungal hydrolysis has been previously reported by Madian et al. [16] and El-Gendy et al. [92] and it can be attributed to its consumption as nutrients source for fungal growth and biological performance.

Rice straw has been reported to have a prodigious energy potential [14, 93], and it is reported to be used a solid biofuel [94, 95]. It has been estimated that upon open burning of approximately 3.24 Mt rice straw, about 3.82 Mt CO_2, 301 Gg CO, 29.5 Gg PM_{10}, and 27 Gg of $PM_{2.5}$ are produced [96]. To prevent such open burning, its application as solid biofuel with sufficient calorific value range between 13 and 14 MJ/kg in industrial boilers, cement industry, and electricity generation is recommendable [15, 97]. The ash can also be used as fertilizer [95].

The FESEM images of rice straw before fungal hydrolysis (Figures 9(a) and 9(c)) showed a smoothed surface with a compacted surface layer. But those after hydrolysis (Figures 9(b) and 9(d)) showed rough, cracked, and irregular shaped surface, with the removal of covering smooth layer. This might prove the good hydrolyzing capabilities of *T. longibrachiatum* DSMZ 16517. The recorded delignification (Table 8) might be a contributing factor in the observed fractured structure (Figure 9(b)) and the development of porosity in the fibrous matrix (Figure 9(d)). Similar observation is reported by Phitsuwan et al. [98].

3.6. Bioethanol Fermentation. The sugars profile in the obtained *T. longibrachiatum* DSM 16517-rice straw hydrolyzate (Table 10) is comparable to those previously reported for the *T. reesei* RUT C-30, *T. virens* FEC161, and *T. harzianum* FEC 755 hydrolyzates after 3 d-SSF of alkali-treated rice straw [87]. Lignocellulosic wastes are known to act as a reservoir of fermentable sugars, so it can act as a potential substrate for enzymes and ethanol production [99]. Cellulose is mainly composed of glucose, while hemicellulose is mainly composed of xylose with other monomers, for example, glucose, galactose, arabinose, and mannose [24, 61, 98]. According to El-Gendy et al. [92], the presence of disaccharides maltose and sucrose is mainly attributed to the hydrolysis of hemicellulose.

Although of the recorded silica (Table 5) and ash (Table 9) contents of utilized rice straw in study, however, the applied separate fungal hydrolysis using *T. longibrachiatum* DSM 16517 and yeast fermentation processes using *C. tropicalis* DSM 70156, *C. shehatae* ATCC 58779, and *S. cerevisiae* ATCC 64712 produced considerable amount of bioethanol of 10.5, 11.13, and 11.04 g/L (Table 10). Thus, rice straw is considered as a resource of bioethanol production although of its relatively high silica and ash content, which is considered according to Ibrahim [95] as one of the obstacles for rice straw bioprocessing into bioethanol. The produced amount of bioethanol (Table 10) is comparable to those reported by Belal [35]. The separate hydrolysis and fermentation of acid and ultrasound pretreated rice straw applying *T. reesei* cellulase and *S. cerevisae* produced 10-11 g/L bioethanol [35]. But it was lower than that reported by

Table 8: Biochemical analysis of rice straw before and after fungal hydrolyzes.

	Biochemical constituents (%) (w/w)			
	Hemicellulose	Cellulose	Lignin	Ash
Rice straw before hydrolysis	31.12 ± 2	36.50 ± 2	14.34 ± 2	16.77 ± 2
Rice straw after hydrolysis	18.5 ± 2	21.2 ± 2	9.5 ± 2	14.5 ± 2

Table 9: Proximate analysis of rice straw before and after fungal hydrolyzes.

	Moisture content	Volatile content	Fixed carbon	Ash content	Calorific value (MJ/kg)
Rice straw before hydrolysis	6.9 ± 2	73.78 ± 2	2.55 ± 2	16.77 ± 2	15.28 ± 2
Rice straw after hydrolysis	7.0 ± 2	73 ± 2	5.5 ± 2	14.5 ± 2	16.05 ± 2

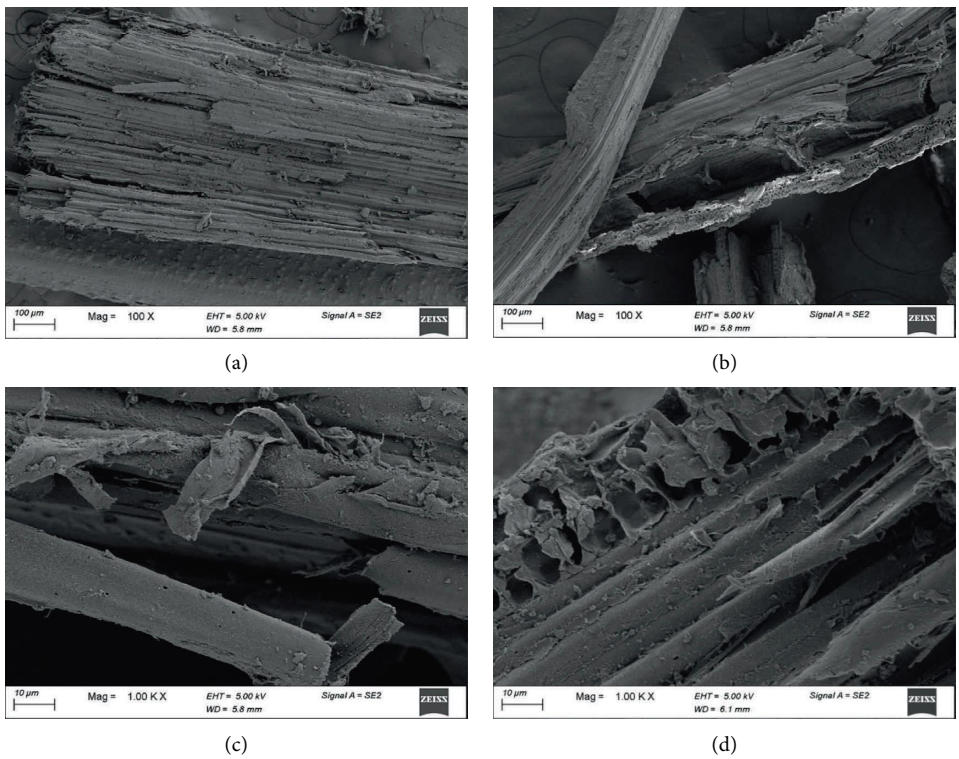


Figure 9: FESEM images of rice straw before (a and c) and after (b and d) fungal hydrolysis by *T. longibrachiatum* DSMZ 16517.

Christopher and Felix [37]; who produced approximately 16 g/L bioethanol from alkali and acid pretreated rice straw using *T. viride and S. cerevisiae*.

The three yeast strains were capable of utilizing hexoses, pentoses, and disaccharides available in rice straw hydrolyzate (Table 10 and Figure 10). According to El-Gendy et al. [92], galactose can be fermented by a combination of the Leloir pathway and glycolysis, whereas glucose, fructose, maltose, and sucrose can all be fermented using the Embden-Meyerhof pathway. The bioethanol yield and productivity from rice straw in this study (Table 10) were comparable to those reported by Abbi et al. [100], Srilekha Yadav et al. [101], and Yuvadetkun and Boonmee [102] using *Candida shehatae* NCL-3501, *Saccharomyces cerevisiae*, and *Candida shehatae* TISTR 5843, respectively.

The suggested bioprocess in this study produced 317.51, 337.47, and 335.48 gallon bioethanol/ton rice straw, applying *C. tropicalis* DSM 70156, *C. shehatae* ATCC 58779, and *S. cerevisiae* ATCC 64712, respectively. Madian et al. [16] reported the valorization of rice straw into bioethanol using *Trichoderma* viride F-94 for fungal hydrolysis and saccharification followed by bioethanol fermentation using *Saccharomyces cerevisiae* Y-39 and *Candida tropicalis* Y-26, which yielded 39 and 50 gallon bioethanol/ton rice straw, respectively. Nassar et al. [11] reported 110 gallon bioethanol production per ton rice straw by means of a sequential bioprocesses of SSF using *Trichoderma longibrachiatum* DSM 16517 followed by bioethanol fermentation by *Saccharomyces cerevisiae* ATCC 76621.

Table 10: Fermentation performance of different yeast strains on the obtained rice straw hydrolyzate.

	Rhamnose (g/L)	Xylose (g/L)	Arabinose (g/L)	Fructose (g/L)	Mannose (g/L)	Glucose (g/L)	Galactose (g/L)	Sucrose (g/L)	Maltose (g/L)	Lactose (g/L)	Total sugars (g/L)	TRS (g/L)	Produced bioethanol (g/L)	Bioethanol productivity (g/L/h)	Bioethanol yield (g/g sugars)	Theoretical yield (%)	Fermentation efficiency (%)
Rice straw hydrolyzate	2.5 ± 0.5	3.97 ± 0.5	4.69 ± 0.5	2.63 ± 0.5	5.35 ± 0.5	6.45 ± 0.5	1.1 ± 0.5	3.55 ± 0.5	1.25 ± 0.5	2.44 ± 0.5	33.93 ± 2	28.29 ± 2	—	—	—	—	—
C. tropicalis DSM 70156	1.00 ± 0.5	0.61 ± 0.5	ND	1.38 ± 0.5	ND	ND	ND	0.64 ± 0.5	0.47 ± 0.5	0.44 ± 0.5	4.54 ± 2	12.84 ± 2	10.5 ± 2	0.22 ± 0.6	0.36 ± 0.6	52.65 ± 2	48.35 ± 2%
C. shehatae ATCC 58779	2.00 ± 0.5	0.43 ± 0.5	ND	0.19 ± 0.5	ND	ND	ND	0.33 ± 0.5	0.65 ± 0.5	0.40 ± 0.5	4.00 ± 2	12.7 ± 2	11.13 ± 2	0.23 ± 0.6	0.37 ± 0.6	55.80 ± 2	51.25 ± 2%
*S. cerevisiae*ATCC 64712	ND	0.90 ± 0.5	1.91 ± 0.5	0.13 ± 0.5	0.6	ND	0.54	ND	0.59 ± 0.5	0.5 ± 0.5	5.17 ± 2	13.12 ± 2	11.04 ± 2	0.23 ± 0.6	0.38 ± 0.6	55.35 ± 2	50.84 ± 2%

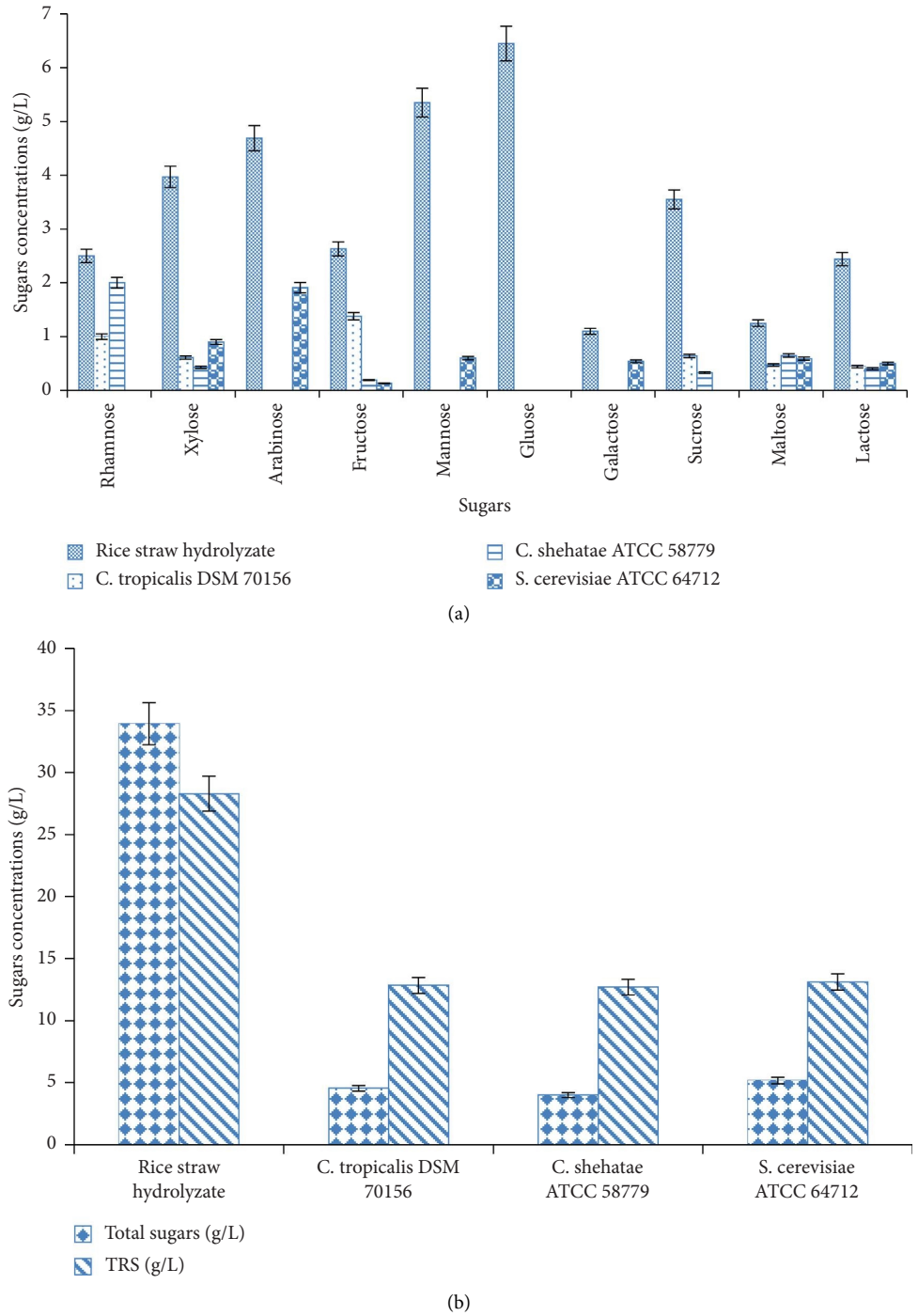


Figure 10: Sugars profile of rice straw hydrolyzate before and after bioethanol fermentation using different yeast strains.

The recorded bioethanol yields ranged between 0.36 and 0.38 g/g sugars with a corresponding bioethanol productivity ranged between 0.22 and 0.23 g/L/h (Table 10). This was comparable to those reported for *C. tropicalis* TISTR 5306, *C. shehatae* TISTR 5843, and *S. cerevisiae* TISTR 5606, which upon the bioethanol fermentation of hydrolyzate obtained alkali treatment of rice straw [103]. The recorded bioethanol yield of 0.38 g/g in the case of bioethanol fermentation by *S. cerevisiae* ATCC 64712 (Table 10) is comparable to that reported for *S. cerevisiae* NCIM 3186 in the batch fermentation of TRS produced from alkali-pretreated rice straw saccharified by *T. reesei* NCIM-1052 [1]. *S. cerevisiae* is known to be the most preferable yeast in bioethanol production for its tolerance to elevated ethanol and inhibitors concentrations [21, 32].

The fermentation efficiencies ranged between 48.35% and 51.25% (Table 10). This was higher than that recoded by El-Metwally et al. [57]; which stated that separate hydrolysis

and fermentation of rice straw by *P. purpurogenum* MM70 and *Saccharomyces cerevisiae* RTL543 yielded 12 g/L ethanol, with fermentation efficiency of approximately 46.38%.

4. Recommendation and Conclusion

This study showed that the readily available rice straw, which causes waste management problems and adds to the problem of climate change, can act as a win-win solution for many energy, industrial, environmental, and economic issues via the whole production chain process.

It can be used for the production of biofertilizer, primary solid biofuel, (hemi) cellulolytic enzymes, and secondary biofuels such as bioethanol and solid biofuel. Thus, rice straw can be considered a resource for cleaner production instead of being a polluting waste disposed of in landfills, resulting in a consequent net reduction in emissions and enrichment in the industrial sector and economy with the safeguarding of fossil fuels and currency.

This study revealed that solid-state fermentation of rice straw via fungal hydrolysis and saccharification using *T. longibrachiatum* precultured and enriched with cost-effective and readily available C- and N-rich agroindustrial wastes is very promising to produce fermentable sugars and (hemi) cellulolytic enzymes on an industrial scale. Moreover, the suggested fungal hydrolysis and saccharification followed by separate bioethanol fermentation could also be applied to produce a high yield of bioethanol. These bioprocesses are completely green and sustainable and do not require any chemicals. They operate under mild operating conditions, with lower waste effluents and lower consumption of water and energy. The valorization of the spent waste rice straw into solid biofuel would also lower the process waste, decrease the overall cost of the process, and make it more feasible.

Further work is being conducted now in the EPRI Biotechnology Lab to purify the produced (hemi) cellulolytic cocktail enzymes and study their stability, storage life, and the effect of different physicochemical parameters on their activities. Other related work is also being undertaken now for the optimization of bioethanol fermentation using different yeast strains.

Governmental strategies and policies are required to encourage the valorization of rice straw into value-added products, e.g., biofertilizer, animal fodder, (hemi)cellulolytic enzymes, bioethanol, and solid biofuel, in a way to achieve the concept of green and circular economy. Develop facilities and construct local stations for rice straw collection, volume reduction, baling, transportation, and storage. Put in place national programmes to increase awareness about the problems of climate change, its negative impact on the environment, health, society, and economy, and all the possible ways to utilize our available resources, including lignocellulosic wastes to overcome these problems

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

The authors are responsible for the content and writing of the article.

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