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Research Article

Schizophyllum commune Lipase Production on Pretreated Sugarcane Bagasse and Its Effectiveness

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Schizophyllum commune UTARA1 was used for lipase production under solid state fermentation (SSF) of sugarcane bagasse (SB) impregnated with used cooking oil medium. Pretreatments of steam, microwave, hydrochloric acid (HCl), sodium hydroxide (NaOH), and their combinations, such as steam-assisted HCl, steam-assisted NaOH, microwave-assisted HCl, and microwave-assisted NaOH, on the milled SB, were done prior to SSF to investigate their effects on lipase production via SSF. The highest lipase activity among the pretreated SB was 0.200 U/g_{SB}, using steam-assisted HCl treated SB, which is lower than the lipase activity produced from the untreated SB, which was 0.413 U/g_{SB}. Scanning Electron Microscope (SEM) imaging showed significant rupture of the SB structure after steam-assisted-HCl pretreatments where the thin walls of the SB pith were wrinkled and collapsed, with no distinctive cell wall structure. The HCl pretreated SB gave the highest crystallinity index (CrI), 91.43%, compared to the untreated, 61.90%. Conversely, microwave and NaOH pretreatments reduced the CrI, which were 46.15% and 43.36%, respectively. In this study, the results obtained indicated that pretreated SB did not improve the lipase production of *Schizophyllum commune* UTARA1 under SSF.

1. Introduction

Agrowaste is composed of plant based materials, with cellulose as the major polymer component of plant cell wall [1]. Cellulose is converted by cellulases, mainly endoglucanases (EC 3.2.1.4), exoglucanases (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21), into simple sugars [2] (Figure 1), which will be utilized by most microorganisms to support their growth. To produce glucose and cello-oligosaccharides, endoglucanase will randomly cleave the carboxy methyl cellulose. Meanwhile exoglucanase attacks on the nonreducing end of the microcrystalline cellulose to produce cellobiose. β-Glucosidase will hydrolyze cellobiose to glucose. To release the glucose, these three enzymes need to act synergistically [3].

Sugarcane bagasse (SB), an agrowaste, is abundantly found in Malaysian night markets. After juice extraction by night market vendors, the SB is being disposed to landfill. This agrowaste mainly consists of two carbohydrate fractions (cellulose and hemicellulose) embedded in a lignin matrix [4]. Rezende et al. [4] and Karp et al. [5] reported that the chemical composition of SB was cellulose (32–44%), hemicelluloses (25–32%), and lignin (19–24%). Cellulose is a linear β (1,4)-glycosidic linked chain of D-glucose molecules forming dimer cellobiose, with their basic fine fibers normally composed of a crystalline form and a small amount of nonorganized cellulose chains forms the amorphous regions [5, 6].

Cellulose, a polysaccharide composed only by D-glucose units [5], will be degraded into simple sugars and consumed rapidly by *S. commune* [7]. Hemicellulose is a polysaccharide made up of C5 and C6 sugars with a lower molecular weight than cellulose [5, 6]. Lignin, found in the cell walls, usually offers structural support, impermeability, resistance against oxidative stress, and microbial attack but it is the most difficult for enzymatic digestion [5]. In addition to that, lignin may prevent the degradation of cellulose by acting as an enzymatic barrier or by inhibiting their function, causing unproductive adsorption of the cellulases [4]. This renders the problem of lignocellulosic residues easily accessible but with limited values in biological processing due to the difficulty in their digestibility [8]. Thus pretreatment is carried out

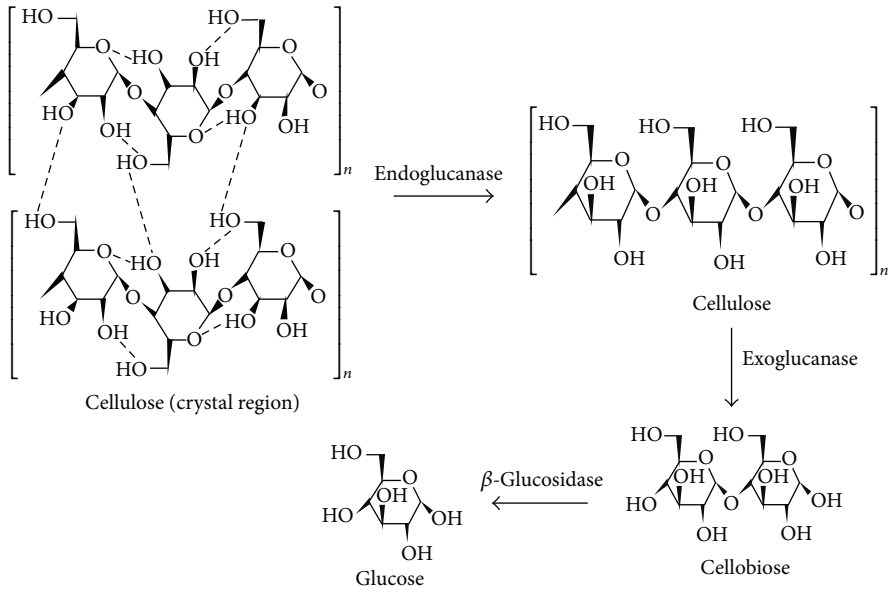

Figure 1: Enzymatic degradation of cellulose. Adopted from Karmakar and Ray [3].

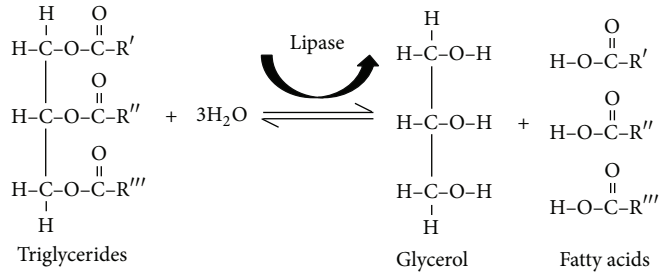

Figure 2: Triglyceride hydrolysis by lipase and its reversible reaction.

to eliminate lignin and sever the lignocellulose structure that may cause the increase of accessible surface areas, decrease in cellulose crystallinity, and/or the degree of polymerization, or selectively remove hemicellulose and lignin in order to expose the cell wall structure for better hydrolytic enzymes accessibility for their hydrolysis [4–6].

Pretreatments such as physical or chemical treatments are normally performed on the lignocellulosic agrowaste before they can be used as substrates for a particular fermentation process. The effectiveness of pretreatment methods depends on the conditions acquired to support the fungal growth and also to produce the desired products. Physical pretreatments are normally used to disrupt the lignocellulosic material, while chemical pretreatments engaged in chemical solutions to degrade lignins, celluloses, and hemicelluloses presence in the agrowaste [9].

Triglycerides, a natural component in fats and oils, are hydrolyzed to glycerol and fatty acids with the aid of lipases [10] as shown in Figure 2. Up to date, varieties of agrowaste consist of oily substances that were routinely used to produce lipase through solid state fermentation comprised of wheat bran, rice bran, almond meal, neem oil cake, gingelly oil cake, mustard oil cake, gingerly seed, groundnut oil cake, and groundnut kernel [11]. In Malaysia, there is no proper channel for the disposal of used cooking oil, mostly being thrown into the drainage system or landfill. Thus, used cooking oil is utilized as an inducer for lipase production in this study. The cooking oil used is palm oil based, with their triacylglycerols containing myristic acid, stearic acid, palmitic acid, linoleic acid, and oleic acid as the major fatty acids which are known to support bacteria and fungi growth in the presence of moisture [12]. The supplied agrowaste was utilized as carbon source (glucose) and support for microbial growth whereas oils induce lipase production.

The sugarcane bagasse will be utilized by the fungus as carbon source and support for its growth under solid state fermentation, whereas the used cooking oil will be served as inducer for the lipase production by the fungus. Hence, the objective of this study was to examine the effects of various pretreatments on SB to be used as carbon source for lipase production by *Schizophyllum commune* UTARA1.

2. Materials and Methods

2.1. Sample Collection and Preparation. Sugarcane bagasse (SB) without its outer rind was collected from a night market located in Ipoh, Perak, Malaysia. It was washed with tap water and distilled water to remove sand and impurities. Then,

the SB was dried at 70°C for 24 hours before milled and sieved to particle sizes ranging from 0.85 to 1.5 mm. On the other hand, the used cooking oil was collected from a night market in Kampar, Perak, Malaysia. The used cooking oil was left at room temperature for sedimentation before further use.

2.2. Fungal Cultivation and Identification. Isolate UTARA1 was maintained at 30°C on potato dextrose agar incorporated with 1% of used cooking oil. The extraction of fungal DNA was conducted according to Liu et al. [13]. The extracted genomic DNA was subjected to polymerase chain reaction (PCR) with the following PCR profile: initial denaturation at 94°C for 5 min, followed by denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec, for 35 cycles, and final extension 72°C for 5 min using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′- TCCTCCGCTTATTGATATGC-3′) primers [14].

2.3. Pretreatments of Sugarcane Bagasse. The milled SB were subjected to physical pretreatment, that is, autoclave at 121°C at 15 psi for 60 minutes and domestic microwave oven (Panasonic NN-ST557M) with the operating frequency of 2450 MHz at medium heat of 600 W for 5 minutes and chemical pretreatment such as soaking in 2 N HCl and in 3 N NaOH for 1 hour at room temperature, respectively. A combination of physical and chemical pretreatments was also carried out which were steam-assisted HCl pretreatment, steam-assisted NaOH pretreatment, microwave-assisted HCl pretreatment, and microwave-assisted NaOH pretreatment. After these pretreatments, the SB was washed with tap water and then soaked in distilled water overnight to remove the acid or alkali residues. Then, the SB was dried at 70°C until its weight was constant.

2.4. Solid State Fermentation. Four grams of treated or untreated SB were placed separately in different 250 mL flasks. Used cooking oil (4 mL) was added to each flask and mixed before the flasks were sent for sterilization. Fermentation medium, with slight modification from Ganguly and Banik [15] (w/v), glucose, 0.2%, urea, 0.2%, $MgSO_4$, 0.01%, and K_2HPO_4, 0.1%, was autoclaved separately before being added into each flask and maintaining the substrate to moisture ratio (g : mL) at 1 : 2. The flasks were then mixed before inoculation with 7 mycelia discs (5 mm diameter) and then incubated at 30°C for 5 days, according to previous studies (data not published).

2.5. Crude Enzyme Extraction. Phosphate buffer (0.1 M, pH 7) was added to extract the crude enzyme at the buffer to SB ratio of 10 : 1. The flasks were agitated in orbital shaker at 200 rpm, 30°C for 30 minutes. Then, it was filtered through muslin cloth. The filtrate was later centrifuged at 13 000 rpm for 5 min. The supernatant will be subjected to lipase assay.

2.6. Lipase Assay. The lipase was assayed and carried out using 2.5 mM 4-nitrophenyl laurate (NPL) (Sigma) as substrate with slight modification from Winkler and Stuckmann [16]. The assay mixture was prepared with 0.2 mL of isopropanol containing NPL, mixed with 1.6 mL of 0.1 M phosphate buffer (pH 7) and 0.2 mL of crude enzyme extract. The substrate was freshly prepared before the assay. The absorbance was read at 410 nm every minute for 15 minutes against an enzyme-free control. One unit of lipase activity is defined as the amount of enzyme which releases 1 μmol of p-nitrophenol per minute under the assay conditions. Under the conditions described, the extinction coefficient of NPL is $\varepsilon = 1.6\,\text{mM}^{-1}\,\text{cm}^{-1}$.

2.7. Scanning Electron Microscopy. Field Emission Scanning Electron Microscope (JEOL JSM-6701F) was used to observe the modifications on the pretreated SB particles to compare with the untreated SB particles. Samples were adhered to carbon tape before observation through an acceleration voltage of 3.0–5.0 kV and working distance of ~19 mm.

2.8. X-Ray Diffraction (XRD). X-ray diffraction was used to evaluate the crystallinity of the cellulose fibers by means of X-ray Diffractometer (XRD-6000 Shimadzu). The X-ray unit was operated at 40 kV and 30 mA. The pretreated and untreated SB were scanned over the angular range of 2° to 40° 2θ. The crystallinity index (CrI) was calculated based on the following formula proposed by Segal et al. [17]:

$$\text{Crystallinity index (CrI)} = \frac{I_{002} - I_{\text{am}}}{I_{002}} \times 100, \quad (1)$$

where I_{002} is the intensity for the crystalline segment (cellulose) at 2θ between 22° and 23° and I_{am} is the amorphous segment (cellulose, hemicelluloses, and lignin) at 2θ between 18° and 19° [18].

3. Results and Discussion

3.1. Fungal Identification. PCR amplification of the fungal DNA, with the band size of 650 bp, was successfully obtained. The amplified DNA was sequenced, aligned against sequences in GenBank at National Center for Biotechnology Information (NCBI), and was found to be *Schizophyllum commune*, with an identity of 99% and 0% of gaps. The sequence was then deposited into GeneBank and named as *S. commune* UTARA1 (Accession number: KJ608191).

3.2. Lipase Production with Schizophyllum commune UTARA1. Among the physical pretreatment investigated, lipase obtained from the microwave treated SB is 15-fold higher compared to steam treated SB (0.010 U/g_{SB}). Their lipase productions were considered relatively low when compared with untreated SB, which was 0.413 U/g_{SB} (Table 1). The SB CrI has increased after being treated with steam (67.38%), comparing to the untreated SB (61.90%). In the presence of high temperature, the crystalline part of cellulose is exposed due to degradation of hemicelluloses and lignin transformation [19, 20]. However, the reduction of the CrI for microwave treated SB, which was 46.15%, can be observed. The low CrI indicates high amorphous regions in the agrowaste. By comparing the lipase production from

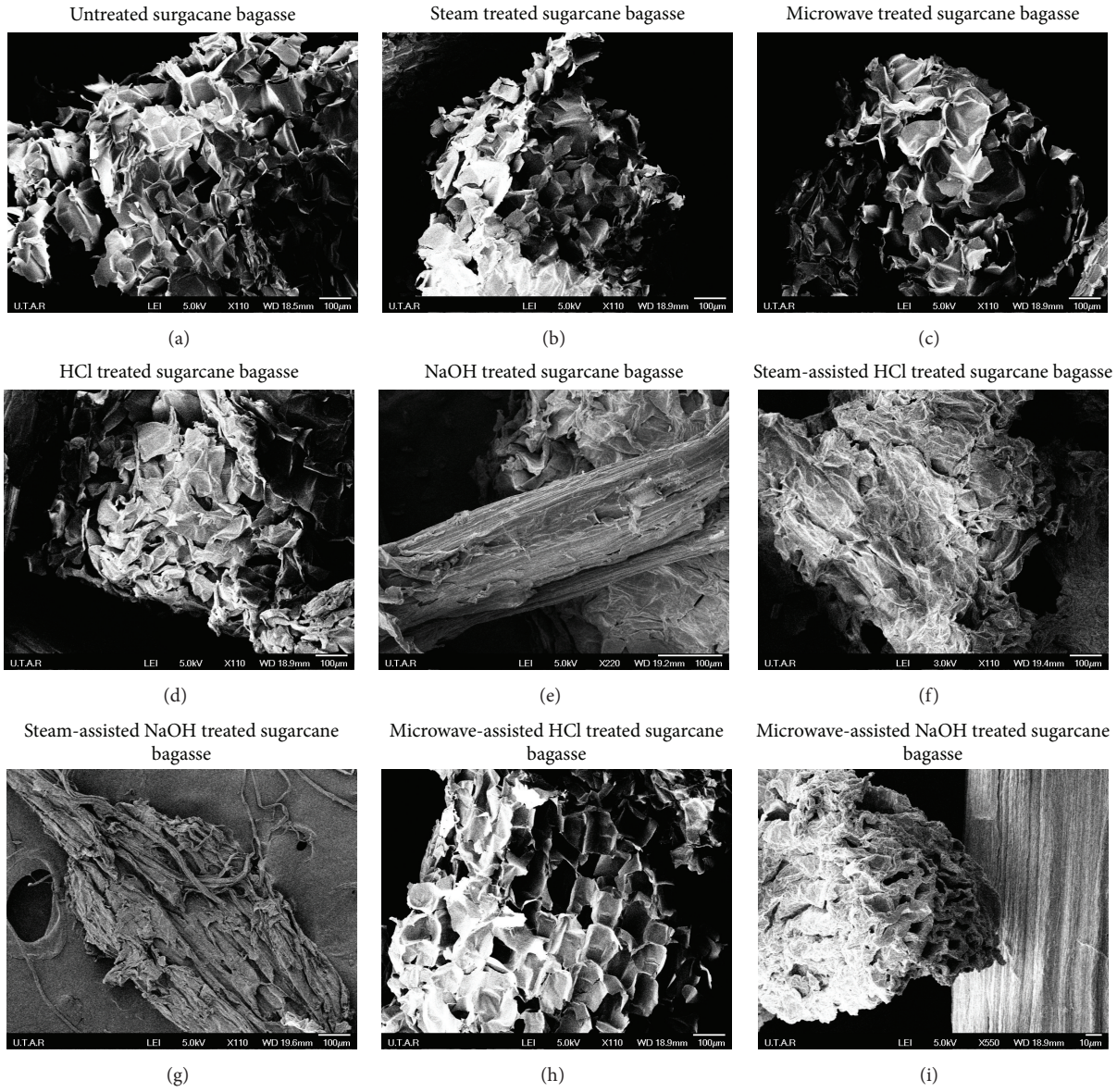

Figure 3: SEM images of sugarcane bagasse. (a) Untreated, (b) steam, (c) microwave, (d) HCl, (e) NaOH, (f) steam-assisted HCl, (g) steam-assisted NaOH, (h) microwave-assisted HCl, (i) and microwave-assisted NaOH.

Table 1: Effects of various pretreatments on SB crystallinity index (CrI) and fungal lipase activity.

Pretreatments	SB crystallinity index (CrI) (%)	Lipase production (U/g_{SB})
Untreated SB	61.90	0.413
Steam	67.38	0.010
Microwave	46.15	0.150
HCl	91.43	n.d.
NaOH	43.36	n.d.
Steam-assisted HCl	88.89	0.200
Steam-assisted NaOH	Incalculable	n.d.
Microwave-assisted HCl	0	n.d.
Microwave-assisted NaOH	Incalculable	n.d.

n.d.: not detected.

the steam treated and microwave treated SB, *S. commune* UTARA1 prefer the SB with high amorphous regions. Park et al. [21] indicated that an amorphous region was considered as "easy and presumed" material for enzymatic digestion before the digestion of the difficult crystalline cellulose. In spite of this, no distinct morphological changes can be observed under SEM, as shown in Figures 3(a), 3(b), and 3(c) for untreated, steam treated, and microwave treated SB, respectively.

Strong acid treatment, such as HCl, hydrolyzes cellulose and hemicelluloses to produce sugars [22]. It was reported that HCl degrades hemicelluloses into pentoses and hexoses and acts as catalyst to convert them to furfural and 5-hydroxymethylfurfural (HMF) which are inhibitors to most microorganisms during fermentation process [5, 23, 24]. In

this study, the levels of HMF in samples were not established. From the findings from Tsigie et al. [24], 0.39 g/L of HMF was produced using 1% HCl, with the SB to acid solution ratio of 1 : 15, at 121°C for 45 minutes. Besides, they also found that the increment of HMF production is directly proportionate with the increment of HCl concentration, which was from 0.39 g/L (1% HCl) to 0.83 g/L (3% HCl) during SB treatment. Shu and Hsu [25] also found that poor fermentation performance, such as cell growth inhibition and product formation limitation of *S. commune* ATCC 38548, might be due to furfural, HMF, and acetic acid. When HMF is present, the fungal growth will be influenced and thus affect the lipase production. Hence, no lipase activities were detected for system using HCl and microwave-assisted HCl treated SB. However, a combination of steam and HCl treatment increased the lipase activity to 20-fold compared to steam treatment. Flash point is defined as the lowest temperature where the fuel can vaporize to appear as an ignitable mixture in air [26]. Since the flash point for furfural is 68°C [27] and HMF is 79°C [28], they were eliminated by the steaming process under pressure in the autoclave, 121°C at 15 psi for 60 minutes. This is in concurrence with Figure 3(d), as the organized structure of the pith was unstructured and has a twisted appearance, and also Figure 3(f), where the thin walls of the sugarcane bagasse pith were wrinkled and collapsed, with no distinctive cell wall structure. The hemicelluloses degradation had occurred in the SB when treated with HCl and steam-assisted HCl, with the CrI of 91.43% and 88.89%, respectively. As for microwave-assisted HCl treatment, the SB structure did not differ much (Figure 3(h)) from the untreated (Figure 3(a)).

Alkaline treatments would remove lignin and also slight hemicellulose fractions; however, it will incorporate inhibitor formation, such as aldehydic, phenolic, aromatic, and polyaromatic that may be released from the delignification process [29]. Phenolic compounds have a significant inhibitory effect and are more toxic than furfural and HMF [29]. Thus, the lipase activities were not detected for fermentation using NaOH-related treated SB. As shown in Figures 3(e), 3(g), and 3(i), the SB bundles were found apart and the fibers disengaged from each other, indicating the removal of lignin from SB. Furthermore, the organized structures of the SB pith were seen collapsed and wrinkled, as shown in Figure 3(i), as the amorphous regions (lignin and hemicellulose) were removed. As for the NaOH-related treated SB, only CrI of NaOH treated SB was detected, which was 43.36%. The decrease in value is in accordance to Mohkami and Talaeipour [18], stating that the hydrogen bonds in crystalline cellulose will be cleaved during NaOH treatment. Due to the efficiency of alkaline treatment in lignin and slight hemicelluloses removal [29], there were no amorphous peaks for steam-assisted NaOH and microwave-assisted NaOH treatments.

Even though pretreatments of agrowaste are reported useful in delignification of lignocellulosic materials and enhance the accessibility of microbe in fermentation process, nevertheless, this study shows that CrI has no direct correlation with the lipase production by *S. commune* UTARA1. But the CrI may influence fungal growth, depending on the crystalline and amorphous regions. Lipase yield basically depended more on the cooking oil as an inducer and not the changes in SB components. This was also supported by Kim and Holtzapple [30] in another study, whereby the removal of the amorphous components in the corn stover did not negatively affect the yield of enzymatic hydrolysis.

4. Conclusions

The various pretreatments carried out on the SB had significant effects on the lipase production, nevertheless, the untreated SB was more suitable over pretreated SB as the substrate for SSF for lipase production. This is due to the present of inhibitors that are toxic to *S. commune* during the chemical pretreatment process which indirectly influence its growth and lipase production. SEM images showed that some pretreatments disrupt the morphological structures of SB. Due to the changes in the SB structure, the CrI of each substrate also differed.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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References

[1] P. Baldrian and V. Valášková, "Degradation of cellulose by basidiomycetous fungi," *FEMS Microbiology Reviews*, vol. 32, no. 3, pp. 501–521, 2008.

[2] S. K. Soni, N. Batra, N. Bansal, and R. Soni, "Bioconversion of sugarcane bagasse into second generation bioethanol after enzymatic hydrolysis with in-house produced cellulases from *Aspergillus* sp. S_4B_2F," *BioResources*, vol. 5, no. 2, pp. 741–757, 2010.

[3] M. Karmakar and R. R. Ray, "Current trends in research and application of microbial cellulases," *Research Journal of Microbiology*, vol. 6, no. 1, pp. 41–53, 2011.

[4] C. A. Rezende, M. De Lima, P. Maziero, E. Deazevedo, W. Garcia, and I. Polikarpov, "Chemical and morphological characterization of sugarcane bagasse submitted to a delignification process for enhanced enzymatic digestibility," *Biotechnology for Biofuels*, vol. 4, article 54, 2011.

[5] S. G. Karp, A. L. Woiciechowski, V. T. Soccol, and C. R. Soccol, "Pretreatment strategies for delignification of sugarcane bagasse: a review," *Brazilian Archives of Biology and Technology*, vol. 56, no. 4, pp. 679–689, 2013.

[6] R. C. N. R. Corrales, F. M. T. Mendes, C. C. Perrone et al., "Structural evaluation of sugar cane bagasse steam pretreated in the presence of CO_2 and SO_2," *Biotechnology for Biofuels*, vol. 5, article 36, 2012.

[7] S. Horisawa, H. Ando, O. Ariga, and Y. Sakuma, "Direct ethanol production from cellulosic materials by consolidated biological processing using the wood rot fungus *Schizophyllum commune*," *Bioresource Technology*, vol. 197, Article ID 15382, pp. 37–41, 2015.

[8] R. Rodriguez-Vazquez, G. Villanueva-Ventura, and E. Rios-Leal, "Sugarcane bagasse pith dry pretreatment for single cell protein production," *Bioresource Technology*, vol. 39, no. 1, pp. 17–22, 1992.

[9] T. Y. Ding, S. L. Hii, and L. G. A. Ong, "Comparison of pretreatment strategies for conversion of coconut husk fiber to fermentable sugars," *BioResources*, vol. 7, no. 2, pp. 1540–1547, 2012.

[10] N. A. Serri, A. H. Kamarudin, and S. N. A. Rahaman, "Preliminary studies for production of fatty acids from hydrolysis of cooking palm oil using *C. rugosa* lipase," *Journal of Physical Science*, vol. 19, no. 1, pp. 79–88, 2008.

[11] D. S. Kumar and S. Ray, "Fungal lipase production by solid state fermentation-an overview," *Journal of Analytical & Bioanalytical Techniques*, vol. 5, article 230, 2014.

[12] M. Koushki, M. Nahidi, and F. Cheraghali, "Physico-chemical properties, fatty acid profile and nutrition in palm oil," *Journal of Paramedical Sciences*, vol. 6, no. 3, pp. 117–134, 2015.

[13] D. Liu, S. Coloe, R. Baird, and J. Pedersen, "Rapid mini-preparation of fungal DNA for PCR," *Journal of Clinical Microbiology*, vol. 38, no. 1, p. 471, 2000.

[14] I. C. Anderson, C. D. Campbell, and J. I. Prosser, "Potential bias of fungal 18s rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil," *Environmental Microbiology*, vol. 5, no. 1, pp. 36–47, 2003.

[15] S. Ganguly and A. K. Banik, "Metabolic flux of L-valine as a function of suitable micro-environment," *International Journal of Deccan Pharma and Life Sciences*, vol. 3, no. 2, pp. 81–87, 2012.

[16] U. K. Winkler and M. Stuckmann, "Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*," *Journal of Bacteriology*, vol. 138, no. 3, pp. 663–670, 1979.

[17] L. Segal, J. J. Creely, A. E. Martin Jr., and C. M. Conrad, "An empirical method for estimating the degree of crystallinity of native cellulose using the x-ray diffractometer," *Textile Research Journal*, vol. 29, no. 10, pp. 786–794, 1959.

[18] M. Mohkami and M. Talaeipour, "Investigation of the chemical structure of carboxylated and carboxymethylated fibers from waste paper via XRD and FTIR analysis," *BioResources*, vol. 6, no. 2, pp. 1988–2003, 2011.

[19] P. Kumar, D. M. Barrett, M. J. Delwiche, and P. Stroeve, "Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production," *Industrial and Engineering Chemistry Research*, vol. 48, no. 8, pp. 3713–3729, 2009.

[20] J. Gabhane, S. P. M. Prince William, A. N. Vaidya, K. Mahapatra, and T. Chakrabarti, "Influence of heating source on the efficacy of lignocellulosic pretreatment—a cellulosic ethanol perspective," *Biomass and Bioenergy*, vol. 35, no. 1, pp. 96–102, 2011.

[21] S. Park, J. O. Baker, M. E. Himmel, P. A. Parilla, and D. K. Johnson, "Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulose performance," *Biotechnology for Biofuels*, vol. 3, no. 10, pp. 1–10, 2010.

[22] M. J. Taherzadeh and K. Karimi, "Acid-based hydrolysis processes for ethanol from lignocellulosic materials: a review," *BioResources*, vol. 2, no. 3, pp. 472–499, 2007.

[23] F. K. Kazi, A. D. Patel, J. C. Serrano-Ruiz, J. A. Dumesic, and R. P. Anex, "Techno-economic analysis of dimethylfuran (DMF) and hydroxymethylfurfural (HMF) production from pure fructose in catalytic processes," *Chemical Engineering Journal*, vol. 169, no. 1–3, pp. 329–338, 2011.

[24] Y. K. Y. A. Tsigie, C.-Y. Wang, C.-T. Truong, and Y.-H. Ju, "Lipid production from *Yarrowia lipolytica* Po1g grown in sugarcane bagasse hydrolysate," *Bioresource Technology*, vol. 102, no. 19, pp. 9216–9222, 2011.

[25] C.-H. Shu and H.-J. Hsu, "Production of schizophyllan glucan by *Schizophyllum commune* ATCC 38548 from detoxificated hydrolysate of rice hull," *Journal of the Taiwan Institute of Chemical Engineers*, vol. 42, no. 3, pp. 387–393, 2011.

[26] M. H. Al-Abdullah, G. T. Kalghatgi, and H. Babiker, "Flash points and volatility characteristics of gasoline/diesel blends," *Fuel*, vol. 153, pp. 67–69, 2015.

[27] A. H. A. Mohammed and M. J. Y. Kheder, "The effect of extraction temperature and solvent to oil ratio on viscosity index of mixed-medium lubricating oil fraction by using solvents extraction," *Iraqi Journal of Chemical and Petroleum Engineering*, vol. 10, no. 2, pp. 35–42, 2009.

[28] S. Kowalski, M. Lukasiewicz, A. Duda-Chodak, and G. Zięć, "5-Hydroxymethyl-2-furfural (HMF) -heat-induced formation, occurrence in food and biotransformation—a review," *Polish Journal of Food and Nutrition Sciences*, vol. 63, no. 4, pp. 207–225, 2013.

[29] P. F. H. Harmsen, W. J. J. Huijgen, L. M. Bermúdez López, and R. R. C. Bakker, *Literature Review of Physical and Chemical Pretreatment Processes for Lignocellulosic Biomass*, Wageningen UR, Food & Biobased Research, Wageningen, Netherlands, 2010.

[30] S. Kim and M. T. Holtzapple, "Effect of structural features on enzyme digestibility of corn stover," *Bioresource Technology*, vol. 97, no. 4, pp. 583–591, 2006.