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Lignocellulolytic Materials-as a Raw Material for the Production of Fermentable Sugars via Solid State Fermentation

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ABSTRACT

The aim of this study was to explore the possibility of utilizing agrowastes effectively to convert them into fermentable sugars by the production of *in situ* enzyme in solid state fermentation, which agrowaste can be used as substrates for the microbial fermentation in the production of commercial viable products. To enhance the usage of abundant agrowaste generated in Malaysia a study was conducted in view of exploring the possibility of utilizing it effectively for the conversion to fermentable sugars. Agrowaste is rich of lignoselulolytic material which can serve as good substrate in solid state fermentation to produce the fermentable sugar. In this study, solid state fermentation was carried out through the flask system in lab skill. We evaluated the production of fermentable sugars by various fungal cultures namely *A. niger* USM AI1, *A. niger* II, *Trichoderma* sp., *A. niger* F4 and *Phanerochaete chrysosporium* via solid state fermentation system. Nine different types of lignocellulolytic materials paddy husks, coconut fibre, wood dust, coconut meal, palm kernel cake, sugarcane baggase, tapioca meal, oil palm trunk and oil palm frond were examined. The highest productivity, 57 mg of fermentable sugars per gram substrate was obtained when *A. niger* USM AI1 was grown on tapioca meal and the biomass of fungus showed about 1.7 mg glucosamine/g substrate. However, about 30.0 mg of fermentable sugars per gram of substrate was obtained when *A. niger* USM AI1 was grown on oil palm frond and the biomass of fungus showed 1.2 mg glucosamine/g substrate. Lesser yields of fermentable sugar were obtained when paddy husk, coconut fibre, wood dust, coconut meal, palm kernel cake and sugarcane bagasse were used as solid substrates, each yielding less than 6.6 mg of fermentable sugars per gram of substrate. Thus, the nature of the substrate and the suitability of fungi used in the solid state fermentation are important variable contributing to product yield of fermentable sugars in solid state fermentation and can presumably be more economical process for agrowaste utilization.

Key words: Biomass, biodegradability, fermentation, fungus

INTRODUCTION

Lignocellulolytic materials are abundant in nature and have great value as alternative energy sources. The compositions of this biomass vary. The major component is cellulose (35-50%), followed by hemicelluloses (20-3%) and lignin (10-25%), in addition to minor components such as proteins, oils and ash that make up the remaining fraction of lignocellulosic biomass (Sjostrom, 1981). The

biodegradation and bioconversion of lignocelluloses into useful products and biological alleviation of pollution from lignocelluloses waste is an enormous environment challenge (Panagiotou *et al.*, 2003). A great variety of fungi can degrade these macromolecules by using a battery of hydrolytic or oxidative process (Perez *et al.*, 2002). Degradation of lignocellulosic materials to monomeric sugars through the concerted action of cellulolytic enzymes is of great importance because sugars can serve as the raw material for a number of biotechnological production processes (Juhasz *et al.*, 2005). For example, the sugars produced can be converted to ethanol (Lawford and Rousseau, 2003), lactic acid (El-Hawary *et al.*, 2001) and biohydrogen (Taguchi *et al.*, 1996; Kaparaju *et al.*, 2009).

The technique of solid state fermentation (SSF) has been used for decades to convert moist agricultural polymeric substrates, example wheat, rice, soy cassava, etc, into fermented products (Rahardjo *et al.*, 2005). SSF is an attractive technique for enzyme production because it presents many advantages, especially for fungal cultivation (Weiland, 1988). In SSF, the productivity per reactor or fermenter volume is much higher compared with that of submerged culture (Grajek, 1987). Also, the operation cost is lower, because simple design machinery and less energy usually are required (Roche *et al.*, 1994).

Over the past decades, enzyme-based technologies have aroused worldwide research interest. Finding economically suitable substrates has always been of particular interest. An ideal lignocellulosic substrate is cheap, easily processed with a high yield and is suitable both for hydrolysis and for production of enzyme. Production of enzymes *in situ* instead of inoculating with commercially available enzymes can improve the economics of a process. However, lack of report has been seen on hydrolyzing agrowaste by using the production of enzymes *in situ* through solid state fermentation. Thus, the objective of this study proposed to seriously explore the possibility of utilizing agrowastes effectively to convert them into fermentable sugars by the production of *in situ* enzyme in solid state fermentation, which agrowaste can be used as substrates for the microbial fermentation in the production of commercial viable products.

MATERIALS AND METHODS

This study was carried out in June 2006 at Industrial Biotechnology Research Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. The duration taken to conduct this work was about 6 months.

Microorganisms and culture condition: The fungi used in this study consisted of *A. niger* USM AI1, *A. niger* II, *Trichoderma* sp., *A. niger* F4 and *Phanerochaete chrysosporium* which obtained from Industrial Biotechnology Research Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. The fungi were grown on potato dextrose agar (Oxoid, England) slants at 37°C until sporulation (5 days) and then were maintained at 4°C until used. The inoculum was prepared by adding 4 mL of sterile distilled water to an agar slant and adjusting the spore suspension to 1×10^6 spores per mL.

Substrate preparation: To be used as substrate, the lignocellulosic material (paddy husks, coconut fibre, wood dust, coconut meal, palm kernel cake, sugarcane baggase, tapioca meal, oil palm trunk and oil palm frond) was thoroughly dried and milled with grinder machine (Rong Tsong Precision Technology Corporation, Taiwan) to 1 mm particle size. Five grams of lignocellulosic material was then weighed and place into a 250 mL Erlenmeyer flask and autoclaved at 121°C for 20 min.

Solid-State Fermentation (SSF): The sterilized solid substrate was inoculated with 1.0 mL of inoculums (1×10^6 spores mL^{-1}) and the moisture content was adjusted to 80% (v/w) with sterile distilled water. The contents were mixed thoroughly by using a sterile spatula and incubated at room temperature (30±2°C). Sample as a whole flasks in triplicates, were withdrawn after one week of cultivation.

Fermentable sugars extraction: The crude fermentable sugars from the fermented materials were extracted by a simple contact method. The fermented substrate was added with 100 mL distilled water. Contents were mixed by shaking for 1 h at room temperature (30±2°C) in a rotary shaker at 150 rpm. At the end of the extraction, the suspension was filtered through filter paper (Whatman No. 1, England) and the supernatant was collected and used as the crude fermentable sugar suspension for analysis.

Analysis: Fermentable sugars were measured by the Nelson and Somogyi (Breuil and Saddler, 1984) procedure. One milliliter of crude filtrate solution was added to 1 mL of cuprum reagent. The solution was placed in boiling water for 15 min and then cooled to room temperature and 1 mL of Nelson reagent added. The solution was then made up to 10 mL with distilled water and OD 540 measured at spectrophotometrically.

Fungal biomass was measured by estimating the N-acetyl glucosamine released by the acid hydrolysis of the chitin, present in the cell wall of the fungus (Sakurai *et al*., 1977). Glucosamine released from the chitin by the acid hydrolysis was mixed with 1 mL of acetyl acetone and incubated in a boiling water bath for 20 min. After cooling to room temperature, 6 mL of ethanol was added followed by the addition of 1 mL of Ehrlich reagent and the mixture incubated at 65°C for 10 min. After cooling, OD 530 was taken against a reagent blank.

RESULTS AND DISCUSSION

In this study, the used of various lignocellulosic materials for the production of fermentable sugars were studied. Figure 1 shows that the highest yield of fermentable sugars occurred when *A. niger* USM AI1 was cultivated on tapioca meal, producing as much as 57 mg of fermentable sugars per gram substrate, followed by oil palm frond which produced about 30 mg of fermentable sugars per gram of substrate (Fig. 1). The best fungal growth also occurred on tapioca meal with about 1.7 mg glucosamine/g substrate. The remaining substrates produced significant fungal growth (biomass) but produced little fermentable sugars.

When *A. niger* II grown on oil palm frond as substrates in SSF, the highest fermentable sugars obtained and was about 18 mg compared with other lignocellulosic materials (Fig. 2). The highest biomass produced was on tapioca meal with about 1.8 mg glucosamine per gram substrate but only a low yield of fermentable sugars obtained. For example only 2.0 mg of fermentable sugars were produced per gram of tapioca.

When *Trichoderma* sp., was grown on various lignocellulosic materials, oil palm frond again yielded the highest fermentable sugar production of about 39 mg g^{-1} substrate with a biomass of 0.51 mg glucosamine per gram of substrate (Fig. 3). Again when tapioca meal was used as the substrate, the highest biomass was produced (1.6 mg glucosamine/g substrate) but there was only a low yield of fermentable sugars (13 mg g^{-1} substrate) obtained (Fig. 3).

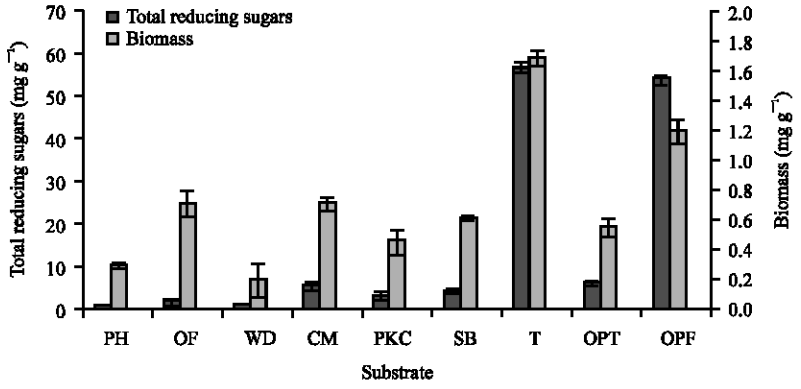

Fig. 1: Total reducing sugars (fermentable sugars) production by *A. niger* USM AI1 on different lignocellulolytic materials. Data are presented in triplicates (n=3) and the error bars show the standard deviation. WD: Wood dust, PH: Paddy husk, PKC: Palm kernel cake, CM: Coconut meal, CF: Coconut fibre, SB: Sugarcane bagase, T: Tapioca meal, OPT: Oil palm trunk, OPF: Oil palm frond, serve as substrate in solid state fermentation system

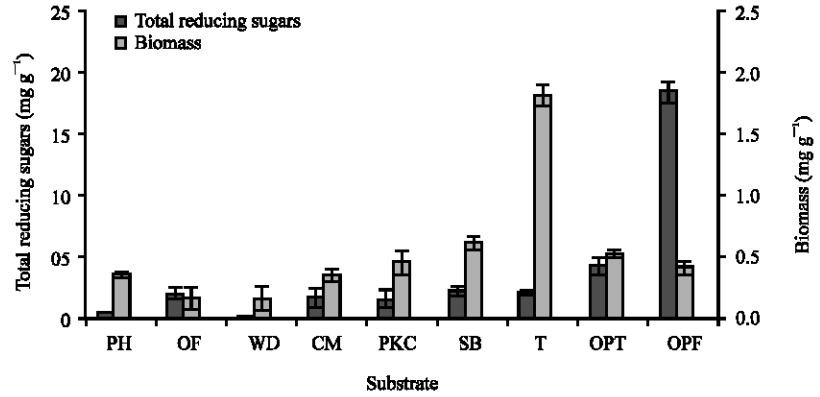

Fig. 2: Total reducing sugars (fermentable sugars) production by *A. niger* II on different lignocellulolytic materials. Data are presented in triplicates (n=3) and the error bars show the standard deviation. WD: Wood dust, PH: Paddy husk, PKC: Palm kernel cake, CM: Coconut meal, CF: Coconut fibre, SB: Sugarcane bagase, T: Tapioca meal, OPT: Oil palm trunk, OPF: Oil palm frond, serve as substrate in solid state fermentation system

For *A. niger* F4 the tapioca meal again produced the highest biomass of about 1.7 mg glucosamine per gram of substrate and 35 mg fermentable sugars per gram of substrate (Fig. 4). However, *A. niger* F4 was not able to grow on.

When *P. chrysosporium* was grown on oil palm frond as a substrate, the highest fermentable sugar production achieved was 54 mg per gram substrate, but the biomass production was only about 0.46 mg glucosamine per g substrate (Fig. 5). The highest biomass, 1.2 mg glucosamine per gram of substrate but a lower fermentable sugar yields (36 mg per gram of substrate). One explanation for these results is that the fungus was growing exponentially during the seventh day of cultivation and resulted in the maximum enzyme production. Xia and Cen (1999) reported that the enzymes produced were capable of degrading polymeric lignocellulosic materials into fermentable sugars.

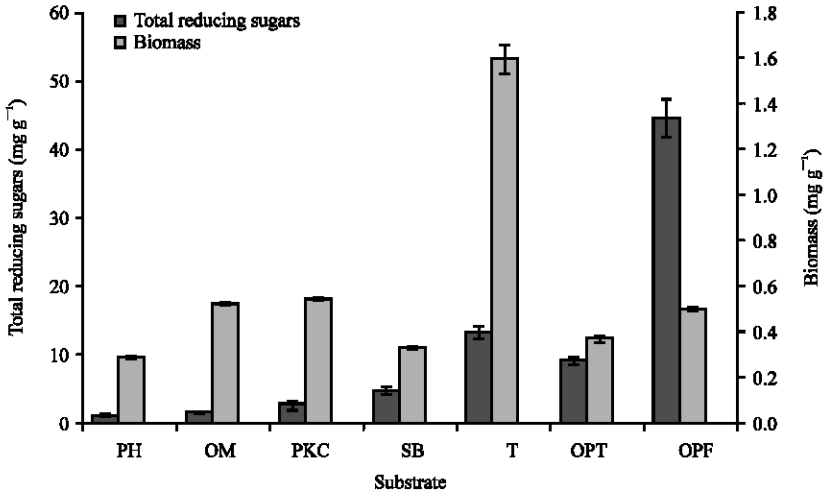

Fig. 3: Total reducing sugars (fermentable sugars) production by *Trichoderma* sp. on different lignocellulolytic materials. Data are presented in triplicates (n=3) and the error bars show the standard deviation. PH: Paddy husk, PKC: Palm kernel cake, CM: Coconut meal, SB: sugarcane bagase, T: Tapioca meal, OPT: Oil palm trunk, OPF: Oil palm frond, serve as substrate in solid state fermentation system

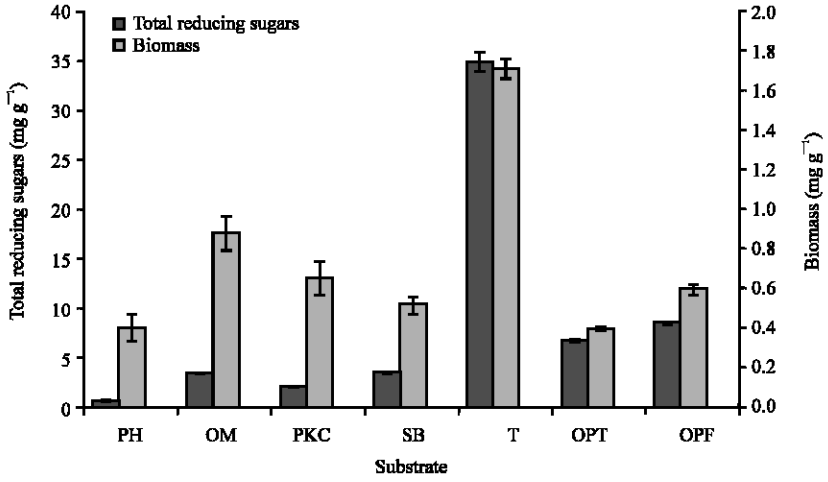

Fig. 4: Total reducing sugars (fermentable sugars) production by *A. niger* F4 on different lignocellulolytic materials. Data are presented in triplicates (n=3) and the error bars show the standard deviation. PH: Paddy husk, PKC: Palm kernel cake, CM: Coconut meal, SB: Sugarcane bagase, T: Tapioca meal, OPT: Oil palm trunk, OPF: Oil palm frond, serve as substrate in solid state fermentation system

In summary, the highest fermentable sugars yield was 57 mg per gam substrate when *A. niger* USM A11 was grown on tapioca meal. It was followed by *P. chrysosporium* growing on oil palm frond, which produced about 54 mg g^{-1} substrate.

Solid state fermentation (SSF) was carried out for the production of total fermentable sugars from various types of lignocellulosic materials by different fungi. Selection of an appropriate

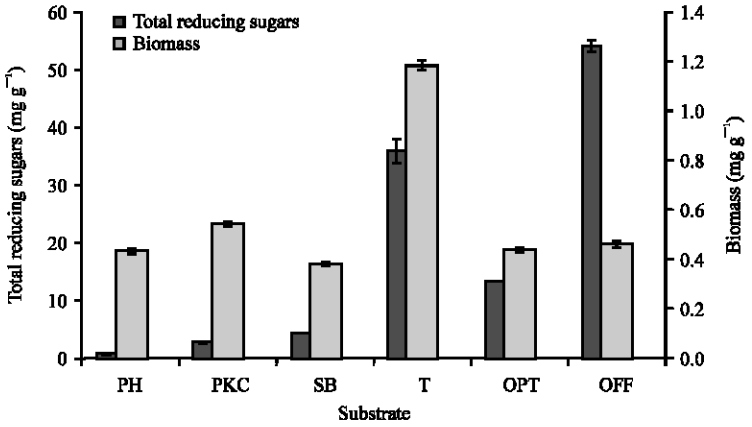

Fig. 5: Total reducing sugars (fermentable sugars) production by *Phanerochaete chrysosporium* on different lignocellulolytic materials. Data are presented in triplicates (n=3) and the error bars show the standard deviation. PH: Paddy husk, PKC: Palm kernel cake, SB: Sugarcane bagase, T: Tapioca meal, OPT: Oil palm trunk, OPF: Oil palm frond, serve as substrate in solid state fermentation system

substrate is a key factor in SSF which determines the success of the process (Niladevi *et al*., 2007). It is a common practice to use lignocellulosic agrowastes for the production of carbon source like fermentable sugars.

In this study maximum fermentable sugar was obtained when cultivated *A. niger* USM AI1 on tapioca meal, which produced 57 mg g^{-1} substrate. The used of lignocellulolytic materials for the production of fermentable sugars has been reported widely from fungal as well as *Aspergillus* genus have previously been reported (De Souza *et al*., 2001). Maximum value of fermentable sugar obtained in this study was much higher than previous finding which reported by Saravanan *et al*. (2009) and Teoh *et al*. (2008), it only shows 20.02 mg fermentable sugars/g substrate and 9.88 mg g^{-1} substrate respectively. Behera *et al*., (1996) reported the fermentable sugar produced by cultivated *Trichorderma reesei* on the stem of *Colotropis procera* as solid substrate, which only shows about 0.5 mg g^{-1} substrate. This finding demonstrates that *A. niger* USM AI1 was attempted on various natural lignocellulosic substrates among which tapioca meal emerged as most suitable.

However, paddy husk, coconut fibre, wood dust, coconut meal, palm kernel cake, sugarcane baggase and oil palm trunk yield lower fermentable sugars among the lignocellulosic materials used as substrate, which each yielded less than 6.6 mg of fermentable sugars per gram substrate used. Presumably the fungi used in this study do not grow well on those substrates and could not able to produce the enzymes needed to degrade those lignocellulosic materials. In addition, the growth of fungi on native and very impure form of lignocellulosic materials may decrease the production of superior enzyme (Knapp and Legg, 1986) eventually affect the effectiveness of the degradation of the lignocellulosic materials. It was noted that SSF process is significantly affected by various factors. Among these, selection of a suitable strain, substrate and process parameters are crucial (Pandey *et al*., 2001). The nature of solid substrate is a major factor for solid state fermentation. It is not only supplies the nutrients to the culture but also serves as an anchorage for the fungus growth during the process (Sharma *et al*., 2008).

We also noted that some lignocellulosic materials did not support the growth of some of the fungi grown on them. For example, *Trichoderma* sp. and *A. niger* F4 did not grow on coconut fibre

and wood dust, respectively. *Phanerochaete chrysosporium* also did not growing on coconut fibre, wood dust or coconut meal. Cowan (1999) reported the lignocellulosic material which contain the secondary metabolite compound like antimicrobial agents can inhibit the growth of microorganisms on them. Kang *et al.* (2004) also reported that the growth of fungus was affected by the nature of the substrate used in fermentation. Therefore, the choice of an appropriate inducing substrate is of importance in order to obtain maximum fermentable sugar yield.

The finding of the current study shows that factors such as diffusion within the solid substrate, mass transfer to and from the solid substrate and the hydrophilic and hydrophobic nature of the solid particles are important factors in the adherence of the fungus to the solid substrate. These factors which in turn affect enzyme production and may as well affect the yield of the fermentable sugars in this study (Mulimani and Patil Ramalingam, 2000). These results also emphasize the importance of the nature of the substrate and the fungi used in SSF for obtaining good yield of fermentable sugars.

CONCLUSION

In conclusion, we evaluated the production of fermentable sugars in different types of lignocellulolytic materials in SSF. Fermentable sugars obtained depended on the substrate composition and the fungi grown on them. *A. niger* USM AI1 might be useful for the production of fermentable sugars when grown on tapioca meal.

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