Maximizing Glucose Production From Palm Kernel Cake (Pkc) From Which Residual Oil Was Removed Supercritically Via Solid State Fermentation (Ssf) Method Using Trichoderma Reesi Isolate Pro-A1

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Citation

Abstract

The present trial was carried out to evaluate the effects of incubation temperature, pH and time during solid state fermentation (SSF) of PKC substrates using Trichoderma

Isolate Pro-A, on glucose production from the substrates before and after the residual oil is extracted via Supercritical Fluid Extraction (SFE) technique. Residual oil was removed from one portion of 250μM-sized Palm Palm Kernel Cake (PKC) substrate, using $SC\text{-}CO_2$ as the solvent in supercritical fluid extraction (SFE) technique (Oil-less treatment), whereas no residual oil was removed from the other portion of the substrate (Oily treatment). Both treatments (Oil-less and Oily) were subjected to SSF, using Trichoderma

Isolate Pro-A incubated at three temperatures (20, 30 and 40°C), three pHs (3, 5 and 7) and at four time intervals (4, 8, 12 and 16 hours) accordingly, and the glucose concentration produced during SSF was quantified. Results showed that glucose production by the fungus from both treatments was significantly higher at 30°C, at pH 5 and at incubation time of 16 hours accordingly. However, the glucose production by Trichoderma reesi was significantly ($p < 0.01$) higher in the Oil-less PKC treatment, from which the residual oil had been removed through the SFE technique. The results of this trial suggest that the removal of residual oil from PKC enhances its use as substrate for glucose production via SSF, using Trichoderma reesi. The results also indicate that temperature, pH and incubation time are important factors to consider during SSF with the green fungus (Trichoderma reesi). It is concluded here, based on the results of this study, that SSF at temperature of 30°C, at pH 5 and incubation time of 16 hr are ideal for higher glucose production by Trichoderma reesi on PKC substrates.

INTRODUCTION

The agricultural industry in Malaysia generates large quantities of wastes, which has been estimated at approximately 5 million tons per annum and this is expected to increase by two-fold or more by the year 2010 (Pang et al. 2006). One notable feature of these generated wastes however, is that majority originate from the palm industry including; oil palm trunks and fronds, palm kernel and palm kernel cake (PKC), among many other materials.

PKC is a waste product generated after crushing the palm kernel, to extract the oil from the fruit using the screw-press extraction (expeller) technique (Alimon 2004; Akpan, et al., 2005; Soltan, 2009). The global production of this by-product has consistently increased in volume with the

expansion of the oil palm industry in many countries in Asia (particularly Malaysia and Indonesia) and Africa in recent years. The global production of PKC in the year 2002 was estimated at over 4 million metric tons (Atasie and Akinhanmi, 2009). The proximate composition of PKC suggests that it could be classified as an energy feed, as it is richly composed of nutrients containing (on the average); 59.9% carbohydrate, 10.2% fat, 2% sugar and 391 kcal of energy. It is also an important source of protein (18.6% crude protein, consisting of high quality amino acids), contains crude dietary fiber of 37% and average residual oil content of 10% (Alimon 2004). The analysis of PKC showed that over 60% is cell wall components, with the fiber content composed of mainly insoluble, mannose-based polysaccharides (Alimon 2004). Jaafar and Javis (1994) also

noted that PKC cell wall consists of 58% mannan, 12% cellulose and 4% xylan.

The use of large quantities of cell wall components like cellulose, mannan and ligno-cellulose in agricultural wastes available in the environment as raw materials for solid state fermentation (SSF) processes for use in animal feedstock continue to receive global attention (Alimon 2004; Soltan, 2009; Wallace et al., 2010). The potential of PKC as a feed ingredient for ruminant animals in the livestock feeds industry after fungi fermentation is well documented (Akpan et al., 2005). However, the major existing problem in the use of PKC for such purposes remains the residual oil content after extraction, where little is known about the process involved in the removal of this residual oil from the PKC matrixes. The residual oil in the cake is partly responsible for PKC's characteristic unpleasant taste and odor, due to incidents of mould and rancidity during storage, thus hindering its use (Pearson 1985).

From residual oil-free PKC, the lignocellulose could be converted to fermentable sugars, after they have been broken down, to be used as carbon sources by several microorganisms. This is because fermentable sugars are known to be produced by fungi in their natural habitats via SSF processes (Ibrahim, 2007). Among the wood-degrading fungi however, Trichoderma reesei has been reported as one of the most successful and extensively studied cellulase systems (Claeyssens and Tomme, 1989; Goyal et al., 1991; Teeri et al., 1998). Cellulolytic enzymes from a filamentous fungus, Trichoderma sp. has also been a subject of intensive research because this organism secretes large amounts of enzymes required for the complete hydrolysis of crystalline cellulose (Kubicek, 1992; Teeri et al., 1992). To maximize the use of PKC as animal and human feed components therefore, alternative methods are needed to remove the residual oils, with supercritical fluid extraction being one such method currently receiving global attention.

Supercritical fluid extraction (SFE) was shown to be a powerful extraction method for the removal of oils from fibers (Lau et al., 2006). In the SFE technique, supercritical carbon dioxide (SC-CO_2) is employed as the solvent and has replaced other traditional solvents in the food industries because of its characteristic advantages of being non-toxic, non-flammable and its availability at reasonably low costs (Nik Norulaini et al., 2004). Therefore, the principal objective of the present study was to evaluate the effects of temperature, pH and time used in SSF of Oily and Oil-less

PKC substrates, using Trichoderma Isolate Pro-A on the potential of maximizing glucose production from the substrates before and after the residual oil is extracted via SFE. This paper describes the physico-chemical parameters explored, to produce glucose from the PKC substrates.

METHODOLOGY

ISOLATION AND IDENTIFICATION OF TRICHODERMA ISOLATE PRO-A1, THE POTENTIAL PRODUCER OF CELLULASE

The solid agar media used for this experiment comprised of 163 mL of V8 mixed vegetable juice added to 87 mL of distilled water and 3 g of Nutrient Agar and mixed thoroughly, using a glass rod. The pH of this mixture was determined to be 5.00 at room temperature, using a bench top pH meter (Hach sensION3 CO, USA) fitted with a gel-filled platinum electrode. The solution was heated to boil at 121°C for 15 min and then allowed to cool to ambient temperature. After cooling, the media was poured into petri dishes. Commercial Trichoderma Isolate Pro-A1was purchased from Pro-Fil Industrial Resources Sdn Bhd., Penang-Malaysia in its liquid form. A few pieces of sterile wood chips were dipped into the liquid solution and cultured on the V8 mixed vegetable juice solid agar media prepared as described above. After 5 days, the Trichoderma culture which was incubated at 28°C, with 8 hours of lighting per day was noticed to have grown to fill up the entire surface of the media. A small fungal piece (measuring 1.0 mm³) from the 5-day old culture was sub-cultured onto petri dishes consisting of the V8 agar prepared earlier. All the culturing procedures were carried out in the laminar flow hood (Model Zenith 4FT, India).

SAMPLING AND PREPARATION OF SUBSTRATES (PKC)

Freshly produced PKC was obtained from a local palm kernel mill in Pinang, Malaysia. The fresh samples were divided into two equal portions; one portion was immediately packed and stored in a refrigerator maintained at 4°C until used later and was labeled as Oily PKC treatment. The other portion was defatted using SFE method, with SC-CO_2 as the extraction solvent as explained in the subsequent section. Residual hexane from this extraction procedure was then removed and the defatted PKC was stored at 4°C until ready for use; where the treatment was labeled Oil-less PKC. Both PKC treatments were dried in an oven maintained at 60°C for 24 hours before use.

Dried PKC substrates were properly ground and screened through a sieve shaker, to obtain substrate particles of three mesh sizes (250μM, 500μM and 1mM), which were used for subsequent evaluations. Substrates of mesh size 250μM were chosen for use in subsequent evaluation.

SUPERCRITICAL FLUID EXTRACTION (SFE), USING C0□ AS THE EXTRACTION SOLVENT

Initial study was conducted using the equipment of Supercritical system; SFX 220 extraction system (ISCO, Lincoln, NE, USA). This consisted of carbon dioxide cylinder, chiller (B-L;730, Yih Der, Taiwan), C0□ syringe pump (ISCO, Model 100DX), modifier syringe pump (ISCO, Model 100 DX), extraction chamber (ISCO, SFX 220), extraction cartridge, controller (ISCO, SFX 200) and Restrictor Temperature Controller (ISCO) (Nik Norulaini, 2004a). From PKC samples with particle size 250 □M, residual oil was super critically removed, at a pressure range of between 27 and 41 MPa, a temperature range of 50°C to 80°C and with the substrate flow rate set at 1.5 mL/min. The substrate yielding the highest residual oil after the SFE extraction was used in subsequent evaluation (as Oil-less treatment).

SOLID STATE FERMENTATION (SSF) OF OILY AND OIL-LESS PKC, USING TRICHODERMA ISOLATE PRO-A1

Solid state fermentation of substrates using the fungus was performed on 5 g PKC and PK as the solid substrates (Oily and Oil-less treatments), with the addition of 2 mL of Mandel's medium. The Mandel's medium was prepared containing the following components (g/L); urea, 0.3; peptone, 0.75; yeast extract, 0.25; $(NH4)_2 SO_4$, 1.4; KH_2PO_4, 2.0; $CaCl_2$, 0.3; $MgSO_4.7H_2O$, 0.3 and trace elements (mg/L): $FeSO_4.7H_2O$, 5; $MnSO_4. 4H_2O$, 1.6; $ZnSO_4.7H2O$, 1.4 and $CoCl_2.6H_2O$, 20.0 (Mandels et al., 1976). The medium and the trace elements were autoclaved separately at 121°C for 15 min, in flasks. After the flask containing the medium had cooled to room temperature, a known amount of the sterilized trace elements was added. The substrate was also autoclaved at 121°C for 15 min and cooled to room temperature as well. The PKC substrates were inoculated with fungi filtrates, containing; $2x10^8$ (spores/mL) of inoculate filtrates. A control was also prepared the same manner as the sample except that no fungi were inoculated. All the samples and controls were incubated for 5 days at ambient temperature. Subsequently, the test was repeated, using Bacteria sp. instead of the Trichoderma sp. as inocula; where the control and Bacteria sp. inocula plates showed no Trichoderma colonies growth.

OPTIMIZATION OF PH, INCUBATION TEMPERATURE AND INCUBATION TIME

The SSF was conducted at 3 different pH which included; 3.0, 5.0 and 7.0. As for the incubation temperature, the tests were also performed at 3 different temperatures as follows; 20°C, 30°C and 40°C. The differences in the incubation times were measured at 4 different time intervals of 4 hours, 8 hours, 12 hours and 16 hours, accordingly. All experimental procedure was maintained as same, except the variations in the pH, temperature and time as mentioned earlier. Experiments were carried out in triplicates.

MEASUREMENT OF GLUCOSE CONCENTRATION

To measure the concentration of glucose produced in the culture medium, a standard curve was plotted, using standard glucose solution (Sigma MO, USA). Approximately 10 mL containing 10 mg/mL of glucose stock solution was prepared. From this stock solution, dilutions were made with distil water to prepare concentrations of 20, 40, 60, 80 and 100 □g/mL of glucose solutions, following standard laboratory protocols. From each of these concentrations, 0.5 mL of glucose solution was measured out into separate vials with a pipette. To each solution in a vial, 0.5 mL of 5% phenol and 0.5 mL of concentrated H_2SO_4 were added. The vials and contents were allowed to stand for 10 min for reactions to take place, after which they were shaken to allow the solutions to mix. Finally, the mixed solutions in the vials were left for 30 min for color development and their absorbance was read in a spectrophotometer at 490 nm. The result was used to plot a standard curve.

Approximately 0.5 mL of the sample solution was also measured with a pipette into a vial. To this 0.5mL of 5% phenol was added, followed by the addition of 0.5 mL of concentrated H_2SO_4 and the solution also left to stand for 10 minutes for the reaction to take place. Subsequently the vials were shaken to mix the solutions and the solutions were also left for 30 minutes for the color to develop and the absorbance of the colored mixture was read at 490 nm in a spectrophotometer. All the procedure was carried out in triplicates and results are reported as mean ± SD of 3 determinations.

STATISTICAL ANALYSIS

Statistical mean of data for the effects of pH, temperature

and time on the concentration of glucose produced in the different culture media were compared using 1 and 2 way ANOVA in the SPSS statistical software package; where all values of P<0.01 were considered significant.

RESULTS

As could be observed in the SEM micrographs shown in Figure 1, Trichoderma reesei colonies covered the surface of the Oily and Oil-less PKC substrate, when incubated for 5 days at 30°C, with 8 hours of lighting per day, although the conidiophores were thicker on the Oil-less substrates. This indicates that the culture conditions were favorable for the growth of the fungi, although the substrates containing less oil seem to support better growth. The particle size of 250 𝜇m for the PKC substrates was selected based on preliminary experiment carried out earlier, which showed this particle size to yield significantly the highest amount of residual oil during SFE.

Figure 1

Figure 1: SEM micrographs of grown on substrates (A) Oily PKC and (B) Oil-less PKC, 5 days after incubation at 30°C with 8 hours of lighting per day. Notice the codia and coniophores of the colonies on the surface of the culture media.
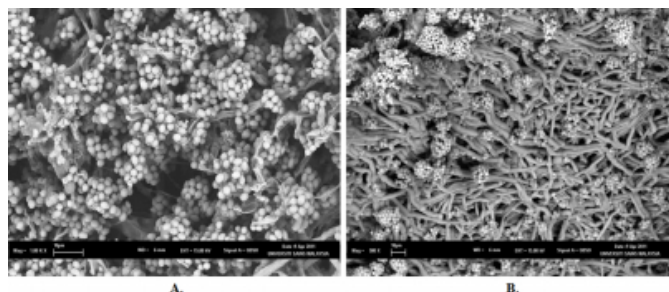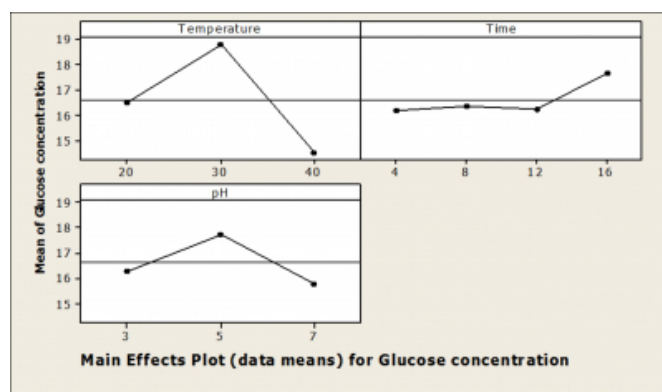

Result of incubation temperature, pH and time of SSF on glucose concentration produced by T. reesei colonies in the Oily PKC substrates is shown in Figure 2. Increase of incubation temperature from 20 to 30°C led to a significant (P<0.01) increase in glucose concentration from ~16.5 to over 18.5 𝜇g/mL, while further increase in temperature to 40°C led to a significant decline in glucose concentration to levels recorded at 20°C (below 15 𝜇g/mL). At pH 3, glucose concentration was recorded at ~16.3 𝜇g/mL. Increasing the incubation pH to 5 also led to significant increase in glucose concentration to ~18 𝜇g/mL; whereas a further increase in incubation pH manifested in a decline in the glucose concentration back to 16 𝜇g/mL. On the other hand, changing the incubation time from 4 to 8 and further to 12 hours led to no significantly (P>0.01) observed changes in the glucose

concentration in the media. However, further increase in incubation time from 12 to 16 hours led to a sharp and significant increase in glucose concentration from ~16.4 to 18 𝜇g/mL.
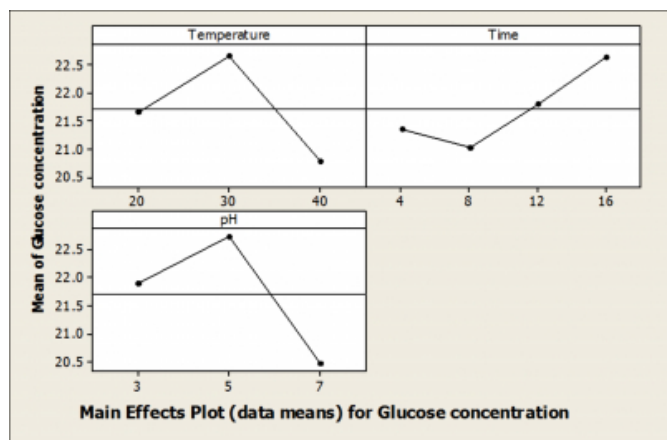
Figure 2

Figure 2: The effects of incubation Temperature, Time and pH of SSF of Oily PKC substrate with on glucose concentration (𝜇g/mL).

Main Effects Plot (data means) for Glucose concentration

In comparison, result of incubation temperature, pH and time of SSF on glucose concentration produced by T. reesei colonies on the Oil-less PKC substrates is shown in Figure 3. The trends observed in the concentration of glucose in the media following increase in incubation temperature and pH were similar to those noted in the Oily PKC substrate. However, the concentration of glucose was significantly higher compared to that in the Oily PKC substrate, at each of the temperatures and pHs tested. In the case of the incubation times tested however, glucose concentration in the media was noticed to slightly (but insignificantly) decline when the time was increased from 4 to 8 hr. Further increase in the incubation time from 8 to 12 and then to 16 hr, led to significantly (P<0.01) higher glucose concentrations, respectively.

Figure 3

Figure 3: The effects of incubation Temperature, Time and pH of SSF of Oil-less PKC substrate with on glucose concentration (g/mL).

Main Effects Plot (data means) for Glucose concentration

Two-way ANOVA showing the interactions of incubation Temperature, pH and time during SSF of the Oily PKC substrate (Table 1) or that for Oil-less PKC substrate (Table 2) all clearly show the significant (P<0.01) interactions of incubation temperature and time, temperature and pH, time and pH and temperature, time and pH, respectively suggesting the effects of all the variables tested.

Figure 4

Table 1: Types and sources of error, degree of freedom and significance of variation (P-values) of the effects incubation Temperature, Time and pH and their interactions during SSF of PKC substrate with on glucose concentration (g/mL)

Source	DF	SS	MS	F	P
Temperature	2	5.99	2.99	62646.17	<0.0001
Time	3	0.75	0.25	5245.60	<0.0001
pH	2	1.36	0.68	14310.23	<0.0001
Temperature*Time	6	4.64	0.77	16184.33	<0.0001
Temperature*pH	4	0.37	0.09	1937.97	<0.0001
Time*pH	6	0.86	0.14	3024.45	<0.0001
Temperature*Time*pH	12	2.40	0.20	4190.36	<0.0001
Error	72	0.003	0.0005		
Total	107	16.41			

Figure 5

Table 2: Types and sources of error, degree of freedom and significance of variation (P-values) of the effects incubation Temperature, Time and pH and their interactions during SSF of PKC substrate with on glucose concentration (g/mL)

Source	DF	SS	MS	F	P
Temperature	2	62.96	31.48	21851.80	<0.0001
Time	3	39.69	13.23	9184.36	<0.0001
pH	2	94.70	47.35	32865.08	<0.0001
Temperature*time	6	30.89	5.14	3574.05	<0.0001
Temperature*pH	4	62.06	15.51	10769.26	<0.0001
Time*pH	6	20.35	3.39	2354.52	<0.0001
Temperature*time*pH	12	25.78	2.14	1491.36	<0.0001
Error	72	0.10	0.001		
Total	107	336.56			

DISCUSSIONS

Trichoderma reesei colonies were noticed to have completely covered the surface of the PKC substrates after 5 days of incubation at 30°C (the best temperature among those tested, which yielded the highest concentration of glucose in the Oil-less substrate), with 8 hours of lighting per day. This was a good indication, suggesting that the culture conditions provided at that temperature favored the growth of the fungi. In Singhania et al. (2006), the use of Trichoderma reesei strains (NRRL 11460) on lingo-cellulose substrates was also reported, using SSF and shown that the temperature favorable for the growth of the fungi was at 28°C, for the highest cellulose enzyme activity to produce cellulose. However, in a study which reported the physiological characteristics of Trichoderma spp. isolated from oyster mushrooms (Pleurotus spp.), Choi et al. (2003) demonstrated that the optimal growth temperature of Trichoderma spp. was between 27 and 30°C, consistent with the conditions observed in the present study. This supports that the choice of 30°C as one of the incubation temperatures tested in this experiment was within the range acceptable for optimal growth of the fungi.

Temperature is a very important factor in the growth of fungi, particularly Trichoderma spp. Studies of the influence of different temperatures on the mycelial growth of Trichoderma revealed that the growth of Trichoderma was favored by higher temperatures, reaching a maximum at 30°C, whereas no growth was observed at 15°C (Lorant, 2008), but growing at wider range between 20-28°C (Woo et al. 2004). In describing the effects of temperature on growth patterns of Trichoderma, Denesh et al. (2000) reported that at temperature of 25°C, growth patterns of T. longibrachiatum, T. harzianum, T. virens and Trichoderma sp. collected from mushroom farms showed significant differences in their type of growth and sporulation patterns between various species and isolates. However, Choi et al. (2003) reported that the optimal growth temperature of Trichoderma spp. was between 27 and 30°C. Trichoderma species are asexual, soil-inhabiting filamentous fungi with teleomorphs. According to Chen and Moy (2004), a fluctuation in such important parameters which are ideal for mushroom cultivation, such as the sources of carbon and nitrogen, high relative humidity, warm temperatures and the absence of light during most of spawn run, are ideal conditions in the environment for the green moulds as well, which can easily lead to their contamination.

In a similar development, pH plays a critical role in the growth of the fungus. According to Lorant (2008),

Trichoderma prefer acidic-neutral conditions (pH 5-7) for optimal growth. The mycotoxins produced by Trichoderma have been reported and identified as gliotoxin, viridin, trichodermin and peptide type (Kim, 1985). Furthermore, Trichoderma species secrete hydrolytic enzymes including chitinases, ⃞-glucanases and cellulases which are kind of mycotoxins and they lyse the fungal cell walls and are speculated to play key roles in the myco-parasitic activity of this fungus (Goltapeh and Danesh, 2000). Certain species of Trichoderma are well known to possess the ability of antagonizing a series of plant pathogenic fungi (Papavizas 1985). Their proposed mechanisms of antagonism include mycoparasitism by the action of cell-wall degrading enzymes, antibiosis by the production of antibiotics, competition for space and nutrients through rhizosphere competence, facilitation of seed germination and growth of the plants via releasing important minerals and trace elements from soil and induction of the defense responses in plants (Herrera-Estrella and Chet 2003; Howell 2003; Benítez et al. 2004).

Recently, the emergence of solid state fermentation (SSF) as a promising technology for the development of several bioprocesses and products, including the production of therapeutic enzymes on a commercial scale cannot be over-emphasized (Pandey et al., 1999). The primary advantage of SSF is the fact that many metabolites could be produced at very high concentrations. From analysis of the literature with regards to enzymatic hydrolysis, it was revealed that high cellulase activity per unit volume of fermentation broth is the most important factor in obtaining sugars (such as glucose as fermentation products) in concentrations of 20 to 30%, from hydrolysis of cellulose for ethanol production from cellulosic materials (Chahal, 1982). Usually, glucose production by Trichderma occurs via acidic or enzymatic hydrolysis of starch present in the substrates which the fungus acts upon. In America and many other parts of the world, glucose is produced mainly by using enzymatic hydrolysis of grains starch (Van de Veen et al., 2005). Similarly, Silva and colleague (2010) used two microorganisms extracted from the soil; Aspergillus niger and Strepotmyces sp., to produce glucoamilase and glucoisomerase enzymes, respectively, through the conversion of cassava starch to glucose and fructose syrup, further justifying the use of micro-organisms for sugar production.

The large scale availability of PKC, which according to

estimates of Atasie & Akinhanmi (2009) stood at over 4 million tons in 2002, has been used as a feed ingredient for ruminant animals in the livestock feeds industry (Akpan et al., 2005). This great advantage of PKC holds a great potential when properly harnessed, for the commercial production of glucose and other sugars through SSF method (Ab Rashid et al. 2011), as was also demonstrated in this study, after the removal of the residual oil via SFE technique. When properly harnessed, the menace created by the accumulation of PKC wastes could be converted to cash, through the combination of a cheap residual oil extraction technique (SFE) and an efficient glucose production method (SSF) using Trichderma, respectively, as was demonstrated in the present experiment.

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References

r-0. Ab Rashid, S., Darah, I. & Omar, I. C. (2011) Utilization Of Palm Kernel Cake For The Production Of Mannanase By An Indigenous Filamentous Fungus, Aspergillus Niger USM F4 Under Solid Substrate Fermentation. The Internet Journal of Microbiology 9(1)

r-1. Akpan, H. D., Udofia, A. A., Akpan, E. J. & Joshua, A. A. (2005) The effect of phytase and zink supplementation on palm Kernel Cake toxicity in sheep. Pakistan Journal of nutrition 4(3), 148-153

r-2. Alimon A. R. (2004) The nutritive value of Palm Kernel Cake for animal feed. Palm Oil Developments 40, 12-14

r-3. Atasie, V. N. & Akinhanmi, T. F. (2009) Extraction, Compositional Studies and Physico-Chemical Characteristics of Palm Kernel Oil. Pakistan Journal of Nutrition, 8 (6), 1680-5194

r-4. Chahal, D. S. (1982) Enzymatic hydrolysis of cellulose: state-of the-art, p. 74. National Research Council of Canada report no. 20283. National Research Council of Canada, Ottawa.

r-5. Chen, A.W. & Moy, M. (2004) Mushroom cultivation: building mold contamination. At: [http://www.mushworld.com] Accessed on 2 December 2011

r-6. Choi, I-Y., Joung, G-T., Ryu, J., Choi, J-S. and Choi, Y-G. (2003) Physiological Characteristics of Green Mold (Trichoderma spp.) isolated from Oyster Mushroom (Pleurotus spp.). Mycobiology 31(3), 139-144

r-7. Claeyssens, M. & Tomme, P. (1989) Structure-activity relationships in cellulolytic enzymes In: Coughlan, M.P. (Ed.), Enzyme Systems for Lignocellulose Degradation. Elsevier, London, pp. 37–49

r-8. Danesh, Y. R., Goltapeh, E. M. & Rohani, H. (2000) Identification of Trichoderma species causing green mold in

button mushroom farms, distribution and their relative abundance. In Science and Cultivation of Edible fungi: Mushroom Science XV(2), 653-659

r-9. Goltapeh, E. M. & Danesh, Y. R. (2000) Studies on interaction between Trichoderma species and Agaricus bisporus mycelium. In Science and Cultivation of Edible fungi: Mushroom Science XV(2), 661-666

r-10. Goyal, A., Ghosh, B. & Eveleigh, D. (1991) Characteristics of fungal cellulases. Bioresour. Technol. 36, 37–50

r-11. Herrera-Estrella, A. & Chet, I. (2003) The biological control agent Trichoderma from fundamentals to applications. In: Arora D. K., Bridge P. D. and Bhatnagar D. (eds), Fungal Biotechnology in Agricultural, Food, and Environmental Applications, Marcel Dekker, New York, 147-156

r-12. Howell, C. R. (2003) Mechanisms employed by Trichoderma species in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis. 87, 4–10

r-13. Ibrahim, C.O. (2007) Development of applications of industrial enzymes from Malaysian indigenous microbial sources. Bioresource Technology 99, 4572–4582

r-14. Jaafar, M.D. & Jarvis, M. C. (1992) Mannans of oil palm kernels. Phytochemistry 31(2), 463-464

r-15. Kim, M. K. (1985) Influences of antibiotic components produced by Trichoderma spp. to oyster mushroom. Kor. J. Mycol. 13, 105-109

r-16. Kubicek, C. P. (1992) The cellulose proteins of Trichoderma Reesei: structure, multiplicity, mode of action and regulation of formation. Adv Biochem Eng Biotechnol. 45, 1-27.

r-17. Lau, H. L. N., Y. M. Choo, Ma, A. N. & Chuah, C. H. (2006) Quality of Residual Oil from Palm-Pressed Mesocarp Fiber (Elaeis guineensis) Using Supercritical CO2 With and Without Ethanol. Journal of the American Oil Chemists' Society (JAOCS) 83(10)

r-18. Lorant, H. (2008) Mushroom pathogenic Trichoderma species: occurrence, biodiversity, diagnosis and extracellular enzyme production. Ph.D. dissertation; Department of Microbiology, University of Szeged, Szeged

r-19. Nik Norulaini, N. A., Md Zaidul, I. S., Anuar, O. & Omar, M. A. K. (2004) Supercritical enhancement for separation of lauric acid and oleic acid in palm kernel oil (PKO). Journal of Separation and Purification Technology 35, 55–60

r-20. Pandey A., Soccol C. R., Selvakumar P. & Nigam P.(1999) Solid-state fermentation for production of industrial enzymes. Current Science 77, 149-162

r-21. Pang, P. K., Darah. I., Poppe, L., Szakacs, G. & Ibrahim, C.O. (2006) Xylanase Production by a Local Isolate, Trichoderma spp. FETL c3-2 via Solid State Fermentation Using Agricultural Wastes as Substrates. Malaysian Journal of Microbiology 2(1), 7-14

r-22. Papavizas, G. C. (1985) Trichoderma and Gliocladium: biology, ecology and potential for biocontrol. Annu. Rev. Phytopathol. 23: 23–54

r-23. Pearson D. (1985) The Chemical Analysis of Foods. 6th Edition. J & A Churchill; Gloucester place, London

r-24. Teeri, T. T., Penttila, M., Keranen, S., Nevalainen, H. & Knowles, J.K.C., (1992) Structure, function and genetics of cellulases, In: Finkelstein, D.B.; Ball, C. (Eds.), Biotechnology of filamentous fungi: technology and products, Butterworth-Heinemann., Boston, pp 417-445

r-25. Teeri, T. T., Koivula, A., Linder, M., Wohlfahrt, G., Divne, C. & Jones, T. A. (1998) Trichoderma reesei cellobiohidrolases: why so efficient on crystalline cellulose? Biochem. Soc. Trans. 26, 173–178

r-26. Silva, R. N., Quintino, F. P., Monteiro, V. N. & Asquieri, E. R. (2010) Production of glucose and fructose syrups from cassava (Manihot esculenta Crantz) starch using enzymes produced by microorganisms isolated from Brazilian Cerrado soil. Tecnol. Aliment. 30 (1), 27-31

r-27. Singhania, R. R., Sukumaran, P. K., Pilli, A. et al. (2006) Solid state fermentation of lignocellusic substrate for cellulose production by Trichoderma reesei NRRL460. Indian J Biotech5, 332-6

r-28. Soltan, M. A. (2009) Growth performance, immune response and carcass traits of broiler chicks fed on graded levels of palm kernel cake without or with enzyme supplementation. Livestock Research for Rural Development 21(3)

r-29. Van der Veen, M. E., Van der Goot, A. J. Boom, R. M. (2005) Production of glucose syrups in highly concentrated systems. Biotechnol Progress 21 (2), 598-602

r-30. Wallace, P. A., Adu E. K. & Rhule S. W. A. (2010) Optimal storage conditions for cocoa cake with shell, palm kernel cake and copra cake as poultry and livestock feed in Ghana. Livestock Research for Rural Development 22 (32)

r-31. Woo, S. L., Di Benedetto, P., Senatore, M., Abadi, K., Gigante, S., Soriente, I., Ferraioli, S., Scala, F. & Lorito, M. (2004) Identification and characterization of Trichoderma species aggressive to Pleurotus in Italy. Journal of Zhejiang University, Agriculture and Life Sciences 30, 469-470.

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