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Upgrading of Animal Feed by Solid State Fermentation by *Pleurotus sajor-caju*

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Abstract: Solid state fermentaton using cellulolytic fungus, *Pleurotus sajor-caju with* pretreatment of irradiation and lime for upgradation of four agro-wastes such as pulse husk, rice bran, wheat bran and sugarcane bagasse to animal feed were investigated. The fermentation of the substrates was done at 30°C. Total bacteria and fungi were reduced effectively by 10 kGy of radiation dose. Pretreatment with lime and irradiation seemed to be an excelent option for upgradation of animal feed. The amount of reducing sugar and soluble protein were found to be the almost same in 5 and 10 kGy irradiated and unirradiated substrates. Soaked samples showed to be equally good for complete fermentation, which was achieved around 8 weeks of incubation time at ambient temperature. The highest amount of reducing sugar obtained after fermentation was 18.0 mg/g and the amount of soluble protein is 26.92 mg/g. The results of the present experiment clearly indicate that *Pleurotus sajor-caju* could be a potential microorganism for the production of enriched and safe animal feed. Further research are, however, indeed necessary to develop upgraded animal feed to overcome the socioeconomic problems of developing countries, including Bangladesh.

Key words: Solid State Fermentation · *Pleurotus sajor-caju* · Animal Feed · Reducing Sugar · Protein

INTRODUCTION

In villages of agro-based countries, farmers use cattle for pulling cart. In many cases, the health conditions of these animals are not satisfactory. Most of them suffer from different diseases due to nutritional deficiency. So it is now the highest concern of livestock scientist to develop upgraded animal feed.

Lignocellulose is the major structural component of woody plants and non-woody plants such as grass and represents a major source of renewable organic matter. The chemical properties of the components of lignocellulosics make them a substrate of enormous biotechnological value [1]. Unfortunately, much of the lignocellulose waste is often disposed of by biomass burning, which is not restricted to developing countries alone, but is considered a global phenomenon [2]. The use of cellulase has been most promising in the degradation of lignocellulosic biomass into simpler forms of nutrients [3]. Because the bulk of all trees, plants and other vegetation have a large amount of lignocellulose mass, it is an excellent source of fuel for energy, especially since it is not edible [4]. Lignocellulosic materials are composed mainly of cellulose, hemicellulose, lignin and also other polysaccharides such as starch, pectin and proteins [5].

Enzymatic degradation of native cellulose is a complex process requiring participation by a sequential operation of several basic cellulase components that contribute to the degradation of cellulose to glucose [6]. Some bacteria (such as *Clostridium thermocellum*) and fungi (like *Trichoderma*) are among the mostly utilized sources of cellulose enzyme which convert cellulose to glucose [7]. Among these, fungi have been studied extensively because they are filamentous organisms and their elongated hyphae create mechanical pressure on the protective cellulose structure and they excrete large amount of cellulase [8]. Most fungal cellulases are complete containing all the components required to digest cellulose and they also produce enzymes

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required to hydrolyze lignin and hemicelluloses [9], since mushrooms like *Pleurotus sajor-caju* (edible fungi) obtain their energy by decomposing wood especially sapwood and heartwood of broad-leaved or rarely coniferous trees [10]. This process can greatly improve the nutritional value of cellulosic substrates like straw, bagasse etc. The hydrolytic enzymes can be produced either by solid-state fermentation (SSF) or submerged culture of the enzyme producing microorganisms [11]. SSF offers potential advantages over submerged liquid fermentation (SLF) such as, low energy consumption, process simplicity, superior enzyme productivity, low capital investment, negligible liquid waste product and ease in product recovery [12-14]. The main focus of this study is to highlight the significant aspects of lingnocellulose biotechnology with emphasis on demonstrating the potential value from an application rather than basic research perspective.

MATERIALS AND METHODS

This study was done in the research laboratory of the Department of Biochemistry and Molecular Miology at Jahangirnagar University and at Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Bangladesh during July 2008 and June 2009.

Biological Samples and Treatment: The pure fungal culture (*P. sajor-caju*) was used for fermentation and routinely sub-cultured and maintained on potato dextrose agar (PDA) slants and was stored at 4°C. The natural cellulosic agro-wastes selected for the present study were pulse husk (*Cicep arictinum*), rice bran, wheat bran and sugarcane bagasse. Substrates used in this study were sugarcane bagasse (0kGy, 5kGy, 10kGy irradiated) with rice bran, wheat bran and pulse husk (lime treated) respectively.

Pretreatment: 250 g of substrate such as pulse husk and sugarcane bagasse were taken and then soaked with a lime solution (5 g $CaCO_3$ in 1875ml distilled water). The substrates were left in soaking condition overnight. Then the lime solution was drained out by tap water [15]. Treated substrate was spread over aluminum foil and allowed to dry overnight at 60°C. Sugarcane bagasse was taken in sealed sterile polythene bag and irradiated by 60co source (50000ci). The dose rate was 340 Gy/h and the radiation doses used ranged from 5 to 10 kGy. The number of surviving bacteria and fungus were enumerated on nutrient agar and PDA plates respectively.

Fermentation: *Pleurotus* sajor-*caju* was subcultured from stock PDA slant to PDA plate. After two weeks of incubation at 30°C three pieces of mycelial growth (about 1 cm in diameter) were taken with two loops full of a hollow borer for inoculation in the fermentation medium in 100 ml conical flask in each containing 50 ml PDA broth. Then each flask was shaked in shaker for one week and transferred each 50 ml inoculums in substrates and incubated at 30°c for 10 weeks with a control where there was no inoculum for each differently treated substrate.

Assay: Collected cellulosic materials were first cleaned off all darts and unwanted materials. Then, these were cut into tiny pieces, washed with water, sun-dried and then crushed in a grinder. The ground substrates were investigated for the determination of their moisture content and percentage of soluble protein and reducing sugar. Moisture content of the substrate was determined by dry oven method, protein was determined by Lowry *et al.* [16] and reducing sugar was determined by method developed by Miller [17].

RESULTS AND DISCUSSION

Figures 1-3 show the accumulation of reducing sugar at different interval of fermentation by *P. sajor-caju* in rice bran, wheat bran and pulse husk mixed with sugarcane bagasse. In every substrate, the amount of reducing sugar was found highest after 8 weeks. In lime treated rice bran (LT-RB), the highest amount of reducing sugar (17.6 mg/gm) was found when mixed with 5KGy sugarcane bagasse (SB); in lime treated wheat bran (LT-WB), the highest amount of reducing sugar (18.0 mg/gm) was found when mixed with 10KGy SB; in lime treated pulse husk (LT-PH), the highest amount of reducing sugar (17.54 mg/gm) was found when mixed with 5KGy SB. The change of reducing sugar content in control substrates (without *P. sajor-caju*) was ignorable.

Figures 4-6 show the accumulation of soluble protein at different interval of fermentation by *P. sajor-caju* in rice bran, wheat bran and pulse husk mixed with sugarcane bagasse. In every substrate, the amount of reducing sugar was found highest after 8-9 weeks. In LT-RB, the highest amount of protein (26.5 mg/g) was found when mixed with 5KGy SB; in LT-WB, the highest amount of protein (25.1 mg/g) was found when mixed with 0KGy SB; in LT-PH, the highest amount of protein (26.92 mg/g) was found when mixed with 5KGy SB. The change of protein content in control substrates (without *P. sajor-caju*) was ignorable.

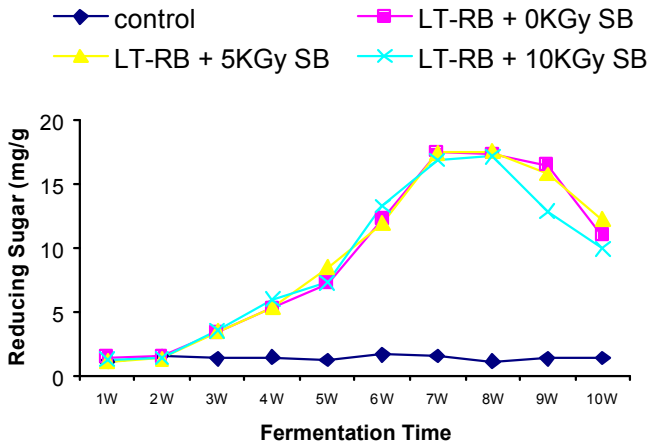

Fig. 1: Reducing sugar (mg/g) content of lime treated rice bran mixed with 0, 5, 10kGy irradiated sugarcane bagasse fermented by *P. sajor-caju. LT-RB: lime treated rice bran, SB: sugarcane bagasse, W: week, Control: (LT-RB+0KGy SB) with no P. sajor-caju fermentation.*

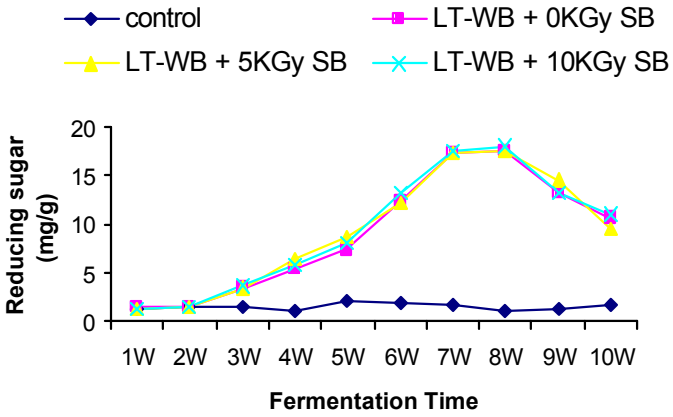

Fig. 2: Reducing sugar (mg/g) content of lime treated wheat bran mixed with 0, 5, 10kGy irradiated sugarcane bagasse fermented by *P. sajor-caju. LT-WB: lime treated wheat bran, Control: (LT-WB+0KGy SB) with no P. sajor-caju fermentation*

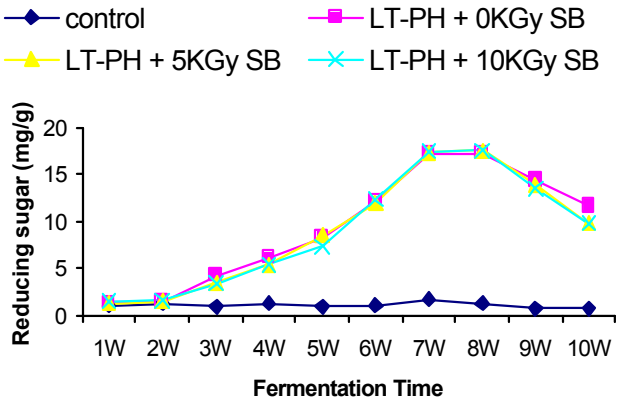

Fig. 3: Reducing sugar (mg/g) content of lime treated pulse husk mixed with 0, 5, 10kGy irradiated sugarcane bagasse fermented by *P. sajor-caju. LT-PH: lime treated Pulse Husk, Control: (LT-PH+0KGy SB) with no P. sajor-caju fermentation*

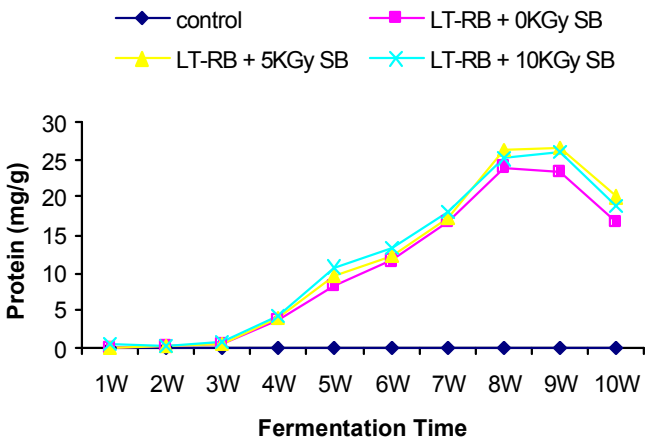

Fig. 4: Protein (mg/g) content of lime treated rice bran mixed with 0, 5, 10kGy irradiated sugarcane bagasse fermented by *P. sajor-caju. LT-RB: lime treated rice bran, SB: sugarcane bagasse, Control: (LT-RB+0KGy SB) with no P. sajor-caju fermentation*

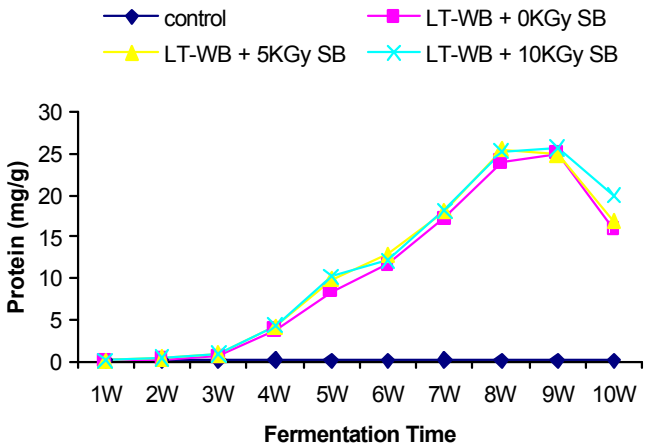

Fig. 5: Protein (mg/g) content of lime treated wheat bran mixed with 0, 5, 10kGy irradiated sugarcane bagasse fermented by *P. sajor-caju. LT-WB: lime treated wheat bran, Control: (LT-WB+0KGy SB) with no P. sajor-caju fermentation.*

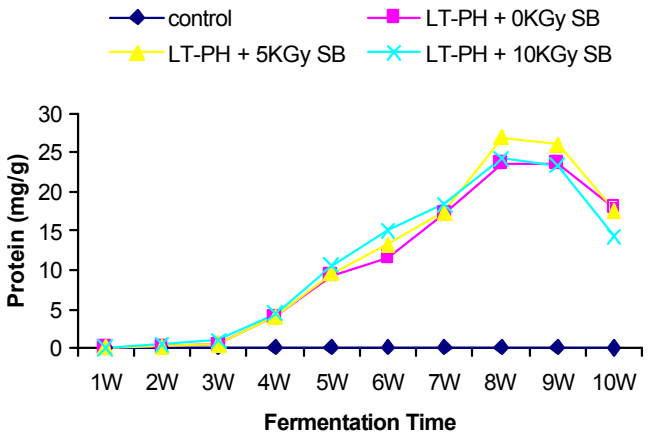

Fig. 6: Protein (mg/g) content of lime treated pulse husk mixed with 0, 5, 10kGy irradiated sugarcane bagasse fermented by *P. sajor-caju. LT-PH: lime treated Pulse Husk, Control: (LT-PH+0KGy SB) with no P. sajor-caju fermentation*

The energy and environmental crises which the world is experiencing is forcing us, among other things, to reevaluate the efficient utilizations or finding alternative uses for natural, renewable resources, especially organic "waste", using clean technologies. Large quantities of cellulosic agro-wastes are produced every year throughout the world, including Bangladesh. Among these wastes pulse husk and sugarcane bagasse are produced in the highest quantities [18]. In the era of declining forests, global climate changes, continuing expansion of industrialization, it is reasonable to consider the consequences of an alternative source of cellulose. Sugarcane bagasse is not currently being used as animal feed because of its relatively low nutritive value, in particular, low in protein content, high in fiber content and low digestibility. Pulse husk is used as animal feed although they are nutritionally very poor. The present study describes physical and chemical pretreatment of some agro-wastes (pulse husk and sugarcane bagasse), irradiation sterilization of these substrates and solid state fermentation of the substrates with a cellulolytic fungi, *P. sajor-caju* and assessment of the nutritional quality of the fermented substrates. Pulse husk and sugarcane bagasse are natural lignocelluloses. The enzymatic hydrolysis through solid state fermentation of these native lignocellulosic materials is very slow; mainly due to compositional heterogeneity and structural complexity. The same problem is also responsible for their poor digestibility by ruminant animal. Association between cellulose, hemicelluloses and lignin in the cell walls, cellulose crystallinity and accessibility of surface area to enzymes is generally recognized as the determinants of the extent of degradation by enzymes or microorganisms.

Cellulose can be effectively hydrolyzed and depolymerized into fermentable sugars by the enzyme cellulase. Cellulase based strategies make the process of bio-refinery more economical by means of utilizing cheaper substrates for enzyme synthesis [19]. The cellulose and hemicellulose are cemented together by lignin. Lignin is responsible for integrity, structural rigidity and prevention of swelling of lignocelluloses [20]. Thus, lignin content and distribution constitute the most recognized factor which is responsible for recalcitrance of lignocellusosic materials to enzymatic degradation by limiting the enzyme accessibility [21]; therefore, the pretreatment of lignocellulosic materials can improve the rate and extent of enzyme hydrolysis. Moreover, solid-state fermentation is often simpler and requires less processing energy than the corresponding liquid fermentation. In the current experiment, for these reasons, SSF was the choice. The increase of reducing sugar occurred rapidly up to 6th week in all substrates and then tended to slow down. In this study, the change of reducing sugar level was determined before and after solid state fermentation. The results suggest that the chemical pretreatment have effect not only in the initial reducing sugar content but also they contribute to increase the reducing sugar during fermentation. *P. sajor-caju* is a mushroom with high protein value [22-23] and substrates with *P. sajor-caju* also become rich with protein. The order of increase of soluble protein in different substrates was found to have the similar trends as that of reducing sugar. From the result, it seems that, the increase of soluble protein was attributed to the increase of mycelia growth and the increase of enzyme production.

On the basis of all the results, it can be concluded that sugarcane bagasse mixed with rice bran, wheat bran and pulse husk can be upgraded to animal feed by irradiation and solid-state fermentation by *P. sajor-caju* as i) the products contained high amount of soluble protein and reducing sugar and were free from toxin. So these fermented agro-wastes can be used as upgraded animal feeds. ii) The tendency of the fungus *P. sajor-caju* to grow at nearly ambient temperature (30°C), at a relative humidity of around 85% and requirement of short time for complete fermentation gave a scope for the production of enriched animal feed in industrial scale.

REFERENCES

1. Malherbe, S. and T.E. Cloete, 2003. Lignocellulose biodegradation: fundamentals and applications: A review. Environ. Sci. Biotechnol, 1: 105-114.
2. Levine, J.S., 1996. Biomass burning and global change. In: Levine JS (eds) (v-1). Remote sensing and inventory development and biomass burning in Africa. The MIT Press, Cambridge, Massachusetts, USA, pp: 35.
3. Demain, A.L., M. Newcomb and J.H. David, 2005. Cellulase, clostridia and ethanol microbiol. Mol. Biol. Rev., 69: 124-154.
4. Ingram, L.O., H.C. Aldrich, A.C.C. Borges, T.B. Causey, A. Martinez, F. Morales, A. Saleh, S.A. Unverwood, L.P. Yomano, S.W. York, J. Zaldivar and S.D. Zhou, 1999. Enteric bacterial catalysts for fuel ethanol production. Biotechnol. Prog, 15: 855-866.
5. Das, K., M. Anis, B.M.N. Azemi and N. Ismail, 1995. Fermentation and recovery of glutamic acid from palm waste hydrolysate by ion-exchange resin column. Biotechnol. Bioeng, 48: 551-555.

6. Adams, J.J., G. Pal, K. Yam, H.L. Spencer, Z. Jia and S.P. Smith, 2005. Purification and crystallization of a trimodular complex comprising the type II cohesin-dockerin interaction from the cellulosome of Clostridium thermocellum. Acta Crystallogr Sect F Struct Biol Cryst Commun, 61: 46-48.
7. Bisaria, V.S. and T.K. Ghose, 1981. Biodegradation of cellulosic material: substrate, microorganism, enzyme and products. Enzyme Microbiol. Technol., 3: 91-104.
8. Schwarz, W.H., 2001. The cellulosome and cellulose degradation by anaerobic bacteria. Appl. Microbiol. Biotechnol, 56: 634-649.
9. Singh, A., A.B. Abidi, N.S. Darmwal and A.K. Agrawal, 1989. Production of protein and cellulase by *Aspergillus niger* AS101 in solid state culture. Mircen J, 5: 451-456.
10. Bonatti, M., P. Karnopp, H.M. Soares and S.A. Furlan, 2004. Evaluation of *Pleurotus ostreatus* and *Pleurotus sajor-caju* nutritional characteristics when cultivated in different lignocellulosic wastes. Food Chem, 88: 425-442.
11. Ishida, N., S. Saitoh, T. Ohnishi, K. Tokuhiro, E. Nagamori, K. Kitamoto and H. Takahashi, 2006. Metabolic engineering of *Saccharomyces cerevisiae* for efficient production of pure L-(+)-lactic acid. Appl. Biochem. Biotechnol., 129: 795-807.
12. Gunju, R.K., P.J. Vithayuthil and S.K. Murthy, 1990. Factors influencing production of cellulases by *Chaetomium thermophile* var. coprophile. Indian J. Exp. Biol., 28: 259-264.
13. Gupte, A. and D. Madamwar, 1997. Solid state fermentation of lignocellulosic waste for cellulose and â-glucosidase production by co-cultivation by *Aspergillus ellipticus* and *Aspergillus fumigatus*. Biotechnol. Prog., 13: 166-169.
14. Mukhopadhyey, S. and B. Nandi, 1999. Optimization of cellulose production by *Trichoderma reesei* ATTCC 26921 using a simplified medium on water hyacinth biomass. J. Sci. Ind. Res., 58: 107-111.
15. Awang, M.R., W.B.W. Husin, T. Osman, M.S. Mahmud, N. Zainal, Z.U.W. Mahmod, H.H. Muttat and Y. Atan, 1994. Evaluation palm empty fruit bunch and its fermented products as for ruminant animal by nutritional values characterization and in-vitro dry matter digestibility. A report of the seminar at Malaysian Institute for Nuclear Technology.7-9 Nov.
16. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem., 193: 265-275.
17. Miller, G.L., 1959. Use of dinitrosalisylic acid for determination of reducing sugar. Annual Biochem., 31: 426-428.
18. Leng, R.A., 2001. In Final Consulting Report, Dairy & Beef Cattle Nutrition Specialist, BLRI, Savar, Dhaka.
19. Mane, V.P., S.S. Patil, A.A. Syed and M.M. Baig, 2007. Bioconversion of low quality lignocellulosic agricultural waste into edible protein by *Pleurotus sajor-caju* (Fr.) Singer. J. Zhejiang Univ. Sci. B, 8: 745-751.
20. Smith, D.C. and T.M. Wood, 1991. Xylanase production by *Aspergillus awamori*, development of a medium and optimization of the fermentation parameters for the production of extracellular xylanase and â-xylosidase while maintaining low protease production. Biotechnol. Bioeng, 38: 883-890.
21. Martinez, A.T., M. Speranza, F.J. Ruiz-Duenas, P. Ferreira, S. Camarero, F. Guillen, M.J. Martinez, A. Gutierrez and J.C. del-Rio, 2005. Biodegradation of lignocellulosics: microbial, chemical and enzymatic aspects of the fungal attack of lignin. Int. Microbiol., 8: 195-204.
22. Alam, N., R. Amin, A. Khan, I. Ara, M.J. Shim, M.W. Lee and T.S. Lee, 2008. Nutritional Analysis of Cultivated Mushrooms in Bangladesh- pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus florida and Calocybe indica. Mycobiology, 36(4): 228-232.
23. Khan, M.A., S.M.R. Amin, M.N. Uddin, M. Tania and N. Alam, 2008. Comparative Study of the Nutritional Composition of Oyster Mushrooms Cultivated in Bangladesh. Bangladesh J. Mushroom., 2(1): 9-14.