J. Appl. Environ. Biol. Sci., 4(4)95-101, 2014

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Single Cell Protein Production from Pretreatment Wheat Straw by Pleurotus Ostreatus Var Florida in Solide-State Fermentation

[1]*Ahmadi, Ali-Reza; [3]Arash, Mehri; [2]Ghourchian, Hedayatollah; [3]Lamerad, Behzad

[1]Dep. of Biomedical Sciences, Alzahra University, Tehran, Iran
[2]Research Center for Biochemistry and Biophisic, Tehran University
[3]Dep. Of Biology, Payam Nour Uiniversity, Tehran Shargh Center

Received: December 5 2013
Accepted: March 9 2014

ABSTRACT

With the increasing world population, the necessity of using lingo-cellulosic wastes for production of single cell protein (SCP) as animal feed seems to be important. Microbial protein was produced by physical, chemical and biological treatment of wheat straws under solid state fermentation.
The effects of alkali and heat pretreatment of wheat straw and urea concentration in solid state fermentation was studied. Pretreated wheat straw was fermented with *Pleurotus ostreatus var florida* and then protein and glucose concentration was analyzed. Fermentation of 2% NaOH solution at 100 ^{0}C on 0.3 g/l urea in mandels medium by inoculation with wheat grain based inoculate with *Pleurotus ostreatus var florida* for 30 days have a product containing 63.24% protein. .

KEY WORDS: lingocellulosic wastes, wheat straw, *Pleurotus ostreatus var florida*, Cellulose, alkali, pretreatment

1.INTRODUCTION

A growing global need of protein necessitates the use of unconventional protein sources such as cultured microbial biomass, generally referred to as single cell protein (SCP) [1].

Lignocellulosic materials such as wood and straw are renewable resources [2]. These vast resources are the potential source of SCP [3].

Lignocelluloses are generally composed of 30-56% cellulose, 10 -24% or more hemicelluloses, 3-30 % lignin and 3-7.2% protein. The major limitations of straw as an animal feed are low digestibility and low protein content [4]. Close associations of cellulose with lignin prevents full microbial digestion of straw in the rumen [1]. Efforts have been made to increase the feed value of cereal straws by chemical and physical treatments [4]. Recently agricultural wastes containing lignocelluloses can be upgraded by solid state fermentation with *Pleurotus ostreatus*. Solid state fermentation (SSF) requires less energy input than liquid fermentation [5].

The edible mushroom *P. ostreatus* has been shown to degrade lignin and generally cultivated on wheat straw [5].

This fungus synthesizes hydrolytic and oxidative enzymes. The major hydrolytic enzymes are endo-1,4-β – D–glucanase (EC 3.2.1.4), Exo-1,4 – β – D-glucanase (EC 3.2.1.91) and Xylanase (EC 3.2.1.8) [6].

There are three extracellular enzymes that are essential for lignin degradation : two glycosylated heme-containing peroxidase, lignin peroxidase (EC 1.11.1.14) and Mn–dependent peroxidase (EC 1.11.1.13) and a copper – containing phenol oxidase, laccase (EC 1.10.3.2) [6,7].

The mushroom (*Pleurotus florida*) is widely used in the delignification of several substrates to improve the digestibility and feeding value of wheat straw in most cases with parallel protein enrichment [8, 9, 10, 11, 12].

The results of Kutlu *et al.*,[8] indicated that *Pleurotus florida* inoculation increased digestibility and nutrient content of wheat straw (P<0.05). *Pleurotus florida* increased digestibility, crude protein, crude oil, nitrogen-free extract about 22, 60, 20, 5%, respectively, while reducing crude fiber content about 16%. Urea supplementation inhibited mushroom growth (fruiting Body) on the wheat straw.

There have been studies on the amino acid and protein content of fruit bodies of *Pleurotus species* [13]. Mycelium like the fruiting body has been shown to be valuable nutritionally as a source of amino acids and B complex vitamins. They can therefore serve as a protein supplement for human beings or as an animal feed [14]. Wheat straw is the most widely used natural substrate for *P. ostreatus* (16 % glucose, 11 % xylose together with other monosaccharide's).

This study dealt with the cultivation *of P. ostreatus var florida* on wheat straw. The objective of the study was to obtain the maximum yield of SCP from pretreated wheat straws.

***Corresponding Author:** Ahmadi AR, Biomedical Sciences Department, Alzahra University, Tehran, Iran.
Tel: +98-21- 85692803 Fax: +98-21- 88049809 Email: aahmadi1970@gmail.com

2. MATERIALS AND METHODS

This study carried out on department of biomedical sciences, Alzahra University and also on institute of Biochemistry and Biophysic, Tehran University.

2.1. Microorganisms

Non-toxic fungi *P. ostreatus var florida* were kindly provided by Dr. Mohammadi Goltape, at Tarbiat Modares University , Agricultural Collage , Fungi collection. *P. ostreatus var florida* culture was maintained by sub-culturing on Potato-dextrose agar (PDA) medium.

2.2. Pretreatment of wheat straw

Wheat straw was obtained from a farm in a local harvest in Karaj. It was milled and pretreated during 30 min. at 100 °C (treatments 1, 3, 5) or 120 °C (treatments 2, 4, 6) with a NaOH 1% or 2% solution in an autoclave. After cooling down the pretreated wheat straw filtered and washed with water free of alkali, dried at 80°C and weighted (Table 1).

Table 1 Pretreatment Conditions

Sample	NaOH %	Temperature	Time
1	2 %	100 °C	30 min.
2	2 %	120 °C	30 min.
3	1 %	100 °C	30 min.
4	1 %	120 °C	30 min.
5	0 %(distilled water)	100 °C	30 min.
6	0 % (distilled water)	120 °C	30 min.

2.3 Inoculums

The method of Surinder [15], was used for preparation of *P. ostreatus var florida* inoculum.1 Kg wheat grains per 1.5 liter water were boiled for 15 min. and maintained in boiled water for 15 min. The excess water drained off and the grains were aerated, Gypsum and Calcium carbonate (1.2 gr. and 3 gr. respectively) were mixed with the boiled grains.

The 0.5 liter bottles were filled with the 250-300 gr. grains mixture and were autoclaved at 121°**C** for 2 hr, cooled and inoculated separately with a small amount of *P. ostreatus var florida* from PDA slants and incubated at 22 -24 °**C** for 30 days in the dark room until thick white growth of the grains was observed.

2.4 Effects of different media

Mendel's medium as described by Mandels and Weber [16] as essential media has been used. The media contained (per liter of distilled water): $(NH_4)_2$ SO4 1.4 g, K_2HPO_4 2 g, $CaCl_2$ 0.3 g, Mg $SO_4.7H_2O$ 0.3 g, protease peptone 1 g, yeast extract 0.25 g, $FeSO_4.7H_2O$ 5 mg, $MnSO_4.7H_2O$ 1.6 mg, $ZnSO_4$. $7H_2O$ 1.4 mg, $CoCl_2.6H_2O$ 2 mg. with 0.3g/l urea (medium A1) and $(NH_4)_2$ SO4 1.4 g, K_2HPO_4 2 g, $CaCl_2$ 0.3 g, Mg $SO_4.7H_2O$ 0.3 g, protease peptone 1 g, yeast extract 0.25 g, $FeSO_4$. $7H_2O$ 5 mg, $MnSO_4.7H_2O$ 1.6 mg, $ZnSO_4.7H_2O$ 1.4 mg, $CoCl_2.6H_2O$ 2 mg. with 2.1 g/l urea (medium A2) and distilled water only without Mendel's medium (A3) were compared for effects on the efficacy of SCP production.

2.5 Solid state fermentation (SSF)

20 grams of pretreated wheat straw in a 500 ml bottles were supplemented with 100 ml medium A1 or medium A2 and autoclaved at 100 °**C** (for samples of 1, 3, 5) and 120°**C** (for 2, 4, 6 samples) at 1 at for 15 min., cooled, inoculated from the inoculums cultures (5% of substrate weight) and incubated in the dark room at 25 °**C** for 28 days. The cultures in glass bottles were checked for mycelia growth. The solid straw residues which are colonized by the fungus were collected, dried at 60 °**C** for 24 hrs. Dried samples were powdered then samples were soaked with 1 ml of NaOH 1N at 100 °**C** and boiled for 10 min, the mixture was centrifuged at 5000 g rpm for 30 minute.

2.6 Estimation of protein and Glucose

The protein was estimated in the supernatant by Bradford assay [17] and Lowry method using bovine serum albumin as the standard protein [18]. Reagent C was prepared by adding reagent A (2% Na2CO3 in 0.1N NaOH) and reagent B (50mg CuSO4 and 100mg potassium sodium tartrate in 10 ml) in a ratio of 50:1 The sample (0.1 ml) was added to 1ml of reagent C. After 10 min, 0.1 ml of Folin reagent (diluted 1:1 with deionized water) was added and mixed. After 30 min, 3.8 ml of distilled water was added and the absorbance was measured at 660 nm. Glucose content of samples was analyzed by Glucose oxidase assay [19].

3. RESULTS

3.1. Bioconversion of straw and protein yield

When *P. ostreatus var florida* was grown on wheat straw substrate utilized depended on the Nitrogen content in media, concentration of NaOH and heat temperature and so SCP production was varied. In table 2 the

protein and glucose contents on dry basis are reported. SCP production of pretreated wheat straw by *P. ostreatus var florida* varies from 13.2 % (by 5A3 test) to 63.24 % (by 1A1 test) .In particular 1A1 shows the highest content of protein.

3.2 The effects of heat and NaOH pretreatment

Pretreatment with 1% NaOH at 120 °C produced 63.24% protein. The results showed that the temperature and NaOH concentration affected each other (fig.1). Generally pretreatment with NaOH and heat yielded better results perhaps because during this step the lignocellulosic material is heated to break down the lignin and carbohydrate structure, solubilize most of the hemicelluloses and make the cellulose fraction accessible to cellulase enzymes. This heating is done directly with steam and also a catalyst such as sodium hydroxide [20]. NaOH alkali pretreatment improves the movement of molecules in the pores of the substrate due to the substrate swelling and hydrolysis of lignin and hemicelluloses [21]. Fig.1 compared the results.

3.3The effect of urea content in media

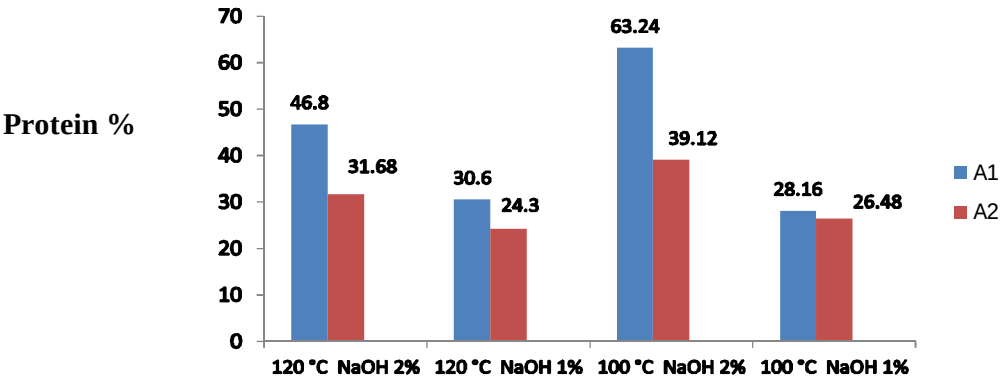
In order to determine the effects of urea concentration on the nutrient content in Mendel's medium, SCP production was analyzed. The results with respect to dry matter content (25 mg dry basis) showed that the higher SCP yield corresponded to the lower urea concentration on the medium that means 0.3 g/l urea (medium A1) is better than medium A2 (2.1 g/l urea in medium) and A3 (without urea and medium).The elemental analysis results with three types of medium (0.3 g/l urea ,medium A1) ,(2.1 g/l urea in medium,A2), (without urea and medium, distilled water,A3), are shown in table 2.

The SCP yield of P. ostreatus var florida was markedly affected by urea concentration. This could be due to the lack of growth of mushroom in the alkali condition [21]. Günay reported that the optimum pH for the growth of *P. ostreatus* is 6.4 – 7.0 [22].

Table 2. Protein and glucose contents in 25 mg dry weight

Sample	Protein concentration *mg/ml*	Glucose concentration *mg/ml*	Protein %	Glucose%
1A1 NaOH 2% 100 °C	5.96	0	63.24	0
1A2 NaOH 2% 100 °C	2.43	2.37	39.12	9.48
2A1 NaOH 2% 120 °C	3.39	3.4	46.8	13.6
2A2 NaOH 2% 120 °C	1. 66	2.74	31.68	10.96
3A1 NaOH 1% 100 °C	1.34	3.63	28.16	14.52
3A2 NaOH 1% 100 °C	1.20	0.85	26.48	3.4
4A1 NaOH 1% 120 °C	1.56	1.12	30.6	4.48
4A2 NaOH 1% 100 °C	1.02	0	24.3	0
5A1 NaOH 0% 100 °C	1.11	3.11	25.28	12.44
5A3 NaOH 0% 100 °C	0.368	4.62	13.2	18.48
6A1 NaOH 0% 120 °C	3.44	0.68	47.18	2.72
6A3 NaOH 0% 120 °C	3.67	1.22	48.86	3.88

Fig1. The effects of heat (100 °C, 120 °C) and NaOH (1%, 2%) pretreatment in media A1 (Mendel's medium with 0.3 g/l urea) and A2 (Mendel's medium with 2.1 g/l urea)



3.4 Discussion

Lignocellulose is the only direct source of carbon for wood-inhabiting species of white-rot fungi, and its capture and degradation are thus of crucial important for the establishment and development of fungal mycelia and reproduction [18]. Lignocellulosic agricultural residues enriched in microbial protein by SSF may be used as animal feed [3].

In this study wheat straw that is the best substrate for *P. ostreatus* var *florida* [23, 24] was used by SSF. Pretreatment methods used to increase the protein production. Both heat and NaOH pretreatment have studied for many years and the positive effects of these methods have been shown [25, 26, 27, 28].

We found that there was a negative correlation between heat temperature and NaOH concentration .As the heat temperature increased (120 °C), the low NaOH concentration (1 %) yield more SCP (fig.1).

Sodium hydroxide (NaOH) has been extensively studied for many years, and it has been shown to disrupt the lignin structure of the biomass, increasing the accessibility of enzymes to cellulose and hemicellulose [29–30]. The effect of alkaline pretreatment depends on the lignin content of the materials [31, 29]. Alkali pretreatment processes utilize lower temperatures and pressures than other pretreatment technologies [32].

NaOH can increase the internal surface of cellulose and decrease the degree of polymerization and crystallinity, which accelerates the lignin structure disruption [33-34].

The hydrothermal pretreatment method subjects the material to high pressures and temperatures for a short duration of time after which it rapidly depressurizes the system, disrupting the structure of the fibrils. The disruption of the fibrils increases the accessibility of the cellulose to the microbial enzymes during hydrolysis [35].

It seems that these factors have positive effects alone but together can cause new results. Heat temperature may help to release different phenolices for their metabolic conversion. Balazs [36] has also reported heat treatment of corn cobs and straw for getting better yields in *P. ostreatus* and *P. florida* [37].

The various heat treatments of the wheat straw before inoculation affected the bacterial populations and the substrate and to produce extracellular lignocellulolytic enzymes [7].

The effect of alkali pretreatment on cellulase hydrolysis of wheat straw was studied by Carrillo [21]. They found that alkali pretreatment increase the sugars yield obtained comparing untreated and treated wheat straw about 3 times.

Pamment [38] reported that dilute sodium hydroxide pretreatment effects a major improvement in sawdust degradability by *Chaetomium cellulolyticum.* The product from fermentation of the pretreatment solids may have some value as a feed for ruminants and this product appears to be a better source of SCP [38].

SCP yield of *P. ostreatus var florida* was studied on 3 type media, i.e. A1 (Mendel's with 0.3 g/l urea), A2 (Mendel's with 2.1 g/l urea) and A3 (distilled water). Data in table 2 show that A1 was the best tested media for *P. ostreatus var florida.* The highest in 1A1 test (63.24%), this was followed by 6A3 test (48.86 %), 6A1 (47.18%) and 2A1 (46.80% of dry basis).

This medium A1 is suitable for fungal growth and could also be used as growth stimulator for *P. ostreatus var florida*. This data show that when the urea is high on the media, the growth of fungus decreased.

Concentration of Nitrogen had considerable influence on yield as well as on the protein production by *P. flabellatus.* Yield of the mycelium increased from 5.75 to 12.87 g/l of the medium as the ammonium citrate concentration was increased from 0.25 to 2 g/l [14].

The effect of varying Nitrogen concentrations on enzyme production by *P. dryinus* IBB 903 and *F. trogii* IBB 146 was studied. By gradually increasing the Nitrogen concentrations from 0 to 10-40 mM, fungal protein content in the final biomass was about doubled [6].

In the literature contradictory evidence exists for the effects of the nature and concentration of the Nitrogen source on lignolytic enzyme production.

While high Nitrogen media gave the highest laccase activity in *L. edodes, Rigidoporus lignonus* and *Trametes pubescens* , nitrogen-limited conditions enhanced the production of the enzyme in *Pycoporus* , *P. sanguineus* and *Phlebia radiata.*

Tekere [39] showed that some *Trametes species* produced the highest manganese peroxidase (MnP) activities in a medium containing high Carbon and low Nitrogen conditions. Kachlishvili [6] also showed that sample fungus grew well and produced significant enzyme activities when no Nitrogen source was present in the medium because of the existence of Nitrogen in the

lignocellulosic substrate and we know that the C/N ratio in wheat straw is high (81.08 %,Table 3). These findings are in agreement with our results about 6A3 test that produced high yield of protein (48.86 %).

Table 3.Carbon and nitrogen content in some lignocellulosic wastes

Material	(*C* %)	(*N* %)	*C/N*
Sorghum straw	36/54	1/45	25/13
Peanut straw	27/04	0/76	35/2
Soybean straw	36/96	0/54	71/76
Wheat straw	42/16	0/52	81/08

The results also remind clear that A2 medium is not suitable for *P. ostreatus var florida* cultivation. Kutulu reported that *P. florida* did not grow on 2% urea treated straw. They suggested that *P. florida* and urea treatment could not be combined in terms of the improving feeding value of straw [22].

In summary of this experiment ,the results suggest that we can produce much more protein yield of wheat straw by pretreatment with heat , NaOH and *P. ostreatus var florida* but use of this product for animal feeding needs more investigations like amino acid analysis .

Acknowlegment

Special thanks go to Department of Biomedical Sciences, Alzahra University and Institute of Biochemistry and Biophysics, University of Tehran, for financial support of this research.

REFERENCES

1. **Thanikachalam,A.,Rangarajan,M.** 1986.Protein enrichment of rice straw by fermentation with cellulomonas sp. *Agricultural wastes*;15:215-224.
2. **Sun,X.F.,Xu,F.,Sun,R.C.,Geng,Z.C.,Fowler,P.,Baird,M.S**. 2005.Characteristics of degraded hemicellulosic polymers obtained from steam exploded wheat straw. *Carbohydrate Polymers*; 60:15-26.
3. **Tengerdy,R.P., Szakacs,G**.2003.Bioconversion of lignocellulose in solid substrate fermentation. *Biochemical Engineering Journal*; 13:169-179.
4. **Han,Y.W.** 1975.Microbial fermentation of rice straw: nutritive composition and in vitro digestibility of the fermentation products. *Applied Microbiology*; 29:510-514.
5. **Kerem, Z., Friesem, D., Hadar, Y.** 1992.Lignocellulose degradation during solid-state fermentation: *Pleurotus ostreatus* versus *Phanerochaet chrysosporium*. *Applied and Environmental Microbiology*; 58(4):1121-1127.
6. **Kachlishvili, E., Penninckx, M. J., Tsiklauri, N., Elisashvili, V.** 2005. Effect of nitrogen source on lignocellulolytic enzyme production by white-rot basidiomycetes under solid-state cultivation. *World Journal of Microbiology and Biotechnology;* 22(4): 391-397.
7. **Velàzquez,M.A.,Farnet,A.M.,Ferré,E.** 2004.Variations of lignocellulosic activities in dual cultures of *Pleurotus ostreatus* and *Trichoderma longibranchiatum* on unsterilized wheat straw. *Mycologia*; 96(4):712-719.
8. **8. Kutlu, H.R., Go¨ rgu¨ lu¨, M., Baykal, L. & O¨ zcan, N. 2000.** Effects of *Pleurotus florida* inoculation or urea treatment on feeding value of wheat straw. *Turkish Journal of Veterinary and Animal Science.* 24: 169–175.
9. **Valmesada, M., Almendros, G. and Martinez, A.T.** 1991. Substrate dependent degradation patterns in the decay of wheat straw and beech wood by ligninolytic fungi. *Appllied Microbiol*ogy 33: 481-484.
10. **Hadar, Y., Kerem, Z. and Gorodecki, B.** 1993. Biodegradation of lignocellulosic agricultural wastes by *Pleurotus ostreatus. Journal of Biotechnology* 30: 133-139.
11. **Streeter, C.L., Conway, K.E., Horn, G.W. and Mader, T.L. 1982.** Nutritional evaluation of wheat straw incubated with the edible mushroom *Pleurotus ostratus. Journal Animal Sciences* 54: 183-188.
12. **Adamovic, M., Grubic, G., Milenkovic, I., Jovanovic, R., Protic, R., Sretenovic, L. & Stoicevic, L.J.** 1998. The biodegradation of wheat straw by *Pleurotus ostreatus* mushrooms and its use in cattle feeding. *Animal Feed Science and Technology* 71: 357–362.
13. **Yildiz, A., Karakaplan, M., Aydin, F.** 1998. Studies on *Pleurotus ostreatus* (Jacq.ex Fr.) Kum.Var.Salignus (pers. ex Fr.) konr.et Maubl.: cultivation, proximate composition, organic and mineral composition of carpophores. *Food Chemistry*; 61(1/2):127-130.

14. **Srivastava, H.C., Bano, Z.** 1970. Nutrition requirements of *Pleurotus flabellatus*. *Applied Microbiology*; 19:166-169.

15. **Surinder S. K Suman K. D.** 1987. Biological conversion of paddy straw into feed. *Biological Wastes*, 22 (1): 11–21.

16. **Mandels, M. and J. Weber,** 1969. The production of cellulases. *Advances in Chemistry Series*, 95: 391-414.

17. **Bradford, M.M.** 1976.A rapid and sensitive method for the quantitation of the microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*; 72:248-254.

18. **Lowry OH, Rosebrough NJ, Farr AL, Randall RJ.** 1951. Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*; 193 (1): 265–75.

19. **Ghalambor, M.A.,** 1971. Enzymatic determination of blood glucose. *Experimental Biochemistry* 2:111-115. Shiraz University Publication.

20. **Nguyen; Quang A.** 1999. Tower reactors for bioconversion of lignocellulosic material. *US Patent* No. 5,888,806.

21. **Carrillo, F., Lis, M.J., Colom, X., López-esas, M., Valldeperas, J.** 2005. Effect of alkali pretreatment on cellulase hydrolysis of wheat straw: kinetic study. *Process Biochemistry*; 40(10):3360-3364.

22. **Kutlu,H.R.,Görgülü,M.,Bykal,L.,Ozcan,N.** 2000.Effects of Pleurotus florida inoculation or urea treatment on feeding value of wheat straw. *Turkish Journal of Veterinary and Animal Sciences;* 24:169-175.

23. **Baldrian,P.,Valášková,V.,Merhautová,V.,Gabriel,J.** 2005. Degradation of lignocellulose by *Pleurotus ostreatus* in the presence of copper, manganese, lead and zinc. *Research in Microbiology*; 156:670-676.

24. Ahmadi A.R., Ghoorchian H., Haji Hosaini R. and Khanifar J. 2010. Determination of the Amount of Protein and Amino Acids extracted from the Microbial Protein (SCP) of Lignocellulosic Wastes, *Pakistan Journal of Biological Sciences*, **13 (8): 355-361.**

25. **Philippoussis, A., Zervakis, G., Diamantopoulou,P.** 2001. Bioconversion of agricultural lignocellulosic wastes through the cultivation of the edible mushrooms *Agrocybe aegerita,Volvariella volvacea* and *Pleurotus spp*. *World Journal of Microbiology and Biotechnology*; 17:191-200.

26. **Sun, R. Lawther J. M. and Banks W. B**. 1995. Influence of alkaline pre-treatments on the cell wall components of wheat straw. *Industrial Crops and Products***,** 4 (2): 127–145.

27. **Zhao, Y. Wang, Y. Zhu, J. Y. Ragauskas, A. and Deng, Y.** 2008. "Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature," ***Biotechnology and Bioengineering*****;** 99 (6):1320–1328.

28. Khanifar, J., Ghoorchian, H., Ahmadi, A. R. and Hajihosaini, R. 2011. Comparison of essential and non essential amino acids in the single cell protein (scp) of white rot fungi from wheat straw. **.** *African Journal of Agricultural Research;* 6(17): 3994-3999**.**

29. **MacDonald, D. G. Bakhshi, N. J. Mathews, F. Roychowdhury, A. Bajpai, P. and Moo-Young, M.** 1983. Alkaline treatment of corn stover to improve sugar production by enzymatic hydrolysis. *Biotechnology and Bioengineering***, 25(8):** 2067–2076.

30. Zhu, J. Wan, C. and Li, Y. 2010. Enhanced solid-state anaerobic digestion of corn stover by alkaline pretreatment. *Bioresource Technology***,** 101(19): 7523–7528.

31. Sun, YE. and Cheng, J. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, 83(1): 1–11.

32. Yang B. and Wyman, C. E. 2008. Pretreatment: the key to unlocking low-cost cellulosic ethanol. *Biofuels, Bioproducts and Biorefining***,** 2 (1): 26–40.

33. Taherzadeh, M.J., Karimi, K., 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *International Journal of Molecular **Sciences***; 9:1621–1651.

34. **Alizadeh, H., Teymouri, F., Gilbert, T.I., Dale B.E.,** 2005. Pretreatment of switchgrass by ammonia fiber explosion (AFEX). *Applied Biochemistry and Biotechnology*; 124 (1): 1133-1141.

35. Ballesteros, I. Oliva, J. M. Negro, M. J. Manzanares, P. and Ballesteros, M. 2002. Enzymic hydrolysis of steam exploded herbaceous agricultural waste (Brassica carinata) at different particule sizes, *Process Biochemistry*, 38 (2): 187–192.

36. **Balazs, S.** 1995. Mushroom cultivation: the past and present of oyster

mushroom. *Kert Szo leísz*, 34: 8-17.

37. **Upadhyay, R.C., Vijay, B., Verma, R.N.** 1996. Use of industrial tea leaf waste for cultivation of Oyster mushroom. *Mushroom Biology and Mushroom products* 423-428.
38. **Pamment, N., Moo-Young, M., Hsieh, F.H., Robinson, C.W.** 1978. Growth of *Chaetomium cellulolyticum* on alkali-pretreated hard wood saw dust solids and pretreatment liquor. *Applied and Environmental Microbiology*; 36(2):284-290.
39. **Tekere M, Zvauya R, Read JS** .2001. Ligninolytic enzyme production in selected sub-tropical white rot fungi under different culture conditions. *Journal of Basic Microbiology*; 41:115–129.