*Cell. Mol. Biol.* 2014; 60 (5): 2-6 Published online December 24, 2014 (http://www.cellmolbiol.com) Received on May 27, 2014, Accepted on August 11, 2014. doi : 10.14715/cmb/2014.60.5.2



# Bioremediation of vegetable and agrowastes by *Pleurotus ostreatus*: a novel strategy to produce edible mushroom with enhanced yield and nutrition

V. K. Singh<sup>1</sup> and M. P. Singh<sup>2</sup>

<sup>1</sup> Department of Zoology, Banaras Hindu University, Varanasi – 221005, India <sup>2</sup> Centre of Biotechnology, University of Allahabad, Allahabad – 211002, India

**Corresponding author:** M. P. Singh Department of Zoology, Banaras Hindu University, Varanasi – 221005, India. Email: mpsingh.16@gmail. com

#### Abstract

*Pleurotus ostreatus* was grown on paddy straw as well as other vegetable and agricultural wastes i.e. pea pod shell, cauliflower leaves, radish leaves and brassica straw in various combinations with paddy straw. The mushroom did not grow on the vegetable wastes separately. The cumulative yield and biological efficiency of the edible oyster mushroom *P. ostreatus* grown on substrate containing paddy straw in various combinations with different vegetable wastes i.e. 20% and 30 % vegetable wastes mixed with 80% and 70% (w/w) of paddy straw was found to be better, when compared with yield and biological efficiency obtained on paddy straw (100%) alone. The protein content and six essential amino acid contents (Leu, Ile, Val, Thr, Met, Phe) showed a significant increase and total sugar and reducing sugar contents showed decrease in the mushroom fruit bodies grown at different combinations of vegetable wastes with paddy straw as compared to paddy straw alone. However, there was not any significant change in moisture content of mushroom cultivated on different groups of wastes. Hence, results of this investigation suggest that the vegetable wastes which are generally left to rot *in situ* in many cities and villages causing outbreak of diseases can be bioremediated by edible mushroom *P. ostreatus*. The added advantage is that we get edible mushroom fruit body with improved nutrition.

Key words: Paddy straw, Biological efficiency, nutritional analysis, Pleurotus ostreatus.

#### Introduction

Huge quantities of agricultural, vegetable, lignocellulosic and other organic wastes are generated annually through the activities of agricultural, forest, wood and food processing industries (1, 2, 3). India is one of the major producers of cereals, fruits and vegetables which lead to generation of heavy amount of agro and vegetable wastes. Efficient management of these wastes can help in preserving vital nutrients of our foods and feeds, and bringing down the cost of production of processed foods, besides minimizing pollution hazards. Recycling of fruit and vegetable waste is one of the most important means of utilizing it in a number of innovative ways yielding new products and meeting the requirements of food, feed and energy. These wastes can be utilized for nutritionally rich food production through cultivation of oyster mushroom (4). Mushrooms are very useful in bioconversion of organic wastes in valuable food, pharmaceuticals production, bioremediation of xenobiotics and recalcitrant substances. Besides, mushroom can be alternative food source which do not depend on agricultural land which is dwindling due to population explosion.

*Pleurotus ostreatus* is an edible mushroom which is commonly known as oyster mushroom. The oyster mushroom confers advantages over other mushrooms for its ability to grow on non-fermented lignocellulosic wastes and produce in turn fruit bodies with higher nitrogen content (3). Due to several other qualities, oyster mushrooms are studied in different parts in the world. This mushroom have high gastronomic value and require shorter growth time as compared to other edible mushrooms; they also demand few environmental controls, their fruiting bodies are not very often attacked by diseases and pests and they can be cultivated in a simple and cheap way (5, 6).

Various workers have reported wide variations in the nutritional and medicinal attributes of mushroom. The difference may be due to variation in the genetic makeup, substrates, cultivation technologies and conditions at the stages of harvest as well as post harvest, which affect the composition. In the present investigation experimental studies on bioremediation of the wastes were performed to grow mushroom. We achieved the best out of wastes through production of edible mushroom with enhanced yield and nutrition.

# Materials and methods

To achieve the objectives of work one of the well studied edible mushroom *Pleurotus ostreatus* was grown on four selected vegetable wastes viz. brassica straw (BS), cauliflower leaves (CF), radish leaves (RL) and pea pod shell (PS) in various combinations with paddy straw (PS). The mushroom was cultivated during winter months (December to February) of year when temperature varied from 10 to 25° C in northern part of India.

## The culture and its maintenance

The pure culture of *P. ostreatus* was procured from National Research Centre on Mushroom, Solan (HP), India and maintained on malt extract agar (MEA) medium at temperature  $25 \pm 2^{\circ}$ C and pH 6 - 6.5 and subcultured at periodic interval of three weeks.

#### Collection of agricultural and vegetable wastes

Five different agrowastes were collected from the different agricultural fields, households and Sabji mandi of district Jaunpur, Uttar Pradesh, India. Details of methods of collection of wastes have been described elsewhere (4).

## Preparation of spawn

Spawn is referred to as the vegetative mycelium of the fungus, which is grown on cereal grains. Wheat grain spawn was prepared by the following method of Singh *et al* (3) which is well established in author's laboratory and has been detailed in the paper (4).

#### **Preparation of substrate**

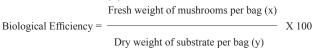
The collected vegetable wastes i.e. cauliflower leaves, radish leaves and pea pod shell were spread in open area for sun drying for 30-40 days. These dried substrates were autoclaved at the temperature 121°C and pressure 15 psi for 40 minutes. Vegetable wastes were used separately as well as in various combinations with paddy straw for cultivation experiment. The paddy straw before mixing of vegetable wastes was completely dipped in water (50 litres for every 10 kg dry chopped paddy straw) in a drum or big bucket and was allowed to stay in water for 12 hours. After that excessive water was drained out, the paddy straw was again completely dipped in hot water (temperature 70-80°C) for an hour. The excess water was drained out and paddy straw was evenly spread on platform and mixed with dried autoclaved vegetable wastes (radish leaves, cauliflower leaves, brassica straw and pea pod shell) in two combinations i.e. paddy straw and vegetable wastes (70 and 30%; 80 and 20%, respectively, w/w).

#### Spawning

Spawning is the process of mixing spawn in the sterilized substrates. 3% wet weight basis spawn grain was mixed with the substrate and filled into polythene bags. The mouth of the bag was tied with rubber band and 12 holes of about 1cm diameter were made; two at each corner of the base, four each on the broader area and one each on the narrow, rectangular side to drain out extra water and for proper aeration. Five bags of each combination of substrates (equivalent to 300 g of dry substrate) spawned with *Pleurotus ostreatus* were filled and kept in mushroom house on the iron racks on the bricks.

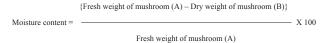
## **Biological efficiency**

The five bags for each substrate were used for evaluation of yield performance and biological efficiency of *P. ostreatus* kept in mushroom house under *in vivo* condition. The yield was expressed as of fresh fruit bodies produced per bag. Biological efficiency (B.E.) was calculated as the percentage conversion of dry substrates to fresh fruit bodies (4).



## Moisture content

It was done by picking fresh fruit body of the *P. os-treatus* and dried in hot air oven at 60°C for 15 hours.



#### Sugar, amino acids and protein estimation

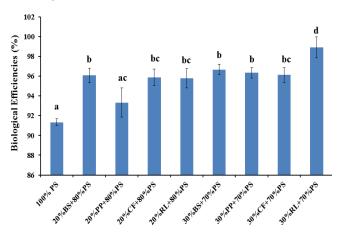
Total sugar estimation was done by using sulphuric acid phenol method (8) and the reducing sugar estimation was done by Dinitro salicylic acid method of Sumner and Graham (9) with some modifications. Amino acids were estimated by following the method of Moore and Stein (10). Protein estimation was done by the method of Lowry *et.al* (11).

#### Statistical analysis

The data were presented as mean  $\pm$  standard error of mean (n=5). Statistical significance was analyzed by ANOVA following Duncan's multiple comparison test (P<0.05). In the given figures the mean bar bears same superscripts has not significant differences.

## Results

The edible mushroom *Pleurotus ostreatus* was grown on radish leaves, pea pod shell and cauliflower leaves separately. The mushroom failed to grow on these vegetable wastes. However, when the mushroom was cultivated on paddy straw and paddy straw in combinations with vegetable wastes, the fructification occurred.

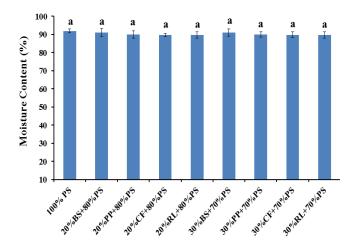


**Figure 1.** Biological efficiency of *P. ostreatus* during its cultivation on different combinations of paddy straw with vegetable wastes.

Figure 1 shows the biological efficiency of *Pleurotus ostreatus* on different agro and vegetable wastes. Paddy straw and vegetable waste combinations supported higher bioefficiency than paddy straw alone. 70% paddy straw and 30% vegetable wastes supported maximum mushroom yield and bioefficiency followed by 80% paddy straw and 20% vegetable wastes.

The yield of first flush fruit bodies was more than second and subsequent flushes. Maximum biological efficiency was reported on 30% radish leaves and 70% paddy straw.

The moisture content of *Pleurotus ostreatus* on different agro and vegetable wastes is presented in figure 2. There was not any major difference in the moisture content of mushroom grown on different combination of paddy straw with vegetable wastes. The moisture content of fresh mushroom fruit bodies grown on various substrates ranged from 89.7 to 91.95%.



**Figure 2.** Moisture content of *P. ostreatus* during its cultivation on different combinations of paddy straw with vegetable wastes.

The observations of protein, total sugar and reducing sugar is presented in figure 3. The protein content of mushroom fruit bodies ranged from 42.1 mg to 53.9 mg per 100 mg of dried fruit bodies. Protein content of mushroom fruit bodies harvested on paddy straw alone was low which increased when harvested on other wastes in combination with paddy straw. The mushroom grown on 70% paddy straw and 30% vegetable wastes showed more protein content than grown on 80% paddy straw and 20% vegetable waste combinations.

However, the total sugar and reducing sugar content decreased in all waste combinations as compared to paddy straw alone. Minimum sugar content was recorded in fruit bodies cultivated on 30% radish leaves and 70% paddy straw combinations. Maximum total and reducing sugar was observed in mushroom fruit bodies produced on paddy straw alone. Total sugar content recorded in the mushroom fruit bodies ranged from 36.3 mg to 48 mg per 100 mg of dried mushroom.

The amino acid content of *P. ostreatus* grown on paddy straw alone and combination of paddy straw and vegetable wastes are given in figure 4. The six amino acids i.e. leucine, isoleucine, valine, threonine, methio-

nine and phenylalanine determined from the fruit bodies of *P. ostreatus* grown on paddy straw alone exhibited lower amount than that on the paddy straw and other agrowastes combination. Among the six amino acids amount of valine was found to be the maximum, followed by threonine and then other amino acids.

### Discussion

In the present study the edible oyster mushroom *P.* ostreatus failed to grow when cultivated separately on radish leaves, pea pod shell and cauliflower leaves. The reason for failure of mushroom crop on 100% aforementioned vegetable wastes may be due to retention of large amount of water by vegetable wastes. This could have prevented the proper aeration of *Pleurotus* mycelia, hence, proper spawn run and fructification failed to occur. However, when these vegetable wastes were mixed with paddy straw, these shortcomings were overcome and adequate spawn run and fructification took place. As presented in figure 1, the first flush of fruiting bodies gave maximum yield than second and subsequent flushes. Similar results were obtained in *P. ostreatus*, (12, 7), *P. sajor-caju* (13) and in *P. florida* (4).

The cumulative yield and bioefficiency of P. ostreatus was found to be better when cultivated on paddy straw mixed with vegetable wastes. The better yield and bioefficiency of P. ostreatus on brassica straw, radish leaves, pea pod shell and cauliflower leaves in combination with paddy straw may be due to presence of various macro and microelements supplied by the vegetable wastes, which are not available in the paddy straw alone (4). The composition of substrates affects the nutritional value of mushroom fruit bodies. The mushroom mycelia secret extracellular enzymes (14) and these enzymes may be helpful in enhancing the nutritional value of fruiting bodies (4). Das and Mukheriee (15) reported that the biological efficiency of *P* ostreatus remain unchanged when it was grown on a weed Leonotis sp and paddy straw, but there was significant increase in biological efficiency if both the wastes were mixed in

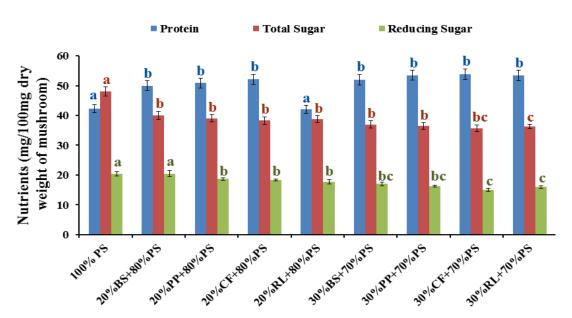


Figure 3. Protein, Total Sugar and Reducing sugar content of *P. Ostreatus* during its cultivation on different combinations of paddy straw with vegetable wastes.

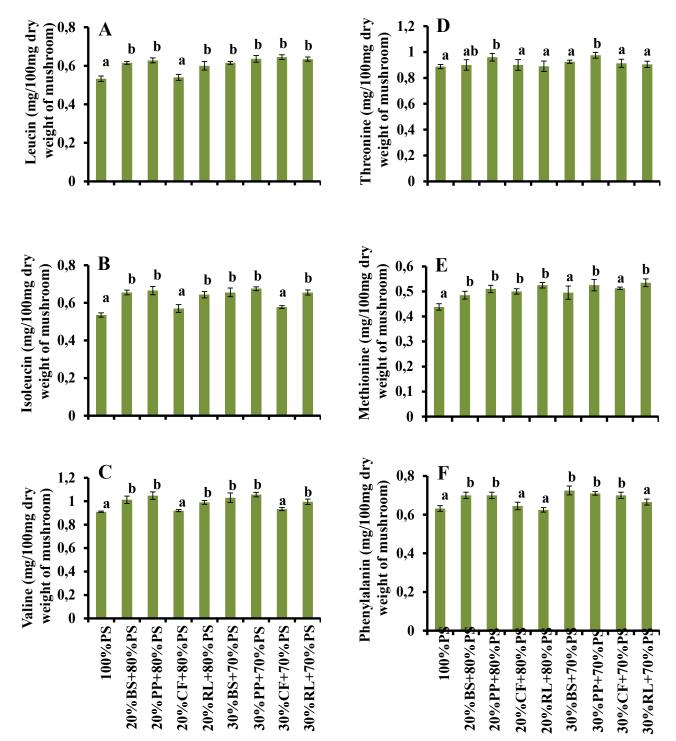


Figure 4. Amino acids content of *P. ostreatus* during its cultivation on different combinations of paddy straw (PS) and vegetable wastes.

1:1 ratio.

The observations of present investigation suggest that the edible mushroom *Pleurotus ostreatus* grown on paddy straw mixed with brassica straw, pea pod shell, cauliflower leaves and radish leaves gives fruit bodies with enhanced protein and amino acid contents. This work can be a step forward in the journey of thousand miles to mitigate the malnutrition problem and enhance the nutritional value through eco-friendly measure.

Other articles in this theme issue include references (16-31).

## References

1. Bushwell, J.A., Chang, S.T., Edible mushrooms: attributes and

applications. In: Genetics and Breeding of Edible Mushrooms. 1993, 297-324.

2. Singh, M.P. and Gautam, N.C., An overview of lignocellulose biotechnology. In: Recent advances inbiotechnology , N.C. Gautam and M.P. Singh (eds.), Shree Publishers, New Delhi, 2004, 3-20.

3. Singh M.P., Pandey, V.K., Srivastava, A.K. and Vishwakarma, S.K., Biodegradation of brassica haulms by white rot fungus P. eryngii. *Cell. Mol. Biol.* 2011, **57** (1): 47-55.

4. Singh, M.P. and Singh, V.K., Biodegradation of vegetable and agrowastes by pleurotus sapidus: a novel strategy to produce mush-room with enhanced yield and nutrition. *Cell. Mol. Biol.* 2011, **58** (1): 1-6.

5. Jwanny, E. W., Rashad, M. M. and Abdu, H. M., Solid-state fermentation of agricultural wastes into food through Pleurotus cultivation. *Applied Biochem. and Biotechnol.* 1995, **50** (1): 71–78. 6. Patrabans, S., and Madan, M., Studies on cultivation biological efficiency and chemical analysis of Pleurotus sajor-caju (Fr) Singer on different bio waste. *Acta Biotechnologia*. 1997, **17**: 107-122.

7. Chang, S.T., Lau, O.W. and Cho, K.Y., The cultivation and nutritional value of Pleurotus sajor-caju. *Eur. J. Appl. Microbiol. Biotechnol.* 1981, **12**: 58-62.

8. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 1956, **26**: 350-356.

9. Sumner, J.B. and Graham, V.A., Dinitrosalicylic Acid: A reagent for the estimation of sugar in normal and diabetic urine. *J. Biol Chem.* 1921: **47**: 5-9.

10. Moore, S. and Stein, In: Methods Enzymol. Colowick, S.P. and Kaplan, N.D. (eds.) Academic Press, New York. 1948, pp. 468.

11. Lowry O.H., Protein measurement with folin-phenol reagent. J. Biol. Chem. 1951, **193**: 265-275.

12. Block, S.S., Tsao, G. and Han, L., Experiments in the cultivation of Pleurotus ostreatus. *Mush. Sci.* 1959, 4: 309-325.

13. Bisaria, R., Madan, M. and Bisaria, V.R., Biological efficiency and nutritive value of Pleurotus sajor-caju cultivated in different agrowastes. *Biol. Wastes.* 1987, **19**: 239-255.

14. Singh, M.P., Pandey, V.K., Pandey, A.K., Srivastava, A.K., Vishwakarma, N.K. and Singh V.K., Production of xylanase by white rot fungi on wheat straw. *Asian Jr. of Microbiol. Biotech. Env. Sc.* 2008, **10** (4): 859-862.

15. Das, N., Mukherjee, M., Cultivation of Pleurotus ostreatus on weed plants. *Bioresour Technol.* 2007, **98**: 2723-2726.

16. Vishnoi, N., Singh, D. P., Biotransformation of arsenic by bacterial strains mediated by oxido-reductase enzyme system. *Cell. Mol. Biol.* 2014, **60 (5)**: 7-14. doi: 10.14715/cmb/2014.60.5.3

17. Srivastava, A. K., Vishwakarma, S. K., Pandey, V. K., Singh, M. P., Direct red decolorization and ligninolytic enzymes production by improved strains of *Pleurotus* using basidiospore derived monokaryons. *Cell. Mol. Biol.* 2014, **60** (5): 15-21. doi: 10.14715/ cmb/2014.60.5.4

18. Kumari, B., Rajput, S., Gaur, P., Singh S. N., Singh D. P., Biodegradation of pyrene and phenanthrene by bacterial consortium and evaluation of role of surfactant. *Cell. Mol. Biol.* 2014, **60** (5): 22-28. doi: 10.14715/cmb/2014.60.5.5

19. Pandey, V. K., Singh, M. P., Biodegradation of wheat straw by *Pleurotus ostreatus. Cell. Mol. Biol.* 2014, **60** (5): 29-34. doi: 10.14715/cmb/2014.60.5.6

20. Pathak, V. V., Singh, D. P., Kothari, R., Chopra, A. K., Phycoremediation of textile wastewater by unicellular microalga *Chlorella pyrenoidosa. Cell. Mol. Biol.* 2014, **60** (5): 35-40. doi: 10.14715/ cmb/2014.60.5.7

Pandey, A. K., Vishwakarma, S. K., Srivastava, A. K., Pandey, V. K., Agrawal, S., Singh, M. P., Production of ligninolytic enzymes by white rot fungi on lignocellulosic wastes using novel pretreatments. *Cell. Mol. Biol.* 2014, **60** (5): 41-45. doi: 10.14715/cmb/2014.60.5.8
Ayaz E., Gothalwal, R., Effect of Environmental Factors on Bacterial Quorum Sensing. *Cell. Mol. Biol.* 2014, **60** (5): 46-50. doi: 10.14715/cmb/2014.60.5.9

23. Singh, M. K., Rai, P. K., Rai, A., Singh, S., Alterations in lipid and fatty acid composition of the cyanobacterium *Scytonema geitleri* bharadwaja under water stress. *Cell. Mol. Biol.* 2014, **60** (5): 51-58. doi: 10.14715/cmb/2014.60.5.10

24. Singh, M. P., Pandey, A. K., Vishwakarma, S. K., Srivastava, A. K., Pandey, V. K., Singh, V. K., Production of cellulolytic enzymes by *Pleurotus* species on lignocellulosic wastes using novel pretreatments. *Cell. Mol. Biol.* 2014, **60** (5): 59-63. doi: 10.14715/ cmb/2014.60.5.11

25. Chandra, P., Singh, D. P., Removal of Cr (VI) by a halotolerant bacterium *Halomonas* sp. CSB 5 isolated from sāmbhar salt lake Rajastha (India). *Cell. Mol. Biol.* 2014, **60 (5)**: 64-72. doi: 10.14715/ cmb/2014.60.5.12

26. Tewari, S., Arora, N. K., Talc based exopolysaccharides formulation enhancing growth and production of *Hellianthus annuus* under saline conditions. *Cell. Mol. Biol.* 2014, **60** (5): 73-81. doi: 10.14715/cmb/2014.60.5.13

27. Kumar, M., Singh, P., Tripathi, J., Srivastava, A., Tripathi, M. K., Ravi, A. K., Asthana, R. K., Identification and structure elucidation of antimicrobial compounds from *Lyngbya aestuarii* and *Aphanothece bullosa. Cell. Mol. Biol.* 2014, **60 (5)**: 82-89. doi: 10.14715/ cmb/2014.60.5.14

28. Arun, N., Vidyalaxmi, Singh, D. P., Chromium (VI) induced oxidative stress in halotolerant alga *Dunaliella salina* and *D. tertiolecta* isolated from sambhar salt lake of Rajasthan (India). *Cell. Mol. Biol.* 2014, **60 (5)**: 90-96. doi: 10.14715/cmb/2014.60.5.15

29. Prakash, S., Singh, R., Lodhi, N., Histone demethylases and control of gene expression in plants. *Cell. Mol. Biol.* 2014, **60 (5)**: 97-105. doi: 10.14715/cmb/2014.60.5.16

30. Singh, A. K., Singh, M. P., Importance of algae as a potential source of biofuel. *Cell. Mol. Biol.* 2014, **60** (5): 106-109. doi: 10.14715/cmb/2014.60.5.17

31. Dixit, S., Singh, D. P., Role of free living, immobilized and nonviable biomass of *Nostoc muscorum* in removal of heavy metals: An impact of physiological state of biosorbent. *Cell. Mol. Biol.* 2014, **60** (**5**): 110-118. doi: 10.14715/cmb/2014.60.5.18