Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 5

Substrate

SUNFLOWER SEED HULLS

N.R. Curvetto, R. Gonzalez Matute, D. Figlas and S. Delmastro
Universidad Nacional del Sur, Argentina

What is There in Sunflower Seed Hulls?

Figure 1. Sunflower whole seeds and hulls (Average size of hulls is 12mm.)

An important portion of the energy invested in producing plant seeds is directed to the seed coatings. These are highly stable in nature as would be expected from their function of protecting seeds against water, providing thermal isolation, serving as a line of defense against pathogens. The coating or hull of the sunflower seed (Fig. 1) is an abundant and cheap lignocellulosic residue of the local (Argentine) edible oil-seed industry. Though the sunflower seed hull (SSH) is not used in human nutrition, SSH composition in organic and mineral substances could theoretically supply a source for other nutritional purposes, but the presence of a high content of lignin renders hulls unmarketable as a dietary supplement for animal feeding or other valuable uses. So, SSH remains an abundant side product of scarce economical value that is usually burned or left in the fields, either of practices that pose an environmental pollution problem.

During the oil extraction procedure raw sunflower seeds are transformed into oil and flour, and seed hulls are produced as a by-product. SSH constitute about 18-20% of the raw seeds. The main organic macro-nutrients of SSH are lipids, carbohydrates and proteins, with the highest percentage of the content being in the lignin and cellulose-hemicellulose portion, with lignin comprising about 20-25% of the total weight (Dorrel and Vick, 1997). Reduced sugars are also an important part of the seed coating, amounting to about 25%. Lipids and protein content are around 5% and 4% respectively, and almost 3% of the lipids are waxes (Cancalon, 1971). This chemical composition makes SSH an attractive material for growing microorganisms.

The high lignin content, however, limits the possibility of rapid biodegradation. The white rot fungi, basidiomycetes, are considered as the primary agents in nature for lignin degradation (Buswell and Oider, 1987; Zadrazil and Reinger, 1988).

Figure 2. Sunflower field

Could Sunflower Seed Hulls be Used for Cultivation of *Pleurotus ostreatus*?

Sunflower seed hulls composition in organic macronutrients (4% protein, 5% lipids and 50% carbohydrates) is as appropriate as that of other substrates commonly used for the cultivation of oyster mushrooms, such as cereal straws, corn husks, used tea leaves and cotton wastes (2-5% proteins, 0.4-2.2% lipids and 32-37% carbohydrates). Oyster mushrooms (*Pleutorus* spp.) possess an extracellular enzymes system and a strategy via free radicals that make them able to degrade lignocellulosic material of SSH as well as others, thus exhibiting a great adaptability to different kind of lignocellulosic materials.

We initially tested whether SSH contain water extractable compounds which could affect mycelial growth of *P. ostreatus*, and found that aqueous sunflower seed hull extract did not affect its mycelial growth in culture medium. On the contrary, when the inoculated mycelium has been previously grown in a medium with a moiety of hull aqueous extract, mycelial growth significantly increased its rate. We concluded that under adequate conditions sunflower seed hulls could be used for mycelial growth, and that the phenomenon of mycelial growth stimulation, in response to the presence of some substances coming from the substrate in agar medium, needs to be further examined (Darjania *et al.*, 1997) since it could provide a useful tool for an advantageous adaptation of mushroom to a particular substrate (Chang, 1978).

Figure 3. Oyster mushroom cultivated on sunflower seed hull substrate

For cultivation of *P. ostreatus*, the most common supplement added to the end-substrate is cereal bran, a protein-rich substance, which is known to be an additive that stimulates mycelial growth and mushroom yield (Siddiqui and Khan, 1989; Kinugawa *et al.*, 1994). On the SSH substrate supplemented with cereal bran, the mycelial growth of *P. ostreatus* was not increased. Increased percentages of wheat bran to the end-substrate did not markedly influence the rate of substrate colonization. Moreover, substrate colonization was suppressed by the presence of 50% wheat bran or more. At this time, results suggested that non-supplemented sunflower seed hulls could be considered as a complete nutritive substrate to be colonized by *P. ostreatus* (Darjania *et al.*, 1997).

What about the Size of the Hull?

Mycelial growth did not show any significant differences when the sunflower seed hulls were used in three particle sizes averaging 7, 10 and 12mm, with the higher being the waste size from oil-seed factories. Complete substrate colonization by *P. ostreatus* was observed after 18 days of spawn running in all bags. However, there were marked differences in fruiting and crop yield. In these tests mushrooms grew better on substrates of the highest hull size, giving rise to about 65% biological efficiency (B.E., kg fresh mushroom weight/kg dry substrate weight x 100) at the 1st flush, and represented about 85% of the total accumulated B.E. at the 3rd flush. It was concluded that particle size did not affect the colonization rate of this mushroom and that additional hull chopping, which implies an extra cost, is unnecessary (Darjania *et al.*, 1997).

Are Sunflower Seed Hulls an Adequate Substrate?

These first approaches to introduce this new substrate indicated that SSH, as coming from oil-seed factories and without any nutritional supplementation, could be used as an adequate substrate for cultivation of oyster mushrooms, as biological efficiency for the first flush was within the commercially acceptable range. An increase of about 15% in B.E. was accumulated following the second and the third flushes. Thus, a prolongation of cropping did not produce a remarkable increase in the B.E., thus lowering the yield production cycle of *P.*

ostreatus. Therefore, for this kind of very low cost substrate, it does not seem economically reasonable to keep *P. ostreatus* cultivation for more than 1 flush, mainly due to the high cost of electric energy resulting from the heating and cooling equipment needed to maintain control of the environmental conditions. However, the production cycle can be extended to 2 flushes in 40-50 days, by using optimized formulas containing growth limiting mineral nutrients such as Nitrogen (as ammonia sulfate) and Manganese (II) (manganese (II) sulfate, a co-factor for the lignolitic activity of some peroxidase enzymes), which resulted, for each *P. ostreatus* strain used in this study, in a marked yield increase–up to 100%–over the corresponding control (Curvetto, *et al.*, 2002). In practical terms and for these *P. ostreatus* strains the production was in the range of 1-1.8kg (60-112% B.E.) mushrooms per 4kg substrate bags.

In summary

1) Sunflower seed hulls can be used as a substrate for the cultivation of *Pleurotus ostreatus*; using a simple formula containing 37.5% SSH, 2% calcium sulfate ($CaSO_4$), 0.5% calcium carbonate ($CaCO_3$), 60% water (H_2O) and pH 6.
2) Under favorable conditions for mycelium growth as described here, addition of wheat bran is not needed.
3) The largest particle size corresponding to sunflower seed coatings as they come from the local edible oil-seed industry produced maximum B.E. in fructification, in comparison with smaller particle sizes.
4) First flush produces about 85% of the total B.E. accumulated through 3 flushes.

Other Mushrooms

We found that SSH-based substrate is also adequate to grow other fungi. For *Lentinula edodes*, the basal formula (37.5% SSH, 0.5% $CaCO_3$, 2% $CaSO_4$, 60% water) produced 2kg shiitake/100kg dry substrate per day for a 55 days production cycle with an accumulated biological efficiency of 108% (Curvetto *et al.*, 2002b), a higher yield of shiitake in a shorter cycle of production than is reported with other substrates. Good results were also obtained for *Ganoderma lucidum* on SSH-based substrates supplemented with 2.5 or 5.0% wheat bran or 5.0% malt, and the productivity was similar or even higher than the one reported in literature (Gonzalez Matute *et al.*, 2002). At present, we are developing protocols to use SSH for the production of *Trametes versicolor*, *Hericium erinaceus*, *Stropharia rugoso-annulata*, *Coprinus comatus*, *Flammulina velutipes*, and brown *Agaricus bisporus*.

A Simple Production Protocol for *Pleurotus ostreatus* on SSH-based Substrate

- This low-cost method is suggested by the authors. Detailed explanation on each equipment and each step is provided on Bag Cultivation in Chapter 7.

Spawn production

We prepare grain spawn in thermoresistant plastic bags or in 1L bottles using wheat (*Triticum durum*) grain mixed with 0.1% (w/w) $CaCO_3$, 0.8% (w/w) $CaSO_4$, and 40% water (1kg wheat, 2g $CaCO_3$, 16g $CaSO_4$ and 0.7L water). Calcium salts are used to adjust and buffer the pH of the substrate near 6. Additionally, these salts avoid the formation of clumps either of the grains used for spawning or of the sunflower seed coatings used for the mushroom substrate. Otherwise, those salts provide sulfur and calcium which are essential mineral macronutients. The mixture is sterilized at 15 psi for 1.5 hours. Each bag or bottle is then inoculated under aseptic conditions with oyster mushroom mycelium (two wedges per bag or bottle) (Fig. 4), and

Figure 4. ***P. ostreatus*** spawn prepared on wheat grain in 1L bottle or small plastic bag

incubated at 25±1℃ in darkness for 15-20 days, with periodical shaking of the bags or bottles to maximize colonization and minimize grain clumping.

Substrate preparation and decontamination

For a 36kg substrate mass with a final composition of 37.5% SSH, 2% $CaSO_4$, 0.5% $CaCO_3$ and 60% tap water, these components are introduced into the drum as follows : 13.5kg SSH, then 10L water containing 720g $CaSO_4$ and 180g $CaCO_3$, and finally 11.6L water (Fig. 5). The decontamination process is always initiated with the gas heater on and the drum in a stationary position, during the first 15 minutes. Heating is provided for 2.5 hours with the drum alternately rotating for fifteen minutes and then stopping for fifteen minutes.

Figure 5. Introduction of sunflower seed hull into drum

Figure 6. Spawning the decontaminated substrate after cooling

Figure 7. Filling the bags

Spawning and spawn running

Figure 8. Puncture of bags for an adequate gas exchange

Figure 9. Colonization of substrate bags 3, 8, and14 days after spawning

The temperature of the substrate is allowed to fall to 35-40℃, with the drum rotating for about 2 hours. The spawn is then added to the substrate at a rate of 5-8% (w/w) (Fig. 6), and keeping the open end of the drum covered, rotation is continued during 15-20 minutes until homogeneous mixing of spawned substrate is obtained. With care

and decontaminated rubber gloves on, the operator fills plastic bags of 0.25m diameter with substrate to make blocks of 4-10kg (Fig. 7). The substrate is compressed by repeatedly tapping the bag on the floor thus obtaining a density of about 0.5kg/L and then, the bag is tightly closed. To assure an adequate O_2 and CO_2 concentrations and gas exchange, they are aseptically punctured on the whole surface using a rod with sharp pointed nails (we recommend an ad hoc device with pins (Fig. 8) that finally gives approximately 7,000 micro-holes per square meter, each one separated by 1.2cm from the surrounding mini-holes). The bags are placed in a growing room at 24±1℃, and after 15-18 days the substrate becomes completely colonized by the mycelium (Fig. 9). During this stage, the bags are daily observed for possible contamination.

Fruiting

Once the substrate in the bags is completely colonized by mycelia, the blocks are transferred to a fruiting room and the plastic covers are evenly punctured using a device with attached archery broadheads (Fig. 10) (Stamets, 1993) to expose a fructification surface of ca. 1% of the total bag surface to the following environmental conditions: 20±1℃, 80-90% R.H., and 12-hour photoperiod (150-200 lux). Observations for possible contamination are also done. Pinning (Fig. 11) and subsequent growth of fruiting bodies in a first flush (Fig. 12) occurred 15-20 days after spawning. A second crop is obtained between 10-15 days after the first one, and usually for this second flush a new set of punctures on the surface of the bags is needed.

Figure 10. A device with attached arrowhead to expose substrate surface for fruiting

Figure 11. Pinning of oyster mushroom

Figure 12. ***P. ostreatus*** on sunflower seed hull

REFERENCES

- Buswell, J.A., and O. Oider. 1987. Lignin biodegradation. Crit. Rev. Biotechnol. 6:1-60.
- Cancalon, P. 1971. Chemical composition of sunflower seed hulls. J. Amer. Oil. Chem. Soc. 48:629-632.
- Chang, S.T. 1978. The biology and cultivation of edible mushrooms. New York, U.S.A.: Academic Press. 819 pp.
- Curvetto, N.R., S.E. Delmastro, R.J. Devalis, and D. Figlas. 1997. A low cost method for decontaminating sunflower seed hull-based substrate in the cultivation of *Pleurotus* edible mushroom. Mushroom Res 6:25-28.
- Curvetto, N.R., D. Figlas, R.J. Devalis, and S.E. Delmastro. 2002a. Growth and productivity of different *Pleurotus ostreatus* strains on sunflower seed hulls substrate supplemented with N-NH4+ and/or Mn(II). Bioresource Technology 84(2):171-176.
- Curvetto, N.R., D. Figlas, R.J. Devalis, and S.E. Delmastro. 2002b. Sunflower seed hulls as substrate for the cultivation of shiitake (*Lentinula edodes*) mushrooms. HortTechnology 12(4):652-655.
- Darjania, L., N.J Curvetto, M. Schapiro, D. Figlas, and D. Curvetto. 1997. Sunflower seed hulls as a substrate for cultivation of *Pleurotus ostreatus* . Mushroom News 45:6-10.
- Dorrel, D.G., and B.A. Vick. 1997. Properties and process of oilseed sunflower. In: A.A. Schneiter (ed.). Sunflower technológy and production. Agron. Monogr. 35:709-745.

- Duncan, K.W. 1997. An ecophysiological approach to the evaluation, modification and production of mushroom growing media. Mushroom News 45:12-27.
- Earanna, N., and K.S. Shetty. 1994. National Symposium on Mushrooms. Mushroom Society of India and National Centre for Mushroom Research and Training, Solan.
- González Matute, R., D. Figlas, R.J. Devalis, S.E. Delmastro, and N.J. Curvetto. 2002. Sunflower seed hulls as a main nutrient source for cultivating *Ganoderma lucidum*. Micologia Aplicada International 14(2):19-24.
- Kaal, E.E.J., J.A. Field, and T.W. Joyce. 1995. Increasing ligninolytic enzyme activities in several white-rot basidiomycetes by nitrogen-sufficient media. Bioresource Technol 53:133-139.
- Kinugawa, K., W. Phusawang, S. Chinbenjaphol, S. Fukada, E. Tanesaka, M. Okada, and H. Tsutsui. 1994. Progress Report (1991-1993) of joint research program of Kinki and Chiang Mai Universities on the promotion of mushroom research. Mem Fac Agr Kinki Univ 27:93-113.
- Lelley, J.I., and A. Janßen. 1993a. Productivity improvement of oyster mushroom substrate with a controlled release of nutrient. Mushroom News 41:6-13.
- Lelley, J.I., and A. Janßen. 1993b. Interactions between supplementation, fructification-surface and productivity of the substrate of *Pleurotus* spp. In: Chang, S.T., J.A. Buswell, S.W. Chiu (eds). Mushroom Biology and Mushroom Products. Hong Kong: The Chinese University Press. Chapter 9.
- Siddiqui, M.A., and S.M. Khan. 1989. Some studies on the cultivation of oyster mushroom (*Pleurotus* spp.) on ligno-cellulosic by-products of textile industry. Proceedings of the 12th International Congress on the Science and Cultivation of Edible Fungi. Braunschweig, Germany. 121-128.
- Stamets, P. 1993. Growing Gourmet and Medicinal Mushrooms. Berkeley, CA: Ten Speed Press Ltd. 352-369.
- Zadrazil, F., and R. Reinger. 1988. Treatment of Lignocellulosics with White Rot Fungi. London, England: Elsevier Applied Science.