MUSHROOMS OF THE *Pleurotus* GENUS: A REVIEW OF CULTIVATION TECHNIQUES

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SUMMARY

The cultivation of edible mushrooms in agro-industrial residues is considered a millennial activity, spread and practiced all around the world. Besides their excellent taste, edible mushrooms have a great biotechnological potential, due to their capacity to produce enzymes and medicines, to act in bioremediation, and other uses. The goal of the present work was to carry out a bibliographic review of the different cultivation techniques used in the production of *Pleurotus* spp. A wide variety of residues may be used as substrate in the cultivation of this mushroom. The use of agro-industrial byproducts represents an economically viable and very promising alternative for small producers, due to the low aggregate value and great availability.

Although there are more than 2000 species of edible mushrooms nowadays, only the champignon (*Agaricus bisporus*), the giant mushrooms (*Pleurotus ostreatus* and *Pleurotus ostreatoroseus*) and shiitake (*Lentinula edodes*) are among the most cultivated ones (Bononi et al., 1999) and are well appreciated.

Mushrooms are saprophyte fungi belonging to the class of the Basidiomycetes. They grow in moist places with decomposing organic matter and are very important in nutrient cycling (Subramanian, 1995).

According to Furlani and Godoy (2007), mushrooms are considered as food with delicious taste and high nutritional value because their contents (g/100g) of protein (23.22), carbohydrate (63.17), phosphorus (104.13) and fiber (34.0) are high, and the amount of lipids (4.71) is low. Mushrooms are also ideal for use in diets, due to their extremely low caloric value. Moreover, they produce a series of metabolites of pharmacological and medicinal interest, such as antioxidants, antitumourals, immunostimulants and antimicrobials (Elmastas et al., 2007; Kitzberger et al., 2007; Moradali et al., 2007; Israilides et al., 2008).

The world production of mushrooms (FAOSTAT, 2008) was...
Techniques Used in the Production of Edible Mushrooms

Usually 6 stages make up *Pleurotus* production: matrix preparation, composting, pasteurization, seeding, incubation and harvest (Eira, 2004).

**Matrix preparation**

The matrix or “spawn” is produced by transferring small parts of the mycelium of the mushroom of interest (previously cultivated in culture medium) to tubes containing grains and/or cooked fibers or sawdust, properly sterilized at 121°C. These inoculated tubes are sealed and usually incubated at 25°C until total colonization of the substrate by the fungi (Bononi *et al*., 1999).

**Composting**

Composting is used for the elaboration of the substrate and pasteurization is carried out to eliminate contaminant microorganisms which might keep a competitive relationship with the fungi of interest (Eira, 2000). According to Moda (2003), composting takes 15-20 days, varying according to the substrate used. In order to correct pH, calcium carbonate is added to the compost, which may also be supplemented with other materials (bran, wheat bran, ammonia nitrate, etc.). Compost humidity must be controlled not to exceed 75%.

**Pasteurization and incubation**

Pasteurization is usually performed in tunnels where water steam passes through the compost during 2 to 3 days, at temperatures >55°C. However, some cultivation systems adopt substrate sterilization in stoves at 121°C for ~2h (Moda, 2003). After that process, the substrate must be cooled to 25°C and stored in plastic bags. Next, the previously prepared spawn must be inoculated. Finally, the bags are placed inside an incubation stove for ~50 days under controlled conditions (Bono- ni *et al*., 1999).

**Biological Efficiency**

The main parameter used to evaluate mushroom yield is called biological efficiency (BE). It mainly depends on the characteristics of the material and the circumstances in which the growth process occurs. It is estimated by means of the formula

$$BE(\%) = \frac{\text{Total fresh weight of mushrooms}}{\text{Dry weight of the initial substrate}} \times 100$$
TABLE 1
SUBSTRATES USED IN THE CULTIVATION OF Pleurotus spp.

<table>
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<th>Species</th>
<th>Substrate</th>
<th>Reference</th>
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<td>Pangola grass</td>
<td>Hernández et al. (2003)</td>
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<tr>
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<td>Crushed sugar cane</td>
<td>Moda et al. (2005a)</td>
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<tr>
<td>P. sajor-caju</td>
<td>Cotton textile processing residue</td>
<td>Castro et al. (2007)</td>
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<td>P. sajor-caju</td>
<td>Cotton processing residue, wheat straw, soy straw, pea stem, peanut stem</td>
<td>Mane et al. (2007)</td>
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<td>P. sajor-caju</td>
<td>Coast-cross hay and crushed sugar cane</td>
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<td>P. ostreatus</td>
<td>Tannery leather sawdust</td>
<td>Bernardi et al. (2008)</td>
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<tr>
<td>P. ostreatus</td>
<td>Banana tree straw</td>
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<td>Olive oil processing effluent</td>
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<td>P. sajor-caju</td>
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<td>P. ostreatus</td>
<td>Rice straw and wheat straw</td>
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<td>P. ostreatus</td>
<td>Rice straw, banana tree straw, corn forage, elephant grass, wheat straw</td>
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<td>P. ostreatus</td>
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<td>P. djamor</td>
<td>Leaves, stem and fruit of the banana tree (<em>Musa paradisiaca</em>) and jequirita sawdust (<em>Cariniana pyrifromis</em>)</td>
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<td>P. ostreatus</td>
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<td>P. florida</td>
<td>Corn cob</td>
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24h. Next, inoculation and incubation were performed during six weeks. It was observed that both treatments resulted in satisfactory BE (55.76% in T1 and 55.39% in T2), thus cotton textile residue being an excellent alternative for *P. sajor-caju* cultivation.

Mane et al. (2007) grew *P. sajor-caju* in several agro-industrial residues: cotton processing residue, wheat straw, soy straw, pea stalk and peanut stalk. Residues were washed and pasteurized at 80ºC for 2h. Afterwards, substrates were cooled and inoculated with the fungus. Composts were stored inside polyethylene bags and incubated for 15 days at 27ºC. The best result was obtained when using cotton residues, pea stalk and wheat straw as substrates.

Silva et al. (2007) analyzed *P. sajor-caju* cultivation in coast-cross hay and crushed sugar cane supplemented with wheat bran and urea. The experiment was divided into five treatments, T1 (control): coast-cross (500g) + crushed sugar cane (500g), T2: coast-cross (450g) + crushed sugar cane (450g) + wheat bran (100g), T3: coast-cross (450g) + crushed sugar cane (450g) + wheat bran (100g) + urea (10g), T4: coast-cross (450g) + crushed sugar cane (450g) + wheat bran (100g) + urea (20g), and T5: coast-cross (450g) + crushed sugar cane (450g) + wheat bran (100g) + urea (30g). The compost was stock in bags and sterilized twice for 1h at 121ºC. Each bag was inoculated with the fungus and submitted to incubation at room temperature until "frutification": the highest yields were obtained in T1 (35.1%), T2 (35.9%) and T3 (34.0%). The supplementation with urea, therefore, did not provide any yield increase of the mushroom tested.

Bernardi et al. (2008) used elephant grass as substrate, supplemented with tannery leather sawdust for *P. ostreatus* cultivation, in concentrations of 0, 5, 10, 15 and 20% in relation to the weight mass of elephant grass. The compost was inserted into glass bottles and then the inoculum was added. Bottles were sealed, sterilized twice for 40min at 121ºC, and incubated at 26ºC for 37 days. Then, the bottles were kept in the incubation room for 60 days more. Only treatments supplemented with 0% and 5% of tannery residue obtained "frutification", with BE of 76 and 64%, respectively. Substrates with more than 5% of supplementation were not colonized by the fungi. The authors proposed further studies in order to analyze the physical and chemical properties of the tannery residue and the bromatological properties of the mushrooms grown in a substrate supplemented with tannery residue.

Pedra and Marino (2006) evaluated *Pleurotus* spp. yield using coconut bark supplemented with rice and wheat bran. Their experiment consisted of six treatments, T1: coconut sawdust (100%), T2: coconut sawdust (80%) and rice bran (20%), T3: coconut sawdust (80%) and wheat bran (20%), T4: coconut sawdust (60%) and wheat bran (20%), T5: coconut sawdust (60%) and rice bran (40%), and T6: coconut sawdust (60%) and wheat bran (40%). The composts were stored inside sealed containers and, next, submitted to sterilization twice during 40min at 120ºC. The substrate was cooled soon after sterilization and then inoculated. Incubation was performed for 30 days at 25ºC. The substrate was not colonized by the fungus in T1, but treatments T4, T5 and T6 presented BE values of 14.32, 15.69 and 15.61%, respectively, showing that the addition of coconut bark supplementation to the substrate favors the development of the mushroom.

*P. sajor-caju* and *P. ostreatus* were grown in banana tree straw, supplemented with rice bran (5%) by Bonatti et al. (2003). The substrate was inserted into sealed polyethylene containers and submitted to sterilization for 1.5h at 121ºC. Next, containers were cooled, being ready to receive the inoculum. Incubation was performed for 20 days at 25ºC. “Frutification” was then induced, followed by an additional 40 days of incubation. Moisture, fat, carbohydrate, ash, protein and raw fiber analyses were performed. Significant differences in the nutritional values of both species analyzed were not found. However, *P. sajor-caju* presented higher biological efficiency (7.51%) than *P. ostreatus* (6.34%).

Dias et al. (2003) grew *P. sajor-caju* using coffee husk, corn straw, corncob and bean straw as substrates, supplemented with wheat bran. Composts were inserted into bottles and sealed. Sterilization at 121ºC for 1h was performed and, following cooling to room temperature, the spawn was introduced and incubated at 24ºC until total colonization. Induction to "frutification" was subsequently performed by keeping bags open for ~90 days. Among the tested residues, bean straw without the supplement ob-
tained the highest BE (87.5%). Coffee husk obtained the lowest performance (25.1%).

The effect of caffeine and tannin in the cultivation and “frutification” of *P. ostreatus* and *P. sajor-caju* in coffee husk was analyzed by Soccot et al. (2006). For the experiment, substrates consisting of coffee husk were added with different concentrations of caffeine and tannin in the amounts of 30, 50, 100, 500, 1000 and 2500mg·l⁻¹, and 100, 500, 1000, 5000 and 10000mg·l⁻¹, respectively. It was found that mycelium growth does not take place in the concentration of 500mg·l⁻¹ of caffeine or higher. Substrates supplemented with tannin at concentrations below 100mg·l⁻¹ stimulated fungus growth.

The results of the analyses of caffeine and tannin in the substrates showed a reduction of 39.3% and 20.8% in the concentrations, respectively. These results point out to good perspectives for *Pleurotus* spp. cultivation using coffee husk as substrate, making unnecessary any pre-treatment.

Akyüz and Yıldız (2008) grew *P. eryngii* using soy straw and wheat straw as substrates. The experiment was divided into three treatments: Treatment 1: soy straw + wheat straw, Treatment 2: bean straw, and Treatment 3: wheat straw. All of them were supplemented with rice bran in proportions of 5 and 10%. The experimental period was 100 days. Treatment 1 presented the most satisfactory biological efficiency (93%), with supplementation of 5% of rice bran. Treatment 3 showed the lowest BE (7%) with 10% rice bran supplementation.

*P. ostreatus* cultivation was also carried out (Baysal et al., 2003) using paper residues supplemented with peat from the region of Bolu, Turkey, hen manure and rice bran, according to treatments arranged as T1 (control): paper residue (100%), T2: paper residue (90%) + peat (10%), T3: paper residue (80%) + peat (20%), T4: paper residue (90%) + hen manure (10%), T5: paper residue (80%) + hen manure (20%), T6: paper residue (90%) + rice bran (10%), and T7: paper residue (80%) + rice bran (20%). Yield was significantly increased by adding rice bran to paper residue. On the other hand, production was drastically reduced due to supplementations with peat and hen manure. Thus, it was concluded that the substrate used in the study may be used for the cultivation of oyster mushrooms.

Weeds were also used as substrates for *P. ostreatus* cultivation (Das and Mukherjee, 2007). The following species were used: *Leonotis* sp. (Lamiaceae), *Sida acuta* (Malvaceae), *Parthenium argentatum* (Asteraceae), *Ageratum conyzoides* (Asteraceae), *Cassia sophera* (Caesalpinaceae), *Teephrosia purpurea* (Papilionaceae) and *Lantana camara* (Verbenaceae). The plants were completely sun-dried, sectioned into small pieces and soaked in water, and, subsequent-ly, excess water was drained. Each species was inoculated with and without supplementing with wheat straw and afterwards submitted to incubation. *Leonotis* sp. supplemented with wheat straw significantly increased *P. ostreatus* yield (1.30kg/kg). It was concluded that the use of weeds as substrates is an efficient method for the cultivation of edible mushrooms.

Kalmis and Sargin (2004) evaluated *P. cornucopiae* and *P. sajor-caju* yield using wheat straw as substrate, supplemented with wheat bran at a 9:1 ratio, moistened with olive oil processing resulting solution, diluted in several concentrations according to the following five treatments, T1 (control): wheat straw + 0% residue, T2: wheat straw + 25% residue, T3: wheat straw + 50% residue, T4: wheat straw + 75% residue, and T5: wheat straw + 100% residue. Composts were stored in containers and sterilized. After cooling to room temperature, inoculation and incubation were performed. The concentrations with the highest BE were 25 and 50%, with yields of 33.7 and 30.6%, respectively, being judged appropriate for *Pleurotus* spp. cultivation.

A similar study was carried out by Kalmis et al. (2008) with *P. ostreatus*, using wheat straw as substrate, supplemented with wheat bran and moistened with the effluent of olive oil processing, in the same ratios used in the former experiment. The authors reported 25% as the only viable oil residue concentration to be used, with BE of 45%. Higher concentrations caused bad mushroom formation. Considering the serious environmental damages caused by the residue of olive oil processing, its use as substrate for edible fungi production provides an economically and environmentally viable solution for the problem.

Zhang et al. (2002) analyzed *P. ostreatus* cultivation in rice straw and wheat straw in two different processing methods, cut into pieces and ground. Higher growth rates were observed in mushrooms cultivated in cut straw. Comparing yields between both residues used, the cultivation carried out in rice straw presented 10% higher yield than wheat straw. Obdai et al. (2003) verified that substrates made up with rice straw also presented higher BE (50.64%) when compared to banana tree straw (37.15%), corn forage (16.50%), wheat straw (29.26%) and elephant grass (0%). Shashirekha et al. (2005) obtained an increase in protein, amino acid and lipid concentrations, and a significant decrease in fiber, free sugar and carbohydrates by supplementing rice straw with cotton seed in *P. floridana* cultivation.

Sainos et al. (2006) used wheat straw supplemented with wheat grains in *P. ostreatus* cultivation, in proportions of T1: 100/0, T2: 75/25; T3: 50/50, T4: 25/75, and T5: 0/100. The experiment was carried out until complete colonization of the substrate by the fungus, during 10 days. T1 was the only treatment with a significant difference in yield (31.7%). The others presented an average of 39.77%. It was concluded that wheat straw as substrate is an excellent alternative for *P. ostreatus* cultivation.

Motato et al. (2006) grew *P. djamor* using leaves, stalk and fruit of the banana tree (*Musa paradisiaca*) and jequitiba (*Cariniana pyriflora*) sawdust, according to seven treatments, T1: sawdust (100%), T2: leaves (100%), T3: stalk (100%), T4: sawdust + stalk (50/50), T5: sawdust + leaves (50/50), T6: sawdust + fruit (50/50), and T7: sawdust + leaves + stalk + fruit (25% each), with three repetitions each. It was reported that fungal growth was more successful in T2, with a BE of 24.1%. Concerning the presence of lignocellulitic enzymes, laccase and peroxidase activities were higher in T2 and T3. Leaves and stalk of the banana tree were also used in the cultivation of *P. ostreatus* and *P. sajor-caju*, in order to verify the action of lignocellulitic enzymes during substrate fermentation. Reddy et al. (2003) verified that both organisms presented the same activity in the production of laccase, peroxidase, lignin, xylanase, endo-1-4-b-D-glucanase (CMCase) and exo-1-4-b-D-glucanase enzymes during the 40 days of cultivation.

Alexandro et al. (2007) aimed to verify the production of lignocellulolytic enzymes by *P. ostreatus*. Dry, ground orange residue was used as substrate for the cultivation. Laccase and manganese peroxidase presented highest activity. Furthermore, the use of this sort of residue provided the necessary nutritional conditions for fungus growth.

Coffee dregs combined with wheat straw and sawdust from *Picea abies* (a tree from the family of the conifers, found in the cold highlands in Northern Europe) were also used to grow *P. ostreatus*. The experiment consisted of six treatments, control (60g sawdust / 125g wheat straw), and five other treatments made up with coffee dregs, sawdust and wheat straw in the following proportions, CB1: 36/60/106, CB2: 74/60/106, CB3: 110/60/93, CB4: 146/60/74, and CB5: 186/60/88. The results showed that the “frutification” capacity and the biological efficiency of the production of the fungus were not decreased by the use of coffee dregs as substrate. Analyses to verify the caffeine content present in the basidiomata of *Pleurotus* spp. showed that 59% is not incorporated by the mushroom, demonstrating its degradation capacity (Job, 2004).

Vetayasuporn (2007) grew *P. ostreatus* using coconut shell, sawdust, crushed sugar cane and leaves of *Typha angustifolia* (a typical tree from North America) as substrates, added with
15% of efficient microorganisms (EM), lactic acid producing bacteria, aiming to eliminate harmful microorganisms present in the substrate, in a 100/15 (residue/EM) ratio. The substrate with the highest BE value was the crushed sugar cane (103.56%), representing an efficient alternative for the production of oyster mushrooms.

Naraian et al. (2008) used corncobs (CC) as substrate for P. florida cultivation, supplemented with urea (U), ammonium sulfate (AMS), grass (G), bran (B), cotton seed (CS), mustard seed (MS), nuts seed (NS) and molasses (M), in three different combinations each: CC + U (0.5, 1 and 1.5%), CC + AMS (0.5, 1 and 1.5%), CC + G (2, 3 and 5%), CC + B (2, 3 and 5%), CC + CS (2, 3 and 5%), CC + MS (2, 3 and 5%), CC + NS (2, 3 and 5%), and CC + M (2, 3 and 5%). All treatments presented higher productivity than the control; however, substrates with higher supplement levels presented significant reductions in yield. The most efficient supplement was 2% cotton seed (93.5%), followed by 2% bran (93%).

Sales-Campos (2008) grew P. ostreatus in wood (Simarouba amara and Ochroma pyramidalare) sawdust, in agroindustrial crushed sugar cane (Saccharum officinarum), and in stem of punheira palm tree (Bactris gasipaes Kunth), all of them residues occurring in the Amazon region. All residues were supplemented with rice bran, wheat and CaCO₃, in a 80:10:8.2 ratio, respectively. Axenic cultivation was performed during 100 days. All treatments obtained high biological efficiency: S. amara sawdust (94.0%), O. pyramidalare sawdust (64.6%), S. officinarum (125.6%) and B. gasipaes strips (99.8%). The mushrooms produced presented high protein (14.67-21.16%) and fiber (18.89-31.30%) levels, as well as low lipids content (1.27-2.14%). Due to the satisfactory results, it was concluded that the residues used represent a great alternative for P. ostreatus cultivation, being the Amazonian region a very favorable environment for that practice.

**Conclusions**

Mushrooms of the *Pleurotus* spp. genus are among the most cultivated ones in the world due to a number of characteristics (medicinal, nutritional, bioremediation of contaminated environments, enzyme production, etc.). Moreover, they are easily manipulated and present a fast development due to the use of decomposing organic matter for growth, thus presenting a wide variety of alternatives for use in cultivation, as are the utilization of agro-industrial residues, leaves, sawdust, fruit peels and industrial effluents according to availability, varying among places.

The development of those fungi in these substrates is possible due to their capacity of synthesizing hemicellulose and lignin-degrading lignocellulolytic enzymes, components that are responsible for the structure and rigidity of vegetables. Due to such constitution, these residues suffer a very slow decomposition, being able to remain disposed in the environment for a very long time.

Edible fungi are also used in the pre-treatment of byproducts employed in the feeding of ruminant animals, in order to make them viable for consumption.

The methodology employed in cultivation, as well as the supplementations to be used, depend on the species and nutritional facts of the residue utilized.

The studies analyzed showed the viability of the use of several byproducts as substrates for mushrooms of the *Pleurotus* genus, such as elephant grass, coast-cross, crushed sugar cane, processing residues (cotton, paper, olive oil, tannage), sawdust, straws (wheat, soy, banana tree, corn, bean) and stalks (banana tree, pea, peanut). Several kinds of materials were used as supplementation, and the highest biological efficiency was obtained with wheat bran and rice bran.

It was verified that mushrooms grown in supplemented substrates presented higher biological efficiency than the ones grown in substrates without nutritional sources, making the addition necessary in order to lead to a more satisfactory production.

Among the residues used as substrate in cultivation, bean straw was the only one to present efficiency in yield without the need of supplementation.

It was observed that edible fungi also degrade most of the organic matter used as substrate, thus representing a very useful way to eliminate toxic products for the environment, by turning them into a nutritional source for their development.

The cultivation of edible mushrooms represents a promising alternative for small producers because of low-cost labor and raw material, once residues, most of times, have low or null aggregate value.

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Pedra WN, Marino RH (2006) Cultivo axênico de Pleurotus sajor-caju cultivado em diferentes lignocellulosic materials as substratos para o cultivo de este hongo. La utilización de subproductos agroindustriales representa una alternativa económicamente viable y bastante promisoria principalmente para pequeños productores, en virtud de su bajo valor agregado y gran disponibilidad.


**HONGOS DEL GÉNERO Pleurotus: UNA REVISIÓN DE LAS TÉCNICAS DE CULTIVO**

Cristiane Suely Melo de Carvalho, Ceci Sales-Campos y Meire Cristina Nogueira de Andrade

RESUMEN

El cultivo de hongos comestibles en residuos agroindustriales es considerado una actividad milenaria, difundida y practicada en todo el mundo. Además de poseer excelente sabor, los hongos comestibles presentan un gran potencial biotecnológico debido a su capacidad de producir enzimas y fármacos, y de servir para biorremediación, entre otros usos. Este trabajo tuvo como finalidad realizar una revisión bibliográfica de las diferentes técnicas de cultivo utilizadas en la producción de Pleurotus spp. Gran variedad de residuos pueden ser utilizados como sustratos en el cultivo de este hongo. La utilización de subproductos agroindustriales representa una alternativa económicamente viable y bastante promisoria principalmente para pequeños productores, en virtud de su bajo valor agregado y gran disponibilidad.

**COGUMELOS DO GÉNERO Pleurotus: UMA REVISÃO DAS TÉCNICAS DE CULTIVO**

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RESUMO

O cultivo de cogumelos comestíveis em resíduos agroindustriais é considerado uma atividade milenar, difundida e praticada em todo o mundo. Além de possuírem um sabor excelente, os cogumelos comestíveis apresentam um grande potencial biotecnológico, devido à sua capacidade de produzir enzimas e fármacos, e de atuarem na biorremediação, entre outros usos. Esse trabalho teve como objetivo realizar uma revisão bibliográfica das diferentes técnicas de cultivo utilizadas na produção de Pleurotus spp. Uma grande variedade de resíduos podem ser utilizados como substrato no cultivo desse cogumelo. A utilização de subprodutos agroindustriais representa uma alternativa econômica viável e bastante promissora principalmente para pequenos produtores, em virtude do baixo valor agregado e grande disponibilidade.