Utilization of wastes from grain processing industries

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Abstract. Waste materials resulting from the grain processing industries are harmless and easily amenable to enzymatic and microbial bioconversions. Basidiomycetous mushrooms grown in vitro are promising producers of protein and essential amino acids. Mushroom biomass production under appropriate cultivation conditions using agricultural by-products is a cheap and affordable way to obtain a high-quality food product. The present work explores the possibility of using grain wastes for the cultivation of golden oyster mushrooms under deep cultivation conditions. For these purposes, cornmeal was used as a source of carbon waste and gluten as a source of nitrogen. A high substrate colonization rate was observed. The fruiting cycle lasted about 26 to 28 days on the studied substrates. The most prolonged cycles were obtained on the medium supplemented with sawdust. The maximum and the minimum yields were obtained on wheat straw substrate (171.2 g per kg of substrate) and a grain waste substrate (113.4 g per kg) respectively. On birch sawdust substrate and on a substrate of grain waste and birch sawdust mixture, the yields reached 143.2 g and 130.3 g in this order. The average yielding crop attained 113.4 g of fresh mushrooms grown on 1 kg of substrate under a relative humidity of 70-75%. This promising work stream not only proposes a waste disposal method, but also, a significant source of income for the grain processing enterprises.

1. Introduction

The increasing economic growth rate of the agriculture and food industry has aggravated the problem of waste management, including grain wastes disposal [1, 2].

Waste materials resulting from the grain processing industries are harmless and easily amenable to enzymatic and microbial bioconversions as well as to various types of preprocessing [3, 4]. Therefore, it would be relevant to continue exploring this research area.

Bioconversion is a natural way to utilize cellulotic waste. It is based on the destruction of an organic substrate by microorganisms and fungi. In this way, it allows to solve two main tasks: the creation of an economically advantageous production process of a target product and, a method of waste disposal for potential environmental pollutants [5 -7].
According to the study of mushroom growing techniques worldwide, different types of wastes are used for substrate preparation, among which: corn stalks, cabbage stalks, rice straw, sunflower husks, coconut shells, cotton and wood processing waste. For the growth of oyster mushrooms, mixtures of straw, soybeans, flax, potato peel, cocoa bean processing waste, sugar cane, coffee, tobacco and grapes are also used as a substrate [8 - 12]. In previous studies, industrial waste has been successfully used for the cultivation of blue oyster mushrooms. In fact, the use of cardboard as a substrate not only increased the growth yields, but also provided greater biological efficiency correlated with a greater productivity of mushroom with larger cap diameter [13].

Under the same cultivation conditions, the spawn rate of oyster mushrooms on substrate dry weight basis will differ depending on the used substrate [14]. In previous studies, winter wheat straw provided a yield of 64.6% of fruiting bodies, corn cobs - 46.7%; cotton waste - 68.4%; cotton waste and straw (4:1) mixture - 75.8% [15-18].

The main substrate that could be used for the cultivation of oyster mushrooms are wastes such as vegetable wastes from the agriculture industry. However, in order to expand the resource base of industrial production of mycelium of higher fungi and to increase the yield of a biomass enriched with protein, deep cultivation on starch containing plant substrates, including grain waste, is proposed. Yet, grain waste requires a mandatory pretreatment using acid or enzymatic hydrolysis [19].

The pretreatment of raw materials contributes directly to an increase in the yield of mycelium and its protein content. Enzymatic hydrolysis is an environmentally friendly process, since there is no threat to human health. On the other hand, acid hydrolysis requires the use of strong inorganic acids that emit harmful emanations. In this regard, grain processing wastes were pretreated using "Alfalad" and "Glyukolad" enzyme preparations produced by Ladyzhensky factory.

Basidial mushrooms grown under artificial conditions (including Pleurotus ostreatus) are promising producers of environmentally friendly proteins and essential amino acids. Mushroom biomass production using deep cultivation conditions with the optimal growth environment and favorable nutritional medium, is a cheap and affordable way to obtain high-quality food products [19]. The mycelium of the Pleurotus genus cultivated on liquid nutritional medium contains 40-50% of crude protein and 30-40% of true protein. These proteins are considered valuable biological compounds and are characterized by a good digestibility, and the mycelium contains all the essential amino acids. The digestibility of proteins of mushroom biomass obtained by deep cultivation represents 59.3-79.4%.

Thus, one of the potential plant substrates for mushroom cultivation is starch-containing grain waste from the grain processing industry. Therefore, the purpose of this work is to study the potential use of grain waste for the cultivation of golden oyster mushroom (Pleurotus citrinopileatus).

2. Methods

The study of the use of grain waste for the cultivation of golden oyster mushroom (Pleurotus citrinopileatus) was carried out in laboratory conditions with the following experiment scheme: 1. Control - wheat straw; 2. Grain waste; 3. Grain waste + birch sawdust (in equal proportions); 4. Birch sawdust. The experiment was performed in triplicate.

The studied substrates were filled into cotton bags and placed in fermentation tanks at a temperature of 60–70º C for 23–24 hours while basifying water with quicklime at a rate of 0.7–1 kg per 100 liters of water. After that, the substrates were autoclaved. Oyster mushroom mycelium was inoculated after cooling the substrate to 28º C [19]. The object of this study was the mycelial culture of golden oyster mushroom.

The grain spawn planting was performed by uniform mixing with the substrate (moistened to 70%) in the amount of 3% by weight. Then, polyethylene bags were tightly packed, tied and placed in a thermostat environment at + 25º C to colonize the substrate with mycelium. After that, 6 cuts of 4-5 cm longs were made with a sterile scalpel in a staggered manner along the entire length of the bags. The bags overgrown with mycelium were placed in a room where humidity was maintained at 85-90% and the temperature between + 18º and 19º C under natural light. Water tanks and a fan were used in order to maintain the high humidity in the room. At the beginning of the mushroom fruiting, the actively
liberated carbon dioxide was removed from the room by aerating and turning on the fan. During the month, we monitored the growth, fruiting bodies formation and the yield of golden oyster mushroom Pleurotus citrinopileatus were monitored for a month [15–17]. If infected sites appeared in the bags, they were removed and treated with a solution of sodium chloride at 250 g/1 liter of hot water [22].

3. Results and observations
Wastes of the grain processing industry include: bran, wastes associated with the cleaning and sorting of grain mass (grain waste), grain weed impurity, injured grains, feeble and germinated grains, seeds of wild plants, substandard grains [21].

During the analysis of the studied grain processing wastes, we isolated two fractions: a fraction with a predominant starch content and another with a predominant fiber content (Table 1). The starch-containing fraction represented 58.2% in 1 kg of waste, and, the fraction containing a predominance of fiber - 41.8% by weight in 1 kg of waste.

| Fraction | Fraction composition | Content in 1 kg of waste, % |
|----------|----------------------|---------------------------|
| Predominantly starchy | Grain waste, damaged grains, puny and sprouted grains, wild plants seeds, substandard grains | 58,2 |
| With a predominance of fiber | Bran, chaff, remnants of straw and weeds | 41,8 |

Substrates for mushroom cultivation must have a number of properties, most important of which are biotechnological - first of all, the ability of the substrate to meet the nutritional needs of the mushroom. Easily available substances are less significant for oyster mushrooms than for its competitor, mold fungi; especially in the first days after grain spawn planting. Oyster mushroom refers to fungi that are equally capable of destruction, both cellulose and lignin, which are highly resistant to biodegradation. Among the competitors of the oyster mushroom, a special place is given to such fungi as Trichoderma green mold. In the course of its vital activity, it can utilize cellulose and very quickly develop on substrates in the presence of easily accessible nutrients.

The selectivity of the substrate is one of the most important biological properties. It is determined by the chemical composition of the raw material and accordingly, by the activity of the beneficial microflora located on its surface. The biological selectivity of a substrate is the ability of a thermostreated substrate to acquire the most favorable conditions for the development of oyster mushrooms and to put the competitive microflora into an inactive state.

In various degrees, any type of vegetal raw material may acquire these properties that are of key importance for the production process. The biochemical mechanism of the acquisition of selective properties by substrates is based on a change in the substrate content in readily available sugars. The effect high-temperature on the substrate leads, upon prolonged exposure, to the chemical hydrolysis of polysaccharides and the accumulation of readily available substances and principally sugars.

When selecting the appropriate substrate for the cultivation of basidiomycetes, a great attention is paid to the carbon and nitrogen contents. The most suitable substrates are hay clover, wheat straw and sunflower husk. Grain waste requires the conversion of its polysaccharide complexes into easily digestible di- and monosaccharides in order to increase its protein content. This is achievable for example, by acid or enzymatic hydrolysis [6] as well as thermal or chemical treatments of the substrate for the suppression of the microflora development.

A comparative analysis of the chemical composition of traditionally used vegetable substrates in the mushroom production industry (Table 2) shows that this raw material (grain waste) contains 12.5% protein, 3.0% of fat; fiber -7.2% and therefore can be used for the cultivation of mushrooms.
The rate of substrate coverage strongly depends on the seeding rate of oyster mushrooms. The higher it is, the faster the biofouling of the substrate. The development of mycelium also depends on the quality of the substrate, since competitive organisms inhibit and sometimes completely inhibit the growth of mycelium. In the first week of incubation, the mycelium grows in thin threads covering the entire substrate block- this is the stage of colonization. At the end of this stage, a small thickened cushion of mycelium can already be seen around the perforations: the primordia mushrooms will be formed on this roller in the future. During the second week of incubation, the nutrients of the substrate are being consumed and the mycelium network starts developing and thickens - the block becomes whiter and whiter, firmer to the touch. The best time to transfer the substrate blocks to the mushroom fruiting workshop is when the whole substrate is covered by mycelium, but fungi in the form of stroma has not yet formed a strong seal and the mushrooms from perforation did not appear yet. The dynamics of mushroom biofouling of the substrate are presented in table 3.

| Substrate type | Date of occurrence | Primordium formation | 1st mushroom harvest | 2nd mushroom harvest |
|----------------|--------------------|----------------------|----------------------|----------------------|
| Control - wheat straw | 2.12 | 15.12 | 21.12 | 28.12 |
| Grain waste | 2.12 | 19.12 | 23.12 | 29.12 |
| Grain waste + birch sawdust | 2.12 | 17.12 | 24.12 | 30.12 |
| Birch Sawdust | 2.12 | 18.12 | 24.12 | 30.12 |

Full biofouling of the substrate is determined visually. If necessary, cross-sections of the substrate into units help determine the uniformity of the growth of mycelium in the thickness of the substrate. The overexposure of blocks or reincubation cause either the appearance of stroma or the appearance of oyster mushroom primordia.

In our study, the golden oyster mushroom showed a high colonization rate of the substrate. Primordia began to form at 13-17 day. The first substrate completely colonized was the wheat straw substrate, and the last was the grain waste substrate on day 17. A possible reason for this is a relative decrease in the intensity of gas exchange in the volume of the starch-enriched substrate, which led to a slower formation of primordia and later, of fruiting bodies and accordingly, an increase in the consumption of the substrate to support the vital processes of the mycelium [22].

Oyster mushroom under high levels of carbon dioxide (as, for example, in the incubation chamber) forms a larger number of fungal primordia than with an excess of fresh air. When overexposing primordia for 1-2 days, deformed and damaged fruiting bodies appear.
The yield of the oyster mushroom Pleurotus citrinopileatus grown in laboratory conditions is presented in Figure 1.

According to the obtained data, the fruiting period of golden oyster mushrooms on the studied substrates represented 26-28 days. This duration was more stretched on the variant auditioned of sawdust. The maximum and the minimum yields were obtained on wheat straw substrate (171.2 g per kg of substrate) and grain waste substrate (113.4 g per kg) respectively. On birch sawdust substrate and on the substrate of grain waste and birch sawdust mixture, the yields reached 143.2 g and 130.3 g in this order.

The most intense strain recovery was reached during the first wave of fruiting on grain waste and wheat straw with 70.0 and 56.7% of the total yield, respectively. Such a high product recovery yield obtained on the substrate of grain wastes is probably associated with a high content of available starch. The product recovery was the lowest on the birch sawdust substrate (47.3%) due to its high cellulose content. The mushroom yield in the first and second waves was approximately the same on the substrate with on the substrate of grain waste and birch sawdust mixture (49.3 - 50.7%).

Under industrial conditions of oyster mushroom production, the most profitable is the two-week harvest window, which shortens the production cycle duration and makes it possible to increase the number of crop turns per year.

In the course of the experiment, the initial dry masses of all organic substrates were taken into account; at the end, the mass of the fruiting bodies and the amount of undigested (residual) substrate were taken into consideration (Fig. 2).
The largest amount of undigested substrate residue was obtained when using grain waste in its pure form - 79.0%, which was related to a mushroom yield of 11.3%. When using grain wastes with the addition of birch sawdust, the degree of digested substrate increased to 69.6%, and the mushroom yield increased to 13.0%, which is lower than the control by 2.9 and 23.9% respectively.

In this way, it can be stated that increasing the nutritional value of the substrate by adding grain processing waste results in a limited increase in the mushroom yield of Pleurotus citrinopileatus, since at the same time, the gas exchange intensity decreases as well as the degree of substrate conversion. Therefore, cellulose-containing components must be added to the substrate with grain waste. Thermal or chemical treatments are also needed to suppress the development of microflora.

4. Conclusions
Grain wastes and grain processing wastes are harmless and easily amenable to enzymatic and microbiological bioconversion. Golden oyster mushroom (Pleurotus citrinopileatus) grown under laboratory condition on grain processing wastes demonstrated a high rate of substrate colonization. The average yielding crop attained 113.4 g of fresh mushrooms grown on 1 kg of substrate under a relative humidity of 70-75%. This promising work stream not only proposes a waste disposal method, but also, a significant source of income for the grain processing enterprises.

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