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Mushroom cultivation on substrates with addition of anaerobically digested food waste

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Abstract

Cultivation of mushrooms is an important biotechnological process which converts a wide range of agro-industrial residues into mushroom growing substrates and produces healthy food. Conventional cultivation of *Agaricus* spp. is performed in composted straw, with chicken manure as the main source of nitrogen triggering the composting process. Anaerobically digested organic waste, i.e. food waste, is comprised of fibrous waste residues and anaerobic bacteria, high in nutrients. The digestate is a rich source of nitrogen, with high pH (8.2) which is favorable for mushroom substrate preparation. Recent focus on renewable energy production has increased the interest in anaerobic digestion – recovering energy, rendering nutrients available for use in agriculture, which makes digestate an easily accessible waste material. The objective of this work was to evaluate usefulness of digestate as a high nitrogen supplement for mushroom cultivation, and waste paper as a carbon source. Yield of three species were compared: *Agaricus arvensis*, *A. bitorquis* and *A. subrufescens*. Mushrooms were cultivated on substrates with increasing amounts of dewatered digestate, ranging from 0 to 40% (DW/DW), with intervals of 10%. For the control, commercial compost was used. Dewatered digestate was added at the start of phase I composting. Substrate preparation was performed according to Stoknes et al. (2013). Results of the present study suggest composted Straw-Paper-Digestate substrate can be used for mushroom cultivation of investigated species compared to control compost.

Keywords: *Agaricus subrufescens*, *Agaricus bitorquis*, *Agaricus arvensis*, yield, biological efficiency, digestate, paper

INTRODUCTION

Cultivation of common edible button mushroom, *Agaricus* spp., is conventionally conducted on a substrate composted from straw, straw-bedded horse manure with chicken manure and gypsum. Composts are prepared in three phases: phase I, mixing and thermophilic composting at up to 80°C; phase II, pasteurisation (60°C) and conditioning (48°C) in controlled tunnels; and phase III, spawning and growth of the mycelium. However, due to some disadvantages with this traditional compost preparation process, like long processing time (approx. 3 weeks), high water demand and high CO_2 footprint there is a demand for alternative methods and substrates to improve efficiencies of production.

Anaerobic digestion (AD) treatment processes are suited for the utilization of organic wastes from agriculture, industry and food waste from households. AD is a cost-effective method for treating biogenic wastes because the produced biogas can be used for electricity and heat and effluent from the digester can be used as a secondary fertilizer (Haraldsen et al., 2010). Anaerobically digested organic waste is comprised of the more resistant fibrous waste fractions and the anaerobic bacteria, high in nitrogen, phosphorus and sulfur, with a pH of 8.2 which is favorable for mushroom substrate preparation. Digestate from AD was successfully used in previous composting studies for *Agaricus bisporus* and *Agaricus subrufescens* by Stoknes et al. (2013). Availability of the cultivation substrate components is one of the most important factors deciding their commercial utilization. In Poland 93 biogas

plants operate at sewage treatments plants, but the number of agricultural biogas plants is not yet very large (ca. 60 working agricultural biogas plants) (Igliński et al., 2015; Urząd Regulacji Energetyki, 2015).

Moreover current interest in improving mushroom compost production technology has focused on waste utilization, such as pulp and paper waste use. Shredded paper, an inevitable element of modern society is difficult to recycle but is a good option for composting to use as a cellulose rich source for mushroom growing substrates.

Three different mushroom species of the *Agaricus* genus were investigated within the scope of this research. The choice was triggered by focusing on less commonly cultivated mushrooms with individual features such as tolerance for lower relative humidity in the cultivation chamber; higher temperature demands; pests and diseases resistance, as an alternative for commonly used *Agaricus bisporus*. The Almond mushroom- *Agaricus subrufescens* prefers warm temperatures of 23-27°C (Chang, 2008). This species is considered as a medicinal mushroom containing bioactive polysaccharides and protein complexes (PSPC) which have been shown to function as potent antioxidants, antitumor, and anticancer agents (Endo et al., 2010; Ishii et al., 2011; Lima et al., 2011). More resistant to viral diseases and bruising, the Pavement mushroom – *Agaricus bitorquis*, can also grow at warmer temperatures (22-30°C) and higher CO_2 concentrations (up to 2000 ppm during cropping). *Agaricus arvensis* – the Horse mushroom, was found to secrete efficient cellulases for saccharification of woody biomass (Jeya et al., 2010). The carpophores have pleasant aniseed-like odor. This species is shows more robustness to pest damage and performs better in lower temperature (15-22°C) and lower humidity (75-85%).

The main goal of our investigation was to combine waste from renewable anaerobic biogas production with recycled waste office paper as a basic component in mushroom substrate for mushroom cultivation.

MATERIALS AND METHODS

Spawn of the three mushroom species was purchased from the mushroom spawn laboratory MYCELIA bvba, Belgium. Five composts were used, one a control of conventional compost and four composts with different levels of digestate, straw and paper were compared (Table 1). The composition was based on dry matter of substrate before processing.

Table 1. Composted substrate composition [%] used in the study. Substrate COM0 is conventional commercially available substrate used as a control.

Substrate	Straw	Paper	Chicken manure	Aenorobic digestate	Gypsum	C:N ratio
COM0	82	0	15	0	3	20:1
SPDG10	28	56	3	10	3	60:1
SPDG20	25	50	3	20	3	40:1
SPDG30	21	42	3	30	3	30:1
SPDG40	18	36	3	40	3	20:1

The digestate from AD of source separated household food waste was separated using a decanter centrifuge. The resulting digestate solid was added to phase I substrate, and further prepared according to commercial *Agaricus* production, as reported by Stoknes et al. (2013). For the controls, cultivation compost was purchased from commercial compost producer HAJDUK, Poland.

Compost preparation

1. Phase I – the composting process.

The substrate was composted in rotating composter drums. After few days the compost loses volume due to heat generation and was mixed and moved to insulated

containers with controlled air flow through the substrate (to obtain similar conditions as in commercial bulk systems; 70-80°C and 6-9% O_2 v/v). The substrate was turned subsequently again twice at an interval of 2 days, giving total of 10 days for phase I.

2. Phase II – pasteurization.

The compost was loaded into a miniature phase II tunnel and the temperature was gradually increased to 60°C over 24 hours and then maintained for 6 hours of pasteurization, then decreased to 55°C, for another 6 hours and subsequently to 50°C where it was maintained for conditioning until ammonia (NH_3 gas) had dissipated (below 10 ppm). More detailed description of Phase I and Phase II processes are described by Stoknes et al. (2013).

Experiment set-up and measurements

For all cultivation experiments, 50 micron polypropylene, autoclavable bags (capacity of 7 L, flat size 38 cm wide × 57 cm high), with four linear ventilation filters were used. Bags were filled with approximately 3 kg of pasteurized compost and left to cooling to room temperature (22°C). The compost was then inoculated with granular spawn on wheat grain according to the method of Lemke (1971), applied at an amount of 3% of fresh substrate weight. The bags were sealed with adhesive tape and shaken by hand until the spawn was evenly distributed.

The spawning occurred in stable, controlled conditions, at 25°C. After the spawn overgrew the substrate, bags were opened and a 5 cm layer (700 g) of casing was applied and moved into the growing chamber (bubble green house). Mushrooms were placed in the chambers located under the tables used for plant cultivation. The temperature inside the chambers was held according to the recommendation for each species. The air humidity for fruit body development was held at 85-95%. The cultivation chambers received indirect natural light from the sides. The cultivation room was aired so that CO_2 concentration did not exceed 1000 ppm.

The first flush was harvested 5 weeks after inoculation. For *A. subrufescens* 4-5 flushes were harvested; for *A. bitorquis* and *A. arvensis* 3-4 flushes were harvested. Yields were determined as weight of harvested fresh mushrooms from the whole cropping period per fresh weight of substrate at inoculation.

Each mushroom species was treated as a separate experiment. Experiments were established in fully randomized design, in 4 replications and 2 cultivation cycles. When comparing the experimental results, the analysis of variance for randomized block with 5 composts treatments was applied (level of significance α=0.05). The results of cultivation experiments were discussed based on mean values from cultivation cycles.

RESULTS AND DISCUSSION

An aim of the present study was to evaluate the possibility of utilizing waste from renewable anaerobic biogas production with recycled waste office paper as a basic component in substrate for mushroom cultivation. The yield response of the three species used in this study differed depending on the composition (amount of digestate, paper and straw) of the cultivation substrate (Figures 1-3).

Yield of *Agaricus arvensis* was highest when produced on commercial compost. Other investigated composts performed significantly lower in terms of yields. The second best yield was obtained on substrate SPDG40. Two substrates, SPDG10 and SPDG30 had similar yields, but were significantly lower compared to SPDG40 and the Control substrate. The lowest yield was obtained using the SPDG20 substrate (Figure 1).

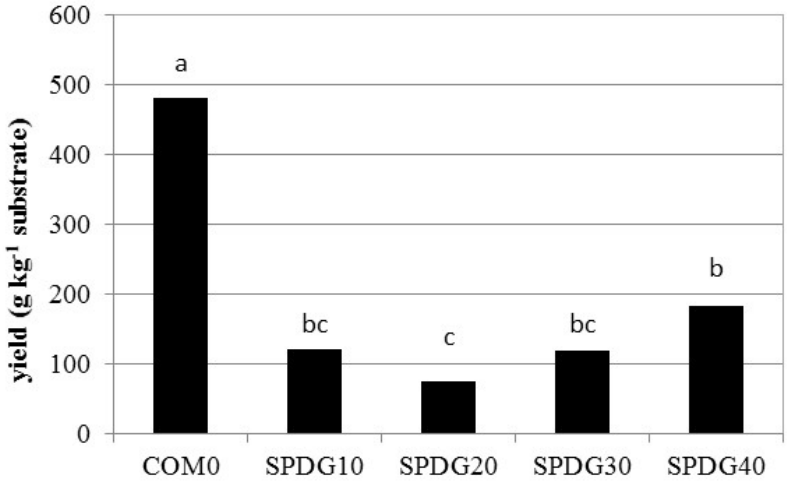

Figure 1. Yield *A. arvensis,* on different compost types (g kg^{-1} FM substrate). Details of composts can be found in Table 1. Different letters indicate significant differences by Duncan's test (α=0,05).

Yields of *Agaricus bitorquis* increased with the amount of anaerobic digestate in the cultivation substrate (Figure 2). Yields were significantly higher on substrates with 30 and 40% AD, compared with the control substrate and other substrates. However, there was no significant difference in yield between SPDG30 and SPDG40. The yield from substrate SPDG20 was similar to that grown with the Control compost. The lowest yield of *A. bitorquis* was obtained on substrate SPDG10.

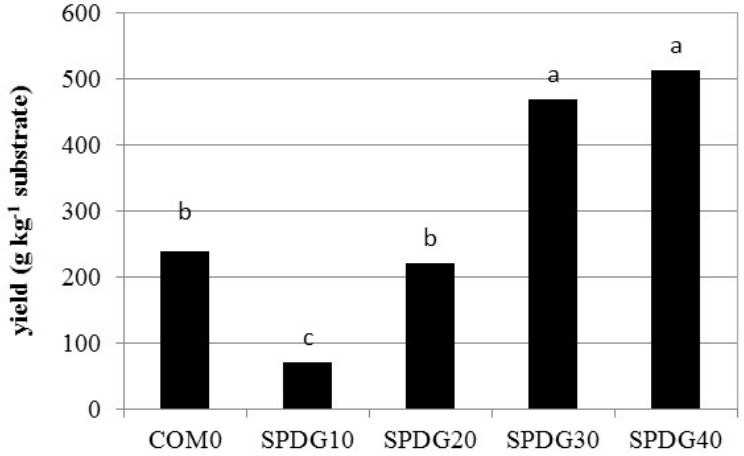

Figure 2. Yield of *A. bitorquis* on different compost types (g kg^{-1} FM substrate). Details of composts can be found in Table 1. Different letters indicate significant differences by Duncan's test (α=0,05).

Yield of *Agaricus subrufescens* differed significantly between the substrates with increasing amount of digestate and the control compost. The yield of *A. subrufescens* was highest on control compost and the second highest yield was obtained on SPDG30. The lowest yields were harvested from substrate SPDG20 (Figure 3).

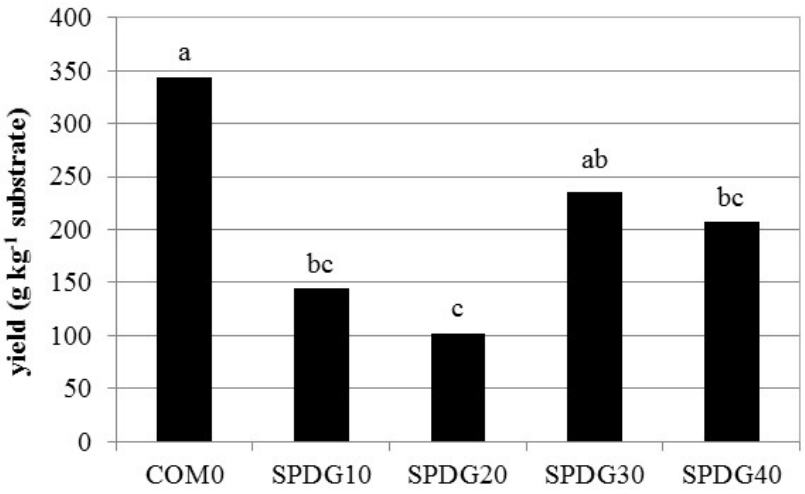

Figure 3. Yield *A. subrufescens* on different compost types (g kg^{-1} FM substrate). Details of composts can be found in Table 1. Different letters indicate significant differences by Duncan's test (α=0,05).

All compost types supported growth and produced mushrooms. For the digestate composts, yield of fruiting bodies of the investigated species depended highly on the substrate composition. Yields tended to increase with increased digestate in the substrates, however the amount of straw and paper were decreasing. Variations in yield with substrate types are expected and differ between species (Philippoussis and Diamantopoulou, 2000).

In comparison to other species from the genus *Agaricus*, yields of *Agaricus subrufescens* are reported to reach 80-200 g kg^{-1} fresh substrate (da Eira et al., 2005; Llarena-Hernandez et al., 2013), which is relatively low yield. *Agaricus bitorquis* yield is reported to range from 170-220 g kg^{-1} (Kariaga et al., 2012) and *Agaricus arvensis* from 170-230 g kg^{-1} (Noble, 1995). The average yield of the commonly used *Agaricus bisporus* is reported to range from 138-420 g kg^{-1} (Sharma et al., 2004; Kariaga et al., 2012). In the present study yields ranged from: 74-481 g kg^{-1} for *A. arvensis*, 70-512 g kg^{-1} for *A. bitorquis* and 102-344 g kg^{-1} for *A. subrufescens*.

Adequate addition of nitrogen to a substrate rich in C considerably improves mycelium growth and quality of fruiting bodies and an optimal nitrogen content should be 1-1.5% (Andrade et al., 2007; Siqueira et al., 2011). The selection of the nitrogen source is essential, since mushrooms from the division *Basidiomycetes*, such as *Agaricus* mushroom, do not produce nitrate reducing enzymes (Gerrits, 1998). C:N ratios in the cultivation substrate vary for different mushroom species though a C:N ratio of 20:1 is suitable for mycelial growth of most fungi (Chang and Miles, 1989). For *A. subrufescens*, urea is the best source of nitrogen and the most advantageous C:N ratio ranges from 10:1 up to as much as 50:1 (Mantovani et al., 2007). Substrate SPDG40 contained the highest amount of digestate (lower in straw and in paper) and a C:N ratio of 20:1, exactly the same as for commercial compost. As a C:N ratio of 20:1 is appropriate for most mushrooms, it is understandable that all of the investigated species yielded better when the substrate contained close to that C:N ratio i.e. the commercial compost and substrate SPDG30 and SPDG40. This effect was most visible within *A. subrufescens* and *A. bitorquis* (Figures 2 and 3).

De-watered food waste digestate is a nutrient rich organic material similar to chicken manure (Noble et al., 2002; Savoie et al., 2011; Stoknes et al., 2013). However, AD contained more compact lumps than chicken manure, which influenced the physical mixing properties. After subsequent addition of water and extended manual mixing, an acceptable structure was achieved, comparable to structure of commercial compost (Stoknes et al., 2013). High digestate content saves straw, which makes it an environmentally friendly option.

In previous investigations made by Stoknes et al. (2013) mushroom production on compost using AD biogas production was also successful with *A. subrufescens* yielding on the

average flush 187 g kg^{-1}. This compares with average yield of *A. subrufescens* reported in this present study of 172 g kg^{-1} grown on digestate composts. In the present study, the highest yield of *A. subrufescens* of 236 g kg^{-1} was obtained when grown on substrate SPDG30 with 30% digestate, comparable with reported yields of 200 g kg^{-1} in substrate with 36% of digestate (Stoknes et al., 2013). Lower yields were obtained when grown on substrate with the highest amount of digestate in both studies (207 g kg^{-1} for SPDG40 in the present study and 161 g kg^{-1} for 47% of digestate reported by Stoknes et al., 2013). There is limited data on the cultivation of *A. arvensis* and *bitorquis* mushrooms on dewatered digestate from anaerobic digestion of biogas production. Our study shows that higher amounts of digestate (40% AD) can be successfully used for cultivation of *A. bitorquis* yielding better than when grown in the Control substrate (512 and 238 g kg^{-1} respectively) but further study is needed to identify the best composition of cultivation substrate for *A. arvensis* (Figures 2 and 3). The demand for finding disposal methods of digestate will be increasing since the number of biogas stations is growing, and mushroom cultivation, with its high nitrogen demand has high potential in using this digestate.

CONCLUSIONS

- Composted Straw-Paper-Digestate substrate can be used for mushroom cultivation in comparison to conventional commercial composts.
- *Agaricus arvensis* and *Agaricus subrufescens* yields are lower when using paper-digestate based compost compared to the conventional compost.
- *Agaricus bitorquis* yields are higher in the paper-digestate based compost compared to the conventional compost.
- Digestate from anaerobic biogas production seems to be a good alternative source of nitrogen for mushroom cultivation.

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