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Original Article

Growth of Mycelium of Different Edible and Medicinal Mushrooms on Medium Supplemented with Digestate from AD Biogas Plant

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Abstract

Digestate is remaining material after anaerobic digestion (AD) of a biodegradable feedstock. The AD process produces two main products: digestate and biogas; digestate is considered as a waste; however, it found application in agriculture i.e. as a soil conditioner or cultivation substrate component. Digestate is rich in nitrogen and phosphorus and can be used as a substrate component for mycelial growth of mushrooms. Aim of the study was examination of agar media supplementation with digestate from AD of foodwaste material on mycelial growth of cultivated and medicinal mushrooms. Mycelial growth of four mushroom species was investigated: *Coprinus comatus*, *Ganoderma lucidum*, *Agaricus subrufescens* and *Laetiporus sulphureus*. First experiment investigated four mushroom species and 3 digestate-only based agar media with growing amount of digestate extract. Later fastest growing mushrooms from exp. 1, *C. comatus* and *G. lucidum,* was chosen, mycelial growth was performed on 2 digestate-only based agar media and 2 what/digestate extract based agar media. Wheat-based and manure-based agar media was used as control medium. Study confirmed positive effect of digestate from AD as an addition or main component of agar media culture for mushroom mycelium growth. The amount of digestate extract affected mycelial growth of investigated species. The best agar media was digestate-based only with 25 g of extract and wheat/digestate based (150/50 g extract). The fastest and the best growth were obtained for *C. comatus.* The residue from AD biogas production could have found additional application as media product for mushroom mycelia production and further as a component for mushroom cultivation substrate.

Keywords: agar media, anaerobic digestion sludge, cultivated fungi, spawning, substrate, utilization of waste

Introduction

Many species of mushrooms are prized for their nutritional value, flavour, and wide use: in medicine, bakery, food industry and cosmetology. The nutritional value of individual species of mushrooms determines their chemical composition. Mushrooms are characterized by high content of water, which causes their energy value to be low, i.e. about 50-70 kcal/100 g (Rajewska and Balasińska, 2004; Dadakova *et al*., 2009), whereas their dry matter consists mainly of proteins (Chang and Miles, 1989). Several species, such as *Ganoderma lucidum* – Reishi; *Agaricus subrufescens*; *Coprinus comatus* or *Laetiporus sulphureus*, besides culinary and gourmet features characterizes with numerous health and medicinal properties.

Mushrooms are saprotrophic organisms. The source of nutrition for saprotrophic organisms is organic matter, living or dead. In nature mushrooms grow on very diverse substrates, such as hardwood, coniferous wood, recombined plant and animal residues. In cultivated conditions, as a substrate, the most common types of waste are agricultural, horticultural or forestry waste. One of such waste is post-fermentation mass. Digestate is the remaining material after anaerobic digestion (AD) of a biodegradable feedstock. The process of AD produces two main products: digestate and biogas, whereas digestate is considered as a waste, however it found application in agriculture i.e. as a soil conditioner or cultivation substrate component, stand-alone fertilizer. Digestate is especially rich in nitrogen and phosphorus, it can be used as a substrate component for mycelial growth of mushrooms. Whereas, conventional cultivation of mushrooms is performed bags or bed cultures, and the substrate used is composted wheat straw, cotton waste, corncobs, rice straw, with chicken manure supplemented by

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solid sisal waste as the main source of nitrogen triggering the composting process. Recent interest in improving production technology focused on various kinds of waste utilization.

Source separation of household bio-waste was introduced as a strategy to reduce the amount of organic waste deposited in landfills, stimulated by European ban on land filling of wastes containing more than 100 g kg^{-1} total organic carbon. The organic waste should be recycled on agricultural land. Anaerobic digestion (AD) is a good way of utilizing organic wastes including food waste; this treatment reduces CO_2 emissions from landfills and the biogas can be used in power production (Weiland, 2000; 2010). Moreover, AD extracts contain the nutrients available for use in agriculture, and reduces odors by a closed process (Singh *et al*., 2010). Anaerobically digested organic waste is comprised of the more resistant fibrous waste fractions and the anaerobic bacteria, a rich source of carbon (340 g kg^{-1}) and nitrogen (15-18 g kg^{-1}), phosphorus (5-13 g kg^{-1}), calcium (13-43 g kg^{-1}), magnesium (2.8-4.2 g kg^{-1}) and sulfur (4.6-6.9 g kg^{-1}), with a pH of 7 to 9.8 which is favorable for mushroom substrate preparation (Govasmark *et al*. 2011; Hanc *et al*., 2011). Digestate from AD was successfully used in previous studies on *Agaricus bisporus* and *Agaricus subrufescens* by Stoknes *et al*. (2013; 2016) and Jasińska *et al*. (2016a and 2016b). Availability of the cultivation substrate components is one of the most important factors deciding on their commercial utilization. In Poland 93 biogas plants operate at sewage treatments plants, however the number of agricultural biogas plants is not yet very large, ca. 60 working agricultural biogas plants (Igliński *et al*., 2015, after Urząd Regulacji Energetyki, 2015).

The first phase of mushroom production is to obtain a healthy mycelium on agar media. Mediums are artificially prepared substrates with a chemical composition and physical properties suitable for the development of micro-organisms. According to Gołębiowska *et al*. (2005), medium must contain the necessary nutrients and growth factors, as well as appropriate osmotic pressure and proper pH. The authors also divides media by the chemical composition of the medium based on natural (organic), synthetic or semisynthetic components.

Organic media are substrates with not fully defined chemical composition. They include medium such as wheat and manure. Organic substrates can also be obtained from wort, milk (whole milk or skimmed milk), eggs or nutrient broth (Różalski, 1996). Synthetic substrates are mainly substrates whose qualitative and quantitative composition is well known and based on chemically defined components (Trojanowska *et al*., 1996). After adding artificial extracts from plant or animal tissues to the substrates, we will obtain a semisynthetic substrate. Trojanowska *et al*. (1996) states that the media can be divided by consistency: liquid and solid. Agar, polysaccharide derived from marine seaweed, is most commonly used to solidify the media, is a constituent only solidifying because it is not broken down and absorbed by microorganisms, except in rare cases (Drewniak and Drewniak, 1994). Media should be clear and all ingredients should be completely dissolved. Substrates for microbial culture should have easy access to the source of biogenic elements (C, O, H, N, P, S) and energy and mineral salts (Różalski, 1996). Mediums for mushroom inoculation are significantly different, but sugar, glucose, agar, cereal grains such as wheat or horse dung and distilled water are still essential ingredients. Good media should be properly prepared. Prior to inoculation, the medium should be sterilized in an autoclave (Sobieralski *et al*., 2011). The choice of medium depends on the type of microorganisms and depending on the intent and purpose of mycelial growth (Englbrecht, 2008). Suitable media for a particular species of fungus is to guarantee the best conditions for rapid growth of mycelium. The fast growth of good quality mycelium is crucial for successful mushroom cultivation.

The aim of the study was to demonstrate the effect of addition of digestate to growing media on mycelial growth of selected species of edible and medicinal fungi, i.e. *Coprinus comatus* (OF Mull.) Pers. *Ganoderma lucidum*, *Agaricus subrufescens* (Wesserr), *Laetiporus sulphureus* (Bull.) Murrill).

Materials and Methods

Biological material

Mycelial growth of five mushroom species was investigated: *Coprinus comatus* (O.F. Mull) Pers, *Ganoderma lucidum* (Curtis) P. Karst; *Agaricus subrufescens* (Wesser) and *Laetiporus sulphureus* (Bull.) Murril. Mycelium of the above species came from the collection of the Department of Vegetables of the University of Life Sciences in Poznan.

Coprinus comatus, known as shaggy mane, is the valuable culinary ingredient, nutritious and healthy (Liu and Zhang, 2003). As a member of *Agaricales* family, is frequently seen on the lawns, along gravel roads and in waste areas all over the world (Park and Lee, 2005), although in some areas limited by seasonal occurrence. The fruit is characterized by a floury, sweet taste and light in pleasant aroma. Fruiting bodies are very unstable, easy to decompose into black liquid, reminiscent of ink. Carpophores of *C. comatus* are valued source of nutrients and medicinal substances showing various health properties such as: antibacterial, anti-inflammatory antitumor action, lowering the blood sugar, and triglycerides cholesterol (Liu and Zhang, 2003; Han *et al*., 2006, 2008; Zaidman *et al*., 2008; Zhou and Han, 2008; Yu *et al*., 2009; Sabo *et al*., 2010).

Ganoderma lucidum W. Curt. called in Japanese Reishi, it is currently one of the most well-known and respected species of medicinal mushrooms. It exhibits medicinal properties, supports the treatment of digestive tract diseases (gastritis, intestines, liver), helps fight rheumatism, relieves nervous tension, is used for chronic inflammatory conditions. It has been shown that extractors from the Reishi can be used as a supplement to radio and chemotherapy for cancer, to prevent recurrence of cancer and to reduce the likelihood of metastases (Kwieciński, 2004). Active substances are found in mycelium and in fruiting bodies (carbohydrates, proteins, amino acids, fats, steroid compounds, volatile oils, vitamins B2, riboflavin and vitamin C, or ascorbic acid). The fruitbody also contains inorganic compounds - Fe, Cu, Ca, Mn, Zn (Chen, 2011).

The carpophore is extremely rich in polysaccharides, which account for 10 to 50% of the fruiting bodies (Siwulski *et al*., 2012). More than 200 different polysaccharides (Wasser, 2010) were isolated from this species. *G. lucidum* is a fungus widely used in the medicine of the Far East. The biggest producer of lacquer is China, but also in Korea, Japan, Taiwan and Malaysia. In China, the healing properties of Reishi have been known for nearly two thousand years (Kwieciński, 2004).

Brazilian button mushroom *Agaricus brasiliensis* (Wesser) syn. *Agaricus subrufescens* is valued not only for its taste but also for health reasons. Antifungal properties (Bernardshaw *et al*., 2005), antivirals (Sorimachi *et al*., 2001), antioxidant properties (Oliveira *et al*., 2007; Soares *et al*., 2009) and antiallergic properties (Ellertsen and Hetland, 2009; Mizuno, 2010). In addition, it lowers blood cholesterol, stimulates the immune system, and is effective for treating AIDS (Mizuno, 2010; Liu *et al*., 2008; Lima *et al*., 2011). Watanabe *et al*. (2002), Kim *et al*. (2005) and Liu *et al*. (2008) have shown that mushroom extract can also be used to treat diabetes, hypertension and viral hepatitis.

Laetiporus sulphureus (Bull.) Murrill is a saprotrophic fungus that causes decay of deciduous and coniferous trees. It appears in many countries and areas in America, Canada, China, Japan, Korea and Poland (Manzi *et al*., 1999). This fungus grows in large concentrations on live and dead stems of deciduous species (Alquini, 2004). In the last decades, this species is widely consumed for its nutritional and therapeutic value (Alquini, 2004). *L. sulphureus* is useful in the treatment of breast cancer and prostate cancer (Lee *et al*., 1975; List, 1958; Rapior *et al*., 2000). The carpophores appear mainly in the spring, May until June; however, it can grow until autumn in the woods, but most often in parks, by the roads, also in orchards and gardens. It often occurs in large concentrations up to 1 m^2 (Weber *et al*., 2004). Young fruiting bodies are edible, but in Poland are very rarely collected. This is also a dangerous parasite that kills deciduous trees, especially in parks (Gumińska *et al*., 1983).

Agar medium preparation procedure and materials

Digestate from source-segrated biodegradable foodwaste material was used. The digestate was obtained from Norwegian company Lindum AS, from Pilot food waste Biogas Plant under the EU project called F2W2F. Digested food wastes from biogas production were separated for solid and liquid fraction by filtration and centrifuging. Only solid fraction of the digestate was used in the experiment. The digestate was analyzed prior the experiment for the content of some important, from the mushroom cultivation point of view, macro and microelements. Digestate was dried at 105±5 °C for 72 h, ground for 5 minutes in laboratory mill and three representative dried subsamples were weighed. Microwave technique were used for decompose plant material. After mineralization all, the samples were subjected to HNO_3 and filtered into the 15 mL volume flask, after those samples were diluted with deionized water to a final of volume. Macro elements content of was analyzed by flame emission spectrometry. The dry matter of analyzed digestate was 36.83% of high pH-7.82 and conductivity ranging 171.67 mS/m. Content of elements was as follows: total nitrogen 17.9 g kg^{-1} (Kjeldahl), total carbon 340 g kg^{-1}; P – 9900 mg kg^{-1}; Fe – 6830 mg kg^{-1}; K – 5566 mg kg^{-1}; Ca 58166 mg kg^{-1}; Mg 5650 mg kg^{-1}; S – 5466 mg kg^{-1}.

Experimental design

Investigation was divided in two parts. In first experiment all four mushroom species were investigated and 3 digestate-only based agar media with growing amount of digestate extract were used (D1-50, D2-75 and D3-100 g).

For the second experiment fastest growing mushrooms from exp. 1, *C. comatus* and *G. lucidum* were chosen, mycelial growth was performed on 2 digestate-only based agar media of D4-25 g and D5-50 g of extract and 2 what/digestate extract based agar media in relation WD1-150/50 g and WD2-175/25 g respectively.

Wheat-based (W) (extract from 200 g of wheat grain) and manure-based (M) (extract from 50 g of dried, pasteurized horse manure) agar media were used as control medium in both parts of experiment.

Medium preparation procedures

The base for all mediums was: agar-agar (22 g); glucose (3 g) solved in 1 dm^3.

Wheat Medium: In order to obtain wheat medium 200 grams of wheat grains were rinsed under running water, 1dm^3 of distilled water was added and boiled for about 35 minutes until cracked grains were obtained.

Manure Medium: Dried horse manure was rinsed with water, then boiled for 30 minutes.

Digestate Medium: The fresh digestion was dried for 2 days in a laboratory drier. The dried mass was grounded, than 1 dm^3 of distilled water was added and boiled for about 35 minutes.

After cooking, the precipitates were poured through a sieve in an aqueous separation of the grains, wheat and undissolved components of the digestate. Clear liquids were supplemented with distilled water up to 1 dm^3. Then the agar-agar was dissolved in the prepared decoctions and other ingredients were added. The solutions prepared as described above were poured into 0.5 dm^3 flasks. The flasks were placed in a water bath for about 35 minutes, stirring until complete dissolution of the ingredients and a homogeneous solution. The flasks were closed with lignin plugs and protected with aluminum foil. The media was sterilized in an autoclave at 121 °C for 45 minutes and then spilled at 0.02 dm^3 into a sterile 9 cm diameter Petri dishes. The next day after preparation of the media, mycelium was inoculated with piece of overgrown mother spawn (about 5 mm diameter) was transplanted on agar medium under sterile conditions on a laminar flow table.

Incubation

The growth of mycelium was performed in the dark, in the incubators, where the temperature was maintained at 25 °C +/−1 °C. The incubation period lasted 9 days.

Measurements

A minimum of three mycelium measurements were taken at intervals of three days. In order to determine the effect of addition of digestate on growth of mycelium, measurements of diameter of mycelial colonies were made to the accuracy of 1 mm.

Statistical analysis

Both experiments were established in fully randomised design, in four replications and 2 inoculation cycles. When comparing the results, the analysis of variance for randomised block was applied, level of significance α=0.05; according to the Newman-Keuls. The results of mycelium growth were discussed based on the mean values from the cycles. The data obtained from the study were statistically analysed using the computer program STAT.

Results

Experiment 1: Mycelial growth of five mushroom species on investigated agar media after 9 days of incubation

The composition of growing media, various amount of digestate, influenced the growth rate of investigated mushroom species. After nine days of incubation, the fastest growth was demonstrated for two species of mushrooms, *Ganoderma lucidum* and *Coprinus comatus*, regardless growing media. Mycelium of *Agaricus subrufescens* showed moderately fast growth. The slowest growth was demonstrated by mycelium of *Laetiporus sulphureus* (Fig. 1).

Mycelium of all investigated mushrooms demonstrated the best growth on both control media: what medium and horse manure medium. Second best growth was shown on the digestate based medium with the lowest amount of digestate – 50 g (D1). The worst growth was demonstrated on digestate based medium with the amount of digestate 75 g and 100 g-D2 and D3 (Fig. 2).

Interaction between two factors of the experiment was found. After nine days of incubation mycelium of *G. lucidum* demonstrated the fastest growth on wheat and horse manure medium, also *L. sulphureus* showed the best growth on wheat and manure medium. *C. comatus* was growing fastest on horse manure medium and digestate based medium with 100g of digestate – D3. The slowest growth of *C. comatus* was demonstrated on digestate based medium D1. Mycelium of *Agaricus subrufescens* demonstrated best growth also on control media, on digestate-based media the growth was moderate. The worst growth rate was showed by mycelium of *L. sulphureus* on all digestate-based media (Table 1).

Experiment 2: Mycelial growth of 2 strains of Coprinus comatus and Ganoderma lucidum on investigated agar media after 9 days of incubation

Significant differences were demonstrated in the growth rate of investigated mushroom species their cultivated and wild strains. Regardless of used growing media the fastest growth was showed by wild strain of *C. comatus* CC01, next was the cultivated strain CC02, there were no statistical

Table 1. Growth of mycelium depending on the growing medium and fungal species after 9 days of incubation [mm]

Species/ Medium	*Laetiporus sulphureus*	*Agaricus subrufescens*	*Coprinus comatus*	*Ganoderma lucidum*
Wheat	82 a*	61 d	82 a	84 a
Manure	84 a	70 c	84 a	84 a
D1	10 fg	49 e	53 e	80 ab
D2	5 g	46 e	81 a	76 def
D3	15 f	49 e	84 a	72 de

* Note: Different letter between means denote significant differences (Newman-Keuls Test, α<0.05)

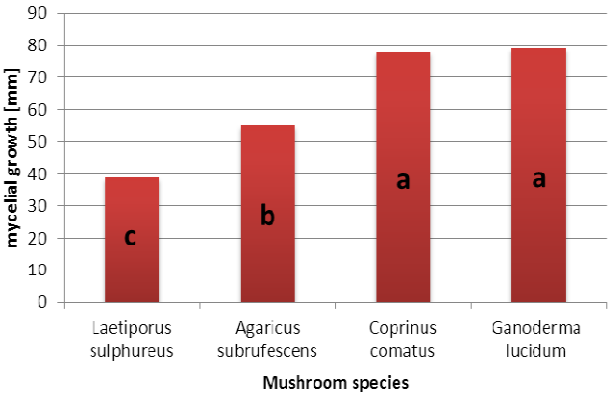

Fig. 1. Diameter of agar media overgrown by mycelium of five cultivated mushrooms after 9 days of cultivation [mm]. Note: different letter between means denote significant differences (Newman-Keuls Test, α<0.05)

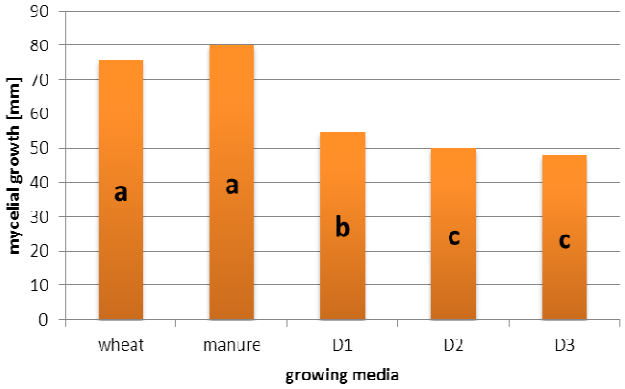

Fig. 2. Mycelium growth of investigated species on different growing media after 9 days of incubation [mm]. Note: different letter between means denote significant differences (Newman-Keuls Test, α<0.05)

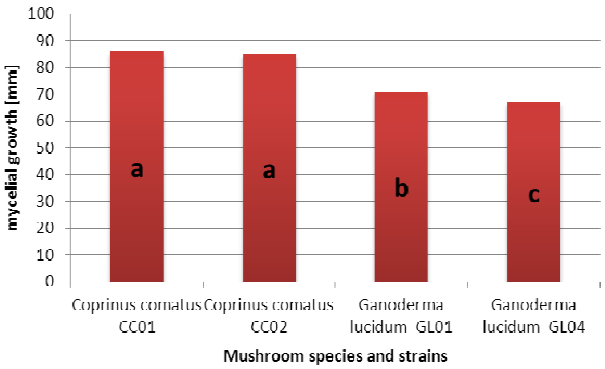

Fig. 3. Diameter of growing media occupied by mycelium of selected species and cultivars after 9 days of incubation, irrespective of medium used [mm]. Note: different letter between means denote significant differences (Newman-Keuls Test, α <0.05)

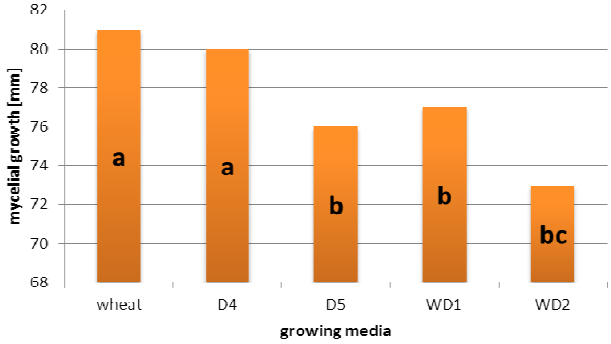

Fig. 4. Growth of mycelium of fungal species tested on agar media after 9 days of incubation, regardless of species and variety [mm]. Note: different letter between means denote significant differences (Newman-Keuls Test, α<0.05)

differences between two strains of *C. comatus*. Mycelium of *Ganoderma lucidum* cultivar showed moderate growth rate, whereas the wild strain demonstrated the slowest growth (Fig. 3).

The best growth of mycelium of investigated species and their strains was demonstrated on control – wheat media and digestate based media D4, with no significant statistical differences between both means. The average growth rate was demonstrated by two medium wheat-digestate based WD1 and digestate-based D5. The slowest growth was demonstrated on wheat-based medium WD2 (Fig. 4).

Interaction between two factors of experiment was found (Table 2). After nine days of incubation both *C. comatus* strains: cultivar and wild one fully overgrown the surface of Petri dish, 90 mm, on all investigated growing media. For *G. lucidum* the cultivar the best media was wheat and digestate based medium where the mycelium growth was the fastest. The worst growth of *G. lucidum* wild strain was showed on wheat-digestate based medium WD2. For cultivar the interaction was quite similar as for wild strain, the best growth was demonstrated on wheat medium and digestate based medium, however the slowest growth was shown on digestate based medium D4 and wheat-digestate based medium WD2.

Discussion

In the experiment, the influence of addition of digestate slurry after anaerobic digestion from biogas production on the mycelial growth and development of selected edible mushrooms and medicinal mushrooms was investigated. It was found that the mycelial growth of investigated mushrooms was dependent on the species of fungus and the composition of substrate used. The mushroom species used in the studies differed significantly in the growth of mycelium on investigated media.

In mushroom cultivation, the good quality mycelium biomass and fruiting bodies are most sought features. To achieve high yields, optimal substrate composition and conditions that influence mycelial growth and its enzymatic activity must be provided (Kalbarczyk and Jamroz, 1989). The rate of mycelial growth and the amount of biomass accumulated depends on the activity of lignin-cellulase enzymes (cellulose, hemicellulose and lignin), and the development of mycelium depends on the nutrient content of the substrate and the degree of their bioavailability, which translates into the time of the overgrowth of medium by the mycelium (Kalbarczyk and Jamroz, 1989).

Growth of mycelium depends on species of fungus, composition of medium and medium (Lomberh *et al*., 2002). The growth rate of mycelium is a very good and very common indicator of the identification and selection of growing media with the appropriate nutritional composition for every species. This indicator is not always consistent with the highest yield of fungi, but the high rate of mycelium overgrowth is associated with a reduced risk of growing media being inhabited by competing organisms (Stamets, 2000; Siwulski *et al*., 2010).

The conducted experiment confirmed positive effect of digestate from AD as an addition or main component of agar media culture for mushroom mycelium growth. The

Table 2. Growth of mycelium of selected species and their cultivars depending on the medium and species after 9 days of incubation

Species/ Medium	*Coprinus comatus* CC01	*Coprinus comatus* CC02	*Ganoderma lucidum* GL01	*Ganoderma lucidum* GL04
Wheat	90 a*	90 a	83 b	77 b
D4	90 a	90 a	84 b	70 b
D5	90 a	90 a	68 b	61 c
WD1	90 a	83 b	77 b	63 c
WD2	90 a	90 a	55 c	62 c

* Note: Different letter between means denote significant differences (Newman-Keuls Test, α<0.05)

amount of digestate extract affected mycelial growth of investigated mushrooms species. The best agar media for mycelial growth and development was digestate-based only with 25 g of extract and wheat/digestate based (150/50 g extract) (Fig 2 and 4). Increasing amounts of digestate in the growing medium provided to weaker growth of mycelium. All investigated mushroom species performed growth on digestate based-media however after 9 days of cultivation the fastest and the best growth was obtained for *Coprinus comatus* and *Ganoderma lucidum* (Fig 1 and 2).

An important factor determining the growth rate of mycelial fungi is the pH of the medium. Park, (2001) states that the most appropriate pH medium for rapid growth of *A. subrufescens* is pH 6 to 7. According to Colauto *et al.* (2008), for *A. subrufescens* optimal initial pH range from 5.5 to 6 and optimal initial pH value of 5.56 ± 0.05. Mycelial growth is inhibited with pH of 3 or lower, 8 or higher. For the *C. comatus* the pH of the medium should be in the range of 6 to 8 (Jang *et al*., 2009). According to Zeng, (2005) the pH of the *L. sulphureus* should be 2.8 to 3.2, while for the *G. lucidum* pH 4 to 5.5 (Vukojević *et al*., 2006; Kapoor *et al*., 2014). The pH of digestate used in the experiments was in the range of 7.82. This pH favors the development of most cultivated mushroom species. The experiment demonstrated that fungi tolerant to high pH of the substrate, ie the *C. comatus*, grew quite well with the substrate from digestate only. The lower the tolerance for high pH, the growth of the mycelium was weaker as in the *A. subrufescens*, or completely stopped as for *L. sulphureus*. Interesting dependency has been demonstrated for *G. lucidum*, for which the recommended optimum pH was 4.5 to 5.5, while in performed experiment the mycelium of *G. lucidum* grew very well, despite high pH of the medium (Fig. 3).

The digestate is rich in organic compounds necessary for proper mycelial growth very similar to the manure chicken, but contains more water and pellets of organic matter. According to Grzebisz (2009) liquid part contain mineral and water-soluble organic compounds, macronutrients: N, P, K, Ca, Mg, S and micronutrients: Fe, Mn, Zn, Cu, Mo. Nitrogen and phosphorus are mineralized to NH_4 and PO_4, and in this form they are much more available to plants and mushrooms than in pre-fermented form (Pontus, 2014). However, depending on the input substrate used, fermentation mass can have beneficial or toxic effects on plants (Schenknel, 2009).

As stated by Siqueira *et al*. (2011), 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 10-15 g kg^{-1} (Andrade *et al*., 2007; Siqueira *et al*., 2011). The selection of the nitrogen source is essential, since mushrooms from the division *Basidiomycetes*, do not produce nitrate reducing enzymes (Gerrits, 1988). The values analyzed in the used digestate show optimal content of total nitrogen, N – 17.9 g kg^{-1}, with higher amount of ammonium nitrogen, NH_4 - N – 2 g kg^{-1}, than nitrate nitrogen NO_3 - N – 416.67 mg kg^{-1} – which explains good growth of mycelium of investigated mushroom species on all media contained digestate. However, when higher, over 50 g l^{-1}, amount of digestate was used the growth rate of mycelium was lower. This might be due to the growing conductivity, making the medium to rich to force mycelium penetrate substrate for nutrients (when digestate only based medium was used – D2, D3 and D5). When mixed medium, wheat-digestate, was used higher amount of digestate, WD1-150 wheat/ 50 digestate extract, was used the mycelium growth was very good, this was due to more different sources of nutrients. However when more wheat extract was used (WD2-175/25), the growth of mycelium was significantly slower, most likely due to lowered amount of ammonium nitrogen (Figs. 2 and 4). Usefulness of mixed media or substrates was confirmed by numerous authors due to the nutrient richer environment, varied sources of nitrogen and carbon (Philippoussis *et al*., 2000; Uhart *et al*., 2008; Amin *et al*,, 2009; Jasińska *et al*., 2012; Jasińska *et al*., 2014).

C:N ratios in the cultivation substrate vary for different mushroom species: C:N ration 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). The research conducted by Möller and Müller, (2012) confirms appropriate pH of digestate, from 6 to 9, and that its C/N ratio is most suitable for the fungal environment. Which makes it proper material for media and substrate production, because it will not be necessary to change the pH or be concerned about the absorption of available nitrogen from soil, so called immobilization of nitrogen by microorganisms contained in biogas slurry. The C/N ratio of digestate used for growing media preparation was 18:1, which is of the same range as in commercial cultivation compost used for *Agaricus ssp*. cultivation, 20:1. Which is, as mentioned before appropriate C:N ratio for most of the mushrooms. Therefore, it is understandable that all of the investigated species performed with good mycelium growth when digestate was added to the growing medium. The higher amounts of digestate or higher amounts of wheat extract influenced the higher C/N ration which might decrease the mycelium growth rate. As mentioned earlier, good mycelium growth comprises often with good fruiting bodies production. In previous investigations made by (Stoknes *et al*., 2013; Jasińska *et al*., 2016a and 2016b) mushroom production on compost using digestate from AD biogas production was successful. *A. subrufescens* yielded on the average flush 187 g kg^{-1} in that study on digestate composts which encourages reusing the digestate not only in agriculture field production but also for mushroom production. The demand on 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

The residue from AD biogas production could have found additional application as media product for mushroom mycelia production and further as a component for mushroom cultivation substrate. The digestate is a waste product which subsequent use or utilization is a significant problem for companies producing biogas. Exploitation of digestate as a mushroom growing media/ substrate

compound is an environmental friendly and sustainable way of disposing the residue which goes along with assumptions policy of circular economy. Based on the experience, it was found that: 1. The composition of agar medium influenced the growth of mycelial mushroom species. 2. Addition of AD digestate to agar medium influenced significantly mycelial growth of the examined mushroom species. 3. The best growth of mycelium was found on digestate-based only with 25 g of extract and wheat/digestate based (150/50 g extract), regardless of species of fungus. 4. Regardless of the medium, the fastest growth of the mycelium was found in the *C. comatus* and *G. lucidum*.

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