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Production of oyster mushroom (*Pleurotus ostreatus*) on sawdust supplemented with anaerobic digestate

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ABSTRACT

Anaerobic digestion of organic waste results in production of biogas and a nutrient-rich digestate that has an established use as fertilizer in plant production. This study evaluated use of anaerobic digestate based on a high concentration of organic household waste as a fertilizer in sawdust-based production of oyster mushrooms (*Pleurotus ostreatus*). Inclusion of 0.5 L of anaerobic digestate (AD) per kg sawdust gave similar productivity in terms of biological efficiency (79.5 ± 5.4 %), and protein concentration (24.7 ± 2.4 % of dry weight (dw)) as standard mushroom substrate (78.1 ± 5.3 %, and 21.9 ± 3.0 % of dw, respectively). However, mushroom growth was impaired at the highest concentration of anaerobic digestate tested, 1 L digestate per kg dw sawdust. Comparison of the AD-fertilized substrate with a mushroom substrate with standard components (sawdust, wheat bran, calcium sulfate) and with similar C/*N*-ratio revealed some differences in elemental composition of the fruiting bodies, with an major increase in sodium concentration for the AD-fertilized substrate compared with the standard substrate (413.3 ± 28.9 and 226.7 ± 30.6 mg kg^{-1} dw, respectively). This difference can be explained by high sodium concentration in the anaerobic digestate, most likely due to inclusion of food scraps from households and restaurants in the biodigester feedstock. Screening of both substrates for a total of 133 micropollutants revealed that total sum of micropollutants was significantly higher in the AD-fertilized substrate (258 ± 12 ng/g dw substrate) than in the standard substrate (191 ± 35 ng/g dw substrate). Nitrogen losses during preparation of the AD-fertilized substrate were negligible.

1. Introduction

Effective waste treatment and circular production systems are key elements in a future sustainable and bio-based society. Anaerobic digestion of organic waste, a well-established technology that results in production of biogas (a renewable energy source) is performed on a large scale in several European countries (Scarlat et al., 2018). In parallel with production of the biogas, a nutrient-rich digestate is obtained as an end-product of the process. Use of this digestate as an organic fertilizer in crop production enables nutrient recycling in a circular system allowing for production of food and energy together with waste treatment. However, uptake of this technology on a wider scale has been slow and a driver for increased implementation has been identified as potential for co-operation between farms and energy companies (Winquist et al., 2021).

Anaerobic digestate is rich in important plant nutrients, such as nitrogen (N) and phosphorus (P), and has a mainstream use as a fertilizer for arable land (Walsh et al., 2012). However, it is of interest to develop additional areas of use, as long-term transport of the liquid digestate to agricultural fields must be avoided for economic and environmental reasons. Therefore use of the digestate for horticultural crop production in soilless cultivation systems and for production of microalgae is being researched (Fuldauer et al., 2018). However, few studies have evaluated use of anaerobic digestate as a fertilizer in mushroom production, despite N supplementation of mushroom substrate being critical for maximization of fruiting body production (Carrasco et al., 2018). From a nutritional perspective, mushrooms are an excellent foodstuff (Kalac, 2013), with the additional benefit of potential use as a meat substitute (Kumar et al., 2017). Internationally, there has been a huge increase in production of cultivated mushrooms in recent decades, with annual production increasing from 1 million ton in 1978 to 27 million tons in 2010, and with China as the main producer (Royse et al., 2017). Thus, using anaerobic digestate not only for plant production but also for production of edible mushrooms could further increase implementation

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of anaerobic digestion technology.

Cultivated edible mushrooms are the fruiting bodies of basidiomycetes. The mushroom production process is based on solid state fermentation, where the fungal mycelium grows through a substrate. The substrate used for the fermentation differs depending on the nutritional needs of the fungal species under cultivation. The most commonly produced mushroom in the Western world is button mushroom (*Agaricus bisporus*), a species that is a secondary decomposer, and its fruiting bodies are formed on composted substrate produced according to certain procedures (Grimm and Wösten, 2018). In an international perspective, however, oyster mushrooms (*Pleurotus* spp.) and shiitake mushrooms (*Lentinula edodes*) are produced in larger volumes than button mushrooms (Grimm and Wösten, 2018). These two species are primary decomposers and can be cultivated for fruiting body production directly on lignocellulosic residues, such as straw and sawdust. Oyster mushroom in particular is well-known for fast growth and ability to produce fruiting bodies on a wide array of substrates (Fernandes et al., 2015). This opens the way for a sustainable local production process where lignocellulosic harvest wastes can be used as mushroom substrate and the residues left after mushroom harvest can be reused in an agricultural context, e.g., as animal feed (Ivarsson et al., 2021) or for soil improvement.

The aim in the present study was to explore the potential for developing a mushroom substrate fertilized with anaerobic digestate in production of oyster mushrooms (*P. ostreatus*). A standard mushroom substrate composed of sawdust and wheat bran was used as a control. Fungal colonization of the substrates was monitored, as was production and quality of the fruiting bodies. In a previous study by our research group on a similar anaerobic digestate as used in the present study, we detected various micropollutants in the digestate (Golovko et al., 2022). Therefore, mushroom substrates in the present study were analyzed for the presence of organic contaminants such as pesticides and pharmaceuticals.

2. Materials and methods

2.1. Fungal strain and mushroom substrate

Grain spawn of the oyster mushroom strain *Pleurotus ostreatus* M2191 was obtained from Mycelia BVBA, Belgium, and used for inoculation of the different mushroom substrates. In the experimental treatments, mushroom substrate composed of alder (*Alnus glutinosa*) sawdust (particle size 2–4 mm) was fertilized with different amounts of anaerobic digestate (AD) obtained from a local Swedish biogas plant where the biogas reactor is fed with organic household waste (37 %), manure (31 %), slaughter residues (19 %), and other organic food waste (13 %). The process is certified according to SPCR 120, which ensures that the wastes included originate in the food and/or feed chain (Avfall Sverige, 2020). Before use in the experiments, the anaerobic digestate was filtered (0.8 mm) to remove large debris and samples were sent for analysis at an accredited laboratory (Eurofins, Lidköping, Sweden). The results showed that total solids (ts) content was 2.5 % (EN 12880:2000), Kjeldahl-nitrogen was 21 % of ts (SS 028101), ammonia–nitrogen was 15 % of ts (standard methods 4500 mod, 1998), and pH was 8.1 (EN 15933:2012).

The filtered digestate was added in different concentrations to the sawdust (see Experimental set-up). The mushroom substrate that served as a control was standard mushroom substrate based on alder sawdust (particle size 2–4 mm), wheat bran, and calcium sulfate (2 % of dry weight (dw)). In an initial experiment, the standard ratio of sawdust (75 %) to wheat bran (23 %) was used (Stamets, 2000), while in a second experiment the concentration of wheat bran was decreased to 11 %, in order to obtain similar carbon to nitrogen (C/N) ratio as in the substrate fertilized with anaerobic digestate. The moisture content of all substrates was set to 65 % by addition of distilled water.

2.2. Mushroom production

The substrates were packed in boxes suitable for mushroom production (TP1600 #30 WH, Sac O2, Nevele, Belgium) and pasteurized at 65 °C for 8 h. After cooling, spawn of oyster mushroom was added to the substrate in a concentration of 10 % of dw. The boxes were incubated in a climate chamber at 22 °C and humidity of 65 % for 20 days, at which point the substrates were densely colonized with mycelium. The boxes were then incubated at 10 °C for three days to induce fructification, followed by incubation at 22–24 °C at 85 % humidity until harvest of the first flush of fruiting bodies. The fruiting bodies were harvested 5 days after pins were observed.

2.3. Experimental set-up

2.3.1. Experiment 1. Inclusion level of anaerobic digestate in the substrate

Filtered anaerobic digestate was added in a concentration of 1.0, 0.5, 0.2, 0.1 or 0 L per kg dw sawdust. A standard mushroom substrate with 75 % sawdust (C/N ratio 194 ± 23), 23 % wheat bran (C/N ratio 18 ± 0.1), and 2 % calcium sulfate (Stamets, 2000) was used as the control in this experiment (Table 1). The C/N ratio of the AD-fertilized substrate was calculated based on previously determined total C and total N concentrations in the sawdust and a total nitrogen content (determined as Kjeldahl-nitrogen) of 5.25 g per L in the anaerobic digestate, while ignoring the effect of addition of total C on adding anaerobic digestate. The obtained C/N ratios ranged from 194 to 60 and are presented in Table 1. Distilled water was added to the substrates to reach a moisture content of 65 % in the substrate and pH in the substrate was determined according to the standard EN 13037:2011.

Mushroom cultivation was performed as described in section 2.2. and the amount of mushrooms (fresh weight and dw) produced in the first flush was determined for each treatment. The dry weight was recorded after lyophilization. Mushroom production (fresh weight) was then related to the amount of substrate (dw), in order to determine the biological efficiency (BE) of the substrate, calculated as:

$$BE = (\text{Amount of mushroom (fresh weight)}/\text{Amount of substrate (dw)}) \times 100.$$

Total protein content in the fruiting bodies was analyzed by the Dumas method (Bellomonte et al., 1987), using a Vario Max CN and a

Table 1
Carbon/nitrogen (C/N) ratio and biological efficiency (BE) of the six substrates tested in experiment 1, and protein concentration (% of dw) in fruiting bodies of oyster mushroom (*Pleurotus ostreatus*) produced on these substrates: standard mushroom substrate (75% sawdust, 23% wheat bran, 2% calcium sulfate), alone or fertilized with increasing proportion of liquid anaerobic digestate (AD, L per kg sawdust dry weight).

Mushroom substrate	Amount of AD	C/N ratio	BE	Protein concentration
Fertilized with AD	0	194 ± 23	18.2 ± 6.5c*	24.8 ± 1.8a
Fertilized with AD	0.1	160**	61.2 ± 10.6b	18.2 ± 2.4a
Fertilized with AD	0.2	135	68.1 ± 7.8ab	19.3 ± 2.7a
Fertilized with AD	0.5	94	78.1 ± 5.3a	21.9 ± 3.0a
Fertilized with AD	1.0	62	0.0c	–
Standard substrate***	0	60	79.5 ± 5.4a	24.7 ± 2.4a

*Values within columns followed by different letters are significantly different ($p \leq 0.05$).
**Based on calculated values for all treatment containing AD. The AD was estimated to contain 5.25 g of total N (measured as Kjeldahl-nitrogen) per liter. Addition of total C from the anaerobic digestate was not accounted for. The wheat bran had a total C content of 42 % and total N content of 2.3 %.
***The standard substrate was amended with wheat bran to decrease C/N ratio.

conversion factor of 4.38 for total nitrogen (Barros et al., 2008).

2.3.2. Experiment 2. Detailed study of mushroom substrate fertilized with anaerobic digestate

Based on the results obtained in experiment 1, a detailed study was performed on substrate fertilized with 0.5 L anaerobic digestate per kg dw sawdust. The C/N ratio in this substrate was determined as 94 through the calculations performed in experiment 1. However, in substrate fertilized with anaerobic digestate, part of the nitrogen will be present as ammonia–nitrogen and some volatilization can be expected during pasteurization and during the drying process (while preparing the sample for analysis). Thus, the C/N ratio of 94 was only an approximation. In order to obtain a more correct value and to determine the actual risk of ammonia volatilization, the substrate was pasteurized as described above, acidified to pH 3.0 with HCl (to avoid further volatilization of ammonia), and lyophilized. The samples were milled and total N and total C were analyzed with a Vario Max CN. Based on this analysis a control substrate fertilized with wheat bran was designed to obtain a similar C/N ratio. This control substrate contained 87 % sawdust, 11 % wheat bran, and 2 % calcium sulfate. Mushroom cultivation was performed as described in section 2.2 and the amount of fruiting bodies produced and the protein content of the fruiting bodies were determined as described above. Additionally, the elemental composition of the substrates, before and after mushroom harvest, and of the fruiting bodies were determined. For this analysis lyophilized substrates samples were milled and wet-combusted in HNO_3 (65 %) using a microwave technique (CEN Mars 5), and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). The elements Al, B, Ca, Cd, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, Si and Zn were analysed.

Before use in mushroom cultivation, the two substrates were also screened for contamination with organic micropollutants. For this analysis lyophilized and milled samples of the substrates before mushroom cultivation were screened for a total of 133 micropollutants, comprising 60 pharmaceuticals, 58 pesticides, three industrial chemicals, one drug, three parabens, two stimulants, one food additive, two vitamins, one personal care product, one fatty acid, and one sweetener. Details of the substances analyzed and of the analytical procedure can be found in Golovko et al. (2022). Extraction of the samples before analysis was performed as described in Golovko et al. (2021).

Additionally, mycelial colonization of the two substrates was studied by monitoring carbon dioxide emissions from the boxes. The fungal respiration was measured during the mycelial colonization phase (day 1–20) using carbon dioxide loggers (Extech CO210, Nashua, USA), which were placed directly above the gas exchange filter on each inoculated box. Carbon dioxide emissions were measured once every hour. The boxes were placed with a distance of 2 m from each other and each box was enclosed in a plastic cone with height 45 cm, a closed base, and an open top with diameter 25 cm.

2.4. Statistics

All experiments were established with three replicates, and mean values and standard deviation are reported. The data were analyzed by analysis of variance followed by Tukey's multiple comparison test, or by *t*-test. Differences were considered significant at $p < 0.05$ (Minitab, version 19).

3. Results and discussion

3.1. Mushroom production

In the substrate fertilized with the highest concentration of anaerobic digestate (1.0 L AD/kg dw sawdust), mycelial colonization was impaired to the extent that it was almost invisible to the naked eye, and no fruiting body production was observed on that substrate (Table 1). In the other treatments in experiment 1, fruiting bodies emerged slightly earlier on the mushroom substrate fertilized with anaerobic digestate (day 24–26) compared with the standard substrate (day 28–30), irrespective of the concentration of anaerobic digestate used. In a substrate based on sawdust only, formation of fruiting bodies was delayed and mushrooms were harvested between day 35 and 45. It is commonly reported that there is a time period of 3–4 weeks required from inoculation to harvest of the first flush for *P. ostreatus* in commercial production (Sánchez, 2010). This time span has also been observed in less conventional substrates, e.g. recycled diapers and food waste (Ma et al., 2020). This demonstrates the versatility of this species. For the treatments amended with 0.2 or 0.5 L AD per kg dw sawdust, there was no significant difference in BE or protein content compared with the standard substrate (Table 1). The substrate amended with 0.5 L of anaerobic digestate per kg of sawdust (dw) was chosen for further studies as the highest BE and protein concentration were observed in this treatment.

As mentioned, oyster mushrooms have the capability to grow and produce fruiting bodies on a wide array of substrates, with suitable C/N ratio reported to be within a wide range (34–120) (Hoa et al., 2015; Osunde et al., 2019). In experiment 1, the C/N ratio ranged between approximately 200 (unfertilized sawdust) and 60 (standard substrate and the substrate fertilized with the highest concentration of anaerobic digestate). However, when anaerobic digestate was added in a concentration that provided similar N levels as in the standard substrate, it became evident that anaerobic digestate is detrimental to fungal growth when the inclusion rate exceeds a certain level, as less mycelial growth and no production of fruiting bodies were observed in that treatment (Table 1). This is in line with findings in an earlier study on fungal growth on anaerobic digestate, based on a high concentration of food waste, where less fungal growth was observed with increasing inclusion rates of the digestate in agar medium (Jasinska et al., 2017). Thus, other aspects of anaerobic digestion, besides lack of oxygen, appear to be detrimental to fungal growth. Phenolic compounds naturally present in anaerobic digestate (Levén et al., 2010) can potentially be involved in fungal growth repression, as some of these compounds are known to have an antifungal effect (Simonetti et al., 2020).

In experiment 2, the control substrate amended with wheat bran had a C/N ratio of 79 ± 12, while the substrate fertilized with anaerobic digestate had a C/N ratio of 88 ± 14. This value agrees well with the estimated value of 94 for the AD-fertilized substrate and suggests that volatilization of ammonia during pasteurization was low. The substrate fertilized with anaerobic digestate had a pH of 7.7 ± 0.2 after pasteurization, while the control substrate had a pH of 7.5 ± 0.1. Similar carbon dioxide emissions were observed in both treatments during spawn run (Fig. 1). These findings suggest that fungal growth, and thus fungal

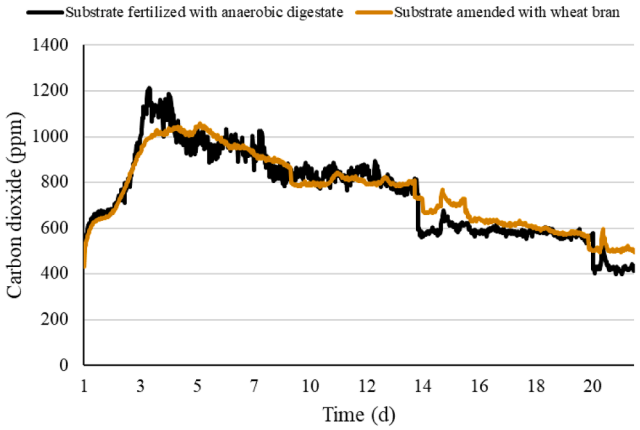

Fig. 1. Carbon dioxide emissions (ppm) over time during spawn run in the mushroom substrates used in experiment 2. Emissions were recorded every hour, points in the graph are based on mean of three replicates (standard deviation was within 10% of the mean).

colonization rate of the two different substrates, was similar and that the amount of anaerobic digestate added was below the level detrimental to fungal growth. Substrate productivity, measured as BE, was also similar (71.6 ± 8.2 for the control substrate, 72.9 ± 10.1 for the AD-fertilized substrate). Further, no significant differences were observed between the treatments with regard to protein content, which was 17.8 ± 2.2 % of dw (control substrate) and 18.6 ± 2.1 % of dw (AD-fertilized substrate).

Several studies have examined the use of white-rot fungi such as *Pleurotus* spp. in the initial phase of biogas production (Pečar et al., 2020; Elissen et al., 2021). The aim in such cases is generally not fruiting body production, but increased lignocellulosic degradation of the feedstock and thereby increased methane production potential. A few studies have evaluated a similar application as in this study, i.e., use of anaerobic digestate for fertilization of mushroom substrate (Banik and Nandi, 2004; Isikhuemhen and Mikiashvili, 2009; Chanakya et al., 2015; Zhou et al., 2018). Similar results as the present study have been reported, with anaerobic digestate showing potential to increase fruiting body production when added to lignocellulosic waste with high C/N ratio, such as straw (Banik and Nandi, 2004; Chanakya et al., 2015) or sawdust. However, compared with substrates with lower C/N ratio, no significant increase in fruiting body production has been observed (Zhou et al., 2018). It should be noted that those studies all involved addition of dried or composted solid residues of anaerobic digestate to the substrate, and thus there was a risk of ammonia volatilization while preparing these solid residues (Whelan et al., 2010). In the present study, the losses of nitrogen through volatilization were observed to be negligible. Additionally, our method of directly adding the liquid slurry to the sawdust substrate is labor-saving compared with mixing solid components, and would facilitate large-scale production of mushroom substrate. Hypothetically, substrate production could take place within biogas plants, which often have equipment for hygienization. Thus AD-fertilized substrate could be mixed, pasteurized, and possibly also inoculated with mushroom spawn, and then collected by a local mushroom farm.

However, fungi generally prefer nitrogen in organic form and it can therefore be argued that food waste preferably should be included in mushroom substrate before anaerobic digestion. This approach would mean that high ammonium concentration and accumulation of metabolites that impairs fungal growth could be avoided. On the other hand, the fast decay of the nutrient-rich food waste renders this approach unpractical. Anaerobic digestion of organic waste is a well-established technology for production of renewable energy (biogas), and developing additional uses for the digestate remaining after biogas production could increase uptake of anaerobic digestion technology and thereby energy production. In this context it should also be pointed out that the spent mushroom substrate remaining after mushroom production is composed of mycelium and partly degraded substrate, and may have several applications that can benefit the development of a biobased society (Grimm and Wösten, 2018). Considering a spent substrate composed of sawdust, anaerobic digestate and mycelium, a potential use could be development of biomaterials as discussed by Khoo et al. (2020) or recirculating it back to the biogas process.

3.2. Elemental composition of substrates and fruiting bodies

Analysis of important nutrients (C, N, P) in the different substrates in experiment 2 revealed similar concentrations of C and N, while P concentration was significantly lower in the AD-fertilized substrate than in the standard (control) substrate (Table 2). This difference was reflected in the elemental composition of the fruiting bodies, where significantly lower P value was observed in the fruiting bodies produced on the AD-fertilized substrate. A previous compilation of data on P concentration in fruiting bodies of *P. ostreatus* indicated that the concentration varies between 6 and 13 g kg^{-1} dw fruiting body (Siwulski et al., 2017). Thus, the P concentration in the AD-fertilized substrate (7.5 g kg^{-1} dw fruiting body) seemed to be sufficient to support production of fruiting bodies. In a recent study on accumulation on macronutrients in edible mushrooms (Malinowski et al., 2021), high accumulation of P was observed in the fruiting bodies. In the present study, the P bioconcentration factor, defined as P concentration in the fruiting bodies relative to P concentration in the substrate, ranged between 9 (control substrate) and 12.5 (AD-substrate). Thus, our data demonstrate that the content of P in fruiting bodies varies in relation to the concentration in the substrate and that uptake (bioconcentration factor) will vary accordingly.

Table 2
Elemental composition of the standard mushroom substrate (control) and substrate fertilized with 0.5 L anaerobic digestate per kg dry weight (dw) sawdust in experiment 2, and composition of the fruiting bodies produced on these substrates. Carbon (C) and nitrogen (N) content are presented as percentage of substrate dw, other elements as mg/kg dw substrate. Substrates and fruiting bodies were compared separately. Mean ± standard deviation, n = 3.

Element	Control substrate	AD-fertilized substrate	Fruiting bodies on control substrate	Fruiting bodies on AD-fertilized substrate
C (%)	45.4 ± 2.2a*	47.5 ± 1.0a	43.8 ± 4.5a	47.2 ± 4.3a
N (%)	0.6 ± 0.1a	0.6 ± 0.1a	4.1 ± 0.5a	4.2 ± 0.5a
Ca	9687.5 ± 1134.5a	4052.5 ± 525.1b	344.0 ± 146.6a	344.7 ± 144.7a
Cd	<0.2	<0.2	0.4 ± 0.1	<0.2
Cu	2.6 ± 0.3a	3.2 ± 0.2b	11.7 ± 0.6a	7.3 ± 0.7b
Fe	35.5 ± 2.1a	197.5 ± 18.9b	57.3 ± 4.0a	57.7 ± 4.6a
K	1875.0 ± 150.0a	1450.0 ± 57.7b	22000 ± 1000a	16333.3 ± 1154.7b
Mg	658.3 ± 63.6a	331.8 ± 25.7b	1516.7 ± 61.1a	1420.0 ± 70.0a
Mn	33.5 ± 3.1a	33.8 ± 2.9a	9.9 ± 0.2a	13.0 ± 1.1b
Mo	<0.2	<0.2	<0.2	<0.2
Na	252.5 ± 12.6a	585.0 ± 20.8b	226.7 ± 30.6a	413.3 ± 28.9b
Ni	1.1 ± 0.3a	1.3 ± 0.6a	<0.5	<0.5
P	1245.0 ± 123.7a	602.3 ± 62.3b	11666.7 ± 665.8a	7533.3 ± 135.0b
S	1450 ± 387.3a	1325.0 ± 250.0a	2000 ± 100a	2066.7 ± 57.7a
Zn	14.5 ± 1.7a	15.8 ± 1.3a	44.7 ± 1.1a	75.0 ± 1.0b

* Values within rows followed by different letters are significantly different ($p \leq 0.05$).

For potassium (K), another important macronutrient that is bioaccumulated in mushrooms (Malinowski et al., 2021), the concentration was lower in the AD-fertilized substrate than in the standard substrate, and this was reflected in lower K concentrations in the fruiting bodies on AD-fertilized substrate (Table 2). Potassium concentration has been studied in an large number of *P. ostreatus* strains and a concentration range of 16–50 g kg^{-1} dw fruiting body has been reported (Golian et al., 2022). The value observed in the present study (16–22 g kg^{-1} dw fruiting body) was at the lower end of this range (Table 2). The macronutrients magnesium (Mg) and calcium (Ca) behaved differently, with the concentration in the standard substrate being significantly higher than that in the AD-fertilized substrate, although the concentration in the fruiting bodies were in similar amounts in both treatments. This is in agreement with findings by Malinowski et al. (2021) that mushrooms do not accumulate Ca and, in nutritional terms, are not a good source of this element. From a nutritional perspective, it is of interest to note that the concentration of the micronutrient iron (Fe) was substantially higher in the AD-fertilized substrate compared to the control substrate. This increase can be attributed to the use of process additives during the anaerobic fermentation process, where Fe is used to decrease emissions of hydrogen sulfides (Kutter et al., 2015). However, the concentration of Fe in fruiting bodies was similar between the treatments. In contrast, Almeida et al. (2015) found that *P. ostreatus* had the capability for bioaccumulation of Fe and suggested Fe-fortified mushrooms as a non-

animal food source of iron. However, fungal Fe uptake is complex, considering the speciation (Fe(II) and Fe(III)) and its presence in a wide variety of forms (Philpott, 2006). In other studies, considerable variation in total concentration of Fe in fruiting bodies has been reported (Siwulski et al., 2017; Golian et al., 2022). Thus *P. ostreatus* has potential capability for accumulation of Fe, but if the aim is to produce fruiting bodies with elevated levels of Fe, consideration needs to be given to the form of this element in the substrate.

Overall, it can be concluded that despite differences in the elemental composition of the substrates, that of the fruiting bodies was similar in the two treatments (Table 2). The concentration ratios of the different elements in the fruiting bodies produced on the two different substrates ranged between 0.6 and 1.8 (Fig. 2). The largest difference was observed for sodium (Na), which is often regarded as a macronutrient for fungi (Vetter, 2003). Considerably higher concentration of Na was observed in the AD-fertilized substrate, reflecting the inclusion of food waste (food scraps from households and restaurants) in the anaerobic digestion feedstock. This elevated Na concentration was clearly reflected in the composition of the fruiting bodies produced on the AD-fertilized substrate, although it should be clearly stated that, in comparison with other foodstuffs, mushrooms are low in Na. Our measured value, of approximately 400 mg kg^{-1} dw fruiting body, is in line with other published values (Vetter, 2003; Siwulski et al., 2017; Golian et al., 2022).

3.3. Content of organic micropollutants in substrate

As discussed in relation to the elemental composition of the substrates and fruiting bodies, fungi are well-known for uptake and bioaccumulation of certain elements (Golian et al., 2022). For some of these elements, mainly the heavy metals, bioaccumulation is a health issue and substrates with high concentrations of these metals may produce fruiting bodies that are less suitable as a food source.

Fungi also have capability for uptake of organic substances, although this has not been widely studied. One exception is a study by Navarro Ramalho et al. (2018), examining caffeine uptake in *Pleurotus ostreatus* grown on coffee grounds, which reported an approximate concentration of 80 mg caffeine per 100 g dw mushrooms. Another study observed occurrence of the plant growth regulator chlormequat in fruiting bodies of *P. ostreatus* (EFSA, 2019). A wide range of persistent organic chemicals are used extensively worldwide and spread into the environment in low concentrations. From a food safety perspective, presence of these substances (commonly referred to as micropollutants) in mushroom substrates is of interest, especially when using a recirculated nutrient source such as anaerobic digestate (Ali et al., 2019; Govasmark et al., 2011; Sharma et al., 2020). In experiment 2, the control substrate and the AD-fertilized substrate were screened for a broad range of micropollutants and 10 (AD-fertilized substrate) and nine (control substrate) micropollutants were detected (Table 3).

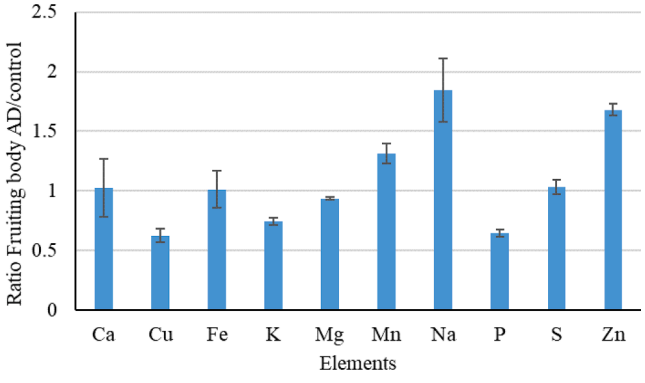

Fig. 2. Concentration ratio of different elements in fruiting bodies produced on mushroom substrate fertilized with anaerobic digestate (AD) and in fruiting bodies produced on standard (control) mushroom substrate (AD-fertilized substrate/control substrate). Mean and standard deviation shown, n = 3.

Clozapine, diazepam, fexofenadine, sulfamethoxazole, clotrimazole, and miconazole were detected in low concentrations in both substrates, indicating their presence in the sawdust. The stimulants nicotine and caffeine and the pesticides DEET and imazalil were only detected in the AD-fertilized substrate. Nicotine, caffeine, and imazalil have previously been identified in similar anaerobic digestate (Golovko et al., 2022). The total sum of micropollutants was significantly higher in the AD-fertilized substrate (258 ± 12 ng/g dw substrate) than in the standard substrate (191 ± 35 ng/g dw substrate). Considering the concentrations in the substrate, there is thus a risk of uptake of micropollutants in fruiting bodies and thereby risk for exposure to the consumers. More studies are needed to estimate the uptake rate and the actual risk of micropollutants being transferred to fruiting bodies.

4. Conclusions

This study demonstrated good potential for using anaerobic digestate as a fertilizer for growth substrate in mushroom production. The digestate tested was produced in a certified process based on feedstock components with known origin. For oyster mushroom (*P. ostreatus*), the productivity of the AD-fertilized substrate and the protein content of the fruiting bodies produced did not differ from those obtained with a standard mushroom substrate. Minor differences were observed in the elemental composition of the fruiting bodies, reflecting differences in the composition of the substrates. The anaerobic digestate was added to the substrate without pretreatment and volatilization of ammonia was almost negligible during substrate preparation. However, anaerobic digestates show high variation in their properties depending on the technology and the feedstock used. The origin of the feedstock, in particular, determines the suitability of the digestate for use in a short nutrient loop such as mushroom production. The mushroom substrates used in this study were screened for presence of micropollutants and it was found that, compared with the standard substrate, total micropollutant concentration was significantly higher in the substrate fertilized with anaerobic digestate. Provided that the anaerobic digestate has a high quality, our results suggest that co-operation with mushroom producers is a commercial possibility and potential new market that could be of interest for the biogas production industry.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 3
Micropollutant concentrations (ng/g dw substrate) detected in mushroom substrate fertilized with anaerobic digestate (AD) and in the standard substrate (control) in experiment 2. Level of quantification (LOQ, ng/g dw substrate) in the analysis is also shown. bd = below detection limit.

Micropollutant	AD-fertilized substrate	Control substrate	LOQ
Nicotine	104.8 ± 6.1	bd	0.5
DEET	15.8 ± 1.3	bd	5.3
Clozapine	23.5 ± 3.3	43.0 ± 11.9	5.2
Diazepam	22.0 ± 5.8	12.5 ± 6.9	5.6
Fexofenadine	6.4 ± 4.5	15.0 ± 4.1	5.3
Caffeine	15.3 ± 6.7	bd	2.2
Sulfamethoxazole	35.8 ± 11.7	40.5 ± 14.2	11.0
Metconazole	bd	5.6 ± 0.9	3.7
Prochloraz	bd	6.4 ± 5.2	4.5
Clotrimazole	10.8 ± 1.6	11.7 ± 6.0	4.6
Miconazole	6.7 ± 1.4	11.3 ± 2.6	4.6
Imazalil	21.0 ± 6.6	bd	8.6
Metazachlor	bd	45.3 ± 15.9	25

Data availability

Data will be made available on request.

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Ethical approval

This work did not involve any studies with human participants or animals performed by any of the authors.

Informed consent

All authors have the authority to publish this material and have agreed to submit it to *Waste Management*.

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