Biomethane digestate from horse manure, a new waste usable in compost for growing the button mushroom , Agaricus bisporus ?

Jean-Michel Savoie, R. Vedier, Frederic Blanc, Nathalie Minvielle, T. Rousseaut, Jean-Philippe Delgenès

BIOMETHANE DIGESTATE FROM HORSE MANURE, A NEW WASTE USABLE IN COMPOST FOR GROWING THE BUTTON MUSHROOM, *AGARICUS BISPORUS* ?

SAVOIE J.-M.[1], VEDIE R. [2], BLANC F. [3], MINVIELLE N. [1], ROUSSEAU T. [2], DELGENES J.-P. [3]
[1] INRA, UR1264, MycSA,
BP81, F-33883 Villenace d'Ornon, France
[2]CTC, Mushroom Technical Centre,
22 rue bizard, Munet F-49400,Distré, France
[3]INRA, LBE Narbonne,
France
savoie@bordeaux.inra.fr, vedie.ctc@wanadoo.fr, delgenes@supagro.inra.fr

ABSTRACT

Mushroom cultivation is a direct utilization of their ecological role of organic matter degradation in the bioconversion of solid wastes generated from industry and agriculture into edible biomass, which could also be regarded as a functional food or as a source of drugs and pharmaceuticals. Significant changes are expected in the integrated management of wastes streams in the future due to the use of plant biomass for biofuel and energy production and other non-food crops. On the one hand these activities may use the same wastes than those allowing mushroom cultivation today and problem of competition for supplying may occur. On the other hand these new activities will generate new wastes to be treated and valorise by the mushroom industry.

Horse manure is a component of the compost used to grow the button mushroom, *Agaricus bisporus* and other *Agaricus* species. We studied the methanisation of horse manure and utilisation of the digestate produced by the biogas reactor as waste for growing *A. bisporus*. Methanisation was efficient and the compost obtained from the digestate in mixture with wheat straw and low quantities of mushroom compost as source of aerobic and thermophilic microflora allowed to grow *A. bisporus*, but with lower yields than in conventional mushroom composts. Horse manure methanisation digestate was also used without composting by incorporation into the compost at spawning compared to a proteinic supplementation and in the preparation of casing soil in substitution of a part of peat moss. No beneficial effect was observed and with increasing rate of digestate incorporated, negative effect appeared (unproductive area).

Keywords: composting, button mushroom, horse manure, methanisation.

INTRODUCTION

Because many natural resources are limited, sustainability becomes an important concept in maintaining the human population, health, and environment. Mushroom cultivation is a direct utilization of their ecological role in the bioconversion of solid wastes generated from industry and agriculture into edible biomass, which could also be regarded as a functional food or as a source of drugs and pharmaceuticals. In Europe, mushroom industry mainly focuses in the cultivation of only few species under standardised cultivation conditions, the button mushroom, *Agaricus bisporus*, being largely dominant. Today, wheat straw and horse or poultry manures are the main wastes used for the button mushroom cultivation, but changes in climatic conditions and agricultural practices may results locally in difficulties for supplying, with the limitation of transportation as an objective. Concerning the integrated management of wastes streams in the future, significant changes are expected due to the use of plant biomass for biofuel and energy production and other non-food crops. On the one hand they may use the same wastes than those allowing mushroom cultivation and problems of competition for supplying may occur. On the other hand these new activities will generate new wastes to be treated and valorise by mushroom industry.

In the present investigation, the possibility to develop an integrated process of biogas production and mushroom cultivation from horse manure was studied. The objective is to state how Anaerobic Digestion of horse manures can be used to produce both renewable energy as methane and an edible biomass as button mushroom.

MATERIALS AND METHODS

Anaerobic Digestion of horse manure. Horse manure on wheat straw was purchased by a racing stable of horses. The optimal conditions for methane productions were studied in a pilot reactor of 30 L filled with 90 % of Horse manure and 10 % of garden wastes and load at a rate of 5.5 g organic matter per L per day during the process. The inoculum was from two reactors working with horse manures on wheat straw and corn. A process of thermophilic (55°C) dry (moisture content of less than 75%) anaerobic fermentation was used to produce biogas. Digestate was collected every week from the reactors and stored at 4°C before being used for growing *A. bisporus*.

Composting. Digestate collected every week in 5L or 30L reactors were stored at 4°C before being used for composting experiments. Composting was performed in reactors for aerobic solid state fermentation containing approximately 3 kg of raw materials in which temperature is managed [1]. The studied parameters of composting were the composition of the mixture raw ingredients: digestates, wheat, straw, horse manure, mushroom compost ready to be spawned, wheat bran, gypsum. The microbial quality of the obtained composts was estimated by measuring the metabolic activity through the hydrolysing activity of fluorescein diacetate and the number of fungal and bacterial colony forming units at both 28°C and 45 °C as in Libmond & Savoie [2]. Analyses of the chemical composition of composts were performed by a private laboratory with normalised methods. The efficiency of the produced compost as cultivation substrate for *A. bisporus* was evaluated by measurement of mycelial growth rates in Petri dishes and small boxes inoculated with commercial spawn.
After the optimisation of the formulation in the reactors, an experiment was performed at a larger scale with a conventional process of composting in two phases for estimation of mushroom yield in 0.09 m² trays filled with 8 Kg of compost spawned at 0.8 % as in previous works [3].

A commercial substrate (Renaud Champignon, Pons, France) obtained with wheat straw and horse manure as main ingredients was used as control.

Digestates as supplement in button mushroom cultivation. Phase II mushroom compost was obtained from France Champignon Substrate Company for all experiments. It was compost made from wheat straw and horse manure. The plots consisted of plastic trays (0.09 m²) filled with 9 kg of pasteurized compost (100 kg/m^2) set in a randomized block experimental design with six replicates. Hybrid white strain of *Agaricus bisporus* (Sylvan A15) was inoculated at the rate of 0.8 %. The compost temperature during spawn-run (13 days) was maintained around 25°C. The casing soil was made of 50 % limestone and 50 % peat. Digestates collected from INRA-LBE Narbonne reactors were stored at 4°C before being used for compost and casing experiments. They were mixed manually with compost or casing at the different rates used in experiments. Mushrooms were grown under French standards of culture with controlled environmental conditions in CTC facilities. Mushrooms were harvested (without trimming the stipe) daily, at market maturity i.e. veil unbroken and gills not exposed, for the crop duration of three weeks, and this for each plot. In this way, the weight of mushrooms was registered daily. The yield was expressed as kg/m^2.

RESULTS AND DISCUSSION

Anaerobic Digestion of horse manure. The average production of biogas containing 50 % of CH_4 was 80 $^+$/- 20 L.j^{-1}. The transformation yield of the dry matter to biogas was of 40 %.

Composting. Digestates may not be used directly as cultivation substrate for *A. bisporus*. There microbial and chemical compositions resulting from anaerobic digestion are not favourable to the mushroom. Mycelium inoculated directly in digestates does not grow from the spawn grain. During various experiments of composting in laboratory scale reactors we observed that one of the difficulties with this material used as main component was the production of ammonia that necessitated long time of composting. Consequently we tested mixtures of digestate and wheat straw in which digestate did not represent more than 60 % (dry weight) of the raw ingredients and where commercial compost was used as an inoculum. Based on the mycelial growth rate tests, analyses of the microbial quality, pH and organic matter measurements and ammonia odour, the selected mixture was (dry weight basis): 45 % wheat straw, 30 % digestate, 11 % gypsum, 9 % wheat bran, 5 % mushroom compost. Composting of this formula at laboratory scale for 20 days followed the temperature patterns presented in Fig 1, and allowed to obtain compost on which the mycelial growth rate was at mean 2/3 that measured in commercial compost used as control.

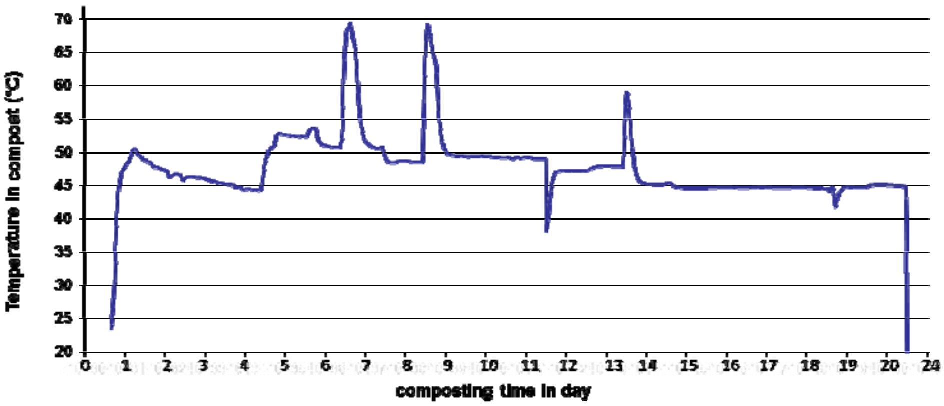

Figure 1: Temperature recorded in the substrate during composting at laboratory scale in fermenters.

Table 1 shows their chemical compositions and that of the initial mixture before composting. The differences in mineral composition may be noticed and is due to the high quantity of gypsum we used. The values of the other parameters of the chemical compositions are close.

The above mixture of raw ingredients was used for the production of 50 Kg of compost with a conventional composting process performed in trays. The recorded mean temperature during composting is reported in Fig 2. The yields of mushroom for 5 weeks of harvest were 25.9 ± 5.68 and 15.2 ± 1.64 Kg/m² (means of 6 replicates) for the control (commercial mushroom compost) and the compost from digestate respectively. The mushroom mean weights were not different in the treatments.

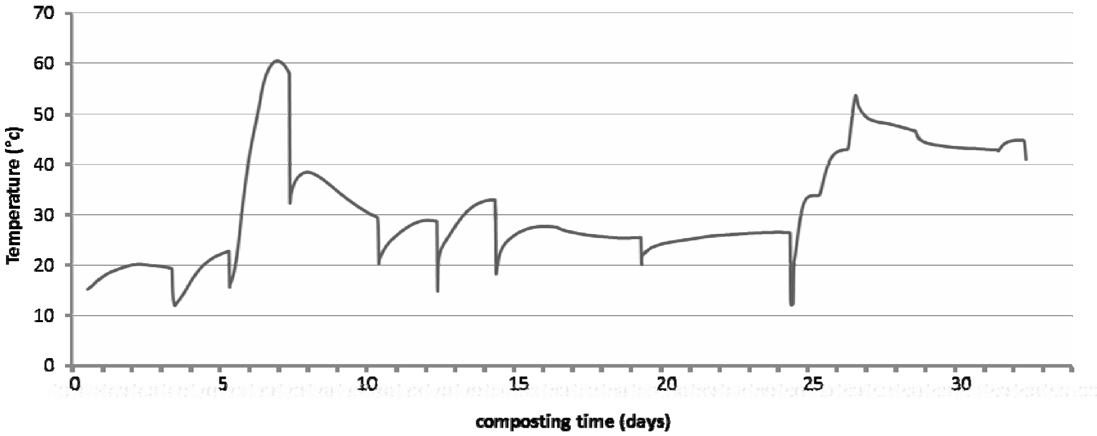

Figure 2: Temperature recorded in the substrate during conventional composting at pilot scale

Table 1: Chemical composition of the mixture of raw ingredients including digestate, of obtained compost, and commercial compost as control

	mixture of raw ingredients	compost from digestate	commercial compost
Minerals (g Kg-1)	288.6	345.5	303.3
K	25.9	35.5	32.1
Mg	5.5	7.11	6.4
Ca	66.4	80.1	55.8
Na	1.8	2.3	2.8
S	65.5	84.4	35.8
Organic C	356	323	348
N (Kjeldahl)	17.8	21.5	23.1
C/N	20.0	15.0	15.1
water soluble OM (% of total OM)	29.5	29.8	32.5
% of OM non soluble in acid detergent	45.8	52.1	48.8
Hemicelluloses (% of total OM)	24.7	18.1	18.7
Cellulose	5.1	5.1	2.9
lignin and cutin	40.7	47.1	45.9

The present work shows that digestates from dry anaerobic digestion of horse manure for production of biogas might be used as raw ingredients of composting in place of horse manure for the cultivation of *A. bisporus*. However it is necessary to add an inoculum of aerobic thermophilic microbial communities. This inoculum can be obtained by introducing mushroom compost obtained with conventional ingredients or by inoculation of pure cultures of useful microorganisms either bacteria [4] or fungi [5]. We only obtained half of the yield of a commercial cultivation substrate, but the simple composting process used here could be optimised.

Digestates as supplement in button mushroom cultivation. Incorporation of digestates into the compost at spawning is compared to the effect of a proteinic supplementation. In that case, compost was supplemented with Promycel 600 at spawning at the rate of 0.8 %. One trial was conducted to investigate the effect of two rates of digestates (2% and 5%). Non-supplemented compost produced 23.48 kg/m² of mushrooms (Fig. 3). With commercial supplementation, yield increased very significantly to 33.38 kg/m² (+ 42 %). In the same time lower increases in yield were observed with addition of digestates to the compost (+ 12 % to + 21 %). Digestates can't be a substitute to commercial supplementation to improve mushroom yield, but might be used with some beneficial effects.

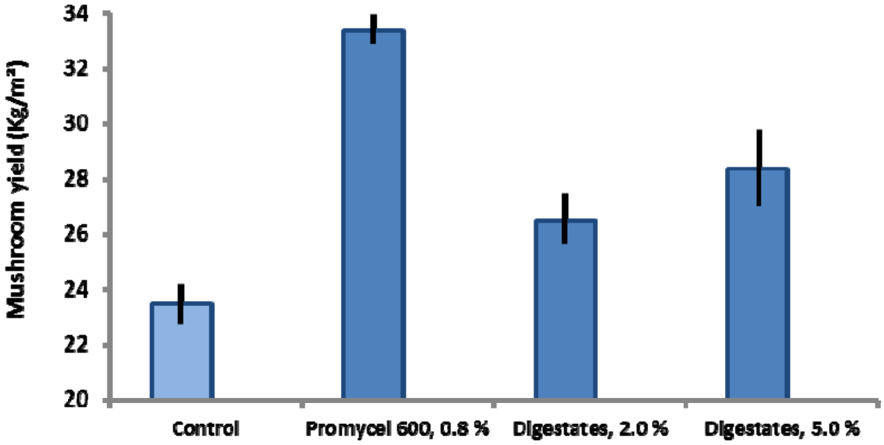

Figure 3 : Effect of 2 rates of digestates, used as supplementation at spawning, on total yield

Incorporation of digestates into the standard casing soil was made at different rates (5-10-15 and 20%). Compost cased with commercial casing (control) produced 32.79 kg/m² of mushrooms (Fig. 4). On casing with digestates, we noted the same level of mushroom production compared to the control, despite the presence of "black" area in the casing soil. Only the highest rate of digestates (20%) gave a significant lower yield (28.24 kg/m²). In our conditions, we can conclude that digestates may be added in casing soil, but without beneficial effect on *A. bisporus* mushroom production.

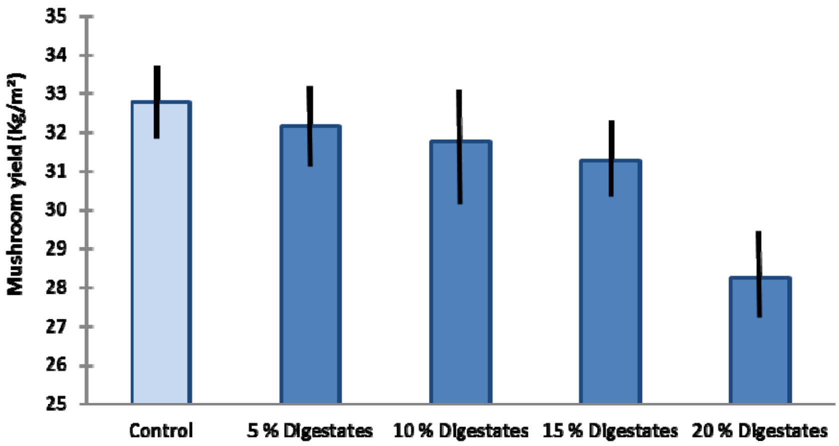

Figure 4: Effect of 4 rates of digestates incorporated into casing on total yield.
Error bars are standard deviations

CONCLUSION

Dry and thermophilic anaerobic digestion of horse manure on wheat straw is efficient for the production of Methane and digestate produced as waste of this process proved to be a raw material that can be used in mixture with others for the composting process aiming at producing a cultivation substrate for the button mushroom *A. bisporus*. However mushroom yields obtained in this study were lower than those obtained with compost used in Europe and further improvements are needed. The use of digestate as supplement, without composting was less efficient for the mushroom production.

Integrated management of horse manure for biomethane and mushroom production might have an economic interest if it is used in parallel with the current way of compost production from horse manure.

ACKNOWLEDGEMENT

The authors want to acknowledge research funding from Ministère de l'Agriculture, de l'Alimentation, de la Pèche, de la ruralité et de l'Aménagement du Territoire, Cas Dar n°8068.
We are grateful to Ludovic Devaux for pilot scale composting and mushroom cultivation.

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