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Mushroom Cultivation Using Compost Produced in the Garbage Automatic Decompose-extinguisher (GADE)

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Abstract

To improve cultivation of edible fungi, we investigated hyphal incubation and mushroom cultivation using media based on compost produced in the garbage automatic decompose-extinguisher (GADE) machine. Growth rates of hyphae of *Pleurotus ostreatus*, *Lentinula edodes*, *Lyophyllum decastes*, and *Agaricus bisporus* on media made from GADE compost were estimated. *P. ostreatus* and *A. bisporus* had the highest growth rates on the GADE compost media. Hyphal growth rates were also investigated on agar plates with water extractives of the GADE compost. The optimum concentration of the extractives and pH of media varied among species of fungi. Mushroom cultivation of *P. ostreatus* was conducted using bottled media of the GADE compost. Nutrient concentration and pH were adjusted using media made from a mixture of the GADE compost and sawdust of todomatsu fir (*Abies sachalinensis*). Fruit bodies formed normally on all the media including the GADE compost.

Key words: sawdust, GADE machine, compost, edible fungi, mushroom cultivation

Introduction

The garbage automatic decompose-extinguisher (GADE) machine, which degrades garbage biologically with aerobic bacteria, has recently been used widely in Japan (Terazawa *et al*. 1999,Kawamoto and Nochi 1998, Fujino *et al*. 1994). The GADE machine degrades garbage aerobically using a reactor matrix such as sawdust, which serves as a habitat for microbes and functions as an air keeper, water conditioner, heat insulator, and absorber for odor and degradation products (Horisawa *et al*. 1999). Minerals and low molecular weight organic compounds produced by the biodegradation of garbage are absorbed in the sawdust matrix. Previous reports showed that GADE compost, the sawdust generated after using this machine for a several months, can be utilized as an organic fertilizer and a soil conditioner (Terazawa *et al*. 1999, Horisawa *et al*. 1999). The GADE machine results in waste reduction and organic resource recycling.

Mushrooms are an important forest product in Japan. Many kinds of mushroom species are cultivated, not only by the log cultivation method but also by a sawdust-based cultivation method. The latter has recently been used more extensively due to some advantages such as shortening the cultivation time and the constant production throughout the year. Sawdust-based media such as rice or wheat bran are added as a nitrogen resource. However, a shortage of sawdust for mushroom cultivation has become a serious problem. GADE compost includes inorganic components such as nitrogen, phosphorous and potassium, so it is expected to be beneficial in mushroom cultivation.

In this study, we investigated use of GADE compost for mushroom cultivation media. Four edible mushroom spcies, *Pleurotus ostreatus*, *Lentinula edodes*, *Lyophyllum decastes*, and *Agaricus bisporus*, were examined. *P. ostreatus* is cultivated widely because of its short cultivation period and easy cultivation with conifer sawdust. *Le. edodes* is grown in the greatest quantity throughout the world. *Ly. decates* is a good edible mushroom that is saprobic and wood degradable (Kinuta *et al*. 1995). *A. bisporus* is also an edible fungus cultivated all over the world and grows on compost media.

Materials and methods

Fungal strains and GADE compost

Four edible mushrooms strains, *P. ostreatus*, *Le. edodes*, *Ly. decastes*, *and A. bisporus*, cultivated in Japan, were tested (Table 1). These strains were precultured on potato dextrose- agar (PDA) (Nissui Pharmaceutical, Tokyo, Japan) plates at 25ºC. The sawdust matrix used in a nursery school for 8 months was employed as the GADE compost. The GADE compost was kept in dark place at room temperature for 2 months until analysis and experiment. The total nitrogen, phosphate, and potassium contents in the GADE compost were analyzed by the Japan Fertilizer and Feed Inspection Association (Table 2) and the water content of it was 27.5%. Inorganic ion contents such as Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, NH^{4+}, Cl^{-}, NO^{3-}, $PO4^{3-}$, and $SO4^{2-}$ were evaluated by capillary electrophoresis (Quanta 4000, Waters, Massachusetts, US). A 5-gram sample of the GADE compost was suspended in 50 ml of deionized water and boiled for 20 min. The supernatant of the suspension was analyzed (Yonekubo and Terazawa 1994).

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Table 1. Mushroom strains tested.

	Origin	Notes
Pleurotus ostreatus	Stock culture in HFPRI[a]	Cultivated strain
Lentinula edodes	Mori465 (Mori Inc.)	Cultivated strain
Lyophyllum decastes	Isolated in Shintoku, Hokkaido	Wild strain
Agaricus bisporus	M2 (Mori Inc.)	Cultivated strain

[a] HFPRI: Hokkaido Forest Products Research Institute.

Table 2. Chemical characteristics of the residual sawdust (dry weight basis).

Analysis	unit	content
Total nitrogen (N)	%	1.95
Total phosphoric acid (P_2O_5)	%	0.83
Total potassium (K_2O)	%	1.32
Sodium chloride (NaCl)	%	1.00
Mercury (Hg)	mg/kg	0.03
Arsenic (As)	mg/kg	12.4
Cadmium (Cd)	mg/kg	0.05

Table 3. Inorganic ion contents in the GADE compost analyzed by capillary electrophoresis.

	Raw sawdust (ppm)	Residual sawdust (ppm)
Ca^{2+}	3.6	78.0
Na^{+}	2.5	139.1
Mg^{2+}	0.7	15.6
K^{+}	14.9	336.6
NH_4^{+}	0.9	116.6
Cl^{-}	10.8	1546.7
NO_3^{-}	4.0	264.4
PO_4^{3-}	5.1	733.0
SO_4^{2-}	1.3	326.4

Preparation of GADE compost-based media

The media for estimating hyphal growth rates of fungi in glass Petri dishes (diameter of 90 mm) were prepared as follows. Water content of the GADE compost was adjusted at 70% on a wet weight basis. An 18-gram aliquot of the conditioned GADE compost was placed in a Petri dish, and was autoclaved for 90 min (GC). For comparison, a todomatsu (*Abies sachalinensis*) medium (TD) and a mixture medium of todomatsu and wheat bran (in a ratio of 4:1 (v/v)) (TW) were also prepared.

Agar plates including water extractives of the GADE compost were also prepared. A 100-gram aliquot of the GADE compost was suspended in 1-liter of deionized water for 2 h and the supernatant was obtained as a water extractives solution. Each agar plate was made from the extractives solution diluted in 1%, 10%, 50%, and 100% without adjustment of pH. The 100% solution plate adjusted to pH of 5.5 was also prepared.

Measurement of growth rates

Fungi strains precultured on the PDA plates were punched into inocula plugs with a diameter of 5 mm and were inoculated on the compost plates and the extractives-agar plates. Each plate was incubated in the dark at 25ºC and colony diameter was measured with time.

Cultivation test of P. ostreatus

The GADE compost and todomatsu sawdust were mixed in ratios of 1:0, 3:1, 1:1, and 1:3 by dry weight. Water contents of mixtures were adjusted to 65% on wet weight basis. A 100-g aliquot of mixture was put in a glass pot (200 ml) and autoclaved for 90 min, twice within 24-h intervals. *P. ostreatus* precultured on a PDA plate was inoculated into cultivation media and was incubated at 25ºC. After hyphae spread entirely in the pot, the following operations for induction of fruit body budding were conducted: removal of hyphae at the surface around the pot opening, soaking the pot in water for 2 h, and incubating at 12ºC and 100% RH. Fresh weight of fruit body was measured to evaluate the yield.

Results and discussion

Inorganic compounds and pH of the compost media

The inorganic ion contents are shown in Table 3. The result of ion content analysis showed that the inorganic compounds were almost not included in the raw sawdust but were in the GADE compost. The inorganic compounds in the GADE compost were presumed to come from the nursery school's kitchen waste and left-overs. Imbalance between anion and cation were shown in this result. One reason for such imbalance is thought that ammonium ion flowed out from the GADE compost as ammonia. Nitrogen, phosphate, and potassium, to which the effects as fertilizer or nutrient source are expected in cultivation of field crops and mushroom, increased through the garbage process. Chloride ion was increased remarkably. The similar result was reported (Yonekubo *et al*. 1994), although the reason for this are not clear. Utilization of the compost with high chloride ion content as fertilizer could have negative influences on environment. However, this problem would not occur in the application of the GADE compost to the media of mushroom because of the closed process. The pH levels of the experimental media are shown in Table 4. The GC media had a high pH of 8.12, but of pH level of TD and TW were low: pH 4.08 and 5.05, respectively. The pH levels of the GADE compost-based media should also be adjusted by mixing GADE compost and raw sawdust.

Hyphal growth on compost media and water extractives agar (WEA) plate

Hyphal growth curves of the four edible mushrooms on the GC, TD, and TW media and the PDA plates are shown in Fig. 1. Growth rates of *P. ostreatus* on the GC, TW and the PDA were similar, while little growth occurred on the TD. The growth rates of *Le. edodes* on TW and PDA were similar, but it grew little on GC and the TD. *Ly. decastes* showed a similar growth rate on TW and PDA but little or no growth on GC and TD. *A. bisporus* growth tended to be similar to that of *P. ostreatus* on these media. The GADE compost should contain sufficient nitrogen to grow hyphae of *P. ostreatus* and *A. bisporus* because the growth rate on the GC was almost equal to that on the TD and PDA. However, growth of *Le. edodes* and *Ly. decastes* were inhibited by the GADE compost. From these results, it was found that growth test on the GADE compost media is necessary for each of fungal species.

The hyphal growth rates of fungal strains on the WEA and PDA plates were estimated (Fig. 2). These results suggest the effects of the concentration of water extractives and pH adjustment on growth rates of the fungi tested. The growth rate of *P. ostreatus* was larger on the WEA with higher concentration than lower. *Le. Edodes* showed the almost equal growth rate regardless of the concentration of WEA except for 100% and the growth rate at the 100%-WEA was improved by pH adjustment. *Ly. decastes* exhibited the highest growth rate at the 50%- WEA and the growth rate on the 100%-WEA was improved by pH adjustment. The growth rate of *A. bisporus* was highest at the 10%-WEA and was not influenced by pH adjustment. These results suggested that *Le. edodes* and *Ly. decastes* required pH adjustment, while *P. ostreatus* and *A. bisporus* did not. *Ly. decastes* and *A. bisporus* required

Table 4. Moisture contents and pH levels of experimental media.

	pH of media
Residual sawdust media	8.12 [b]
Todomatsu[a] sawdust media	4.08 [b]
Todomatsu sawdust media added wheat bran	5.05 [b]
PDA (potato dextrose agar)	5.60[c]
Water extractive stock solution	9.53 [c]
Water extractive stock solution (pH adjusted)	5.50 [c]
Residual sawdust−todomatsu sawdust (3 : 1)	8.26 [b]
Residual sawdust−todomatsu sawdust (1 : 1)	7.97 [b]
Residual sawdust−todomatsu sawdust (1 : 3)	7.48 [b]

Notes: [a]: todomatsu; (*Abies sachalinensis* (Fr. Schm.) Masters),
[b]: after autoclaving, [c]: before autoclaving

control of the concentration of water extractives. However, the growth of *Le. Edodes* and *Ly. decastes* were lower than PDA though the dilution of the extractives concentration and the pH adjustment. Therefore, it was found that GADE compost is an appropriate cultivation media for *P. ostreatus* and *A. bisporus*.

Cultivation of P. ostreatus using GADE compost

In the pot cultivation of *P. ostreatus*, hyphae of fungi spread entirely in each medium (Fig. 3). TW media (todomatsu and wheat bran) was used as control. The mycelial density decreased in the media with lower contents of the GADE compost. The cultivation time and fruit body yield of *Pleurotus ostreatus* on the GADE compost and todomatsu sawdust medium are shown in Fig. 4. Time until hyphal spreading and budding induction were not significantly different among the different kinds of media, but time to budding and harvest was delayed in the mixture media with the ratio of 1:3. Normal fruit bodies were produced in all media (Fig. 5). Yield of fruit bodies grown on the mixture media of the GADE compost and sawdust were almost equal among the different mixture ratios, but did not reach the yield of produced on the TW. The void volume of the GADE compost seems to affect the fungal growth because the GADE compost has little space for air and water (Horisawa 1999). The air and water holding capacities of the GADE compost are expected to be increased by mixing the compost with raw sawdust. The GADE compost had no promoting effect on the fruit body yield of *P. ostreatus* but there was no inhibition, too. Further improvement may increase the fruit body yield using GADE compost. The chemical component included in the GADE compost may be effective in incubating the fungi that is cultivated using compost, such as *A. bisporus*, A. blazei, and *Volvariella volvacea*.

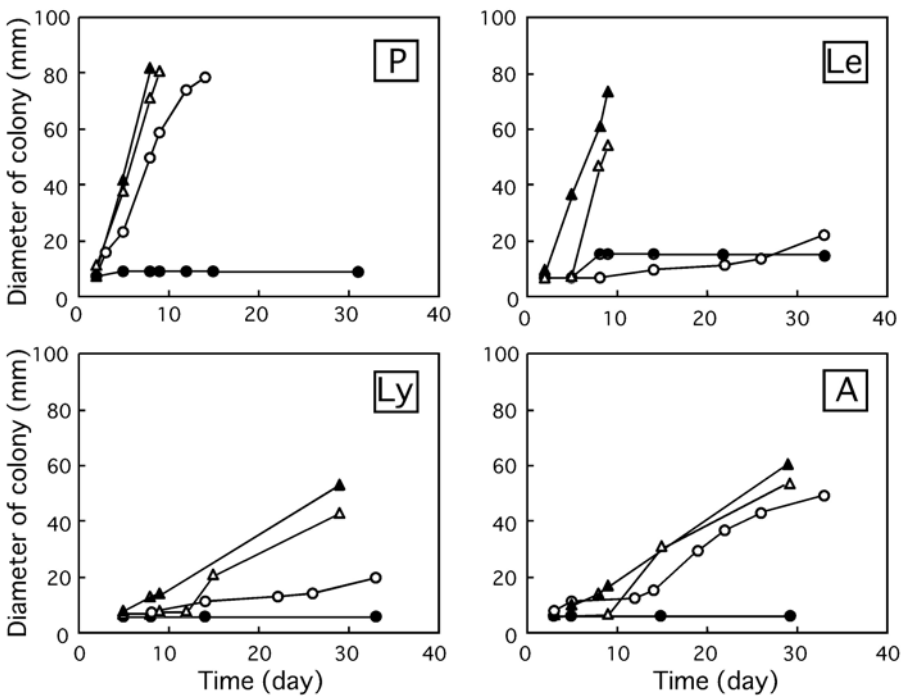

Fig. 1. Hyphal growth of *Pleurotus ostreatus* (P), *Lentinula edodes* (Le), *Lyophyllum decastes* (Ly), and *Agaricus bisporus* (A), on the GADE compost-based media and todomatsu media.

Legend —○—: The GADE compost (GC), —●—: Todomatsu sawdust (TD), —△—: Todomatsu sawdust with wheat bran (TW), —▲—: PDA.

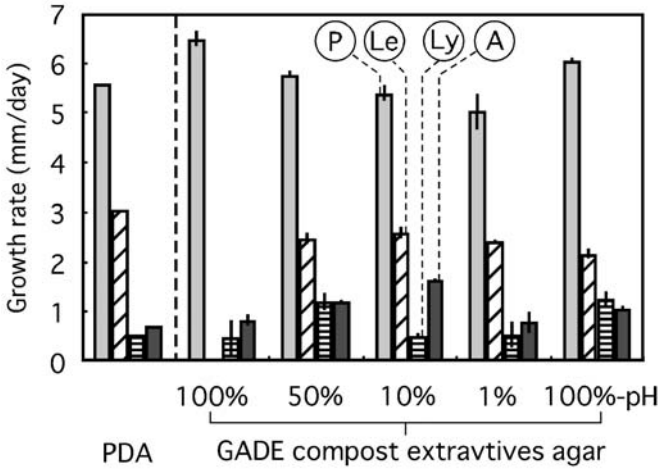

Fig. 2. Hyphal growth rates of 4 strains, *Pleurotus ostreatus* (P), *Lentinula edodes* (Le), *Lyophyllum decastes* (Ly), and *Agaricus bisporus* (A), on the water extractives agar and the potato dextrose agar (PDA) plates. The extractives solution was obtained as the supernatant of the GADE compost suspension and dilluted in 1%, 10%, 50%, and 100%. The 100% solution plate adjusted to pH of 5.5 was also prepared.

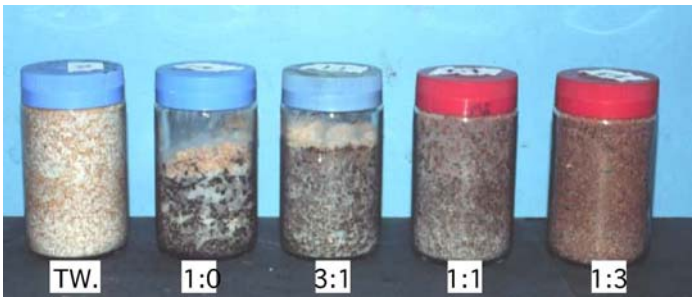

Fig. 3. Pot cultivation of *Pleurotus ostreatus* at day 14 after inoculation. TW shows control media (todomatsu and wheat bran) and the ratios show mixing ratios of the GADE compost and todomatsu sawdust.

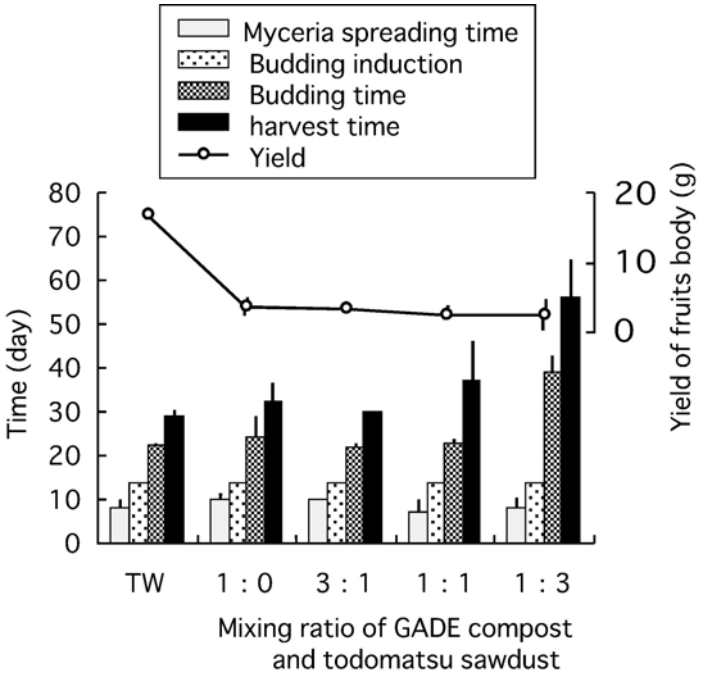

Fig. 4. The cultivation time and fruit body yield of *Pleurotus ostreatus* on the GADE compost and todomatsu sawdust medium. The TW medium was made from todomatsu sawdust and wheat bran (20%). Bars show standard deviations.

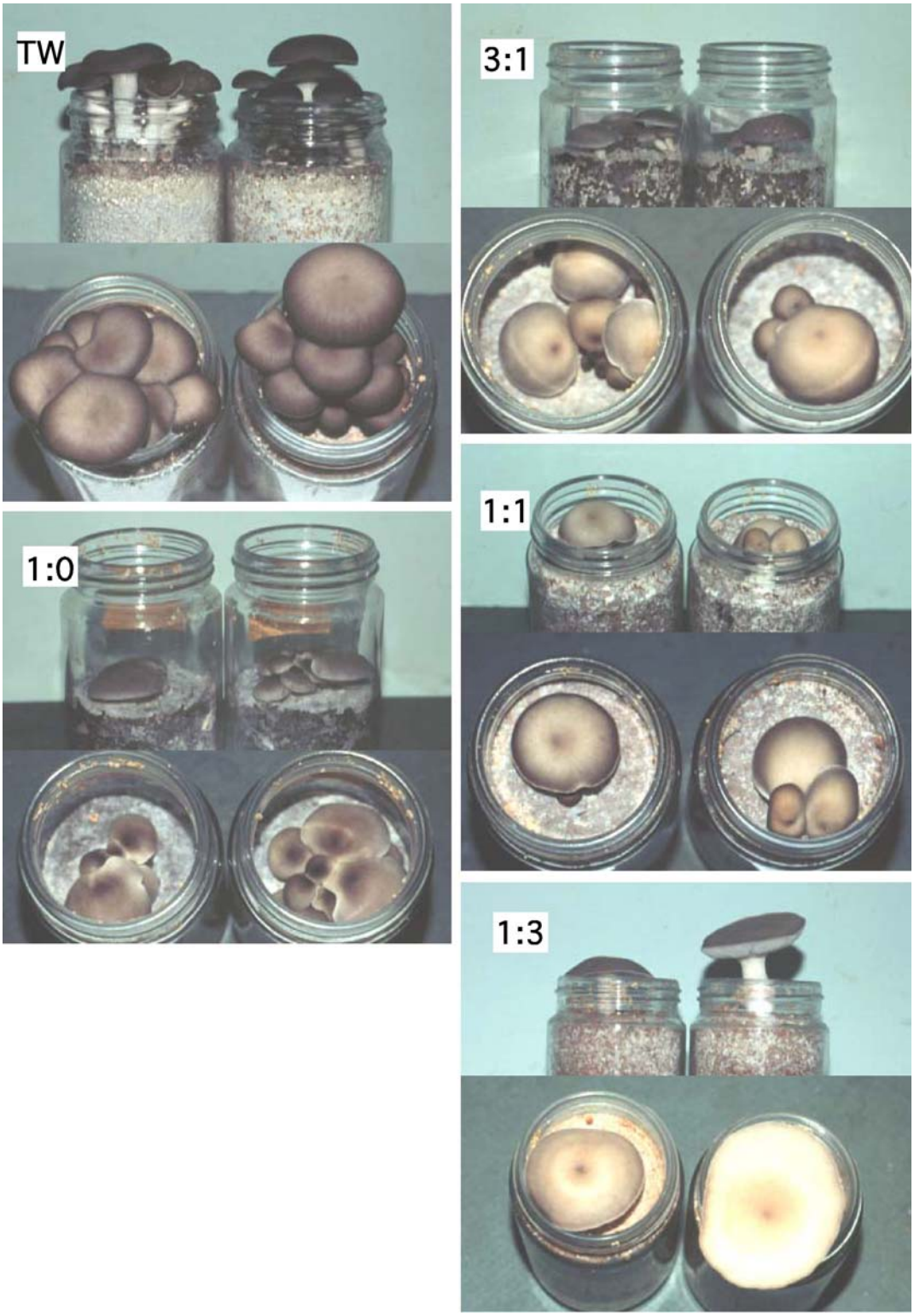

Fig. 5. Fruit bodies of *Pleurotus ostreatus* on the TW media, and the mixture media of the GADE compost and todomatsu. The ratios show the mixture ratio of the GADE compost and todomatsu sawdust.

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References

Fujino, E., Shinbo, H., Minoura, H., Kumada, K. (1994) Garbage Decomposer. Matsushita electric works technical report, No. 48 September pp 81-85 (in Japanese).

Horisawa, S., Sunagawa, M., Tamai, Y., Matsuoka, Y., Miura, T. and Terazawa, K. (1999) Biodegradation of Non-lignocellulosic Substances II: The changes in physical and chemical properties of sawdust before and after using as an artificial soil. J. Wood Sci. 45: 492-497.

Kawamoto, K. and Nochi, K. (1998) The characterization and estimation of performance of the small scale bio-degradation machine. Proceedings of the 8th Annual Conference of the Japan Society of Waste Management Experts, pp.283-286 (in Japanese).

Kinuta, M., Furuno, T., Takahashi, A., and Furukawa I. (1995) Echology and Wood Decay of Lyophyllum decastes. Mokuzaigakkaishi 41(5): 511-515.

Terazawa M, Horisawa S, Tamai Y, Yamashita K (1999) Biodegradation of non-lignocellulosic substances I. J Wood Sci, 45: 354-358.

Yonekubo, J., and Terazawa, M. (1994) Capillary Ion Analysis for Garbage Automatic Decompose Extinguisher System. J Environ. Chem. 4(1): 45-53.

Yoneyama, S., Togashi, I., Oikawa, H., and Aoyama, M. (1990) An Antifungal Substance in the Volatile Wood-oil of Todomatsu, Abies sachalinensisMast. Mokuzaigakkaishi 36(9): 777-780.