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Enhancement of Biodegradability of Paperboard in Soil by Supporting *Trichoderma* Cells and Nutritional Constituents

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When paperboard made from used paper was supported by *Trichoderma reesei* NBRC31137, a cellulolytic basidiomyces, the weight decrease of the paperboard in soil was enhanced. Number of viable microorganisms around the paperboard in the soil increased and reached 10^7 after 2 weeks. Nutritional medium by itself, constituted of yeast extract and peptone, also stimulated the degradation in soil. Cells of *T. viride* NBRC31326 and *Trichoderma reesei* NBRC 31327 were less effective. These results suggested that we could control biodegradation rare of cellulosic material in an agricultural field.

INTRODUCTION

Many kinds of fibers and polymers especially those from petroleum have been used as one–way plastic: plastic items once used have been immediately discarded as refuse. And recycle of such materials were generally in consideration for establishing 'Recycle–Based Society' in Japan (http://law.e–gov.go.jp/htmldata/H12/ H12HO110.html). To whatever they were intended to recycle, however, their collection and treatment (processing) require certain energy input, i.e. fossil fuel consumption. Especially, recycle of such materials in agriculture was badly evaluated in their Life Cycle Assessment (LCA) (Tan and Khoo, 2005). Reuse and recycle of these materials requires many steps for chemical transformation and reproduction of products. Furthermore, as they have been inevitably soiled during their usage and sometimes distributed in wide area, repetitive usage of plastic materials in agricultural field, such as transplantation pots, sometimes requires rather higher energy, i.e., fossil fuel, to collect and wash than making the new ones from virgin petroleum. Thus it is sometimes better to recycle them thermally than to reuse or recycle materially (Consonni *et al*., 2005).

In such situation, intentional operation of biodegradable bio–based materials, that would be degraded to carbon dioxide and water after their usage and mineralized in soil would be preferable rather than returning to reproduction cycle. Biodegradable natural fibers such as cellulose and bio–based plastics such as poly–L–lactate are recognized as environmentally–friendly materials, because carbon atoms in their molecule have been fixed by plant before harvest, net generation of carbon dioxide occurred by their mineralization would be zero, even if they incinerated or landfilled after usage (carbon neutral) (Sakai *et al*., 2003). Hence it would reduce energy cost for material recycle and help for environmental protection (Sakai *et al*., 2001). In spite of that, their biodegradation rate are sometimes slow. Such biodegradable materials should be required to maintain functions, i.e., their shape and strength, during certain period in their usage, and should be degraded quickly after finishing their initial role. So that biodegradability of such materials have to be controlled and sometimes enhanced should begin being mineralized in the soil. To solve the above problems, we tried to introduce the timely–biodegradable characteristic to paperboard, by supporting cell suspensions of cellulolytic basidiomyces (Wyk and Mohulatsi,2003, Lo *et al*., 2005) with nutritional components for the growth of microorganism in soil (Gaint *et al*., 2005).

MTERIALS & METHODS

Three kinds of bacidiomyces, *Trichoderma viride* NBRC31326, *Trichoderma reesei* NBRC31327, *Trichoderma reesei* NBRC31137 were obtained from NITE Biological Resource Center (Chiba, Japan). They were cultured in 1000 ml yeast extract–peptone medium (0.3% yeast extract, 0.5% peptone, pH 6.5 ; YP medium) with adding 1% cellulose at 30 °C for 24 hs, with shaking at 120 rpm. After being harvested by centrifugation (8,000×g, 20 min), the cells were washed with and suspended to fresh YP medium (0.5 mg wet cells/ml). To support cells on the paperboard, the cell suspension (10 ml) was immersed onto a paperboard sample (2×70 ×40 mm, ca 8 g, 4 pieces in each experimental run). Then the paperboard were dried in a desiccator at room temparature for 3 days. After measuring the dry weight, the dried paperboard was put and keep between stainless nets (8 mm mesh), and the nets with paperboard samples were laid in the soil (2–4 cm depth). As a control, paperboard soaked into water or YP medium without cells was also laid under ground. Sample soil for gardening was commercially purchased, and stands at room temperature with sprinkling periodically (every 3 days). During thee experiment, the room temperature ranged 18 °C–28 °C. Paperboard made from 100% used–newspaper was obtained from Nippon Seishi Co. (Oita, Japan).

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Two pieces of paperboards in each experimental runs were dig up after 2 and 4 weeks, and their surfaces were washed gently. After drying up at room temperature in a desiccator for 3 days, the stainless nets were taken off and the morphology and surface condition of samples were recorded and changes in dry weights of paperboard were measured. Soil samples attached on the paperboard surface (ca 0.5 g) were collected, and microflora in the soil samples were analyzed: after diluting appropriately with saline, the samples (0.1 ml) were spread out onto standard agar plates. Number of colonies appeared were counted after incubation of the plate at 30 °C for 2days.

RESULTS & DISCUSSION

After 2 weeks incubation in soil, surface of all buried paperboards became rougher configuration, (errosion, and cracking), and changed their color to spotty of black and brown. Surface of samples supported by *Trichoderma* cells were slightly whitish by their grown hyphae. These changes were more significant after 4 weeks than after 2 weeks. Figure 1 shows change in dry weight of the paperboard during incubation in soil. Even in control run (without microorganism nor nutrition), linear weight loss of the paperboard was observed and the decrease was 5% after 4 weeks. Support of YP medium alone slightly enhanced the degradation rate (7.2% decrease after 4 weeks). Most significant degradation was observed in the sample supported by *T. reesei* NBRC31137 with YP medium. Weight loss of the sample was accelerated during incubation in soil (5.5% and 16.5%, after 2 and 4 weeks, respectively). Support of *T. reesei* NBRC31327 cells was less effective than the strain NBRC31137, and that of *T. reesei* NBRC31326 scarcely stimulated the degradation.

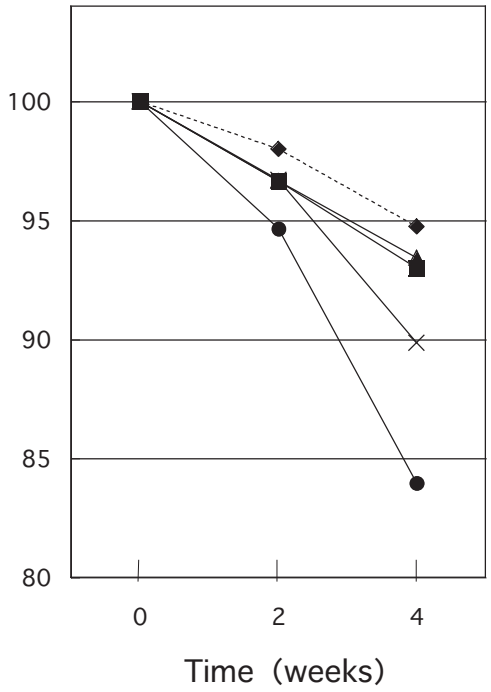

Fig. 1. Change in dry weight of paperboard buried in soil. Paperboard was soaked with water (diamond), 10 ml YP medium (square), YP medium containing 500 mg cells of *T. viride* NBRC 31326 (triangle), *T, reesei* NBRC 31137 (circle), or of *T. reesei* NBRC 31327 (cross), and after drying they were buried in soil for 2 or 4 weeks. Dry weight of paperboard samples (%) are represented relatively to those before buried.

Table 1. Cell numbers in soil burying paperboard with YP medium constituents and Trichoderma cells

Immersed with	Cell number ($\times 10^5$ cells/g)	
	After 2 weeks	After 4 weeks
Water	2.5±1.1	29±0.7
YP medium	42±2.3	43±6.8
T. viride NBRC31326	68±12	39±3.6
T. reesei NBRC31327	108±15	45±2.9
T. reesei NBRC31137	110±17	44±7.8

Concentrations of viable cells in the soil around the buried paperboard after 2– and 4–weeks were shown in Table 1. After 2 weeks, the soil around the paperboard immersed into YP medium contained 17–times higher concentrations of bacterial cells, compared with soil in control run (no medium and nor cells). Support of *Trichoderma* cells resulted further higher increase in viable cell number after 2 weeks, and the number was highest in the soil supported by *T. reesei* NBRC 31137 (1.1×10^7 cells/g). This means that microorganisms naturally habitat in soil, as well as bacidiomyces cells supported exogenously, proliferate well by support of YP medium constituent and involved in biodegradation of paperboard in soil. On the other hand, the viable cell numbers after 4 weeks were similar to those after 2 weeks on all experimental runs and were 3– 4.5×10^6 cells/g.

As a conclusion, it was demonstrated here that immersion of *Trichoderma* cells enhanced the degradation rate of paperboard in soil, associated with nutritional elements for their growth. As paperboard supported by *T. reesei* strains NBRC 31137 and NBRC 31327 showed different degradation rates, we could control biodegradability by selecting microorganism to be supported. Otherwise the paperboard pot for seeding and transplantation have been kept their shape in the soil and would inhibit primary growth of plant root. While by quick degradation of pot by support of the basidiomyces, plant root would be able to grow faster. We confirmed that paperboard cup supported by *T. reesei* strains NBRC 31137 did not cause any inhibitory effect in germination of seed and initial growth of Komatsuna (*Brassica camperstris* var. *peruviridis*) (data not shown). *T. reesei* and *T. viride* are recognized as safe to human (Nevalainen *et al*., 1994). In addition cellulolytic enzymes from the microorganisms have reported to show inhibitory effect to plant–pathgenic fungus (Picard *et al*., 2000). These properties would be rather favorable to cultivation of plants (Turoczi *et al*., 1996). Further investigation on effect of these microorganisms on growth of plant should be done for long term cultivation.

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