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NOTE

Sakae Horisawa · Yoh Sakuma · Yutaka Tamai
Shuichi Doi · Minoru Terazawa

Effect of environmental temperature on a small-scale biodegradation system for organic solid waste

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Abstract The optimum environmental temperature for a biodegrading machine using wood particles as a matrix was investigated using a small-scale degradation reactor and model waste. The biodegradation rate was evaluated by weight loss of waste and CO_2 evolution. The degradation reaction was restricted only by adjusting the environmental temperature while sufficient oxygen and substrates were supplied. Results suggested that the optimum temperature for degradation was 30°–40°C for exploiting biological activity effectively with the lowest use of energy. Bacteria from the environment propagated in the reactor with no inoculum added. The microbial flora changed during the operation time but had no effect on the biodegradation rate.

Key words Biodegradation · Food waste · Optimum temperature · Wood particles · Microorganism

Introduction

A garbage automatic decomposer-extinguisher (GADE) machine that processes organic waste, especially food waste, at a site where it is produced has been developed.[1–4] Microorganisms propagate within a wood matrix in a container of this machine, degrading garbage aerobically when it is put in the machine.

Aerobic degradation using a wood matrix has high performance, but the optimum operating conditions must be determined to use the machine effectively. We have reported that the moisture content of the wood matrix has an effect on the degradation rate using a small-scale degradation reactor and uniform model waste.[5] The environmental temperature is also an important factor. Several studies were conducted to determine the optimal temperature for composting. Kuter et al. reported that the optimal temperature for composting cow manure was 40°–60°C.[6] Suler and Finstein reported that 56°–60°C was optimum for composting food waste;[7] and Nakasaki et al. reported that the optimal temperature for composting sewage sludge was 60°C.[8] There has been no report about the optimal temperature for small-scale biodegradation machines such as the GADE. The temperature mentioned above is higher than that of the matrix in the GADE.[2] In our experiment, the degradation rate was accurately estimated in a small-scale degradation reactor. When using such a reactor, the temperature of the matrix can be considered equal to the environmental temperature.

The number and community of bacteria that change with the environmental temperature may influence the degradation rate of the waste. Multiplication of the microorganism was confirmed at an environmental temperature of 30°C in our previous report.[5] It was expected that microorganisms adapting to the environmental temperature propagate at higher or lower temperatures.

In this study, we examined the effect of the environmental temperature on the degradation rate by determining the rate of weight loss and CO_2 evolution to estimate optimum conditions more accurately. The number of bacteria in the wood matrix was monitored continuously throughout the experiment. A profile of the bacterial community was also investigated by a Gram stain test and utilization of carbon sources.

S. Horisawa (✉) · S. Doi
Institute of Wood Technology, Akita Prefectural University, Noshiro, Akita 016-0876, Japan
Tel. +81-185+52-6986; Fax +81-185-52-6975
e-mail: horisawa@iwt.akita-pu.ac.jp

Y. Sakuma
Biological Resources Division, Japan International Research Center for Agricultural Sciences, Tsukuba 305-8686, Japan

Y. Tamai · M. Terazawa
Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

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Materials and methods

Degradation experiment

The wood particles used as a matrix were prepared from sapwood of Japanese cedar [*Cryptomeria japonica* (L. fil.) D. Don]. They were ground, and a grain size of 0.5–1.0 mm was sieved. The moisture content of the wood particles was adjusted to 60% on a wet weight basis. A formula feed for rabbits, made from alfalfa, flour, delipidated soybean, and wheat germ (Hi-Pet Co., Osaka, Japan), was applied as the model waste because its content ratios of crude protein (19%), fat (2%), fiber (10%), and ash (9%) were similar to that of real food waste.[1,9–11] The carbon/nitrogen (C/N) ratio of the model waste was 13.9.

A 1000-ml Kjeldahl flask was used as a small-scale degradation reactor.[5] Each setup was established in a separate incubator adjusted to temperature of 10°, 20°, 30°, 40°, or 50°C. The moisture content of the matrix was kept at 60%. Model waste (3.5 g) was added to each reactor every 24 h. Each of the three experiments lasted 30 days at 10°, 20°, 30°, 40°, and 50°C, respectively.

Initially, 40 g of dried wood particles were placed in the reactors where the moisture contents were adjusted to 60%. Each reactor was rotated at 30 rpm for 2 min every 15 min. The model waste (3.5 g) was added to each of the reactors every 24 h. The ratio of model waste to wood particles was greater than the ratio of the actual waste to the wood particles used in the previous study.[1,2] The moisture contents of the wood matrices were checked with an infrared moisture content meter every 24 h by using a 7-g sample from each treatment. The moisture contents of the matrices were then adjusted to maintain a constant level.

Weight loss, evolved CO_2, pH level, and viable count of bacteria of the model waste

The total weight of the residual model waste was determined by weighing the model reactor flask, including the wood matrix, and the model waste, assuming that the matrix was not degraded. The total weight loss of the degraded model waste was calculated by determining the difference between the original weight and the residual weight.

The concentration of carbon dioxide (CO_2) in the exhaust gas was monitored by a gas detector (Gastec, Kanagawa, Japan). The rate of CO_2 evolution was calculated by determining the difference in the concentration of CO_2 in the inflowing air and the outflowing air, airflow rate, temperature, and gas constant using the equation for the ideal gas state. The pH of the supernatant, 30 ml deionized water mixed with the 3 g wet matrix, was measured using a pH meter. All of these measurements were performed each day just before the new model waste was added.

The number of viable bacteria in a matrix attained from each treatment every 5 days was counted[5] by a serial dilution method following standard methods.[12] The medium was composed of yeast extract (0.25%), peptone (0.5%), glucose (0.1%), and agar (1.5%). The plates on which about 20 colonies appeared were selected, and the dominant bacteria were isolated. They were classified as gram positive or negative using SMA-added crystal violet (5 ppm).[13] The bacteria were isolated and identified by a Biolog system, which determines the utilization pattern of 95 carbon sources.[14,15]

Results and discussion

Rate of weight loss and CO_2 evolution

The time courses of total weight loss, CO_2 evolution, pH, and number of viable bacteria in the matrix at 10°C and 40°C are shown in Fig. 1, as an example. The variation of weight loss in the time charts was small at the examinations except for the results at 10°C. Degradation could be stabilized by restricting environmental conditions such as temperature, moisture content of the matrix, and aeration rate.

A lag phase of the degradation test showed no weight loss in the waste at the various temperatures. The lag phase at higher temperatures became shorter. The relation between the incubation time and weight loss was linear after the lag phase. The rates of weight loss, determined from the slopes of the lines, were 1.45, 1.77, 1.95, 2.01, and 2.15 g/day at 10°, 20°, 30°, 40°, and 50°C, respectively. In the range of temperatures used, the higher temperatures showed a higher rate of weight loss. From those values, the rates of weight loss ($R\text{-}_w$) in the supplied waste weight, which were quotients of the rate of weight loss to the rate of model waste added (3.5 g/day), were calculated to be 298, 502, 556, 556, 590, and 631 mg/g at environmental temperatures of 10°, 20°, 30°, 40°, and 50°C, respectively (Fig. 2). The sample at the lowest environmental temperature, 10°C, showed the lowest rate of weight loss; but samples at other temperatures, 20°–50°C, showed nearly the same rates of loss.

The speed of CO_2 evolution increased gradually from the initial stage and became almost constant (steady state) at each temperature condition except 10°C. At 10°C, the lag phase was longer. The constant levels of CO_2 evolution were determined from the average levels at steady state to be 1.12, 2.15, 2.52, 2.65, and 2.24 g/day at 10°, 20°, 30°, 40°, and 50°C, respectively. Based on these values, the rates of CO_2 evolution in the samples ($R\text{-}_{CO_2}$), which were quotients of the speed of CO_2 evolution to the speed of adding model waste (3.5 g/day), were determined to be 320, 565, 613, 705, 745, and 626 mg/g, respectively (Fig. 3). Samples at the upper and lower ends of the temperature range showed lower rates of CO_2 evolution.

The $R\text{-}_w$ value increased with an increase in environmental temperature, whereas the $R\text{-}_{CO_2}$ peaked at 40°C. This supposed volatilization of low-molecular-weight compounds compensated for the decline in CO_2 evolution and made the rate of weight loss higher at 50°C. This finding suggests that heating is not necessary to exploit biological activity effectively. However, the GADE machine should

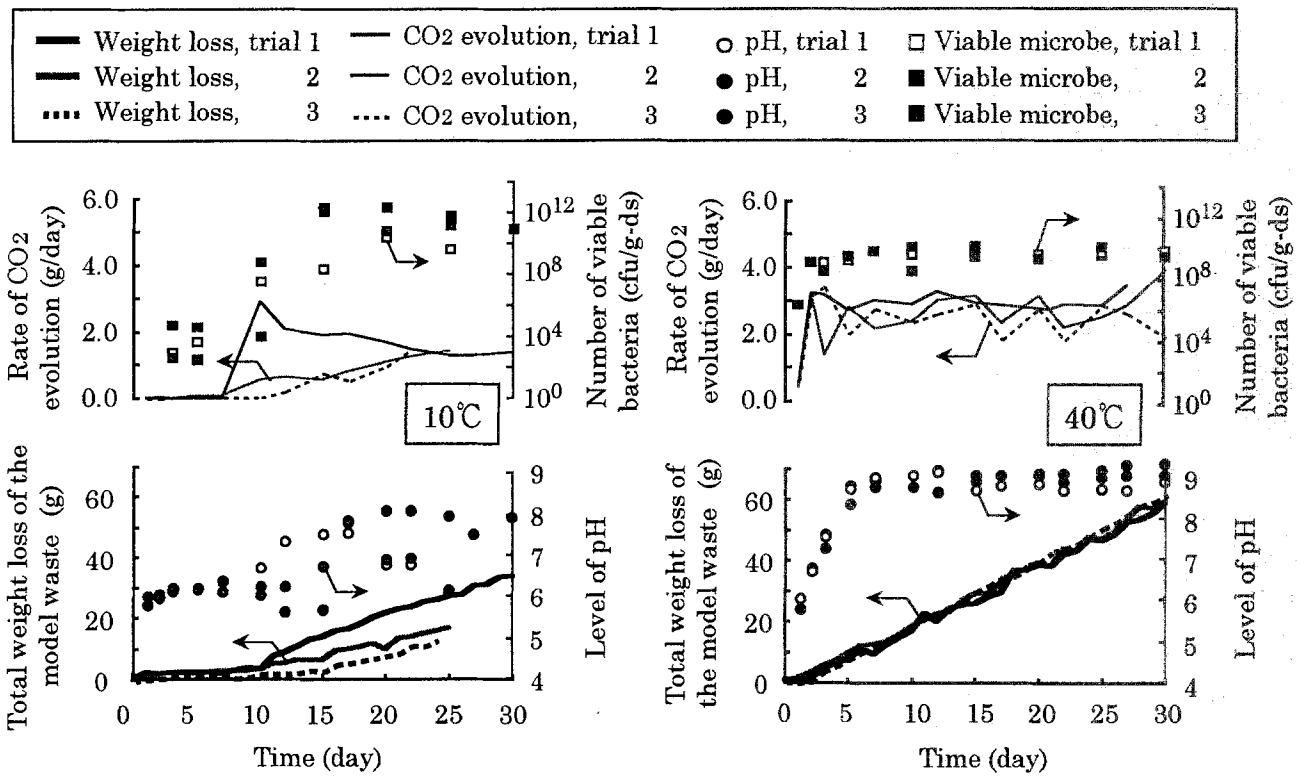

Fig. 1. Time course of total weight loss of the model waste, rate of CO_2 evolution, number of bacteria, and level of pH of matrix during the biodegradation operation of model waste at 10°C and 40°C

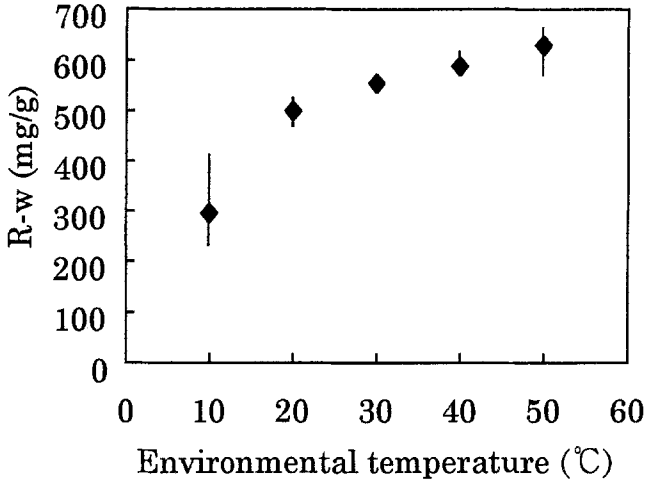

Fig. 2. Relation between environmental temperature and the ratio of weight loss of the model waste, $R\text{-}_w$, in the steady state. *Vertical bars* show maximum and minimum values in three plots

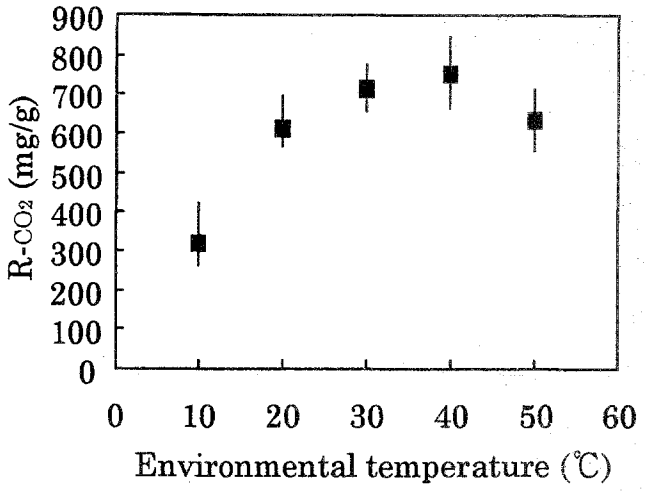

Fig. 3. Relation between environmental temperature and the rate of CO_2 evolution, $R\text{-}_{CO_2}$, in the steady state. *Vertical bars* show maximum and minimum values in three plots

be equipped with a heater or a heat insulator when it is used at a lower temperature.

Viable microbial concentration and pH of matrix

Bacterial growth was observed in all trials despite the fact that no inoculum was added. No bacterum was detected at the initial stage of the experiments, but the number of bacteria increased logarithmically during the early stages until a steady state was reached (Fig. 1). It is believed that the bacteria came from the air or the wood particles and multiplied in the reactor. Similar results were reported by Inoue et al.[16] and by us.[5] The higher temperatures took a shorter time to reach a steady state and allowed a smaller number of viable bacteria at the steady state. An inverse relation was shown between the number of viable bacteria and the environmental temperature (Fig. 4).

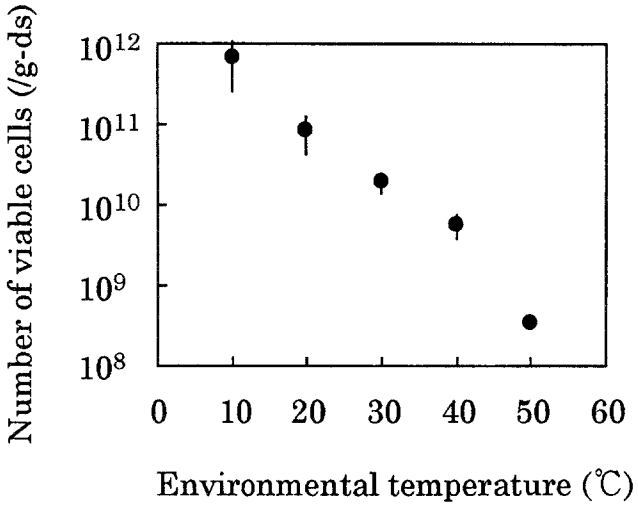

Fig. 4. Relation between the environmental temperature and the number of viable microorganisms in the steady state. *Vertical bars* show maximum and minimum values of three plots

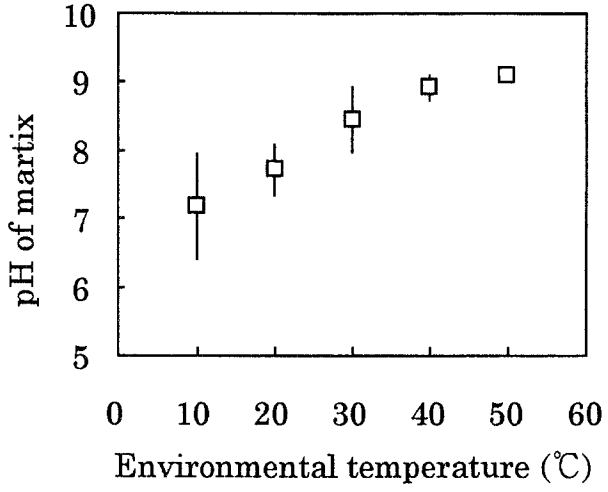

Fig. 5. Relation between the environmental temperature and average pH of the matrix in the steady state. *Vertical bars* show standard deviations in the steady state

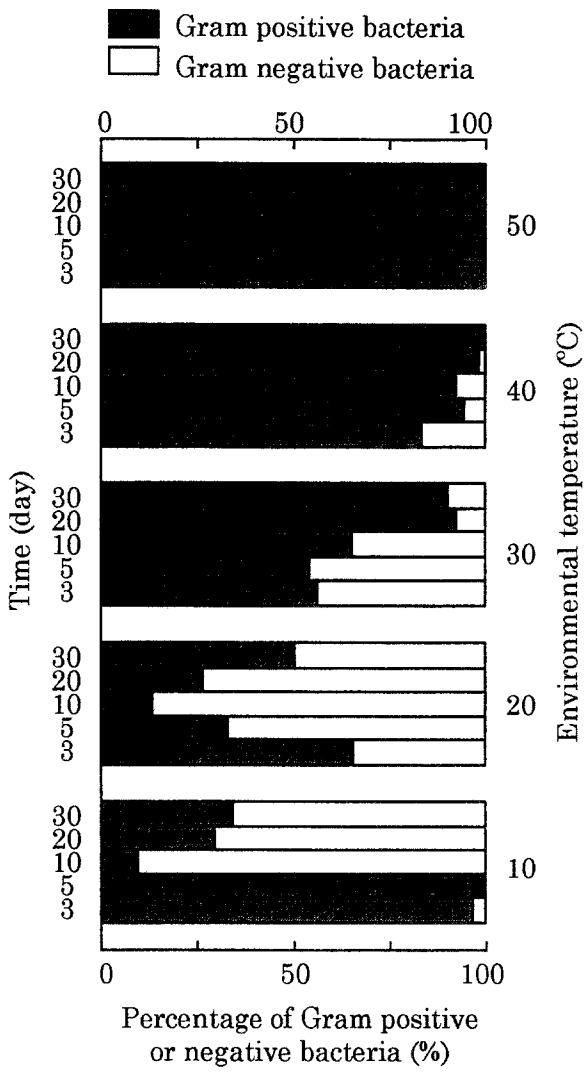

Fig. 6. Transitions of gram-stained bacteria due to temperature and elapsed time

The pH levels of the original sawdust and the raw model waste were 5.50 and 5.72, respectively. The pH of the matrix changed from acidic to alkaline at each temperature condition after reaching the steady state (Fig. 1). These results are similar to those of our previous experiment.[2] The pH increased more rapidly at higher temperatures. After the pH reached 9, it maintained a constant level. It was reported that biodegradation during composting proceeds in largely alkaline conditions.[17] The average pH at a steady state increased from the environmental temperature of 10°C to 50°C (Fig. 5). The pH increases were due to the generation of ammonium ions,[18] which is believed to be more active at the higher temperature.

The results of the pH tests and the number of viable bacteria suggest that the degradation reaction equilibrates without adjusting the pH or microbial concentration, and it is restricted only by changes in the environmental temperature when oxygen and substrate are sufficiently supplied. The pH level also changed markedly, but there was no effect on R_{-w}. The pH level is likely to be closely related to the bacterial community.

Transition of bacterial flora

The results of the transition of the bacterial community are shown in Fig. 6. Gram-positive bacteria were dominant during the early stages, and Gram-negative bacteria appeared during the later stages at 10°C and 20°C. Gram-positive bacteria were dominant in conditions of comparatively higher temperatures. Moreover, at 50°C all isolates were Gram-positive. Almost all of the Gram-positive bacteria were identified as *Bacillus* sp. and *Cellulomonas turbata* from the Biolog system. These species are dominant in sewage treatment facilities.[19] These results suggest that bacterial flora in the reactor changes in direct correlation with environmental conditions. The bacterial community changed as the process progressed, but it did not signifi-

cantly influence the rate of weight loss (slopes of the total weight loss) or the rate of CO_2 evolution (constant CO_2 evolution rate) (Fig. 1).

Conclusions

The degradation reaction, using a small-scale degradation reactor and model waste, was restricted only by adjusting the environmental temperature while sufficient oxygen and substrates were supplied. To exploit biological activity effectively with the lowest use of energy, the optimum temperature for biodegradation of the model waste was 30°–40°C.

Bacteria from the environment propagated in the reactor at all of the temperatures tested, with no inoculums added. The microbial flora changed during the operation time but did not affect the biodegradation rate. As a result, inoculation was found to be unnecessary.

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