

TREATMENT OF FOOD WASTE MATERIAL BY EFFECTIVE MICROORGANISMS AND ITS USE IN CROP PRODUCTION

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Abstract

A study evaluated the usefulness of Effective Microorganisms (EM) in treating food wastes by fermentation, and the benefits of this compost in crop production. The fermentation was carried out in plastic buckets which were designed as garbage bins with a leachate removal system at the base. The fermentation process was monitored for microbial populations, pH, and salinity, while the effect of the leachate on crop growth was measured.

The use of EM reduced the offensive odor of fermenting garbage within 4 days. The development of esters and alcohol in the process of fermentation was evident in two days. The development of Lactic acid bacteria observed, along with the increase in acidity of the leachate. These parameters were used as indicators of fermentation. The leachate removed approximately 35% of the salt content of the waste food. However, this leachate enhanced germination of crop seeds, with no adverse effect on seedling growth. The addition of the compost increased soil properties which could promote crop growth. The potential of EM in fermenting food wastes and its use in crop production presented.

Introduction

The total solid waste in Korea has increased by 8% annually but reached culmination in 1991 and decreased afterwards. The food waste, however still maintains a high percentage (31.6%) and the waste composition is projected to be about 41% of total municipal solid wastes^{5,7}. The reduction and recycling of food wastes through an efficient treatment is therefore highly important considering enormous cost of the waste treatment and the secure of its landfill site which becomes increasingly difficult recently. Especially the food wastes cause not only a visual discomfort but also problems in their handling and storage. Moreover they are one of the major sources for leachate generation in most landfill sites and can be readily degraded to produce various putrefying gases and offensive odor, eventually causing the subsidence of the landfill site. Notwithstanding, the recycling rate of the wastes in Korea is significantly low (2.1%) and most of them (95.4%) becomes buried¹⁸. To combat the disposal problem their recycling through composting and feedstock production by fermentation is seriously considered recently.

The current composting techniques were initially employed as a contingency plan to alleviate the problems associated with waste reduction at the sources and land filling. It is, therefore, necessary to establish and develop an efficient collection and composting system for the food wastes that allows solution of problems coped with collection (offensive smell and sanitation) and production of quality composts. Accordingly, Effective Microorganisms (EM) has been introduced by Pusan Metropolitan City Government and other local governments in Korea and used for composting of food wastes a few years ago. The mechanistic basis of EM action

during composting, however, was barely pursued. EM was originally developed by Dr. Higa (Ryukyu University, Okinawa, Japan) in 1982 and composed of several microbial groups such as lactic acid bacteria, filamentous fungi, yeasts, *streptomycetes*, and photosynthetic bacteria (10 genera include 80 species), and the recent improved products include quite less number of representative species. These microbial groups work in the ways beneficial to the environment, and live interactively and synergistically, and can enhance the anti-oxidation capability in soil, making possible the organic farming. EM products, moreover, can prevent putrefaction and hence suppress the generation of offensive odor, are reported to be effective in treating the wastewater^{3,9}. The lactic acid bacteria can rapidly decrease pH through production of lactic acid in the early fermentation stage, suppressing growth of putrefying bacteria and enhancing the availability of the inorganic compounds (i.e., phosphoric salts). The filamentous fungi can degrade macromolecules whose monomers are utilized by all the microbial groups, and yeasts can synthesize bio-active materials (vitamins, hormones, etc.), stimulating growth of other EM organisms. *Streptomycetes* can produce various antibiotics, inhibiting growth of soil-borne pathogens, and photosynthetic bacteria can fix and utilize CO₂ and H₂S generated in putrefaction process, resulting in removal of the pollutants and offensive odors⁸.

At present several EM products (EM Bokashi, EM-cerarnic, EM-X, etc.) are available in the market and mainly supplied as microbial agents for agriculture (crop production and husbandry) and wastewater treatment uses in U.S.A. Japan, and Southeastern Asian countries as well as Korea. In a recent EM symposium held in Thailand a large number of research works were presented regarding the EM application to agricultural production and prevention of environmental contamination and their effects. Herein the effectiveness of EM culture solutions as pesticides and fertilizers was rarely acknowledged¹⁰ but the fertility of composts manufactured from agricultural wastes treated by EM was proven to be compatible to chemical fertilizers in terms of crop productivity¹³. When an EM culture solution was applied to the treatment of domestic wastewater, there was little difference in the treatment efficiency compared with the indigenous activated sludge¹⁹. By the way generation of H₂S was significantly inhibited when *Proteus vulgaris* was grown on peptone iron agar²⁰. In these studies, however, the aspect of EM application was emphasized while little explanation was made about mechanisms supporting the phenomena. Aerobic composting of food wastes has been studied^{1,12} but very few researches have been performed on composting of the wastes by using EM domestically and internationally.

The objectives of this study were to analyze the EM products used in Pusan Metropolitan City, to investigate physicochemical and biological aspects of the EM fermentation process for food wastes and to evaluate effectiveness of the fermentation leachate as a fertilizer.

Materials and Methods

1. Isolation and identification of EM organisms from EM products

Isolation and identification of EM organisms from EM products and measurement of their population density were performed according to the manual⁴ and the procedure recommended by EM Research Organization (Okinawa, Japan). Analyzed organisms were lactic acid

bacteria, filamentous fungi yeasts, *Streptomyces*, and non-EM organisms while photosynthetic bacteria were not measured throughout this study. All the analyses were done by diluting samples (products and fermented materials) in phosphate buffer (pH 7.0) at appropriate levels (10^{-10}), and then spread-plated onto an appropriate agar media and incubated at 25-30°C at least one week before observing the morphological and physiological characteristics. Lactic acid bacteria (LAB) and non-EM organisms were isolated using Bacteriological trypticase soy agar (Difco) while LAB were further identified on MRS agar containing trace amount of bromophenol blue. Filamentous fungi, yeasts, and *Streptomyces* were isolated and identified by using Rose Bengal, YM agar, and glucose-asparagine agar.

2 Fermentation of food wastes

Fermentation experiments were performed in EM bucket (10 L) with a leachate collection system on the bottom and waste sample (5.9Kg; moisture content 80%) used for fermentation was taken in the bucket after its maceration up to 3-5mm in diameter to secure the representations of samples for analysis. EM Bokashi used was products from Pusan Red Cross (named "PRC") and from Pusanjin Office (named "PJ") which were treated at 3% (w/w) of the waste. Each treated waste was left under ambient room temperature and light conditions for 10 days. The control did not contain EM Bokashi. The wastes were collected at kitchens of dormitory restaurants and student cafeteria, and their composition was: grains (20-50%, w/w), fruit and vegetables (32-67%) and fish and meat (14-17%).

3. Analysis of fermentation process

Solid waste (40-50g) and leachate (50ml) were collected every other day for 10 day-period to do the fermentation process analysis. Moisture content, pH, salinity, leachate volume, color and morphological change of food waste were measured or observed as physical and chemical indicators for monitoring the fermentation. In addition biological and biochemical parameters were smell, microbial population density, and acidity.

(1) Measurement of moisture content

Sample (10-20g) was taken in aluminum foil and dried at 103-105°C for 3-4 h, and the moisture content was calculated based on the weight loss due to evaporation.

(2) Measurement of pH and salinity

For pH measurement of solid waste, sample (10-20g) was taken into 100-200ml of distilled water and incubated at shaking incubator (25°C; 200rpm) for 1 h and then measured using Orion PerpHect Meter (Model 350; Analytical Technology Inc., Boston, MA). The leachate was directly measured without dilution. Salinity was measured by Salinometer (Model LC 84; TRS Pty. Limited, Brisbane, Australia) at room temperature after diluting the above extracted samples or leachate.

(3) Measurement of smell

Smells occurring during fermentation were differentiated by two categories such as non-offensive smells (esters alcohol etc.) and offensive smells (ammonia, hydrogen sulfide, etc.) and measured based on sensual method⁶⁾.

(4) Measurement of acidity

The total acidity of leachate was measured as an indicator of lactic acid fermentation by EM. The acidity was measured by titrating the diluted sample (50ml) using 0.05N-NaOH¹⁷⁾.

4. Crop germination test and young plant growth test

Germination rates of Chinese cabbage (Seoul cabbage Seoul Seed Co., Seoul), radish (Changpyung radish, Kyungsin Seed. Co., Euisung) and Garland chrysanthemum (Seoul Seed Co., Seoul) were measured to elucidate the effect of EM leachate on crop growth. Germination rates were measured according to Abridged Guide to Compost Analysis published by Advanced Research Institute for Agriculture¹⁴⁾.

Growth test of radish and Chinese cabbage seedlings was also done according to the above guide to examine fertility components in leachate and maturity of the EM food waste compost. For leachate experiment for the crops, sand culture and water culture systems were employed while compost maturity test was performed by seeding Chinese cabbage in sandy soil carrying the food waste composted for 1 month. Hoagland solution 1(H-1 solution)¹¹⁾ was used as a basal solution which received the leachate at 500-1000 times dilution. For the sand culture system fine beach sand (less than 1 mm in diameter) was washed 3 times with tap water and finally with distilled water once and was then autoclaved. Miniature pots (diameter 10 cm height 10 cm; 500 ml) were filled with the sand, where radish and Chinese cabbage seeds (10 seeds) were planted in each pot with three replications for each treatment. For the first 5 days sterilized distilled water was used to irrigate the crops until they reached a seedling stage and then 10 ml of Hoagland solution-1 with or without EM leachate was irrigated to each pot daily for 3 weeks. For the water culture system a rectangular 3MM paper (10 x 12 cm) was rolled 2-3 times and stapled, and then 6 seeds of Chinese cabbage were put in the slots and held 2-3 cm from the top. The filter papers were put into test tubes (diameter 2.5 cm height 14 cm) containing 20 ml of Hoagland solution-1 with or without EM leachate and soaked. The crops were then grown for 2 weeks when shoot height was measured every week and dry weight at the final day of the crop.

Results and Discussion

1. Microbial analysis of EM products and others

EM microbes and other non-EM organisms were isolated from EM products and other microbial agents in order to elucidate whether efficacy of the products were due to the activity of the microbes. The major target organisms were lactic acid bacteria, filamentous fungi, yeasts, *Streptomyces*, and non-EM organisms found together. The microbial species and their population density were shown in Table 1. Here EM products did carry lactic acid bacteria, filamentous fungi, yeasts and *Streptomyces* (at least 10⁴ viable cells/g) while they were not isolated from other companies' products (10-10000 times dilution rates). Other products, therefore, may not work in a way that the EM products function. This was supported by the fact that Product A (manufactured by "K" Co.) showed a suppression effect of putrefaction smell during food waste fermentation but did not produce a sweet smell (esters) peculiar to the EM fermentation and the leachate showed very low acidity (pH 3-4).

Growth of the mixed culture from EM stock cultural solution (PRC) and pH change during the growth were shown in Fig. 1. The mixed culture appeared to have little difficulty in growing at room temperature (around 25°C) and pH of the medium decreased up to 4 within 2-3 days.

Table 1. Microbial groups and their population density in the commercially available microbial agents used in the fermentation of food wastes.

Microbial groups ¹	Population Density (c.f.u./g or c.f.u./ml) ²			
	EM Bokashi (Pusan Red Cross)	EM Bokashi (Pusanjin)	Product A (“K” Co.) ³	Product B (“S” Co.)
Lactic acid bacteria	5 x 10 ⁴	5 x 10 ⁵	0 (10)	0 (10 ⁴)
Filamentous fungi	4.1 x 10 ⁶	0 (10 ³)	0 (10)	0 (10 ⁴)
Yeasts	4.5 x 10 ⁶	5.1 x 10 ⁶	0 (10)	0 (10 ⁴)
Streptomyces	3.0 x 10 ⁴	0 (10 ³)	0 (10)	0 (10 ⁴)
Others	0 (10 ³)	0 (10 ³)	5.1 x 10 ³	4 x 10 ⁵

¹Photosynthetic bacteria were not measured; “Others” indicates non-EM bacteria;

²The number in the parenthesis indicates dilution rate;

³Formulated as liquid.

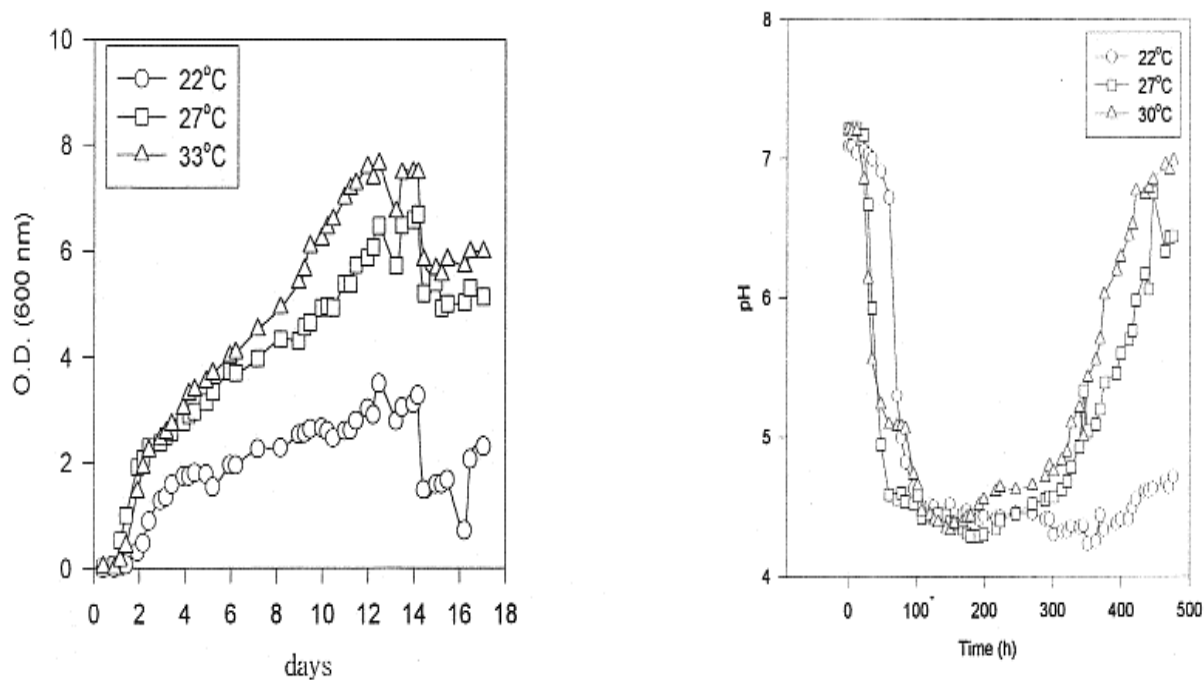


Fig. 1. Temperature effect on the growth of EM (effective microorganisms) stock (Pusan Red Cross: PRC) in trypticase glucose soy broth (A) and pH change during the growth (B).

2 Effect of EM fermentation on treatment of food wastes

EM fermentation was performed under ambient conditions (similar conditions found at a household) to eventually investigate effect of compost from the EM-treated food wastes on soil physicochemical and biological characteristics.

(1) Suppression effect of offensive odors⁶⁾ The results based on the sensual method showed that little of non-offensive smells (esters and alcohol unique to EM fermentation) were detected in the non-treatment throughout the experimental period while the moderate level of offensive smells (ammonia and hydrogen sulfide) were consistently observed in the EM-treatment (PRC) during the period. This appeared to result from a certain activity of indigenous lactic acid bacteria and the slow putrefaction process in the top portion of the food wastes that were relatively aseptic due to cooking. Strong offensive smells, however began to occur after 2 weeks. On the other hand, the non-offensive and sweet smells were increasingly observed (up to the very strong level) in the EM treatment during the fermentation while the offensive smells gradually decreased and little was detected in 4 days. This effective fermentation was maintained at least for 3 weeks. This was presumed to result from the EM microbial activity so that a few important parameters including lactic acid bacteria involved in the fermentation were examined.

(2) Population dynamics of lactic acid bacteria

Higa⁸⁾ reported that lactic acid bacteria could inhibit growth of putrefying bacteria and hence suppress the production of offensive smell in the fermentation of organic materials. Therefore, the population densities of lactic acid bacteria and putrefying bacteria first measured (Fig. 2). Here total population density of lactic acid bacteria (2 species of lactic acid bacteria were observed for both treatment and non-treatment) generally increased regardless of EM treatment as the fermentation proceeded. Particularly the increase was significant in non-EM treatment whose density showed 4-5 times as high as that of the EM-treatment (Fig. 2, left). The density increase, however, appeared to be caused by domination of the lactic acid bacterial population which turned out to be weak in lactic acid production (refer to Fig. 4, right). In contrast the population density of putrefying bacteria decreased in all treatments (Fig. 2, right). This seemed to echo Higa's result⁸⁾. The relative population density of lactic acid bacteria in the EM treatment (PJ) was generally higher than non-treatment within one week but no difference was observed after 8 days (Fig. 3). Although quite a high number of lactic acid bacteria (10^{10} cells/g) were also detected in the non-treatment, this population was relatively lower in lactic acid productivity and considered as an indigenous population from the food wastes themselves. In non-EM treatment, there was little flavor that was usually observed in the EM-treatment and the leachate volume was much less.

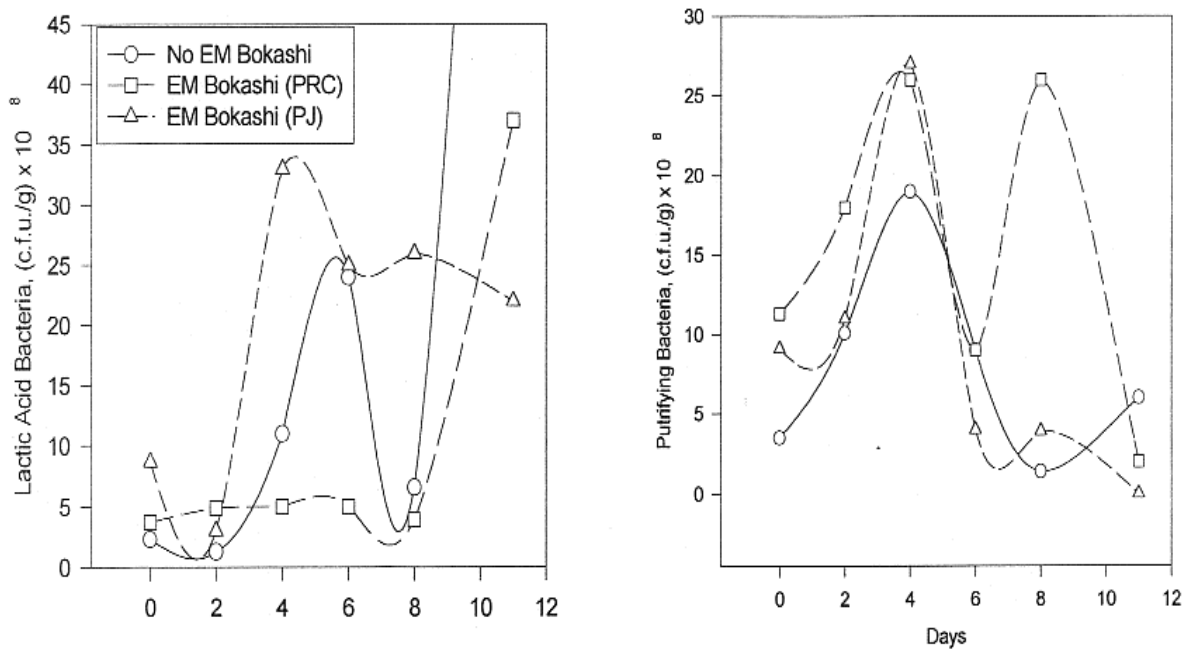


Fig. 2 Population dynamics of lactic acid bacteria and putrefying bacteria during the EM fermentation in bucket carrying food waste (5.9kg) treated with EM Bokashi, PRC and Pusanjin (PJ) (3% w/w).

(3) Comparative analysis in lactic acid production

To take a close look at the above phenomena a comparative analysis of total acid production¹⁷⁾ as an indicator of lactic acid production was made for each treatment. Time course of the total acid production was shown in Fig. 4 (left). The EM treatments (PRC and PJ) produced at least 50 % more acid than the non-EM treatment. This results indicated that low acid production in the non-EM treatment might be reflected by the weak acid production rate of lactic acid bacteria. Thus pure cultures of lactic acid bacteria were isolated from each treatment and evaluated for their lactic acid production (Fig. 4, right).

As shown here acid production of the strains (LAB2-PRC and PP2-PRC) from the Bokashi (PRC) treatment was relatively higher than that of the dominant strain (PP1-None; 97%; 11 days) from the non-EM treatment. Besides the non-dominant strain (LAB1-None) from the non-EM treatment had an excellent acid production capacity in the pure culture form but the relative population density was significantly low (3%). It was, therefore, assumed that a successful EM fermentation would be secured when lactic acid bacterial population(s) of a better acid-production capacity took a niche at the first stage of the fermentation, which resulted in suppression of putrefying bacterial population, and then other EM species (yeasts, filamentous bacteria and photosynthetic bacteria) would take the later successional stage.

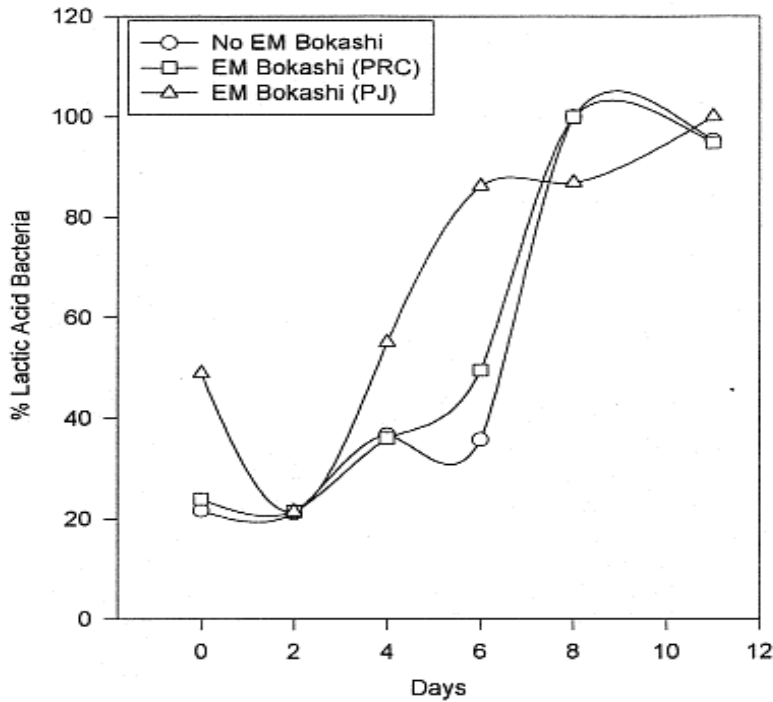


Fig. 3. Percent distribution of lactic acid bacteria in the total bacterial population during EM fermentation in bucket carrying food waste (5.9kg) treated with EM Bokashi, PRC and PJ (3% w/w).

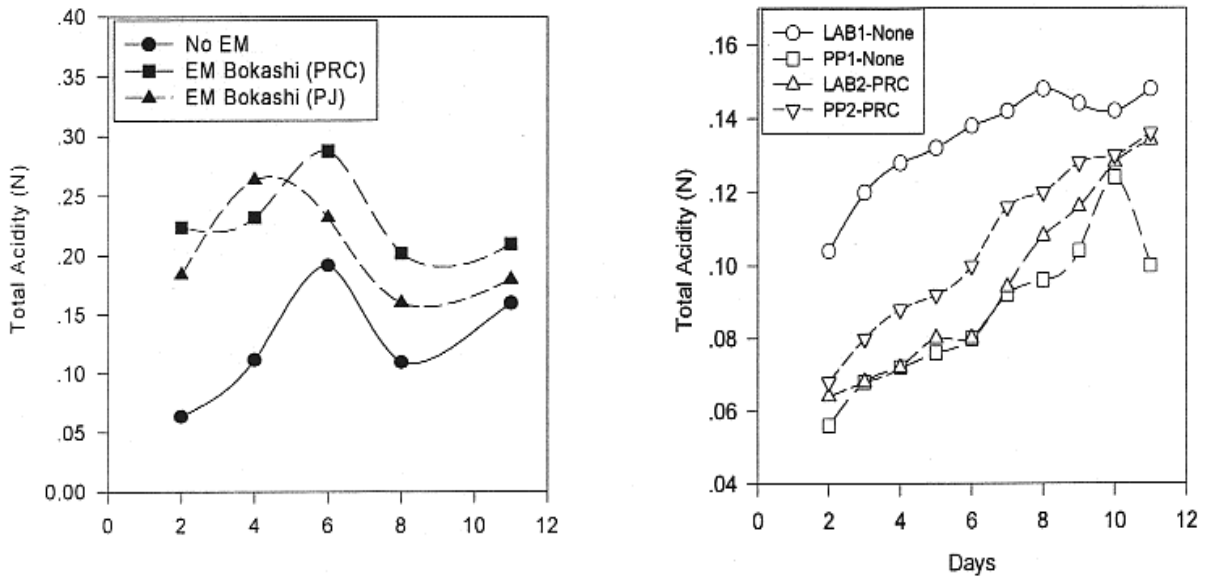


Fig. 4. Dynamics of total acidity in the leachate during EM fermentation in bucket carrying food waste (5.9kg) treated with EM Bokashi, PRC and PJ (3%, w/w) (A), and ability of total acid production by pure culture of lactic acid bacteria isolated from each EM-fermented food waste (B). Each culture was grown in trypticase glucose soy broth and the total acid was titrated with 0.05 N NaOH.

(4) Change of moisture content

Change of moisture content during the fermentation was shown in Fig. 5. Moisture removal effect (5-8%) was observed in the EM-treatments. Little change in moisture content of non-EM treatment throughout the period appeared to result from the very slow leaching of water as the putrefaction process was slow (leachate, 3.4% in 11 days). In contrast most of leachate was generated in the EM-treatment in 7 days and reached 25% in 11 days. The observation of texture and color changes of the EM-treated food wastes showed that destruction of the food waste by the activity of EM organisms could allow the leakage of water present in tissues (unpublished data). By the way EM Bokashi-treatments caused the initial moisture content to be 2% less than the control, and a little increase in 6 days was attributed to the sprinkling of each leachate on the top of the wastes by the experimenter's mistake. Moisture removal during the fermentation can make the subsequent composting procedure easier in terms of waste handling and reduction in bulking agent used as a moisture controller.

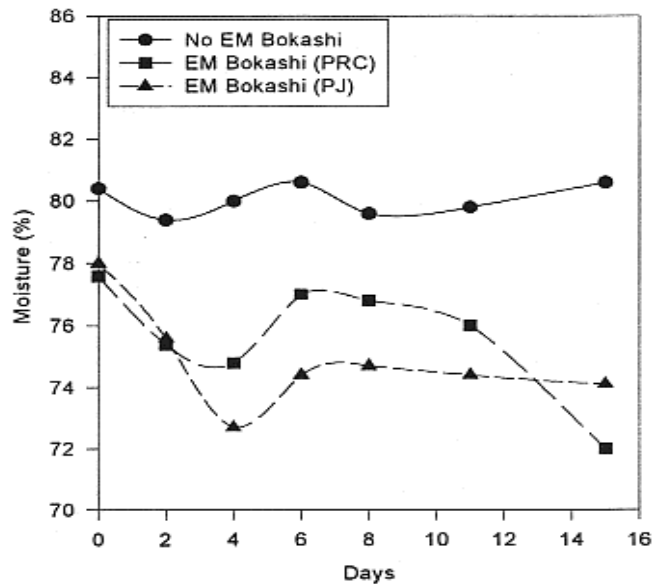


Fig. 5. Change of moisture content in the food waste during EM fermentation in bucket carrying the waste (5.9kg) treated with EM Bokashi, PRC and PJ (3%, w/w).

(5) Change of salinity in leachate

The salinity increase (30-60%; Fig. 6) in the leachate of EM-treatments appeared to be related to the effective production of leachate by working of EM organisms. As mentioned above, the destruction of food waste tissues was obviously observed in the EM-treatments while little structural changes were observed in the non-EM treatment. The destruction of tissues might be ascribed to the functions of EM organisms (particularly, filamentous fungi and yeasts) in the wastes whose major components were grains, fruits and vegetables.

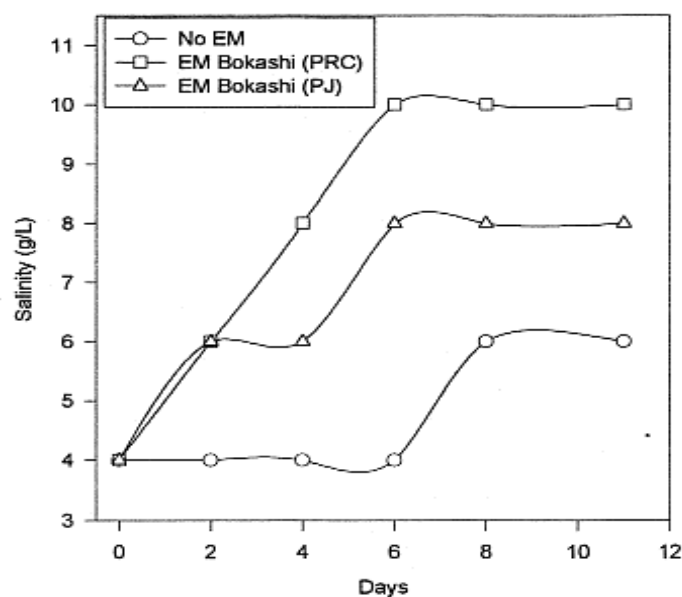


Fig. 6. Change of salinity in the leachate during EM fermentation in bucket carrying food waste (5.9kg) treated with EM Bokashi, PRC and PJ (3%, w/w).

Salt removal through generation of the leachate was measured (Table 2). The removal rates in the treatments were 7-8 times higher than control. The salt removal effect will be beneficial in view of application of the composted could be introduced to the soil, otherwise the equivalent amount of salt could be introduced to the soil.

Table 2. Effect of EM fermentation on the removal of salts through the leachate generated from the food waste.

Leachate measurements	Fermentation condition ¹		
	No EM	EM Bokashi (Pusan Red Cross)	EM Bokashi (Pusanjin)
Volume (mL)	20 1	1491	1486
Salinity (g/L)	6	10	8
Total salts amount (g)	1.21	14.9	11.9
Salt removal rate (%) through leaching ²	4.1	34.1	30.4

¹The fermentation of food waste (5.9 kg) using 3% (w/w) EM Bokashi was carried out in each bucket (10 L) at room temperature for 11 days.

²The rate was based on the initial salt content of the food waste.

3. Effect of EM leachate on growth of crops

Germination and young plant tests of crops were performed in order to confirm fertilizer components in the EM leachates.

(1) Germination test

As shown in Fig. 7, the germination of Chinese cabbage and garland chrysanthemum was stimulated by treating the EM leachates (PRC and PJ) (about 5% and 10%; increase respectively). In radish, however, little effect was shown, which was probably due to the generic high germination rate of the crop's itself.

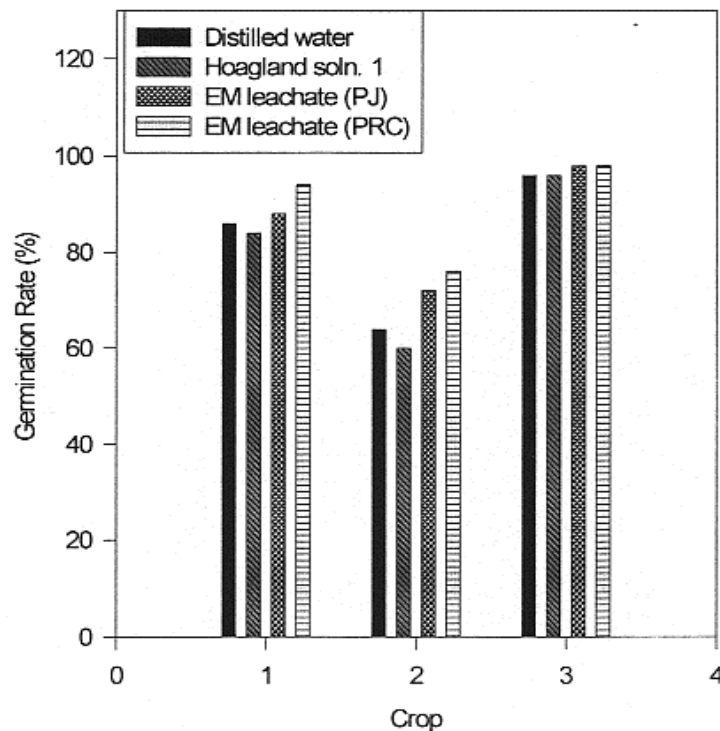


Fig. 7. Effect of EM leachate on the germination of crop. EM leachate (PRC and PJ; 8 days old) was diluted up to 1000 times and applied to the seeds of the following crops: crop 1, Chinese cabbage; crop 2, Garland chrysanthemum; crop 3, radish.

(2) Effect on young plant growth

Fertilizer effect of the leachates on young plant was investigated using water and sand culture systems. The water culture system used Hoagland solution-1 as a basal medium to which a certain concentrations of EM leachates were added for the growth stimulation test (Fig. 8). Here there was no statistically significant difference between the treatments and control.

To test fertilizer effect of the leachates using the sand culture system, Chinese cabbage, radish and garland chrysanthemum were seeded in a sterilized sand and dilutions (500-1000x) of leachate were applied to these crops daily for 3 weeks, and then shoot growth was measured. In 3 weeks, a little growth stimulation effect was detected in radish while growth of the chrysanthemum was inhibited and little effect was shown in the cabbage. By the way there was no statistical significance in the treatment when the radish was harvested and measured for root and shoot dry weight (unpublished data). It was concluded that the growth inhibition of chrysanthemum was caused by certain component(s) of leachate (especially, lactic acid).

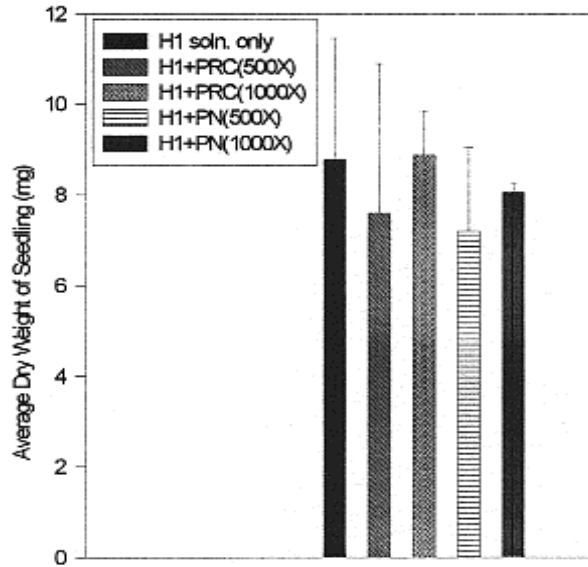


Fig. 8. Effect of EM PRC and PN (an improved PRC) leachate on the growth of Chinese cabbage seedling grown in the water culture system for 2 weeks. The treatments were H-1 (Hoagland solution 1) that contained 500x or 1000x dilutions of each EM leachate (11 days old).

This was supported by the fact that pure lactic acid (several hundred ppm) was shown to significantly inhibit the germination of the crop (unpublished data). Therefore, the growth stimulation effect of EM leachate on the young crops tested was hardly realized. In this study, however, a certain level of effect on germination stimulation was acknowledged for the crops tested (crysanthemum and cabbage). Moreover, Dr. Nishizawa (Tokyo University) has recently reported that organic compounds of low molecular weight and macromolecules (amino acids and proteins) could be directly taken up through roots or leaves as a nutrient and the growth stimulation effect of these compounds was better than the inorganic (ammonia and nitrates)¹⁶. This may indicate a necessity for a systematic approach for investigation of the EM leachate as a fertilizer.

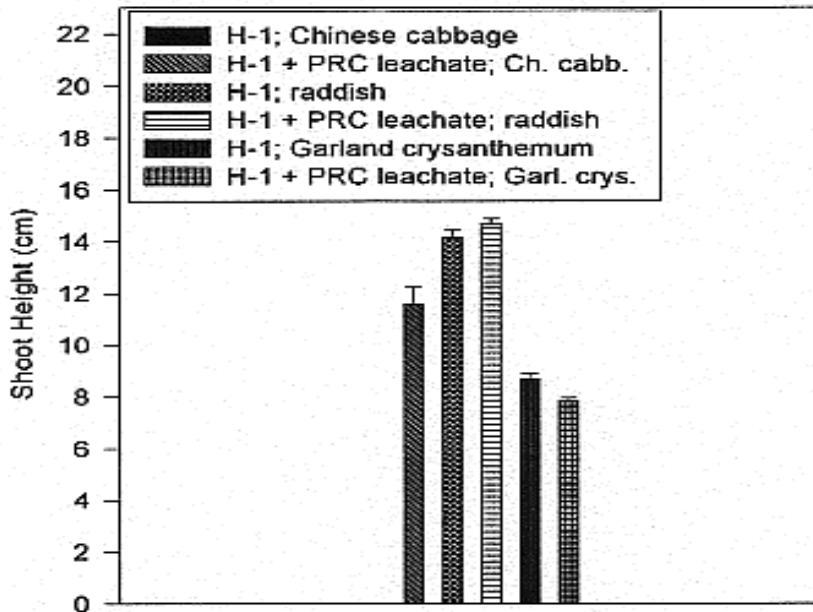


Fig. 9. Effect of EM (PRC) leachate on the growth of crop grown in the sand culture system after 3 weeks' application. The treatments were H-1 (Hoagland solution 1) that contained 500x and 1000x dilutions of the EM leachate (8-11 days old).

Acknowledgements

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