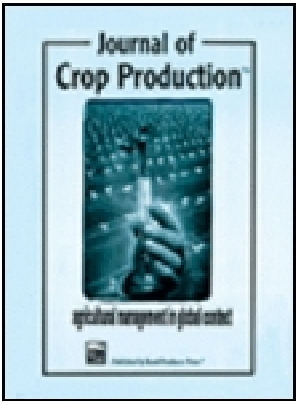


Journal of Crop Production
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/wzcp20

Properties and Applications of an Organic Fertilizer Inoculated with Effective Microorganisms

Kengo Yamada [a] & Hui-Lian Xu [a]
[a] International Nature Farming Research Center, 5632 Hata, Nagano, 390-1401, Japan

Version of record first published: 20 Oct 2008

Properties and Applications of an Organic Fertilizer Inoculated with Effective Microorganisms

Kengo Yamada
Hui-lian Xu

SUMMARY. Research studies were conducted to elucidate the chemical, physical and microbiological properties of an organic fertilizer that was inoculated and fermented with a microbial inoculant (Effective Microorganisms or EM). The quality estimation methods employed addressed the mechanistic basis for beneficial effects of soil improvement and crop yield. Effective Microorganisms or EM was utilized as the microbial inoculant that is a mixed culture of beneficial microorganisms. Tests showed that the fermented organic fertilizer contained large populations of propagated *Lactobacillus* spp. Actinomycetes, photosynthetic bacteria and yeasts; high concentrations of intermediate compounds such as organic acids and amino acids; 0.1% of mineral nitrogen mainly in the ammonium (NH_4^+) form, and 1.0% of available phosphorus; and a C:N ratio of 10. The quality of the fermented organic fertilizer depends on the initial water content; addition of molasses as a carbon and energy source; and the microbial inoculant. The medium pH appears to be reliable fermentation quality criterion for producing this organic fertilizer. Beneficial effects of the fermented organic fertilizer on soil fertility and crop growth will likely depend upon the organic fraction, direct effects of the introduced microorganisms, and indirect

Kengo Yamada is Research Agronomist, Hui-lian Xu is Senior Crop Scientist, International Nature Farming Research Center, 5632 Hata, Nagano 390-1401, Japan.

Address correspondence to: Hui-lian Xu at the above address (E-mail: huilian@janis.or.jp).

[Haworth co-indexing entry note]: "Properties and Applications of an Organic Fertilizer Inoculated with Effective Microorganisms." Yamada, Kengo, and Hui-lian Xu. Co-published simultaneously in *Journal of Crop Production* (Food Products Press, an imprint of The Haworth Press, Inc.) Vol. 3, No. 1 (#5), 2000, pp. 255-268; and: *Nature Farming and Microbial Applications* (ed: Hui-lian Xu, James F. Parr, and Hiroshi Umemura) Food Products Press, an imprint of The Haworth Press, Inc., 2000, pp. 255-268. Single or multiple copies of this article are available for a fee from The Haworth Document Delivery Service [1-800-342-9678, 9:00 a.m. - 5:00 p.m. (EST). E-mail address: getinfo@haworthpressinc.com].

effects of microbially-synthesized metabolites (e.g., phytohormones and growth regulators).

KEYWORDS. Effective Microorganisms, EM, EM bokashi, fermentation, microbial inoculant, nature farming, organic farming, plant nutrients

INTRODUCTION

The concept of nature farming was first introduced in 1935 by Mokichi Okada, a Japanese naturalist and philosopher (Anonymous, 1993). While nature farming is somewhat similar to organic farming, e.g., both advocate the non-use of synthetic chemicals, there are conceptual and ideological differences. The use of Effective Microorganisms or EM has become an important part of nature farming (Arakawa, 1985; Suzuki, 1985; Higa, 1998). EM consists of mixed cultures of naturally-occurring, beneficial microorganisms applied as inoculants to soil and plants which are widely documented to improve soil quality and the growth and yield of crops (Higa and Parr, 1994; Iwahori and Nakagawara, 1996; Iwaishi, 1994; Suzuki, 1985). Although EM is comprised of a large number of microbial species, the predominant populations include lactic acid bacteria, yeasts, actinomycetes and photosynthetic bacteria. Because most of the microorganisms in EM cultures are heterotrophic, i.e., they require organic sources of carbon and nitrogen, EM has been most effective when applied in combination with organic amendments to provide carbon, nitrogen and energy.

Consequently, there has been considerable interest in applying EM as a component of organic fertilizers. One such product is EM bokashi in which a mixture of rice bran, oil mill sludge or cake and fish meal is inoculated with EM and fermented, often under poorly-defined conditions. While researchers have often shown EM bokashi to be effective in improving soil quality and crop growth, some results have not shown consistent beneficial effects (Kato et al., 1997; Noparatraraporn, 1996). The reasons for these discrepancies can likely be attributed to (a) the wide range in type and quality of organic materials used to produce bokashi, (b) fluctuations in environmental conditions, (c) variable conditions of fermentation, and (d) differences in practical application technology. Moreover, research is needed to determine the mechanisms or modes-of-action on how EM bokashi actually elicits beneficial effects on soil quality and on crop growth and yield.

Therefore, the purpose of this paper was to assess the properties of EM bokashi produced under standardized conditions as well as by farmers themselves, to evaluate methods for estimating product quality; and to determine the mechanistic basis for the effects of EM bokashi on soil improvement and crop production.

MATERIALS AND METHODS

Experiment 1: Aerobic Fermentation of Organic Materials with EM Added

EM bokashi was prepared by adding molasses (8 ml), water (800 ml) and EM-1 (8 ml) to the mixed materials of rice bran (3.5 kg) and rice husk (2.0 kg), rapeseed oil mill cake (1.5 kg), and fish meal (1.0 kg) in a closed container. The treatment was repeated three times with the mixed non-EM materials as a control. The microbiological and chemical properties were examined 7, 21, 42 and 84 days after the beginning of fermentation. The numbers of aerobic and anaerobic microorganisms, fungi, aerobic dye tolerant bacteria, *Lactobacillus* spp. and yeast were evaluated using the dilution plate method with media of YG, VL, rose bengal, crystal violet added YG, GYP agarose, and YM, respectively (Kanbe, 1990; Koto, 1992; Uchimura and Okada, 1992). EC and pH were measured with glass electrodes (CM-20E and F-7AD, TOA Electrics Ltd., Tokyo, Japan). The anaerobes and *Lactobacillus* spp. were cultured in a nitrogen gas exchange incubator (BNR-110, TABAI ESPEC Corp., Tokyo). C:N ratios were determined with a carbon-nitrogen analyzer (MT-700 Yanaco Analytical Industries Ltd., Kyoto, Japan). Total and available phosphorus as well as NO_3^- and NH_4^+ were extracted with vapor distillation methods (Bremner, 1965). Organic acids such as lactic, acetic and butyric were measured by a high performance liquid chromatography.

Experiment 2: The Quality of Bokashi as Affected by EM and Molasses Additions and Water Content

EM bokashi was prepared similarly to that in Experiment 1 by adding water (800 ml), molasses (8 ml) and EM 1 (8 ml) to mixed materials of rice bran (2.7 kg), rapeseed oil mill cake (1.0 kg) and fish meal (1.0 kg). Treatment varied with or without additions of EM and molasses, and with water content (20% and 30%). Because material properties were observed within 21 days in Experiment 1, chemical properties and microbial numbers were evaluated on days 7 and 21 in Experiment 2. Items determined and methods used were the same as in Experiment 1.

Experiment 3: The Standards of EM Bokashi Prepared by Farmers

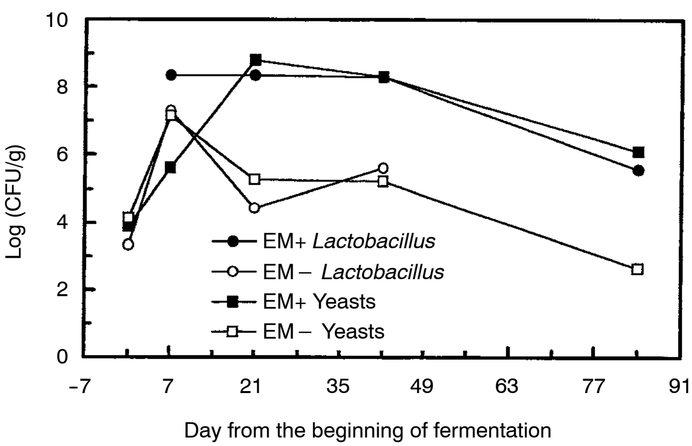
Although EM bokashi is commercially-marketable, most bokashi products are produced and used by farmers themselves. Therefore, chemical analyses were conducted for 9 different EM bokashi products made by farmers. EC, pH, C:N ratios, inorganic N and available P were determined as in Experiment 1.

RESULTS

The Characteristics of Bokashi During Fermentationwith EM Added

As shown in Figure 1, the *Lactobacillus* spp. population was only 10^3 CFU g^{-1}, at the beginning of fermentation, but increased to 10^8 CFU g^{-1} after seven days. Yeast populations increased from 10^4 CFU g^{-1} at the beginning to 10^8 CFU g^{-1} after three weeks of fermentation. The fermentation of bokashi with EM resulted in different trends for microbial numbers. *Lactobacillus* and yeast showed higher populations and lasted longer in EM bokashi than in non-EM bokashi. On day 84, the population of *Lactobacillus* was lower than 10^5 CFU g^{-1} and yeast was about 10^2 CFU g^{-1} in EM bokashi. Fungi were never detected at levels higher than 10^4 CFU g^{-1}.

FIGURE 1. Changes in *Lactobacillus* and yeast concentrations during the fermentation period.



Electrical conductivity was higher and pH was lower in EM bokashi than non-EM bokashi. The ratios of population of actinomycetes to fungi and of bacteria to fungi changed during the fermentation period (Figure 2). However, it is not clear what the trends mean and why the ratios change in these ways.

The changes in concentration of organic acids are shown in Figure 3. Of the three organic acids analyzed, L-lactic acid showed the highest concentration, increasing steadily from day 7 to 21, and remaining high until the end of fermentation. This pattern was amplified by EM addition. An increase in acetic acid concentration was also observed for EM bokashi but not for non-EM bokashi.

Changes in pH, EC, and inorganic nitrogen (NO_3^--N and NH_4^+-N) are shown in Figure 4. In EM bokashi, pH decreased significantly from day 42 and reached 4.5 on day 84. However, pH for non-EM bokashi remained at a high level of 6.0 until day 84. EC increased rapidly until day 21 for EM bokashi and then decreased slowly while a slow and steadily increase in EC was observed for non-EM bokashi. NH_4^+-N concentration was higher than NO_3^--N that was only 1/2 to 1/10 that of NH_4^+-N. On day 42, the total

FIGURE 2. Changes in actinomycetes/fungi and bacteria/fungi ratios during the fermentation period

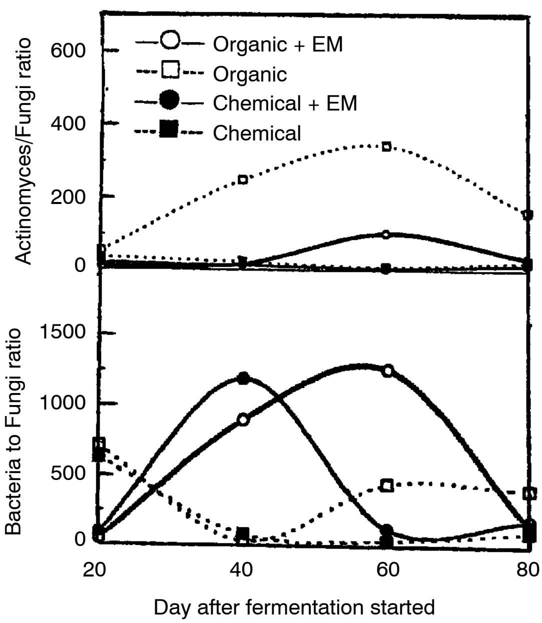
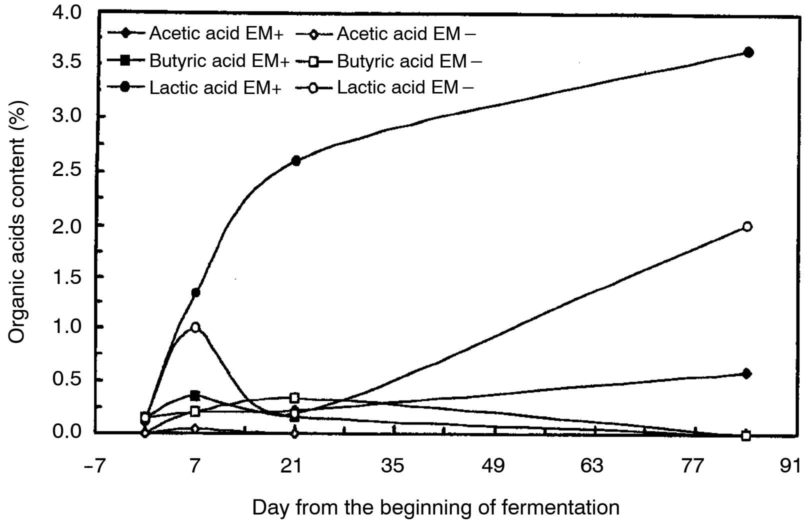


FIGURE 3. Changes in concentration of organic acids during the fermentation period.



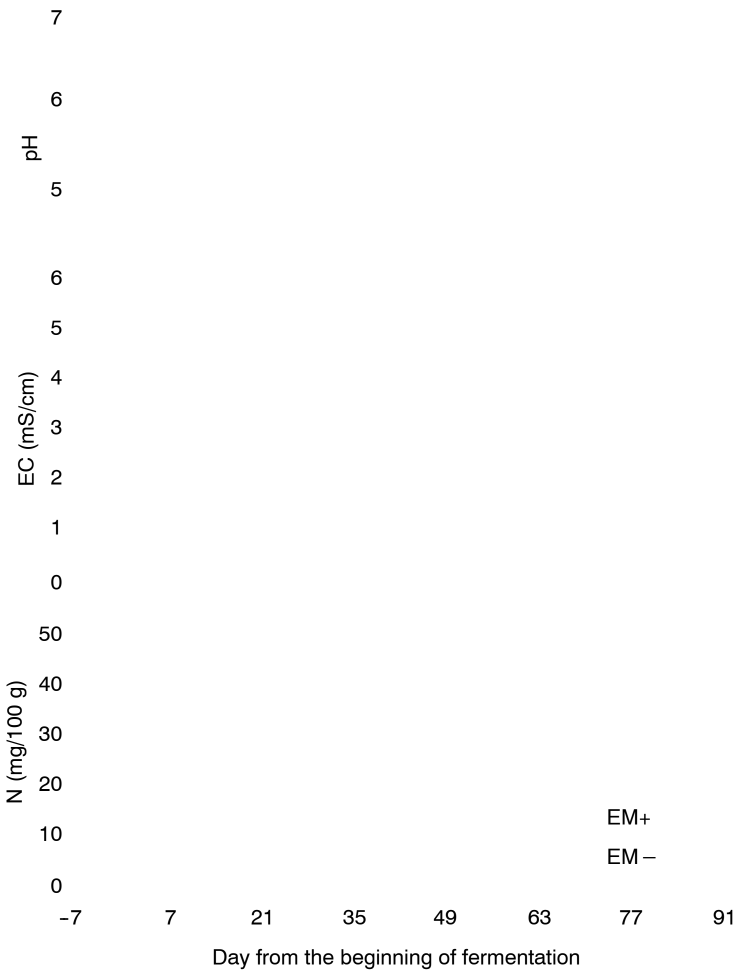
nitrogen content was higher in EM bokashi, but on day 84 it was higher for non-EM bokashi.

Fermentation Quality as Affected by EM, Molasses and the Water Content

In the present study, only when the water content of the materials was maintained at 30% during fermentation was lactic acid production observed (Figure 5). Addition of molasses did not show any effect on lactic acid production. As shown in Figures 5 and 6, pH at the beginning of fermentation was 6.1 in EM bokashi with a water content of 20%, while it was 4.8 for EM bokashi with a 30% water content. If no EM was added, pH was always about 6.0 whether the water content was low or high. EC was 3.2 mS cm^{-1} in bokashi with a 20% water content, but 5.1 with a water content of 30%. EC was 5.0 for EM bokashi without molasses added and only 3.4 for non-EM bokashi.

The population of *Lactobacillus* was 10^7 CFU g^{-1} in bokashi with a 30% water content and EM added (Figure 5). However, the number declined markedly when EM was not added, and was only 10^3 CFU g^{-1} when the water content was 20%. There was no fixed trends observed for the numbers of aerobes and yeast. Moreover, only when the water content was 30% with EM added did L-lactic acid reach a high concentration.

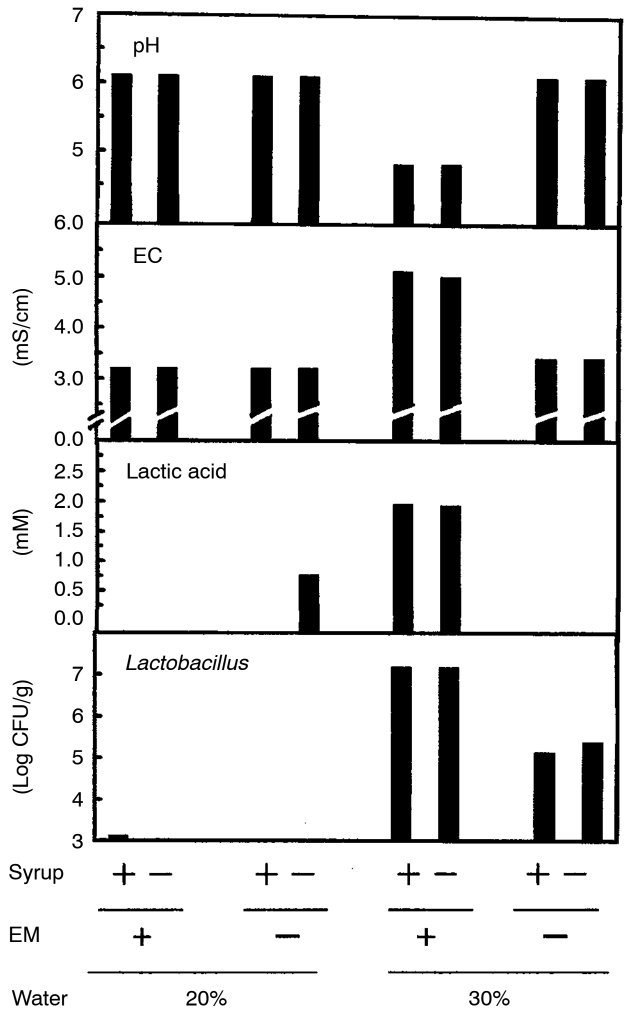
FIGURE 4. Changes in chemical properties during the fermentation period.



Properties of EM Bokashi Produced by Farmers

The farmer's EM bokashi was made mainly from rice bran that was locally available. The range in quality parameters for 9 different bokashi products produced by farmers is presented in Table 1. The lowest pH was 4.5 and the highest was 6.8 with a mean of 5.5 and standards deviation (SD) of 0.76. The lowest EC was 2.5 mS cm^{-1} and the highest was 6.5 with a mean of 4.9 and

FIGURE 5. Effects of preparing factors on the quality of EM bokashi.



SD of 1.38. The total carbon and total nitrogen contents were 44.5% and 4.5%, respectively, with a C:N ratio of 10.3. The average NH_4^+-N and NO_3^--N contents were 1007 and 85 mg kg^{-1}, respectively, both having large variations. The available phosphorus was as high as 9934 ± 1549 mg kg^{-1}, but variable among the products. Even so, the survey showed that these bokashi products are all good nutrient resources.

FIGURE 6. Effects of water content on pH of EM bokashi.

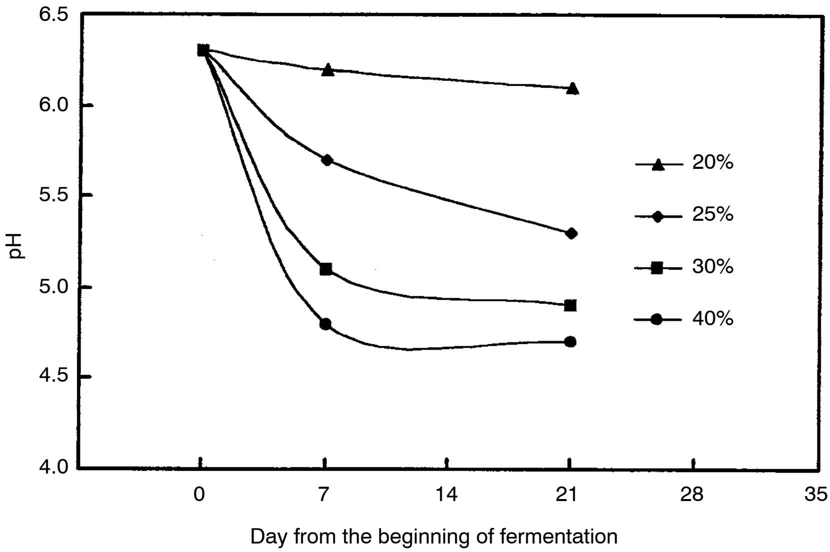


TABLE 1. Quality Parameters (EC [mS cm^{-1}], Electrical Conductivity; Av.-P [g kg^{-1}], Available Phosphorus; TC [g kg^{-1}], Total Carbon; TN [g kg^{-1}], Total Nitrogen; C:N, Carbon to Nitrogen Ratio) of the Commonly Used EM Bokashi.

pH	EC	NH_4^+-N	NO_3^--N	Av.-P	TC	TN	C:N
5.5	4.9	1.007	0.085	9.934	445	45	10.3
±0.7	±1.3	±0.6	±0.076	±1.549	±26.9	±6.1	±2.3

DISCUSSION

The beneficial effects of EM bokashi for improving soil quality and crop production have been widely reported. However, some experimental results have not shown a clear effect of EM because of fluctuations in environmental conditions and a lack of practical technology (Kato et al., 1997; Noparatraraporn, 1996). The application of EM bokashi is vital for the adoption of EM technology and sustainable crop production in nature farming systems. Considerable research on cultivation technology with EM application has been conducted (Iwahori and Nakagawara, 1996; Iwaishi, 1994; Suzuki, 1985). However, the various properties of EM bokashi and application characteristics have not been elucidated clearly. The farmer's bokashi is aerobically fermented by mixing the organic materials with soil to provide beneficial microorganisms, while EM bokashi is characterized by anaerobic fermentation in enclosed containers with EM added. EM bokashi produced and used in the present study is quite different in materials and fermentation conditions than the common bokashi produced by farmers. Therefore, the material properties of EM bokashi for both chemical and microbiological aspects will likely be different from the common bokashi.

Results of the present research suggest that lactic acid fermentation does occur during the incubation period. *Lactobacillus* propagated rapidly under anaerobic conditions that resulted from the activities of microorganisms at the early stage. The pH decreased as the lactic acid concentration increased. This low pH suppressed the propagation of many other microbes and enabled yeast to reproduce dominantly. Consequently, the intermediate substances like lactic acid, amino acid and others increased due to the activities of *Lactobacillus* and yeast. The principles of lactic acid fermentation technology are extensively utilized by industries that process foods and agricultural products (Kanbe, 1990; Uchimura and Okada, 1992), but rarely used for soil improvement and crop production. However, EM bokashi is now considered as an organic fertilizer that is uniquely different from other organic fertilizers.

As mentioned earlier, the quality of EM bokashi depends on whether or not lactic acid fermentation is predominant. One of the most important conditions is the water content of the materials at the beginning of fermentation. If the water content is too low, the aeration of the materials will increase and activities of anaerobic microbes will be suppressed, resulting in a poor quality bokashi as a consequence. The research presented in this paper found that maintaining the water content at a proper level was critical to producing high quality bokashi. The water content must be maintained at 30% or a little higher to ensure the desired decrease in pH, increase in EC, synthesis of lactic acid, and propagation of *Lactobacillus*. In general, farmers tend to use little water in preparing bokashi that may result in incomplete fermentation and poor product quality.

Even so, under a wide range of conditions, a decrease in pH is usually indicative of lactic acid synthesis and propagation of *Lactobacillus*. Therefore, pH can be a reliable indication and a simple criterion of the quality of EM bokashi. This is supported by results of the present research.

The chemical properties of EM bokashi are characterized by high NH_4^+-N and very low NO_3^--N levels, which result from suppressed aerobic nitrification because of anaerobic conditions. The high available phosphorus content suggests that EM bokashi can be a good nutrient source for plants.

Based on the present research, the following conclusions can be drawn for EM bokashi prepared according to stated directions: (1) EM enhances anaerobic fermentation of organic materials, increases the production of lactic acid and decreases the media pH; (2) EM bokashi is an organic fertilizer that contains 0.1% mineral N, 1% available P and has a C:N ratio of 10; (3) the quality and maturity of EM bokashi can be simply estimated by changes in pH and EC. Although it was not determined, it is likely that photosynthetic bacteria and actinomycetes might also exist in EM bokashi along with *Lactobacillus* spp. and yeast. Therefore, EM bokashi can be considered as a "living fertilizer" or "microbial fertilizer."

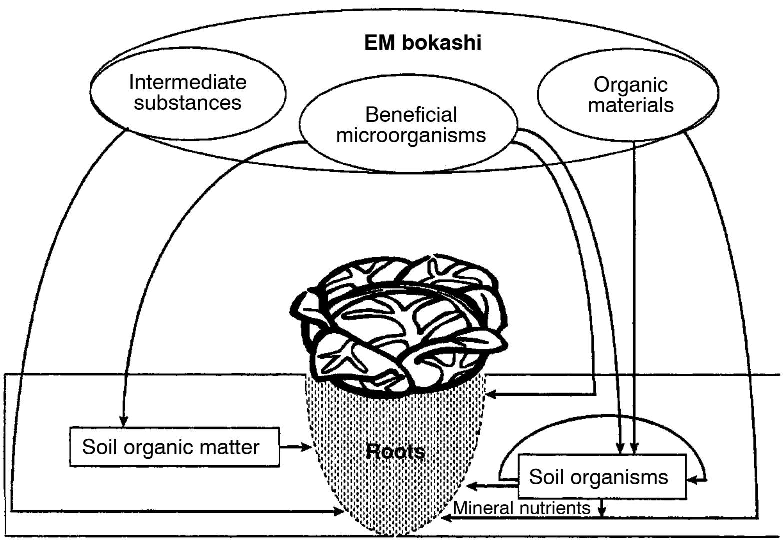
Research is needed to elucidate the exact mechanisms and modes-of-action whereby EM bokashi elicits beneficial effects on soils and plants. As mentioned earlier, EM bokashi includes propagated EM microbes; intermediate bioactive products from fermentation and other metabolic processes; and inorganic nutrients and undecomposed organic substances. Obviously, there will be individual effects and interactive effects when EM bokashi is applied to soils and plants. The occurrence and magnitude of these effects may depend on soil conditions. According to a recent study the (Kato et al., 1997), most NH_4^+-N was nitrified within 20 days when EM bokashi was applied to an Andosol soil. Such rapid nitrification was also observed in the field where EM bokashi was applied. The release of available plant nutrients from EM bokashi depends on the activities of ammonium-oxidative microbes and nitrite-oxidative microbes in the soil. Therefore, rapid nitrification does not occur in degraded and infertile soils because these nitrifying microbes do not thrive there. The nutrient availability of EM bokashi is comparable to clover leaves and chicken manure because the C:N ratio is only 10 and the undecomposed materials are quickly mineralized.

Extensive research has been conducted on the effects of bokashi on plant growth, photosynthesis and grain yield compared with chemical fertilizers (Fujita et al., 1997; Xu et al., 1997). The total dry matter of plants produced by chemical fertilizer was clearly higher at the early stage of growth, but lower at the later stages. However, plants nourished with bokashi maintained vigorous growth with greater root mass and activity and a higher rate of photosynthesis until harvest, showing a different growth pattern compared with plants treated with chemical fertilizer (Fujita et al., 1997). The well-developed roots of the bokashi-treated plants would likely play an important role in maintaining a higher rate of growth and photosynthetic activity (Yamada et al., 1997). This may largely be the result of the sustained nutrient supply of bokashi (Kato et al., 1997). However, the possibility still exists that EM contains phytohormones or other biologically-active substances that can delay senescence of plants. Similar phenomena have been observed for other organic materials with low C:N ratios. It was also found that plants nourished with aerobic bokashi showed a higher growth rate, higher photosynthetic activity, and finally higher grain yields than plants treated with anaerobic bokashi (Fujita et al., 1997; Xu et al., 1997). This was also due to the more developed root system of plants treated with aerobic bokashi. Compared with anaerobic bokashi where nitrification is needed after application to the soil, aerobic bokashi contains available NO_3^--N that is immediately available to plants after soil application. In most cases, however, nitrification can occur rapidly after application of anaerobic bokashi and should provide adequate available N for plants. However, in the case of rapid early plant growth, the anaerobic bokashi should be treated aerobically to promote nitrification and a higher level of NO_3^--N before application.

Because EM bokashi was prepared by fermenting organic materials with the EM inoculant, comparisons between non-EM bokashi and EM bokashi were made (Fujita et al., 1997; Xu et al., 1997). In addition to a higher growth rate, and increased photosynthetic activity, the most obvious effect of EM was enhanced root development and root growth (Yamada et al., 1997). The percentage ratio of root dry mass was clearly higher for the EM treatments than for non-EM treatments. It is also possible that phytohormones or other auxin-type growth regulators produced by EM and present in EM bokashi, could have stimulated root activity (Yamada et al., 1997).

From the foregoing discussion one may conclude that the beneficial effects of EM bokashi can be mainly attributed to (a) the sustained release of available plant nutrients from decomposition of organic materials, and (b) biologically-active substances such as phytohormones and growth factors synthesized by the EM cultures or produced as by-products during organic matter decomposition. This concept is illustrated in the schematic diagram of Figure 7. It is well

FIGURE 7. A speculative illustration of the effect of EM bokashi on soil fertility and plant growth.



known that plant hormones such as auxins, gibberellins and abscisic acid play important roles in root growth and development (Schneider and Wightman, 1974; Kuraishi, 1983). Moreover, research has shown that many soil microorganisms, i.e., bacteria, fungi and actinomycetes produce a variety of bioactive compounds that can enhance plant growth and metabolism (Arshad and Frankenberger, 1992). Some researchers have also speculated that to a large extent, the beneficial effects of EM can be attributed to the biosynthesis of antioxidants, although this has yet to be scientifically proven. Consequently, research is needed to determine the mechanisms or modes-of-action as to how EM elicits "growth-promoting" or "growth-stimulating" effects on plant growth and metabolic processes. Finally it was recently reported that EM can enhance soil aggregation. Research is needed to determine the exact conditions under which this occurs and the mechanisms that are involved.

REFERENCES

Anonymous. (1993). About the name Kyuse Nature Farming. In *The Basis of Paradise*, ed. The Editorial Board of Kyusei Dogma. Tokyo, Atami: Kyusei Publisher.

Arakawa, Y. (1985). Effects of EM application on snow thawing as well as snow rotting and powdery mildew problems of crops (Part V). *Proceedings of Symposium of Applied Soil Microbiology*. November 22, 1985, Urazoe, Okinawa, Japan.

Arshad, M. and W.T. Frankenberger. (1992). Microbial production of plant growth regulators. In *Soil Microbial Ecology*, ed. F.B. Metting. New York: Marcel Dekker, pp. 307-348.

Bremner, J.M. (1965). Inorganic forms of nitrogen. In *Methods of Soil Analysis*, ed. C.A. Black. Madison, Wisconsin: American Society of Agronomy, pp. 1179-1237.

Fujita, M., S. Kato, K. Yamada, H.L. Xu, K. Katase and H. Umemura. (1997). Applications of effective microorganisms in nature farming. VIII. Growth and yield of sweet corn as affected by applications of organic fertilizer with effective microorganisms. *Annual Meeting of Japanese Society of Soil Science and Plant Nutrition*, April 2(4, 1997, Shizuoka, *Proceedings* 43:163.

Higa, T. (1998). Effective microorganisms for a more sustainable agriculture, environment and society: potential and prospects. In *Proceedings of the Fourth International Conference on Kyusei Nature Farming*, eds. J.F. Parr and S.B. Hornick. Washington, DC: U.S. Department of Agriculture, pp. 6-7.

Higa, T. and J.F. Parr. (1994). *Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment*. Atami (Japan): International Nature Farming Research Center, 16 p.

Iwahori, H. and T. Nakagawara. (1996). Studies on EM application in nature farming. V. Applying methods of EM bokashi in vegetable cultures. *Annual Meeting of Japanese Society of Soil Science and Plant Nutrition*, April 1996, Tokyo, Japan.

Iwaishi, S. (1994). Effects of EM bokashi on various paddy-rice varieties. *Annual Meeting of Asia-Pacific Nature Agriculture Network*. October 6, 1994, Seoul, Korea.

Kanbe, M. (1990). Use of lactic acid bacteria. *Journal of Microorganisms* 6:56-65.

Koto, K. (1992). Quantification, isolation, and identification of soil bacteria. In *Experimental Methods of Soil Microbiology*, eds. T. Hittori, M. Nishio, and K. Miyashita. Tokyo: Yokendo, pp. 15-54.

Kato, S., K. Yamada, M. Fujita, H.L. Xu, K. Katase and H. Umemura. (1997). Applications of effective microorganisms in nature farming. IX. Soil fertility and plant nutrient uptake of sweet corn as affected by applications of organic fertilizer with effective microorganisms added. *Annual Meeting of Japanese Society of Soil Science and Plant Nutrition*, April 24, 1997, Sizuoka, *Proceedings* 43:164.

Kuraishi, S. (1983). *Plant Hormones*. Tokyo: University of Tokyo Publisher, pp. 1-142.

Noparatraraporn, N. (1996). Thailand collaborative research on evaluation of EM and EM products, their feasibility testing and effects of their uses on agriculture and environment. Open Symposium: *Present Situations and Prospects of Microorganisms as Agricultural Materials*. August 23, 1996, Tokyo.

Schneider E.A. and F. Wightman. (1974). Metabolism of auxin in higher plants. *Annual Review of Plant Physiology* 25:487-531.

Suzuki, Y. (1985). Effects of effective microorganisms on yield and quality of ginseng herbs. *Symposium of Applied Soil Microbiology.* November 22, 1985, Urazoe, Okinawa.

Uchimura, T. and W. Okada. (1992). Experimental methods for *Lactobacillus*. In *Manual of Lactobacillus Experiments-from Isolation to Identification*, ed. M. Osaki. Tokyo: Asakura Books, pp. 21-125.

Xu, H.L., S. Kato, K. Yamada, M. Fujita, K. Katase and H. Umemura. (1997). Applications of effective microorganisms in nature farming. XI. Photosynthesis of sweet corn as affected by applications of organic fertilizer with effective microorganisms added. *Annual Meeting of Japanese Society of Soil Science and Plant Nutrition*, April 24, 1997, Shizuoka, *Proceedings* 43:164.

Yamada, K., S. Kato, M. Fujita, HL Xu, K. Katase and H. Umemura. (1997). Applications of effective microorganisms in nature farming. X. Root development of sweet corn as affected by applications of organic fertilizer with effective microbes added. *Annual Meeting of Japanese Society of Soil Science and Plant Nutrition*, April 24, 1997, Shizuoka, *Proceedings* 43:163.