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Biofertilizer: Ingredients for Sustainable Agriculture

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13.1 INTRODUCTION

The structure of agriculture has radically changed in the last two decades. Innovations in agricultural systems have been introduced to maximize the potential and opportunity of this vibrant industry to produce adequate supply and accessibility of safe, nutritious and high quality of food to world population.In India, about 2.5 million hectares of land areas are organically managed out of 31 million hectares constitutingabout 600,000 farmers. Other developing countries are also developing several approaches to transform their agricultureinto modern, dynamic and competitive with other industries. The industrial agriculture has gain prominent achievements due to theusage of agrochemicalssuch as high inputs of chemical fertilizers, pesticides, water, as well as the increasing use of machines to extract maximum output in the shortest possible time. Approaches such as the ‘Green Revolution’ that was started in 1940s in Mexico had demonstrated the contribution of new or more attractive varieties, intensive use of agrochemicals, progressive mechanization and increased monocultures to increased cropyields as compared to the use of traditional varieties(Evenson and Gollin, 2003).However, the use of certain

agrochemicals has also been associated with some important environmental and ecological damage issues.Pollution and contamination of soil by excessive and injudicious use of synthetic input have ledscientists to identifynew,harmless and safe alternativeinput products, which is less expensive and sustainable.

In Malaysia, value of gross output generated from the agriculture sector in 2008 was RM 38,379 million with value added amounting to RM22,534 million. The crop sub-sector was the main contributor to value gross output at 85.1% followed by livestock and fisheries with 13.1% and 1.8% respectively. The value added recorded for crops was 96.1%, livestock - 3.0% and fisheries - 0.9% (The Department of StatisticsMalaysia, 2009). The total land use in Malaysia under the agriculture sector trended upward with bigger increase between the year 2000-2005. Between1995-2010,it was estimated that the agricultural land use increased from about 5.5 to 6.9 million hectares. Table 13.1shows the value of gross output by selected industries for crops. Oil Palmrecord the highest contribution with a value of RM31,425 million which accounted for 96.1%of the total value of gross output for crops.

Table 13.1 Malaysia's value of gross output by selected industries in 2008

Industries	Value of Gross Output (RM Million)	% Contribution
Palm oil	31,425.5	96.14
Rubber	671.9	2.03
Growing of flower	172.4	0.53
Growing of vegetables	111.4	0.34
Growing of fruits	95.4	0.29
Growing of sugarcane	21.9	0.07
Growing of cocoa	11.6	0.04
Growing of paddy	10.5	0.03
Other crops	43.2	0.19
Mixed farming	9.5	0.03
Agricultural services	95.1	0.29
TOTAL	32,668.4	100.0

Source: The Department Statistics of Malaysia, 2011.

Malaysia is the third highest Agricultural Importeramongst the South East Asian (ASEAN) Countries as shown in Figure 13.1. During the last 1998 Economic Crisis, the recovery in agriculture output had contributed towards the positive growth in the Malaysian economy (Bank Negara Malaysia, 2000). The 10thMalaysia Plan (2011 to 2015)is intended to expiate agriculture sector which would be contributing as much as 6.6% to the Gross Domestic Product (GDP) in 2015 and the New Economics Model (NEM) is targeting a contribution of as high as 5.2% to GDP in 2020. The emphasis on agricultural development will shift from a commodity-based approach to a product-based approach which focuses on the production of agricultural product based on marketdemand and potential. Complementing the product-based approach, an agro-forestry approach will be adopted to optimise resource use and maximise returns. The rich biological resources of the country will be conserved, managed and sustainably-utilized

for the development of new products and future industries as new sources of growth for the agricultural sector (Bee, 2008).

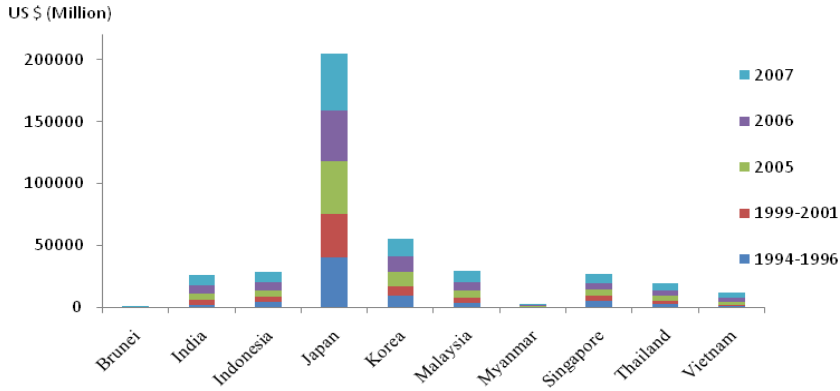

Figure 13.1: Value of Agricultural Imports for Asean Country (Department Statictics of Malaysia, 2009)

In many developing countries including Malaysia, agriculture is considered as one of the main engines for economic growth. The fertilizer industry worldwide is efficient and competitive. Urea and ammonium based fertilizers are the main fertilizer imported to Malaysia with import value averagingaround RM2.5 billion from 2004 to 2006(Figure 13.2). Nevertheless, Malaysia also substantially exports urea to other countries such as (year 2008) Thailand (32.5%), Australia (26.8%), Japan (13.5%), India (14.0%) and the Philippines (6.0%). However, emerging technologies together with global pressures on numerous issues such as social and environmental concerns towards agriculturehas increased the opportunities to promote biopesticide as an alternative to the chemical-based pesticide used.

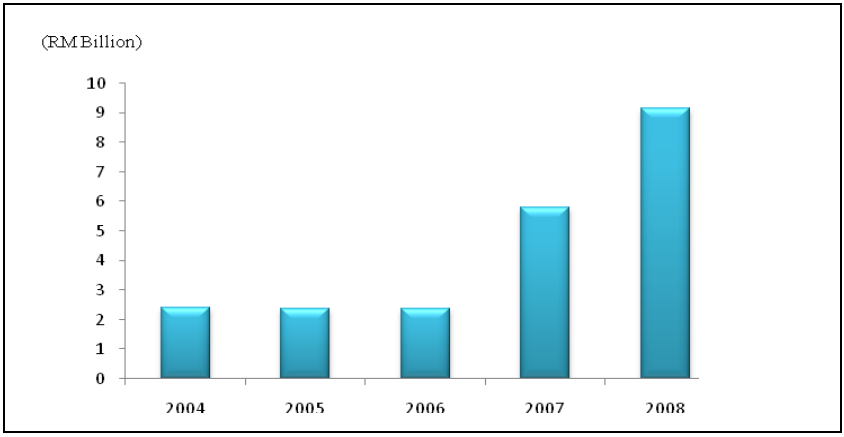

Figure 13.2 Malaysian Fertilizer Import Between 2004-2008 (Sabri, M.A. 2009)

Plant breeders should now turn to the effort of using sustainable, cheap and non-polluting resources as fertilizer, example of which are empty fruit bunches (EFB), palm oil mill effluent (POME) or animal faeces. The use of biofertilizer was introduced to lessen the indiscriminate use of chemical fertilizers in agriculture.Promising consequences offered by biofertilizer such as in improving food security, is boosted from the fact that almost 50 - 74% of thetotal land mass and population in developing countries, notably from the African and Asian regions, had yet reached to be utilized (Ogbo, 2010).Almost with similar reason to chemical fertilizer in providing viable leverage to increase crop productivity, biofertilizers are supposed to be the safe alternative to minimize the ecological disturbance, creating non-pollution agriculture as well as healthier and more vibrant environment.The use of biofertilizers has been determined as one of the main options to address the rising concern on agricultural and environmental sustainability. In Malaysia, the 3rd National Agricultural Policy (1998–2010) has identified some of the potential areasto support sustainability namely agroforestry, mixed farming, rehabilitation of marginal land, recycling of organic waste, mulching, cover

cropping, composting, organic farming as well as soil and water in agriculture activities (Ahmad, 2001).

Opportunities for developing, using and commercializingbiofertilizers are of utmost importance in maintaining the tilth, fertility and productivity of agricultural soils.In fact, biofertilizers can easily be mass-produced and are compatible with Good Agricultural Practice (GAP), food safetyand environmental concerns. With a growing public and private investment in this area, some countries in Asia such as Japan, Korea, Thailand and Taiwan already started in commercializing and accomplishing this momentous technologyafter having big investmentin research and development in that area.

13.2 ENVIRONMENTAL CONCERNS (DECLINING CROP PRODUCTIVITY, DEFICIENCY OF NUTRIENT IN SOIL)

Intensive tillage, exploitive, improper farming practices and massive amounts of agrochemicals usedhad shown negative results for soil health and productivity. Nutrient deficiency, erosion potentiality and dominance of the coarse fractionshave led to low moisture retention and lack of biological activities in the soil. For example, contamination of groundwater with high concentration of nitrate content is a threat toanimal and unfit for human consumption. In addition, the drain-off of agricultural fertilizer such as heavy metals into streams, lakes and other surface waters increased the possibility of eutrophication leading to extensive mortility of fish, overgrowth of nuisance fish and off-taste of drinking water. Transfer of these heavy metals such as Hg, Cd and As from soil to plant definitely affect the plant system and food chain as they are known as global pollutants and potential neurotoxins (Smolinska and Cedzynska, 2007). Chemical fertilization could also cause disturbances in the soil reaction, development of nutrient imbalances in plants, increased susceptibility to pests and diseases, reduction in legume root

nodulation and plant mycorrhizal associations (Thawornchaisit and Polpraser, 2009;Wangstrand*et al.*,2007).

Speculation on the future use of chemical-based agricultural fertilizer has been raised in this region as early as 1989 by the Research Council of the National Academy of Science (Benbrook, 2010).Reduced productivity in the agricultural sector is closely related to poor inter-correlation of energy conversion that isalso influenced by crop physiology, the environment and other biological factors. Since plantation activities above requiresurgent attention to reduce the problems, the use of biofertilizerseems appropriatein ensuring continuous supply of nutrients for plant and soil fertility (Das et al., 2008). Various non-farmingorganic wastes including sewage sludge, municipal solid wastes and agricultural and industrial processing waste could also be used together with the chemical fertilizer or be used as raw materials to produce biofertilizer.

13.3 BASIC CONCEPT OF BIOFERTILIZER

13.3.1 What Is Biofertilizer?

Biofertilizer is a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere of the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey, 2003). Generally, biofertilizer can also be called as microbial inoculants. It contains live and efficient formulates of bacteria, algae and fungi either separately or in combination that is capable offixing atmospheric nitrogen, solubilized phosphorus, decompose organic material or oxidize sulphur and on application will enhance the availability of nutrients for the benefits of the plants. The first generation of biofertilizer was developed from nitrogen fixing rhizobacteria isolated naturally in legumes (Gavrilescu and Chisti, 2005). In Malaysia, the use of microbial inoculum started in the late 1940’s and was in demand since

1970's with the application of *Bradyhizobium* and *Mycorrhizae*to legumes of rubber trees and oil palm.

13.3.2 Role and Mechanisms

The preparation of biofertilizer must not include any chemicals which are detrimental to the living soil. Besides the living content, biofertilizer may compriseof other components of biological materials either from plant or animal source. Application of biofertilizer to soil is extremely beneficial to stimulate plant growth, activate the soil biologically and improve land fertility while maintaining metabolic and nutritional state. Soil enrichment occurred when the complex organic material was transformed into simple compounds making it available for plant uptake (Arun, 2007). Microorganisms in biofertilizers restore the soil's natural nutrient cycle, build soil organic matter thus improves root proliferation due to the release of growth promoting hormones. These biofertilizers harness atmospheric nitrogen making it directly available to the plants.

Microbial interactions in soil and plants increased the ameliorating effect to benefit the agricultural and environmental ecosystem. Most of the functions participated in decomposition, mineralization and nutrient availability, thus reserve the nutrient cycles efficiency, cation exchange capacity, soil acidity and toxicity as well as the soil water holding capacity (Table 13.2). Generally the biochemical pathways for the plant growth promoting rhizobacteria (PGPR) can be classified into two groups namely direct and indirect pathways. The direct pathway involves the synthesis of phytohormones, N_2 fixation, reduction of membrane potentials of roots, synthesis of some enzymes as well as solubilizationand mineralization of inorganic phosphate making phosphorus available to plant (Rodriquez and Fraga, 1999). Indirect effects are commonly associated with decrease or deterrence effect of the pathogenic microorganisms due to the synthesis of antibiotics or siderophores.

Table 13.2: Interaction Between Some Microorganisms and Parts of Crop in Biofertilizers and Soil Conditioners (Pimentel, 2002)

Microorganisms	Mode of Action	Part of Crop	Geographic Region
Rhizobium spp.	N_2 fixation	Legumes	Russia, several countries
Cynobacteria	N_2 fixation	Rice	Japan, several countries
*Azospirilium*spp	N_2 fixation	Cereals	Several countries
Mychorrhizae	Nutrient acquisition	Conifers	Several countries
Penicilliumbilaii	P solubilisation	Cereals, legumes	Canada
Directed Compost	Soil Fertility	All plants	Several countries
Earthworms	Humus formation	Vegetables, Flowers	Cottage Industry

Biofertilizer can be classified into three common groups namely N-based or known as nitrogenous biofertilizers, P-based (phosphatic)biofertilizers and C-based (organic) fertilizers. Various microorganisms consisting of symbiotic nitrogen fixers such as *Rhizobium sp.*, non-symbiotic, free living nitrogen fixers such as *Azotobacter, Azospirillum*, phosphate solubilizing microorganisms (PSM) such as *Bacillus, Pseudomonas, Penicillium,Aspergillus* and*Mycorrhiza*. *Mycorrhiza* is a cellulolytic microorganism widely used in biofertilizer (Mahdi *et al.*, 2010). The symbiotic feature of this fungus playsan important role in nutrient recycling in the ecosystem also as a mode of plant protection against environmental and cultural stress.Azcon-Aguilar*et al.* (1994) explained that the formation of *Mycorrhiza*results from mutualistic symbiosis, where the host plant receives mineral nutrients via the fungal mycelium (mycotrophism) and the heterotrophic fungus obtains carbon from the host's photosynthetic activity. This interaction of root-fungus allows the integration of both organisms and harmonized unity, within the context of soil-plant ecosystem (Gianinazzi-Pearson *et al.*, 1994). Sometimes rock phosphate has

been added as an alternative source for phosphorus in biofertilizer (Ahmed, 2010).

Another symbiotic microorganism associatedwith plants is known as "Diazotrophs". It utilizes elemental nitrogen that is already in organic combination form from the atmosphere. Plant growth promoting *rhizobacteria* (PGPR) and *cyanobacteria* are rhizospheric microbes which produces some bioactive compounds to promote plant growth and retard pathogen diseases. They secrete organic acid, which enhances the uptake of phosphorus by plants by dissolving rock phosphate and tricalcium phosphate. Natural growth response components in plants such as auxins, abscisic acid, gibberelic acid, cytokinins, ethylene and others may be the result of phytohormones production in the rhizosphere. These plant regulators are important in plant's metabolism, seed germination, enhanced root growth and symbiotic nitrogen fixation.

13.4 DEMAND OF BIOFERTILIZER APPLICATION

13.4.1 Concept of Sustainable Agriculture and Alternative Agriculture

In response to the growing concern on the impact of agricultural activities to the environment, sustainable agriculture is the vital concept that fulfills the agricultural needs. Conventional agricultural techniqueswhich have been practised since 1950s are no longer feasible due to low productionyield and the ever escalating operating cost. Similarly, the monoculture farming technique has lose its application potential due to its direct role in the depletion of top soil, soil vitality, ground purity and reducing the population of natural soil microbes due to repeating planting on the same land. Common agricultural practices need to be redress to overcome the problem of the after-use chemicals. According to the Food, Agriculture, Conservation and Trade Act of 1990 (FACTA), sustainable agriculture was addressed as "an integrated system of plant and animal production practices having a site-specific application that will sustain over the long term". Under that law,

sustainable agriculture is intent on (i) satisfying human food and fiber needs, (ii) enhancingenvironmental quality and the natural resource based upon which the agricultural economy depends, (iii) make the most efficient use of non-renewable resources and on-farm resources and integrate, where appropriate, natural biological cycles and controls, (iv) sustain the economic viability of farm operations and (v) enhance the quality of life for farmers and society as a whole.Alternative agriculture emphasized more in optimizing the use of internal production material such as on-farm resources with skilled management practices in order to improve the crops' yields, crop production as well as profit returns.

13.4.2 Integrated Nutrient Management

The realization of such detrimental effects of chemical fertilizers when used continuously in agriculture and fair outcome of organic fertilizer and biofertilizer, has triggered interest approach described as Integrated Nutrient Management (INM) (Gruhn*et al.*,2000).This approach basically suggeststhe optimum combination of different sources of nutrient supply (chemical fertilizers, organic manure, crop residues and biofertilizers) for efficient crop production. Five principles of INM were outlined to have a sustainable crop systems; (i) in the soil, replenishment of chemicals removed by the crop (ii) maintenance of humus level in the soil, i.e. physical texture ofthe soil (iii) avoidance of weeds, pests and diseases(iv) control of soil acidity and toxicity and (v) control of soil erosion.The soil microorganisms that are mostly derived from organic input and soil is undermined in INM approach, since this biota contributes major role in soil organic matter dynamics, nutrient use and *in situ*pest and disease control.New approaches that can optimize the crops and soil management system such as crop rotation, organic amendments, conservation tillage, crop residue recycling, soil fertility restoration, maintenance of soil quality and the biocontrol of plant diseases could be applied to integrate the nutrient for agricultural benefits (Singh *et al.,* 2011).

13.5 KEY STEPS FOR PRODUCTION OF BIOFERTILIZER

13.5.1 Selection of Inoculants

Microorganisms such as *Rhizobium* (Singh *et al.*,2011), phosphate-solubilizing bacterium (Ogbo, 2010; Rodriquez and Fraga, 1999)and mycorrhizal fungi (Azcon-Aguiler and Barea,1997) has previously been identified as potential candidates to considerably fulfill the nutrient requirements of various crop species and are known to be used in biofertilizer. Different strains usedwill exhibit different attributes,such ashost specificity, nodulation potential and nitrogen fixation potential. Selection of strain should also emphasize in its ability to colonize the soil and rhizosphere, compete with native soil microorganisms and sufficient capacity to survive in soil while associating with other soil microbes. It is still uncertain whether the use of mixed or single cultures during composting would lead to higher inoculum density and longer cell protection after application.However, Higa (1994) has strongly demonstrated that high density of inoculums can be achieved when an organic amendment or mixed inoculums were applied repeatability during their high level of growth and activity. Other studies (Abdalla and Omer, 2001, Khan *et al.,* 2007, Galal, 2003) reported that the plant nutrient balance is far more efficient when a combination of N_2 fixing and P-solubilizing bacteria were used as inoculant compared to the use of single strain microbes. In addition, the time of inoculation is also important and affects the performance to the plant (Barea*et al.*,1993).

13.5.2 Composting

Composting biofertilizers are used to hasten the process of composting and for enriching its nutrient value. Composting is the biological decomposition or breakdown of organic material by bacteria, fungi, actinomycetes, worms and beetles favored by enzymes secreted by microorganisms (cellulolytic fungal cultures)

to hydrolyzepectins, xylans, hemicelluloses, cellulose releasing beneficial micronutrient for plant.Bacteria grow and multiply while conditions are right for them, and die-off as they create conditions more favourable to others to thrive. Bacteria, actinomycetes and fungi all consume waste directly and are known as first-level decomposers. They are assisted by larger organisms - earthworms, beetle mites, sow bugs, whiteworms and flies which also consume waste directly. Composting could be an alternative solution in solid waste management as part of bioremediation applications to treat and reprocess of organic waste into useful, safe and beneficial biofertilizers and soil conditioners which is more sustainable, economic and natural (Gavrilescua and Chisti, 2005).

Temperature is vital during the composting process. As temperatures rise and fall in the compost, different bacterial species will become either more active or less active. Psychrophilic bacteria, mesophilic bacteria and thermophilic bacteria each operate best within specific temperature ranges. Psychrophilic bacteria which work at optimum temperature of 13˚C will work first where the oxidation of carbon and heat generatedwould provide suitable growth conditionfor the next group of bacteria called mesophiles. Mesophiles starts to operate in mid-range temperature between 15˚C and 40˚C. During this phase, heat generated from the decomposition process of mesophileswould raise the temperature in the pile and creating more suitable conditions for the next phase to be taken over by thermophilic bacteria. These bacteria bring the temperature up to about 70 ˚C and the process is very fast within three to five days using proper bacterial inoculation. After this period, the temperature started to drop,where the number of thermophilicbacteria reduces while the compost now turns to more mature stage.

Temperature changes occurring during the three phases of composting, accelerate the process of mass reduction, waste stabilization and inactivation of pathogen in the waste. Microbial combination and their enzymatic activities are the factors that facilitate the mineralization of plant nutrients intosimple metabolic substance for plant utilization. Customarily, the production of

biofertilizerfromboth plant and animal by-products are slow acting. However, nutrient losses and non-homogenous product after the composting are now being evaded with the inoculation of certain microbial inocula such as decomposer fungus *Trichodermareesei* and nitrogen fixing bacterium, *Azotobacter*(Gordon and Moore, 1981). Contrary with anaerobic composting, the aerobic composting providerapid and greatest reduction in pathogen level due to high temperature attained. Other factors that affect the composting process (Hornick and Parr, 1987)are listed in Table13.3.

Table 13.3 Factors affecting the composting process during the production of biofertilizers and soil conditioners (Parr and Willson,1980).

Factors	**Process occur during composting**
C :N Ratio	• Rapid composting occurred when C:N ratio between 15 and 35 • <15 resulted in loss of ammonia • >35 resulted in slow composting process
Moisture content	• 40 to 60% (by weight) • <40% - aerobic but slow • >60% - insufficient air space led to anaerobic composting to occur
Temperature	• Temperature shift from mesophilic to thermophilic during composting • Optimum rapid aerobic composting ranges from 55-65 ˚C
pH	• Optimum pH ranges from 5.0 to 9.0
Aeration/ oxygen supply	• At least 30% air space
Particle size/texture	• More favourable surface to volume ratio enhances rate of decomposition

13.5.3 Special Features for Biofertilizer

The nature and characteristics of biofertilizer is highly dependent on the type of raw material used and theproduction process involved.Freshly producedbiofertilizer must contain effective strains in appropriate population and should be free from contaminating microorganisms.The combination of different biofertilizers should be carried out properly and complete study must be done before its expiry date. Special informations such as the method of application and seed treatment must clearly be shown on the label. Additional guidance such as implementation of corrective methods using lime or gypsum pelleting seeds can be proposed for problematic soil and correction of soil pH.Additives may be added to stabilize the biofertilizer or prevent the bacteria from clumping and ready to use. Surfactant, emulsifier or pure oil is examples of additives that can be usedforresuspension after prolonged storage. In some liquid biofertilizer, sunscreen is added to a formulation to protect microorganisms from UV-rays. A good biofertilizer should have basic concepts as followed; stabilization, persistence, good delivery and enhancing activity.

13.5.4 General Concept of Quality Control (Chemical, Microbiological, Physical)

The quality of chemical, microbiological and physical aspects is fundamental toany biofertilizer product. Failuresduring the application of biofertilizer may be due to absence of several of these factors such as ineffective inoculums formulation, high level of contamination, inadequate storage facilities, incorrect use of the recommended dosage and methods,and soil or environmental factors.Saratchandra *et al.* (2001) demonstrated that the interrelations of N and P elementsto the soil microbial and nematode populations showedno effect after the treatments. This study showed that the microbial community in the soil is similar and fertilizer amendments are insufficient to induce changes either directly or indirectly.

Nutrient content of biofertilizer is determined by the quantity of organic substances and chemical elements they contain. All plant nutrients such as N, P, K and Mg as well as vitamins and microelements necessary for plant growth are kept intact in biofertilizer. The C/N ratio (around 1:15) normally hasa favorableeffect on the soil quality. Phosphate (a form of phosphorus, directly assimilated by the plants) content does not change on the process of substrate digestion. In these forms, plants can assimilate around 50% of overall phosphorus content. Fermentation also does not influence the percentage of potassium (75-100%of which can be assimilated by the plants). Unlike phosphate and potassium, the nitrogen content in substrate nitrogen changes during fermentation process. Around 75% of nitrogen, contained in fresh manure becomes a part of an organic macromolecule and the remaining 25% are present in mineral form. After digestion in biogas plant, around 50 % of nitrogen would be in the organic form and another 50% in the mineral form.

13.5.5 Shelf Life

The shelf life of biofertilizerdepends largelyon the raw material used, species and density of cell present in the biofertilizer (Higa and Parr, 1994). Formulation of biofertilizer should contain suitable carrier base that is capable ofholdingthe high population of the specific microorganisms for a sizeable period. Generally either slurry of sugar, jaggary or gum is added to the biofertilizer in order to achieve sufficient shelf life required. The differencebetween liquid and solid biofertilizerspresents a real challenge to the manufacturer in terms of sustaining the shelf life of this product (Ngampimol and Kunathigan, 2008). Solid biofertilizercan be used up to six months compared to liquid fertilizer which has a shelf life of only three months. Sincebiofertilizer is considered as a life product, it cannot be used and stored as easily as chemicals. This has been the major deterrent in the adoption process.Carrier baseis important to determine the successful and failure of biofertilizer and their shelf life. Liquid biofertilizer is more difficult to sustain

than solid biofertilizer. The reason of failures may be derived from the quality of the strains or microbes used such as poor cell protection towards temperatures or other factors such as unavailability of the food carrier in a local area, consistency of carriers, poor moisture retention and problem in proper packing. Therefore, cautions at the stage of manufacture, culture, transportation, distribution and application should be seriouslyconsidered when producing biofertilizer with a long shelf-life.

13.6 CASE STUDY: A COMPARISON STUDY OF MACRONUTRIENTS, MICRONUTRIENTS AND MICROBIOLOGICAL CONTENT OF BIOFERTILIZERS AND THEIR EFFECTIVENESS IN COMMERCIALS APPLICATIONS

13.6.1 Treatments and Experimental Design

13.6.1.1 Fermentation and Temperature Monitoring

Open fermentation was performed for Biofertilizers A, B and C which contains different proportions of the following ingredients; burned soil, nitrogen source meal, saw dust and burned rice husk(Table13.4).Each of the biofertilizers was inoculated with 3% of effective microorganisms (EM) before the fermentation proceeds. Substrate temperature was measured daily at a depth of 50 cm of the compost between the 7 days fermentation period.

Table 13.4 Ingredient for Biofertilizerused in this study

Ingredients	Biofertilizer types		
%	A	B	C
1- Burned soil	41	46	39
2- Nitrogen source meal	7	10	7
3- Saw dust	15	30	30
4- Burned rice husk.	15	-	-

(-) = without addition of burned rice husk

13.6.1.2 Isolation and Identification of Microbial Species

The total microbial population of each sample was determined using the following specific media for each strain (Table 13.5).

Table 13.5 Microorganisms and specific media used for isolation and identification

No.	Microorganism	Specific medium
1	*Lactobacillus* sp.	Acidified MRS
2	Yeast and Mold	CGYE
3	N_2 fixing bacteria	Ashby‘s medium
4	Photosynthetc bacteria	Mineral salts-Succinate Broth
5	Nitrifying bacteria	AOB, NOB
6	*Actinomycetes*	Actinomycetes isolation agar

CGYE = Glucose Yeast Extract Agar, AOB: Ammonia-oxidizing broth and Nitrogen-oxidizing broth (NOB)

For routine enumeration of lactobacilli and to encourage the growth of the lactic acid bacteria such as lactobacilli, enterococci and pediococci, acidified MRS agar medium was used (British Standards Institute, EN 15787: 2009). Enumeration methods of yeasts and especially molds are imprecise because they consist of a mixture of mycelia and spores (Leuschner*et al.*, 2003). Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium. The CGYE agar medium was used for this purpose and inoculated medium is incubated at 37±1°C for five days. Nitrogen fixing bacteria is determined using the nitrogen-freemedium(Ashby, 1907). This method is based on the ability of nitrogen-fixing bacteria to grow in nitrogen-free medium and the total N_2- fixing bacteria were counted after incubation is complete. The colonies that grow on the medium appear as white, off-white, grey and grey to white; circular, flat, raised; serrate in elevation, small and pinpoint in size.

Photosynthetic bacteria are monitoredby trackingthe ability of photosynthetic bacteria to assimilate CO_2 and use light as their energy source in the medium during incubation in a shake flask

under light and dark conditions (Prasertsan*et al.*, 1993). This is followed by plating in the same medium but with the addition of trace elements.The isolates were incubated for four to seven days at 30±1°C until appearance of red pigment (bloom) which indicates the presence of photosynthetic microorganisms. Positive broth was taken for inoculation onto succinate agar and further incubated anaerobically at 30±1°C for four to seven days. Multiple Five Tube method was used to identify nitrifying bacteria using Ammonia-oxidizing broth (AOB) and Nitrogen-oxidizing broth (NOB) (Awad*et al*.,2009 ;Wollum, 1982). Each sets of AOB and NOB tubes was inoculated with 1 ml of sample suspension and incubated at 25-30°C for either 23-28 days (AOB) and 23 (NOB). At the end of the incubation time, one drop of sulfanilic acid and N,N-dimethyl-1-naphthylamine was added into each portionsof the AOB and NOB media inthe tubes.Red colour appears to indicate the presence of active AOB while an absence of any colour changes was a positive result for NOB. Confirmation test was carried out by addingone drop of diphenylamine to a drop of sample on a clean spot plate to determine nitrite/nitrate production. Positive tubes or wells are identified by development of a blue colour and are scored positive and the absence of colour is scored negative.

All results were computed into MPN table. In addition, isolation and enumeration of Actinomycetes colonies were performed by soil dilution plate technique using the Actinomycete isolation agar medium (Difco, NJ, USA) supplemented with 25 µg mL^{-1} penicillin G (Sigma-Aldrich, USA). Results obtained were expressed as the colony forming unit (CFU) and the Most Probable Number (MPN) according to the method used.Actinomycete colonies were characterized morphologically andphysiologically based on the International*Streptomyces* project (Shriling and Gottlieb, 1966)andthe Bergey's Manual of Systematic Bacteriology (Locci, 1989).

13.6.1.3 Chemical Analysis

The total NPK content of samples was analyzed according to the following method; Nitrogen was determined by acid combustion elemental analysis method using a macro Kjeldahl system (Gerhadt, German); phosphorus, potassium and other micronutrients were digested using acid digestion method and analyzedspectrophotometrically (Spectroquant NOVA 60, Merck, USA) using EPA method 3050B (Kimbrough and Wakakuwa,1989). Moisture contents of samples were determined using a moisture analyzer (MX-50, A&D Company Ltd, Japan) to a constant weight. pH was measured in a 5-fold dilution of distilled water equilibrated with sample for one hour with a pH meter (Delta 320, Mettler Toledo, Germany). Ash content in a dried sample was determined at 550°C for 24 hours using (CWF 110, Carbolite, England). Carbon and Nitrogen ratio was analyzed using HACH method (Rossi *et al.,* 2004).

13.6.2 Plant Techniques and Data Collection

The field trial study was carried out for the ladyfinger plant, which was planted from March to August 2010 under a different plot. The following treatments were applied for each plot; Plot 1: Application using Biofertilizer A; Plot 2: Application using Biofertilizer B; Plot 3: Application using Biofertilizer C and Plot 4: for the control (without any application of Biofertilizer). The three treatments were imposed in a randomized block design with five ladyfingers per plot. Care was taken to prevent contamination of the control plot by adopting suitable distance between the plots. Each biofertilizers was applied weekly for the first month and continues to twice a month until the day of harvesting. During harvesting, three plants were carefully removed from each plot and the plant height, length of roots, diameter of leaves and the fruit's weight were recorded.

13.6.3 Physical, Chemical and Microbiological Analysis

The temperature was monitored up to 8 days of fermentation period. The biofertilizers temperatures increased rapidly during fermentation, reaching a maximum temperature of 71°C on day 4 and then decreased gradually for the maturation of the biofertilizers on day 8 as shown in Figure 13.3.

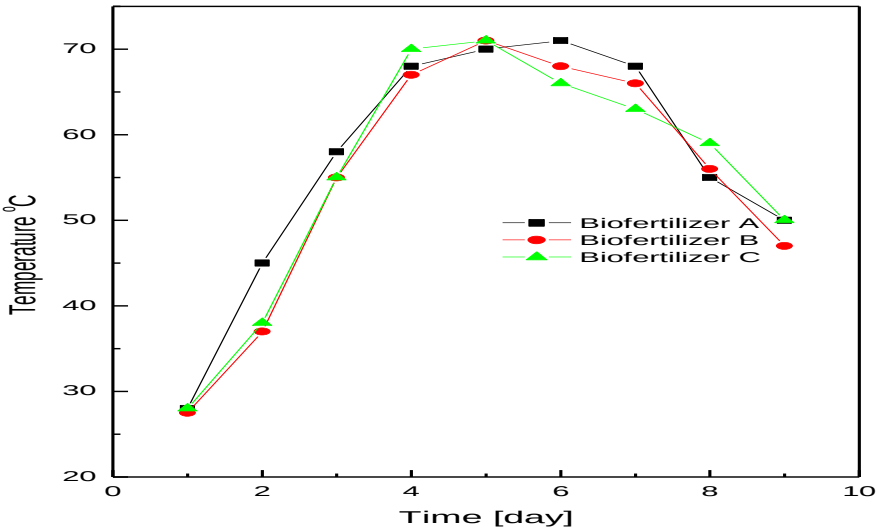

Figure 13.3 Temperature profile during the production of biofertilizers.

During fermentation, the total microbial population in biofertilizersA, B and C were analysed and calculated as CFUg^{-1} biofertilizers and the results are shown in Table 13.6. *Lactobacillus sp.* form the major population and nitrogen-fixers form the minor population in all biofertilizers examined. The results showed that the total count of *Lactobacillussp* was3.3 x 10^5 CFU g^{-1}, 4.9 x 10^5 CFU g^{-1} and 2.3 x 10^4 CFU g^{-1} in Biofertilizer A, B and C, respectively. While, Biofertilizer B and C showed greater growth of yeast total count, which was 3.0 x 10^7 CFU g^{-1} and 3.5 x 10^7 CFU g^{-}1 butlower total count of yeast (2.4 x 10^5 CFU g^{-1}) was recorded from Biofertilizer A. On the other hand, the population of

nitrifying bacteria for Biofertilizer A had shown significant difference from Biofertilizer B, C and ranged from 3.20 x 10^2 to 3.60 x 10^2 CFU g^{-1} for AOB and NOB respectively. Whilst in nitrogen-fixing bacteria count, no significant difference had been observed for all types of biofertilizers formulation. Lastly, no growth was detected in both of photosynthetic bacteria and *Actinomycetes* in all biofertilizers examined.

The pH for all types offormulations was slightly alkaline and ranged between 8.2-8.5 due to the degradation of nitrogen-containing materials to soluble organic nitrogen (Table 13.7). The moisture content differed for biofertilizer to other bases on the formulation composition and ranged from 16.60 to 22.30. The ash content ranged between 0.42-0.61% depending on the composition of biofertilizer. The stability of ash content can be used as a parameter of compost maturity. Biofertilizer B possess the highest final total organic carbon and nitrogen content (19.2) followed by Biofertilizer C (12.0) and BiofertilizerA (6.0).Generally,BiofertilizerB recorded highest content in most macro and micronutrient contents followed by Biofertilizer A and C.

Table 13.6 Population of Effective Microorganisms (EM) examined in Biofertilizer A, B and C in CFU g^{-1}.

Microorganism Total Count		Biofertilizers		
		A	B	C
Lactobacillus sp.		3.30 x $10^{5\,a}$	4.90 x $10^{5\,a}$	2.30 x 10^{4}_{b}
Yeast		2.40 x $10^{5\,b}$	3.00 x $10^{7\,a}$	3.50 x 10^{7}_{a}
Nitrifying Bacteria	AOB	>1.60 x $10^{3\,b}$	3.3 x $10^{2\,a}$	3.5 x $10^{2\,a}$
	NOB	6.20 x $10^{2\,b}$	3.2 x $10^{2\,a}$	3.6 x $10^{2\,a}$
Photosynthetic bacteria		NG	NG	NG
Nitrogen-fixing bacteria		4.5 x $10^{1\,b}$	5.2 x $10^{1\,b}$	1.4 x $10^{1\,b}$
Actinomycetes		NG	NG	NG

Significant difference ($P<0.05$) NG= no growth, AOB=Ammonia-oxidizing bacteria,
NOB= Nitrite-oxidizing bacteria

Table 13.7: Macro- and micronutrient contents and other chemical analysis for Biofertilizer A, B and C.

Biofertilizer	Ash (%)	C/N Ratio	Percentage (%)					Concentration (mgL^{-1})				
			N	P	K	Mg	Ca	B	Fe	Mn	Na	Mo
A	0.61	6.0	1.4	<0.001	4.7	2.9	<0.5	0.6	16.3	0.8	22.0	0.1
B	0.42	19.2	1.7	0.001	6.6	4.7	0.8	1.8	<0.001	4.9	8.5	1.1
C	0.45	12.0	1.8	<0.001	4.9	8.5	1.1	0.5	1.8	0.2	9.0	1.0

The results showed that the plants treated with Biofertilizer C grew vigorously and were taller than the other treatments (Table 13.8). The plants treated with Biofertilizer A that contained burned rice husk exhibit bigger fruitdiameters as well as heavier. On the other hand, plants treated with Biofertilizer B showed better growth compared to plants treated with Biofertilizer A and C based from the leaves diameter. Overall, it was observed that the plants treated with Biofertilizer A, B and C had grown well and had better yields than the plants which were planted without any treatment (control).

Table 13.8 Physical analysis during field trial for ladyfingers fertilized with Biofertilizer A, B and C

.

Physical analysis	Biofertilizer A	Biofertiizer B	Biofertilizer C	Control
Plant height (cm)	185.0±7.00	217.5±6.93	237.6±4.96	79.9±3.53
Root length (cm)	34.4±0.57	36.7±0.47	41.8±1.68	17.1±0.85
Leaves diameter (cm)	34.8±1.50	44.4±0.50	41.8±1.68	17.3±0.32
Fruits diameter (cm)	3.2±0.12	2.8±0.15	2.6±-0.47	1.5±0.38
Fruits weigh (g)	38.5±0.70	36.2±3.63	28.0±2.11	11.4±0.95

Significant difference ($P<0.05$)

13.6.4 Discussion

During biofertilizer preparation, microbial decomposition of organic matter releases significant portion of CO_2 and heat (Yang, 2003). Temperature monitoring was important during fermentation as it indicates activity of the microbes. Data showed that temperature increased from 41°C to 71°C on day 4 possibly due to the active microbial growth occurring during fermentation. The temperature patterns recorded were similar with that of the commercial composting process (Pai*et al,* 2003)with gradual decrement to 50°C after 8 days of fermentation time (indicating maturity of biofertilizer).Proper fermentation also will effectively destroy pathogens and weeds through the metabolic heat generated by microorganismsduring the process (Yang, 2000; Nakasaki*et al,* 1996). This study is in agreement with Tsai *et al*(2007) who reported that the inoculation of appropriate microbes during fermentation will shorten the period of maturity and thus improve the quality of biofertilizers. Most literatures reported the presence of these microorganisms from the environment sample such as soil (Fuentes *et al.,* 2010).

In order to prepare the multi-functional biofertilizer, thermo-tolerant phosphate-solubilizing microbes (including bacteria, *Actinomycetes* and fungi) were isolated from different compost plants and biofertilizers (Cheng-Hsiungand Shang-Shying, 2009). Three different biofertilizers were analysed for its microbiological, chemical and physical components. The presence of certain microorganisms and the nutrient mineralization directly indicate the potential of using biofertilizerto support plant growth and enhancing plant yields (Parthasarathi and Ranganathan, 1999). Edward and Fletcher (1988) stated that the increasing number of microbial populations will increase the performance of biofertilizer value for microbiological, chemical and physical properties. The large number of *Lactobacillus* sp. and yeast isolated from the final product of biofertilizer indicated successful fermentation. The totalnumber of *Lactobacillus sp.* and yeast were between 5.00 x 10^{5-8} CFU g^{-1}, which is consistent with the microbial analysis

result from liquid biofertilizers produced by other studies (Ngampimol and Kunathigan, 2008). Nitrifying and nitrogen fixing bacteria werepresent in low numbers (< 1.00x 10^3 CFU g^{-1}) but still observed in all formulations.

The chemical analysis results during composting showed that biofertilizers inoculated with the tested microbes had a significantly higher temperature, ash content, pH, total nitrogen and soluble phosphorus content. Adding these microbes can shorten the period of maturity, improve the quality, increase the soluble phosphorus content and enhance the populations of phosphate-solubilizing and proteolytic microbes in biofertilizers (Cheng-Hsiung and Shang-Shyng, 2009). The pH readings of each biofertilizer in our experiments were in the range of 8.20-8.50 which were slightly higher from other solid biofertilizers studied (Deboszet *al*, 2002; Tsai *et al*, 2009). The slight alkaline pH of biofertilizer is beneficial in agriculture because of its contribution to the neutralization of acidic agricultural soil (Fageria and Baligar, 2001). The moisture content of compost decreased during the incubation because the use of the inoculation of the biofertilizer with the EM which increased the temperature and decreased the moisture content of biofertilizer. The same phenomenonwas also observed in open field composting (Pai*et al*, 2003). The ash content in the biofertilizer samples can be used as a parameter for the maturity of compost. The ash content increased significantly during preparation since the organic materials was decomposed to form metabolic gases such as CO_2 and NH_3(Pai*et al*, 2003; Chang and Yang, 2006). Total organic carbon content decreased from 19.2 (Biofertilizer B) to 12.0 (Biofertilizer A) and 6.0 (Biofertilizer C).

Trial study is applied to monitor and observe differences of the biofertilizers effectiveness to the plant physical characteristics. The significantly lower physical characteristics observed when a plant grown without the use of any biofertilizersmay be explained by the decrease or low fertility of the soil. All parameters evaluated (plant height, length of roots, diameter of leaves, fruits weights)showed increased values in the presence of biofertilizers.

It was noted that the addition of burned rice husk in Biofertilizer B providesa higher percentage of potassium (6.6%) that contributes to an extra fruit diameter and weight compared to other biofertilizerwithout the addition of burned rice husk. The results are comparable with a study by Seripong(1989) who reported that the dry weight of shoots and fruits increased significantly as more burned rice husk was added. Similarly, with the addition of more than 3% nitrogen source meal from total 7% in Biofertilizer A and C, had resulted in good yield of leave diameter for plant fertilized with Biofertilizer B. High rates of roots and stem lengths for plants usingBiofertilizer C, which is in the range 237.6±4.96 cm had been observed compared to plant with Biofertilizer A and B with each in the range of 185.0±7.00 cm and 217.5±6.93 cm. Total nitrogen content for all biofertilizer (>1%) had no effect on populations of total bacteria, yeast as well as ammonia and nitrite utilising bacteria. This is in agreement with the study carried out by Sarathchandran*et al.*(2001) who reported that when the nitrogen content used was between 0.48-0.69%, no significant difference was recorded for the number of bacterial population.

13.6.5. Conclusions for the Case Study

From the microbiological, chemical and physical properties, Biofertilizer A is suitable to be used as a promoter in flower and fruits development, Biofertilizer B for leaves production and Biofertilizer C for strength of roots and stems. This study also suggests that biofertilizer prepared with the inoculation of special microbes is a feasible product and has a potential for commercialization as well as being environmental friendly.

13.7 CONCLUSION

This report reviews the importance of producing local and natural resources to support the sustainability of environmental and human health, especially in the developing countriesin Asia. Integrated management between biofertilizer and chemical fertilizer might be

adapted by big industry and the effectiveness and efficacy of bio-agents have yet to be established to be comparable if not to exceed the agrochemicals.Extensive marketing, promotion and awareness program must also be implemented coupled with training provided to farmers at all levels of community. Support from government to create legislations and strong policy, especially in the area of standards and regulationsare necessary. Technical partnership and information exchange among countries is not the merely important factor but also a catalyst for the process of adoption of a product. Basic principles of good agricultural practice should be equally important to beunderlined during the application of biofertilizer to an environment. Biofertilizer can be regarded as an important component of the integrated nutrient supply system and hold a great promise to improve crop yields through environmentally better nutrient supplies.The future of biofertilizer largely depends on i) the advancement of research and development of efficient and multifunctional biofertilizer for different crops ii) establishment of systematic quality control of specific strain during biofertilizer production iii) survivability study for biofertilizer using strains under the stress conditions and iv) establishment of Biofertilizer Act to strengthen the policy of quality control in markets and application.

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