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# The Study of Shelf Life for Liquid Biofertilizer from Vegetable Waste

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## *Abstract*

*Liquid biofertilizer is increasingly available in the market as one of the alternatives to chemical fertilizer and pesticide. One of the benefits from biofertilizer is a contribution from population of microorganisms available. Traditionally, liquid biofertilizer produced from fermentation of effective microorganisms (EM) was recommended to be used within three months. This experiment showed that shelf life of the liquid biofertilizer produced from vegetable waste contains high amount of viable microbial population after four months of storage. The two conditions of storage, with and without light, were tested and it was found that there was no significant difference ($p>0.05$) upon viable microbial population, chemical and physical characteristics. However, there was significant difference from batch to batch of production due to raw materials.*

***Keywords:*** *Alternative agriculture, EM microorganisms, plants nutrients, viable microbial population.*

## Introduction

Thailand is an agricultural country (FAO 2004). In the last few decades, there were changes in the agricultural practice from small to larger farming that emphasized in production efficiency using modern agricultural strategy. The extensive use of chemical fertilizer and pesticide according to this strategy caused numbers of deaths and illnesses to the farmers. The poor farm management technique and improper use of agrochemicals has also resulted in both soil quality and environmental degradation (Setboonsarng and Gilman 1999). Several methods of alternative agricultural systems were introduced into Thailand in the last decades according to Setboonsarng and Gilman (1999). Their common objective is to provide socioeconomic and ecological benefits. Among these benefits, improvement of soil quality is one of the interesting aspects since it contributes to a broad attributes including food quality and safety, human and animal health, and also environmental quality (Parr, *et al.* 2002). The use of non-chemical fertilizers and pesticides is one of the common practices that have been introduced with alternative agricultural systems, which include the use of biofertilizer.

Biofertilizer is commonly referred to as the fertilizer that contains living micro-organisms and it is expected that their activities will influence the soil ecosystem and produce supplementary substance for the plants (Parr *et. al*. 2002). However, the species and quantity will vary depending on the source of cultures and raw materials used to produce the fertilizer. These microorganisms and the nutrients obtained from the raw materials are used to improve soil health and nutrition. There are different types of biofertilizer available and their differences are mainly in the raw materials used, forms of utilization and the sources of microorganisms (DOAE 2003; and Higa and Parr 1994).

Among different techniques to produce biofertilizer, the concept of effective microorganisms (EM), which is available in liquid form, has been introduced in 1991 by Dr. Teruo Higa of Japan (Setboonsarng and Gilman 1999). The major groups of microorganisms contained in the EM include filamentous fungi, yeast, lactic acid bacteria, and other soil bacteria (Higa and Parr 1994).

The application of EM aims to function as inoculum of microorganisms to the soil in which it will help to establish or re-establish soil ecosystems. EM is commercially available in concentrated form that needs to be processed before the application (Anon. 2006). According to the preparation suggested by EM manufacturer (Anon. 2006), the concentrated EM (EM Bokashi) can be used directly by mixing with molasses and water. However, the common method is to use EM Bokashi as a starter to ferment the raw materials and produce either liquid or solid biofertilizer. The common raw materials include left-over plant or animal materials in the farms. The fermentation period was suggested to be at least seven days and the product is recommended to be used within three months. Nowadays, the production of ready-to-use liquid biofertilizer from EM is becoming available in the market due to the convenience for small-scale farming or domestic application in which the users do not have space and raw materials available for fermentation. Efficiency of biofertilizer depends on the components available in raw materials as well as contribution from living microorganisms in them. However, as far as the authors know there is no previous published documentation of tests done to analyze the diversity of the microorganisms during the storage. The experiment described in this contribution aimed to assess the microbial and chemical properties of liquid biofertilizer during storage and also to estimate their shelf life.

## Experimental Procedures

### Preparation of Liquid Biofertilizer and Packing

Samples of liquid biofertilizer were produced from plant-based waste material using a commercial method practiced by Varee (87) Co., Ltd. Three independent batches of liquid biofertilizer were produced and packed for the experiment from the factory.

Liquid biofertilizer was packed in one liter plastic (PP) bottles. The samples were then delivered and stored at room temperature for four months in two conditions: with exposure to direct sunlight and without. Temperature of each storage condition was also monitored.

### Microbial Analysis

Microbial analysis for the number of microorganism was done every two weeks for the number of total viable bacteria on plate count (PCA) agar, yeast on yeast extract-malt extract (YM) agar, lactic acid bacteria on MRS agar and mold on red Bengal agar (RBA) agar. All media were prepared from HiMedia (India) dehydrated media.

The samples of each time point were collected from two independent bottles of the same storage condition. All media were prepared as described in Atlas (1993) or indicated by the manufacturer.

### Chemical and Physical Analysis

The liquid biofertilizer was analyzed for their pH using handheld pH meter. Color of the samples was also monitored using Muncell color system. The amount of reducing sugar from each sample of liquid biofertilizer was analyzed using DNS method (Bernfeld 1955). The samples were sent at the Office of Science for Land Development to analyze their total nitrogen (N), phosphorus (P), and potassium (K). The new sample was collected once a month for analysis throughout the course of storage.

Other general characteristics of liquid biofertilizer, including appearance of the package, odor and flocculation were also observed.

## Result and Discussion

### Microbial Analysis

Viable cells counts for total bacteria, yeast, lactic acid bacteria and mold were monitored during storage of liquid biofertilizer. During four months of storage, the number of viable microorganisms was found to reduce from the original number during the first 60-75 days of storage with no significant difference between total bacteria, yeast and lactic acid bacteria (Figs. 1A and B). The microbial

population was also not significantly affected from the condition of storage (Figs. 1A and B). The numbers of total bacteria, yeast and lactic acid bacteria were in between $10^5$ – $10^8$ cfu/ml, which is consistent with the microbial analysis result from liquid biofertilizer produced from different plant-based raw materials during fermentation as published by DOA (2004).

Besides these three groups of microorganisms, mold is another group that is commonly found in liquid biofertilizer from EM. In this experiment, viable mold was found in a small number (less than 30 cfu/ml) in all samples (Fig. 3, complete data not shown). However, their number remained constant over the course of storage.

Some of the bacterial colonies on the plates were also investigated under the microscopes and most of them were rod shape gram positive bacteria in all types of media (Fig. 2).

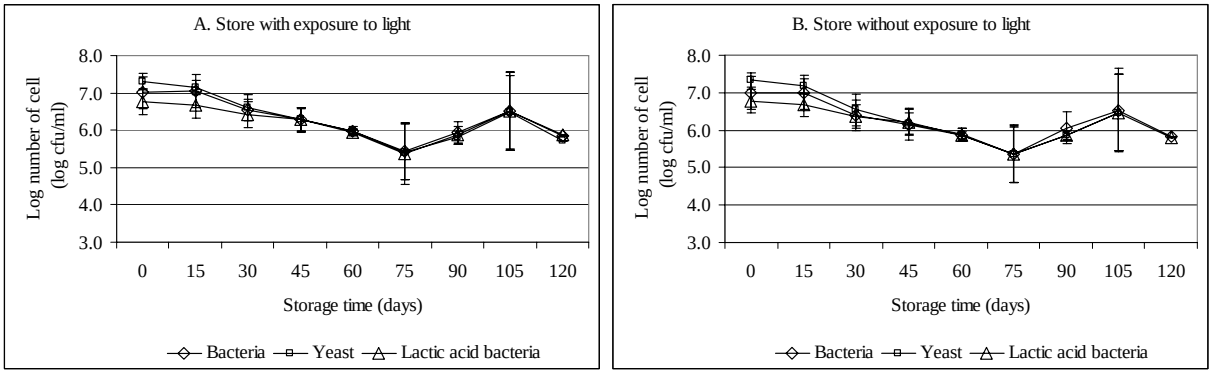
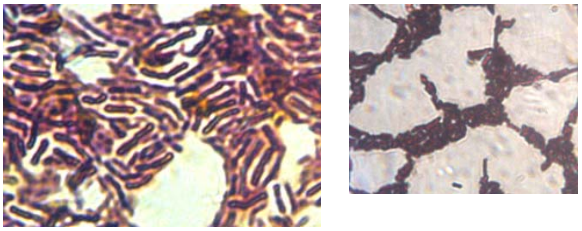



Fig. 1. Microbial profile of liquid biofertilizer showed in log number of viable cells (log cfu/ml) in the two storage conditions (A) with light and (B) without light (—◇— bacteria, —□— yeast, —△— lactic acid bacteria).



A. PCA media B. MRS media

Fig. 2. Gram positive rod bacteria grown on different type of media and observed under light microscope (1000x).

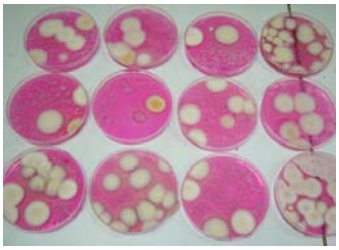


Fig. 3. Mold from biofertilizer in RBA plate.

**Chemical Characteristic**

Besides microbial quality of the liquid biofertilizer, chemical properties of liquid biofertilizer were also monitored. Reducing sugar content in liquid biofertilizer was analyzed to assess carbon source available in liquid biofertilizer mainly for microorganisms. The result showed no significant change in the amount of reducing sugar during storage for both conditions (Fig. 4). However, the amount of reducing sugar was significantly different in different batches of liquid biofertilizer (data not shown) and will be discussed later.

The pH of liquid biofertilizer was monitored and showed average pH range between pH 3.5-3.9. The observed pH was significantly reduced after 30 days of storage compared to the initial pH and increased to similar level after 60 days (Fig. 5). This observation corresponded with the result published earlier by DOA (2004), in which during the first six months of fermentation the pH of liquid biofertilizer from plant materials was in between 3.3-4.0 and increased after 180 days of fermentation. However, the pH was not significantly affected from the condition of storage.

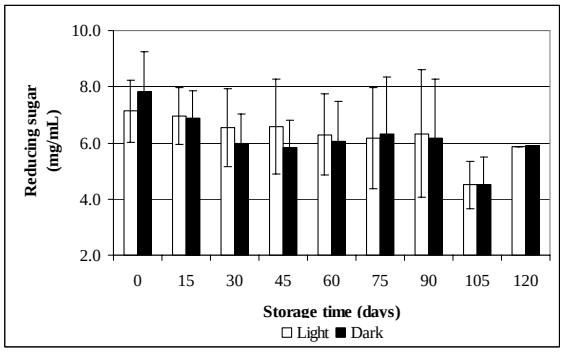



Fig. 4. Reducing sugar (mg/ml) in liquid biofertilizer stored in light and dark conditions.

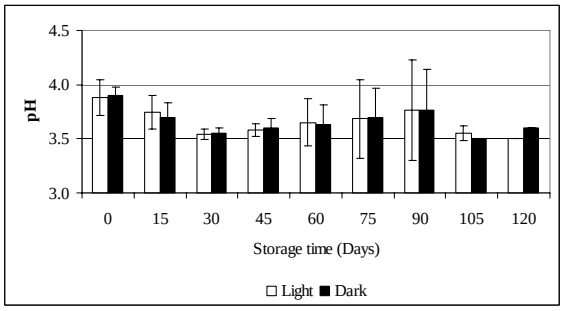
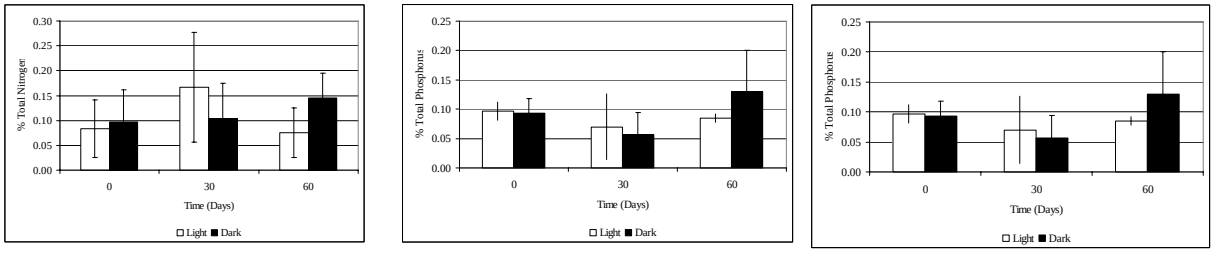



Fig. 5. Average pH of liquid biofertilizer stored in light and dark conditions.




A. %Total Nitrogen B. % total Phosphorus C. % total Potassium

Fig. 6. Plants micronutrients in liquid biofertilizer stored in light and dark conditions.

Major plant nutritional compounds were analyzed and the result showed high variability of these three nutrients from different batches of productions. The amount of total nitrogen (N) was in the range between 0.05-0.28%, total phosphorus (P) was 0.08-0.13% and total potassium was 0.46-0.66. These amounts of N, P and K were in the range that was found in biofertilizer that uses vegetables as raw materials as published by DOA (2003 and 2004).

**Physical Characteristic**

The color of samples changed during storage to a darker and less turbid one (Fig. 7). All three batches of liquid biofertilizer showed similar characteristic in the change of color. However, biofertilizer from Batch 3 showed higher rate of change and also high viscosity. The swelled bottles were also observed to vary differently between the two storage conditions. This swelling characteristic suggested activity of microorganisms in the liquid biofertilizer that causes production of carbon dioxide during storage.

In order to effectively estimate an appropriate shelf life of liquid biofertilizer, physical qualities like color, odor and bottle appearance are also important. From this experiment, the microbial content of liquid biofertilizer indicated longer shelf life than the one suggested earlier (Anon. 2006). However, when considering the appearance, especially of the container, the shelf life was reduced significantly since the swelling characteristic could be observed after the first month of storage. The storage conditions being varied in this experiment showed no effect on the shelf life of the product which will help to ease the handling of the product.


Fig. 7. Liquid biofertilizer after three different durations of storage.

Besides the differences of the temperature between the two storage conditions (4°C, data not showed), liquid biofertilizer showed no significant difference in all qualities tested. However, the variation of qualities tested was significant between batches of biofertilizer, especially Batch 3. This suggested that the quality of raw material used affected the shelf life of the products since the production methods and duration were the same in all batches. The ratio of different groups of microorganisms residing in each batch possibly contributed to the differences of chemical and physical characteristics in each batch of biofertilizer. (DOA 2003 and 2004). The quality control for the production needs to be assessed further for better shelf life estimation.

## Conclusion

When studying the shelf life of liquid biofertilizer, viable microorganisms and chemical characteristic are considered. The experiment described in this contribution showed that the two storage conditions, with and without direct sunlight, have no significant effect on the four months storage of liquid biofertilizer produced from vegetable wastes.

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