CHAPTER V

DISCUSSION

5.1 Isolation and identification of bacteria

Bacteria from various sources were screened for the activities of cellulase, protease and lipase. The selected bacteria identified as *Bacillus cereus, Bacillus subtilis* and *Serratia marcesens*, isolated from the compost provided by The Royal Project, Chitralada, Palace, were found to produce highest cellulase activity. The strains *Bacillus coagulans* from decomposed vegetables and hot spring area, produced the highest protease activity and finally, *Bacillus cereus* and *Pseudomonas aeruginosa*, were found to produce the highest lipase activity. The selected bacteria were later used for compost production.

Cellulase producing bacteria

The selected bacteria, P1, P2, P3, Y1 and Y2, were measured for cellulase activities. The results showed the specific activities as 71.12, 43.72, 38.02, 68.56 and 78.88 (unit/ml/mg. protein), respectively. So, the bacteria P1, P2, Y1 and Y2 were used in the compost pots, because of the highest cellulase specific activities correlated to the total activities. Singh *et. al.* (2004), presented the specific activities of CMCase of *Bacillus sphaericus* JS1 from crude enzyme as 0.20 U/mg. and after purified by Sephadex G-100, the specific activity was 38.4 U/mg. Therefore, 4 isolates of selected bacteria from the compost of The Royal Project, Chitralada Palace, were suitable for compost production. These bacteria were *Bacillus cereus, Bacillus subtilis* and *Serratia marcesens*.

Many strains of *Bacillus* sp. were reported as cellulase producing bacteria. For example, *Bacillus cereus* RW1 and *Serratia marcesens* RW3, isolated from hind-gut of the termites were reported by Thayer in 1978 that it could produce moderate amount of carboxymethylcellulase. In addition, *Bacillus subtilis* (CBTK 106), reported by Krishna (1999) was also used in solid-state fermentation as decomposed agricultural waste.

Protease producing bacteria

Bacteria, A4, B2, D2 and D5 were selected for the biofertilizer pots. When all clear zone producing bacteria on skim milk agar were measured for the protease activities, 4 isolates of those bacteria were selected depending on the specific activities, 17.90, 36.32, 25.29 and 21.49 U/mg protein. Then, 4 isolates of protease producing bacteria were identified as *Bacillus coagulans.*

Many strains of *Bacillus* sp. were known as protease producing strains. Yang *et al.* (2000) produced and purified protease from *Bacillus subtilis* with protease activity of 20.2 U/mg and the bacteria were used to deproteinize crustacean wastes. Singh *et al.* (2001) purified and characterized alkaline and extracellular proteases from *Bacillus* sp. SSR1 and used that enzyme in the laundry detergents industry.

Lipase producing bacteria

Bacterial isolates G1, G2, N26 and J10, were selected from the high values of lipase specific activities which equal to 0.23, 0.21, 0.22 and 0.15 U/mg protein. Together with the degree of fluorescence on Rhodamine B agar, the bacteria were subsequently identified as *Bacillus cereus* and *Pseudomonas aeruginosa.*

In comparison with the other reports, Dhamsthiti and Luchai (1999) reported *Bacillus* sp. THL027 which produced lipase and gave the specific activity as 1.7 U/mg of crude enzyme. That strain was isolated from an oil-contaminated area around a seaside restaurant. Haba *et. al.* (2000) isolated several strains of microorganisms from contaminated soil and water. The strains *Pseudomonas* sp., *Bacillus* sp., *Rhodococcus* sp., *Candida albicans,* etc., were studied for the culture conditions and suitable substrates from used oil for lipase activities. The results showed high activities from the strain *Bacillus subtilis,* and *Pseudomonas aeruginosa.* Many reports have described the use of lipolytic enzymes in wastewater treatment. Dhamsthiti and Kuhasantisuk (1998) studied *Pseudomonas aeruginosa* LP602, a lipase producing strain isolated from a restaurant wastewater sample and applied the enzyme to use as treatment for lipid-rich wastewater.

From the results, some of the selected bacteria were found to be capable of producing more than one type of enzymes. *Bacillus cereus* was found to produce both cellulase and lipase while *Bacillus coagulans* could produce protease and cellulase. Hence, the application of all these bacteria should enhance the activities of each type of bacterium.

Antagonistic test

Twelve isolated bacteria were studied for antagonism in order to test for symbiotic capacity applied for the production of the biofertilizer. The results revealed that there was no antagonistic effect and the bacteria could be used for further experiment.

5.2 Biofertilizer production

Twelve isolates of selected bacteria were used in the compost pots after the antagonistic test. The results of antagonism were shown in Figure 4.9 (p. 40). It could be seen that 12 isolates of bacteria could grow together in the same pot. The 6 experimental pots of biofertilizer consisted of: pot 1: Home food waste, pot 2: Home food waste inoculated with selected bacteria, pot 3: Synthetic waste, pot 4: Synthetic waste with mixed bacteria, pot 5: Autoclaved waste and pot 6: Autoclaved waste inoculated with acteria. After 30 days of biofertilizer production, temperature, pH and moisture content, C:N ratio and nutritional values were measured.

The properties of the liquid biofertilizer product

Temperature

Temperature is the primary factor affecting microbial activity in composting and a good indicator of the various stages of the composting process. Based on microbial activity, the composting process can be divided into four different stages. The first stage is mesophilic stage which is around 25 to 35°C. According to Stoffella and Kahn (2000), the temperature rises past 45°C based on the activity of thermophilic microbial. As the readily available microbial food supply is consumed, the temperature falls to ambient and the material enters the maturation stage. From the results, in the beginning, temperature of the compost pots as found to be increased from 27 to 31°C, and decreased to 27- 28°C at the end of composting (30 days). The maximum temperature in the biofertilizer pots is 31°C. The biofertilizer pots were stirred many times to turn over the substrates, so that the temperature in the pots did not increase.

Since the size of the biofertilizer pots was rather small, the heat could be easily transferred to the environment. If the pot was larger, the flipping over was required in order to decrease the heat with in the compost. In addition, the bacteria were added in the composition of the biofertilizer. Therefore, the water content was higher than normal which resulted in temperature decrease within the obtained biofertilizer. As water has high heat capacity, the absorption of heat was therefore the result. From the result, the highest temperature within the pot was approximately 31°C. This could prevent the total growth of the bacteria within the pots and resulted in less capacity for the decomposition of the solid waste.

pH

From the results, the initial pH of the biofertilizer is slightly acidic (pH 4.8). Although the composting process is relatively insensitive to pH because of the natural buffering capacity of compost material, the pHs of final composting are in the range of 7.5 to 8.5. Usually, compost mixtures with high pH should be avoided because this can lead to loss of N as NH_3, and its associated odor problems. In this study, the pH in each pot increased from 4.8 to about 8.0 at the end of composting similar to that of Stoffella and Kahn (2000)

The values of pH from biofertilizers in pot 1 and 2 were rather different. This might have been from the reason that the samples from the home food waste were not totally identical. However, from the results obtained, it showed that the trends of the pH from both conditions were eventually pointing to the same direction as time passed. This can be explained that there was probably the adjustment of the biofertilizers to approach the equilibrium with in the pots in both conditions. This might be the results from the cooperative activities from the natural bacteria within the samples and the added bacteria. Therefore, it might be concluded that, the added bacteria should be applicable in the production of the biofertilizers since the natural pH is not constant.

Moisture content

Microorganisms need water to survive. Composting works well if the composting material retains water in the range between 40 and 60 percent. If organic decomposition occurs at higher or lower moisture content, the rate will decrease. If the material is too wet, the pores in the compost pile will fill with water and reduce the flow of oxygen necessary for living microorganisms. When moisture is above 60 percent, the compost approaches saturation and anaerobic condition will prevail. When moisture is so high that it flows freely from the compost pile, it may cause dissolved nutrients to leach out, causing potential pollution of ground or surface water. If moisture level is too low, organism populations will decrease and decomposition will be quite slow. Below 30 percent moisture, microbial activity nearly ceases. The moisture content of 80% is for semi-solid compost similar to the compost in this research. From the results (Table 4.8, p. 44), moisture contents were 81.73, 82.60, 82.13, 81.76, 83.70 and 82.18%. The biofertilizer textures were rather semi-solid because the material composition for the biofertilizer contained the used oil in the proportion of 1/3. In addition, the added bacteria were in the media and certainly resulted in more water content. Hence, after the period of the production, the products appeared paste like, semi-solid and the moisture content was high. From this high moisture, it would be consistent with low temperature and neutral pH of the obtained biofertilizer.

Nutritional values

Microorganisms in the composting process must have sufficiently balanced nutrient sources. This is usually described as the carbon:nitrogen ratio (C:N). Microbes rely on carbon in the composting material for their energy source and their cell structure. The C:N ratio is one of the most important considerations in successful composting. An ideal C:N ratio is considered to be in range of 20:1 to 30:1 (Stoffella and Kahn, 2000). Ratios wider or narrower than that range will likely cause material to decompose much more slowly. The C:N ratios in this research were decreased from the beginning, results were shown in Table 4.9 (p. 45). The C:N ratio at the end of composting were 16.80, 13.08, 13.08, 15.74, 8.00 and 7.53, respectively. These might be attributed to loss in total dry mass due to loss of C as CO_2.

At the beginning, C:N ratio obtained in every pot was found to be 19:1 which was quite closed to 20:1. When the time passed, the ratio was decreased resulting from the organic decomposition of the bacteria added into the biofertilizers. As can be seen from Pot 1 and 2, it was found that the decomposition in Pot 2 was higher than in Pot1. However, Pot 3 showed the higher rate of decrease of the C:N ratio (13.08) than in the Pot 4 (15.74) with added bacteria. This might be the results of the antagonistic effect from the natural bacteria against the added. The natural bacteria might work better in that condition and resulted in better decomposition than that of added bacteria. Moreover, when comparing Pot 5 and 6 in which the waste were produced from autoclaved materials, the ratio of C:N in Pot 6 which contained the bacteria showed higher rate of decomposition (7.53). Therefore, it could be concluded that the added bacteria decomposed the waste well in the absence of the natural bacteria to compete with. In addition, the temperature within the Pot wasn't high enough to enhance the bacterial activities and full growth.

The nutritional values of the compost; N, P, K, were measured after 30 days of composting. The values of N, P, K were shown in Table 4.10 (p. 45). The results showed the ratio of nutritional values of around 1:1:1 percent, the maximum percent was detected in pot 6 (autoclaved synthetic waste with isolated bacteria) as 1.42 : 1.20 : 1.23, higher than the report from Satsanakit (2001). The percentage of Nitrogen, Phosphorus and Potassium (N, P, K) were shown as 0.25, 0.05 and 1.4 percent of liquid biofertilizer, and 0.58, 0.10 and 0.55 of fish extract biofertilizer.

Temperature, pH, moisture content and the ratios of C:N represent the important indicators of the nutritional values or the capacities of the decomposition of the bacteria. During the process of the compost production, the temperature has to be increased and later should be stabilized at the end of the composting. Also, pH has to be maintained at neutrality. The moisture could be rather high since there could be water during the process of the decomposition. The ratio of the C:N can reflect the degree of waste decomposition. From this study, the nutritional values obtained from the liquid biofertilizers were higher than that obtained from the fish waste and some other types of biofertilizers. In addition, the rate of decomposition was better. Therefore, the bacteria from this study were appropriate for the decomposition of the waste and hence useful for the production of the liquid biofertilizers.

Since there has been no study on the optimized quantity of the biofertilizers, the amount recommended by the Land Development Department was applied for use in this study. However, the nutrients from the liquid biofertilizers from this study were applicable for use with plants but the proportion or the concentration of the used biofertilizers has to be further investigated. Obviously, the concentration was not suitable for Chinese spinach and should be interesting to investigate further about the supplying method, the appropriate type of plants, the period of plantation and the supplying period of time in the future studies.