Chapter 5. Discussion and Conclusion

5. 1. Discussion

In the following discussion, the concentration of carbaryl was actually the concentration in the supernatant after the samples were centrifuged. The absorption of carbaryl on the cell surface might reduce the carbaryl concentration , but the result of the concentration change in the presence of glucose showed that the recovery of carbaryl was high though the cell mass increased. So the part of carbaryl that attached on the surface of the cell could be neglected, and the concentration of carbaryl in the supernatant could be considered as the concentration in the medium.

The concentration of carbaryl decreased in the enrichment medium containing soil samples but remained at the same level in the control flask indicated the existence of carbaryl degrading microorganisms.

Though there were 7 isolates appeared on the nutrient agar at the beginning, but only the isolate 5 gave the positive result in degradation ability test. So it is possible that only isolate 5 was capable to degrading carbaryl in the enrichment medium, but others are some species which can survive the extreme condition. After several sub-cultures of the initial enrichment medium, the isolates appeared on the nutrient agar was only isolate 5.

Fig 4.1 a and b shows the degradation kinetics. The possibility that bacteria might use acetonitrile as carbon source was eliminated since there was no evidence of growth in the control flask containing only acetonitrile as carbon source. The growth curves followed the pattern of the typical bacterial growth curve (Pelczar and Reid, 1958). It consisted of the lag phase which lasted for 12 hours, accelerate-growth phase, lasted for about 3 hours, the logarithmic phase, lay between 15 to 24 hours, and the stationary phase. Comparing with the degradation curve, the carbaryl concentration steadily decreased in the first three phase, however sharply decreased while the growth slowed down and was about to enter the stationary phase. During the stationary phase, the degradation continued at slower rate. This degradation pattern suggested that the degradation of carbaryl was related to the population of bacteria, once the biomass reached a certain level, the accelerated degradation occurred. It was probable that the degradation of carbaryl needed certain substance the concentration of which might increase while the population of bacteria increased. The results of carbaryl degradation in the presence of other carbon source showed similar pattern except that the carbaryl concentration remained more or less the same when glucose was presented in the medium.

The bacterial growth curve in the medium containing carbaryl as the only carbon source is compared with those supplemented with glucose, sucrose and starch in Fig 5.1 (a and b).

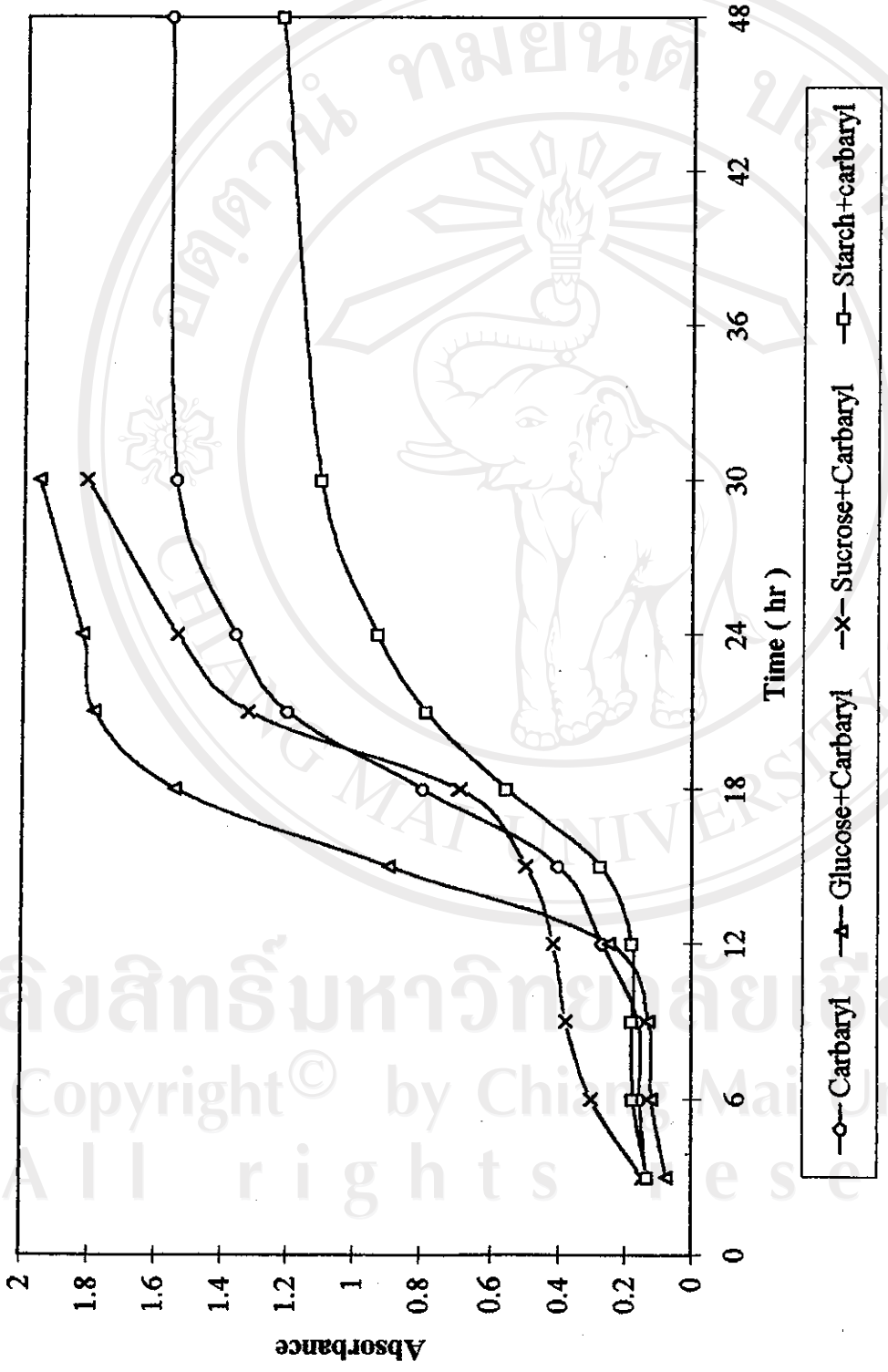

Fig 5.1.a. Bacterial growth in the presence of different carbon source in the minimum mineral medium.

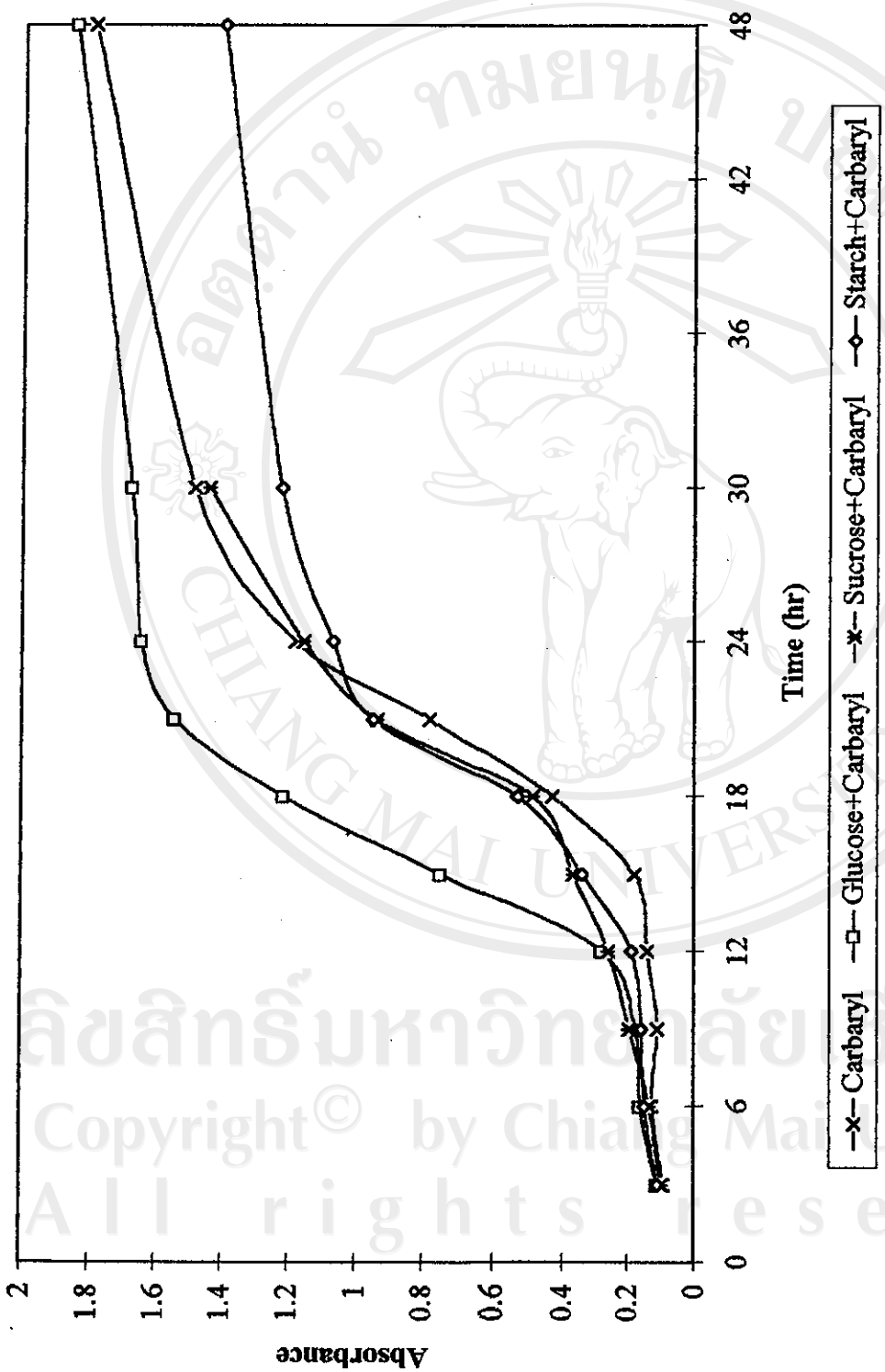

Fig 5.1.b. Bacterial growth in the presence of different carbon source in the minimum mineral medium.

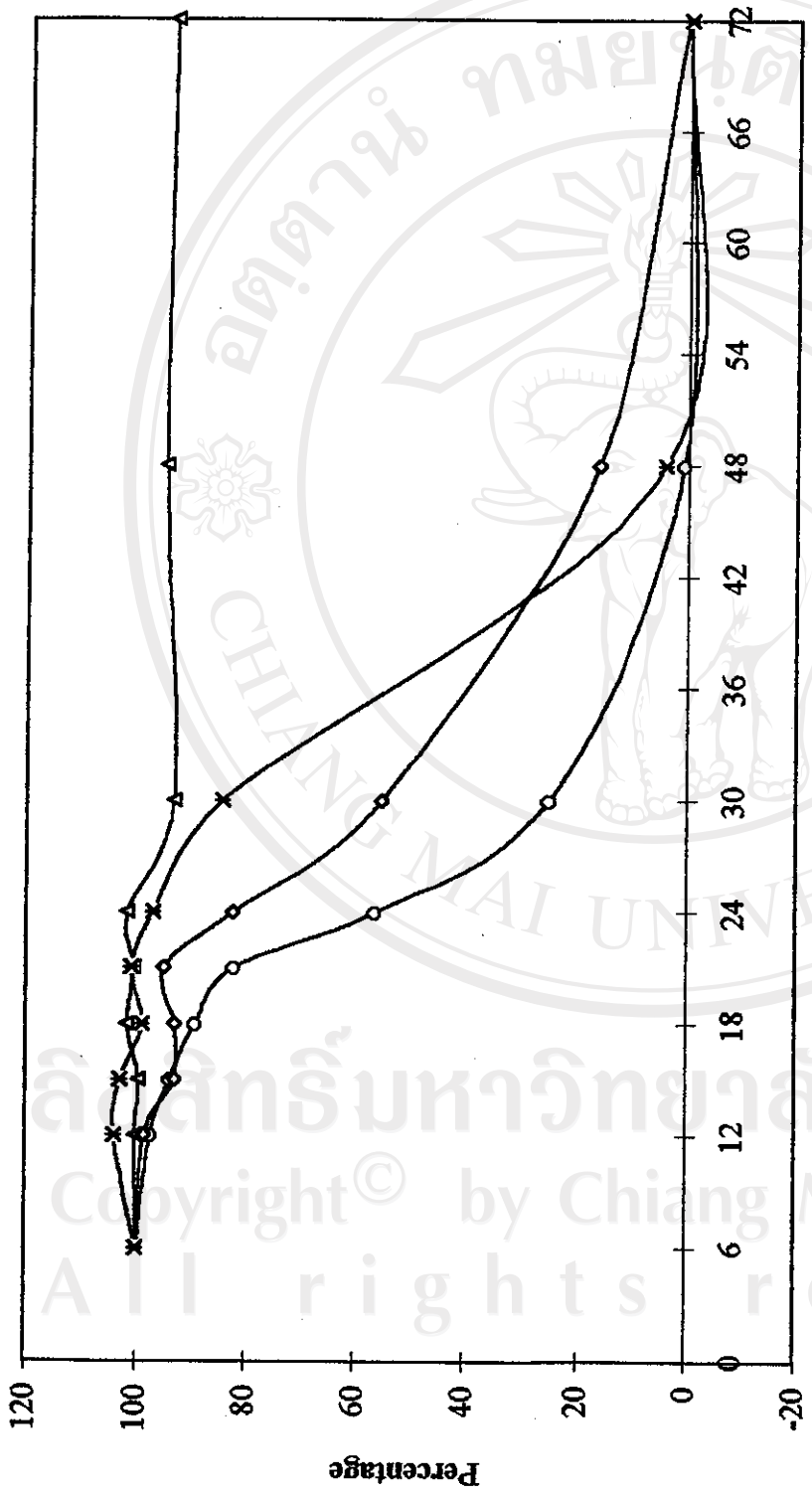

Fig 5.2.a. The percentage of remaining carbaryl in the presence of different carbon source in the minimum mineral medium.

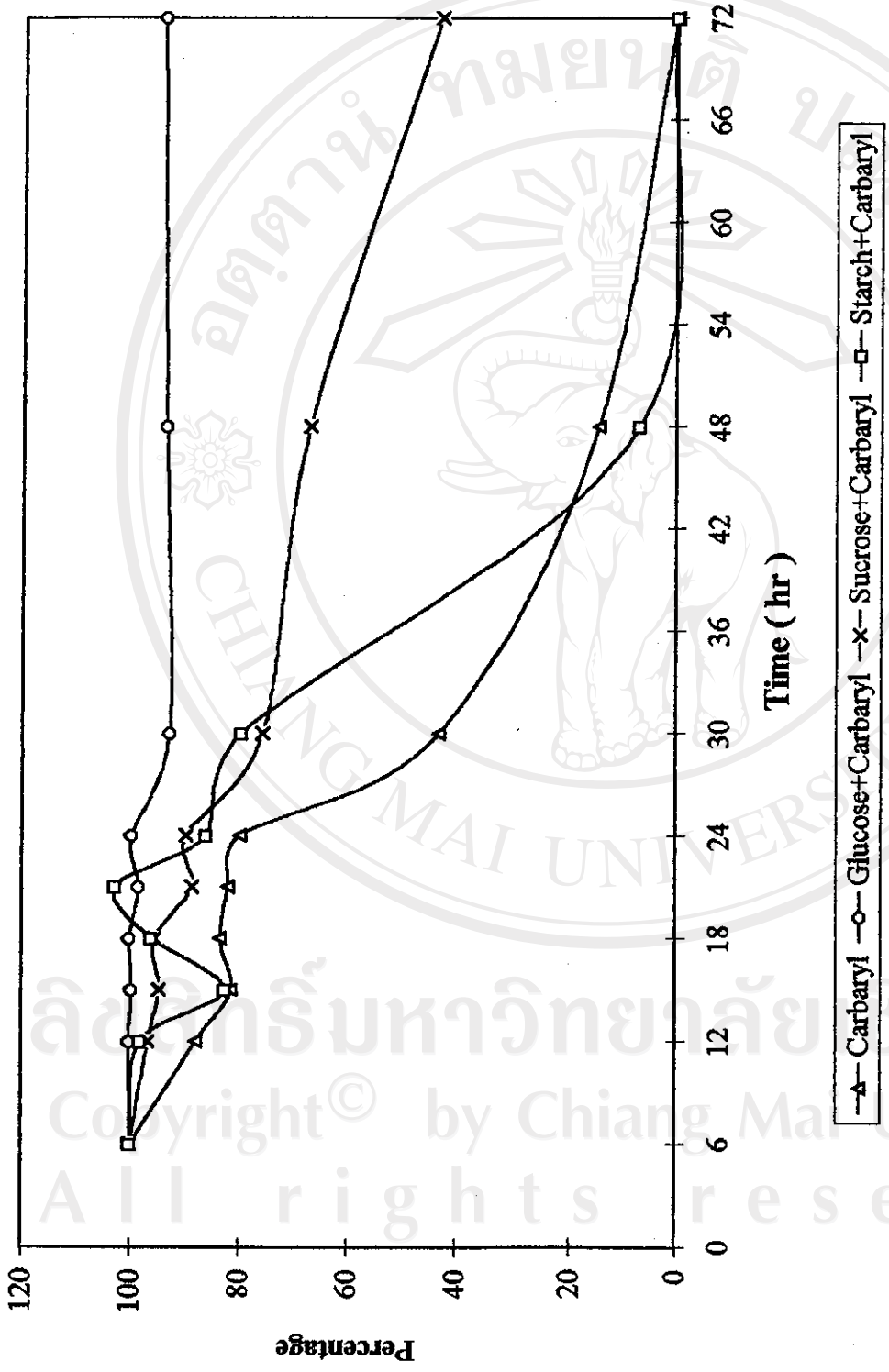

Fig 5.2.b. The percentage of remaining carbaryl in the presence of different carbon source in the minimum mineral medium.

The lag time was more or less the same in all cases, since it might be related to the initial population which was controlled to be the same for all inocula. During the lag period the bacteria need to adapt themselves to the new environment and consequently the reproduction rate is slow. In the logarithmic phase, the growth in the prsence of glucose rose faster than the others, suggesting that glucose was far more efficient than the other carbon source.

The degradation curves were compared in Fig5.2 (a and b). Since the initial concentration of the carbaryl was different in the medium, the remaining carbaryl was coverted to the percentage of the initial concentration. It could be seen that the degradation of carbaryl was faster in the medium containing carbaryl as the sole carbon source. However, a steady decrease of carbaryl concentration in the presence of starch and sucrose was observed. In the presence of glucose the carbaryl concentration decreased slightly at the time interval from 24 to 30 hours and remained stable afterwards.

Generally, the growth rate is a function of chemical concentration. The relationship between growth rate and substrate concentration could be described by the Monod model:

$$\mu = \mu_{max}[S/(K_S+S)]$$

where μ is the specific growth rate, μ_{max} is the maximum specific growth rate, S is the substrate concentration, and K_S is a constant equal to the substrate concentration when $\mu = 0.5\ \mu_{max}$. (Cork and Krueger, 1991). Several degradation patterns were illustrated

in fig 5.3. It can be seen that the degradation pattern in this study resembles to that of logistic model which occurs when a single bacterial species is provided with a mineralizable substrate at concentrations below the K_S value and few cells of the active species are present initially. Under these conditions the bacteria will grow, but at a rate that falls constantly with diminishing substrate concentrations. A growth pattern in which there is an increasing cell number encountering a decreasing nutrient resource.

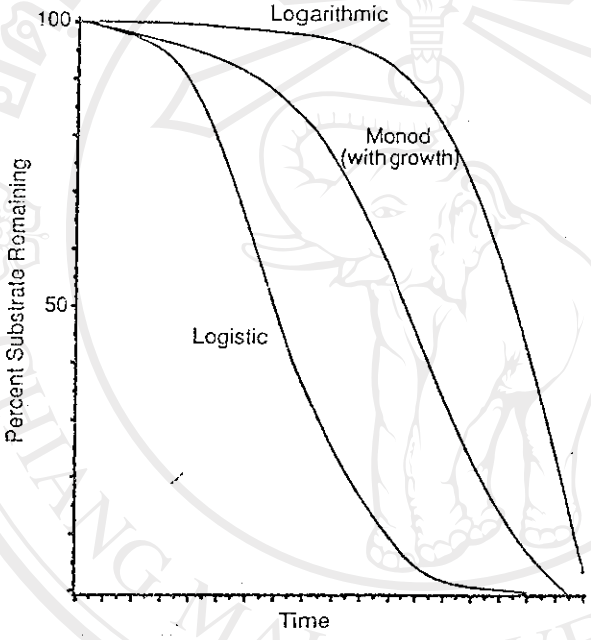

Fig 5.3. Disappearance curves for chemicals that are mineralized as related to logistic, logarithmic, and Monod kinetic models (Alexander, 1985)

However, from the chromatograms of the degradation in the medium containing carbaryl as the only carbon source, a concomitant peak appeared near the carbaryl peak, the retention time was about 0.7 minutes later than the carbaryl peak. (see Fig. 5.4). The unidentified peak was not found in the standard chromatogram, however, it was found in the blank sample but the peak was very small and maintained the same level.

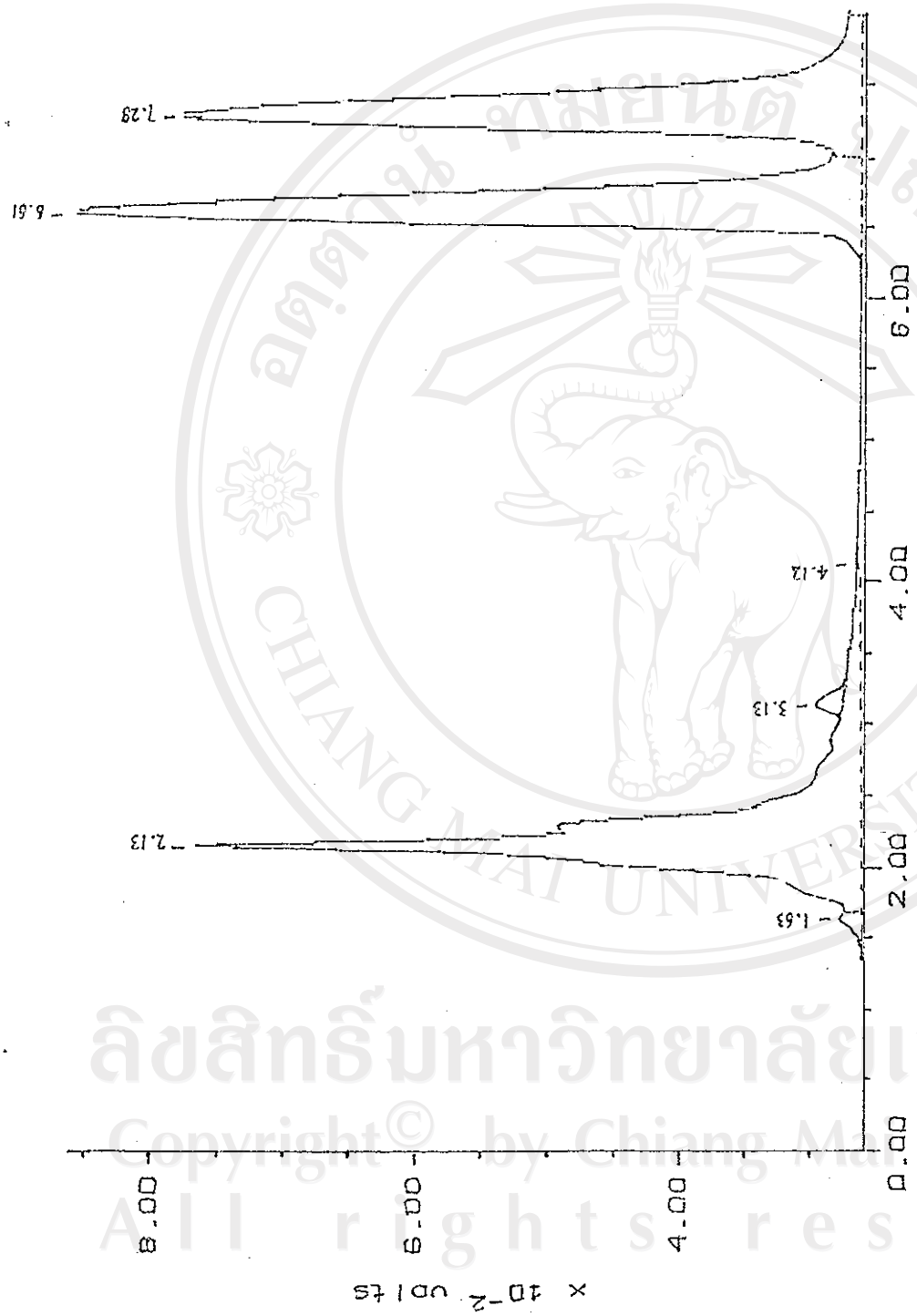

Fig 5.4. Chromatogram of carbaryl and unidentified peak.

When compared the area of two peaks at different time interval, it showed that the area of the unidentified peak was increasing while the area of the carbaryl peak was decreasing (see Fig 5. 5). When it reached a certain level at 30 hours, it decreased simultaneously with the carbaryl peak. The similar phenomenon was found when the sucrose and starch was also presented in the medium (see Fig 5.6 and 5.7) but not found in the presence of glucose (see Fig 5.8). In the case of sucrose and starch, Since the sucrose and glucose are the prior carbon sources, the metabolizing of carbaryl and accumulation of the unidentified product was retarded.

Since the change of unidentified peak was only shown in the case which the degradation of carbaryl occurred, this unidentified peak was probably related to the degradation of carbaryl. There was one possibility that the unidentified peak was the intermediate metabolic product or products. The increasing of the peak area parallel with the decreasing of carbaryl concentration before 30 hours, but both peaks declined after 30 hours, and disappeared at the end of the experiment. This suggested that carbaryl underwent two degradation steps. The unidentified peak was the product of the first step, when its concentration reached a certain level, it might induce its own degradation, and further accelerated the degradation of carbaryl.

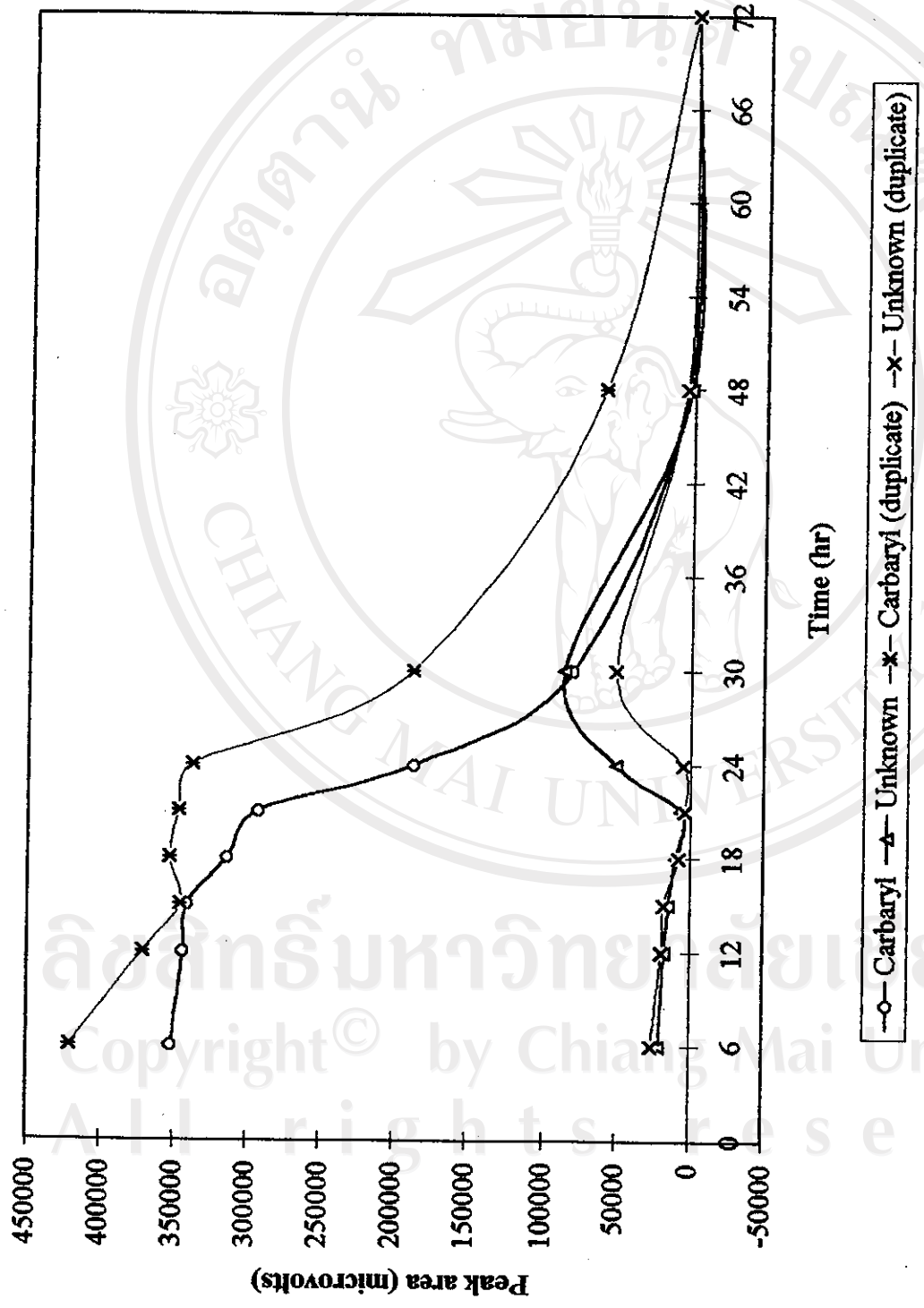

Fig 5.5. Peak area change in minimum mineral medium.

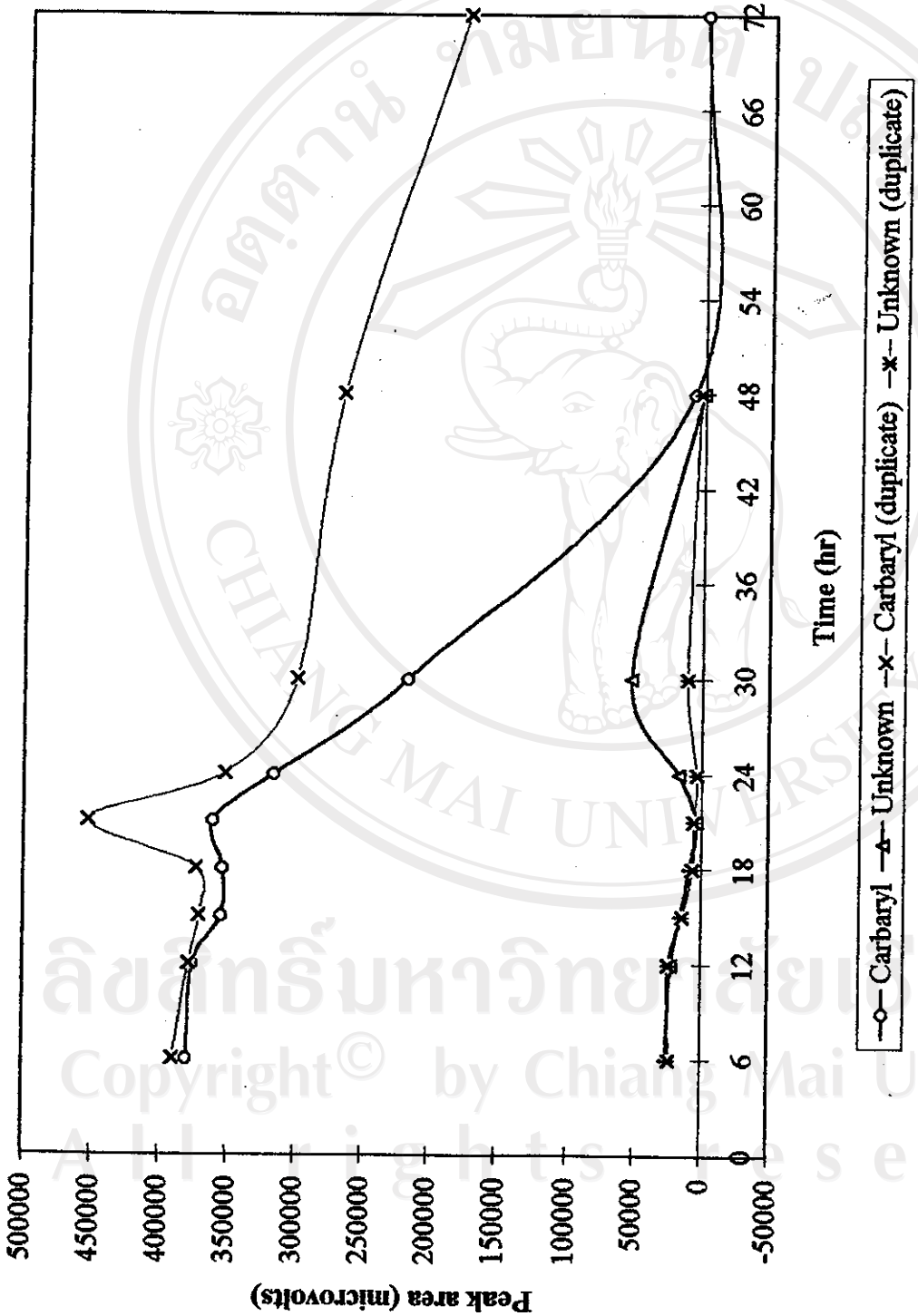

Fig 5. 6. Peak area change in the presence of sucrose.

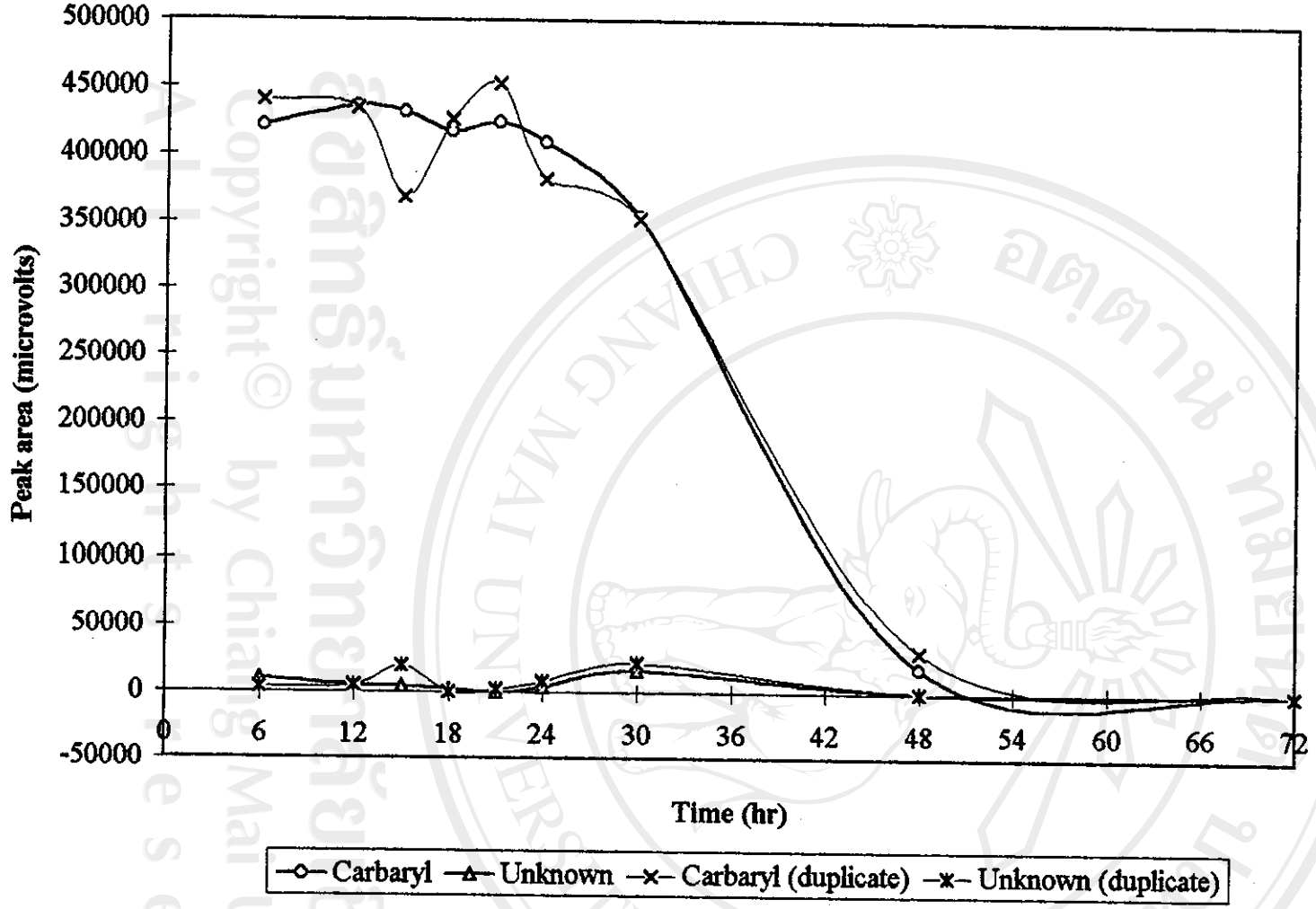

Fig 5. 7. Peak area change in the presence of starch.

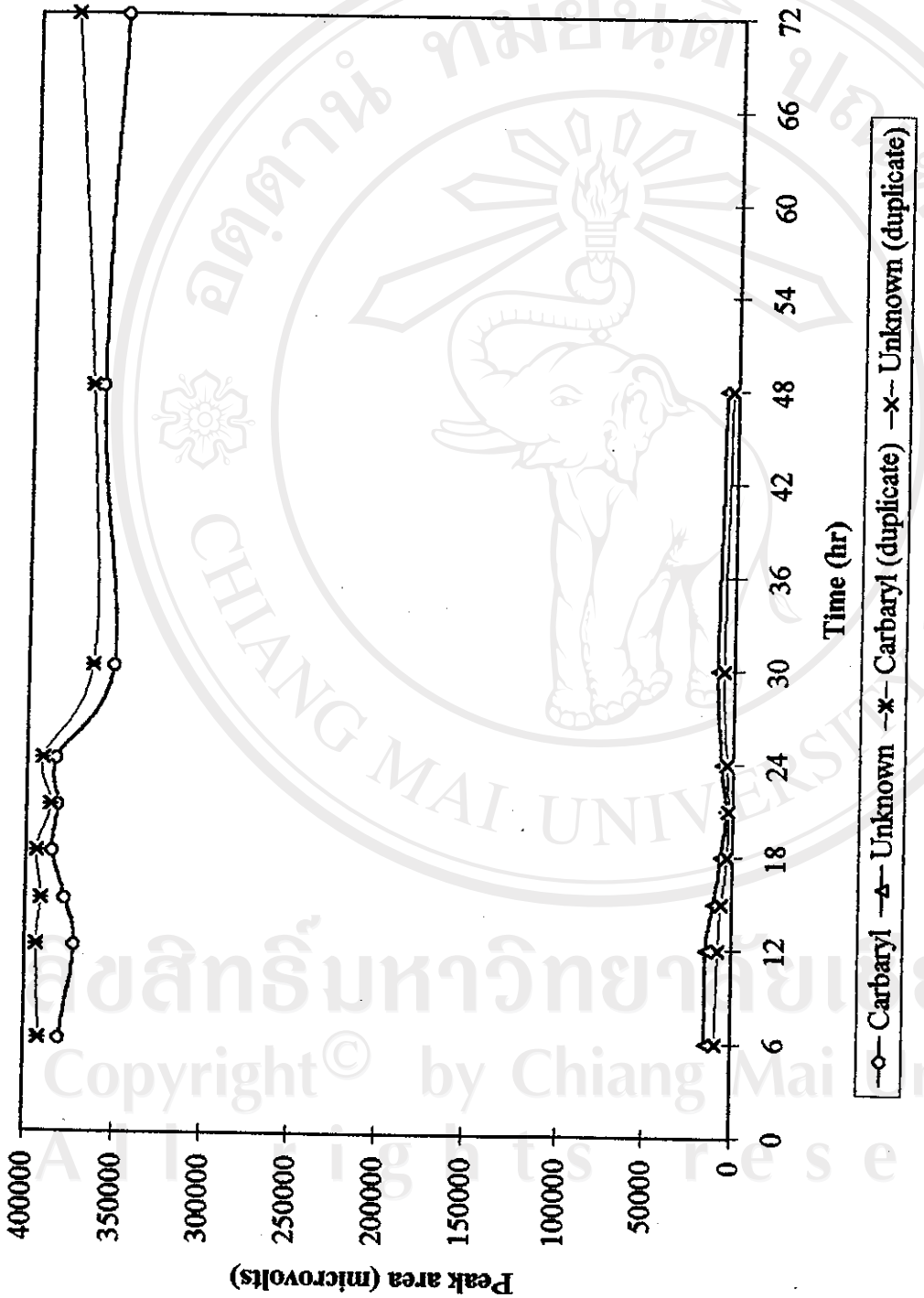

Fig 5. 8. Peak area change in the presence of glucose.

However in the presence of glucose, the first step of carbaryl metabolizing was blocked because the glucose is far more efficient than the carbaryl as carbon source, the bacteria might not be involved in the pathway of carbaryl metabolism, therefore the unidentified product never could accumulate up to the threshold to induce the second step of the degradation. So the concentration of carbaryl remained the same in the medium. The pH change might be taken into account. During the experiment, the pH change in the medium was monitored at a later period of the incubation. It was found that the pH decreased down to 5 in the medium containing glucose, but remained neutral in other flasks. It might explain why the degradation of carbaryl was blocked in the presence of glucose. The rapid growing of bacteria might cause the acidic substance to accumulated in the medium. However the second step of carbaryl degradation might need a neutral or alkaline condition.

The result of the carbaryl degradation in the presence of paraquat indicated that paraquat did have the negative effect on the carbaryl degradation. There were some possibilities: first, the bacteria might use paraquat as a carbon source as well; second, paraquat inhibited the metabolic pathway of carbaryl; and last, paraquat had the effect on the growth and population of the bacteria. The first possibility which was the cross adaptation phenomenon was unlikely because that the cross adaptation was usually found between the pesticides of the same group, normally caused by the similar chemical structure. So far, the cross adaptation found in the microbial degradation of carbaryl was with other methylcarbamate pesticide such as carbofuran. The second possibility was also unlikely since the degradation did occur even when paraquat was

present at a concentration as high as 100 mg/l. So it is more probable that paraquat might have the effect on the growth and population of the bacteria. It is supported by the observation that during the first two days of incubation the medium in the flasks containing paraquat were less turbid than the medium with no addition of paraquat.

Whether or not paraquat would have a negative effect on the biodegradation of carbaryl has not been tested, but it would be interesting to study in the real field condition. Since the two pesticides might be used in the same field for a short time interval. The interaction between the pesticides and microorganisms might as well lead to the longer persistence time of carbaryl. It might be argued that paraquat used in the field may in such a low concentration that it had no effect on other pesticide degradation. So the concentration of paraquat entered into soil was estimated in the following part.

As mentioned before, the recommended amount of paraquat used for weed control in plantation crops is 280 to 560 gram per hectare. If all the paraquat was precipitated on the surface soil, for example 1 cm deep layer, then it is 2.8 to 5.6 g/m^3 soil. Again, if all the paraquat were dissolved in the soil solution, since paraquat is extremely water soluble, and the soil moisture is 17 % (in this case), then the concentration of paraquat in the soil solution is 16 to 33 mg/l., a concentration range to give a possible negative effect on carbaryl degradation. In the case of other control method like stubble cleaning (140 - 840 g / ha) and pasture renovation (140 - 2,210 g/ha), paraquat may be applied at a higher concentration. Alhough paraquat could be

absorbed rapidly on clay and mineral surface, the damage on the microorganisms might occur before it was absorbed.

The analysis of the soil indicated that the soil was a neutral clay soil. There was not much difference between the soils under different vegetation types and surface and sub-surface layers. The only difference is the organic matter content, and the nitrogen in vegetable field is somewhat higher than in the soybean field, it maybe caused by the use of fertilizers. The pour plate counts somehow gave a higher reading in the samples from the vegetable fields. Alexander (1977) indicated that the organic fraction of soil which contained the organic carbon and nitrogen was an important food source for microorganisms in the soil. Schnurer *et al* (1985) reported that the biomass and activity in an agricultural soil showed a positive correlation with soil organic matter content. So, this might explain the higher reading from the pour plate counts in the vegetable field sample.

The same carbaryl degrading bacteria was isolated from all the samples, it was distributed in the whole sampling area, both the upper 10 cm and the lower 10 cm layers. Since the surface layer of the soil was disturbed by ploughing, the distribution in the lower layer might be the result of the disturbance.

Although there is only one species of bacteria proved to be able to degrade carbaryl, it was not the only carbaryl degrading species in the soil. Soil is such a complex matrix that contains numerous substances and microorganisms. The interaction

between the microorganisms and the environment is so complicated and is not well understood. Microorganisms might be capable to degrade carbaryl in different conditions. For example, in the case study about the degradation of triphenyltin pesticides by *Pseudomonas putida* No. C (Visoottiviseth *et al* 1992), it was reported that the bacteria only can degrade pesticides in the presence of glucose. Roberts *et al* (1993) reported that a stable mixed bacterial culture could degrade herbicide linuron. They isolate 124 pure cultures from the mixture but no single culture could degrade linuron. So far, the only conclusion is that the bacteria isolated was the only one favor the condition in the enrichment medium.

5. 2. Recommendations

The carbaryl degrading bacteria was isolated and obtained as pure culture. More chemical and biochemical tests should be done to identify the bacteria to the species level.

Since a possible intermediate metabolite was found but so far not yet identified, the further work could be done to identify the unknown substance. This substance could be separated by HPLC first, and inject into GC-MS. Before the identification, a test should be done to prove whether it is the pure substance. It could be helpful to understand the pathway of degradation. It is expected that biodegradation could lead to the mineralization of the synthetic chemicals in the environment for the safety of human beings and ecosystems. So it could be interesting to know whether or not the bacteria

can mineralize carbaryl thoroughly. If so, the bacteria could be a potential agent to clean the tools, containers, fruits or the soil contaminated with carbaryl.

So far the experiment was done mainly in the laboratory and in man made conditions. For the understanding of carbaryl behavior in the soil environment, further work could be done in the real soil samples. The field study might be affected by too many environmental conditions. The result might be difficult to explain even with better control and management. It is possible that using a soil column with natural profile, some of the physical conditions could be standardized in this way, and mathematical models might be established. The knowledge might be very helpful in the understanding in the real field conditions.

Since the negative effect of paraquat on carbaryl degradation by the isolated microorganisms was approved, it could be interesting to further study the degradation of carbaryl or other pesticides in a semi or real field condition in the presence of paraquat. This may contribute to the optimizing the usage of pesticides and avoiding the possible negative effect on the soil environment.

5. 3. Conclusion

The following conclusion could be drawn from the study :

1. A species of carbaryl degrading bacteria exists in the soil sample collected from the Royal Pang Da Agricultural Station. The bacteria is distributed down to the lower layer (10 cm below the surface), probably by the tillage treatment.

2. The bacteria could metabolize carbaryl in the medium containing carbaryl as the sole carbon source.

3. Glucose presented in the medium could inhibit the degradation of carbaryl, however, sucrose and starch have less effect on the degradation.

4. Herbicide paraquat could retard the degradation of carbaryl when presented more than 10 mg/l in the medium.