# CHAPTER 3. RESULTS

## 3.1. Optimization of Biodegradation of Carbaryl

### 3.1.1. pH

At pH 6.0, the selected bacterial strain of isolate-5 was unable to grow and also the carbaryl concentration remained constant. After 48 hours of incubation the final concentration of carbaryl was 9.54 ppm (Figure 1). Whereas at pH 6.5, the strain growth continuously increased for 32 hours and also the carbaryl degradation followed the same pattern. After 32 hours the growth rate of bacteria dramatically rose up again and enhanced carbaryl degradation was observed within this period. Again, after 40 hours, the bacterial growth and carbaryl degradation fell. Finally, after 48 hours of incubation, the final growth and the remaining carbaryl were 0.176 ($A_{600}$) and 4.0 ppm, respectively (Figure 1).

At pH 6.8, the highest bacterial growth was found after 32 hours whereas the remaining amount of carbaryl was found to be 6.52 ppm and finally after 48 hours of incubation the bacterial growth and the remaining carbaryl were 0.115 ($A_{600}$) and 6.00 ppm, respectively (Figure 1).

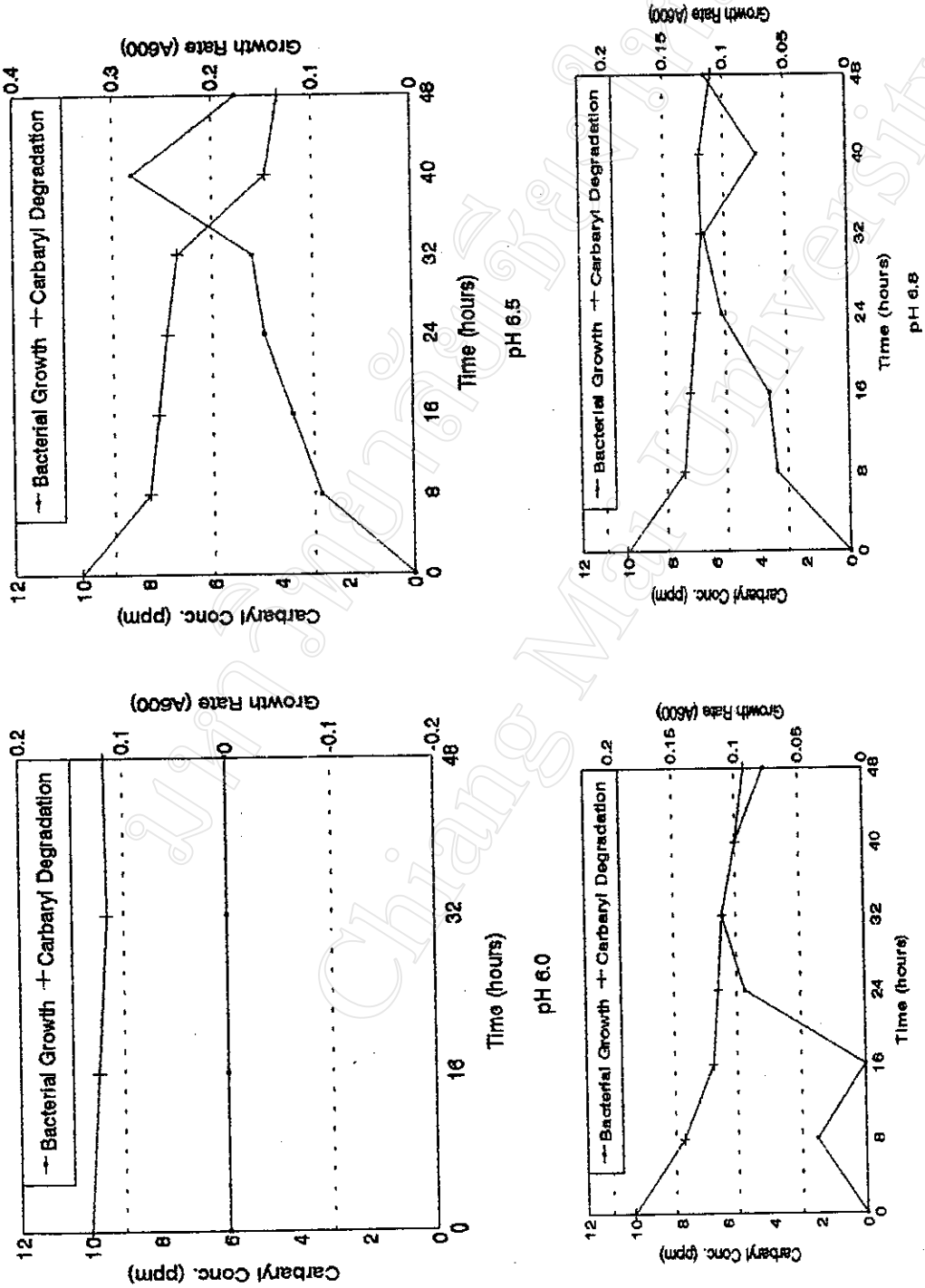


Figure 1. Carbaryl degradation in MM medium at pH 6.0, 6.5, 6.8 and 7.0 at 34 °C.

As at pH 6.8 at pH 7.0 there was the highest bacterial growth of isolate-5 was observed after 32 hours and the remaining carbaryl at that time was 6.07 ppm. At this pH the bacterial growth rate suddenly fell after 8 to 16 hours. Carbaryl degradation did not follow this trend whereas it was reduced from 7.82 ppm to 6.52 ppm. After 48 hours the final bacterial growth in this pH was 0.077 ($A_{600}$) and the remaining amount of carbaryl was 5.04 ppm (Figure 1).

At pH 7.2 there was a good correlation between strain growth and carbaryl degradation was found for 16 hours of incubation and about a half the concentration of carbaryl (5.5 ppm) was degraded within this period. After 48 hours the final bacterial growth was 0.126 ($A_{600}$) and the remaining carbaryl was 2.94 ppm (Figure 2).

At pH 7.5 a higher bacterial growth was found within 16 hours and carbaryl was degraded from 10.0 ppm to 3.06. Finally, after 48 hours of incubation only 1.14 ppm of the remaining carbaryl was found (Figure 2).

At pH 8.0 a continuous bacterial strain growth was observed whereas the carbaryl degradation was higher within a 16 hour period. At this time, the concentration of carbaryl was reduced from 10.0 ppm to 0.940 ppm, and after 48 hours of incubation only 0.05 ppm carbaryl was remained in the medium (Figure 2).

At pH 8.5 and after 16 hours incubation the concentration of carbaryl found in the medium was 0.098 ppm whereas no bacterial growth was found at that time. After 16 hours, bacterial growth started and highest growth ($A_{600}$ 0.042) was found after 32 hours incubation (Figure 2).

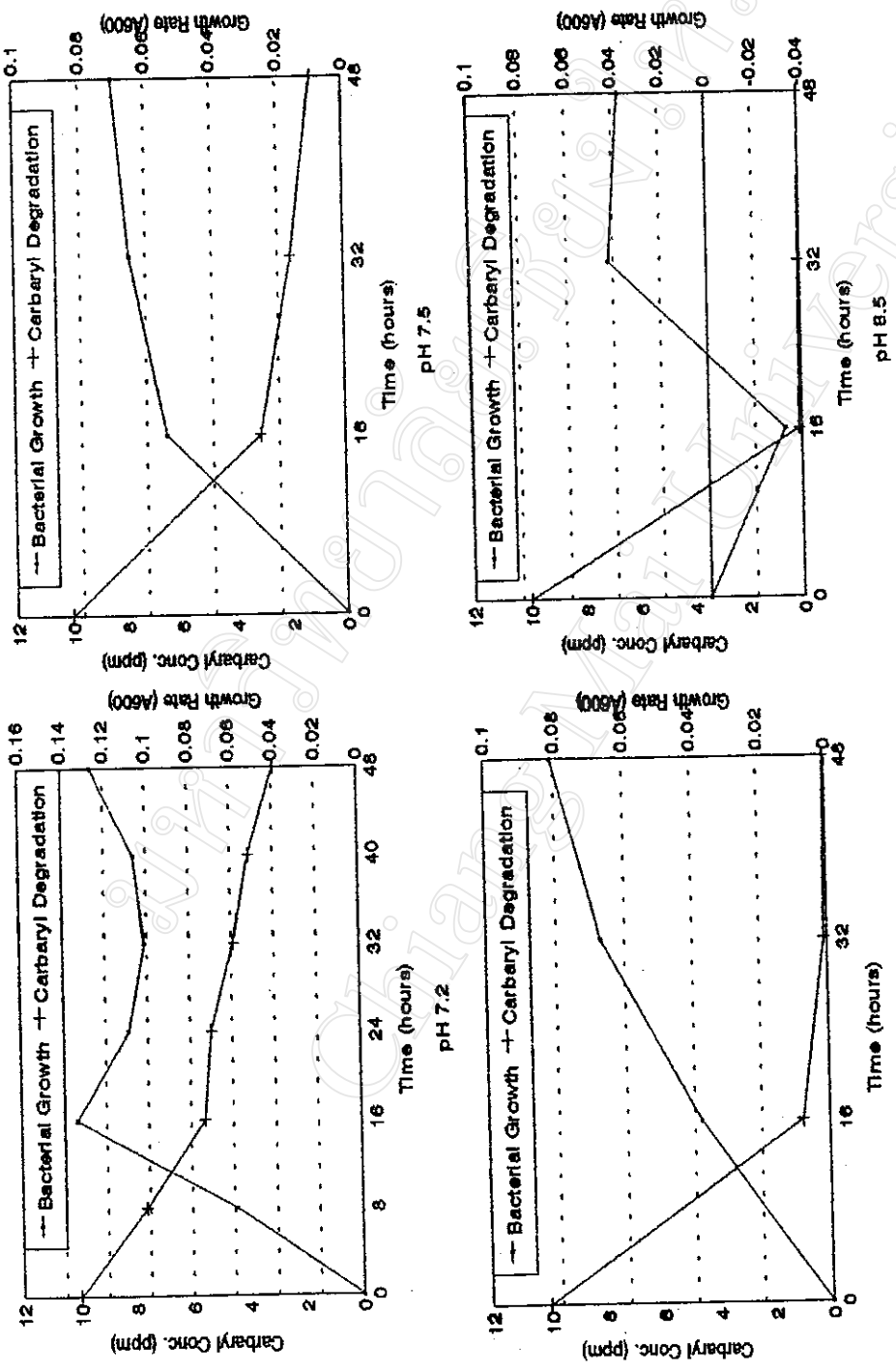


Figure 2. Carbaryl degradation in MM medium at pH 7.2, 7.5, 8.0 and 8.5 at 34 °C

### 3.1.2. Temperature

At 30 °C the highest strain growth of isolate-5 was found after 36 hours of incubation and the remaining carbaryl was 8.13 ppm. After 48 hours the bacterial growth was 0.079 ($A_{600}$), whereas the final concentration of carbaryl was 7.88 ppm (Figure 3).

At 34 °C increased bacterial strain growth was observed for 48 hours and the final concentration of carbaryl was found to be 6.0 ppm (Figure 3).

At 37 °C strain growth increased with time and after 48 hours the remaining amount of carbaryl was found to be 6.47 ppm (Figure 3).

At 41 °C no bacterial strain growth was observed whereas the carbaryl degradation did not follow this trend. It was reduced from 10.0 ppm to 3.65 ppm (Figure 3).

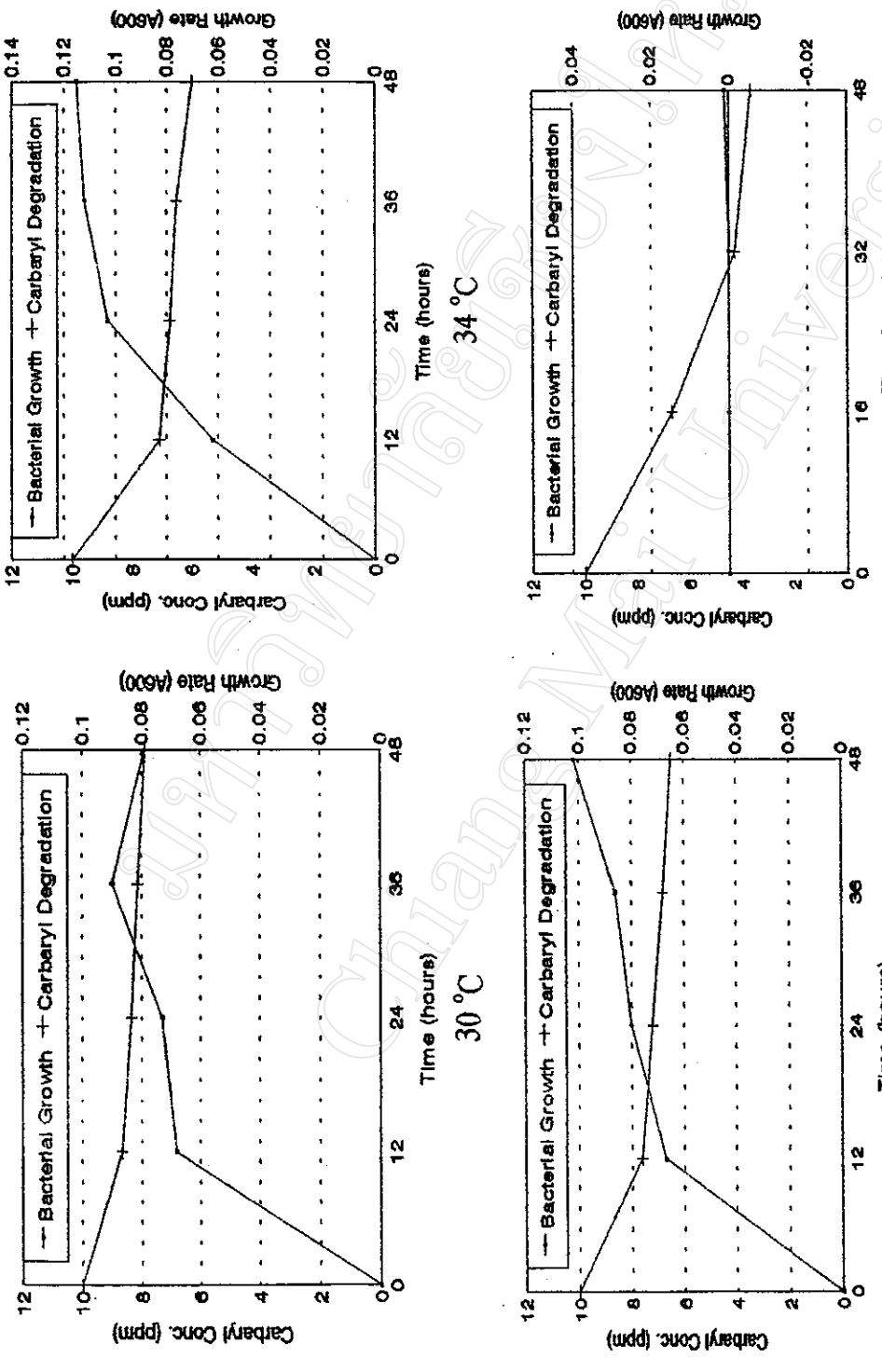


Figure 3. Carbaryl degradation in MM medium at 30 °C, 34 °C 37 °C and 41 °C at pH 6.8

### 3.1.3. Nutrients

Although the selected bacterial strain growth was very low in minimum minerals media with carbaryl, better growth was found in the nutrient broth and in yeast-extract enriched MM medium.

In nutrient broth the highest strain growth was found after 16 hours of incubation and within this period the half of carbaryl (5.36 ppm) was degraded. Later, the bacterial strain growth continuously went down as well as the concentration of carbaryl. After 48 hours of incubation only 0.763 ppm of carbaryl remained in the medium (Figure 4).

In MM with yeast extract the highest strain growth was found after 33 hours and the remaining amount carbaryl was found to be 6.18 ppm. It's concentration after 48 hours was 5.86 ppm and after 81 hours the final concentration of carbaryl was 3.12 ppm (Figure 5).

With the addition of vitamins the highest strain growth was found in vit. $B_6$ supplemented MM medium than the other two vitamins i.e. vit. $B_1$ and nicotinamide (Figure 6). After 48 hours of incubation it was found that the bacterial growth in MM with vit. $B_6$ and without any vit. was same whereas the degradation was higher in presence of vitamin.

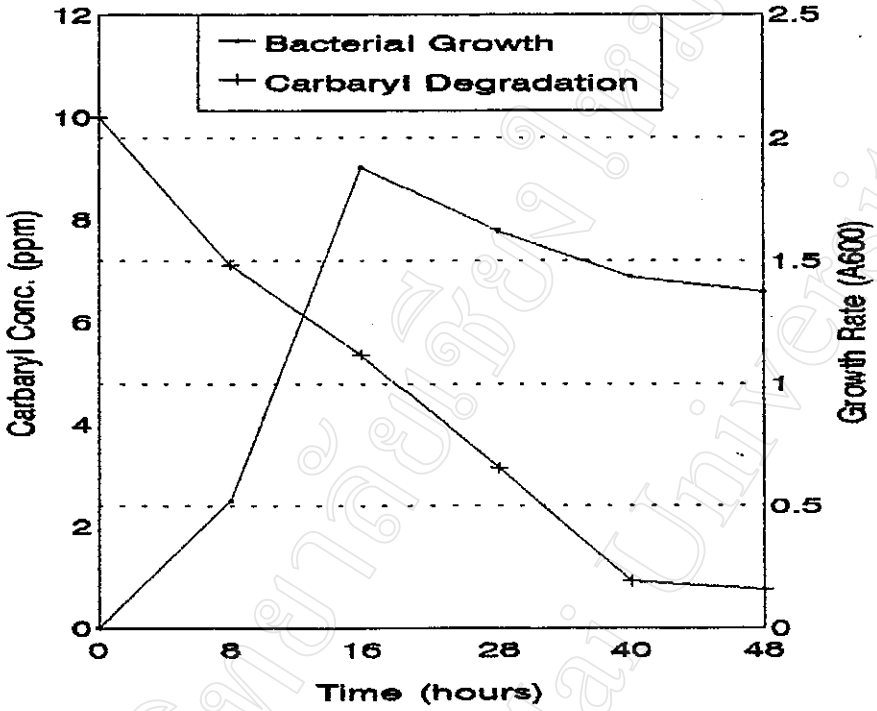


Figure 4. Carbaryl degradation in nutrient broth at pH 6.8 at 34 °C.

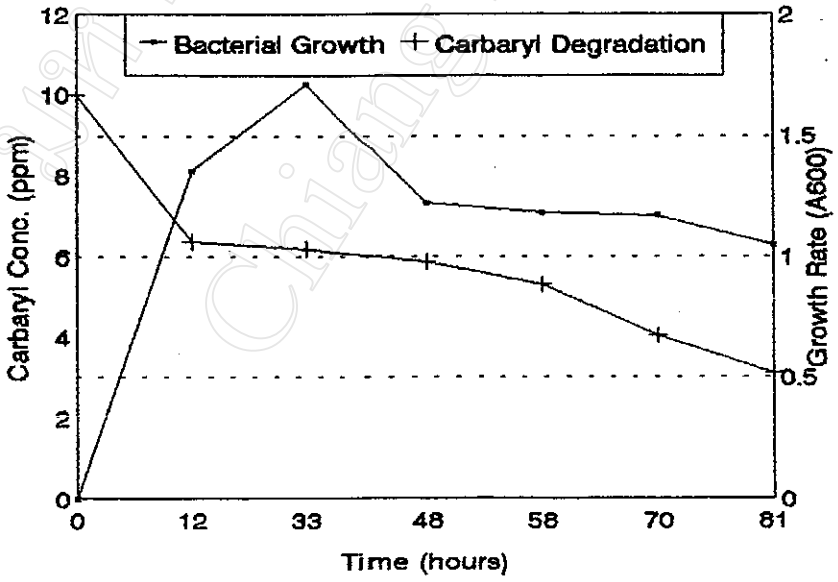


Figure 5. Carbaryl degradation in MM with yeast-extract at pH 6.6 at 34 °C.

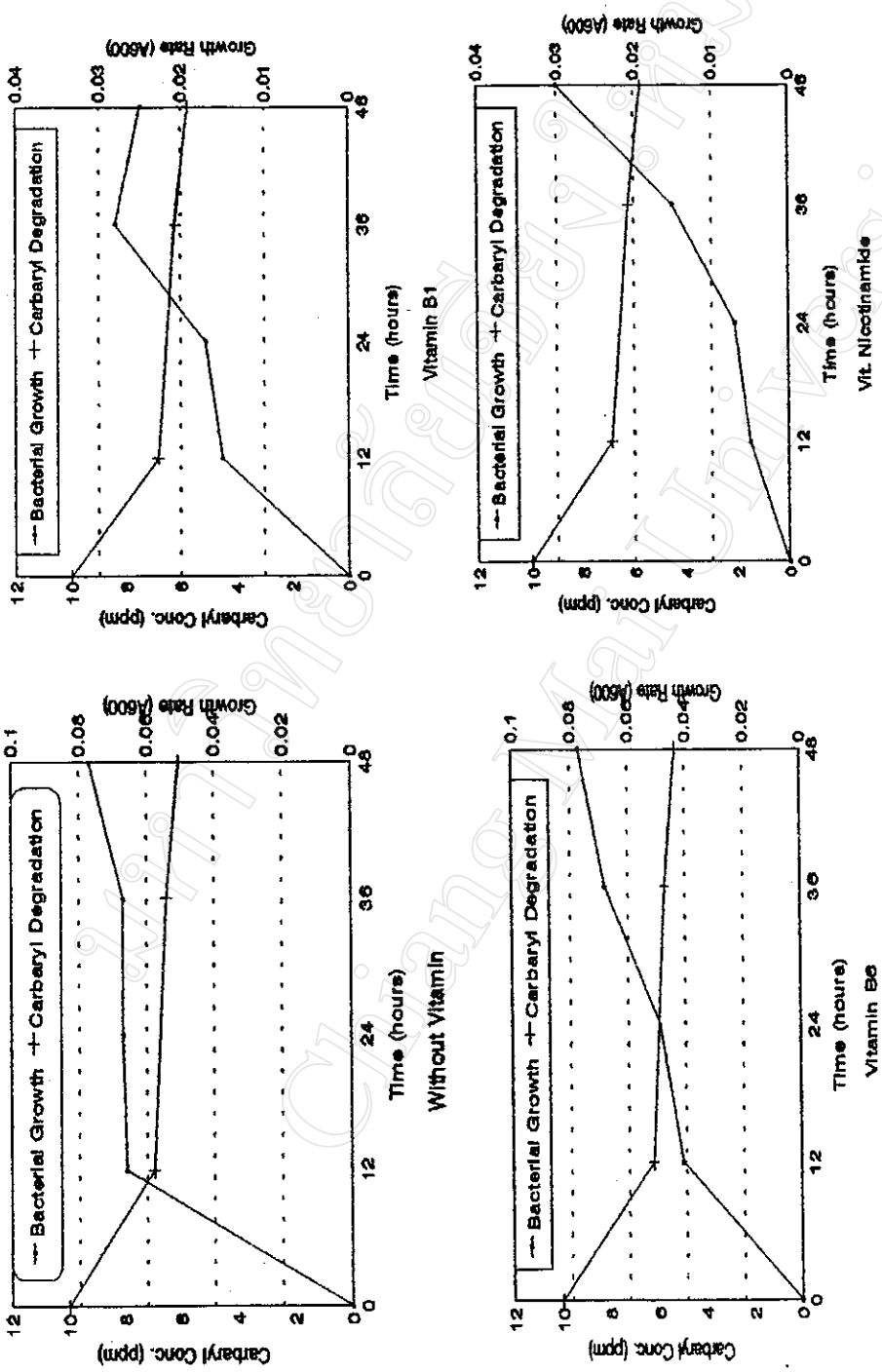


Figure 6. Carbaryl degradation in MM in presence of vit. at pH 6.8 at 34 °C.

### 3.2. Carbaryl Enriched Bacteria

Using carbaryl enriched bacteria of isolate-5, the highest strain growth was found after 20 hours and the remaining amount of carbaryl was 7.22 ppm. Later, the bacterial strain growth increased slowly up to 68 hours and carbaryl degradation followed the same pattern. The final concentration of carbaryl was 6.97 ppm (Figure 7).

### 3.3. Cross-feeding

In cross-feeding the degradation of carbaryl was lower than without cross-feeding. In the presence of carbofuran the highest bacterial strain growth was found after 24 hours and the remaining carbaryl was 8.4 ppm. After 48 hours it was reduced to 7.86 ppm (Figure 8).

In presence of carbosulfan continuous bacterial strain growth was found for 36 hours and the remaining carbaryl was 7.67 ppm. The final concentration of carbaryl was 7.43 ppm (Figure 9).

Continuous bacterial strain growth was observed in MM medium which supplemented with the intermediate product of carbaryl , 1-naphthol. It was found that naphthol concentration was reduced from 10.0 ppm to 6.25 ppm within 48 hours of incubation period (Figure 10).

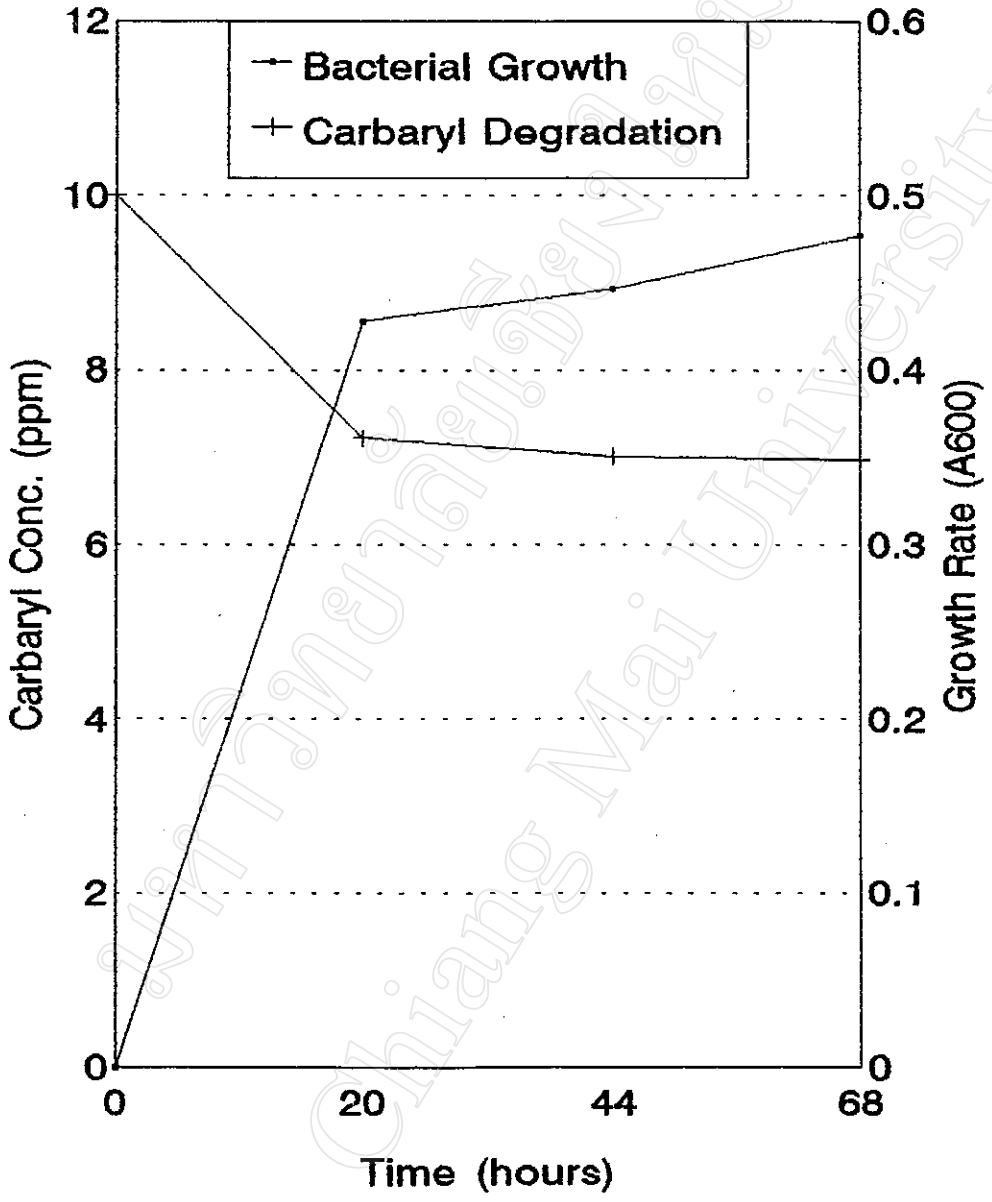


Figure 7. Carbaryl degradation in MM with enriched bacteria at pH 6.8 at 34 °C.

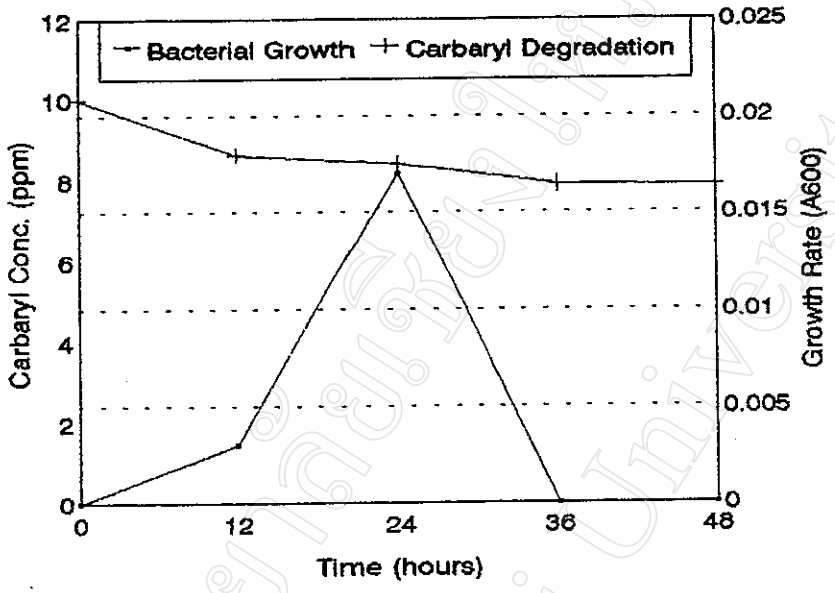


Figure 8. Carbaryl degradation in MM with carbofuran at pH 6.8 at 34 °C

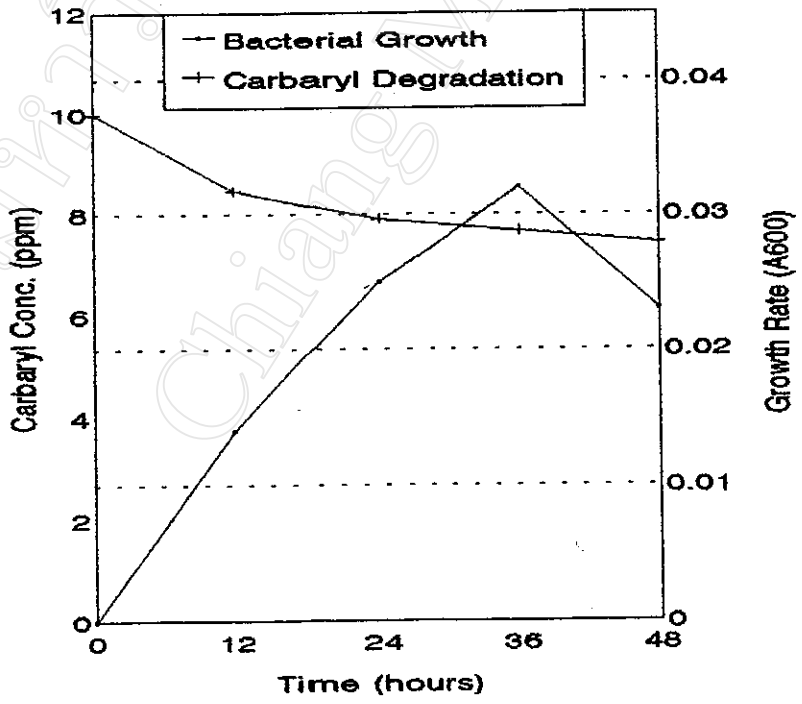


Figure 9. Carbaryl degradation in MM with carbosulfan at pH 6.8 at 34 °C.

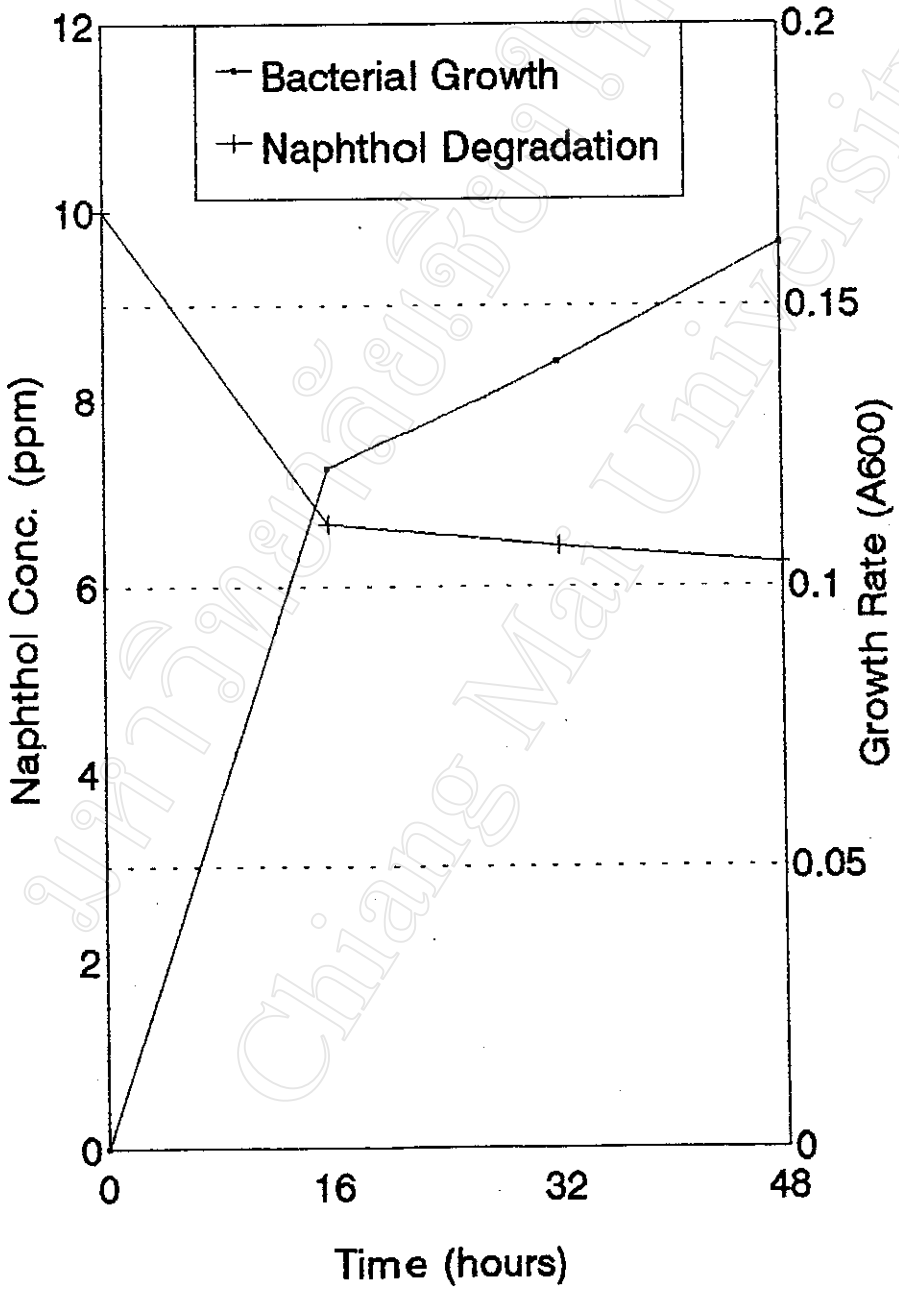


Figure 10. Degradation of 1-naphthol in MM medium at pH 6.8 at 34 °C

### 3.4. Bacterial Mutation

Strain mutation of isolate-5 was done by UV radiation. It was found that UV-60 seconds caused 50% of the bacterial strain population die whereas UV-120 seconds caused most of the bacteria to die. The bacterial colony/ml was counted for UV-0, UV-10, UV-30, UV-60 and UV-120 seconds were 3,790,000, 3,200,000, 2,870,000, 1,960,000 and 83,000, respectively (Figure 11).

Biodegradation of carbaryl was tested with UV-60 seconds exposing bacteria . It was found that only strain U3 can degrade more carbaryl than both normal and other UV-exposed bacteria (Table 1).

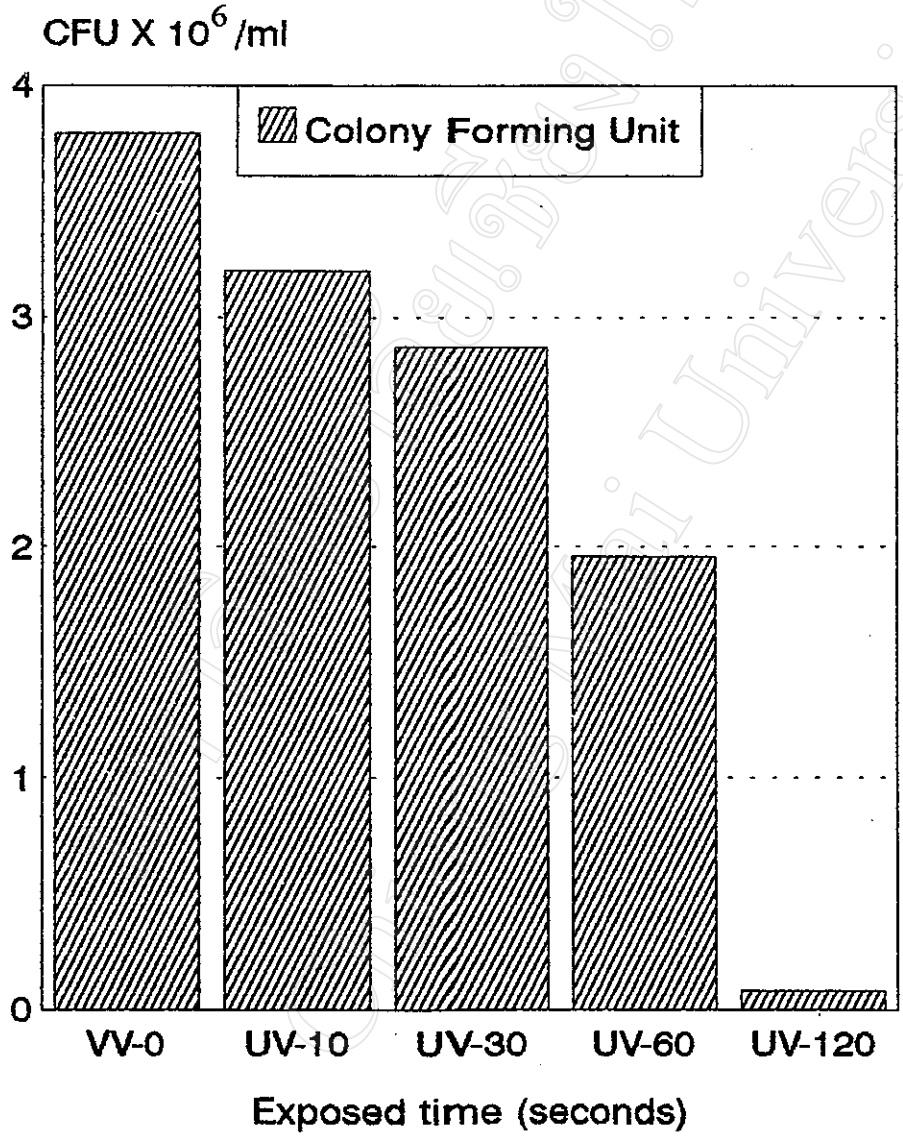


Figure 11. Effect UV-radiation on selected bacterial strain

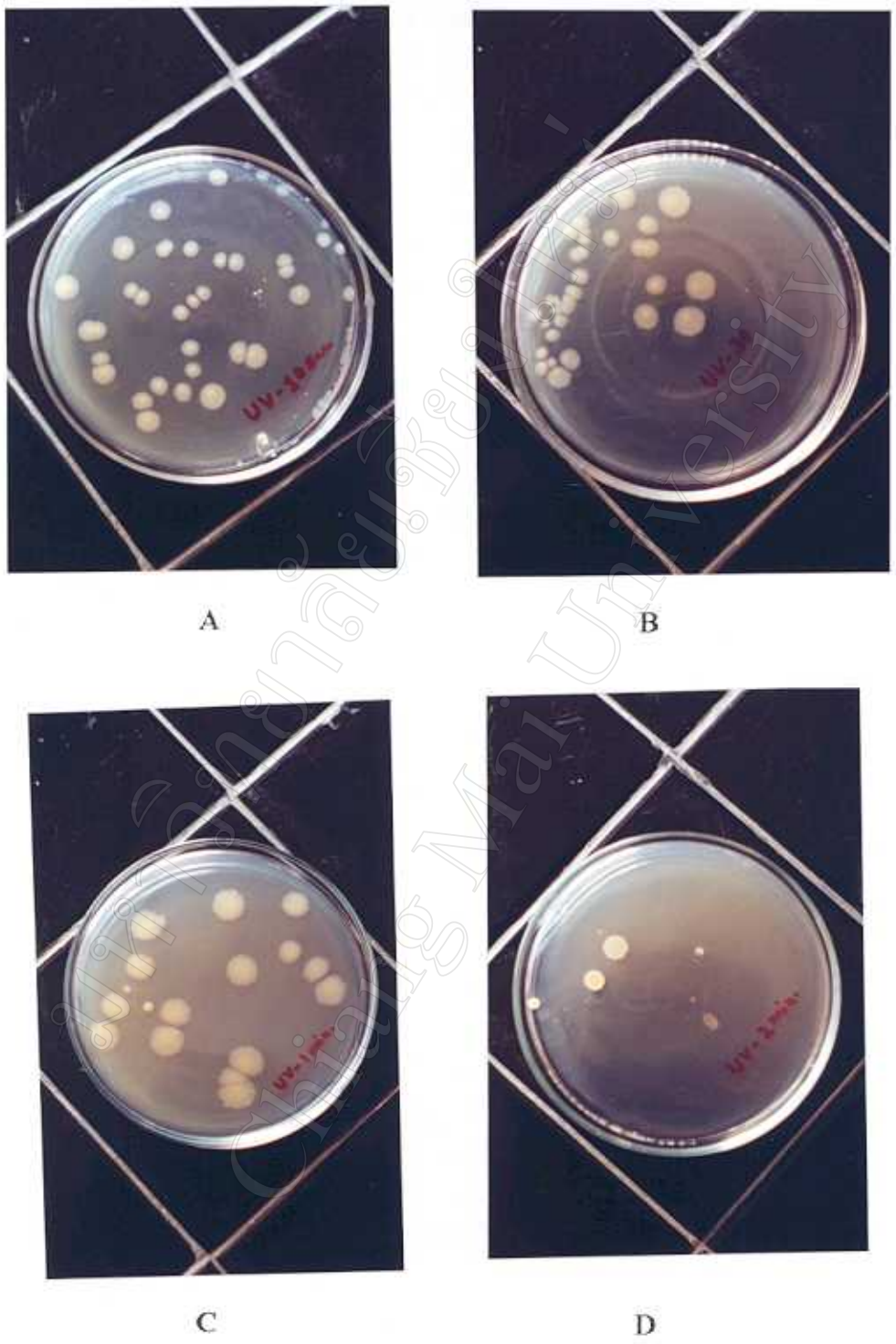


Figure 12. A: bacterial strain with UV-10 seconds, B: bacterial strain with UV-30 seconds, C: bacterial strain with UV-60 seconds, and D: bacterial strain with UV-120 seconds.

Table 1. Carbaryl degradation with UV-60 seconds exposed bacteria

| Sample/Mutant | Bacterial growth ($A_{600}$) | | Remaining carbaryl (ppm) | |
|---|---|---|---|---|
| | 0 hour | 48 hours | 0 hour | 48 hours |
| Blank1 | 0.778 | 0.803 | 10.0 | 7.40 |
| Blank2 | 0.763 | 0.792 | 10.0 | 7.30 |
| Normal1 | 0.803 | 0.810 | 10.0 | 7.40 |
| Normal2 | 0.799 | 0.803 | 10.0 | 7.13 |
| U1 | 0.793 | 0.815 | 10.0 | 6.99 |
| U2 | 0.803 | 0.807 | 10.0 | 7.07 |
| **U3** | **0.849** | **0.840** | **10.0** | **5.96** |
| U4 | 0.784 | 0.788 | 10.0 | 6.74 |
| U5 | 0.806 | 0.837 | 10.0 | 7.05 |
| U6 | 0.797 | 0.799 | 10.0 | 7.17 |
| U7 | 0.809 | 0.815 | 10.0 | 7.07 |
| U8 | 0.806 | 0.820 | 10.0 | 7.17 |
| U9 | 0.713 | 0.700 | 10.0 | 7.13 |
| U10 | 0.766 | 0.782 | 10.0 | 7.22 |

**Table 1. Continued**

| Sample/Mutant | Bacterial growth ($A_{600}$) | | Remaining carbaryl (ppm) | |
|---|---|---|---|---|
| | 0 hour | 48 hours | 0 hour | 48 hours |
| Blank3 | 0.746 | 0.760 | 10.0 | 7.21 |
| Normal3 | 0.774 | 0.788 | 10.0 | 7.05 |
| U11 | 0.776 | 0.782 | 10.0 | 7.03 |
| U12 | 0.766 | 0.778 | 10.0 | 6.99 |
| U13 | 0.737 | 0.747 | 10.0 | 6.93 |
| U14 | 0.747 | 0.782 | 10.0 | 6.99 |
| U15 | 0.731 | 0.767 | 10.0 | 6.89 |
| U16 | 0.755 | 0.776 | 10.0 | 6.89 |
| U17 | 0.770 | 0.770 | 10.0 | 7.09 |
| U18 | 0.765 | 0.776 | 10.0 | 7.13 |
| U19 | 0.777 | 0.795 | 10.0 | 7.17 |
| U20 | 0.741 | 0.767 | 10.0 | 6.99 |
| U21 | 0.818 | 0.805 | 10.0 | 7.03 |
| U22 | 0.740 | 0.748 | 10.0 | 7.17 |
| U23 | 0.779 | 0.783 | 10.0 | 7.11 |

**Table 1. Continued**

| Sample/Mutant | Bacterial growth($A_{600}$) | | Remaining carbaryl (ppm) | |
|---|---|---|---|---|
| | 0 hour | 48 hours | 0 hour | 48 hours |
| Blank4 | 0.766 | 0.761 | 10.0 | 7.02 |
| Normal4 | 0.807 | 0.843 | 10.0 | 6.58 |
| U25 | 0.796 | 0.821 | 10.0 | 7.78 |
| U26 | 0.771 | 0.791 | 10.0 | 6.74 |
| U27 | 0.787 | 0.829 | 10.0 | 6.68 |
| U28 | 0.800 | 0.819 | 10.0 | 6.98 |
| U29 | 0.787 | 0.815 | 10.0 | 6.86 |
| U30 | 0.776 | 0.805 | 10.0 | 6.52 |
| U31 | 0.787 | 0.817 | 10.0 | 6.92 |
| U32 | 0.802 | 0.809 | 10.0 | 6.64 |
| U33 | 0.781 | 0.829 | 10.0 | 6.72 |
| U34 | 0.732 | 0.777 | 10.0 | 6.78 |

### 3.5. Bacterial Identification

The results of different identification tests are presented in Tables 2 and 3.

Table 2. Results of different identification tests of selected bacterial strain of isolate-5

| Tests | Results |
|---|---|
| Cell shape | Short rod |
| Gram staining | -ve |
| Oxidase | +ve |
| Fluorescent pigment production | +ve |
| Citrate | +ve |
| MacConkey | +ve |
| Urea | -ve |
| Gelatin liquefaction | +ve |
| Motility | +ve |
| Nitrate reduction | +ve |
| Flagella | more than one |
| Phenylalanine deaminase | -ve |

**Table 3. Acid and gas production test with different sugars**

| Sugars | Acid production | Gas production |
|---|---|---|
| Glucose | -ve | -ve |
| Sucrose | -ve | -ve |
| Arabinose | -ve | -ve |
| Lactose | -ve | -ve |
| Rhamnose | -ve | -ve |
| Inositol | -ve | -ve |
| Xylose | -ve | -ve |

Comparing all of these results of identification tests with the characteristics of *Pseudomonas* bacteria of Bergey's Manual it was found that the selected bacterial strain of this study was belonged to the *Pseudomonas fluorescens*.

### 3.6. DNA Preparation and Isolation

The DNA was isolated from the selected bacterial strain of isolate-5. This DNA was analysed by gel electrophoresis (Figure 13).

Figure 13. DNA band of the selected bacterial strain

### 3.7. Confirmation of carbaryl

Sample S1 and standard of carbaryl (10.0 ppm) were injected at two wavelengths, viz. 220 and 270 nm. The ratio of peak area of sample and standard was the same at these two wavelengths. This proved that the identified peak only for sample not for other compounds. If this peak for others compounds the ratio would not be the same.

Table 4. Data for confirmation analysis of carbaryl

| Sample | Wavelength (nm) | Retention time (min.) | Peak area (μvolt.sec.) | Ratio |
|---|---|---|---|---|
| S1 | 220 | 6.467 | 4230000 | 13.13 |
| | 270 | 6.517 | 322000 | |
| Std. 10 | 220 | 6.458 | 4830000 | 13.13 |
| | 270 | 6.467 | 367000 | |

### 3.8. Recovery analysis of carbaryl

In recovery analysis about 95% of carbaryl was recovered from the MM medium at pH 6.0. This is satisfactory level because there was no extraction done for carbaryl during this research. Direct injection method was followed.

Table 5. Data for recovery analysis of carbaryl

| Sample | Initial concentration of carbaryl, ppm | Carbaryl recovered, ppm | % recovery |
|---|---|---|---|
| S3 | 10.0 | 9.56 | 95.6 |
| S11 | 10.0 | 9.70 | 97.0 |
| S13 | 10.0 | 94.2 | 94.2 |

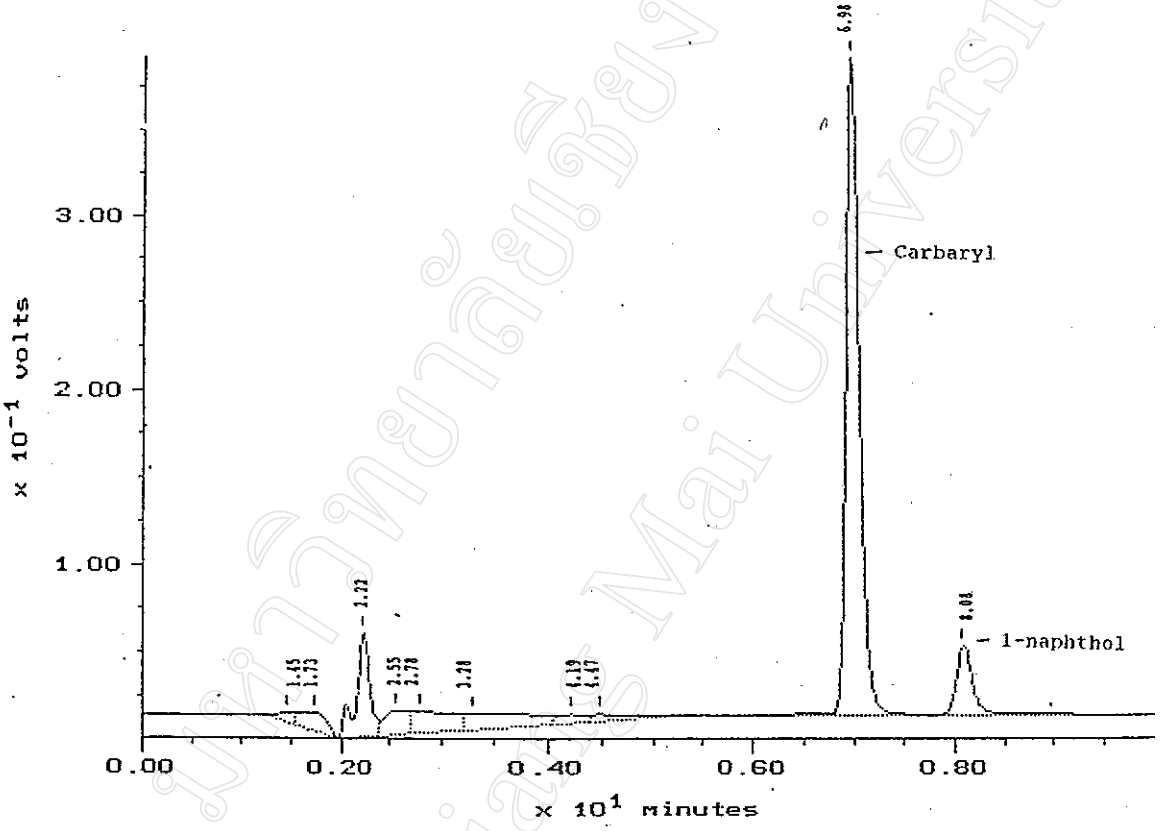


Figure 14. The peak of carbaryl and its intermediate product 1- naphthol