CHAPTER IV

RESULTS AND DISCUSSION

4.1. Analysis of MT by HPLC

MT peak appeared at a retention time of 17.6 min using acetonitrite and water as a mobile phase. Signal/noise (S/N) ratio was calculated by Chemstation software. The limit of detection (LOD) and limit of quantification (LOQ) were 0.008 and 0.030 mg/l for S/N ratios of 3.0 and 10.0, respectively. Percent recovery and percent relative standard deviation (RSD) of MT in methanol, MilliQ® water (18.2 Ω), and IS and Basal medium were analyzed by using solutions with known concentrations of MT. The percent recovery was used to represent the accuracy of the method, and the percent RSD was used to represent the precision of the method. The accuracy and the precision of MT in the solutions ranged from 94 to 104 % and 2.9 to 4.5 %, respectively, except for the IS and Basal medium. In the IS medium, the percent recovery was lower than this range which may be due to sorption of MT onto the surfaces of suspended particles. In order to solve this problem, an equal volume of methanol to the volume in the vial was added before analysis by HPLC.

4.2. Biodegradation of MT by Microorganisms from Wastewater Treatment systems and Sediment under Aerobic and Anaerobic Conditions

4.2.1. Aerobic Biodegradation of MT Using Aerobic Sludge

The results for the aerobic biodegradation tests for the five different initial MT concentrations of 0.3, 1.0, 5.0, 7.0 and 10 mg/l are presented in Figures 4.1 to Figure 4.5. The results showed that MT depleted to close to zero concentration between 2 to 20 days depending on the initial concentration. MT concentrations in the recovery and control tests remained the same throughout the experiment indicating minimum sorption or chemical degradation. There was no lag phase in all cases suggesting that microorganisms in aerobic sludge did not need time to acclimatize to MT. First-order degradation rate constants and half lives were estimated (Table 4.1). First-order

degradation rate constants were found to decrease with increasing initial MT concentrations ranging from 1.43±0.12 day^{-1} for 0.3 mg/l of MT to 0.36±0.17 day^{-1} for an initial MT concentration of 10.0 mg/l. The results showed that microorganisms in the aerobic sludge have higher activities at lower initial MT concentrations than at higher initial MT concentrations. Half lives of MT were estimated to be between 0.8 and 6.50 days for initial MT concentrations of 0.3 and 10.0 mg/l, respectively. (Table 4.1)

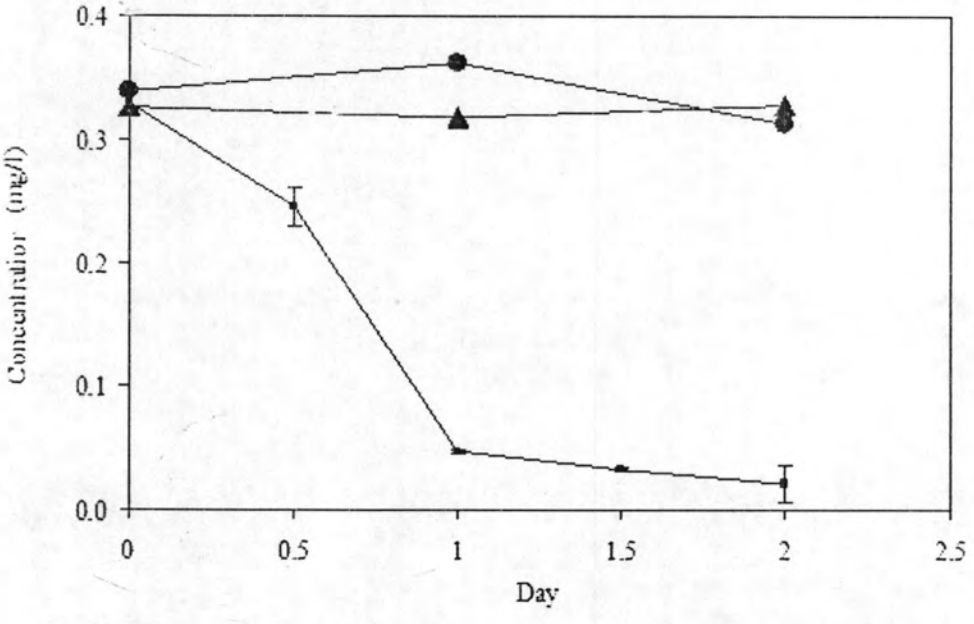

Figure 4.1. Aerobic degradation of MT using aerobic sludge at an initial MT concentration of 0.3 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests.

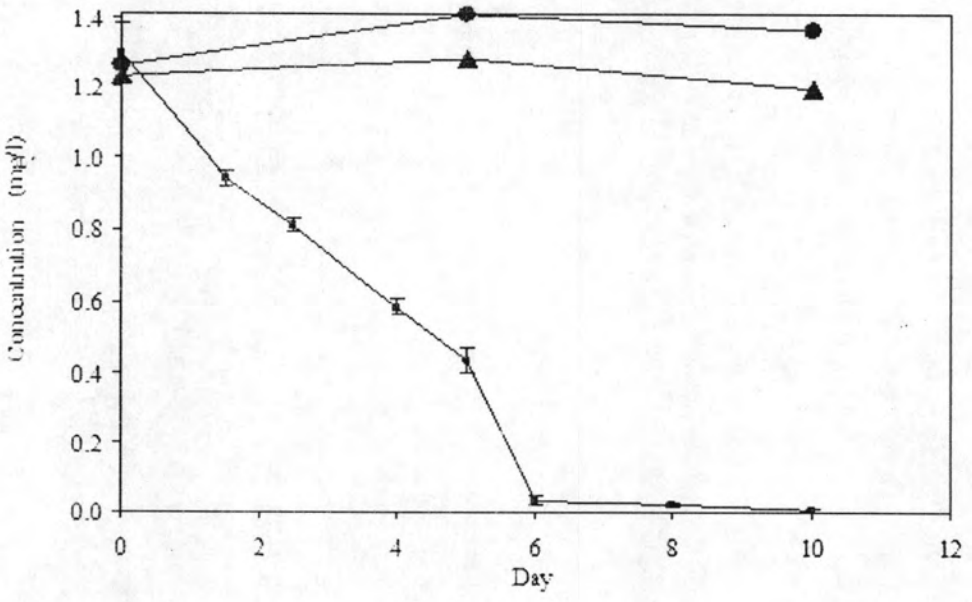

Figure 4.2. Aerobic degradation of MT using aerobic sludge at an initial MT concentration of 1.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

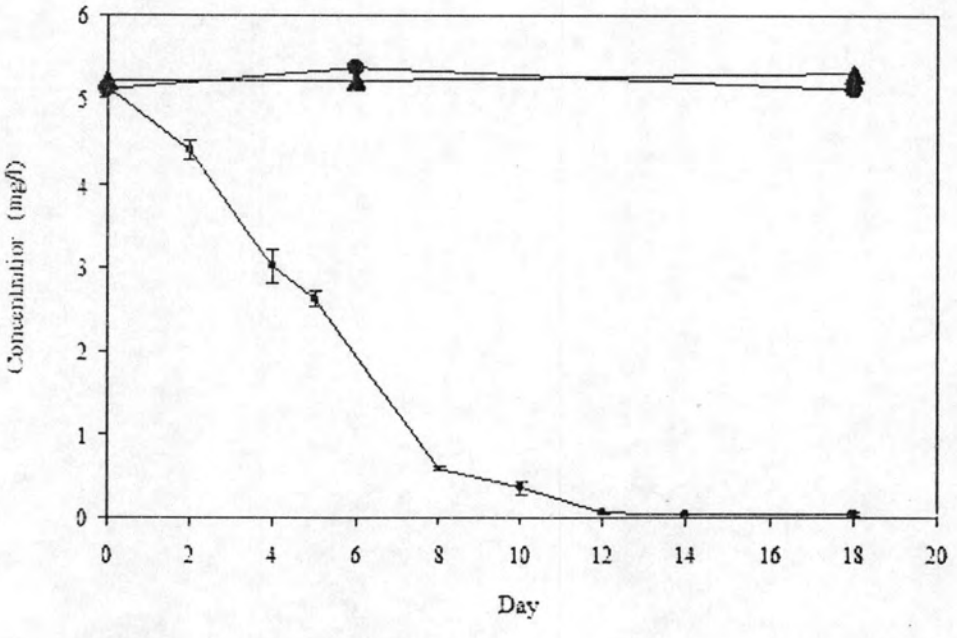

Figure 4.3. Aerobic degradation of MT using aerobic sludge at an initial MT concentration of 5.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) test

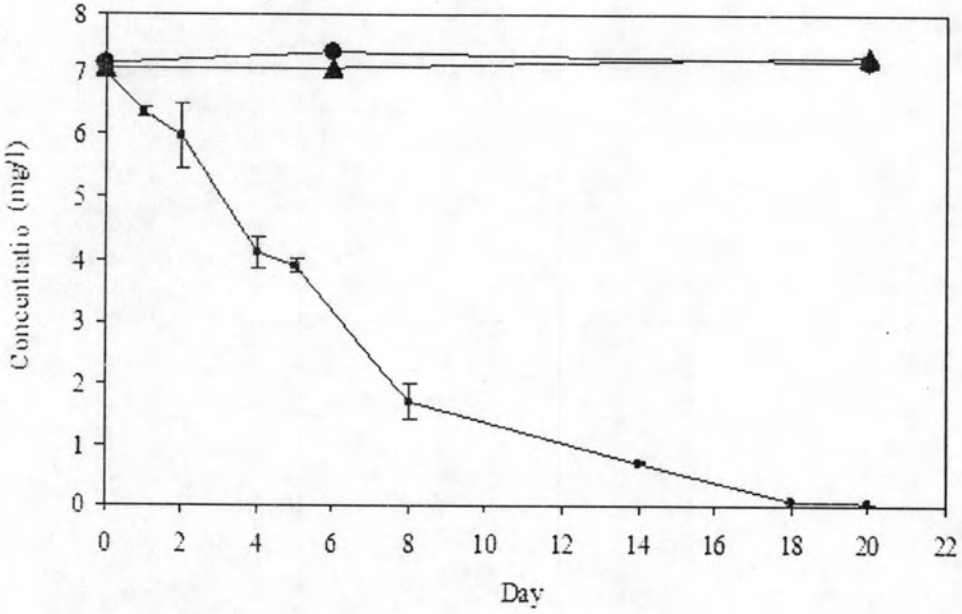

Figure 4.4. Aerobic degradation of MT using activated sludge at an initial MT concentration of 7.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

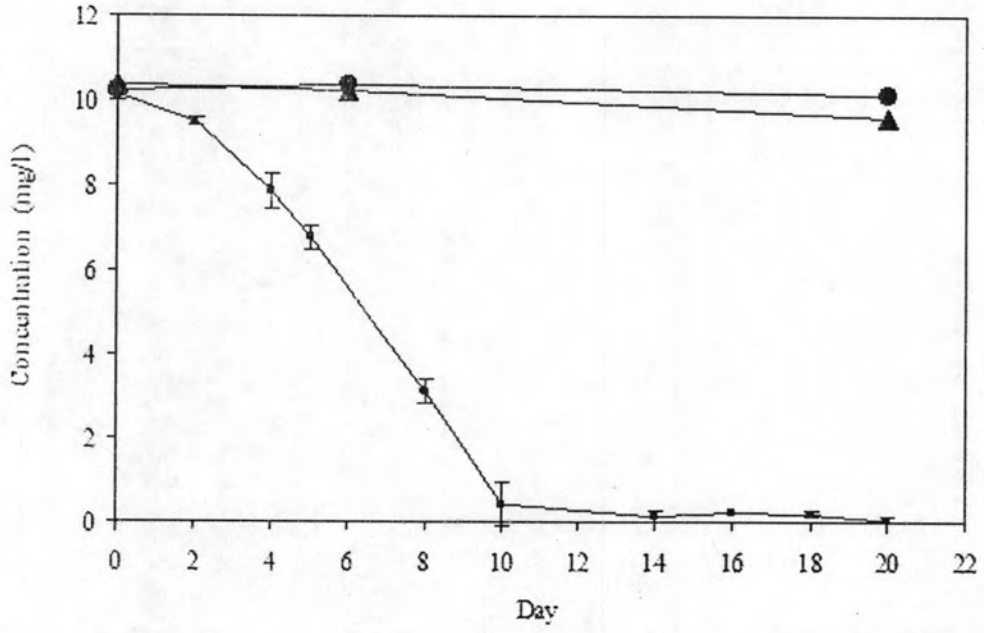

Figure 4.5. Aerobic degradation of MT using aerobic sludge at an initial MT concentration of 10.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

Table 4.1. First-order degradation rate constants (k) and half lives ($t_{1/2}$) of MT using aerobic sludge under aerobic conditions.

Initial MT concentration (mg/l)	k (day $^{-1}$)	t ½ (day)
0.3	1.43±0.12	0.8
1.0	0.49±0.04	4.0
5.0	0.37±0.01	5.5
7.0	0.24±0.01	5.8
10.0	0.36±0.17	6.5

Although, the results suggested that MT is biodegradable, previous study show that very low MT concentrations in a range of 2.8 – 2.9 ng/g were left over in the sediment nearly three months after cessation of treatment (Fitzpatrick et al., 2000).

The retention behavior of MT in C-18 column has been reported to depend greatly on the organic modifier of the mobile phase, column temperature and type of reversed-phase material. Using this method, MT was separated in an end-capped RP C-18 column at ambient temperature using acetonitrile–water gradient. The separation of the substances is presented in Figure 4.6. MT was identified based on its retention time of 17.5 min. With all experiments an intermediate metabolite appeared at a retention time of 16.1 min. The MT peak areas and metabolite peak areas were in similar proportion with respect to the initial concentrations suggesting that this intermediate metabolite came from MT. As shown in Figure 4.6, the intermediate metabolite of MT was released earlier than the MT. This result suggests that metabolite has more polarity than the parental MT. Possible metabolites with more polarity than MT are 17α-methyl-5α-androstan-3α, 17β-diol, 17α-methyl-5β-androstan-3α, 17β-diol, 17α-methyl-5α-androstane-3β,17β-diol and 17α-methyl-5β-androstane-3β, 17β-diol, which are products of hydroxylation of the parent compound and, 17α-methyl-5x-androstane-3x,16x,17β-triol (x = α or β), which a minor oxygenated metabolite of the parent compound.

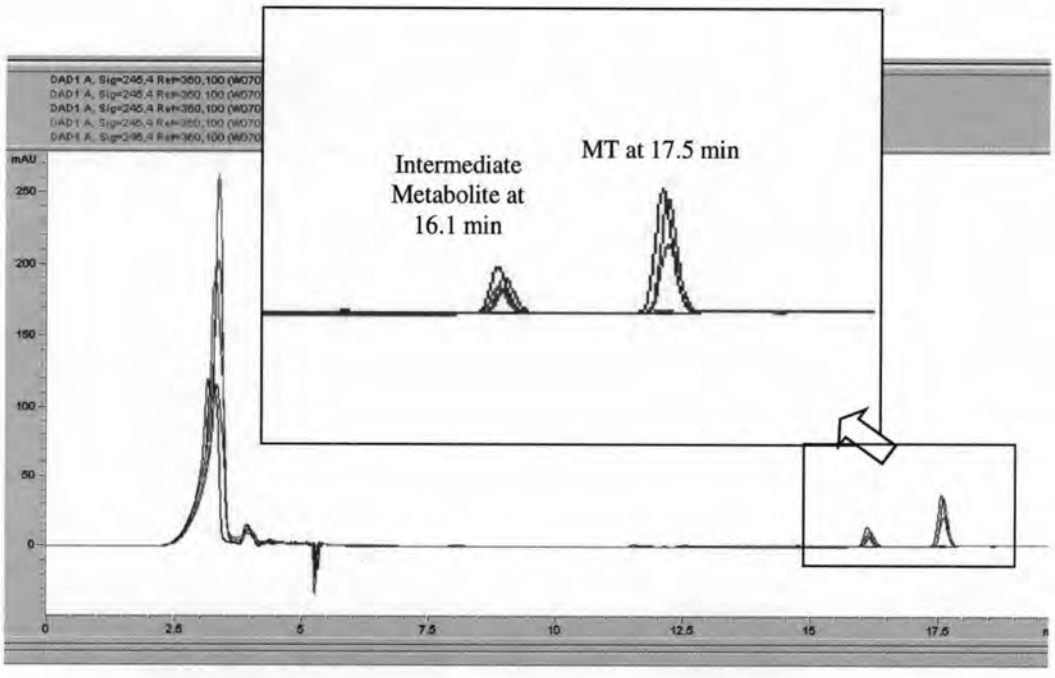

Figure 4.6. HPLC chromatogram of MT and its metabolite during aerobic biodegradation of MT using aerobic sludge

4.2.2. Aerobic Biodegradation of MT Using Sediment

Results of the test using sediment for five different initial MT concentrations of 0.3, 1.0, 5.0, 7.0 and 10.0 mg/l are presented in Figure 4.7 to 4.11. MT was shown to deplete to close to zero concentrations between 5 to 32 days depending on the initial concentration. MT concentrations in the recovery and control tests remained the same throughout the experiment. There were no lag phase in all cases suggesting that microorganisms in the sediment did not need time to acclimatize to A probable reason is that the sediment was collected from the masculinizing pond of Nile tilapia fry which have been exposed to MT-impregnated food. Degradation rate constants and half lives were estimated as in Table 4.2. First-order degradation rate constants ranged from 0.52 ± 0.02 day^{-1} for an initial MT concentration of 0.3 mg/l to 0.10 ± 0.01 day^{-1} for an initial MT concentration of 10.0 mg/l. Half lives were estimated to be between 2.4 and 12.8 days. These results showed that the degradation of MT decreased with increasing initial MT concentrations as in aerobic sludge degradation tests. This indicates that microorganisms in the sediment have higher activities at lower initial MT concentrations

than higher initial MT concentrations. The only study related to this showed that between 2.8 and 2.9 ng/g of MT were found to remain in soils nearly three months after cessation of treatment (Fitzpatrick et al, 2000).

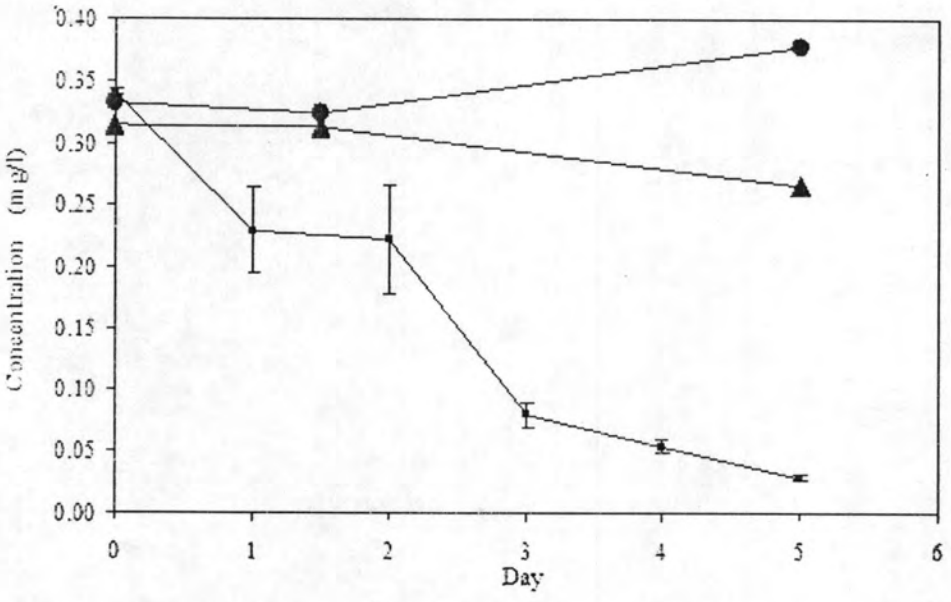

Figure 4.7. Aerobic degradation of MT using sediment at an initial MT concentration of 0.3 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

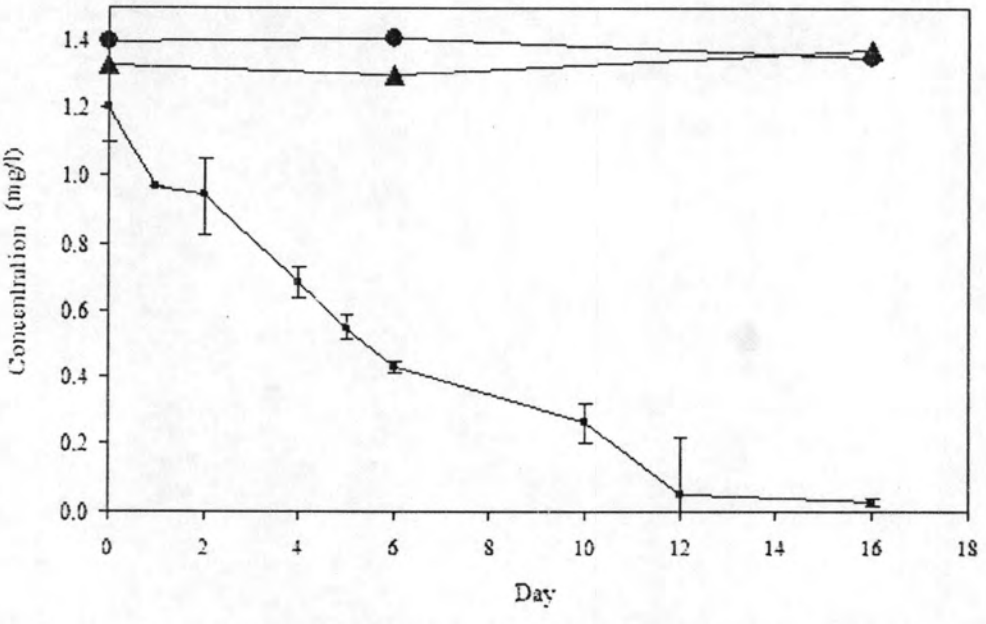

Figure 4.8. Aerobic degradation of MT using sediment at an initial MT concentration of 1.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

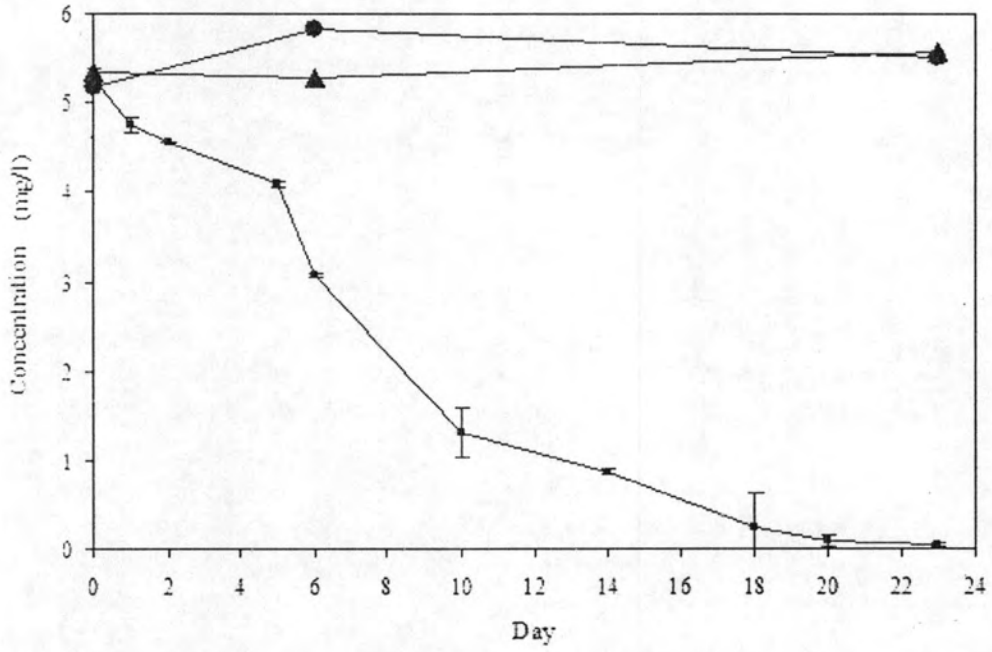

Figure 4.9. Aerobic degradation of MT using sediment at an initial MT concentration of 5.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

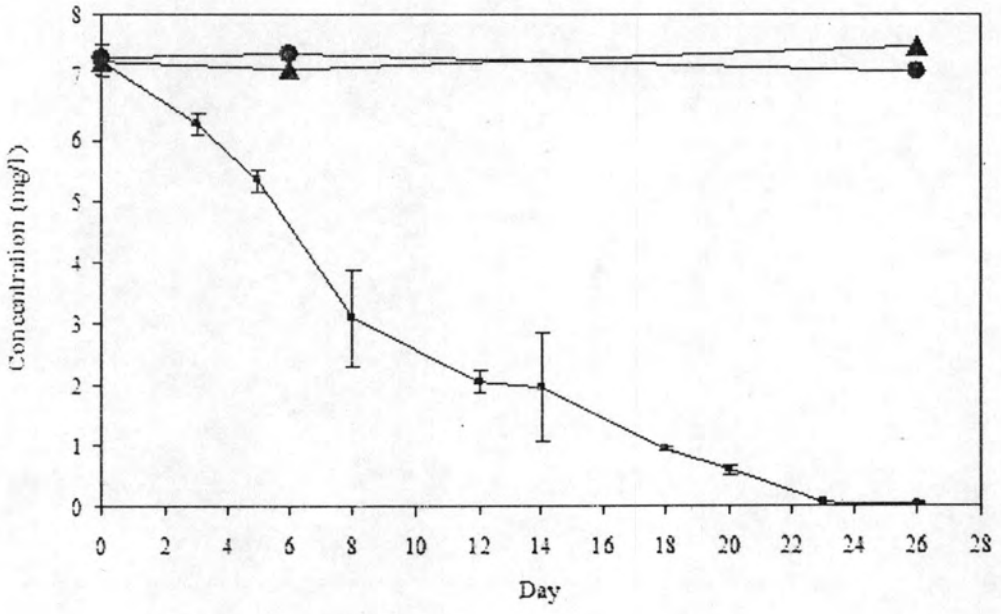

Figure 4.10. Aerobic degradation of MT using sediment at an initial MT concentration of 7.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

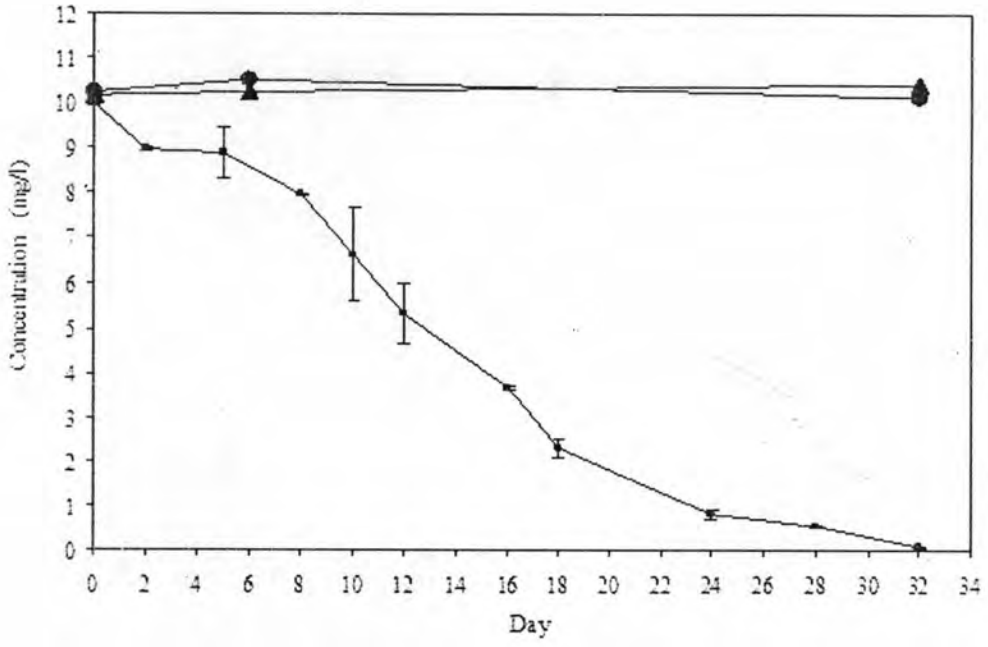

Figure 4.11. Aerobic degradation of MT using sediment at an initial MT concentration of 10.0 mg/l/: Biodegradation (▪), recovery (▲), and control (●) tests

Table 4.2. First-order degradation rate constants (k) and half lives ($t_{1/2}$) of MT using sediment under aerobic conditions

Initial MT concentration (mg/l)	k (day^{-1})	$t_{1/2}$ (day)
0.3	0.52±0.02	2.4
1.0	0.23±0.04	4.5
5.0	0.17±0.02	7.0
7.0	0.13±0.01	7.5
10.0	0.10±0.01	12.8

Separation of MT and its metabolite is presented in Figure 4.12. MT has a retention time of 17.5 min while a metabolite appeared at retention time of 16.10 min. This is the same as the aerobic sludge tests suggesting that the intermediate metabolite in the aerobic sludge and sediment degradation tests were the same compound. As mentioned earlier, possible metabolites with more polarity than MT include 17α-methyl-5α-androstan-3α, 17β-diol, 17α-methyl-5β-androstan-3α, 17β-diol, 17α-methyl-5α-

androstane-3β,17β-diol, 17α-methyl-5β-androstane-3β,17β-diol, and, 17α-methyl-5x-androstane-3x,16x,17β-triol (x = α or β).

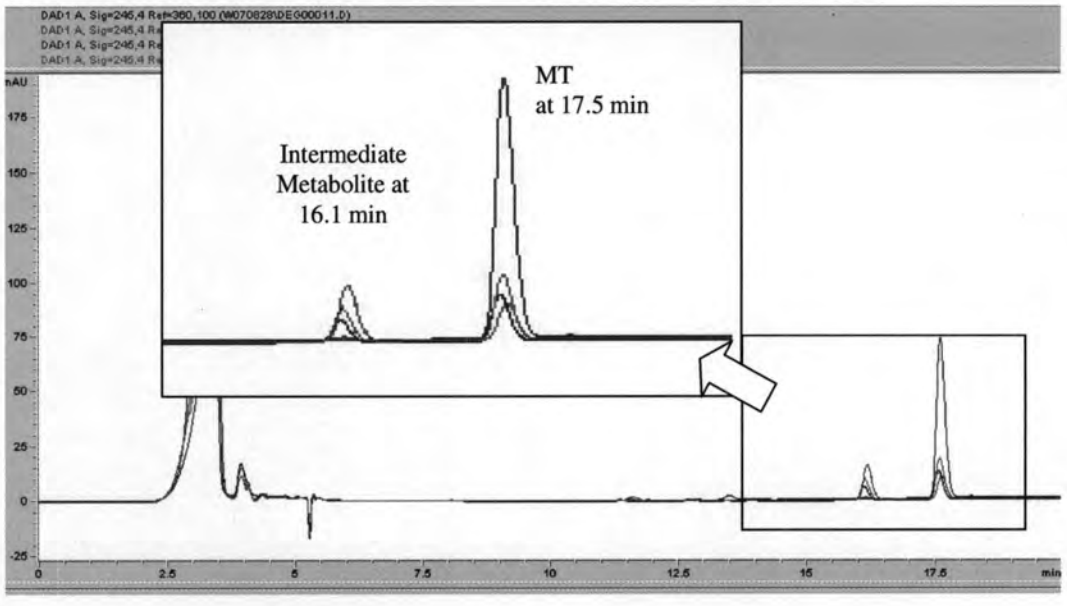

Figure 4.12 HPLC chromatogram of MT and its metabolite during aerobic biodegradation of MT using sediment

4.2.3. **Anaerobic Biodegradation of MT Using Anaerobic Sludge**

Anaerobic degradation tests for initial MT concentrations of 0.1, 1.0, 3.0, 5.0, and 10.0 mg/l are presented in Figures 4.13 to 4.17. Concentrations of MT in biodegradation tests decreased to close to zero within 15 to 35 days whereas those in the recovery and control tests remained the same throughout the experiment. There was no lag phase in all cases suggesting that microorganisms in anaerobic sludge did not need time to acclimatize to MT. First-order degradation rate constants were estimated to be 0.19±0.01 day^{-1} for an initial MT concentration of 0.1 mg/l increasing to 0.28±0.03 day^{-1} at 3.0 mg/l and then decreasing to 0.1±0.01 day^{-1} at 10 mg/l. Half lives were 4.5 and 12.0 days for initial MT concentrations of 0.1 and 10 mg/l, respectively as showed in Table 4.3. These results suggested that the degradation rate constants increased for MT concentration from 0.1 to 3.0 mg/l. but decreased for MT concentration higher than 5 mg/l.

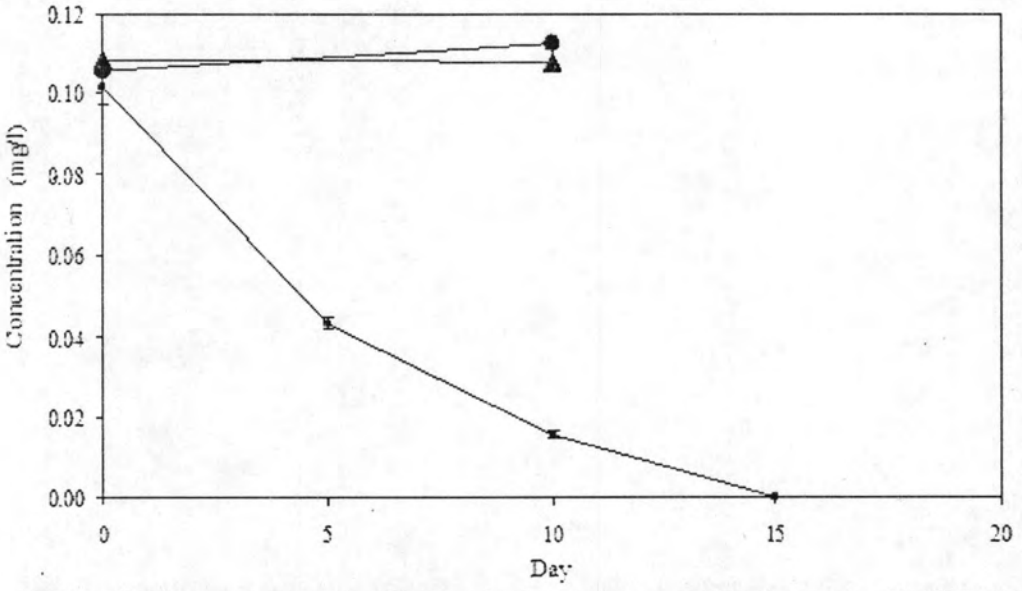

Figure 4.13. Anaerobic degradation of MT using anaerobic sludge at an initial MT concentration of 0.1 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

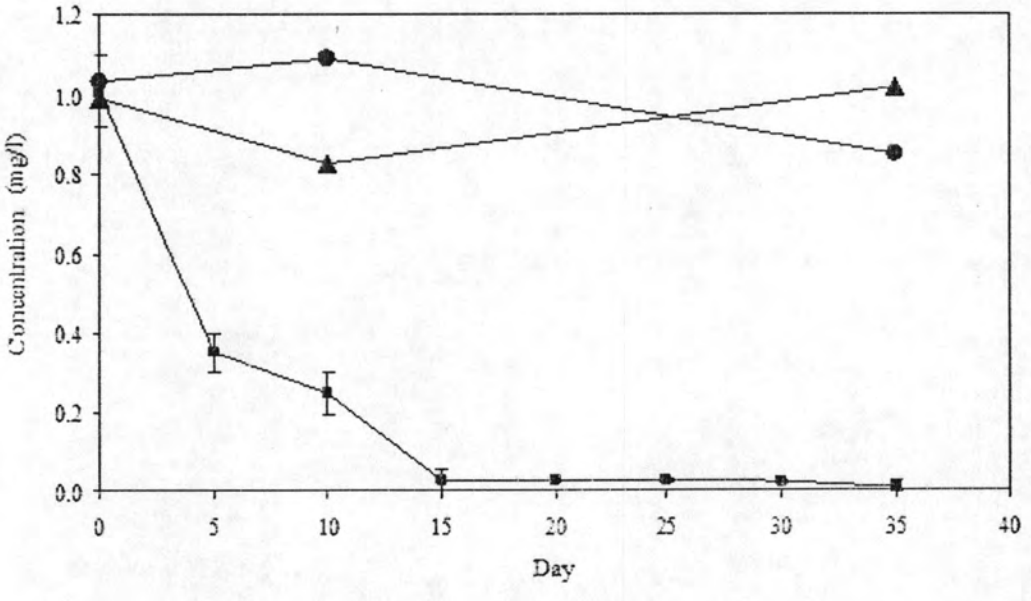

Figure 4.14. Anaerobic degradation of MT using anaerobic sludge at an initial MT concentration of 1.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

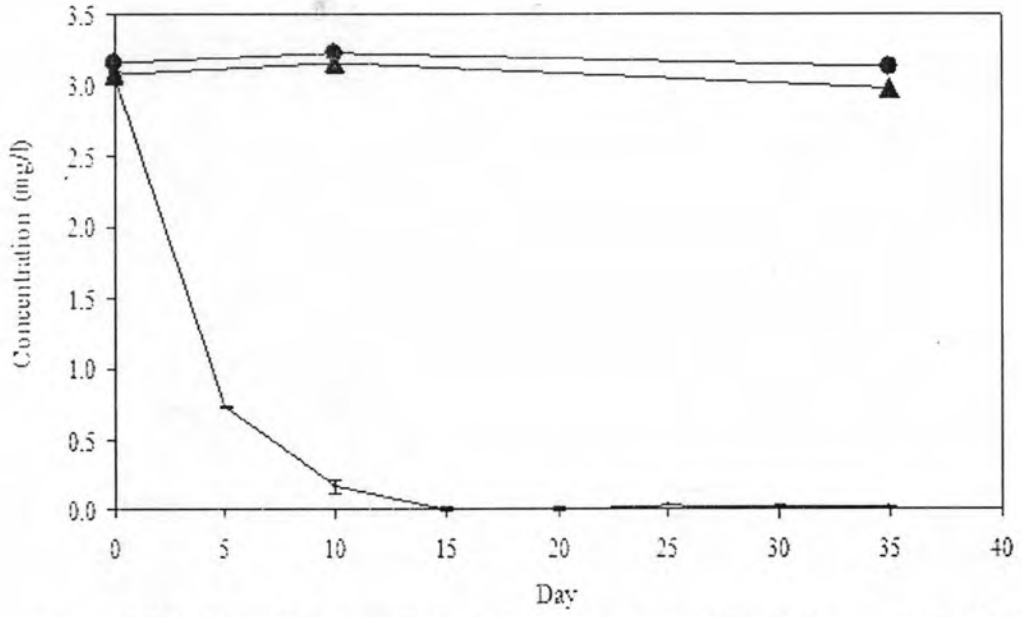

Figure 4.15. Anaerobic degradation of MT using anaerobic sludge at an initial MT concentration of 3.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

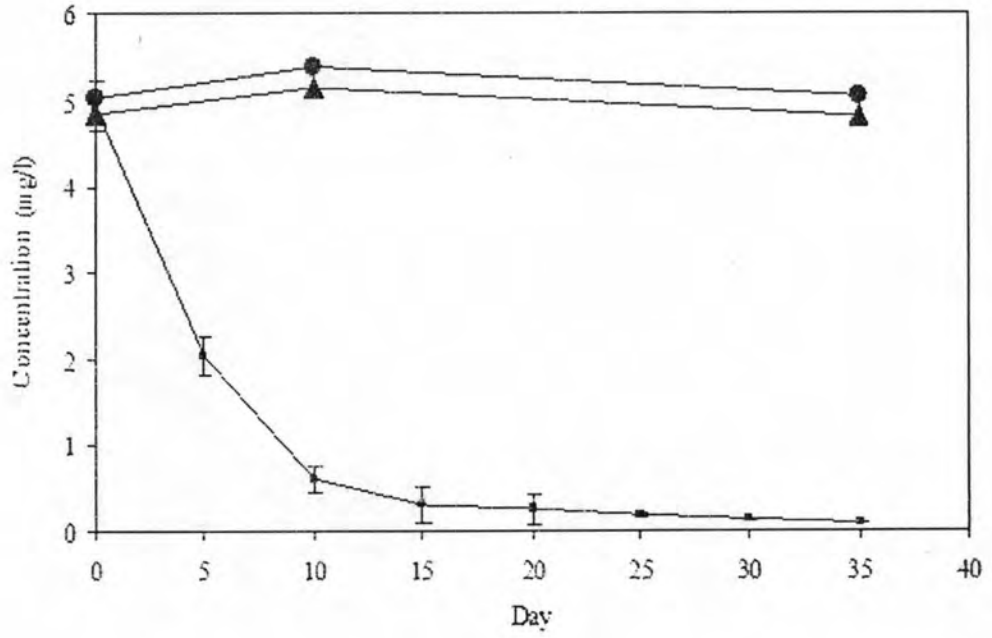

Figure 4.16. Anaerobic degradation of MT using anaerobic sludge at an initial MT concentration of 5.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

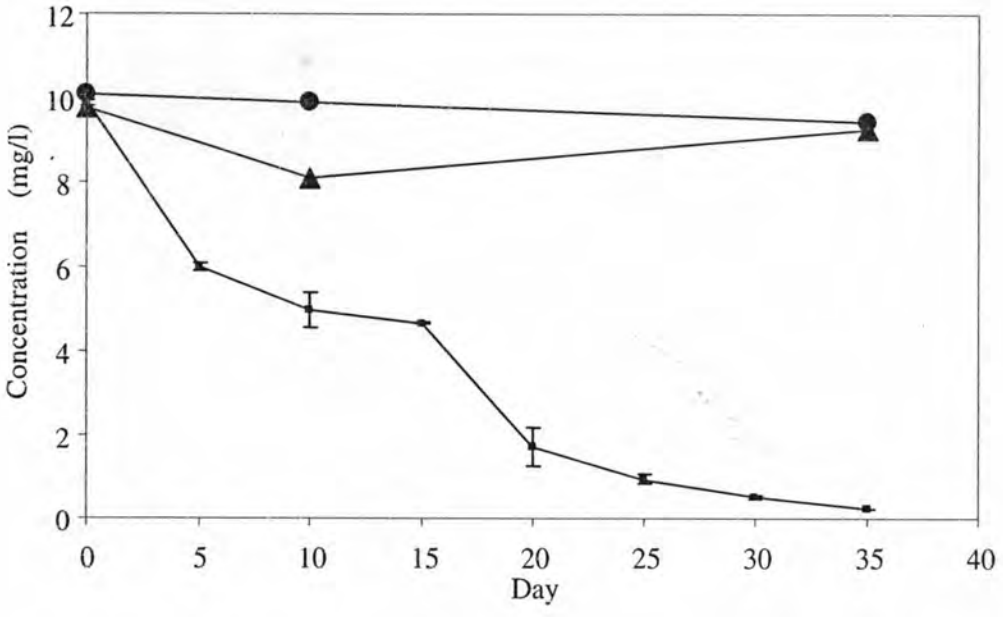

Figure 4.17. Anaerobic degradation of MT using anaerobic sludge at an initial MT concentration of 10.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

Table 4.3. First-order degradation rate constants (k) and half lives ($t_{1/2}$) of MT using anaerobic sludge under anaerobic conditions

Initial MT concentration (mg/l)	k (day^{-1})	$t_{1/2}$ (day)
0.1	0.19±0.01	4.5
1.0	0.21±0.01	4.0
3.0	0.28±0.03	3.5
5.0	0.19±0.01	4.5
10.0	0.10±0.01	12.0

Although, the results suggested that MT is biodegradable, for high initial MT concentrations, the microorganisms need more time to be degrade MT. Moreover, in all cases, a residual concentration of MT of 0.02 mg/l remained even for experiments up to 30 days. These results indicated that microorganisms in anaerobic sludge were unable to degrade very low concentrations of MT.

Three intermediate metabolites were found to appear at retention times of 12.1, 13.4 and 15.7 min as shown in Figure 4.18, suggesting that the degradation pathway was possibly different from that of aerobic degradation. All these three intermediate metabolites found to be more polar than the parental MT. As before possible metabolites with higher polarity include 17α-methyl-5α-androstan-3α, 17β-diol, 17α-methyl-5β-androstan-3α, 17β-diol., 17α-methyl-5α-androstane-3β,17β-diol, 17α-methyl-5β-androstane-3β,17β-diol and, 17α-methyl-5x-androstane-3x,16x,17β-triol (x = α or β).

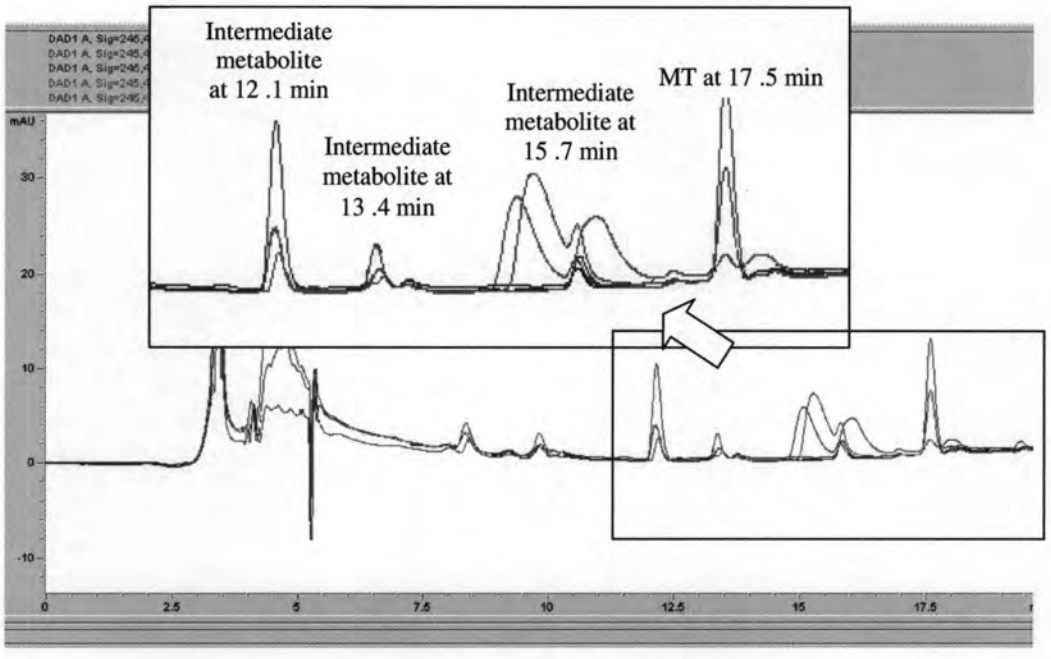

Figure 4.18. HPLC chromatogram of MT and its metabolites during anaerobic biodegradation of MT using anaerobic sludge

4.2.4. **Anaerobic Biodegradation of MT Using Sediment**

Anaerobic degradation tests using sediment as five different initial MT concentrations of 0.1, 1.0, 3.0, 5.0, and 10.0 mg/l are presented in Figures 4.19 to 4.23. MT concentrations in the recovery and control tests remained the same throughout the experiment. There was no lag phase in all cases suggesting that microorganisms in sediment did not need time to acclimatize to MT. This may be due to the fact that the sediment was exposed to MT-impregnated food. Degradation rate constants and half lives were estimated as in Table 4.4. All degradation rate constants obeyed the first-order

reaction. First-order degradation rate constants were 0.09±0.22, 0.34±0.0, 0.33±0.0, 0.23±0.08 and 0.09±0.0 day^{-1} and half lives were 4.00, 3.00, 3.00, 2.80 and 9.00 days for initial MT concentrations of 0.1, 1.0, 3.0, 5.0, and 10.0 mg/l, respectively. The degradation rate constant increased with increasing initial MT concentration but decreased for initial concentration of more than 5 mg/l. Microorganisms can response in the degradation of MT at only low concentration rather than high concentration. Moreover, in all cases a residual MT concentration of 0.02 mg/l remained even for experimentals of more than 30 days. These results indicated that microorganisms in anaerobic sludge were not able to degrade MT at very low concentrations.

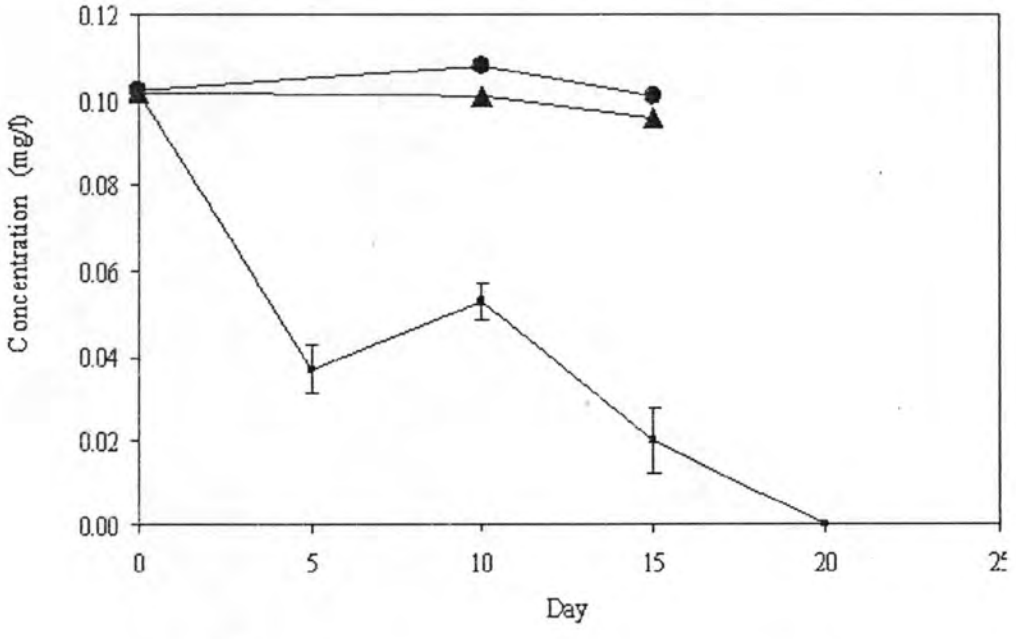

Figure 4.19. Anaerobic degradation of MT using sediment at an initial MT concentration of 0.1 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

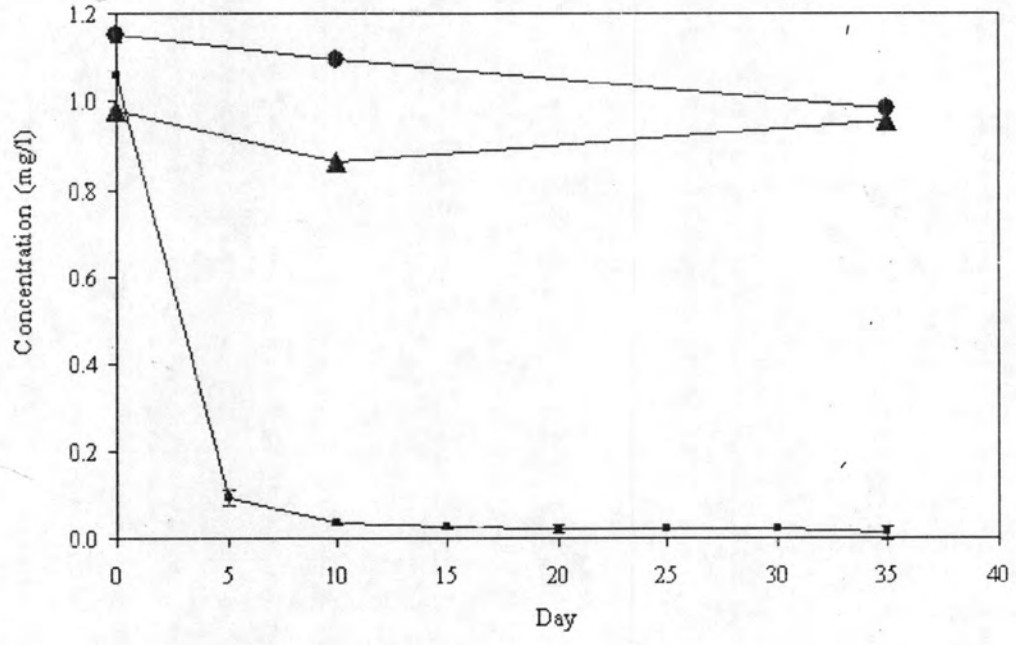

Figure 4.20. Anaerobic degradation of MT using sediment at an initial MT concentration of 1.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

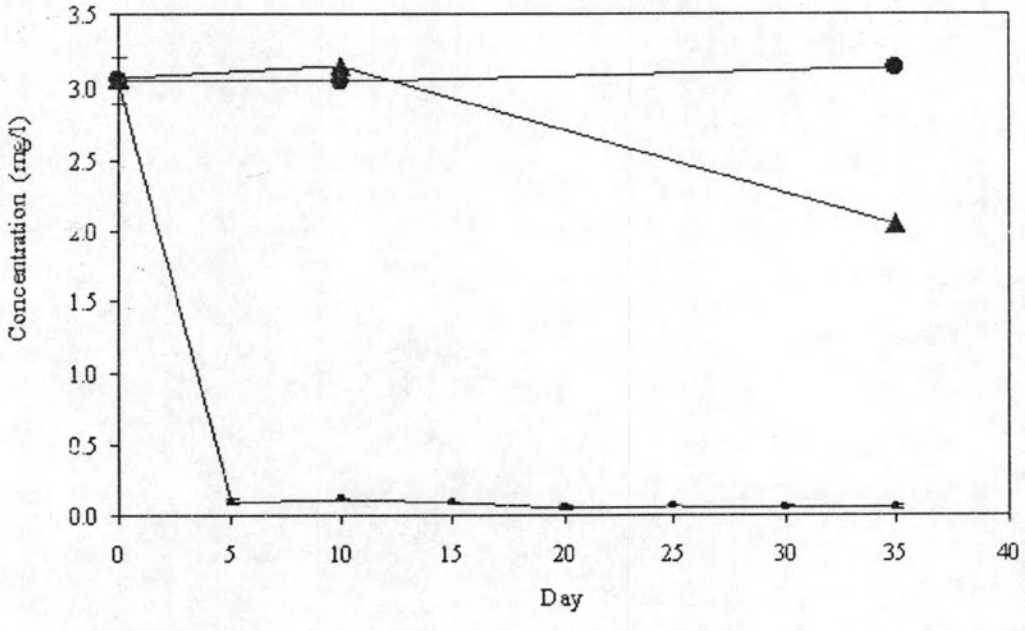

Figure 4.21. Anaerobic degradation of MT using sediment at an initial MT concentration of 3.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

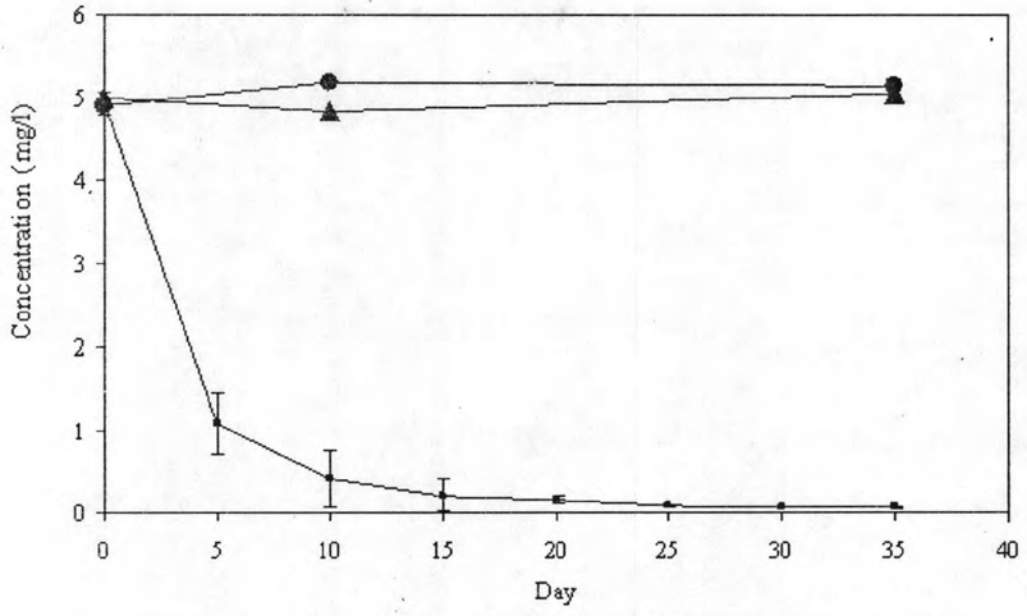

Figure 4.22. Anaerobic degradation of MT using sediment at an initial MT concentration of 5.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

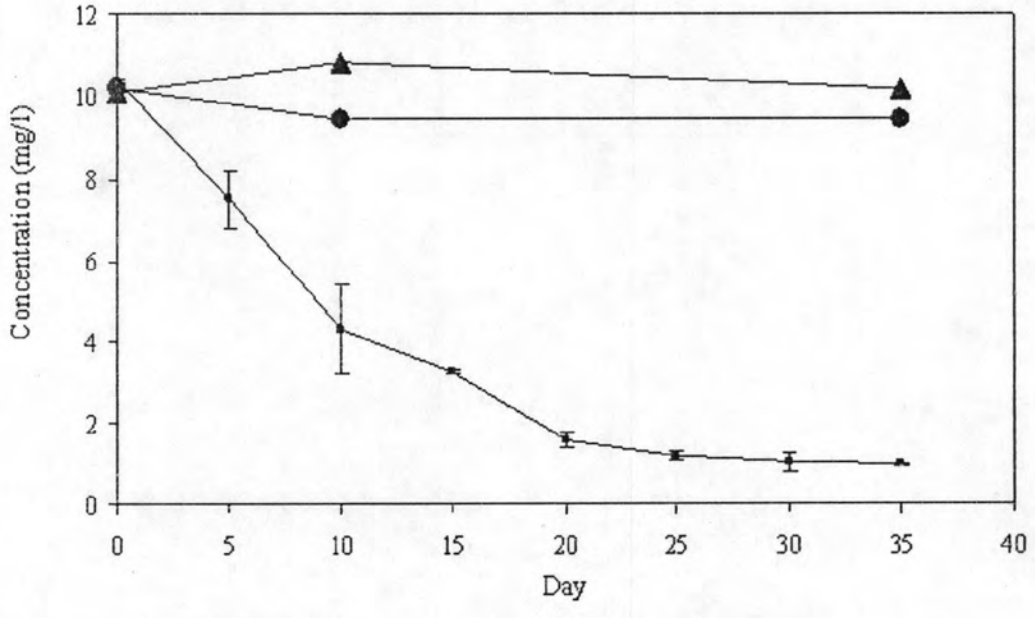

Figure 4.23. Anaerobic degradation of MT using sediment at an initial MT concentration of 10.0 mg/l: Biodegradation (▪), recovery (▲), and control (●) tests

Table 4.4. First-order degradation rate constants (k) and half lives ($t_{1/2}$) of MT using sediment under anaerobic conditions

Initial MT concentration (mg/l)	k (day^{-1})	$t_{1/2}$ (day)
0.1	0.09±0.02	4.0
1.0	0.34±0.01	3.0
3.0	0.33±0.01	3.0
5.0	0.23±0.08	2.8
10.0	0.09±0.01	9.0

Although, the results suggested that MT is biodegradable, at high initial MT concentrations, degradation was slower than low MT concentrations. In this study, MT concentration of about 0.02, 0.055, 0.69 and 0.99 mg/l for initial MT concentration of 1.0, 3.0, 5.0 and 10.0 mg/l, respectively remained in the sediment even though the experimental period was extended for more than 30 days. These results indicated that microorganisms in anaerobic sludge were unable to degrade very low MT concentration in the microgram range.

Three intermediate metabolites were found to appear at retention times of 12.1, 13.4 and 15.7 min suggesting that degradation pathway was possibly different due to the difference in degradation pathway as compared to aerobic degradation. All these three intermediate metabolites were more polar than the parental MT. Possible metabolites with more polarity than MT include 17α-methyl-5α-androstan-3α, 17β-diol, 17α-methyl-5β-androstan-3α, 17β-diol., 17α-methyl-5α-androstane-3β,17β-diol, 17α-methyl-5β-androstane-3β,17β-diol, and, 17α-methyl-5x-androstane-3x,16x,17β-triol (x = α or β).

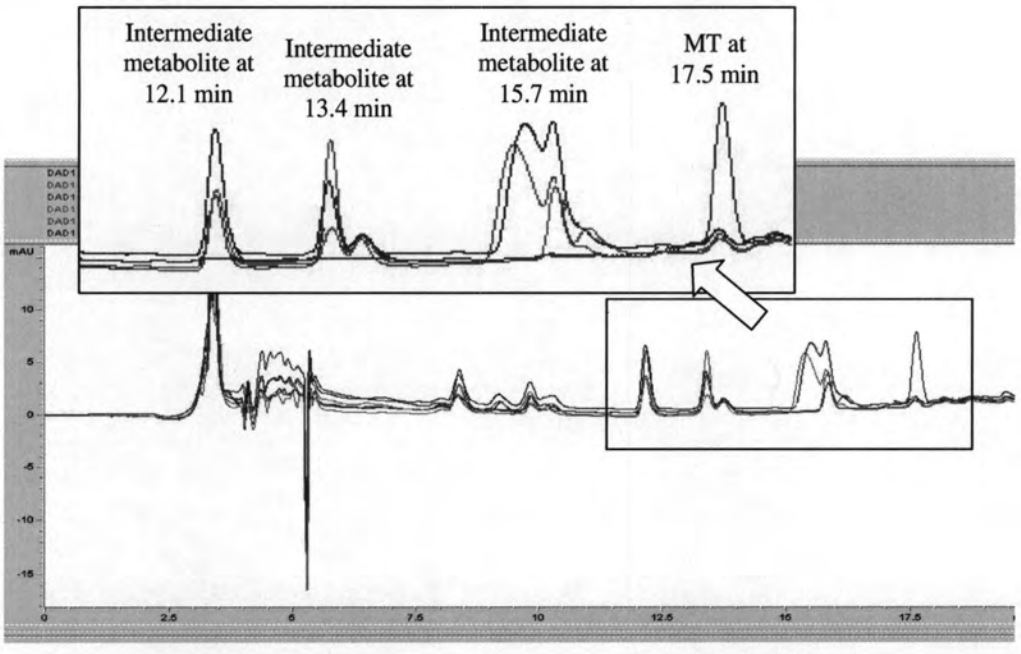

Figure 4.24. HPLC chromatogram of MT and its metabolites during anaerobic biodegradation of MT using sediment

4.3. Isolation of MT-degrading Bacteria from Aerobic Sludge of a Wastewater Treatment System

4.3.1. Pre-screening Isolated MT-degrading Bacteria Using Colony Morphology

Five, three and four different colony types, for the cultures enriched and isolated with initial MT concentrations of 10, 100 and 500 mg/l, respectively, were observed. The results of the different colony morphologies are showed in Tables 4.5 – 4.7

Table 4.5 Colony morphology from cultures enriched and isolated with an initial MT concentration of 10 mg/l

Isolated Name	Colony type	Morphology
MT 1/10	A	Clear in pigment, irregular in form, undulate in margin, flat in elevation, diameter 1 mm
MT 2/10	B	Pink in pigment, circle in form, entire in margin, raised in elevation, diameter 1 mm
MT 3/10	C	Clear in pigment, elliptical in form, entire in margin, flat in elevation, diameter 1 mm
MT 4/10	D	Clear in pigment, 3 elliptical ring in form, entire in margin, flat in elevation, diameter 1.5 mm
MT 5/10	E	Yellow in pigment, circle in form, entire in margin, convex in elevation, diameter 1.5 mm

Table 4.6 Colony morphology from cultures enriched and isolated with an initial MT concentration of 100 mg/l

Isolated Name	Colony type	Morphology
MT 1/100	A	Clear in pigment, irregular in form, undulate in margin, flat in elevation, diameter 0.2 mm
MT 2/100	A	Clear in pigment, irregular in form, undulate in margin, flat in elevation, diameter 1 mm
MT 3/100	C	Clear in pigment, elliptical in form, entire in margin, flat in elevation, diameter 1 mm

Table 4.7 Colony morphology from cultures enriched and isolated with an initial MT concentration of 500 mg/l

Isolated name	Colony type	Morphology
MT 1/500	F	White yellowish in pigment, circle in form, entire in margin, convex in elevation, diameter 1 mm
MT 2/500	G	White in pigment, circle in form, entire in margin, convex in elevation, diameter 0.5 mm
MT 3/500	A	Clear in pigment, irregular in form, undulate in margin, flat in elevation, diameter 1 mm
MT 4/500	B	Pink in pigment, circle in form, entire in margin, raised in elevation, diameter 0.5 mm

4.3.2. Identification of Isolated MT-degrading Bacteria Using 16S rRNA Gene Sequences

Results from the analysis of 16S rRNA gene sequences of isolated MT-degrading bacteria strains MT 3/10, MT 5/10 and MT 1/500 are presented in Table 4.8. The results suggest that isolated MT-degrading bacteria strains MT 3/10 are closely related to *Acidovorax* sp. RCPCd1 (accession number of DQ922761) with 100% identity in the *Acidovorax* genus. Isolated colony MT 1/500 and MT 5/10 were closely related to *Methylophilus leisingeri* strain RCP5 (accession number of DQ922752.1) with 99 % identity in *Methylophilus* genus.

Table 4.8. Closely related neighbor of analyzed sequences from isolated MT- degrading bacteria

Isolate colony	Closely related sequence	Accession No.	Score	Percent identity
MT 3/10	*Acidovorax* sp. RCPCd1	DQ922761.1	425	100
MT 5/10	*Methylophilus leisingeri* strain RCP5	DQ922752.1	625	99
MT 1/500	*Methylophilus leisingeri* strain RCP5	DQ922752.1	440	99

4.3.3. Characterization of MT-degrading Bacteria Strains MT 3/10 and MT 1/500

4.3.3.1. Kinetic Analysis of Isolated MT-degrading Bacteria Strain MT 3/10

Results of aerobic biodegradation using isolated MT-degrading bacteria strains MT 3/10 at different initial MT concentrations of 3.0, 10.0, 15.0 and 115.0 mg/l are shown in Figures 4.25 to 4.28. The first-order degradation rate constants are presented in Table 4.9

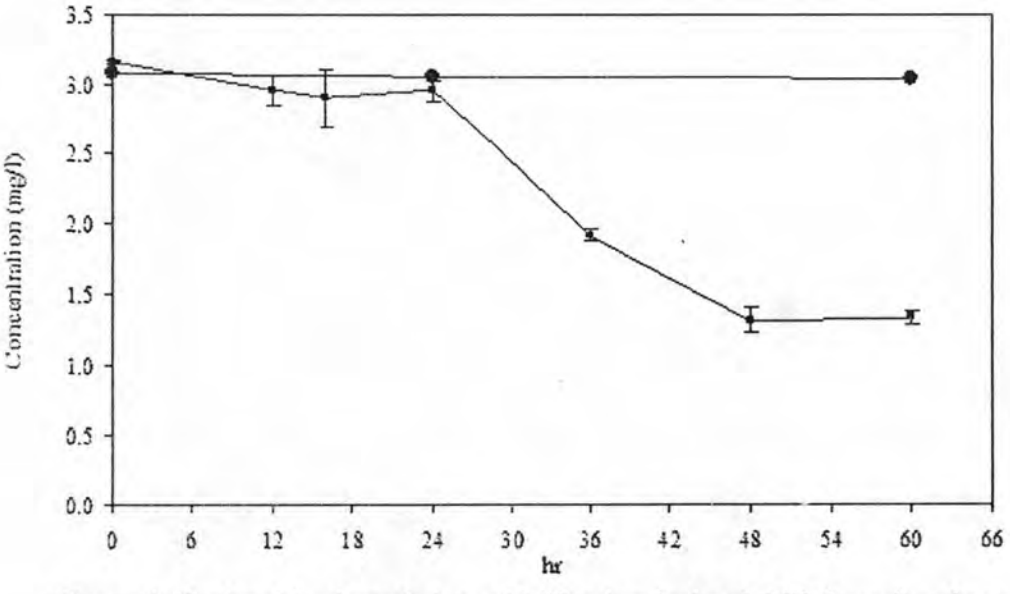

Figure 4.25. Aerobic degradation of MT using isolated MT-degrading bacteria strains MT 3/10 at an initial MT concentration of 3.0 mg/l: Biodegradation (■) and control (●) tests

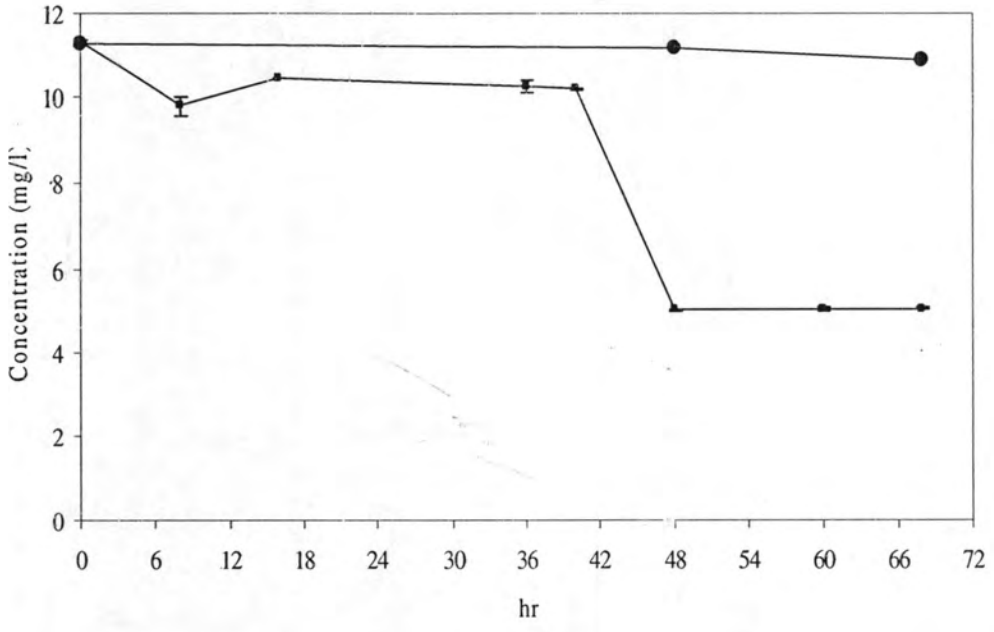

Figure 4.26. Aerobic degradation of MT using isolated MT-degrading bacteria strains MT 3/10 at an initial MT concentration of 10.0 mg/l: Biodegradation (■) and control (●) tests

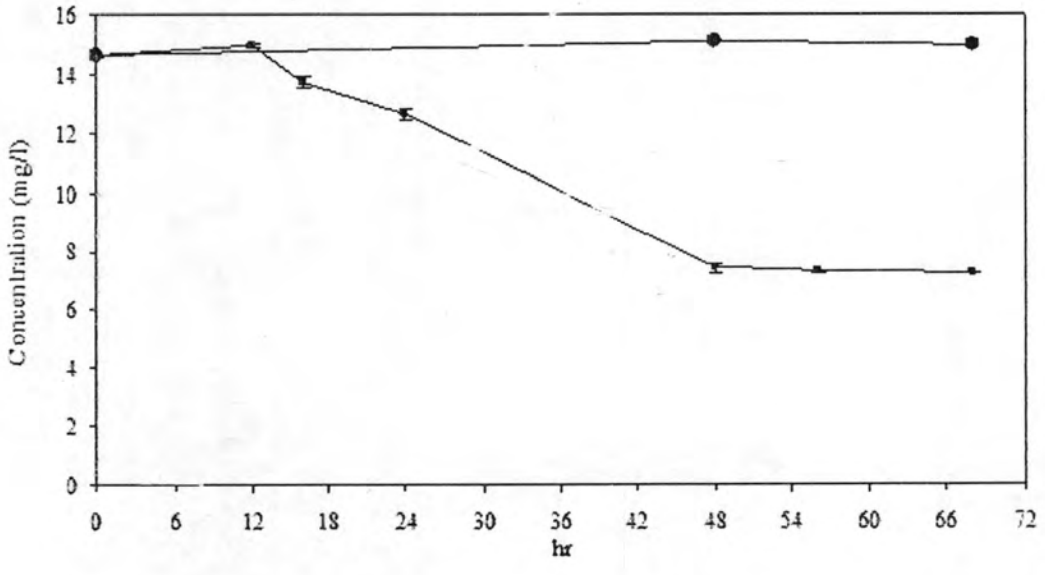

Figure 4.27. Aerobic degradation of MT using isolated MT-degrading bacteria strains MT 3/10 at an initial MT concentration of 15.0 mg/l: Biodegradation (■) and control (●) tests

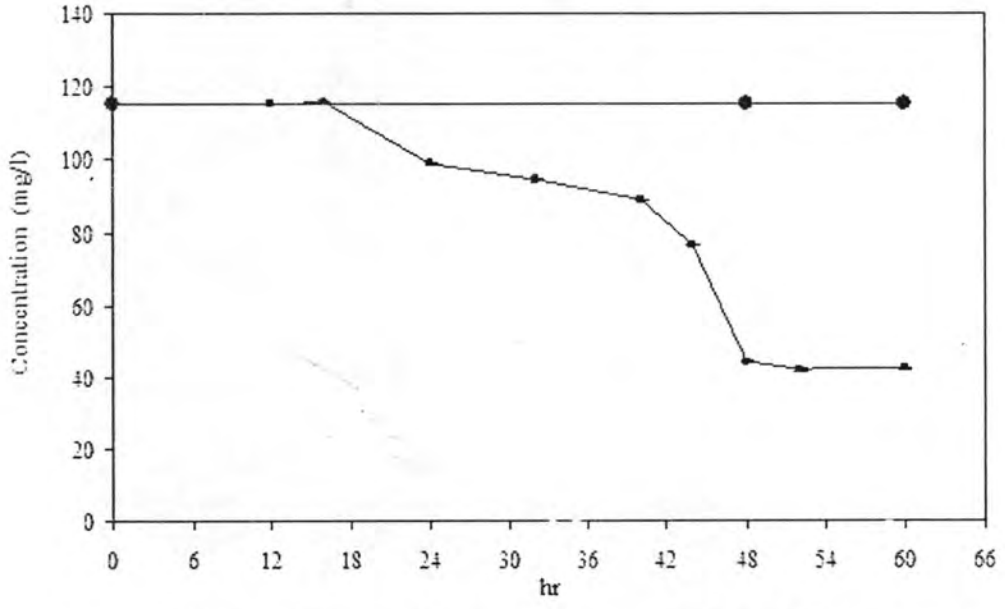

Figure 4.28. Aerobic degradation of MT using isolated MT-degrading bacteria strains MT 3/10 at an initial MT concentration of 115.0 mg/l: Biodegradation (▪) and control (●) tests

Table 4.9. First-order degradation rate constants (k) half lives ($t_{½}$) and lag phase of MT bacteria strain MT 3/10

Initial MT concentration (mg/l)	k (hr^{-1})	$t_{1/2}$ (hr)	Lag phase (hr)
3	0.03±0.01	43	24
10	0.03±0.01	47	42
15	0.02±0.00	48	12
115	0.03±0.00	47	16

Concentrations of MT in biodegradation test decreased whereas MT concentrations in control tests remained the same throughout the experiment. Lag phases occurred in all cases suggesting that isolated MT-degrading bacteria strain MT 3/10 needed time to acclimatize or MT was not readily available since the initial concentration was close or higher than the solubility limit of MT. The first-order degradation rate constant at MT initial concentrations of 3 and 10 mg/l were higher than initial MT concentration of 15 and 100 mg/l because of the possible lack of bioavailability at higher

concentrations.

4.3.3.2. Kinetic Analysis of Isolated MT-degrading bacteria strain MT 1/500

Results of aerobic biodegradation using isolated MT-degrading bacteria strain MT 1/500 at different initial MT concentrations of 3.0, 10.0, 15.0 and 115.0 mg/l are shown in Figures 4.29 to 4.32. First-order degradation rate constants are presented in Table 4.10

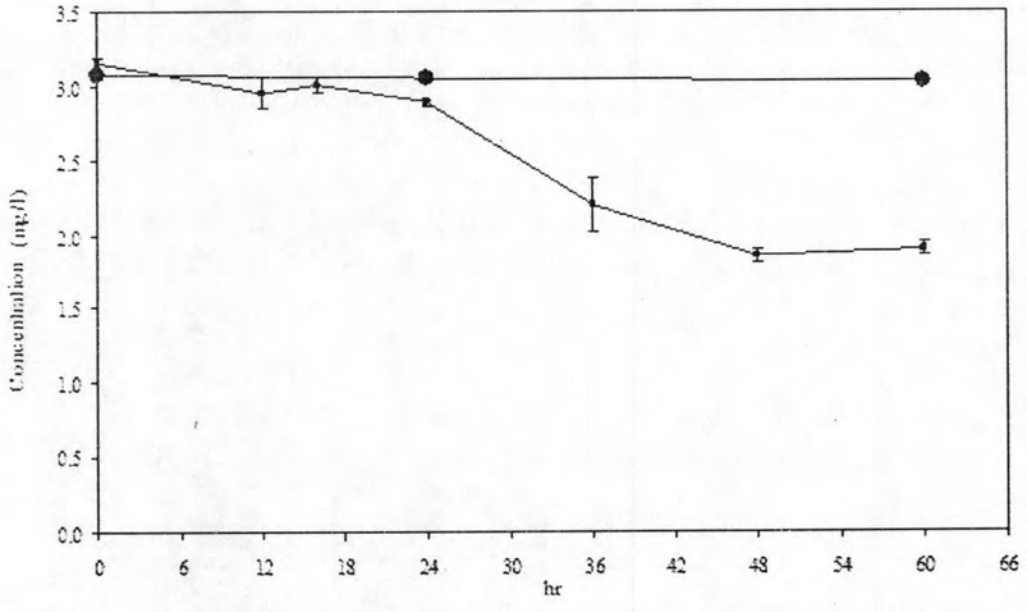

Figure 4.29. Aerobic degradation of MT using isolated MT-degrading bacteria strains MT 1/500 at an initial MT concentration of 3.0 mg/l: Biodegradation (▪) and control (●) tests

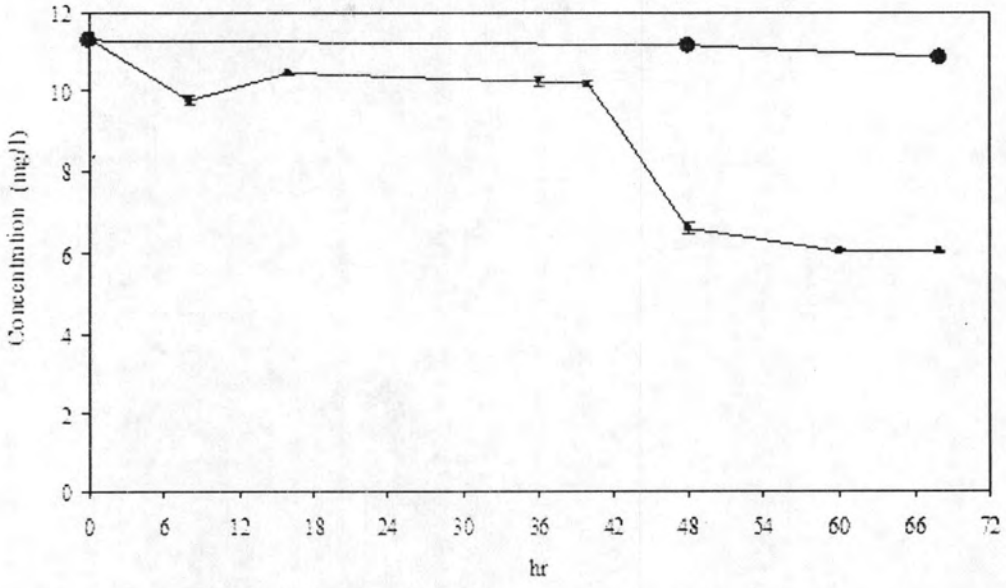

Figure 4.30. Aerobic degradation of MT using isolated MT-degrading bacteria strains MT 1/500 at an initial MT concentration of 10.0 mg/l: Biodegradation (▪) and control (●) tests

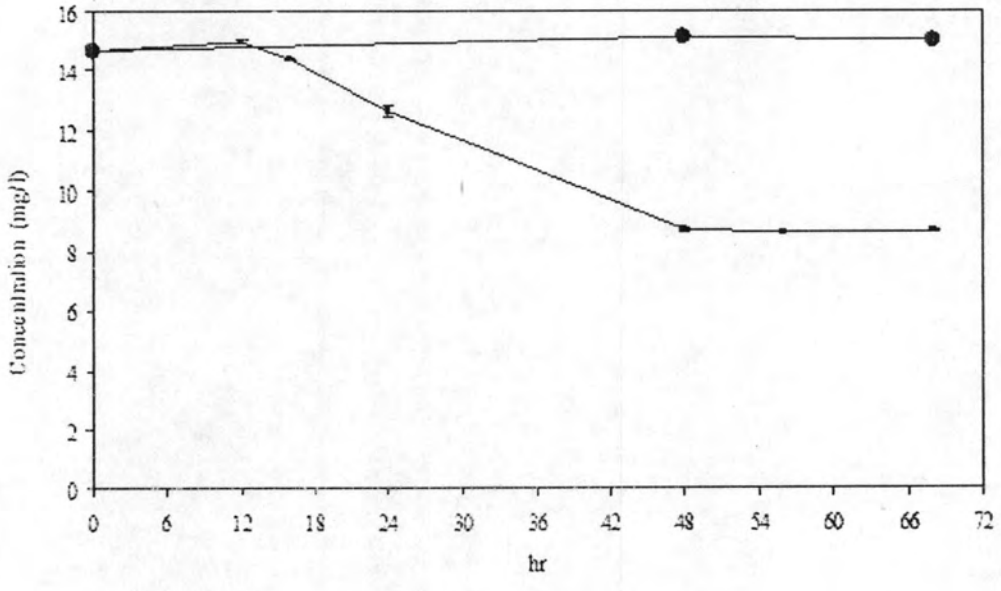

Figure 4.31. Aerobic degradation of MT using isolated MT-degrading bacteria strains MT 1/500 at an initial MT concentration of 15.0 mg/l: Biodegradation (▪) and control (●) tests

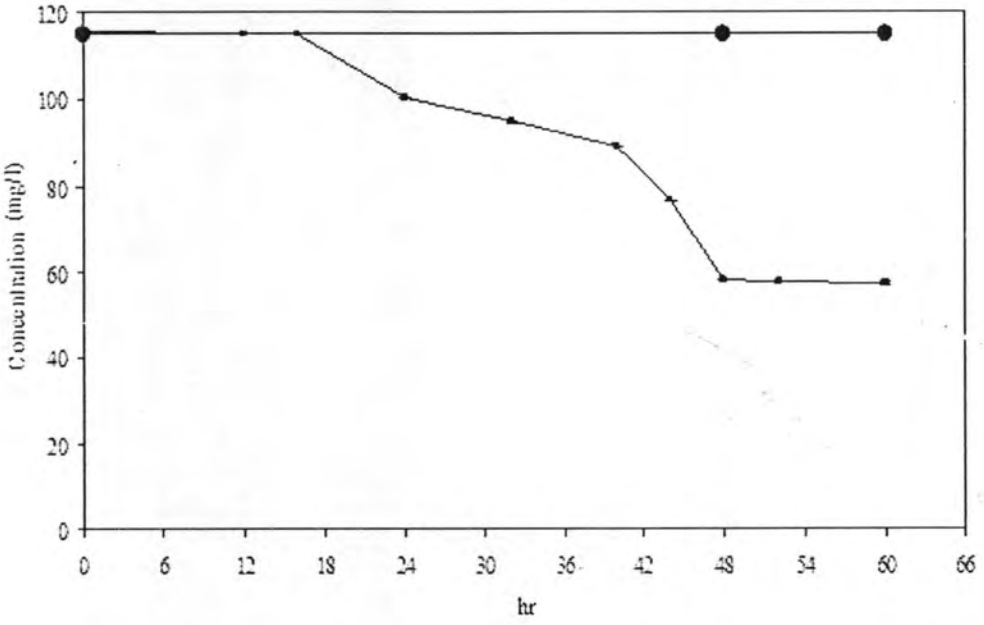

Figure 4.32. Aerobic degradation of MT using isolated MT-degrading bacteria strains MT 1/500 at an initial MT concentration of 115.0 mg/l: Biodegradation (▪) and control (●) tests

Table 4.10. First-order degradation rate constants (k) half lives ($t_{½}$) and lag phase of MT using bacteria strain MT 1/500

Initial MT concentration (mg/l)	k (hr^{-1})	$t_{½}$ (hr)	Lag phase (hr)
3	0.02±0.01	42	24
10	0.03±0.00	> 72	42
15	0.02±0.00	> 72	16
115	0.02±0.00	48	16

As shown earlier there were lag phases in all cases suggesting possible lack of bioavailability. Intermediate metabolites for this study were not observed at any retention time throughout the analysis.

4.3.3.3. Aeroboc Biodegradation Test of MT Using MT-degrading Bacteria Strains MT 3/100 and MT 3/500

This study was done to test the activity of MT-degrading bacteria strain MT 3/100 and strain MT 3/500. Two parallel batch tests comprising of degradation test and control test were carried out in 16 ml amber vial with PTFE screw cap. All tests were done in duplicate. Biodegradation tests were conducted with concentrations similar to their enrichment and isolation at 100 and 500 mg/l, for strains MT 3/100 and MT 3/500, respectively. Inoculums of MT-degrading bacteria, strains MT 3/100 and MT 3/500, were added to the IS medium to obtain a final concentration of 2.4 x 10^7 CFU/ml, estimated using plate count technique.

4.3.3.3.1. Aerobic Biodegradation Test of MT Using MT-degrading Bacteria Strain MT 3/100

Results of aerobic biodegradation using MT-degrading bacteria strain MT 3/100 are presented in Figure 4.33. The degradation rate constants obeyed first order reaction kinetic with a value of 0.06±0.01 hr^{-1} and half life of 16 hr. These results suggested that bacteria strain MT 3/100 did not need time to acclimatize to MT.

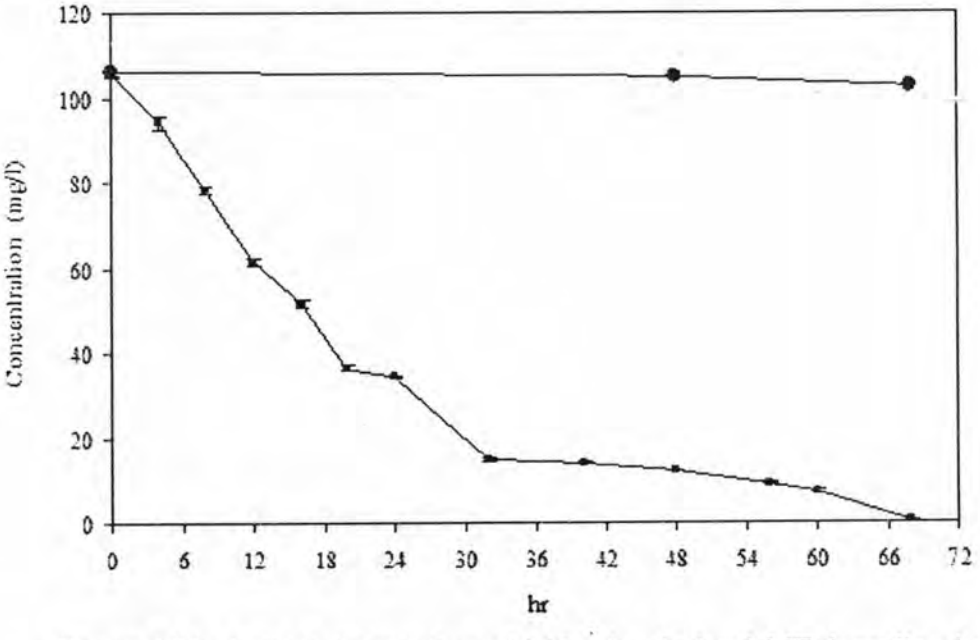

Figure 4.33. Aerobic degradation of MT using isolated MT-degrading bacteria strain MT 3/100 at an initial MT concentration of 100 mg/l: Biodegradation (▪) and control (•) tests

Peak areas of the metabolite were found to rapidly increased within the next 12 hr and then decreased at the degradation time of 32 hr. At the end of experiment, the metabolite peak area disappeared. The relative peak areas of the metabolite and MT were in the opposite direction meaning the peak area of MT increased whlie the peak area of metabolite decreased (see Figure 4.34). This result had the same trend as that of the aerobic sludge. This might be because the bacteria were isolated from aerobic sludge. In addition, the retention time of the metabolite was closely comparable with the metabolite from aerobic sludge and the metabolite has more polarity than parental MT from retention time result.

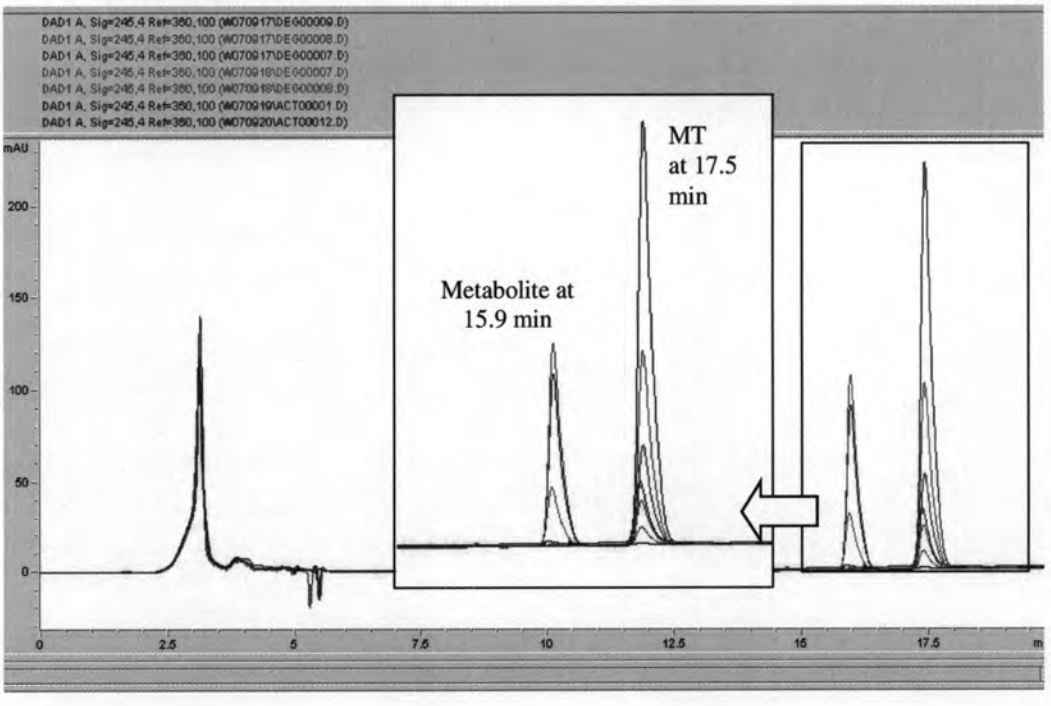

Figure 4.34. HPLC chromatogram of MT and its metabolite during aerobic biodegradation of MT using isolated MT-degrading bacteria strain MT 3/100

From the metabolite chromatogram, the possible metabolites include 17α-methyl-5α-androstan-3α, 17β-diol, 17α-methyl-5β-androstan-3α, 17β-diol., 17α-methyl-5α-androstane-3β,17β-diol, 17α-methyl-5β-androstane-3β,17β-diol, and, 17α-methyl-5x-androstane-3x,16x,17β-triol (x = α or β).

4.3.3.3.2. Biodegradation Test of MT Using MT-degrading Bacteria Strain MT 3/500

Results of the aerobic biodegradation test using isolated MT-degrading bacteria MT 3/500 are presented in Figure 4.35. The degradation obeyed first order reaction with a value of 0.1±0.00 hr^{-1} and a half life of 12.0 hr. The results suggest that bacteria strain MT 3/500 did not need time to acclimatize to MT.

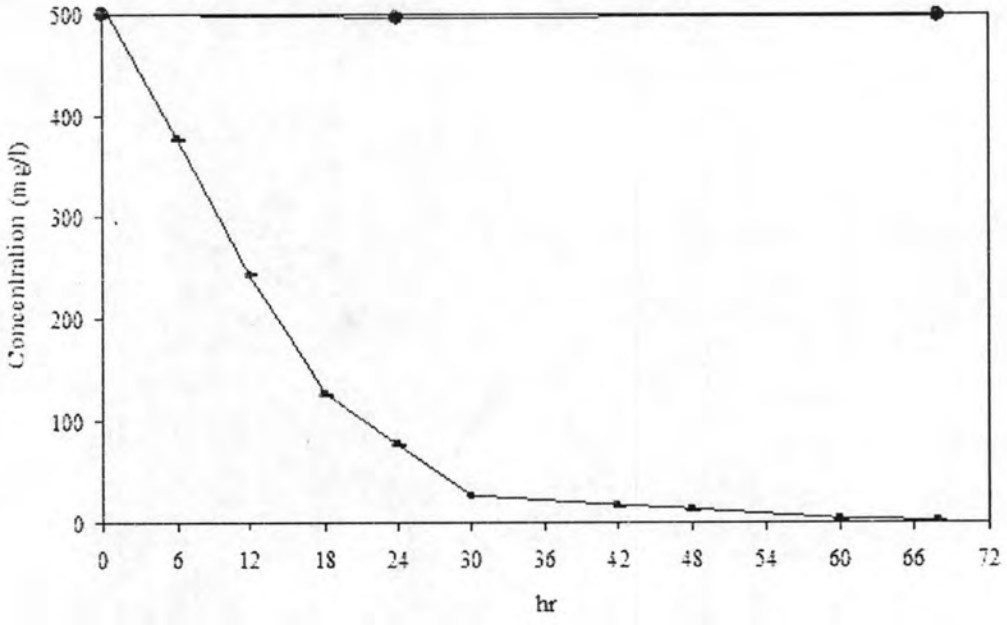

Figure 4.35 Aerobic degradation of MT using isolated MT-degrading bacteria strains MT 3/500 at an initial MT concentration of 100 mg/l: Biodegradation (▪) and control (●) tests

Metabolites were found to appear at a retention time of 15.9 min. The results were close to results from aerobic sludge. Bacteria strain MT 3/100 was isolated from the aerobic sludge. The metabolite has more polarity than parental MT based on the retention times. From the metabolite chromatogram, the possible metabolites include 17α-methyl-5α-androstan-3α, 17β-diol and 17α-methyl-5β-androstan-3α, 17β-diol, 17α-methyl-5α-androstane-3β, 17β-diol, 17α-methyl-5β-androstane-3β, 17β-diol, and 17α-methyl-5x-androstane-3x,16x,17β-triol (x = α or β).

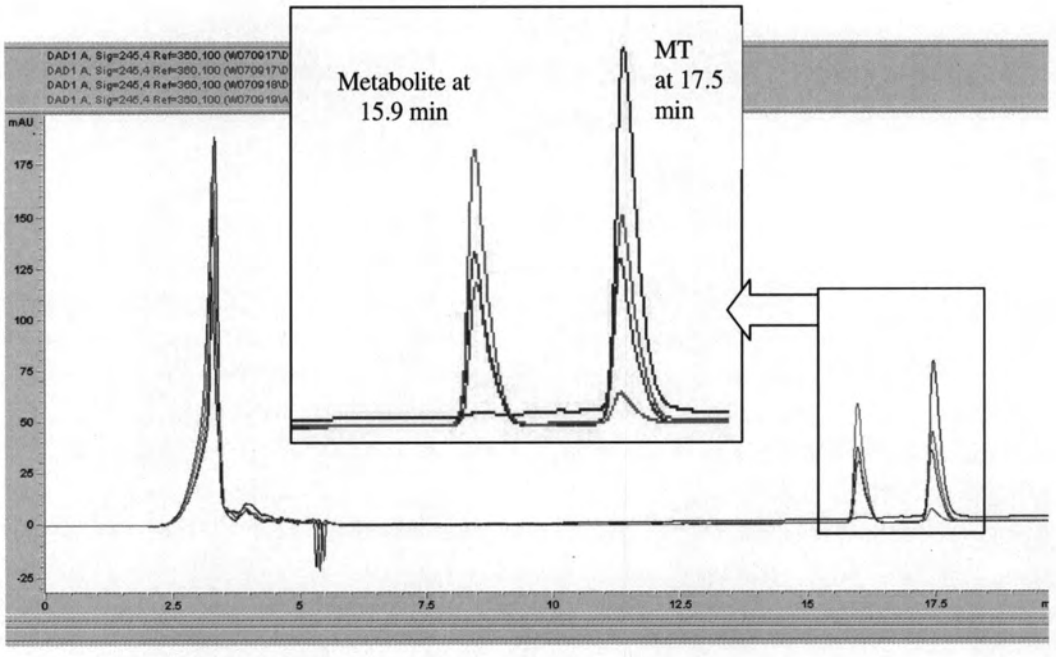

Figure 4.36 HPLC chromatogram of MT and its metabolite during aerobic biodegradation of MT using isolated MT-degrading bacteria strains MT 3/500