CHAPTER V

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

5.1 Conclusions

Three major objectives of this study are; 1) biodegradation of MT by microorganisms from wastewater treatment systems and sediment under aerobic and anaerobic conditions, 2) isolation of MT-degrading bacteria from aerobic sludge of a wastewater treatment system, 3) identification and characterization of the isolated MT degrading bacteria. The findings of this study fulfill all the objectives. Significant details of findings can be summarized as follow.

MT can be degraded by microorganisms from aerobic sludge, and anaerobic sludge collected from wastewater treatment plant and from sediment collected from masculinizing pond of Nile tilapia fry under aerobic and anaerobic conditions.

Aerobic biodegradation were conducted with aerobic sludge from a municipal wastewater treatment plant and sediment from a masculinizing pond of Nile tilapia fry. First-order degradation rate constants of aerobic sludge and sediment followed the same trend whereby the degradation rate constants decreased with increasing in initial MT concentrations. These results suggest that under aerobic condition, microorganisms in aerobic sludge and sediment have higher activities when expose to lower MT concentrations. This may be due to the bioavailability of MT. Comparison of the chromatogram of the intermediate metabolite of aerobic sludge and sediment showed that intermediate metabolite was similar. The intermediate metabolite have more polarity than the parental MT.

Anaerobic biodegradation were conducted with anaerobic sludge from a wastewater treatment plant of a Brewery factory and sediment from a masculinizing pond of Nile tilapia fry. First-order degradation rate constants of anaerobic sludge and sediment followed the same trend whereby the degradation rate constants increased with increasing initial MT concentrations until a certain concentration (> 5 mg/l) where the degradation rate constants decreased. These results suggest that microorganisms in anaerobic sludge and sediment were limited in their activities when exposed to higher MT concentrations and this may be due to the bioavailability of MT. In anaerobic degradation studie, very

low MT concentrations of 0.02-0.09 mg/l remained even for the extend period of more than 30 days. Three intermediate metabolites were found with retention time of 12.1, 13.4 and 15.7 min suggesting a possible different degradation pathway as compared to aerobic degradation. The intermediate metabolites were found to eluded earlier in the liquid chromatograph suggesting the metabolite were more polar than MT.

MT-degrading bacteria were isolated from aerobic sludge under initial MT concentrations of 10, 100 and 500 mg/l. In total, 7 colony types were obtained: 5 from an initial MT concentration of 10 mg/l, 3 from an initial MT concentration of 100 mg/l and 4 from an initial MT concentration of 500 mg/l.

Three colony types from initial MT concentrations of 10 and 500 mg/l were further identified by analysis of 16S rRNA gene sequences. The results suggested that isolated MT-degrading bacteria strain MT 5/10 and MT 1/500 were related closely to *Methylophilus leisingeri* strain RCP5 (accession number of DQ922752.1) with 99% identity and isolated MT-degrading bacteria strain MT 3/10 were related to *Acidovorax* sp. RCPCd1 (accession number of DQ922761) with 100% identity the intermediate metabolite in this study was the same as the metabolite found by using aerobic sludge. In all batch tests, MT concentrations were left over at 46 % for strain MT 3/10 and 55 % for strain MT 1/500.

5.2 Suggestions for future works

As this is one of the pioneer studies in this area, several issues remaining include:

- Study the fate of MT in the environment
  - Sorption of MT to soil including clay, sand and sediment.
  - Biodegradation of MT under anoxic condition.
  - Photodegradation of MT.
- Identification of MT-degrading bacteria
  - Isolation of MT-degrading bacteria from soil under aerobic condition.
  - Isolation of MT- degrading bacteria under anaerobic conditions.
- Study on MT-degrading bacteria isolated in:
  - Identify MT metabolites.
  - Identify Degradation pathways.