CHAPTER VIII

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

8.1 Conclusions

17alpha-methyltestosterone (MT), an anabolic androgenic steroid, is used widely in reversing the sex of Nile tilapia (*Oreochromis niloticus*), an important freshwater aquaculture fish in many developing countries. All male population is desired since it has high growth rate and large body size as compared to a mixed sex or female population. The feeding process of these fish farms would result in accumulatation of residual MT and its metabolites in water and sediment of the masculinizing ponds. If released into the receiving water body and near by environment, MT may impact on human, animals and ecosystem. MT is a suspect human carcinogen and can act as an endocrine disrupting compound interfering with the normal function of endocrine and reproductive systems of animals when exposed at concentrations as low as nanogram per liter level. When untargeted organisms come in contact with MT in the receiving body of water, an unbalance of male and female animals in the ecosystem may occur.

So far, an understanding of the occurrence and fate of MT in the environment has been limited. It is possible that MT is biodegraded in the water and sediments of the masculinization pond. Based on the chemical property of MT, it is probable that MT tends to accumulate in the sediment of the masuclinization pond.

MT was shown to degrade differently by microorganisms from sediment under different electron acceptor conditions. MT was shown to be degraded by aerobic, sulfate-reducing and metahnogenic conditions. The first order degradation rates for aerobic, sulfate-reducing and methanogenic conditions were estimated and were shown to be statically similar. MT was hardly degraded under iron(III)-reducing condition and there was no degradation under nitrate-reducing condition. During the incubation period, an unknown metabolite appeared shortly after the lag period and disappeared at the end of the incubation period for the aerobic condition only and was not found in the other conditions. Measured androgenic activities showed that for the androgenic activities for aerobic and sulfate-reducing bioassays were reduced at the end of the experiment but under methanogenic condition, androgenic activity

continued to persist in the bioassay for more than a month even though the measured MT concentrations were negligible. This implied that MT was transformed to other androgen-like compounds and may have not transformed further under methanogenic conditions. The results suggest that MT has the potential to accumulate in environments such as iron (III)-reducing, nitrate-reducing, and methanogenic conditions.

Five different types of colony morphology (A-E) were obtained from the isolation tests under three initial MT concentrations. The strains isolated from both sediment and water at an initial MT concentration of 1 mg/L and sediment at a initial MT concentration of 100 mg/L were found to be closely related to *Pimelobacter simplex*. The strains isolated from the sediment at an initial MT concentration of 10 mg/L and the strains isolated from water at initial MT concentrations of 10 and 100 mg/L were closely related to *Rhodococcus sp*, and *Nocardioides nitrophenolicus*, respectively. Since all the sequences came from the same group, a representative strains SB100-05, SB010-03, and WB100-05 were selected for each group for further identification and characterization due to their strong MT biodegradability. Based on phylogenetic analysis, physiological properties and cell morphology suggested that the strain SB100-05 and WB100-05 were related closely to *Nocardioides aromaticivorans* and *Nocardioides nitrophelolicus*, respectively which were a member of the genus *Nocardioidaceae* and the strain SB010-03 related closely to *Rhodococcus equi* which is a member of the genus *Nocardiaceae*. During the incubation period, a labile metabolite of MT was found after lag phase which then disappeared at the end of incubation period. The isolated strains were found to be capable of using MT as a sole carbon source and can cleave MT to non-androgenic compounds. The estimated kinetic parameters of three strains fitted with the Haldane's substrate inhibition model. Of the three strains, strain SB100-05 was able to degraded MT faster but had lower resistance to high concentration of MT.

Contaminated sediment and waters from masculizing ponds must be disposed of properly to prevent contamination of MT in nearby receiving waters and environment. An approach in eliminating MT without the androgen-like compounds as byproducts and is to biodegrade MT under aerobic condition.

8.2 Suggestions for future work

Until now, not much is known about the occurrence and fate of MT in the environments. Therefore, first priority should be reserved for the occurrence of MT in the masculinization ponds and nearby environments to reveal the actual remaining levels in such media. Then, fate of MT in those media should be clarified. Two possible significant mechanisms involving fate of MT in the environments include sorption and biodegradation. One of my colleagues has studied sorption of MT onto sediments and soils. Thus, desorption of MT and distribution of MT in sediment and soil should be studied to understand the transportation of MT in ground and underground.

In case of fate of MT, the main fate of MT is sorption and biodegradation. My colleague studied only about the sorption of MT onto sediments and soils. Thus, the desorption of MT and the distribution of MT in sediment and soil should be studied to understand the transportation of MT in underground and groundwater.

In case of biodegradation, MT was found to be hardly degraded under iron (III)-reducing condition and there was no degradation under nitrate-reducing condition. Under iron (III)-reducing condition, the biodegradation of MT with various co-substrate should be studied to improve the MT biodegradability. Under nitrate-reducing condition, there is currently no direct explanation for the lack of degradation of MT under this conditions although the methyl group at the C-17 position in the MT structure has been invoked as a possible explanation. The impact of other parameters such as pH, concentrations of MT, lack of enzyme system, effect of nitrite concentration, and effect of background organic compound in sediment should be studied to confirm the parameters responsible.

During the incubation period, an unknown metabolite was observed. Identifying the metabolite would provide further information on the possible mode of attack of the MT molecule by various enzymes. This would also provide information and identification of the androgen-like compounds under methanogenic conditions. By identifying the metabolites, the degradation pathway of MT under each electron acceptor condition can be elucidated.

In case of MT-degrading bacteria, it is found that each isolated strain was able to use MT as sole carbon source. However, further studies are needed to assess whether these isolated strains can use MT as a sole carbon source in the presence of

other organic compounds such as humic materials. In addition, it would be interesting to study whether these strains can degrade similar compound such as testosterone and estrogens.

To prevent and protect the distribution of MT from MT –contaminated site to other sources, there are two approaches to manage MT including removal of MT and reuse of MT by recirculation water.

To remove MT from MT-contaminated site, treatment under aerobic condition is the best practice due to the high MT removal efficiency without the production of androgen-like compounds as byproducts and easy operation and maintenance. Biodegradation of MT at environmentally relevant concentrations under aerobic condition by isolated MT-degrading bacteria should be studied in lab-scale to confirm the MT biodegradability to remove MT in the real site. Then the application of isolated MT-degrading bacteria in the real site should be studied to know the efficiency to remove MT in reality. Development of effective microorganism (EM) to remove both MT and other organics should be studied to help farmer to discard friendly environmental wastewater to natural resources.

To reduce the risk from residual MT in wastewater in masculinization pond, biodegradation of MT at environmentally relevant concentrations under aerobic, nitrification and denitrification conditions should be studied in both batch and lab-scale experiment to understand the possibility to reuse the residual MT in wastewater and to set the recirculation water system in masculinization fish farm.