CHAPTER I

INTRODUCTION

1.1 Introduction

Nile tilapia (*Oreochromis niloticus*) is a popular aquaculture product of Thailand as they are easy to spawn, highly tolerant over a wide range of environmental conditions, and are able to grow on various types of feed. Nile tilapia are not consumed locally but are exported, giving farmers extra incentives to raise this agriculture product. Production of Nile tilapia is not maximized when a culture of mixed sex population is used due to possible overpopulation, energy loss from reproduction and competition with younger fish (Macintosh and Little, 1995; Green *et al.*, 1997). Therefore, production of all male population of Nile tilapia is practiced where twice the growth rate and larger body sizes can be achieved as compared to raising female tilapia or a mixed sex population (Macintosh and Little, 1995).

There are several masculinizing techniques for the production of all male population. These techniques include manual sexing, interspecific hybridization, use of genetically-altered male tilapia and hormone-induced sex reversal. Among these techniques, hormone-induced sex reversal is more efficient, easier to implement and less expensive than other techniques.

17alpha-methyltestosterone (MT), an anabolic androgenic steroid hormone, is popularly used for masculinizing Nile tilapia (*Oreochromis niloticus*) throughout the world. There are two current techniques for steroid-induced sex inversion of Nile tilapia using MT: oral administration of MT and immersion of Nile tilapia fry in aqueous solution containing MT. For oral administration, MT-impregnated food is used to treat Nile tilapia fry with a masculinzing dose of 60 mg of MT/kg of food for four weeks. For the immersion technique, Nile tilapia fry is immersed in solution containing 1800 ug/L of MT for 48 hr. Oral administration is the most common technique used for masculinizing of Nile tilapia. However, residual MT from uneaten and unmetabolized MT-impregnated food can contaminate the pond environment and receiving waters near the area of masculinizing ponds. Recently, a study on the persistency of MT in a model masculinizing pond of Nile tilapia showed that two

months after cessation of MT treatment, MT continued to persist in the sediment of the pond at concentrations between 2.8 and 2.9 ng/g (Fitzpatrick and Contreras-Sánchez, 2000). Therefore, concerns have been raised with respect to MT leaking from the pond environment into the environment and potentially impacting human, aquatic wildlife and the ecosystem.

MT is a questionable human carcinogen, producing nonmalignant tumors in the liver (Soe *et al.*, 1992). The main risks of excessive exposure to androgens are menstrual irregularities and virilization in women and impotence, premature cardiovascular disease and prostatic hypertrophy in men.

MT is also classified as an endocrine disrupting compound (EDC) because it interferes with the normal function of reproductive and endocrine systems of aquatic organisms and invertebrates. There are some reports on the chronic effects of MT on the reproductive system of medaka (*Oryzias latipes*). Exposure of parental fish to 27.75 ng/L showed secondary male sex characteristics in which no ovary could be discerned (Masanori *et al.*, 2004). This suggests that concentrations of MT as low as nanogram level can affect aquatic organisms.

So far, not much is known about the fate of MT in the environment. MT has a high log K_{ow} value and low water solubility. In a masculinizing pond environment, sediments may act as a sink for MT. Therefore, the fate of MT in sediment is very important. In addition, research work on the microbial transformation/biodegradation of MT in sediments from masculinizing pond of Nile tilapia under aerobic, anoxic and anaerobic conditions has not been reported. Research on biodegradation of MT under pond environment including identification of MT-degrading bacteria will further contribute towards the body of knowledge on the fate and transport of MT in the environment.

1.2 Objectives

The objectives for this study are:

1. To investigate the biodegradation of MT by microorganisms in sediment of masculinizing pond of Nile tilapia fry under different electron acceptors (O_2, NO_3^-, Fe^{3+}, SO_4^{2-} and CO_2)

2. To enrich, isolate, identify and characterize MT-degrading bacteria from two media, sediment and water of masculinizing pond of Nile tilapia fry at different MT concentrations under aerobic conditions

3. To assess the androgenic activities of MT and its metabolites from the biodegradation of MT by microorganismss in sediment under different electron acceptors and by isolated MT-degrading bacteria.

1.3 Hypotheses

1. MT can be degraded rapidly by microorganisms in sediment under aerobic condition and slowly under other electron acceptor conditions.

2. There are various species of MT-degrading bacteria that isolated from sediment and water and these have different biodegradability of MT.

3. MT can be biotransformed to non-androgen-like compounds by MT-degrading microorganisms.

1.4 Scopes of study

1. Biodegradation of MT by microorganisms in sediment was conducted under different electron acceptor conditions including O_2, NO_3^-, Fe^{3+}, SO_4^{2-}, and CO_2.

2. MT-degrading bacteria were isolated from sediment and water in masculinizing pond of Nile tilapia at initial MT concentrations of 1, 10, and 100 mg/L.

3. β-galactosidase assay was used as an indirect measurement to verify the androgenic property of MT and its metabolites.