CHAPTER I

INTRODUCTION

1.1. Background and motivation

During this decade, the presence and potential effects of pharmaceuticals and personal care products (PPCPs) in the environment have been great concern. PPCPs can be categorized as endocrine disrupting chemicals (EDCs) with the potential of subtle effects at low concentrations. Some of the PPCPs include estrogenic compounds, antibiotics, pain killers and antibacterial compounds. Also included are hormones which are the focus of this research. At this time, not much is known about the disposition of these compounds in the environment (Laurence and Mordechai, 2003), i.e., their fate and transport in the environment and their adverse effects on native biota known.

Because steroid hormones and their metabolites are biologically active and potent, there is a potential effect on humans and/or non-target organisms when exposed to these compounds. Whereas, some hormones may not have a long half-life in the environment, the continuous discharge of hormones from sources such as fish farms can result in a "pseudo-persistence" condition in the environment (Edward et al., 2004).

Steroid hormones are used widely in livestock to increase their yield. As such, they can be introduced into the environment through many routes such as wastewater discharges and contaminated sediment. Fish farm industry is an example industry that uses steroid hormone to increase their production yield. Fish farm industry in Thailand has expanded recently due to an increase in demand domestically and aboard (Terence et al., 2003).

Nile tilapia (*Oreochromis niloticus*) is one of the most popular aquaculture products in Thailand because it is easy to grow and harvest, resistant to diseases, and tolerant to a wide range of environmental conditions. In addition, there is a strong export demand. To enhance growth potential and yield in Nile tilapia farming, an all-male population is produced because a culture of mixed sex population often results in precocious maturation and early reproduction. In an all-male population, no energy is shunted toward reproduction, and there is no competition with younger fish (Macintosh and Little, 1995; Green et al., 1997). Moreover, males grow to a larger final size than females (Macintosh and Little, 1995).

Techniques used in masculinizing Nile tilapia include: physical sex characterization between male and female fish (manual sexing); interspecies hybridization; genetically altered male tilapia (supermale tilapia) and; hormone-induced sex reversal by androgenic hormone. Among these techniques use of hormone-treated feeds for the production of all-male populations is the most popular in tilapia aquaculture (Macintosh and Little, 1995). An all-male tilapia can be produced by treating tilapia fry with 17 alpha-methyltestosterone (MT)-impregnated foods at concentrations of 60 mg MT/kg food for 21 days, beginning at 25 days after post hatch (DPH) (Lene et al., 2006). The steroid acts as a sex-inversion agent by functionally masculinizing individual fry in the population (Terence et al., 2007).

MT is a naturally-occurring anabolic steroid and is synthesized from cholesterol via pregnenolone in the testis, ovary and adrenal gland. MT is white or slightly yellowish-white crystals. It is odorless and slightly hygroscopic. It is practically insoluble in water but freely soluble in chloroform and methyl alcohol and sparingly soluble in vegetable oils (Hiroyuki et al., 1998). The only legitimate therapeutic indication for anabolic steroids is replacement of male sex steroids in men who have androgen deficiency. For example as a result of loss of both testes and the treatment of certain rare forms of a plastic anemia which are or may be responsive to anabolic androgens, the drug has been used in certain countries to counteract catabolic states after major trauma (Barry et al., 2005). In addition, MT use as an androgen and as a performance enhancement drug in athletes may result in its release to the environment through various waste streams. Occupational exposure to MT may occur through dermal contact with this compound at workplaces where MT is produced or used. Exposure to the drug among the general population may be limited to administering MT as an androgen (Gustavo and Luis, 2003).

MT is a questionable human carcinogen, producing nonmalignant tumors in the liver (Soe et al., 1992). It is a poison by intraperitoneal route. It causes developmental abnormalities in the urogenital system (Lewis, 1991). The target organs are not at serious risk from acute poisoning, but chronic use can cause harm. The main risks of excessive exposure to androgens include: menstrual irregularities and virilization in women and impotence, premature cardiovascular disease and prostatic hypertrophy in men. The adverse effects of anabolic steroids include weight gain, fluid retention, and abnormal liver function as measured by biochemical tests.

It is highly probable that the remaining and un-metabolized MT-impregnated foods accumulate in the sediment of the masculinization ponds are released into the receiving waters and discharged into certain aquatic environments at low MT concentrations. Consequently, resident aquatic organisms, including fish and invertebrates that are present in aquatic systems near sources of MT are likely to be exposed to these chemicals. MT is biologically active and may impact the metabolic activity of living cells. A recent study showed that MT concentrations in soils were between 2.8 and 2.9 ng/g demonstrating the persistence of MT in soil for nearly three months after cessation of treatment (Mcelwee et al., 2000). MT residues in receiving water may impact humans who consume or expose to contaminated water, affecting their endocrine and reproductive systems. Moreover, using MT for masculinization in Nile tilapia, can have an affect on aquatic organisms. When the residues of MT are released into natural water body, there is the potential for continuous, multigenerational exposure and can induce sex reversal of other fish species living in that area. There are some reports on the chronic effects of MT on reproductive status of medaka (*Oryzias latipes*). Exposure of parental fish to 27.75 ng/l showed male secondary sex characteristics in which no fish with ovary could be discerned (Masanori et al., 2004). This suggests that MT at low concentration can affect aquatic organisms, So far, there is no effluent standard for MT from fish farm.

For reasons listed above, this research is focused on the fate and biodegradation of MT at a laboratory scale and the isolation of MT degrading bacteria from microorganisms obtained from wastewater treatment plants and sediment collected from masculinization ponds of fish farms.

1.2. Objectives

The objectives of the study are:

- To study the biodegradation of MT by microorganisms from wastewater treatment systems and sediment under aerobic and anaerobic conditions.
- To isolate MT-degrading bacteria under different initial MT concentrations.
- To identify and characterize MT-degrading bacteria.

1.3. Hypotheses

The following hypothesis will be investigated:

- MT can be degraded by microorganisms from wastewater treatment systems and sediment under aerobic and anaerobic conditions.

1.4. Scope of work

The main scope of this study includes

1. Biodegradation studies using microorganisms from difference sources including aerobic sludge from a municipal wastewater treatment, anaerobic sludge from an upflow anaerobic sludge blanket and sediment from masculinization pond.
2. Biodegradation studies using batch experiments
3. Isolation of MT-degrading from aerobic sludge of a municipal wastewater treatment under aerobic condition