

BIODEGRADATION OF 17 ALPHA-METHYLTESTOSTERONE  
UNDER AEROBIC AND ANAEROBIC CONDITIONS

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A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science Program in Environmental Management  
(Interdisciplinary Program)

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การย่อยสลายทางชีวภาพของสาร 17 อัลฟาเมทิลเทสโทสเตอโรนภายใต้สภาพที่ใช้ออกซิเจนและไร้ออกซิเจน

นางสาวธีระจิตต์ วัฒนนคร

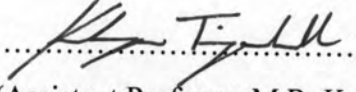
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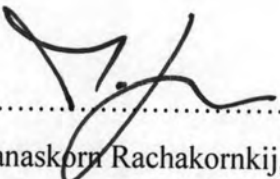
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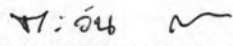
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17 อัลฟาเมทิลเทสโทสเตอโรน(MT) เป็นฮอร์โมนเพศชายสังเคราะห์ ใช้ในการแปลงเพศปลาให้เป็นเพศผู้ MT ถูกนำไปผสมกับอาหารปลาโดยมีความเข้มข้นของ MT 60 มิลลิกรัมต่ออาหาร 1 กิโลกรัม เพื่อใช้ในการแปลงเพศ ปลานิล ฮอร์โมน MT จัดเป็นสารก่อมะเร็ง ดังนั้นได้มีการศึกษาในระดับห้องปฏิบัติการถึงการตกค้างของสาร MT ในน้ำ และในตะกอนดิน ผลการทดลองพบว่าการตกค้างของสาร MT ในตะกอนดินกั้นบ่อแปลงเพศปลานิล ที่ระดับความเข้มข้น 2.8-2.9 นาโนกรัมต่อกรัมตะกอนดิน เป็นระยะเวลา 3 เดือนนับจากการหยุดให้อาหารที่ผสมฮอร์โมน ก่อให้เกิดผลกระทบต่อคนที่สัมผัสกับน้ำที่มีการปนเปื้อนและก่อให้เกิดผลกระทบต่อสิ่งมีชีวิตในแหล่งน้ำที่มีการปนเปื้อน จุดประสงค์ของงานวิจัยทำการศึกษถึงการย่อยสลายทางชีวภาพของสาร MT ด้วยจุลินทรีย์จากกากตะกอนในระบบบำบัดน้ำเสียและตะกอนดินที่ภายใต้สภาวะที่มีและไร้ออกซิเจน จากการทดลองพบว่า MTสามารถย่อยสลายได้ ภายใต้การย่อยสลายทั้งมีและไร้ออกซิเจน จากการย่อยสลายด้วยกากตะกอนจากระบบบำบัดน้ำเสียภายใต้สภาพใช้ออกซิเจนมีค่าอัตราการย่อยสลายเท่ากับ  $1.43 \pm 0.12$   $0.48 \pm 0.04$   $0.37 \pm 0.01$   $0.24 \pm 0.17$  และ  $0.36 \pm 0.17$  ต่อวัน ที่ระดับความเข้มข้นเริ่มต้น 0.3 1.0 5.0 7.0 และ 10.0 มิลลิกรัมต่อลิตรตามลำดับ จากการย่อยสลายด้วยตะกอนดินภายใต้สภาวะมีออกซิเจนมีค่าอัตราการย่อยสลายเท่ากับ  $0.52 \pm 0.02$   $0.23 \pm 0.04$   $0.17 \pm 0.02$   $0.13 \pm 0.01$  และ  $0.10 \pm 0.01$  ต่อวัน ที่ระดับความเข้มข้นเริ่มต้น 0.3 1.0 5.0 7.0 และ 10.0 มิลลิกรัมต่อลิตรตามลำดับ จากการย่อยสลายด้วยการตะกอนจากระบบบำบัดน้ำเสียภายใต้สภาพไร้ออกซิเจนมีค่าอัตราการย่อยสลายเท่ากับ  $0.19 \pm 0.01$   $0.21 \pm 0.01$   $0.28 \pm 0.03$   $0.19 \pm 0.01$  และ  $0.10 \pm 0.01$  ต่อวันวันที่ระดับความเข้มข้นเริ่มต้น 0.1, 1.0, 3.0, 5.0, และ 10.0 มิลลิกรัมต่อลิตรตามลำดับ จากการย่อยสลายด้วยตะกอนดินภายใต้สภาพไร้ออกซิเจนมีค่าอัตราการย่อยสลายเท่ากับ  $0.09 \pm 0.02$   $0.34 \pm 0.01$   $0.33 \pm 0.01$   $0.23 \pm 0.08$  และ  $0.09 \pm 0.01$  ต่อวันวันที่ระดับความเข้มข้นเริ่มต้น 0.1, 1.0, 3.0, 5.0, และ 10.0 มิลลิกรัมต่อลิตรตามลำดับ จากการจำแนกเชื้อได้เชื้อแบคทีเรีย 3 ชนิดคือ strain MT 3/10 มีลักษณะใกล้เคียง *Acidovorax* sp. RCPCd1 และ แบคทีเรีย strain MT 1/500 และ MT 5/10 มีลักษณะใกล้เคียง *Methylophilus leisingeri* strain RCP5 จากการทดลองความสามารถในการย่อยสลายของแบคทีเรียทั้งสองพบว่า แบคทีเรีย strain MT 3/10 และ MT 1/500 มีความสามารถในการย่อยสลาย MT ต่ำ โดยเมื่อเวลาผ่านไป 2.5 วัน พบว่ายังคงมีความเข้มข้นของ MT เหลือร้อยละ 46 จากการย่อยสลายด้วยแบคทีเรีย strain MT 3/10 และ มีความเข้มข้นของ MT เหลือร้อยละ 55 จากการย่อยสลายด้วยแบคทีเรีย strain MT 1/500

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KEY WORD: 17ALPHA-METHYLTESTOSTERONE / 17ALPHA-METHYLTESTOSTERONE-DEGRADING BACTERIA / AEROBIC / ANAEROBIC / AEROBIC SLUDGE / ANAEROBIC SLUDGE / DEGRADATION / SEDIMENT

THEERACHIT WATTANODORN: DEGRADATION OF 17 ALPHA-METHYLTESTOSTERONE UNDER AEROBIC AND ANAEROBIC CONDITIONS. THESIS ADVISOR: TAWAN LIMPIYAKORN, Ph.D. THESIS COADVISOR: PROFESSOR SAY KEE ONG, Ph.D., 152 pp.

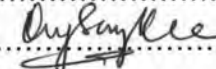
To produce an all-male population, a very common practice is to feed the Nile tilapia (*Oreochromis niloticus*) fry with 17alpha-methyltestosterone (MT)-impregnated food. MT residues in fish feed for the masculinization of Nile tilapia fry may accumulate in masculinizing ponds and be released into natural receiving water. A recent study showed that MT concentration in soils were between 2.8 and 2.9 ng/g for nearly three months after cessation of treatment demonstrating the persistence of MT in soil. The objectives of this study were to investigate the biodegradation of MT under aerobic and anaerobic conditions and isolation of responsible microorganisms. Bacterial seed used was aerobic sludge, anaerobic sludge taken from wastewater treatment plants and sediment taken from a masculinizing pond of tilapia fry. Aerobic and anaerobic biodegradation test suggested that MT was biodegradable. Under aerobic condition, first-order degradation rate constants using aerobic sludge were  $1.43 \pm 0.12$ ,  $0.48 \pm 0.04$ ,  $0.37 \pm 0.01$ ,  $0.24 \pm 0.17$  and  $0.36 \pm 0.17 \text{ day}^{-1}$ , for an initial MT concentrations of 0.3, 1.0, 5.0, 7.0, and 10.0 mg/l, respectively. Under aerobic condition, first-order degradation rate constants using sediment were  $0.52 \pm 0.02$ ,  $0.23 \pm 0.04$ ,  $0.17 \pm 0.02$ ,  $0.13 \pm 0.01$  and  $0.10 \pm 0.01 \text{ day}^{-1}$  for the initial MT concentrations of 0.3, 1.0, 5.0, 7.0, and 10.0 mg/l, respectively. Under anaerobic degradation, first-order degradation rate constants using anaerobic sludge were  $0.19 \pm 0.01$ ,  $0.21 \pm 0.01$ ,  $0.28 \pm 0.03$ ,  $0.19 \pm 0.01$  and  $0.10 \pm 0.01 \text{ day}^{-1}$  for the initial MT concentrations of 0.1, 1.0, 3.0, 5.0, and 10.0 mg/l, respectively. Under anaerobic degradation, first-order degradation rate constants using sediment were  $0.09 \pm 0.02$ ,  $0.34 \pm 0.01$ ,  $0.33 \pm 0.01$ ,  $0.23 \pm 0.08$  and  $0.09 \pm 0.01 \text{ day}^{-1}$  for the initial MT concentrations of 0.1, 1.0, 3.0, 5.0 and 10.0 mg/l, respectively. MT-degrading bacteria strain MT 3/10, MT 5/10 and 1/500 were isolated from aerobic sludge. Analysis of 16S rRNA gene sequences suggested that the strain MT 5/10 related closely to *Acidovorax* sp. RCPCd1 which is in the genus *Acidovorax*. And the strains MT 3/10 and MT 1/500 related closely to *Methylophilus leisingeri* strain RCP5 which is in the genus *Methylophilus*. The activities of both strains were not so effective. After 2.5 days of operation, MT concentrations were left over at 46 % for strains MT 3/10 and 55 % for MT 1/500.

Department: Environmental Management

Student's signature..... 

Academic year 2007

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Co-advisor's signature..... 

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## ABBREVIATIONS

BLAST	=	basic local alignment search tool
CFU/ml	=	colony forming unit/milliliter
cm	=	centimeter
DAD	=	diode array detector
DPH	=	days post hatch
EDs	=	endocrine disruptors
g	=	gram
HPLC	=	high performance liquid chromatography
K	=	degradation rate constant
Kg	=	kilogram
K <sub>ow</sub>	=	octanol-water partitioning coefficient
l	=	litter
m	=	meter
mg/l	=	milligram per liter
min	=	minute
MLSS	=	mixed-liquor suspended solids
MT	=	17alpha-methyltestosterone
ng/g	=	nanogram per gram
ng/l	=	nanogram per liter
PCR	=	polymerase chain reaction
PTFE	=	polytetrafluoroethylene
PPCPs	=	pharmaceuticals and personal care products
UASB	=	upflow anaerobic sludge blanket
WWTS	=	wastewater treatment system