

BIODEGRADATION OF 17ALPHA-METHYLTESTOSTERONE BY
MICROORGANISMS IN SEDIMENT FROM MASCULINIZING
POND OF NILE TILAPIA FRY



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A Dissertation Submitted in Partial Fulfillment of the Requirements
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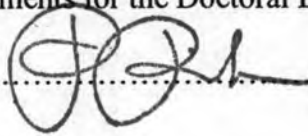
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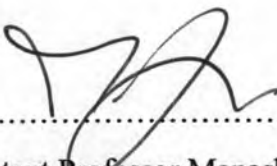
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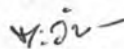
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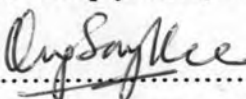
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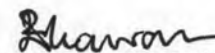
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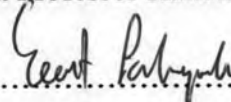
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สุปรีดา นอมกลิน: การย่อยสลายทางชีวภาพของสาร17แอลฟา-เมทิลเทสโทสเตอโรนด้วยจุลินทรีย์ในตะกอนดินก้นบ่อแปลงเพศปลานิล (BIODEGRADATION OF 17ALPHA-METHYLTESTOSTERONE BY MICROORGANISMS IN SEDIMENT FROM MASCULINIZING POND OF NILE TILAPIA FRY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ดร.ตะวัน ลิ้มปิยากร, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: Professor Say Kee Ong, Ph.D., 162 หน้า.

ฮอร์โมน17แอลฟา-เมทิลเทสโทสเตอโรน หรือ MT เป็นฮอร์โมนสังเคราะห์เพศชายที่มีการนำมาใช้อย่างแพร่หลายในการแปลงเพศปลานิล (*Oreochromis niloticus*) ให้เป็นเพศผู้ คาดว่าMTอาจเป็นสารก่อมะเร็ง นอกจากนี้ยังพบว่า MT เป็นสารรบกวนฮอร์โมนทำให้เกิดความผิดปกติต่อการทำงานของระบบสืบพันธุ์ของสัตว์ เมื่อได้รับMT ในระดับนาโนกรัมต่อลิตร อย่างไรก็ตามข้อมูลทางด้าน Occurrence และ Fate ของ MT ในระบบสิ่งแวดล้อมยังมีอยู่อย่างจำกัด ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาการย่อยสลายของMT ด้วยจุลินทรีย์จากตะกอนดินในบ่อแปลงเพศปลานิลและทำการคัดแยกแบคทีเรียที่ย่อยสลาย MT จากตะกอนดินและน้ำในบ่อแปลงเพศปลานิล รวมทั้งประเมินค่า Androgenic activity ที่เกิดจาก MT และผลผลิตที่ได้จากการย่อยสลายของ MT จากการศึกษาพบว่า MT ถูกย่อยสลายได้ภายใต้สภาวะ aerobic, sulfate-reducing และ methanogenic แต่ย่อยสลายได้ยากภายใต้สภาวะ iron (III)-reducing และไม่ย่อยสลายภายใต้สภาวะ nitrate-reducing โดยการย่อยสลายของ MT ภายใต้สภาวะ aerobic, sulfate-reducing และ methanogenic มีอัตราเร็วในการย่อยสลายเป็นแบบ 1st order และมีค่าใกล้เคียงกัน แต่อย่างไรก็ตามค่า Androgenic activity ที่วัดได้ภายใต้สภาวะ methanogenic นั้นมีค่าคงตัวเป็นระยะเวลานานกว่า 1 เดือน ในขณะที่ MT ถูกย่อยจนหมด ในระหว่างการย่อยสลาย MT พบว่ามี metabolite เกิดขึ้นเฉพาะภายใต้สภาวะ aerobic แต่ไม่พบในสภาวะอื่น จากผลดังกล่าวเป็นไปได้ MT และ metabolite ของ MT มีโอกาสที่จะเกิดการสะสมในบ่อแปลงเพศปลานิลเป็นระยะเวลานาน โดยเฉพาะอย่างยิ่งภายใต้สภาวะ nitrate-reducing, iron (III)-reducing และ methanogenic จากการคัดแยกเชื้อที่สามารถย่อยสลาย MT จากตะกอนดินและ น้ำในบ่อแปลงเพศปลานิลพบว่ามีเชื้อจำนวน 30 สายพันธุ์ที่สามารถย่อยสลาย MT ได้ จากเชื้อที่คัดแยกมาทั้งหมดสามารถแบ่งได้เป็น 3 จีนัส คือ *Pimelobacter*, *Rhodococcus* และ *Nocardioidea* โดยเชื้อ SB100-05, SB010-03 และ WB100-05 ถูกเลือกเป็นตัวแทนของเชื้อทั้ง 3 กลุ่ม เพื่อทำการ identification และ characterization ผลของการวิเคราะห์ด้วย phylogenetic, สมบัติทางสรีรวิทยา และ สันฐานวิทยาของเซลล์ พบว่าสายพันธุ์ SB100-05, SB010-03 และ WB100-05 มีลักษณะใกล้เคียงกับ *Nocardioidea aromaticivorans*, *Rhodococcus equi* และ *Nocardioidea nitrophelicus* ตามลำดับ จากการวิเคราะห์ทางจุลศาสตร์ด้วย Haldane's model พบว่า MT มีผลกระทบต่ออัตราการยับยั้งการเจริญของแบคทีเรียจากแบคทีเรียทั้ง 3 สายพันธุ์ดังกล่าวพบว่า SB100-05 สามารถย่อยสลาย MT ได้เร็วกว่าสายพันธุ์อื่นแต่ทนต่อ MT ได้น้อยกว่าสายพันธุ์อื่น

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SUPREEDA HOMKLIN: BIODEGRADATION OF 17ALPHA-
 METHYLTESTOSTERONE BY MICROORGANISMS IN SEDIMENT FROM
 MASCULINIZING POND OF NILE TILAPIA FRY. THESIS ADVISOR:
 TAWAN LIMPIYAKORN, Ph.D., THESIS CO-ADVISOR: PROF SAY KEE
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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, to produce an all male population. 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 to MT levels as low as nanogram per liter. However, an understanding of the occurrence and fate of MT in the environment is very limited. This study investigated the biodegradation of MT and its androgenic potency by microorganisms in sediment under different electron acceptor conditions and by isolated MT-degrading bacteria from the sediment and water of a masculinizing pond. MT was degraded under aerobic, sulfate-reducing and methanogenic conditions, but it was hardly degraded under iron(III)-reducing condition and was not degraded under nitrate-reducing condition. Statistically, the first-order biodegradation rates of MT for aerobic, sulfate-reducing and methanogenic conditions were similar. However, the measured androgenic activities under methanogenic condition continued to persist for more than one month even though the measured MT concentrations were negligible. An unknown labile metabolite of MT was found under aerobic condition but not for other electron acceptor conditions. Based on the biodegradation results, it appeared that MT and its metabolites can potentially accumulate in environments under iron (III)-reducing, nitrate-reducing, and methanogenic conditions. Thirty strains of MT-degrading bacteria were isolated from the sediment and water of masculinization pond. The isolated bacteria were related to three genus including *Pimelobacter*, *Rhodococcus*, and *Nocardioides*. Three isolated strains, strains SB100-05, SB010-03, and WB100-05, were selected for further identification and characterization. Phylogenetic analysis, physiological properties and cell morphology of the three strains SB100-05, SB010-03 and WB100-05 suggested that they were closely related to *Nocardioides aromaticivorans*, *Rhodococcus equi*, and *Nocardioides nitrophenolicus*, respectively. Haldane's model was found to fit the MT degradation kinetics of the three strains indicating the possibility of substrate inhibition. Of the three strains tested, strain SB100-05 degraded MT faster but had lower resistance to high MT concentration than the other strains.

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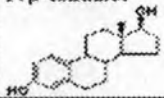
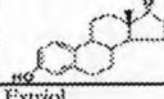
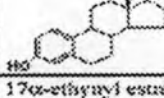
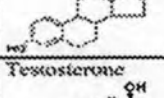
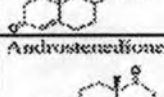
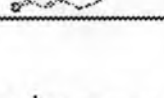
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natural estrogens (estrone, estradiol and estriol) are degraded by microorganisms in activated sludge whereas ethynylestradiol, a synthetic estrogen, has been found to persist in contact with activated sludge (Ternes *et al.*, 1999). Estradiol was found to transform to estrone in activated sludge (Ternes *et al.*, 1999) and the by-products from the biodegradation of estrone were not observed (Ternes *et al.*, 1999). Vader *et al.* (2000) studied the biodegradation of ethynylestradiol by nitrifying activated sludge

Table 2.2: Property of estrogens and androgens

Hormone	Molecular weight (g mol ⁻¹)	Water solubility (mg l ⁻¹)	Log K _{ow} ^a	MP °C ^d
17β-estradiol 	272.4	13 ^e	4.01 ^f 3.10 ^g , 3.90 ^h	173
Estrone 	270.4	13 ^h	3.43 ^d , 3.13 ^h , 3.38 ⁱ , 2.45 ^f	259
Estriol 	288.4	32 ^d	2.81 ^d , 2.6 ^g 2.55 ^e	285
17α-ethynyl estradiol 	296.4	4.8	3.87 ^d , 4.15 ^h	183
Testosterone 	288.4	18 - 25 ^g	3.22 ^e	155
Androstenedione 	286.4	37-41 ^e	NA ^g	173-174

^a Lai *et al.*, 2002; ^b Sagaya *et al.*, 2002; ^c Nuez and Yalkowsky, 1997; ^d Solubility from a control tablet comprising of estriol and alpha-cyclodextrin; ^e measured at 37 °C; ^f Suzuki *et al.*, 2001; ^g not available

(NAS) in batch experiments with different ammonia degradation rates and found that at high ammonia degradation rate (50 mg NH₄⁺g⁻¹.DW.h⁻¹), ethynylestradiol was degraded but no degradation of ethynylestradiol was observed at low ammonia

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LIST OF ABBREVIATIONS

BLAST	= basic local alignment search tool
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_{cw}	= octanol-water partitioning coefficient
L	= liter
m	= meter
mg/L	= milligram per liter
min	= minute
mM	= millimolar
MLSS	= mixed-liquor suspended solids
MT	= 17alpha-methyltestosterone
nm	= nanometer
ng/g	= nanogram per gram
ng/L	= nanogram per liter
PCR	= polymerase chain reaction
PTFE	= polytetrafluoroethylene
TEQ	= testosterone equivalent
WWTS	= wastewater treatment system