CHAPTER III

METHODOLOGY

3.1 Experimental framework

The study is focused on the biodegradation of MT by microorganisms in sediments under different electron acceptors and isolation of MT-degrading bacteria from sediment and water of masculinizing pond of Nile tilapia fry. The experiments were divided into 5 parts as shown in Figure 3.1.

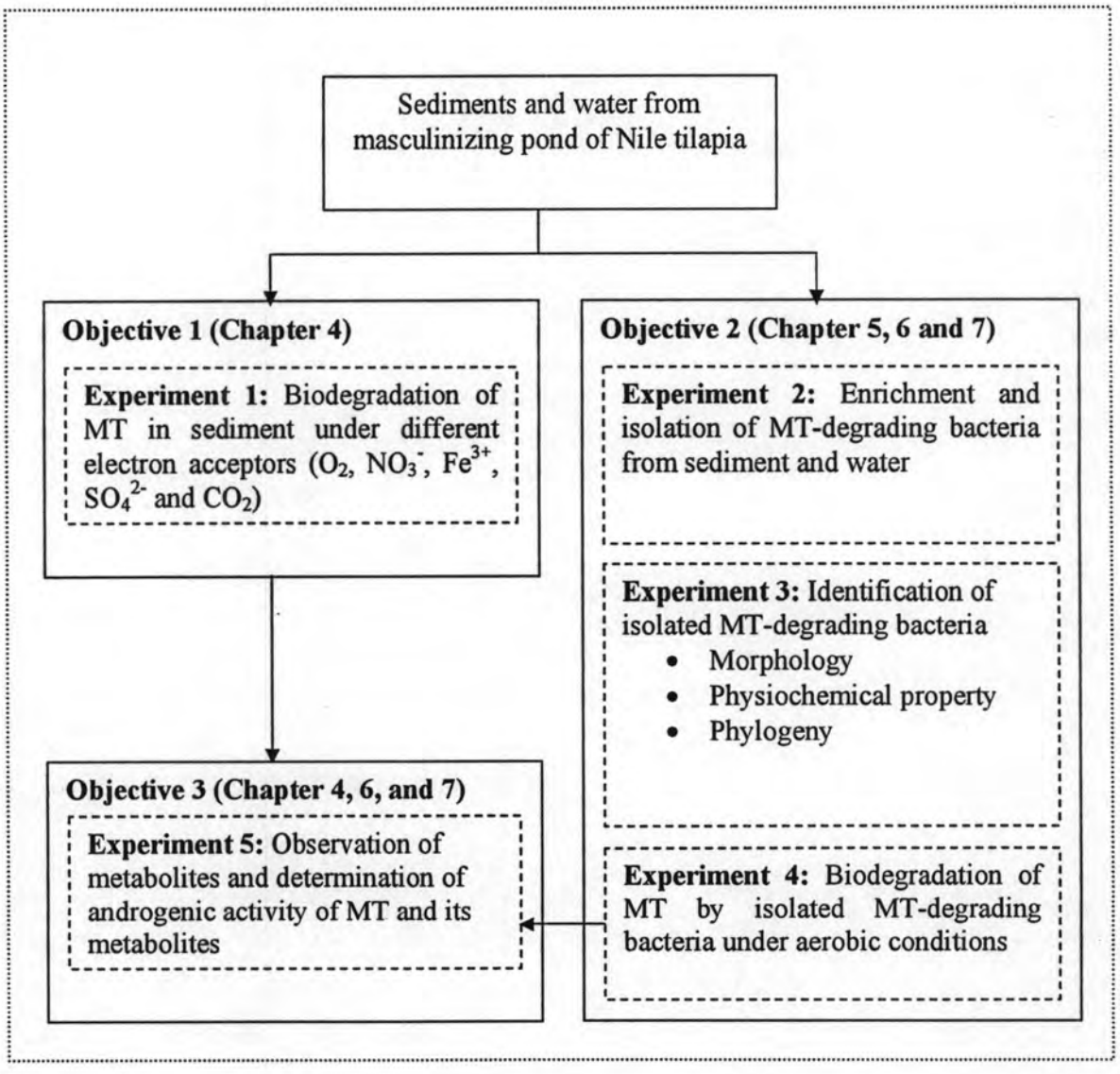

Figure 3.1: Experimental framework of study

Experiment 1 investigates the biodegradation of MT in sediment by using batch bioassays (see Chapter 4). Experiment 1 consisted of six batch experiments conducted in parallel (see Table 3.1).

Table 3.1 Six batch experiments in Experiment 1

Batch	Component	Investigation	Analysis/Collection
T1: Biodegradation test with MT	- MT (10 mg/l) - Sterilized freshwater mineral medium* - Sediment slurry (10% (v/v))	Biodegradability MT by microorganism	- Triplicate - Collect 3 bottles / time - 200-300 bottles
T2: Biodegradation test without MT	- Sterilized freshwater mineral medium* - Sediment slurry (10% (v/v))	Electron acceptor for background organic	- Duplicate - Collect 2 bottles / time - 200-300 bottles
T3: Biodegradation test with glucose	- Sterilized freshwater mineral medium* - Sedimentslurry (10% (v/v)) - Glucose (1 mM)	Available microorganism	- Duplicate - Collect 2 bottles / time - 80-100 bottles
T4: Control test with MT and sterilized sediment	- MT (10 mg/l) - Sterilized freshwater mineral medium* - Sterilized sediment slurry* (10% (v/v))	Chemical and physical transformation of MT	- Duplicate - Collect 2 bottles / time - 20-30 bottles
T5: Abiotic control test with MT	- MT (10 mg/l) - Sterilized freshwater mineral medium*	Abiotic transformation (hydrolysis)	- Duplicate - Collect 2 bottles / time - 20-30 bottles
T6: Abiotic control test with glucose	- Glucose (1mM) - Sterilized freshwater mineral medium*	Abiotic transformation (hydrolysis)	- Duplicate - Collect 2 bottles / time - 20-30 bottles
T7:** Biodegradation test with MT and denitrifying activated sludge	- MT (10 mg/l) - Sterilized freshwater mineral medium* - Sludge (10% (v/v))	Biodegradability MT by nitrate-reducing microorganism	- Triplicate - Collect 3 bottles / time - 200-300 bottles

* Autoclave at 121 °C for 15 min; ** Only nitrate-reducing condition

Then, the samples from biodegradation test (T1) at start, middle and last of incubation were selected to determine the androgenic activity by β-galactosidase assay in Experiment 5 (see Chapter 4).

According to previous experiments, MT was found to be rapidly degraded under aerobic condition. As such, isolation of MT-degrading bacteria was conducted under aerobic conditions (Experiment 2, 3, and 4).

Experiment 2 enriched and isolated MT-degrading bacteria from the sediment and water at three initial MT concentrations (1, 10, and 100 mg/L) (see Chapter 5). There were three criteria to select bacteria for further study including colony morphology, biodegradation of MT in short duration time (3 days), and partial 16s rRNA analysis. As the result from Experiment 2, the strain SB010-03, SB100-05, and WB100-05 represented MT-degrading bacteria species of *Rhodococcus equi*, *Pimelobacter simplex*, and *Nocardioides nitrophelolicus*, respectively, were selected to study in detail in Experiment 3 and 4.

MT-degrading bacterium strain SB010-03 was studied in Experiment 3 and 4 (see Chapter 6) whereas strain SB100-05 and WB100-05 were also studied in Experiment 3 and 4 (see Chapter 7). Experiment 3 identified the selected MT-degrading bacteria by investigating the bacteria morphology, physiochemical properties and phylogeny. Experiment 4 investigated the biodegradation of various initial MT concentrations (0.5, 1, 5, 10, 50, and 100 mg/L) by the isolated MT-degrading bacteria under aerobic conditions.