CHAPTER 3

EXPERIMENT AND METHODOLOGY

3.1 Materials and Method

3.1.1 Synthetic Wastewater

The compositions of synthetic wastewater were modified from van Dongen et al. (2001 a.b) and Isaka et al. (2006) [45], [46], [47], as shown in Table 3.1. By varying nitrite concentration with ammonia per nitrite ratios.

Table 3.1. Compositions of synthetic wastewater

Carbon Sources		**Trace minerals**	
$KHCO_3$	125 g/L	$Na_2O_3Se•5H_2O$	0.4 g/L
Phosphorus Sources		$MoNa_2O_4•2H_2O$	1.1 g/L
KH_2PO_4	25 g/L	$CuSO_4•5H_2O$	1.25 g/L
Nitrogen Sources		$ZnSO_4•7H_2O$	2.15 g/L
$(NH_4)_2SO_4$		$MnCl_24H_2O$	4.95 g/L
$NaNO_2$		$CoCl_2•6H_2O$	1.2 g/L
Basic mediums		$NiCl_2•6H_2O$	0.95 g/L
$MgSO_4•7H_2O$	200 g/L		
$CaCl_2$	300 g/L		
Na_2EDTA	15 g/L		
$Na_2EDTA•2H_2O$	2 g/L		
$FeSO_4•7H_2O$	1 g/L		
Trace mineral	0.1 mL/L		

3.1.2 Sequencing Batch Reactor (SBR)

The experiment used a 2-L acrylic reactor. Impeller was connected with mixer motor inside reactor as shown in Figure 3.1.

3.1.3 Peristaltic pump

Cole-Parmer (model A-07553-85) peristaltic pump was controlled at 1-100 rpm with a flow rate of 0.06-380 mL/min.

3.1.4 Stock anammox culture

The initial seed was obtained from Nongkam Activated Sludge Wastewater Treatment Plant and was screened by feeding with specific substrate for a long time.

3.1.5 Ar/CO_2 gas and regulator

The ratio of mixed gas is 95% of Argon /5% of CO_2. And the regulator model of Cole-Parmer (Argon Regulator) flow rate is 100 mL/min.

3.1.5 Analysis Instrument

N_2O level in off-gas was analysed by Gas Chromatography, Shimudsu 14B, which was equipped with a capillary column (2 columns are Porapack Q and Porapack N) and electron capture detector (ECD) was used. Temperature in column was 65°C, injection temperature was 150°C and detector temperature was 300°C.

Ammonia concentration was analysed by the phenate method, nitrite concentration was analysed by colorimetric method according to Standard Methods for Examination of Water and Wastewater [48].

3.2 Experimental Set-up

Two cylindrical vessels as duplicate with working volume of 2 L installed impeller inside were used as sequencing batch reactor (SBRs) as shown in Figure 3.1and 3.2. The reactors were operated under anaerobic system. The cycle time is 24 hours. The SBRs filled within 10 minutes while flushing with Ar/CO_2 gas. For reaction time, the reactors were operated in 23 hours and settling period was 1 hour. The experiments were done by varying

ammonia to nitrite ratio (NH_4^+-N/NO_2^--N) in the influent. N_2O concentration in gas phase was periodically monitored. Gas sample was taken from the headspace by gas tight glass syringe after 23 hours operating in each cycle and analyzed immediately. During the same day, a sample of effluent was taken, filtrated by 0.45 μm membrane filter, and NH_4^+-N, NO_2^-N were determined.

3.3 Variable parameters

The variable parameters in the SBRs operation as well as these values are shown in Table 3.2.

Table 3.2. The variable parameters used in the experiment

Experiment no.	NH_4^+:NO_2^- ratio	NH_4^+ concentration (mgN/L)	NO_2^- concentration (mgN/L)
R1(100)-1	1:1	100	100
R1(100)-2	1:1	100	100
R1(100)-3	1:1	100	100
R0.75(100)	0.75:1	100	134
R0.75(75)	0.75:1	75	100
R0.5(100)	0.5:1	100	200
R0.5(75)	0.5:1	75	150
R0.5(50)	0.5:1	50	100

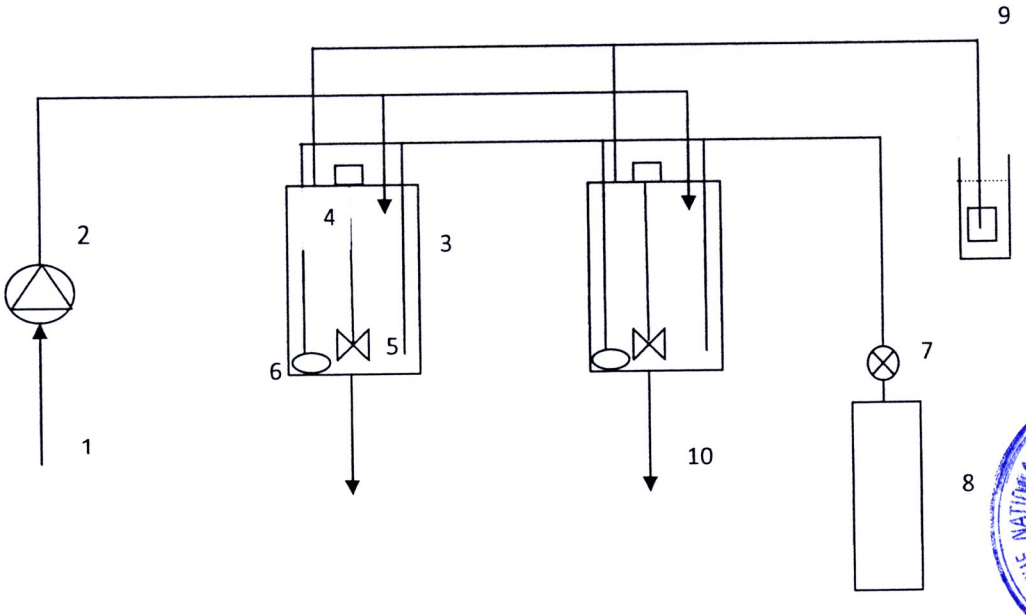

Figure 3.1. Schematic diagram of sequencing batch reactor: (1) influent synthetic wastewater, (2) peristaltic pump, (3) reactor, (4) mixer motor, (5) impeller, (6) diffuser, (7) regulator, (8) Ar/CO_2 gas, (9) gas collector, (10) effluent.

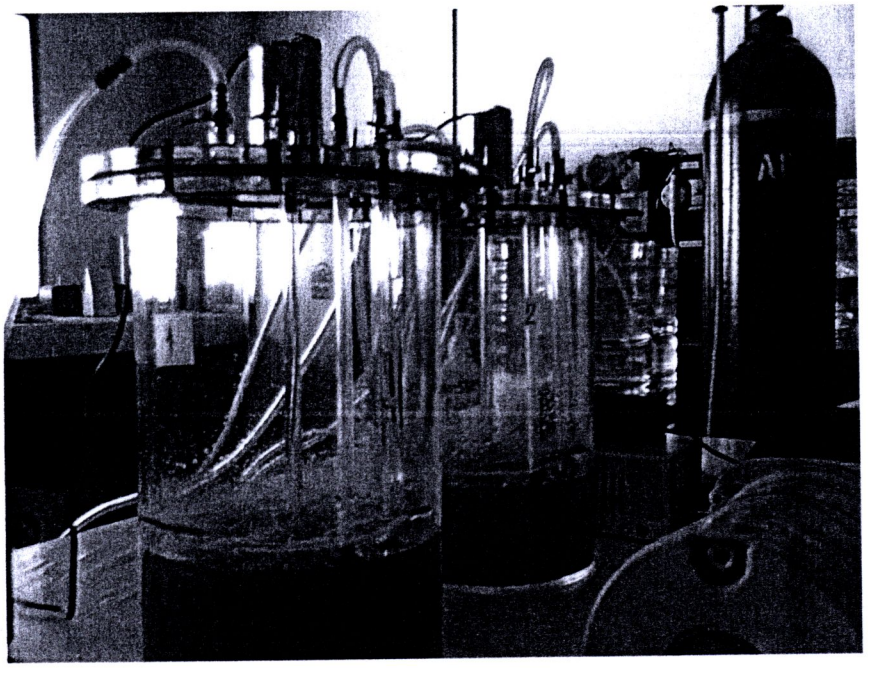

Figure 3.2. Image of sequencing batch reactor (SBR)

3.4 Microbiological Identification

The stock microorganism seed to SBRs were identified by using Fluorescence *in situ* hybridization (FISH) technique. The probes of identification are shown in Table 3.3.

Table 3.3. The probes used in the experiment

Probes	**Sequence (5'-3')**	**Target groups**	**% Formamide**	**Lable**	**Refernces**
Nsm156	TAT TAG CAC ATC TTT CGA	Ammonia oxidizing bacteria (*Nitrosomonas)*	40	Cy3	Mobarry *et al.*(1996) [28]
AMX368	CCT TTC GGG CAT TGC GAA	all Anammox organism	15	FITC	Schmidt *et al.*(2003) [31]