CHAPTER 3
METERIALS AND METHODS

3.1 Experimental setup

In this experiment, nitrification and denitrification in synthetic seawater are performed in a 60 L external loop airlift reactor packed with immobilized nitrified and denitrified plastic bioballs. The packing plastic bioballs as shown in Figure 3.1 has a diameter of 2.5 cm with surface area of 32 cm^2. The packed bed external loop airlift contactor (PBABR) is designed as one aerated column (riser) interconnected with two unaerated columns (downcomer). The heights of both aerated/unaerated columns are 1.2 m. The aerated section (riser) of the PBABR is a cylindrical column with a volume of 8 L and a diameter of 9 cm. Each unaerated column diameter is 20 cm with a volume of 37 L. The cross-sectional area of the unaerated column (A_D) is larger than the aerated (A_R) with a ratio between A_D/A_R of 9.87. This is to ensure adequate retention time for denitrification which should be about 5-10 times larger than that for nitrification (Balderston and Sieburth, 1976; Turk, 1996). The aerated and unaerated columns are connected with a 50 cm long conduits with a diameter of 4.5cm. The aerated section is packed with 200 bioballs whereas each unaerated column is packed with 2000 bioballs. This packing is supported by an aluminum perforated plate installed at the bottom of the column. A gas sparger for air dispersion in the PBABR is located at bottom of aerated column. The air flow rate is determined as a minimum that can induce liquid circulation between aerated and unaerated sections. This was observed to be about 0.66 m^3/h and keep constant throughout all experiments in this work. The dissolved oxygen (DO) in the column is measured by a DO meter (Hanna HI 964400) while the oxidation/reduction potential (ORP) is by an ORP meter (Hanna HI 98240). The schematic diagram of the experimental setup for this experiment is shown in Figure 3.2.

3.2 Experimental procedures for Nitrification and Denitrification experiment

3.2.1 Preparation of immobilized nitrifiers/denitrifiers

Denitrifying bacteria are immobilized on plastic bioballs by immerging plastic bioballs in a shrimp cultured pond for 2 weeks. The immobilized plastic bioballs are tested for denitrifying bacteria by inspecting DO consumption in synthetic seawater. This is performed simply by immerging plastic bioballs into a small bottle with synthetic seawater in which the level of DO is monitored. The decrease in DO in this synthetic seawater indicates that denitrifying bacteria are immobilized on the surface of plastic bioballs.

3.2.2 Nitrification/Denitrification experiment

1) Prepare synthetic seawater with salinity of about 30 ppt.
2) Fill 60 L of synthetic seawater in pack bed airlift bioreactor (PBABR, Fig. 3.2).
3) Add ammonium chloride (NH_4Cl) at 8.7 mg NH_4-N/L or potassium nitrate (KNO_3) at 10 mg NO_3-N/L for initial nitrogen compound.
4) Allow synthetic seawater to mix in PBABR for 1-2 h.
5) Add methanol into the system. This serves as a source of carbon source for the bacteria.
6) Take 50 mL sample from the three sampling port for the determination of initial concentration of ammonium-nitrogen (NH_4-N), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) by Strickland and Parson 1972 method (see 3.2.3).
7) Regularly monitor temperature (^oC), DO (by Hanna HI 964400), pH and ORP (by Hanna HI 98240) in both aerated and unaerated columns.
8) Take samples from both aerated and unaerated columns for the daily measurement of NH_4-N, NO_2-N and NO_3-N.

During the experiment, make-up seawater is added to replace the volume lost in sampling procedure. Evaporation loss is replaced by distilled water.

3.2.3 Analytical methods for measuring nitrogen compounds (Strickland and Parson, 1972)

A. Ammonium-nitrogen concentration measurement

Reagent solutions

1) De-ionized water
2) Phenol solution
- Dissolve 20 g of crystalline phenol in 200 mL of 95 % v/v ethyl alcohol.
3) Sodium nitroprusside solution
- Dissolve 1.0 g of sodium nitroprusside ($Na_2Fe(CN)_5NO.2H_2O$) in 200 mL of de-ionized water. Store in an amber bottle .The solution is stable for at least 1 month.
4) Alkaline reagent
- Dissolve 100 g of sodium citrate and 5 g of sodium hydroxide (analytical reagent grade) in 500 mL of de-ionized water. This solution is stable indefinitely.
5) Sodium hypochlorite solution (1.5N)
6) Oxidizing solution
- Mix 100 mL of reagent 4 and 25 mL. of reagent 5. Keep this solution stoppered while it is not in use. Prepare fresh everyday.

Procedure

1) Add 5 mL of sample to a tube from a 5 mL pipette.
2) Add 0.2 mL of phenol solution, from a pipette, swirl the solution.
3) Add 0.2 mL of sodium nitroprusside solution and 0.5 mL of oxidizing solution.
4) Mixing after each addition.
5) Allow the tube to stand at temperature between 20-27 °C for 1 h. The top of the tube should be covered with aluminum foil at this storage to lessen the contamination by atmospheric ammonia.
6) Read the absorbance at 640 nm in a spectrophotometer (Spectronic 20 Genesys) against distilled water using 10-cm cells.

7) Correct the measured absorbance by that of a reagent blank and read the ammonia-nitrogen concentration from the standard calibration curve.

Blank

- Carry out the method exactly as described in 1) to 6) above using freshly de-ionized water. Blank absorbance using a 10-cm cell should not exceed 0.075.

Calibration

Standard ammonia solution

- Dissolve 0.1 g. of ammonia sulfate in 1000 mL. of de-ionized water.
- Add 1 ml of chloroform.
- Storage this solution in refrigerant (This solution is stable).

The ammonia concentration

$$1 \text{ mL} = 0.021 \text{ mg of } NH_3\text{-N}$$

B. Nitrite-nitrogen concentration measurement

Reagents

1) Sulphanilamide solution

- Dissolve 5 g of sulphanilamide in a mixture of 50 mL of concentrated hydrochloric acid and 300 mL of distilled water.
- Dilute to 500 mL with water (the solution is stable for many months).

2) N-(1-Naphthyl)-ethylenediamine dihydrochloride solution (NNED solution)

- Dissolve 0.5 g of dihydrochloride in 500 mL of distilled water.
- Store the solution in a dark bottle. (The solution should be renewed once a month).

Procedure

1) Add 5 mL of sample to the tube with a 5 mL pipette.
2) Add 0.1 mL of sulphanilamide solution, from automatic pipette to each sample, mix, and allow the reagent to react for between 2 and 8 min.
3) Add 0.1 mL of naphthylenediamine solution and mix immediately. Between 10 min and 1 h afterwards measure the absorbance at 543 nm in a spectrophotometer.

4) Correct the measured absorbance by subtracting reagent blank and read the nitrite-nitrogen concentration from a standard calibration curve.

Calibration

Standard nitrite solution

- Dry anhydrous sodium nitrite ($NaNO_2$) at 110 ^{o}C for 1h.
- Dissolve 0.345 g in 1000 mL of distilled water
- Add 1 mL of chloroform as a preservative.
- Store the solution in a dark bottle (the solution is stable for at least 1-2 months).

The nitrite concentration

$$1\ mL = 0.005\ mgNO_2\text{-}N$$

Blank

Synthetic seawater solution

- Dissolve 310 g of sodium chloride (NaCl), 100 g of magnesium sulphate ($MgSO_4.7H_2O$) and 0.5 g of sodium bicarbonate ($NaHCO_3.H_2O$) in 10 L of distilled water.

C. Nitrate-nitrogen concentration measurement

Reagents

1) Sulphanilamide solution
2) N-(1-Naphthyl)-ethylenediamine dihydrochloride solution
3) Cadmium-copper column
4) Concentrated ammonium chloride solution

- Dissolve 125 g of analytical reagent quality ammonium chloride in 500 mL of distilled water and store in glass or plastic bottle.

5) Diluted ammonium chloride solution

- Dilute 50 mL of concentrate ammonium chloride solution to 2000 mL with distilled water. Store the solution in a glass or plastic bottle.

Procedure

1) Add 2 mL of concentrated ammonium chloride to the 50 mL sample in Erlenmeyer flask. Mix the solution and pour about 5 mL of this sample onto the top of column and allow it to pass through.
2) Add the remainder of the sample to the column and place the drained Erlenmeyer flask under collection tube to collect sample that passes through the column.
3) As soon as possible, use the procedure for nitrite measurement (3.3.5 (B) from 1-4).
4) The nitrate-nitrogen concentration can be calculated from the differential of nitrite concentration before it passes through the column (from the nitrite measurement procedure).

Calibration

Standard nitrate solution

- Dissolve 1.02 g of analytical reagent quality potassium nitrate (KNO_3) in 1000 mL of distilled water (the solution is stable indefinitely in the absence of evaporation).

The nitrate concentration

$$1 \text{ mL} = 0.01 \text{ mg } NO_3\text{-N}$$

Blank

Synthetic seawater solution

- Dissolved 310 g of sodium chloride (NaCl), 100 g of magnesium sulphate ($MgSO_4.7H_2O$) and 0.5 g of sodium bicarbonate ($NaHCO_3.H_2O$) in 10 L of distilled water.

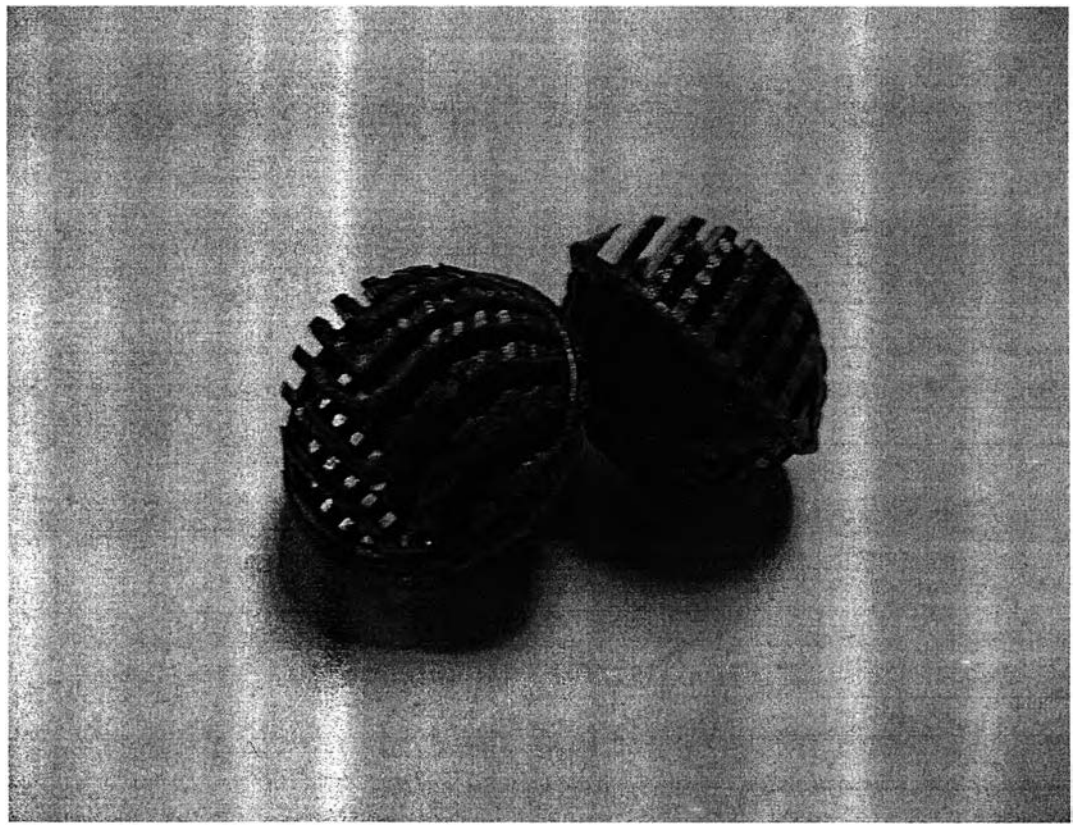

Figure 3.1 Plastic bioballs for immobilized nitrifying/denitrifying bacteria

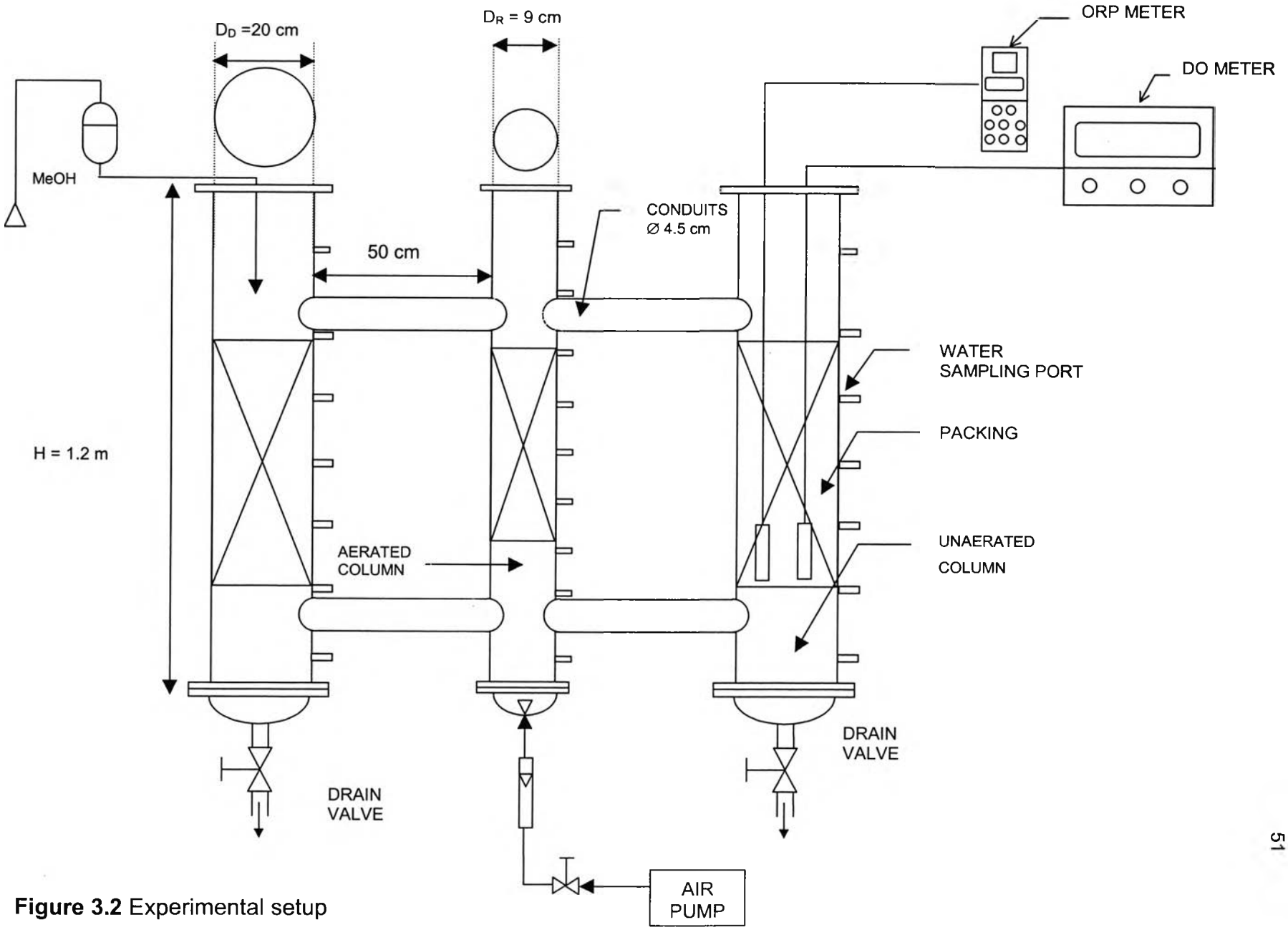

Figure 3.2 Experimental setup