CHAPTER 3

Materials and Method

3.1 Instrument, Apparatus and Chemicals

3.1.1 Instruments

1. Analytical balance , Mettler Toledo AB204-S, USA
2. UV/Visible Spectrophotometer , Lambda 25, Perkin Elmer Instruments, Serial No. 101 N1121004, USA
3. Air ventilation oven , Whatman International Ltd., England
4. Variable peristaltic pump ,Lead fluid BQ80S Micrometer Speed, China
5. Submerged pump , RESUN SP-6000 , China
6. Air Pump , Jeneca AP-12000 , China
7. Gast High-Capacity Vacuum Pump, Cole-Parmer ,USA
8. Vacuum suction filter device , Kicute, China
9. Multi-parameter analyzer , Consort C 533 version 2.2
10. Hot plate magnetic stirrer, Cenco instrument, Netherland
11. Refrigerated Incubators , The Lab Depot, USA

3.1.2 Apparatus

1. Thermometer
2. 300 mL Winkler bottle,
3. 50mL , 100 mL ,250mL ,500mL, and 1000mL Beakers , Pyrex, Witeg, Germany
4. 50 mL , 250mL, and 500mL Volumetric flasks , Thechnico , England
5. 1mL, 5mL, and 10mL Pipette; Pyrex, Germany
6. 1mL and 5mL micropipette , Finnpipette® , Finland
7. 100mL and 250mL Erlenmeyer flasks , Pyrex, Germany
8. 25mL, 50mL,100mL, and 1000mL measuring cylinders, Pyrex, Germa

9. 50 mL Burette , Pyrex, Germany
10. 20 mL Test tube, Witeg, Germany
11. 500mL Plastic sampling bottle
12. Filter papers, No.1, Whatman, Cat No. 1001 090, Whatman International.
13. Laboratory sealing film ,Parafilm –M, Thailand
14. Aluminum foil, Diamond, USA
15. Hand gloves
16. Forceps
17. Scissor

3.1.3 List of Chemicals

3.1.3.1 Chemicals for BOD analysis

1. Manganese (II) sulfate monohydrate Lab ($MnSO_4.H_2O$)
2. Sodium hydroxide 97% Lab (NaOH,)
3. Sodium iodide Lab (NaI)
4. Sodium azide Lab (NaN_3,)
5. Starch soluble Lab $(C_6H_{10}O_5)_n$
6. Salicylic acid, ($C_7H_6O_3$)
7. Sodium thiosulfate pentahydrate ($Na_2S_2O_3.5H_2O$)
8. Sulfuric acid 98%, (H_2SO_4)
9. Potassium iodide (KI)
10. Potassium hydrogen di iodate ($KH(IO_3)_2$)

3.1.3.2 Chemicals for orthophosphate analysis

1. Phenolphthalein indicator aqueous solution.
2. Ammonium molybdate 4-Hydrate ((NH_4) 6 Mo_7O_{24} : $4H_2O$)
3. Tin (iii) chloride hydrated ($SnCl_2$: $2H_2O$)
4. Potassium dihydrogen orthophosphate anhydrous (KH_2PO_4)
5. Refined Glycerin

3.1.3.3 Chemicals for ammonium nitrate analysis

1. Potassium nitrate (KNO_3)
2. Ammonium sulfate (($NH_4)_2$ SO_4)
3. Sodium salicylate ($C_7H_5NaO_3$)
4. Tri-sodium citrate($C_6H_5Na_3O_3$)
5. Sodium nitroprusside ($Na_2[Fe(CN)_5NO].2H_2O$)
6. Dichloroisocyanuric acid Sodium dihydrate ($C_3Cl_2N_3NaO_3.2H_2O$)

3.2 Pilot Scale Reactor Design

Pilot-scale AS- FAMCW was established and maintained in a greenhouse facility at Biology Department, Chiang Mai University. Figure 3.1 shows the schematic diagram of the pilot scale reactor used in this study. The aeration tank, settling tank and FAMCW were made of plastic with a capacity of 5.3, 6.0 and 47.6 L, respectively. PVC pipes were used to connect the system. An air pump and a set of diffusion aerators were used to aerate the aeration tank. Peristaltic pump was used to automatically supply wastewater to the reactor from the feed tank and sludge was 100% recycled. Aerobically digested sludge obtained from a CMU Wastewater Treatment Plant used as inoculum. Wastewater generate from Biology Department cafeteria was used to feed the reactor.

Water hyacinth *(Eichhornia crassipes)* was collected from CMU reservoir and cultured in a plastic bowl at the greenhouse of Department of Biology, Faculty of Science, CMU for two weeks. The whole plants were directly transferred to the reactor. Hence, Sixty percent of the water surface was covered by water hyacinth initially and maintained throughout the experiment.

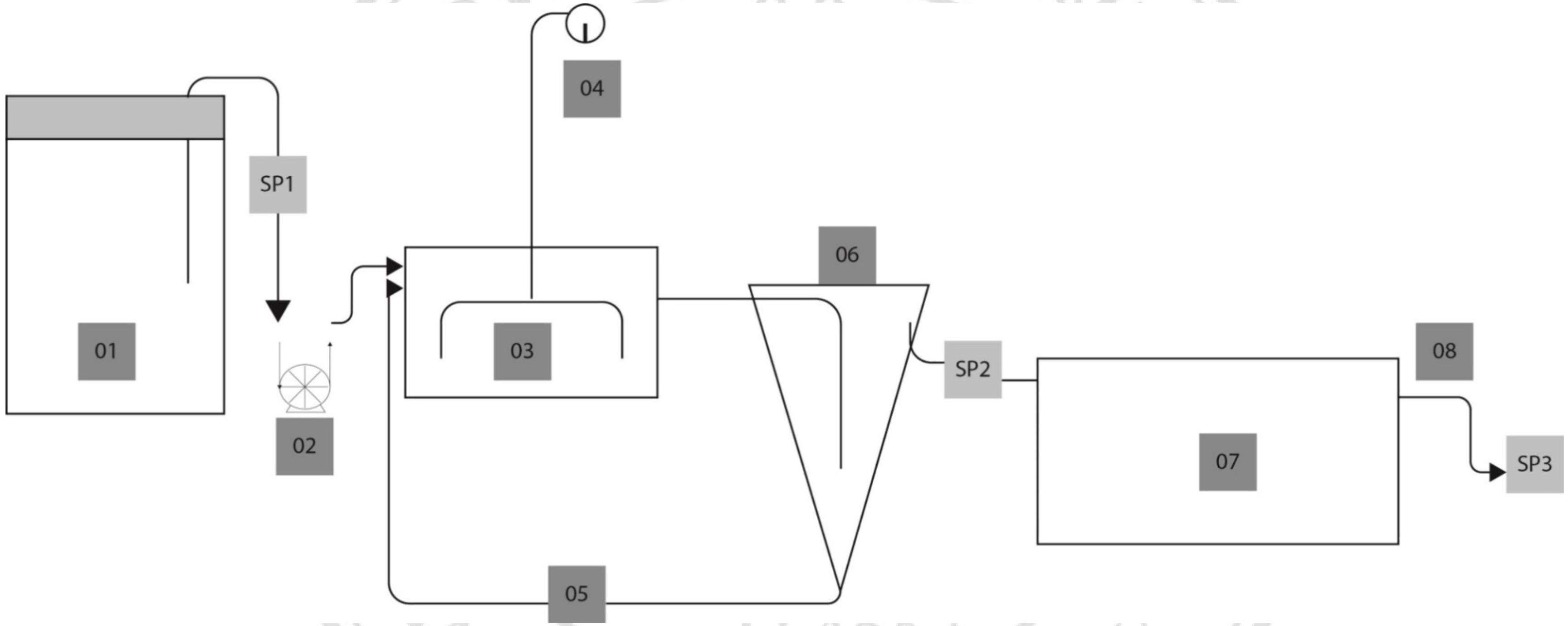

Figure 3.1 Schematic diagram of pilot scale reactor

01. Storage tank	02. Parasitic pump	03. Aeration tank	04. Air pump
05. Return activated sludge (100%)	06. Secondary clarifier	07. Free floating macrophyte constructed wetland	
08. Outlet	SP1 – Sampling point 01	SP2 – Sampling point 02	SP3 – Sampling point 03

Table 3.1 Specifications of aeration tank , clarifier unit and FAMCW unit.

Specification	**Aeration Tank**	**Clarifier unit**	**FFMC wetland unit.**
Shape of the reactor	Rectangular	Conical	Rectangular
Material of construction	Plastic	Plastic	Plastic
Size of the reactor / Diameter	0.2 m x 0.2 m x 0.2 m	0.2m(top); 0.025m(bottom)	1.0 m x 0.5 m x 0.5 m
Height	-	0.25m	-
Inlet	One	One	One
Outlet	One	One	One
Total volume	6.5 L	6.2 L	56 L
Working volume	5.3 L	5.0 L	48 L

3.3 Operation of Reactor and Water sampling

Wastewater was pumped up by using submerged water pump. Posterior end of the plastic pipe was connected to the submerged water pump and anterior end was connected to 50L storage tank. Before pouring the wastewater into the storage tank, wastewater was filtered using 0.5mm mesh to avoid food particles. Then pilot scale reactor was fed by stored wastewater with different flow rates. Flow rates were controlled by peristaltic pump. Flow rate was adjusted by changing of RPM (Round per minute) of motor. Each flow rate was determined by calibration curve (Figure 3.2).

Volume of aeration tank was constant and different HRT was obtained by changing of influent flow rate.

$$HRT(T) = \frac{Volume\ of\ aeration\ tank\ (L^3)}{Influent\ flow\ rate\ (\frac{L^3}{T})} \qquad \text{Eq.3. 1}$$

Where, L = length, T = time

The reactor was operated under the hydraulic loading rates of 14.72, 7.36, and 4.91 mL/min to acquire the 6, 12 and 18 h of HRT, respectively. Peristaltic pump was weekly calibrated to ensure the constant flow rate (Figure 3.2 and Table 3.2) and relatively constant concentration of anti-foaming agent was mixed with raw wastewater (10-20 ppm). Dissolved oxygen concentration in aeration rank was maintained within a range of 2-3 mg/L. Airflow rate was regulated by air control valve attached to the air compressor. 500 mL of water samples were collected from the inlet (SP1), outlet of the AS (SP2) and outlet of FAMCW (SP3) (Figure 3.1). Three replicates (n=3) were collected from each sampling point Water samples were collected at every three days until reactor reach steady-state condition and sampling was sustained from July 2017 to September 2017.

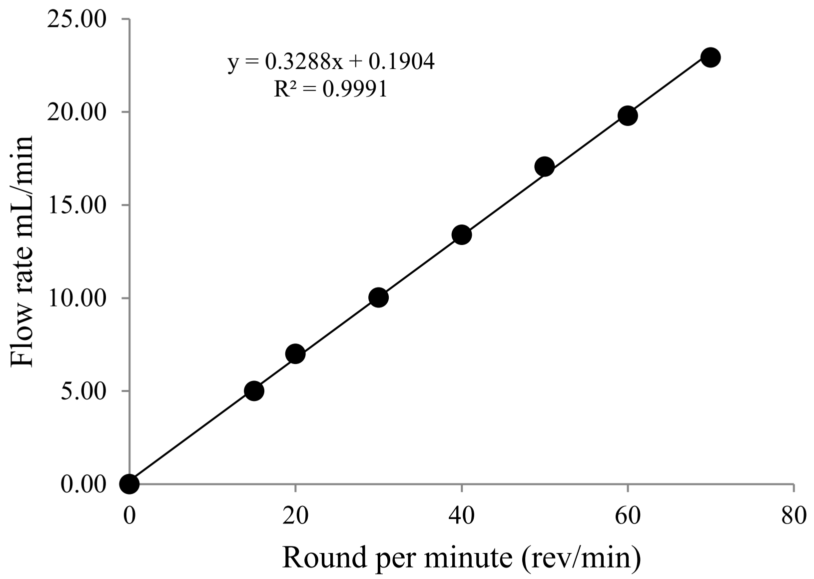

Figure 3.2 : Calibration curve for determine the flow rate

Table 3.2 RPM value set up for each HRT value

HRT	Flow rate (± 0.5) mL/min	RPM
6	14.72	44.2
12	7.36	21.8
18	4.91	14.3

3.4 Water Sample Analysis

3.4.1 Preparation of standard solution

The standard solution for each nutrient parameter was prepared. The concentration of standard solution was depending on the concentration of the water samples. Working standard solutions were prepared by successive dilution of the standard stock solution. All of the standard solutions were kept at 4ºC in refrigerator.

3.4.2 Analysis of water sample and relative growth rate

Collected samples were analyzed for temperature, electrical conductivity, pH, biological oxygen demand (BOD), total suspended solid (TSS), nitrate nitrogen (NO_3-N), ammonium Nitrogen (NH_4 - N) and ortho – phosphate (O-PO_4) using standard methods (APHA, 2005) (Table 3.3). The relative growth rate of water hyacinth was measured fallowed using Jampeetong & Brix, (2009).

Table 3.3 Parameters and methods used to analyze water samples.

Parameter	Methods /Instrument
Temperature	Thermometer
Electrical Conductivity	Multi-parameter analyzer
pH	Multi-parameter analyzer
Total Dissolved Solid	Multi-parameter analyzer
Total Suspended Solid	APHA 2540 B. Total Solids Dried at 103–105°C
Biological Oxygen Demand	APHA 4500-O C - Azide Modification Method
Nitrate Nitrogen (NO_3 - N)	UV Method (Madsen & Cedergreen, 2002)
Ammonium Nitrogen(NH_4 - N)	Modified Salicylate Method , (Quikchem method no. 10-107-06-3-B)
Ortho -Phosphate(o-PO_4)	APHA 4500-P D – SnCl2 Method

3.4.3 Removal Efficiency Calculation

The removal of TDS, BOD, TSS, NO_3-N, NH_4-N and *o*-PO_4 in the AS-FWSCW system refers to the difference between the influent (C_{in}) and effluent (C_{out}) concentrations of wastewater. Thus, the removal efficiency (R_r) of is expressed by (1):

$$R_r = \frac{C_{in} - C_{out}}{C_{in}} \qquad \text{Eq.3. 2}$$

Where, C_{in} = Inlet concentration (mg/L)

C_{out} = Outlet concentration (mg/L)

3.4.4 Statistical Data Analysis

All the statistical analyses were performed with the R statistical software version 3.3.1. All variables were tested for normal distribution and variance homogeneity, using the Kruskal-Wallis (Kruskal & Wallis, 1952) nonparametric statistical test followed by Dunn's (Dunn, 1964) multiple comparison test when assumptions on the normal distribution and variance did not meet. When necessary, logarithmic transformations were used to achieve normal distributions. All statistical tests were performed considering a 5% significance level. The p-values was adjusted using Benjamini-Hochberg method (Benjamini & Hochberg, 1995). Principal component analysis was performed to determine the relative importance of each variable. Principal component analysis (PCA), as a nonparametric method of classification, used to define the linear combination of optimally-weighted observed variables.

3.4.5 Mathematical Modeling for BOD Degradation

Mass balance equation and 4th Order Runge – Kutta Method was used to perform the mathematical model for BOD degradation at activated sludge unit.

3.4.6 Standardizing of Inlet Pollutant Concentrations.

AS- FAMCW reactor was fed by wastewater in different time intervals. Inlet concentrations of pollutant consisted in wastewater varied, hence concentrations were normalizes by calculating area under the concentration fluctuation curves. Results shows that inlet concentrations were ranged at 95% confidence level and average concentrations were used to calculate the removal efficiencies of each pollutant (Table 3. 4).

Table 3.4 Average inlet concentrations of pollutants at 95% confidence range

HRT	Average inlet concentrations (mg/L)				
	BOD	TSS	NO_3-N	NH_4-N	O-PO_4