CHAPTER III
EXPERIMENTAL

3.1 Materials and Equipment

3.1.1 Sludge

Bacterial sludge was collected from the activated sludge wastewater treatment plant treating a biodiesel wastewater of Thai Oleochemical Company Limited (TOL). The pH and total suspended solids (TSS) of the sludge were 7.4 and 64.68 g/L, respectively. This sludge sample can used as the seed sludge to start up SBR units in this study.

3.1.2 Biodiesel Wastewater

In the production process of Bangchak refinery plant, biodiesel was produced form spent cooking oil by reacting with ethanol and NaOH was used as the catalyst. This biodiesel production process is known as tranesterification reaction. After complete reaction, the mixture was washed with water for 5 times. Hence, there were five wastewater streams which their COD values were 278,000, 49,000, 19,000, 5,000, and 465 mg/L. In this study, only the wastewaters from the fourth and fifth rinses were taken and diluted by deionized water to have a COD of about 600 mg/L and BOD of about 280 mg/L which was used as the feed.

3.2 Methodology

3.2.1 SBR Set-up and Operation

To start-up the SBR units, an quantity of the seed sludge was added to obtain a microbial concentration of about 20 g/L. The SBR units were operated at different COD loading rates in the range of 0.05–0.60 kg/m^{3}d and a constant feed COD of 600 mg/L. Each reactor was operated at 4 cycles per day. Each cycle consisted of filling, aeration, settling, and decanting. Table 3.1 shows the time period in each cycle for the SBR operation in this study. There were 3 timers to be used to control each step of operation. These 3 timers were set in sequence, starting with the timer number 1 which was used to control filling process by sending signal to

operate the diaphragm pump. When the volume of mixed solution reached the desired level, the level controller sent the signal to the diaphragm pump to stop filling. The second timer was then started to control the aeration step by turning on the air pump to aerate the system. After the aeration time was over, the air pump was stopped and the system entered the settling step for about 1 hour. Next, the timer number 3 started the decanting step by sending the signal to open the solenoid valve to drain the settled effluent out of the column to the effluent tank. After the time set was over, the solenoid valve was closed. The flow diagram of the studied SBR unit is shown in Figure 3.1 and Figure 3.2.

Table 3.1 The period of time per cycle in SBR operation

Process	Time
Filling (min)	30
Aeration (h)	4
Settling (h)	1
Decanting (min)	30

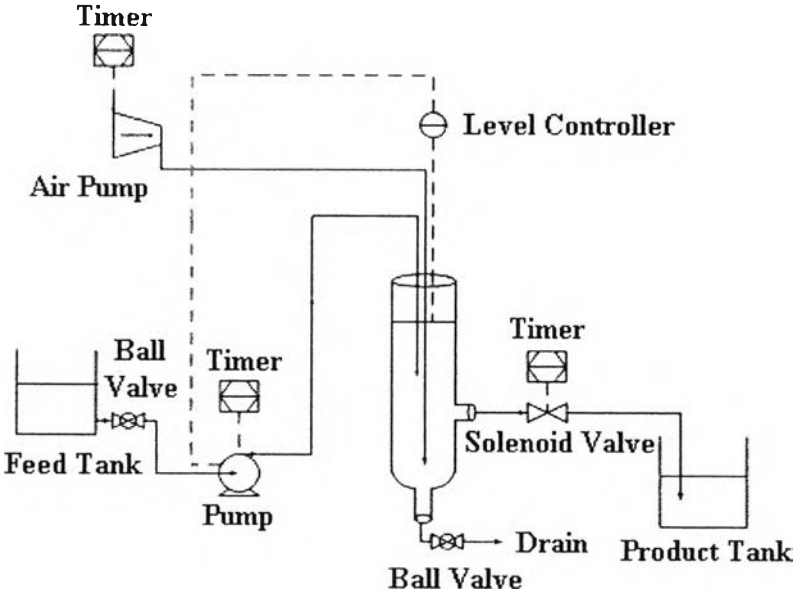

Figure 3.1 Flow diagram of SBR system.

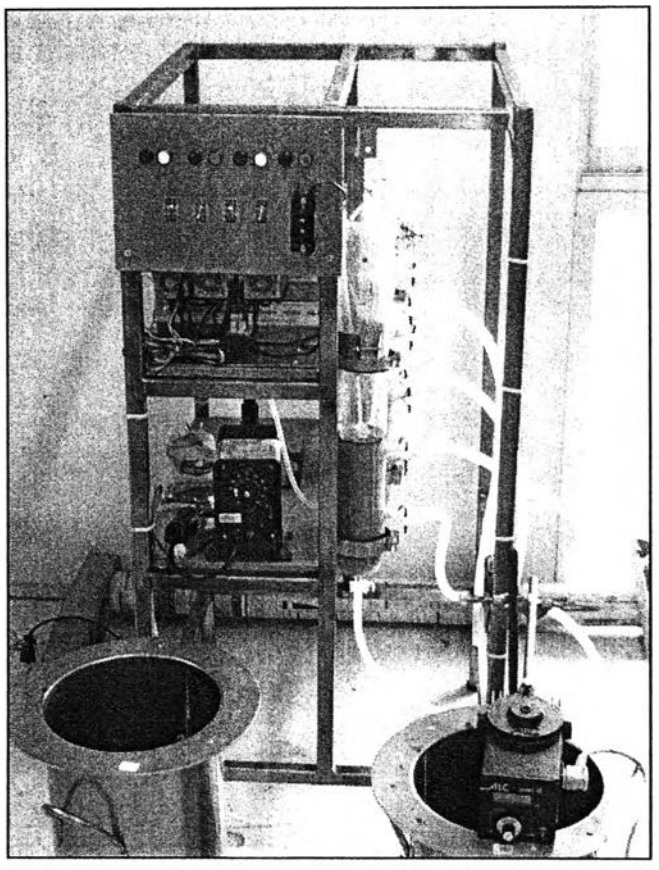

Figure 3.2 Photograph of the studied SBR system.

3.2.2 Aerobic Biodegradation Experiments

The dilute biodiesel wastewater was diluted by deionized water to have a constant COD value of about 600 mg/L which was used as the feed solution to the SBR units. Since this wastewater lacked of nitrogen and phosphorus, ammonium hydrogen carbonate (NH_4HCO_3) and di-potassium hydrogen orthophosphate (K_2HPO_4) were added to have a ratio of COD:N:P equal to 100:2.5:0.5. After that, the prepared wastewater was fed into the reactor during the filling step. The SBR columns already contained the acclimatized sludge. The studied SBR units were operated at different COD loading rates at a constant cycle time of 4 cycles per day and at room temperature (25-27°C). Table 3.2 shows the conditions of different COD loading rates used in the biodegradation study.

Table 3.2 Volume of filling or decanting at each COD loading rate using 4 cycles per day (A constant feed COD of 600 mg/L)

COD Loading Rate (kg/m^3d)	Filling and Decanting Volume (mL/cycle)	Filling and Decanting Flow Rate (mL/d)
0.050	31.25	125
0.075	46.875	187.5
0.100	62.5	250
0.200	125	500
0.400	250	1,000
0.600	375	1,500

For each COD loading rate, the SBR unit was operated to reach steady state before taking samples for analysis. The steady state was assumed when the effluent COD did not vary with time. The feed and effluent samples were taken for analysis of chemical oxygen demand (COD), biochemical oxygen demand (BOD), and suspended solids (SS). The analysis methods used in the present work were followed the standard methods (Hinchee *et al.*, 1991). The analysis data were averaged from at least 3 times and the average values were used to assess the process performance.

3.2.2.1 *COD Analysis Method*

Chemical oxygen demand (COD) is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence. So, COD method is often used as a measurement of pollutants in wastewater and natural waters. A COD reactor (HACH) equipped with a user selectable temperature setting was used for the chemical oxidation reactions at 150°C. The internal temperature sensor was used to prevent over-heating. It was used to digest up to 25 samples simultaneously for 120 min with audible alarm and auto-off feature. For the analysis, 2.5 mL of sample, 1.5 mL of digestion reagent (K_2CrO_7), and 3.5 mL of sulfuric acid reagent

were added to a digestion vial. For the digestion reagent was prepared by adding 10.216 g K_2CrO_7 (primary standard grade, which had been dried at 103°C for 2 h into 500 mL of distilled water. For the acidic reagent, 33.3 g $HgSO_4$ was added into 167 mL 98% H_2SO_4. The mixture was left for complete dissolution, cooled to room temperature, and finally diluted to 1 L. After that, Ag_2SO_4 (reagent grade) was added into the acidic solution to obtain a weight ratio of Ag_2SO_4 to H_2SO_4 of 5.5 g to 1 kg. The mixture was left to stand for 1 to 2 days to completely dissolve Ag_2SO_4.

After adding all reagents, the digestion vial was inverted several times to homogeneously mix the content, and it was then placed in the preheated COD reactor for 2 h and then left for about 20 min to cool. After that, a spectrophotometer (HACH DR 2700) was used to measure the absorbance at the wavelength range of 410 to 610 nm with accuracy of ±1.5 nm to indicate the amount of K_2CrO_7 used to oxidize all organics in the sample.

3.2.2.2 *TOC Analysis Method*

A wastewater sample was first filtered through a filter paper (Whatman 42, size 70 mm) to remove all the microbial cells and suspended solids and the filtrate was injected into a TOC analyzer (Shimadzu, 500A).

3.2.2.3 *BOD Analysis Method*

Biochemical Oxygen Demand (BOD) in a measuring parameter to indicate the amount of biodegradable organic matters present in a wastewater sample. BOD is expressed as weight of oxygen consumed per unit volume of the wastewater sample during 5 days at 20°C. In this study, a manometric BOD analyzer was used to measure the BOD of samples. For all BOD analysis, a sample was added with 4 standard nutrient solutions to ensure the test sample having sufficient amount of all nutrient elements throughout the test period. The preparation methods of the four nutrient solutions were as follows:

Solution A: 0.25 g of ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) was added in 1 L of distilled water

Solution B: 27.5 g of calcium chloride anhydrous ($CaCl_2$) was added in 1 L of distilled water

Solution C: 22.5 g of magnesium sulphate heptahydrate ($MgSO_4 \cdot 7\ H_2O$) was added in 1 L of distilled water

Solution D (buffer): 8.5 g of potassium monobasic phosphate (KH_2PO_4), 33.4 g of di-sodium phosphate heptahydrate ($Na_2HPO_4 \cdot 7\ H_2O$), 21.7 g of di-potassium phosphate (K_2HPO_4), and 1.7 g of ammonium chloride (NH_4Cl) were added in 1 L of distilled water.

A sample size of a sample which depends on its BOD valve was added into a BOD bottle. Table 3.3 shows a volume of sample at different BOD ranges recommended further BOD analysis. A KOH was used to adsorb the CO_2 produced during the incubation period. The BOD valve was directedly measured from the pressure drop shown in the manometer scale.

Table 3.3 The volume of sample each BOD scale

BOD scale (mg/L)	Volume of sample (mL)
0-1,000	100
0-600	150
0-250	250
0-90	400

3.2.2.4 *MLSS Measurement*

Mixed liquid suspended solids (MLSS) can be used to represent a microbial concentration in a bioreactor. A sample taken from the SBR during the aeration step was filtered through a filter paper (PALL type A/E, size 47 mm). The filter paper was dried in an oven at constant temperature 105°C for 1 h according to the standard methods (Eaton *et al.*, 2005). The MLSS is calculated as shown in the following equation.

$$MLSS\ (mg/L) = \frac{(A-B)\ x\ (1{,}000)}{Sample\ Volume\ of\ Wastewater\ (mL)} \quad (3.1)$$

where:

A = Weight of filter paper after drying mg and

B = Weight of filter paper before use mg

3.2.2.5 *SS Analysis*

Effluent suspended solids (Effluent SS) can be used to indicate the microbial cell wash-out from wastewater treatment systems. An effluent sample taken during the decanting step was filtered using a filter paper (PALL type A/E, size 47 mm). The filter paper was then dried in the oven at a constant temperature 105°C for 1 h. The SS value is calculated by the following equation.

$$Effluent\ SS\ (mg/L) = \frac{(A-B)x1{,}000}{Sample\ Volume\ of\ Wastewater\ (mL)} \quad (3.2)$$

where:

A = Weight of filter paper after drying mg and

B = Weight of filter paper before use mg