CHAPTER THREE : MATERIALS AND METHODS

3.1 Materials

3.1.1 Jar Test Apparatus

The Chemix Floc Tester, Model CL6, equipped with mechanical stirrers, speed controller and timer was used as a jar test apparatus in the precipitation/flocculation study for the removal of zinc from the wastewater.

3.1.2 Atomic Absorption Spectrophotometer

The Unicam Model Solar 939 Atomic Absorption Spectrophotometer (AAS) was used to determine the concentration of zinc in the wastewater samples.

3.1.3 Water Bath

The Grant Water Bath, Model W 28 was used to vary the temperature of the wastewater to be fed into the upflow anaerobic filter.

3.1.4 Upflow Anaerobic Filter

A laboratory scale upflow anaerobic filter of 12.0 L working volume (see *Figure 11* and *Plate 1*) was used in the present study. The set up was located at Green & Smart Sdn. Bhd. laboratory. The reactor contains reticulated polyurethane (PU) foam as its packing media. The PU foam which was obtained from Recticel company of Wetteren , Belgium has a porosity of about 97% and a specific surface area of about 600 m^2/ m^3 (Poels *et al.,* 1984).

3.1.5 Precipitant/Flocculant

Sodium sulphide (dark brown crystals) and polyelectrolyte LT 27 (white crystals) were used as a precipitant and a flocculant respectively in the study of zinc removal from the wastewater samples. Polyelectrolyte LT 27 is a high molecular weight (8 - 10 million) polymeric flocculating agent of anionic character. Stock solutions of 10,000 mg/L of sodium sulphide and 500 mg/L of polyelectrolyte LT 27 were prepared separately by dissolving 10.0 g of sodium sulphide and 0.5 g of polyelectrolyte LT 27 respectively in 1L of distilled water. These stock solutions were used to prepare the required dosage of precipitant/flocculant in the study.

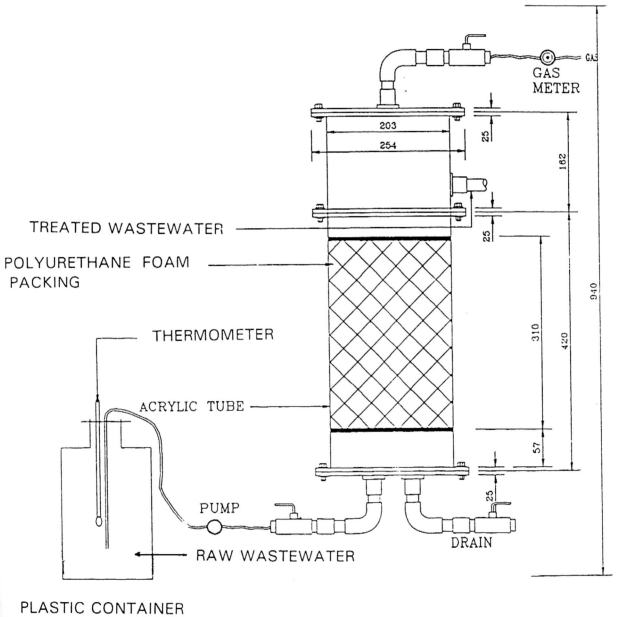


Figure 11. Sketch of the Laboratory Scale Upflow Anaerobic Filter

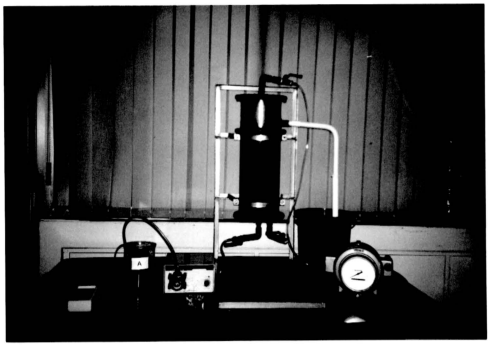


Plate 1.Photo of the Laboratory Scale Upflow Anaerobic Filter

3.1.7 Seed Material

A well digested anaerobic sludge obtained from Pantai Sewage Treatment Plant was used as a seed material for the reactor. For this purpose the sludge was first filtered through a screen to remove the foreign objects and other coarse materials.

3.1.8 Synthetic Wastewater

A synthetic wastewater containing acetate and other nutrients for the microorganisms was used to start up the reactor. The composition of this synthetic wastewater is given in *Table 7.* This wastewater has a theoretical COD level of about 5,000 mg/L (Verstraete, 1984).

3.1.9 Wastewater Samples

In the study on the characteristics of rubber thread manufacturing industry wastewater, composite wastewater samples were collected from the latex compounding stage and the leaching stage from three selected factories. The factories were selected on the basis of nearest distance to Kuala Lumpur. The locations for the sample collection are shown in *Figure 12.* In the chemical precipitation/flocculation study for removing zinc from the wastewater, the combined wastewater from the latex compounding and the leaching stages collected from the equalization tank of one of the selected factories were used. After zinc has been removed, the combined wastewater samples were then used in the studies involving the upflow anaerobic filter.

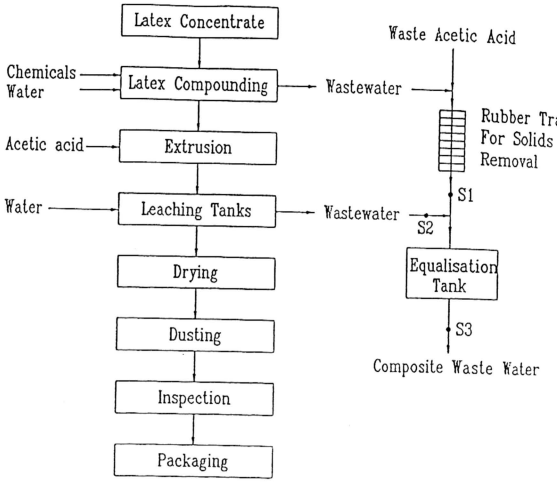
Table 7. Composition of Synthetic Wastewater (Verstraete *et al., 1984)*

Chemical	Amount (g/L)
CH_3COONa	6.0
KH_2PO_4	3.0
K_2HPO_4	1.0
$CaCl_2.2H_2O$	0.3
NH_4Cl	1.0
$FeCl_3.6H_2O$	0.1
$MgCl_2.6H_2O$	0.1
Sucrose	0.1
Tryptic soy	0.2
Yeast extract	0.2
Trace elements solution*	1.0**
COD concentration	5.0

Notes:

* Composition of trace elements solution (in mg/L of tap water): $NiSO_4.6H_2O$, 500; $MnCl_2.4H_2O$, 500; $FeSO_4.7H_2O$, 500; $ZnSO_4.7H_2O$, 100; H_3BO_3, 100; $Na_2MoO_4.2H_2O$, 50; $CoCl_2.6H_2O$, 50 and $CuSO_4.5H_2O$, 5.

** Unit in mL/L.



S 1 - Sample point of Waste Water from Latex Compounding Section after Rubber Trap

S2 - Sample point of Waste Water from Leaching Tanks Common Drain

S3 - Sample point of Composite Waste Water generated by factory after Equalisation Tank

Figure 12. Schematic Flow Diagram of the Rubber Thread Manufacturing Showing Locations of Wastewater Collection

3.2 Methods

3.2.1 Collection of a Composite Wastewater Sample

In the collection of a composite wastewater sample, individual samples of fixed volume of 1.0 L were collected at hourly intervals for a period of 24 hours and poured into a common container. After mixing the samples thoroughly, about 2.0 L was collected as a composite sample.

3.2.2 Precipitation/Flocculation Study

Before the precipitation/flocculation study was carried out, the concentrations of total and soluble fractions of zinc in the combined rubber thread manufacturing industry wastewater were determined according to the methods described in *Section 3.2.14x.*

In the precipitation/flocculation study, equal volumes (500 mL) of combined wastewater samples were transferred into six 1-L beakers of the jar test apparatus. After adding the required amount of precipitant with and without the flocculant, the contents of the beakers were thoroughly mixed by operating the mechanical stirrers at 120 rpm for

one minute after which the stirrers were operated at a low speed of 20 rpm for another 40 minutes. After this, the stirrers were removed and the flocs were allowed to settle for a period of 60 minutes. After recording the appearance of flocs, about 200 mL of sample were withdrawn from each beaker for analysis of zinc by atomic absorption spectrophotometer.

3.2.3 Start-up Procedure

The upflow anaerobic reactor was first filled with 20 pieces of PU foam as packing materials. After that about 1.2 L of seed material (well digested anaerobic sludge) was added into the reactor. The reactor was then started-up by pumping daily 12.0 L of diluted synthetic wastewater using a peristaltic pump at the initial loading rate of 1.0 g COD/L/d. The treated wastewater coming out of the reactor was recycled back to the feed reservoir. The loading rate was gradually increased up to 5 g COD/L/d after more than 80% of COD in the synthetic wastewater were removed at each loading rate. After this, the reactor was then fed daily with the combined rubber thread manufacturing wastewater which contained no zinc (removed by the sodium sulphide and polyelectrolyte LT 27 precipitation/flocculation method) at the initial loading rate of 1 g COD/L/d. The start-up process

was continued until more than 80% of COD was removed at the loading rate of 5 g COD/L/d. When this was achieved, the reactor was ready for other experimental work.

3.2.4 Study on the Influence of Organic Loading Rates

After the start-up period was over, the reactor was fed daily with the combined rubber thread manufacturing wastewater (free of zinc) without effluent recycle at different loading rates. The loading rate was varied from 2.0 to 14.0 g COD/L/d by varying the wastewater flow rate fed into the reactor. In this case the reactor was operated at the room temperatures of 26 – 32 °C. The volume of biogas produced was recorded and the sample was collected daily for analysis of methane content. The wastewater coming out of the reactor was sampled daily and analyzed for pH, COD, BOD and zinc content. The loading rate was changed after a steady state condition was achieved as indicated by the consistent biogas production rate and the COD or BOD removal efficiency.

3.2.5 Study on the Influence of Hydraulic Retention Time (HRT)

In this study, the same upflow anaerobic filter was fed daily with the combined wastewater from the rubber thread manufacturing factory at different HRTs by varying the wastewater flow rates. The biogas and wastewater samples were collected daily and analyzed in the same manner as mentioned in *Section 3.2.3.*

3.2.6 Study on the Influence of Temperature

In the study on the influence of temperature on the performance of upflow anaerobic filter, the same reactor was fed daily with the combined wastewater from the rubber thread manufacturing industry at the fixed wastewater flow rate of 12 L/d. The temperature was varied by placing the feed reservoir (plastic container containing wastewater) in the water bath which was maintained at the required temperature. The biogas and wastewater samples were collected and analyzed in the same manner as described in the previous study.

3.2.7 Study on the Influence of pH

In this study, the same reactor was fed daily with combined wastewater from the rubber manufacturing factory at the wastewater flow rate of 12 L/d. However in this case the wastewater was first adjusted to required pH before feeding into the reactor. The biogas and the wastewater samples were collected and analyzed in the same manner as described in the previous study.

3.2.8 Study on the Effect of Addition of Micronutrients

The experiment with rubber thread manufacturing industry wastewater was carried out to study the effect of inclusion of nickel and cobalt in the feed on treatability and biogas generation in the upflow anaerobic filter. In this experiment, the anaerobic filter was fed with wastewater containing 100 mM nickel and 50 mM cobalt as chloride salts. The results obtained were compared with those obtained from the earlier study involving the same wastewater but without nickel and cobalt additions.

3.2.9 Specific Biogas Productivity

The biogas generated from the anaerobic digestion of rubber thread manufacturing industry wastewater at different organic loading rates was collected and analyzed for its methane content according to the method described in *Section 3.2.13.* The biogas productivity was calculated from the volume of biogas produced for every gramme of COD removed per day.

3.2.10 COD:N:P Requirement

The rubber thread manufacturing industry wastewater was supplemented with nitrogen and phosphorus by adding diammonium phosphate to maintain a COD:N:P ratio of 100:5:1. Results of biogas productivity and pollutants removal efficiencies were compared with those obtained without nitrogen and phosphorus addition.

3.2.11 Study on the Importance of the Attached versus Suspended Growth in an Anaerobic Filter

The same upflow anaerobic filter was used in this study. The reactor was first operated by daily feeding with rubber thread manufacturing industry wastewater for two weeks at the organic loading rate of 6.0 g COD/L/d and at the HRT of 1.0d. After two weeks, the reactor was drained off to remove suspended solids from the unit. This operation was done with utmost care, so that air was not allowed into the unit , by applying the pressure of nitrogen gas from a gas cylinder and by removing the liquid and suspended solids from top to bottom step by step.

The emptied anaerobic filter unit was then filled with the same rubber thread manufacturing industry wastewater and operated at the same organic loading rate of 6.0 g COD/L/d and at the same HRT of 1.0 d. At this stage the anaerobic filter unit contained only the attached biomass. Its performance was then compared with that of earlier operation in which the anaerobic filter contained both attached and suspended biomass.

3.2.12 Gas Recording

The volume of biogas produced was recorded by the Schlumberger gas meter. The volume of biogas produced was normalized to the standard conditions (temperature = 273 °K , pressure = 1 atm).

3.2.13 Gas Analysis

Analysis of biogas was carried out using Orsat apparatus containing absorption facility for carbon dioxide, oxygen and combustion pipette for determining the methane by volume.

3.2.14 Chemical Analyses

The American Public Health Association (APHA) Standard Methods (1992) were adopted for the chemical analyses of wastewater samples. The descriptions of the methods are as follows:

i). PH

The pH values of wastewater samples were estimated using the Radiometer, model RHM 92 pH meter.

ii). Chemical Oxygen Demand (COD)

The COD was determined by refluxing 20 mL of wastewater sample for 2 h in the presence of 0.25M potassium dichromate solution (oxidant) and a mixture of silver sulphate and sulphuric acid as catalyst using TECATOR COD digestion unit. After cooling, the refluxed sample was then titrated with standard 0.1M ferrous ammonium sulphate solution using orthophenanthroline as an indicator. The concentration of COD was determined from the amount of potassium dichromate consumed.

iii). Biochemical Oxygen Demand (BOD)

The wastewater sample was first diluted with specially prepared dilution water which was saturated with oxygen. The diluted sample was then distributed into two Wheaton BOD bottles, one set analyzed for its dissolved oxygen (DO) content

on the same day and the other set was analyzed after incubation at 20 °C for 5 days. The difference in the two DO levels gave the BOD concentration of the sample after correction for the dilution factor. The DO was determined by using YSI Model 57 DO meter which was equipped with the BOD probe and built-in stirrer.

iv). Total Solids

The total solids content was estimated by drying 100 mL of wastewater sample in an oven set at 105 °C. After cooling in a desiccator, the dried residue (total solids) was then weighed to a constant weight by using an analytical balance.

v). Suspended Solids

The suspended solids was determined by filtering 100 mL of wastewater sample through a Gooch crucible and by drying the solids obtained in an oven at 105 °C. After cooling in a desiccator, the suspended solids was then weighed to a constant weight by using an analytical balance.

vi). Volatile Suspended Solids

The determination of this parameter in wastewater samples was carried by ashing the solids obtained from the determination of suspended solids in a muffle furnace at 650 °C. The difference between the weight of suspended solids and the remaining ash gave the concentration of volatile suspended solids of the sample.

vii). Total Nitrogen

The total nitrogen was determined by digesting an appropriate volume of wastewater sample with a mixture of concentrated sulphuric acid and phosphoric acid using a TECATOR digestion block at 550 °C. After digestion for about 1h, the digested sample was cooled and then steam-distilled after the addition of a 40% sodium hydroxide solution by using a TECATOR distillation unit. The distillate which was collected in a boric acid solution was then titrated with standardized 0.01N sulphuric acid solution using screened methyl red indicator. The total nitrogen was calculated from the amount of sulphuric acid consumed.

viii). Ammoniacal Nitrogen

This parameter was determined by steam distillation of an appropriate volume of wastewater sample followed by absorbing the distillate (ammonia) in a boric acid solution and titration with standardized 0.01N sulphuric acid solution using screened methyl red indicator. The concentration of ammoniacal nitrogen was calculated from the amount of sulphuric acid consumed.

ix). Phosphorus

The determination of phosphorus involved conversion of inorganic and organic phosphorus to orthophosphate. Orthophosphate was reacted with ammonium molybdate to form molybdophosphoric acid which was reduced by ascorbic acid to form an intensely coloured complex known as molybdenum blue. The intensity of the complex was measured by an auto-analyzer colorimeter at 880nm. The concentration of phosphorus in a wastewater sample was estimated from the standard curve prepared from standard phosphorus solutions in the same manner.

x). Zinc

The concentration of soluble zinc in wastewater samples was determined by filtering the samples through glass fibre grade B (G/F B) filter papers. The soluble zinc concentration in the filtrate was then read direct from the atomic absorption spectrophotometer. The concentration of total zinc was determined by digesting an appropriate amount of wastewater sample using a mixture of sulphuric acid and perchloric acid followed by determining the zinc concentration in the digested samples by atomic absorption spectrophotometer.