CHAPTER 3

MATERIALS AND METHODS

3.1 GENERAL

The discharge of wastewater without any treatment is a significant risk for public health and environmental condition. The industrial wastewater leads to contamination of water, soil and air. Since they are discharged with inadequate treatments, there is a need for the treatment of industrial wastewater so that, the final disposal to the environment can be made without harmful effects and converts them as an energy source. Over the past 30 years, anaerobic wastewater treatment process became more popular due to its advantages over aerobic processing, including low biomass production, low nutrient requirements and the production of fuel gas, such as methane gas and hydrogen. Studies have shown that anaerobic treatment is a stable process under proper operation. But parameters such as process configuration, temperature, biomass, pH, nutrient, and substrate must be carefully scrutinized in order to make successful anaerobic treatment. Many reactor configurations have been investigated. An improvement in the efficiency of anaerobic treatment can be brought about by either reactor design modification or advanced operating techniques. Literature review revealed that the anaerobic technology for the treatment of wastewater was known in India in recent days. Also the anaerobic baffled reactor systems were not thoroughly studied at laboratory and pilot scale level for the treatment of industrial wastewater. Hence, there is an ample scope to adapt the anaerobic
baffled reactor system for industrial effluent treatment like dairy wastewater treatment.

3.2 MATERIALS

To study about the performance of anaerobic wastewater treatment technology, experiments were conducted in anaerobic baffled reactor and anaerobic reactor. Laboratory studies were under taken to study the treatability of dairy wastewater and to evaluate the kinetic parameters using Monod kinetic Model. For this study, the dairy wastewater was collected from a dairy plant located near Chennai. The analyses were carried out according to the Standard Methods of APHA.

3.3 DAIRY WASTEWATER

The liquid waste in a dairy plant originates from manufacturing process, utilities and service section. The various sources of waste generation from a dairy are spilled milk, spoiled milk, skimmed milk, whey, wash water from milk cans, equipment, bottles and floor washing. Whey is the most difficult high strength waste product of cheese manufacture. This contains a proportion of milk protein, water-soluble vitamins and mineral salt. Wastewater management in the dairy industry is well documented, but the effluent disposal is remains a problematic issue for the dairy industry. A large volume of high organic wastewater is produced during the period of production and normally they are discharged into the land or into the nearby water sources, usually small streams, practically without any treatment. The dairy wastewater is normally characterized as follows:

i. They are mainly diluted milk or milk products

ii. Cleaning compounds and sanitizers are the major constituents of dairy wastewater.
iii. The contaminants are highly organic and having nutrients (N, P).

iv. They have high BOD, COD and total solids.

v. The use of acid and alkaline cleaning compounds may cause high pH variability.

vi. All compounds of dairy wastewater are biodegradable except protein and fat.

The polluted water caused by the heavy discharge of organic wastewater, produce odour nuisance near the dairy plants, which is a very common phenomenon. All the concerned bodies, both industry people and pollution control agencies are aware of these problems and they are trying to find the solution to stop the nuisance in an economical way.

3.3.1 Operations in a Dairy Plant

- Receiving
- Pasteurizing
- Bottling
- Condensing
- Dry milk manufacturing
- Cheese making
- Butter making
- Casein making

Milk solids consist of carbohydrates, fats and proteins. Dairy wastes are made up of dilution of milk and its by-products. The BOD values of dairy wastewater of a dairy plant are,

- Whole milk - 90,000 to 1,05,000 mg/L
- Skim milk - 65,000 to 75,000 mg/L
- Butter milk - 55,000 to 65,000 mg/L
- Whey - 35,000 to 45,000 mg/L
The manufacturing activities in a dairy plant are of the batch type. One batch of milk is processed, the equipments are drained, thoroughly cleaned and the next batch operation are taken for processing. Thus the flow of dairy wastewater is in slugs. General processing flow diagram for a dairy plant is shown in Figure 3.1 (Patwardhan 2008).

The general characteristics of dairy wastewater are given in Table 3.1.

### Table 3.1 Characteristics of Dairy waste water

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour</td>
<td>Grayish white</td>
</tr>
<tr>
<td>2</td>
<td>pH</td>
<td>7.5-11</td>
</tr>
<tr>
<td>3</td>
<td>Total alkalinity as CaCO$_3$ mg/L</td>
<td>1500-3000</td>
</tr>
<tr>
<td>4</td>
<td>COD, mg/L</td>
<td>4000-40000</td>
</tr>
<tr>
<td>5</td>
<td>BOD, mg/L</td>
<td>1900-22000</td>
</tr>
<tr>
<td>6</td>
<td>Oil and grease, mg/L</td>
<td>300-450</td>
</tr>
<tr>
<td>7</td>
<td>TKN, mg/L</td>
<td>150-400</td>
</tr>
<tr>
<td>8</td>
<td>SS, mg/L</td>
<td>200-300</td>
</tr>
<tr>
<td>9</td>
<td>TS, mg/L</td>
<td>2000-3000</td>
</tr>
<tr>
<td>10</td>
<td>TDS, mg/L</td>
<td>1300-1850</td>
</tr>
<tr>
<td>11</td>
<td>VSS, mg/L</td>
<td>3100-8000</td>
</tr>
</tbody>
</table>
Figure 3.1 Flow diagram for a Milk processing plant
3.4 EXPERIMENTAL SETUP

For the study, two set of reactors Anaerobic Reactor and Anaerobic Baffled Reactor of total volume 12 litres each were fabricated at Vishnu Priya Industries, Madurai. The experimental setup was made with 6 mm thick acrylic sheet material having external dimensions of 55 cm long, 12 cm width, and 28 cm height. Figure 3.2 shows the schematic diagram of Anaerobic Reactor without baffle walls. The working volume of AR is 11.22 liters.

![Figure 3.2 Anaerobic Reactor](image)

Figure 3.2 Anaerobic Reactor

Figure 3.3 shows the schematic diagram of Anaerobic Baffled Reactor. The working volume of ABR is 10.137 litres. The ABR was divided into 10 equal compartments by providing vertical standing and hanging baffle walls as shown in the longitudinal section of the Figure 3.3. The photograph view of ABR before loading and after loading is shown in Figure 3.4(a) and 3.4(b) respectively. Each compartment was equipped with sampling ports to draw out the samples for analyzing.
The outlet of the reactor was connected with a U-tube for level control and to dropout the effluent. At the bottom of the reactor, an outlet was provided for sludge withdrawal. The reactor was kept at room temperature. The produced gas was collected via port hole at the top of the reactor and connected to the gas collecting bottle of 10 litres capacity. The daily volume of gas was determined using the gas liquid displacement technique.
Accumulation of gas was identified by the fall of brine solution layer in the gas collecting bottle. Similar arrangements were made for the Anaerobic Reactor AR without the baffle walls.

### 3.5 START UP OF THE REACTORS

The term start-up means, acquiring bio film formation within the reactor. Without proper startup of the anaerobic reactor, the system is not likely to reach its maximum degree of efficient removal of soluble nutrients and organic matters. The biofilm formed within the reactors are influenced by the parameters such as liquid flow rate, scale up of the reactor and organic loading rate.

These bio films are essential for the successful operation of anaerobic reactors. The Anaerobic Reactor and Anaerobic Baffled Reactor were started using the seed of anaerobic ally digested liquid cow dung up to 50 % of the reactors volume for inoculation. Sufficient mixed liquor suspended solids were developed by adding continuously fresh dairy wastewater. The cycle of wasting and feeding was continued till steady state condition was achieved. During this period, the pH and gas evolved were noted. pH was monitored throughout the study. The steady state condition is indicated by the accumulation of equal volume of gas in each day. The reactors took up to 48 days to develop the sufficient MLSS concentration after acclimatization. The dairy wastewater was added to the reactor each day, to promote and sustain bio film growth within the reactor.

### 3.6 OPERATION OF THE REACTORS

The experimental protocol was designed to examine the effect of OLR, HRT and pH on the efficiency of the AR and ABR. The start-up period of the reactor was found to be 48 days. After the start-up period regular wasting and feeding were performed until steady state condition reached by
AR and ABR. The loading was done once in a day after the withdrawal of equal volume of wastewater from the reactor. The steady state level was confirmed by considering consistent COD removal and constant gas production. Then for a particular COD concentration, the reactors were operated for minimum 10 days in order to study its performance. At steady state, daily gas production, BOD, COD of the effluent and the room temperature were recorded. The removal efficiency in terms of BOD, COD and percent stabilization based on methane gas production are calculated. Temperature, pH, concentration, alkalinity of the effluent also be recorded. The treatability studies for both reactors were carried out based on semi-continuous loading at room temperature. The volume of wastewater added daily was calculated based on the COD concentration.

\[
\text{OLR in kg of COD / L / d} = \frac{\text{Concentration of COD (kg / L)} \times \text{Daily flow (L / d)}}{\text{Volume of the reactor (L)}} \tag{3.1}
\]

\[
\text{Hydraulic Retention time in days} = \frac{\text{Volume of the reactor (L)}}{\text{Daily Flow rate (L / d)}} \tag{3.2}
\]

3.7 CHEMICAL ANALYSIS

After attaining steady state, Chemical analyses were performed on the effluents from the reactors. To study the treatability of the dairy wastewater the treated effluent was analyzed for pH, BOD, COD by standard Methods (APHA 1980). For each observation, temperature, gas pressure absolute pressure of mercury and vapour pressure of water were noted. From the observed data, volume of methane gas collected in Litres at STP was calculated using the expression given below: (Rao and Datta 1987)

\[
C = \frac{[0.37 (P_1 - P_w) C_1]}{(273 + T_1)} \tag{3.3}
\]

Model calculation was given in the appendix. The gas collected from the reactor was analyzed for its composition of methane content by
passing it through 1N Potassium Hydroxide solution. Calculation for determination of percentage of methane is given in the appendix.

3.8 PERFORMANCE STUDY OF ANAEROBIC REACTOR USING DAIRY WASTE WATER

To find out the efficiency of Anaerobic Reactor performance study using dairy wastewater treatment was carried out in an Anaerobic Reactor with considering suitable influencing factors and the results were compared with the efficiencies of ABR. The reactor, AR was operated for the following parameters.

3.8.1 Organic Loading Rate (OLR)

The performance study of AR was carried out for various OLR ranges from 0.91 kg of COD /L/d to 3.87 kg of COD/L/d, using dairy wastewater with semi continuous loading and intermittent mixing at room temperature. For the particular organic loading rate the hydraulic retention time was calculated as per the COD concentration and daily volume of flow. Throughout the study the temperature and pH were monitored and recorded.

3.8.2 pH

This study was carried out in AR for various pH levels of 5 to 10 with semi continuous loading at room temperature. The reactor, ABR was operated at an organic loading rate of 1.00 kg of COD/L/d with dairy wastewater. The most common chemicals used for pH control in the reactor are quick lime and hydrochloric acid.

3.8.3 Hydraulic Retention Time (HRT)

In this study, to investigate the effect of hydraulic retention time on organic substrate degradation in the anaerobic treatment of dairy
wastewater, experiments were conducted in AR at varying hydraulic retention time of 3.75 to 7.5 days. The steady state result of the variable parameters were observed and noted. The gas collected from the reactors were analyzed for its composition of methane content by passing it through potassium hydroxide solution.

3.9 PERFORMANCE STUDY OF ANAEROBIC BAFFLED REACTOR USING DAIRY WASTEWATER

The performance study of Anaerobic Baffled Reactor using dairy wastewater treatment was carried out considering suitable influencing parameters. The reactor was operated for the following parameters.

3.9.1 Organic Loading Rate (OLR)

The study was carried out for various organic loading rate ranges from 1.00 kg of COD/L/d to 3.25 kg of COD/L/d in, ABR with semi continuous mode of loading and intermittent mixing at room temperature. For the particular organic loading rate the hydraulic retention time was calculated as per the COD concentration and daily flow volume. Throughout the study the pH was monitored.

3.9.2 pH

The study was carried out in ABR for various pH values of 5 to 10 with semi continuous loading at room temperature. The reactor, ABR was operated with the constant organic loading rate of 1.00 kg of COD/L/d with dairy wastewater. After attaining steady state the experiments were carried out for various pH values from 5 to 10. The most common chemicals used for pH control in the reactor are quick lime and hydrochloric acid.
3.9.3 Hydraulic Retention Time (HRT)

In this study, to investigate the effect of hydraulic retention time on organic substrate degradation in the anaerobic treatment of dairy wastewater, experiments were conducted in ABR at various Hydraulic Retention Time of 3 to 8 days. The steady state result of varying parameters were observed and noted.

3.10 EVALUATION OF BIO-KINETIC COEFFICIENTS

Another part of the research contributes to determination of kinetic coefficients for treatment of the dairy wastewater in the anaerobic treatment system.

The biological treatment system is important to maintain the proper balance of biomass. This is accomplished by controlling the growth of new cells in the biological system. For effective growth control and proper balance of biomass in the system it is essential to have a clear knowledge of kinetics of microbial growth rate, substrate utilization rate, limiting substrate or nutrients that affect the growth of cells and endogenous decay or death rate of microorganisms in the systems. The proportionality constants obtained from the kinetic equation are called bio–kinetic coefficients or growth constants.

The kinetic constants of the anaerobic treatment process are a useful tool which is able to describe and to predict the performance of the system. The bio-kinetic constants depend on the type of microbial species and environmental conditions like pH, temperature, dissolved oxygen, nutrients, inhibitory substances and degradability of the organic substrates in the wastewater.

The common type of model such as Mass balance, reaction rate equation and the most popular model of Monod equation of anaerobic treatment process is applied to evaluate the kinetic coefficients. From the
experimental data the kinetic coefficients of AR and ABR, maximum specific growth rate of bio Mass ($\mu_{\text{max}}$), Monod half-saturation constant ($K_S$), endogenous decay constant ($K_d$) and maximum substrate utilization rate constant ($K$) were estimated as per Monod kinetic model.

3.10.1 Specific Growth Rate, $\mu$

In a biological treatment system, the rate of increase in biomass is directly proportional to the reactor biomass concentration. This proportionality factor is known as specific growth rate constant.

$$R_g = \frac{dX}{dt} = \mu X$$

Where  
- $R_g$ = growth rate of biomass  
- (Mass / unit volume of reactor $\times$ time)  
- $\mu$ = specific growth rate of biomass (d$^{-1}$)  
- = $\frac{\text{growth rate}}{\text{unit of biomass}}$  
- = $\frac{\text{mass of new cells synthesized}}{\text{mass of cells present in the reactor } \times \text{time}}$  
- $dX/dt$ = rate of change of biomass  
- (Mass of microorganisms / unit volume $\times$ time)  
- $X$ = Concentration of biomass in the reactor  
- (Mass / unit volume of liquid) in mg/L

3.10.2 Yield Coefficient, $Y$

The mass of new cells produced per unit of substrate utilized or removed by the microorganisms present in the treatment system is called cell yield or yield coefficient, $Y$
Y = \frac{\text{bacterial growth rate}}{\text{substrate utilization rate}}
= \frac{R_g}{R_{su}}
= \frac{dX/dt}{dS/dt}

Y = \frac{X_t - X_0}{S_0 - S_t} \quad (3.5)

Where

Y = \text{cell yield coefficient (Mass of cell / Mass of substrate)}
\frac{dx}{dt} = \text{rate of change of biomass [Mass / (volume \times time)]}
\frac{dS}{dt} = \text{Rate of change of substrate removal}
[Mass / (volume \times time)]
X_t = \text{biomass concentration at time } t, \text{ mg/L}
X_0 = \text{bio Mass concentration at time } t_0, \text{ (Mass / volume)}
S_0 = \text{influent substrate concentration at time } t_0, \text{ (Mass / volume)}
S_t = \text{Substrate concentration in effluent after time } t,
\text{(Mass / volume)}

For substrate removal and specific growth rate of bacteria, the equation has been referred as Michaelis-Menten equation, proposed by Monod. When the substrate is being used at its maximum rate, the bacteria are also growing at their maximum rate. The maximum specific growth rate of the bacteria is related to the maximum specific substrate utilization rate as follows:

\mu_{\text{max}} = K \cdot Y \quad (3.6)

K = \frac{\mu_{\text{max}}}{Y} \quad (3.7)

Where
\mu_{\text{max}} = \text{maximum specific growth rate of biomass in d}^{-1}
K = \text{maximum substrate utilization rate constant in d}^{-1}

The value of Y depends on the nature of substrate, the type of microbial species present in the system and the temperature.
3.10.3  **Half Velocity Constant, \( K_S \)**

The value of limiting nutrient or substrate concentration of one-half of the maximum growth rate of biomass is termed as half velocity constant, \( K_S \). This value is determined from the plot of \( \mu \) versus \( S \), and using

\[
\mu = \mu_{\text{max}} \times \frac{S}{K_S + S} \quad (3.8)
\]

Where \( \mu \) = specific growth rate of biomass (d\(^{-1}\))

\( \mu_{\text{max}} \) = maximum specific growth rate of biomass in d\(^{-1}\)

\( S \) = growth limiting soluble substrate concentration in mg/L

\( K_S \) = Half velocity constant or Substrate concentration at one half of the maximum specific substrate utilization rate.

3.10.4  **Endogenous Decay Coefficient, \( K_d \)**

When the substrate concentrations in the wastewater are at its minimum, the microorganisms metabolize their own protoplasm, and the concentration of biomass in the reactor decreases due to death of some cells. This phenomenon is called endogenous decay of biomass. The rate of such biomass decay is proportional to the concentration of remaining biomass. The proportionality constant, \( K_d \) is called endogenous decay coefficient.

\[
R_d = -K_d X
\]

\[
K_d = \frac{-R_d}{X} \quad (3.9)
\]

Where \( R_d \) = rate of endogenous decay of biomass

\( R_d \) = [(Mass / (volume \times time))]

\( K_d \) = endogenous decay coefficient in d\(^{-1}\)

\( X \) = Concentration of biomass in the reactor

(Mass / unit volume of liquid) in mg/L
The negative sign in equation (3.9) indicates decrease in substrate concentration.

The kinetic growth coefficients \( Y \) and \( K_d \) may be determined from the laboratory experiments by the measurement of temperature and pressure in the gas collecting bottle.

The Mass of biological solids utilized daily,

\[
\frac{dx}{dt} = \frac{Y \left( \frac{dF}{dt} \right)}{1 + K_d \theta_C} \tag{3.10}
\]

Where \( \theta_C \) is HRT = \( \frac{V}{Q} \)

\[
\frac{dF}{dt} = \text{Rate of substrate utilization} = eF
\]

\( e = \text{Fractional efficiency of waste utilization, ranges from 0.6 to 0.9} \)

\( F = \text{Ultimate COD supplied to the reactor in kg/d} \)

The end products of the anaerobic treatment are methane and carbon dioxide. The quantity of theoretical production of methane gas is 350 liters per kg of ultimate BOD or COD stabilized at standard condition.

\[ \therefore \text{The volume of methane gas produced per day,} \]

\[
C = 350 \left( eF - 1.42 \frac{dx}{dt} \right) \tag{3.11}
\]

Where, \( C = \text{L of methane gas produced /day at standard condition.} \)

\[
C = \frac{0.37 \left( P_1 - P_w \right) C_1}{273 + T_1} \tag{3.12}
\]

In which

\( P_1 = \text{Pressure under which the volume of gas is measured, in mm of mercury.} \)

\( P_w = \text{Vapor pressure of water at } T_1°C \text{ in mm of mercury} \)

\( C_1 = \text{Volume of methane in litres in the collected gas} \)
To design the biological process for treatment of a particular wastewater, the required values of bio-kinetic coefficients are determined by carrying out the laboratory scale studies using AR and ABR as follow: (Karia and Christian 2006)

i. Hydraulic retention of each experiment was recorded as $\theta$.

ii. Determining the mean values of $Q_0$, $S_0$, $X$ and $S$ at steady state conditions of operation.

iii. Calculating the values $K_s$ and $K$ from the Plot of

\[
\frac{X\theta}{S_0 - S} \text{ Versus } \frac{1}{S}
\]

(iv. Computing the values of $Y$ and $K_d$ by plotting the curve of

\[
\frac{1}{\theta} \text{ Versus } \frac{S_0 - S}{X\theta}
\]

3.11. DETERMINATION OF CH$_4$ EMISSION REDUCTION

Another part of the research contributes to determine the CH$_4$ emission reduction from the anaerobic dairy wastewater treatment system.

The amount of methane that would be emitted to the atmosphere in the absence of the project activity is called baseline emission and it can be estimated referring to UNFCCC – approved methodology AMS III. H.Version 9, Methane recovery in wastewater treatment.

According to the UNFCCC-IPCC guidelines,

Baseline emission = $Q \times \text{COD} \times \beta \times \text{MCF} \times \text{GWP}_\text{CH}_4$

Where

$Q$ = volume of wastewater treated per year

COD = Chemical oxygen demand of the treated wastewater in the year in kg
\[ \beta = \] maximum methane producing capacity (or) the specific methane yield in kg of \( \text{CH}_4 \)/kg of COD

\[ \text{MCF} = \] Methane conversion factor (fraction)

\[ \text{GWP}_\text{CH}_4 = \] Global warming potential for methane (value of 21 is used)

The COD is to be directly measured by the project as the baseline activity level, since the effluent that goes in the lagoon in the baseline situation is the reactor in the project work.

As per the IPCC guidelines, MCF values are as below

**Table 3.2. IPCC Default Values for Methane Correction Factor (MCF)**

<table>
<thead>
<tr>
<th>Types of wastewater treatment and discharge pathway or system</th>
<th>MCF lower values</th>
<th>MCF higher values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discharge of wastewater to sea river or lake</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Aerobic treatment well managed</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Aerobic treatment poorly managed or overloaded</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Anaerobic digester for sludge without methane recovery</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Anaerobic reactor without methane recovery</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Anaerobic shallow lagoon (depth less than _ metres)</td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Anaerobic deep lagoon (depth more than – metres)</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Septic system</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Project emission = \( Q \times \text{COD} \times \beta \times \text{MCF} \times \text{GWP}_\text{CH}_4 \)

Where

\[ Q = \] volume of wastewater treated per year

\[ \text{COD} = \] Chemical oxygen demand of the treated wastewater in the year in kg
$\beta$ = maximum methane producing capacity (or) the specific methane yield in kg of CH$_4$/kg of COD

MCF = Methane conversion factor (fraction—Higher value is 0.2)

GWP$_{CH_4}$ = Global warming potential for methane (value of 21 is used)

**Estimation of CH$_4$ Emission Reduction**

CH$_4$ emission reductions are calculated as the difference between the baseline emission and the sum of the project emission and leakage. In accordance with the anaerobic methodology, there is no leakage and the actual calculation of CH$_4$ emission reduction is as follows.

CH$_4$ Emission Reduction kg/yr = Baseline emission kg/yr – Project emission kg/yr

**3.12 CONCLUSION**

Laboratory experiment was conducted for dairy wastewater treasure by considering the factors affecting anaerobic degradation. For seeding the aerobically digested sludge was used. BOD and COD concentration were used as grass substrate parameter for determining anaerobic biodegradability. The volume of wastewater added daily was calculated on the basis of substrate concentration. The reactors usually are operated at room temperature. Hence, the reactor AR and ABR in this study was also operated at room temperature.

To optimize the process, the concentration of the feed was changed. The reactors were operated for various OLR, HRT and pH. To predict the treatability of the reactor, the performance of the reactor AR and ABR were compared.

Using the Monod Kinetic Model the Bio kinetic coefficients were evaluated.