CHAPTER 2

EXPERIMENTAL METHODS

In this chapter, a brief description of the equipments, technique adopted for biological digestions and the reagents used are discussed. The detailed experimental methods followed for the digestion and the methodology for the determination of biokinetic coefficients are also explained.

2.1 EXPERIMENTAL SETUP

The experimental setup used in the present study is shown in Fig 2.1. The experimental setup is described as follows:

i) The digester used for biological digestion is essentially the conventional fixed domed type with a cylindrical shape of 5 lt capacity. The digester is made of glass and is fitted with an inlet and outlet for the purpose of sampling and monitoring physico-chemical properties.

ii) The calibrated aspirator jar made of glass for the collection of biogas.

iii) The glass tube used for sand filtration is a long borosil glass (6 cm x 40 cm) with a provision for sample collection.

iv) Air compressor is used to pass air for aerobic digestion.

v) Atomic absorption spectrophotometer is used for the estimation of chromium.
1. Digested sludge
2. Gas collection tube
3. Feed tube
4. Withdrawal tube
5. Levelling bottle

Fig. 2.1 Laboratory reactor for the conduct of biological treatment studies.
2.2 REAGENTS

All chemicals used during digestion and for analysis are of AR grade. The chemicals used for analyses are given as follows.

Chemicals

Potassium dichromate, ferrous ammonium sulphate, silver sulphate, mercuric sulphate, sodium thiosulphate sodium azide, silver nitrate, barium chloride, sodium hydroxide, ferroin indicator etc.

2.3 EXPERIMENTAL PROCEDURE

The spent chrome tannery effluent samples were collected from a tannery unit near Chennai. Analysis of total solids, TDS, BOD, COD etc. were carried out as per standard methods. Removal of chromium was done using alkalis like lime, NaOH and NH₄OH. The precipitate was allowed to stand overnight for separation of clear liquor and the liquor was then filtered through slow sand filter. The filtrate was analysed for its contents. The chromium content in the filtrate was analysed by Atomic absorption spectrophotometer. The amount of lime required for maximum removal of chromium was noted.

2.4 ANAEROBIC AND AEROBIC DIGESTION OF EFFLUENTS

About 2 lt of effluent was taken in the digester, Cowdung was used as the seed material and was fed with the substrate at varying organic loads on BOD basis. During anaerobic and aerobic digestion of tannery effluent, the organic load supplied were 480, 800, 1360, 1740 and 2250 mg/L and for distillery effluent, the organic load supplied were 820, 1450, 2100, 2850 and 3340 mg/L. The BOD loads were fed with the effluent at regular interval of 12, 18 and 24 hours. The necessary nutrients such as nitrogen and
phosphorus in the form of urea and diammonium phosphate were also added to the feed solution in order to maintain the BOD: N:P ratios of 100:5:1. The pH of the influent samples was adjusted to 7.0 by adding calcium carbonate before feeding. After feeding, the contents in the digester was given a thorough mixing. The digestion was carried out until stabilized condition was achieved as represented by sludge growth on a day-to-day basis and effluent BOD remained constant. The samples of effluents drawn at different loading rates were analysed for TS, TDS, TSS, VSS, BOD, COD etc. and the gas produced during digestion was burnt periodically to confirm the presence of methane which formed a major portion of the gas. The digestion was carried out at room temperature.

For aerobic digestion, compressed air was introduced into the reactor and the digestion was carried out with varying organic loads.

2.5 METHODOLOGY FOR DETERMINATION OF BIOKINETIC PARAMETERS

The modified Monod's equations (Monod 1949) expressing the biological growth and substrate utilization rates has been used to estimate the bio-kinetic coefficients for the anaerobic and aerobic processes.

In both batch and continuous culture systems the rate of growth of bacterial cells can be defined by the following relationship.

\[ r_g = \mu X \]  \hspace{1cm} \ldots (1)

where \( r_g \) = rate of bacterial growth, mass/unit volume time
\( \mu \) = specific growth rate, time\(^{-1}\)
\( X \) = Concentration of microorganism, mass/unit volume.
Since \( \frac{dX}{dt} = r_g \), the following relationship is also valid for a batch culture.

\[
\frac{dX}{dt} = \mu X \tag{2}
\]

Monod has proposed an expression for \( \mu \) in terms of limiting substrate concentrations as

\[
\mu = \mu_m \frac{S_e}{K_s + S_e} \tag{3}
\]

where
- \( \mu_m \) = maximum specific growth rate, time\(^{-1}\)
- \( S_e \) = concentration of growth limiting substrate in solution, mass/unit volume
- \( K_s \) = half-velocity constant mass/unit volume

Substituting eq. (3) in eq. (1) the bacterial growth rate is expressed as

\[
r_g = \frac{\mu_m X S_e}{K_s + S_e} \tag{4}
\]

In both batch and continuous culture systems, a portion of the substrate is converted into new cells and a portion is oxidized to inorganic and organic end products. Since the quantity of new cells produced has been observed to be reproducible for a given substrate, the following relationship has been developed between the rate of substrate utilization and the rate of growth.

\[
r_g = -Y r_{su} \tag{5}
\]
where \( r_g \) = rate of bacterial growth, mass/unit volume, time
\( Y \) = maximum yield coefficient
\( r_{su} \) = substrate utilization rate, mass/unit volume, time.

substituting eq(4) in eq(5),

\[
\frac{\mu_m X Se}{Y(K_s + Se)}
\]

... (6)

where \( \mu_m/Y \) is replaced by \( k \), the substrate utilization rate constant

\[
k = \frac{\mu_m}{Y}
\]

... (7)

Substituting equation (7) in equation (6)

\[
\frac{k X Se}{K_s + Se}
\]

... (8)

The endogeneous decay term can be formulated as

\[
r_d = -K_d X
\]

... (9)

where \( K_d \) = endogenous decay coefficient, time\(^{-1}\)
\( X \) = concentration of cells, mass/unit volume

and the net bacterial growth rate \( r_g' \) is obtained as

\[
r_g' = r_{su} + r_d
\]

... (10)

combining, equations (4) and (5) and (9) the expression for \( r_g' \) is obtained as

\[
r_g' = \frac{\mu_m X Se}{K_s + Se} \cdot K_d X
\]

... (11)
Dividing both sides by $X$,

$$\frac{r_g'}{X} = \frac{\mu_m S_e}{K_s + S_e} - K_d$$  \hspace{1cm} \text{(12)}

$$\frac{dX/dt}{X} = \frac{k Y S_e}{K_s + S_e} - K_d$$  \hspace{1cm} \text{(13)}

The substrate removal rate ($U$) or the food-to-microorganism ratio ($F/M$), is defined as

$$U = \frac{\Delta F/\Delta T}{X}$$  \hspace{1cm} \text{(14)}

where $\Delta F/\Delta T$ is the mass of substrate utilized,

which is approximated per unit time to $r_{su}$ as follows

$$r_{su} = \frac{\Delta F}{\Delta T} = \frac{k X S_e}{K_s + S_e}$$  \hspace{1cm} \text{(15)}

Dividing equation (15) by $X$

$$\frac{\Delta F/\Delta T}{X} = U = \frac{k S_e}{K_s + S_e}$$  \hspace{1cm} \text{(16)}

$\Delta F/\Delta T$ is also expressed in terms of initial and final substrate concentration using mass balance as

$$\frac{\Delta F}{\Delta T} = \frac{Q}{V} (S_0 - S_e)$$  \hspace{1cm} \text{(17)}
Dividing eq. (17) by X,

\[
\frac{\Delta F/\Delta T}{X} = \frac{Q}{VX} (S_0 - S_e) \quad \text{(18)}
\]

ie.,

\[
U = \frac{S_0 - S_e}{\theta X} \quad \text{(19)}
\]

where \(S_0\) and \(S_e\) are the influent and effluent substrate concentrations, \(Q\), the flow rate and \(V\), the volume of the reactor.

Comparing the equations (16) and (19),

\[
\frac{1}{U} = \frac{X\theta}{S_0 - S_e} = \left[ \frac{K_s}{k} \right] + \frac{1}{k} \quad \text{(20)}
\]

A plot of \(1/U\) versus \(1/S_e\) gives a straight line. The slope of the straight line is \(K_s/k\) and the intercept is \(1/k\).

### 2.5.1 Microorganism and Substrate Mass Balances

An unsteady state mass balance for the mass of microorganisms can be written as

\[
\frac{dX}{dt} = QX_0 - QX + V_r r_g' \quad \text{(21)}
\]

where \(\frac{dX}{dt} = \text{rate of change of microorganism concentration}\) in the reactor measured in terms of VSS, mass VSS/unit volume time.

\(Q = \text{flow rate, volume/time}\)
$X_0 = \text{concentration of microorganisms in influent, mass VSS/unit volume}$

$X = \text{concentration of microorganisms in reactor mass VSS/unit volume}$

$r_g' = \text{net rate of microorganism growth, mass VSS/unit volume}$

Substituting equation 10 for $r_g'$ in equation (21)

$$\frac{dX}{dt} = \theta X_0 - \theta X + \frac{\mu_m X S_e}{K_s + S_e} - KdX \quad \cdots (22)$$

It is assumed that the concentration of microorganisms in the influent can be neglected and that steady-state conditions prevail ($dX/dt = 0$), equation (22) can be simplified to

$$\frac{\theta}{V_r} = \frac{1}{\theta} = \frac{\mu_m S_e}{K_s + S_e} \cdot Kd \quad \cdots (23)$$

where $\theta = \text{hydraulic detention time, V/Q}$.

The term $1/\theta$ corresponds to the net specific growth rate is also related to $1/\theta_c$ where $\theta_c$ is the mean cell residence time.

$$\text{i.e., } \frac{V_r}{\theta_c} = \frac{V_r}{\theta} \cdot \frac{X}{X'} \quad \cdots (24)$$

where $X = \text{mass of the solids in the system}$

$X' = \text{mass of the solids leaving the system}$. 
\[
\frac{1}{\theta_c} = \frac{\theta_s}{V_r} = \frac{\mu_m \text{Se}}{K_s + \text{Se}} - K_d \quad \ldots (25)
\]

\[
\frac{1}{\theta_c} = \frac{\text{YkSe}}{K_s + \text{Se}} - K_d \quad \ldots (26)
\]

\[
\frac{1}{\theta_c} = \text{YU} - K_d \quad \ldots (27)
\]

where \( Y = \) Yield coefficient

\( K_d = \) Decay coefficient

A plot of \( U \) versus \( 1/\theta_c \) will give a straight line, the slope gives the value of \( Y \) and its intercept \( K_d \).