CHAPTER-V
THEORITICAL ANALYSIS
V. THEORETICAL ANALYSIS

V.1. Microbiology of bacterial growth

Bacterial growth is the division of one bacterium into two daughter cells in a process called binary fission. Providing no mutational event occurs the resulting daughter cells are genetically identical to the original cell. Hence, "local doubling" of the bacterial population occurs. Both daughter cells from the division do not necessarily survive. However, if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth. The measurement of an exponential bacterial growth curve in batch culture was traditionally a part of the training of all microbiologists; the basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry), direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods. Growth usually refers to changes in the total population rather than an increase in the size or mass of an individual organism. The inoculum contains thousands of organisms and growth denotes the increase in number beyond that present in the original inoculum. Therefore, determination of growth requires quantitative measurement of the total population of the cells or cell crops at the time of inoculation and again after incubation of 24 hours. Thus for microbes, growth is their most essential response to their physiochemical environment. Growth is a result of both replication and change in cell size. Microorganisms can grow under a variety of physical, chemical and nutritional conditions. In a suitable nutrient medium, organisms extract nutrients from the medium and convert them into biological compounds. Part of these nutrients is used for energy production and part is used for biosynthesis and product formation. As a result of nutrient utilization, microbial mass increases with time.

V.2. Phases of bacterial growth in batch culture:

Bacterial growth in batch culture can be modeled with four different phases: lag phase (A), exponential or log phase (B), stationary phase (C), and death phase (D).

1. During lag phase, bacteria adapt themselves to growth conditions, synthesizing enzymes and getting ready for multiplication. It is during this time that the cells carry out such
functions as synthesizing transport proteins for moving the substrate into the cell, synthesizing enzymes for utilizing the new substrate, and beginning the work for replicating the cells’ genetic material. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs. The duration of the lag phase depends upon the growth medium from which the inoculum was taken relative to the reaction medium in which it is placed. If the inoculum is similar to the medium of the batch reactor, the lag phase will be almost non-existent. However, if the inoculum were placed in a medium with a different nutrient or other contents or if the inoculum culture were in the stationary or death phase, the cells would have to readjust their metabolic path to allow them to consume the nutrients in their new environment.

2. Exponential phase (sometimes called the log phase or the logarithmic phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate and both the number of cells and the rate of population increase double in each consecutive time period, called doubling period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time. The actual rate of this growth (i.e. the slope of the line in the figure V.1) depends upon the growth conditions, which affect the frequency of cell division events and the probability of survival of both daughter cells. However, exponential growth cannot continue indefinitely, because the medium is soon depleted of nutrients and gets enriched with wastes.

3. During stationary phase, the growth rate slows as a result of depletion of nutrients and essential metabolites. Accumulation of organic acids and toxic products also slow down the growth rate of the cells. This phase is reached as the bacteria begin to exhaust the resources that are available to them. During this phase number of cells reaches a constant value as the rate of bacterial growth is equal to the rate of decay.

4. At death phase, there is an accumulation of toxic compounds and bacteria run out of nutrients and die.
Batch growth refers to culturing cells in a vessel with an initial charge of medium that is not altered by further nutrient addition or removal. This form of cultivation is simple and widely used both in the laboratory and industrially. Stages of cell growth in a batch reactor are shown in figure V.1. The log of the number of living cells is plotted as a function of time in this figure.

Batch culture is one of the widely used laboratory growth methods in which bacterial growth is studied. It is ideally spatially unstructured and temporally structured. The bacterial culture is incubated in a closed vessel with a single batch of medium. In some experimental regimes, some of the bacterial culture is periodically removed and fresh sterile medium is added. In the extreme case, this leads to the continual renewal of the nutrients. This is a chemostat, also known as continuous culture.

![Figure V.1. Stages of cell growth](image-url)
V.2.1. Rate law for growth of bacteria

Under suitable conditions, microorganisms extract essential nutrients from the medium and convert them into biological compounds (Shuler et al., 2000). Microbial growth is an example of heterogeneous autocatalytic reaction and can be expressed as

\[
\text{Substrates + Cells} \longrightarrow \text{Extracellular products + More cells}
\]

\[
\sum S + X \longrightarrow \sum P + nX
\]

The rate of growth is directly related to cell concentration and cellular reproduction is the normal outcome of this reaction. The rate of microbial growth is characterized by the specific growth rate (\(\mu\)), which is defined as

\[
\mu = \frac{1}{C_X} \frac{dC_X}{dt}
\]

(V.1)

Where \(C_X\) is cell mass concentration (g/l), \(t\) is time (day) and \(\mu\) is the specific growth rate (day\(^{-1}\)).

Microbial growth can also be described in terms of cell number concentration \(N\). This is as follows:

\[
\mu = \frac{1}{N} \left( \frac{dN}{dt} \right)
\]

(V.2)

V.2.2. Stoichiometry of cell growth

Cellular metabolism is highly dependent on microorganism/nutrient system and environmental conditions such as pH, temperature, redox potential, etc. A view of the cell as an open system may be represented as follows,
Figure V.2: A view of the cell as an open system

Number of nutrients present in a growth medium may be large. It is, however, impractical to write the balance equation taking all components into account. Balance equations are usually written in terms of growth limiting component or substrate. The growth limiting substrate is generally the first one to be exhausted when the organism is grown batchwise. From the kinetic viewpoint, it is the substrate which limits the growth rate of organisms. From the stoichiometric viewpoint growth limiting substrate is one which limits the growth in terms of obtainable biomass. In practice, a single substrate limiting growth both kinetically and stoichiometrically is considered as growth limiting substrate. In terms of growth limiting substrate, the metabolic reaction of bacterial growth may be represented as follows,

\[
A \xrightarrow{\text{Cells}} \text{Cells} + \text{Product (P)}
\]

\[
(1-\alpha)C_{S0} \longrightarrow (C_{X0} + Y_{X/S} (\alpha C_{S0})) \quad \alpha C_{S0} Y_{P/S}
\]

Where ‘\(\alpha\)’ is the degree of conversion of substrate, A.

Where the yield coefficients, \(Y_{X/S}\) and \(Y_{P/S}\) may be define as follows,

\[
Y_{X/S} = \frac{\text{Mass of new cell formed}}{\text{Mass of substrate consumed to produce new cells}}
\]
Or, $Y_{s} = \frac{dX}{dt} \frac{dt}{ds} \quad (V.3)$

$Y_{PS} = \frac{\text{Mass of product formed}}{\text{Mass of substrate consumed to form product}}$

Or, $Y_{s} = \frac{dP}{dt} \frac{dt}{ds} \quad (V.4)$

V.2.3. Composition of Cells:

The growth of single cell from inception till the time of its division into daughter cells constitutes cell cycle. Different phases in the cell cycle are M phase, G1 phase, S phase and G2 phase. In the M phase of the cell cycle, nuclear division (mitosis) occurs followed by the interphase. During the interphase daughter cells, formed from the mitosis phase, enter G1 phase which is characterized by a high rate of biosynthesis. This is followed by S phase which starts with DNA synthesis and ends when DNA content of the cells gets doubled. S phase is followed by G2 phase which ends with the initiation of mitosis. Thus the biochemical content of the cell is constantly changing over the cell cycle (Blanch and Clark). That is unbalanced growth prevails. However, balanced growth, characterized by equal rate of change of each cellular component predominates in the exponential growth phase.

V.3. Kinetics of batch study

There are several well-established kinetic models, unstructured and structured, non-segregated and segregated, available to predict the growth kinetics of microorganisms. Although, models containing both structure and segregation are the most realistic ones, they are also computationally complex (Shuler et. al., 2000). In such situation of heterogeneous autocatalytic reaction it is always advisable to select a model which is simple in nature and whose kinetic constants can be evaluated in a convenient way (Levenspiel 1984). The classical Monod model will be attempted to explain the nature of batch type experimental results.
V.3.1. Growth models for uninhibited growth

Monod equation describes substrate-limited growth only when growth is slow and the microbial population density is low. This is applicable in case of microbial growth in absence of inhibition and is very much similar to the Langmuir-Hinshelwood (or Hougen-Watson) kinetics in traditional chemical engineering system or Michaelis-Menten kinetics for enzyme catalyzed reactions in biochemical systems. It falls in the category of unstructured non-segregated substrate limited growth model.

Monod equation can be described as follows:

\[ \mu = \frac{\mu_m C_s}{K_s + C_s} \]  \hspace{1cm} (V.5)

At very high concentration range of substrate,

\[ C_s >> K_s \]

Therefore,\[ \mu = \mu_m \]

Therefore, a zero order correlation between \( \mu \) and \( C_s \) is obtained.

Where \( \mu_m \) is the maximum specific growth rate at saturating concentration.

At low range of substrate concentration,

\[ C_s << K_s \]

Therefore, equation (V.5) reduces to,

\[ \mu = \frac{\mu_m}{K_s} C_s \]  \hspace{1cm} (V.6)

Therefore, 1st order correlation exists between \( \mu \) and \( C_s \).
Other equations are also proposed to describe the substrate-limited growth phase. The following equations are alternatives to the Monod equation,

Blackman equation: \( \mu = \mu_m, \text{ if } C_s \geq 2K_s \)

\[
\mu = \left( \frac{\mu_m}{2K_s} \right) C_s, \quad C_s < 2K_s \quad (V.7)
\]

Tessier equation: \( \mu = \mu_m \left( 1 - e^{-K_i} \right) \quad (V.8) \)

Moser equation: \( \mu = \frac{\mu_mC_s^n}{K_s + C_s^n} = \mu_m \left( 1 + K_sC_s^{-n} \right)^{-1} \quad (V.9) \)

Contois equation: \( \mu = \frac{\mu_mC_s}{K_sC_C + C_s} \quad (V.10) \)

**V.4. Biodegradation of LCFA under anaerobic enviroment**

A series of complex reactions take place when lipid-rich waste undergoes anaerobic degradation leading to biogas generation. Reaction schemes involved during degradation of different lipid sources, namely, mustard oil and soybean oil are described in the following subsections.

**V.4.1. Reaction scheme of Mustard oil degradation**

In case of synthetic effluent containing mustard oil-water mixture, erucic acid (C22:1) is considered as the only growth limiting substrate which breaks down to oleic and stearic acid in the first step of microbial degradation. The acids subsequently produce propanoic, butyric and valeric acid as common volatile fatty acids, which after degradation produce acetic acid leading to biogas generation under the action of methanogenic bacteria. Erucic acid degradation takes place mainly via \( \beta \)-oxidation pathway in presence of enzymes secreted by the microbial cells with the production of energy in terms of ATP molecules. In case of acidogenesis of fatty acids containing even numbered carbon atoms such as erucic acid (C22:1), acetic acid is the main VFA which accumulates in the reaction media. The step for enzymatic hydrolysis of lipid molecules to long
chain fatty acids is considered as an instantaneous step and is not incorporated in the reaction kinetics during the evaluation of kinetic parameters using Monod model.

V.4.1.1. Proposed Reaction scheme

(i) Enzymatic Hydrolysis

Lipid molecules (triglycerides) $\rightarrow$ Erucic Acid (C 22:1)

(ii) Acidogenesis

Erucic Acid + CO$_2$ + $X_{\text{acidogen}}$ $\rightarrow$ LCFAs + n$X_{\text{acidogen}}$

(Eicocenic, Oleic, Arachidic, Stearic)

LCFAs + $X_{\text{acidogen}}$ $\rightarrow$ VFAs + n$X_{\text{acidogen}}$

(Valeric, butyric, propanoic, acetic)

VFAs + $X_{\text{acidogen}}$ $\rightarrow$ Acetic acid + n$X_{\text{acetogen}}$

Methanogenesis

Acetate + $X_{\text{methanogen}}$ $\rightarrow$ CH$_4$ + CO$_2$ + n$X_{\text{methanogen}}$
V.4.2. Reaction scheme of Soybean oil degradation

Linoleic acid is the major LCFA present in soybean oil and the probable series of reactions taking place during the fatty acid degradation are shown below considering linoleic acid as the growth limiting substrate,

\[
\text{Biomass} \quad \text{Linoleic Acid (C18:2) + CO}_2 \quad \rightarrow \quad \text{Oleic (C18:1) + Palmitoleic (C16:1)}
\]

\[
\text{Acidogenic}
\]

\[
\text{Biomass} \quad \text{Oleic acid (C18:1)} \quad \rightarrow \quad \text{Palmitic acid (C 16:0) + Myristic acid (C 14:0)}
\]

\[
\text{Acidogenic}
\]

Palmitic acid + Myristic acid \rightarrow VFAs (Butyric + valeric + propanoic)

\[
\text{Biomass} \quad \text{VFAs} \quad \rightarrow \quad \text{Acetic acid}
\]

\[
\text{(Acetogenic)}
\]

Methanogenic step is identical with that of erucic acid.

V.4.3. Characterization of microbial kinetics using long chain fatty acid as the growth limiting substrate

In order to understand the reaction engineering behavior of the biodegradation of fatty acid, classical Monod type of substrate uninhibited kinetic model (as shown by equation V.5) has been attempted during simulation work.

\[
\mu = \frac{\mu_{\text{max}} C}{K_s + C}
\]
The above equation may be modified as follows,

\[
\frac{1}{\mu} = \frac{1}{\mu_{\text{max}}} + \frac{K_s}{\mu_{\text{max}} C_s}
\]  

(V.11)

From equation (V.11) a Lineweaver-Burk type double reciprocal plot of $1/\mu$ vs $1/C_s$ has been made using the batch-type experimental data generated in Erlenmeyer flasks. The values of kinetic parameters namely $\mu_{\text{max}}$ and $K_s$ have been determined from the intercept and slope of the plot respectively. The values obtained from the batch studies have been compared with that obtained in a chemostat study.

V.4.4. Evaluation of yield coefficient

Substrate is utilized for different purposes during microbial growth. These are as follows,

\[
\Delta S = \Delta S_{\text{Biomass growth}} + \Delta S_{\text{Growth Energy}} + \Delta S_{\text{Maintenance Energy}} + \Delta S_{\text{Extracellular products}}
\]

Yield coefficient, $Y_{X/S}$ of biomass with respect to the utilization of substrate in the exponential growth phase may be defined as follows,

\[
Y_{X/S} = \frac{dX/dt}{ds/dt} = \frac{\Delta X}{-\Delta S}
\]  

(V.12)

This may be termed as apparent yield coefficient since value of true yield coefficient varies with time during batch culture.

The differential mass balance equations for biomass and lipid during batch and continuous Mode of operation in a chemostat are as follows:

Batch mode:
Figure V.3: Operation in a chemostat in batch mode

**Biomass,**

\[
\frac{dC_{\text{Xmethanol}}}{dt} = \mu C_{\text{Xmethanol}} \tag{V.13}
\]

\[
\frac{dC_{\text{Xmethanoll}}}{dt} = \mu C_{\text{Xmethanoll}} \tag{V.14}
\]

**LCFA,**

\[
\frac{dC_{\text{LCFA}}}{dt} = -\frac{\mu C_{\text{Xmethanol}}}{Y_{\text{Xmethanol}}/\text{LCFA}} \tag{V.15}
\]

**VFA,**

\[
\frac{dC_{\text{VFA}}}{dt} = \left( \frac{\mu C_{\text{Xmethanol}}}{Y_{\text{Xmethanol}}/\text{LCFA}} \right)_{\text{LCFA} / \text{VFA}} - \left( \frac{\mu C_{\text{Xmethanol}}}{Y_{\text{Xmethanol}}/\text{VFA}} \right) \tag{V.16}
\]

**Methane,**

\[
\frac{dC_{\text{CH}_4}}{dt} = \left( \frac{\mu C_{\text{Xmethanol}}}{Y_{\text{Xmethanol}}/\text{LCFA}} \right)_{\text{CH}_4 / \text{Xmethanol}} + \left( \frac{\mu C_{\text{Xmethanol}}}{Y_{\text{Xmethanol}}/\text{VFA}} \right)_{\text{CH}_4 / \text{Xmethanoll}} \tag{V.17}
\]
Transient concentration profiles of biomass and LCFA have been simulated by solving equations (V.13 to V.17) through 4th order R-K method using the following initial conditions:

\[
\begin{bmatrix}
C_{\text{methanol}} &= 0.03 \text{ kg} / \text{m}^3 \\
C_{\text{methanol,sf}} &= 0.02 \text{ kg} / \text{m}^3 \\
C_{\text{LCFA}} &= 0.08 - 0.15 \text{ kg} / \text{m}^3 \\
C_{\text{VF4}} &= 0 \text{ kg} / \text{m}^3 \\
C_{\text{CH4}} &= 0 \text{ kg} / \text{m}^3
\end{bmatrix}
\]

At \( t = 0, \)

**Continuous mode:**

Material balance of biomass and substrate in a continuous culture in a chemostat are as follows,

\[
F_{\text{c}} C_{\text{Sin}} - F_{\text{c}} C_{\text{X}} C_{\text{S}} = V \left( \frac{dC_{\text{X}}}{dt} \right)
\]

Figure V.4. Operation of a chemostat under continuous mode

Biomass,

\[
V \frac{dC_{\text{X}}}{dt} = F (0 - C_{\text{X}}) + V \mu C_{\text{X}} \tag{V.18}
\]

Dividing both sides by \( V, \)
\[
\frac{dC_x}{dt} = -\frac{F}{V} C_x + \mu C_x
\]

Under steady state,

\[
C_x \left( \mu - \frac{F}{V} \right) = 0
\]

\[
(\mu - D)C_x = 0 \quad (V.19)
\]

Material balance of LCFA around the reactor leads to,

\[
\frac{dC_s}{dt} = \frac{F}{V} (C_{sw} - C_s) - \frac{\mu C_x}{Y_{XS}} \quad (V.20)
\]

Under steady state,

\[
\frac{F}{V} (C_{sw} - C_s) - \frac{\mu}{Y_{XS}} C_x = 0 \quad (V.21)
\]

Or, \( D(C_{sw} - C_s) - \frac{\mu}{Y_{XS}} C_x = 0 \quad (V.22) \)

Where, \( D = \frac{F}{V} = \mu = \frac{1}{HRT} \quad (V.23) \)

Or, equation (V.22) reduces to,

\[
\frac{1}{HRT} (C_{sw} - C_s) - \frac{\mu}{Y_{XS}} C_x = 0
\]

or,

\[
\frac{C_{sw} - C_s}{HRT C_x} = \frac{\mu}{Y_{XS}} \quad (V.24)
\]
The value of yield coefficient ($Y_{x\mu}$) may be obtained from the slope of the straight line when $(C_{Sin}-C_{SS})/HRT.C_X$ is plotted against $\mu$.

**V.4.5. Reactor Modeling**

The up-flow packed bed bioreactor has been schematically represented in figure V.5. Figures a and b represent the characteristic spheres and their contacting pattern in the bed matrix respectively.
The following assumptions have been made during the development of the mathematical model,

1) Inlet feed stream is sterile;
2) Biofilm thickness remains unchanged throughout the operation;
3) The process is under steady state;
4) Erucic acid is the only growth limiting substrate;
5) Packing material may be represented by equivalent spheres, as shown in figure
6) Reactor packing is random;
7) Both acidogenic and methanogenic bacterial consortia follow balanced growth governed by Monod model;
8) Bed porosity($\epsilon$) remains same during the operation;
9) Both external and internal mass transfer resistances are negligible;
10) Both axial and radial dispersion may be neglected.

Applying the above assumptions the substrate and biomass balance equations over the differential element $\Delta z$ (Figure V.5) in the anaerobic filter may be written as follows,

**Substrate:**

Under steady state,

\[
\text{Input} - \text{Output} - \text{Rate of reaction} = 0
\]

\[
UA\epsilon C_a - UA\epsilon C_a - r_y A\Delta Z(1 - \epsilon)\alpha_i l_i = 0 \quad (V.25)
\]

Where,
\[-r_x = \frac{r_x}{Y_{\frac{Y}{S}}}
\]

\[r_x = \mu C_x\]

or,

\[r_x = \frac{\mu_{\text{max}} C_s C_x}{K_s + C_s}\]  \hspace{1cm} (V.26)

Equation (V.25) reduces to,

\[UAeC_lZ - UAeC_s = \frac{\mu_{\text{max}} C_s C_x}{Y_{\frac{Y}{S}}(K_s + C_s)} A\Delta Z(1 - \epsilon) a_p l_f = 0\]  \hspace{1cm} (V.27)

Dividing both sides by \(AU\Delta Z\) and limiting \(\Delta Z \to 0\)

\[\lim_{\Delta Z \to 0} \frac{C_lZ - C_sZ_{\Delta Z}}{\Delta Z} = \frac{\mu_{\text{max}} C_s C_x}{Y_{\frac{Y}{S}}(K_s + C_s)\epsilon U} (1 - \epsilon) l_f a_p \]  \hspace{1cm} (V.28)

Or,

\[\frac{dC_s}{dZ} = \frac{(1 - \epsilon) \mu_{\text{max}} C_s C_x}{\epsilon U Y_{\frac{Y}{S}}(K_s + C_s) l_f a_p}\]  \hspace{1cm} (V.29)

Biomass:

\[\text{Input} - \text{output} + \text{generation} = 0\]
\[
U \alpha e C x |_{z} - U \alpha e C x |_{z + \Delta Z} + r x A \Delta Z (1 - \varepsilon) a_{j} l_{j} = 0
\] (V.30)

\[
U \alpha e C x |_{z} - U \alpha e C x |_{z + \Delta Z} + \frac{\mu_{\max} C x}{(K_{x} + C_{x})} A \Delta Z (1 - \varepsilon) a_{j} l_{j} = 0
\] (V.31)

Dividing both sides by \(AU\Delta Z\) and limiting \(\Delta Z \rightarrow 0\)

\[
\lim_{\Delta Z \rightarrow 0} \frac{C_{k}}{(1 - \varepsilon) a_{j} l_{j}} = \frac{\mu_{\max} C x}{(K_{x} + C_{x}) \alpha U}
\] (V.32)

Or,

\[
\frac{dC_{k}}{dZ} = \frac{(1 - \varepsilon) \mu_{\max} C x}{\alpha U (K_{x} + C_{x})} a_{j} l_{j}
\] (V.33)

**V.4.6. LCFA (Erucic acid) degradation concentration zone of 0.08 to 0.15 kg/m\(^3\)**

The differential system equations based on Monod kinetics for LCFA degradation, VFA generation and methane production may be written as follows,

\[
\frac{dC_{LCFA}}{dZ} = \frac{1}{Y_{LCFA} \nu_{LCFA}} \frac{\mu_{\max} C_{LCFA} C_{X_{molten}} a_{j} l_{j}}{K_{S1} + C_{LCFA}} (1 - \varepsilon) \frac{(1 - \varepsilon)}{\alpha U}
\] (V.34)

\[
\frac{dC_{VFA}}{dZ} = \left[ \frac{1}{Y_{LCFA_{molten}} \nu_{LCFA}} \frac{\mu_{\max} C_{LCFA} C_{X_{molten}} a_{j} l_{j}}{K_{S2} + C_{LCFA}} \right] \left[ \frac{1}{Y_{VFA_{molten}} \nu_{VFA}} \frac{\mu_{\max} C_{VFA} C_{X_{molten}} a_{j} l_{j}}{K_{S2} + C_{VFA}} \right] (1 - \varepsilon)
\] (V.35)

\[
\frac{dC_{CH4}}{dZ} = \left[ \frac{1}{Y_{LCFA_{molten}} \nu_{LCFA}} \frac{\mu_{\max} C_{LCFA} C_{X_{molten}} a_{j} l_{j}}{K_{S1} + C_{LCFA}} \right] \left[ \frac{1}{Y_{VFA_{molten}} \nu_{VFA}} \frac{\mu_{\max} C_{VFA} C_{X_{molten}} a_{j} l_{j}}{K_{S2} + C_{VFA}} \right] (1 - \varepsilon)
\] (V.36)
Equations (V.34), (V.35) and (V.36) have been solved by Runge-Kutta method using a suitable C program.

Substrate uninhibited Linoleic acid degradation

Equations (V.34) to (V.36) have been used to generate the simulated profiles of LCFA,VFA and methane using linoleic acid as the limiting substrate.

V.4.7. Biochemical methane potential

Computation of cumulative methane production per unit volume of the reactor or biochemical methane potential has been done using the following expressions:

\[
Q_{C,CH_4} = \frac{\sum \left( \frac{C_{CH_4} - S_{CH_4}}{M_{CH_4}} \right) \times \left( \frac{T}{273} \right) \times 22.4}{\sum \Delta t}
\]  \hspace{1cm} (V.37)

Where, \( S_{CH_4} = \) solubility of methane in water at the specified condition in kg/m \(^3\)

V.4.8. Determination of specific surface area

Specific surface area of the particles having irregular shapes (e.g., residues of earthen pots of different sizes) has been determined by screen analysis method and the correlation with bed porosity as obtained from Alonso et al’s concept can be written as follows:

For spherical particles,

\[
a_p = \frac{4\pi \left( R + \frac{1}{2} \right)^2 - nA_i}{\frac{4}{3} \pi R^3}
\]  \hspace{1cm} (V.38)
Where,
\[ A_i = 2\pi(R + l_f) \rho_f \]  \hspace{1cm} (V.39)

Assume \( n = 6 \);

And, \( R = \frac{d}{2} \)

For irregularly shaped particles, particle diameter, \( d \) is to be corrected to average particle diameter, \( d_p \) incorporating sphericity (\( \beta \)) as given below,

\[ \frac{\pi}{6} d_p^3 = \beta \frac{\pi}{6} d^3 \]

\[ d_p = \beta^{\frac{1}{3}} d \]

\[ d = \beta^{-\frac{1}{3}} d_p \]

By replacing \( d \) with \( d_p \) the Equation (V.38) becomes,

\[ a_p = \frac{4\pi}{\frac{4}{3} \pi \left( \frac{\beta^{\frac{1}{3}} d_p}{2} \right)^3} \left( \frac{\beta^{\frac{1}{3}} d_p}{2} + l_f \right) - nA_i \]  \hspace{1cm} (V.40)
V.4.9. Determination of Cold Gas Efficiency

Erucic acid rich oil + water

Figure V.6: Schematic representation of the Anaerobic Filter

Basis = 1 day

Heating value involved with the feed stream = \( QC_{oil} LHV_{oil} \) \( \text{(V.41)} \)

Where, \( Q \) = volumetric flow rate of the feed stream, \( \text{m}^3/\text{day} = \frac{V_F}{HRT} \)

\( C_{oil} \) = concentration of mustard oil in the feed in \( \text{kg/m}^3 \)

\( LHV_{oil} \) = lower heating value of mustard oil, \( \text{kJ/kg} \)

Heating value involved with biogas = \( GV_{CH_4} LHV_{CH_4} \) \( \text{(V.42)} \)

Where,

\( G \) = volumetric flow rate of biogas in \( \text{m}^3/\text{day} \)
$V_{CH4} =$ volume % of methane present in biogas

Therefore,

\[ \% \text{ cold gas efficiency, } \eta_{cg} = \left( \frac{GV_{CH4}LHV_{CH4}}{QC_{oil}LHV_{oil}} \right) \times 100 \]  

(V.43)

**V.4.10. Determination of biofilm thickness ($l_f$):**

Bed porosity of the anaerobic packed bed reactor before and after immobilization has been determined by water saturation method. The thickness of the biofilm developed may be obtained as follows:

\[
\text{Biofilm thickness} = \frac{\text{Bed porosity after immobilization (} \epsilon_0) - \text{Bed porosity before immobilization (} \epsilon_f)}{\text{Surface area of particles}}
\]

or,

\[ l_f = \frac{(\epsilon_0 - \epsilon_f)V}{nv_p a_p} \]

where, \(nv_p = V(1 - \epsilon_0)\)

Therefore,

\[ l_f = \frac{(\epsilon_0 - \epsilon_f)V}{V(1 - \epsilon_0)a_p} \]

or, \(l_f = \frac{\epsilon_0 - \epsilon_f}{(1 - \epsilon_0)a_p} \)  

(V.44)
V.4.11. Modeling of the anaerobic filter (AF) with backwashing

V.4.11.1. Determination of initial biofilm thickness

The initial biofilm thickness can be obtained by determining the bed porosity before and after immobilization using the following equations,

\[ V_n(e_0 - e) = n_r \left[ \frac{4}{3} \pi \left( R + \frac{L_p}{2} \right)^3 - \frac{4}{3} \pi R^3 \right] \]

Or,

\[ V_n(e_0 - e) = n_r \left[ \frac{4}{3} \pi \left( \beta^{1/2} \frac{d_v}{2} + L_p \right)^3 - \frac{4}{3} \pi \left( \beta^{1/2} \frac{d_v}{2} \right)^3 \right] \]

(V.45)

V.4.11.2. Reactor modeling

The up-flow anaerobic filter has been schematically represented in figure .

The basic assumptions for the development of the mathematical model are given as below,

1. Inlet feed stream of the filter is sterile;

2. Biomass produced due to biochemical reaction in the reactor gets adhered to the packing surface and is not transported with the liquid stream. Biofilm thickness is allowed to increase and consequently bed porosity gets altered during the operation;

3. The process is under steady state;

4. Erucic acid is the only growth limiting substrate;
5. The characteristic spheres of the packing materials are used as the immobilization matrix. Some of the spheres, n, is always in contact with one spherical particle leading to loss in biofilm surface area and volume per unit sphere in contact;

6. Reactor packing is random;

7. Growth kinetics of both acidogenic and methanogenic bacterial consortia can be determined by following classical Monod model;

8. Both external and internal mass transfer resistances are negligible.

The system equation based on differential mass balance for the conversion of lipid in terms of LCFA, present in the simulated wastewater, is as follows,

\[
\frac{dC_{\text{LCFA}}}{dz} = -\frac{1}{Y_{\text{LCFA}}} \frac{\mu_{\text{max}} C_{\text{S}}}{K_{S} + C_{\text{LCFA}}} a_{p} l_{f} \left(1 - \varepsilon_{b}\right) \frac{\partial \theta}{\partial u} \quad (V.46)
\]

According to Alonso et al.’s concept, surface area available for a sphere, with which n neighbouring spheres are in contact, is as follows,

\[
a_{p} = \frac{4\pi (R + l_{f})^{2} - nA_{i}}{\frac{4}{3} \pi R^{3}} \quad (V.47)
\]

For non-spherical particles of equivalent diameter, dp, specific surface area, a_{p} may be represented as follows,

\[
a_{p} = \frac{4\pi \left(\frac{\beta^{\gamma} d_{p}}{2} + l_{f}\right)^{2} - nA_{i}}{\frac{4}{3} \pi \left(\frac{\beta^{\gamma} d_{p}}{2}\right)^{3}} \quad (V.48)
\]

Where,
\[ A_i = 2\pi(R + l_j)l_j \]

= loss of contact surface area per unit sphere in contact

*Assume particles in contact, n=6; and, R = d/2*

Making material balance for methanogenic(I) and methanogenic(II) microbial biomass over differential element of thickness \( \Delta Z \) located at position \( Z \), for time \( \Delta t \),

\[ \text{Figure V.7. Schematic representation of anaerobic filter showing solid matrices} \]

\[ A \\Delta Z (1 - \varepsilon) \alpha \rho \ l_j \ C_{XT} \bigg|_{t+\Delta t} - A \ \Delta Z (1 - \varepsilon) \alpha \rho \ l_j \ C_{XT} \bigg|_t = \]

\[ \left[ \mu_{\text{methanol}} \ C_{\text{Xmethanol}} + \mu_{\text{methanone}} \ C_{\text{Xmethano}} \right] A \ \Delta Z (1 - \varepsilon) \alpha \rho \ l_j \ \Delta t \]

(V.49)

Where,

\( C_{X0, \text{Acido}} = \alpha C_{XT} \)

\( C_{X0, \text{Methano}} = \beta' C_{XT} \)

Where \( \alpha \) and \( \beta' \) are the fractions of methanogenic(I) and methanogenic(II) bacteria respectively.

Dividing both sides of equation by \( A \ \Delta Z (1 - \varepsilon) \alpha \rho \ l_j \ \Delta t \), the above equation reduces to,
\[
\frac{1}{L_f} \frac{dL_f}{dt} = \eta \left[ \mu_{\text{max}, 1} \left( \frac{\alpha C_{\text{LCFA}}}{K_{\text{S1}} + C_{\text{LCFA}}} \right) + \mu_{\text{max}, 2} \left( \frac{\beta C_{\text{VFA}}}{K_{\text{S2}} + C_{\text{VFA}}} \right) \right]
\]  \hspace{1cm} (V.50)

Therefore equation 1 can be modified as below:

\[
\frac{dC_{\text{LCFA}}}{dz} = -\frac{1}{Y_{\text{LCFA}} \left( \frac{\mu_{\text{max}} C_{\text{LCFA}} C_{\text{s}}}{K_{\text{S1}} + C_{\text{LCFA}}} \right)} \left[ 4\pi \left( \frac{\beta \sqrt{\varepsilon} d_p}{2} + l_f \right)^2 - 4/3 \pi \left( \frac{\beta \sqrt{\varepsilon} d_p}{2} \right)^3 \right] - n A_\eta f_f (1 - \varepsilon_t) \frac{\varepsilon_t}{\varepsilon_H} \]  \hspace{1cm} (V.51)