CHAPTER –I
INTRODUCTION
I. INTRODUCTION

Water pollution is a major global problem which requires ongoing evaluation and revision of water resource policy at all levels. Industrial and domestic liquid effluents are identified as point sources to water pollution as they are coming from a single, identified source. The main cause of water pollution by these effluents is their high organic load. Wastewater generated from edible oil industries, dairy and food processing industries, fish processing industries are having high BOD and COD values owing to their large lipid content. Among them, environmental problems created by edible oil industries are prominent particularly in India.

India is fourth largest oil seed producing country in the world. It is one of the world’s largest edible oil economies with 15,000 oil mills, 689 solvent extraction units and over 1000 refineries. Liquid effluents from edible oil refineries and food processing industries having high BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) values owing to their large lipid content cause groundwater pollution if disposed off to the water bodies without proper remediation. When mixed with groundwater, this wastewater poses a serious threat to mankind as they affect the water ecosystem adversely. Therefore, these liquid wastes demand a proper way of disposal subject to modern engineering inventions.

I.1. Indian scenario of Edible oil Industries

India has approximately 550 units of edible oil refineries located in different States. The sources of edible oil manufacture are soyabean, groundnut, rapeseed, sunflower, safflower, cotton, sesame, coconut, palm, mustard, rice bran, watermelon, neem etc.

Oilseeds and edible oils are two of the most sensitive essential commodities. India is one of the largest producers of oilseeds in the world and this sector occupies an important position in the agricultural economy and accounting for the estimated production of 24.88 million tonnes of nine cultivated oilseeds during the year 2009-10 (November-October). India contributes about 6-7% of the world oilseeds production.
I.2. Status of the Vegetable Oil Industry

Table I.1: Status of the Vegetable Oil Industries in India

<table>
<thead>
<tr>
<th>Type of Vegetable Oil Industry</th>
<th>Number of units</th>
<th>Annual Capacity (Lakh MT)</th>
<th>Average capacity Utilization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oilseed Crushing Units</td>
<td>1,50,000</td>
<td>425*</td>
<td>10-30</td>
</tr>
<tr>
<td>Solvent Extraction Units</td>
<td>795</td>
<td>419**</td>
<td>34</td>
</tr>
<tr>
<td>Refineries attached with Vanaspati Units</td>
<td>127</td>
<td>51***</td>
<td>45</td>
</tr>
<tr>
<td>Refineries attached with Solvent Units</td>
<td>226</td>
<td>37***</td>
<td>29</td>
</tr>
<tr>
<td>Independent Refineries</td>
<td>590</td>
<td>35***</td>
<td>36</td>
</tr>
<tr>
<td>Total Refineries</td>
<td>943</td>
<td>123***</td>
<td>37</td>
</tr>
<tr>
<td>Vanaspati Units</td>
<td>268</td>
<td>58****</td>
<td>19</td>
</tr>
</tbody>
</table>

* In terms of seeds  
** In terms of Oil-bearing Material  
*** In terms of oil  
**** In terms of Vanaspati, Bakery Shortening & Margarine

I.3. Locations of Major edible oil industries in India

The locations of major edible oil industries in India are shown in Figure I.1. The average refining capacity of a standard edible oil refinery may be in the range of 1200 to 1600 tonnes of crude oil processed per day.
I.4. Major edible oil produced in India

The major edible oils produced in India include soybean oil, sunflower oil, rapeseed oil, mustard oil, coconut oil etc. Although edible oil in most of the countries outside Indian subcontinent is based on soybean oil, olive oil, sunflower oil and so on, in Indian perspective mustard oil and soybean oil are very important. India occupies third position in the list of mustard producing countries.

I.4.1. Mustard oil

Mustard oil is an important vegetable oil consumed mostly in north and eastern India. The oil is produced from black mustard (Brassica nigra), brown Indian mustard (Brassica juncea), and white mustard (Brassica hirta). Mustard oil contains the pungent allyl isothiocyanate and has about 60% monounsaturated fatty acids of which 42% is erucic acid and 12% is oleic acid. It has 21% polyunsaturates of which 6% is the omega-3 alpha-linolenic acid and 15% omega-6 linoleic acid and it has 12% saturated fats.
Indian specifications of crude mustard oil are given in Table I.2.

Table I.2: Specifications for crude mustard seed Oil (Indian)

<table>
<thead>
<tr>
<th>Properties of Mustard oil</th>
<th>Values at standard conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (at 15°C)</td>
<td>900-930 kg/m³</td>
</tr>
<tr>
<td>Flash point ( Pensky-Martenes Method)</td>
<td>220°C</td>
</tr>
<tr>
<td>Calorific value</td>
<td>35,000kJ/kg</td>
</tr>
<tr>
<td>Coke deposit (Conradson procedure)</td>
<td>0.4 wt%</td>
</tr>
<tr>
<td>Iodine value</td>
<td>100-120g/100g</td>
</tr>
<tr>
<td>Saponification number</td>
<td>168-177</td>
</tr>
<tr>
<td>Sulphur content</td>
<td>20 mg/kg</td>
</tr>
<tr>
<td>Neutralization number</td>
<td>2.0mg KOH/g</td>
</tr>
<tr>
<td>Phosphoros content</td>
<td>15mg/kg</td>
</tr>
<tr>
<td>Water content</td>
<td>75mg/kg</td>
</tr>
<tr>
<td>Ash</td>
<td>0.01 wt%</td>
</tr>
</tbody>
</table>

I.4.1.1. Fatty acid composition of mustard oil

The major fatty acids present in mustard oil are erucic acid (54 wt%), palmitic acid, oleic acid, stearic acid, eicosenoic acid, etc. The detailed composition may be given in Table I.3.

Table I.3. Fatty acid composition of mustard oil

<table>
<thead>
<tr>
<th>Saturated fatty acids</th>
<th>Values in wt%</th>
<th>Unsaturated fatty acids</th>
<th>Values in wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td>1.5</td>
<td>Oleic acid</td>
<td>22.0</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>0.4</td>
<td>Eicosenoic acid</td>
<td>7.0</td>
</tr>
<tr>
<td>Arachidic acid</td>
<td>0.5</td>
<td>Erucic acid</td>
<td>54.0</td>
</tr>
<tr>
<td>Behenic acid</td>
<td>2.0</td>
<td>Linoleic acid</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Linolenic acid</td>
<td>6.8</td>
</tr>
<tr>
<td>Total saturated</td>
<td>5.4</td>
<td>Total unsaturated</td>
<td></td>
</tr>
</tbody>
</table>
I.4.2. Soybean oil

Soybean oil is a vegetable oil extracted from soybean seeds. It is one of the most widely consumed cooking oil in India. 100g of soybean oil has 16g of saturated fat, 23g of mono unsaturated fat, and 58g of poly unsaturated fat. The fatty acid composition of soybean oil may be shown in table I.4.

Table I.4. Fatty acid composition of soybean oil

<table>
<thead>
<tr>
<th>Saturated fatty acids</th>
<th>Values in wt%</th>
<th>Unsaturated fatty acids</th>
<th>Values in wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stearic acid</td>
<td>4</td>
<td>α-Linolenic acid(C 18:3)</td>
<td>7-10</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>10</td>
<td>Linoleic acid(C 18:2)</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oleic acid (C 18:1)</td>
<td>23</td>
</tr>
</tbody>
</table>

The major unsaturated fatty acids in soybean oil triglycerides are 7–10% alpha-Linolenic acid (C-18:3); 51% linoleic acid (C-18:2); and 23% oleic acid (C-18:1). It also contains the saturated fatty acids 4% stearic acid and 10% palmitic acid.

I.4.3. Description of essential fatty acids present in edible oil

Erucic acid is the main constituent of mustard oil. It is a monounsaturated omega-9 fatty acid, denoted 22:1 ω-9. It has the formula CH₃(CH₂)₇CH=CH(CH₂)₁₁COOH. Erucic acid can be used as a substitute of mineral oils but it is readily biodegradable than some common oils. Erucic acid is the major component of rapeseed/mustard oil seeds being the members of the brassica family.

Linolenic acid is an unsaturated fatty acid, C₁₇H₂₉COOH, considered essential to the human diet that is an important component of natural drying oils. Linolenic acid is of two types, namely, α-Linolenic acid and γ-Linolenic acid. The first one is an ω-3 fatty acid which is found in many vegetable oils. The unmodified term, \textit{linolenic acid}, most commonly refers to this substance.

Linoleic Acid (also called \textit{cis, cis,-9, 12-octadecadienoic acid}) is an example of a \textit{poly-unsaturated} fatty acid, due to the presence of two C=C double bonds. It is the main fatty acid found in vegetable oils such as soybean oil, sunflower oil, corn oil and rapeseed oil. Linoleic acid belongs to one of the two classes of essential fatty acids that humans require. These acids are called "essential" because they cannot be synthesised by the human body and must be eaten in food.
Stearic acid (C 18:0) is a saturated fatty acid with the formal IUPAC name octadecanoic acid. It is a waxy solid, and its chemical formula is C₁₈H₃₆O₂, or CH₃(CH₂)₁₆COOH. Stearic acid is found in vegetable oils such as sunflower oil, palm oil etc.

Structure of some common fatty acids present in edible oil such as mustard oil, soybean oil etc are shown in Table I.5.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Chemical Structure</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid</td>
<td><img src="image1.png" alt="Chemical Structure" /></td>
<td>C₁₈H₃₄O₂</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td><img src="image2.png" alt="Chemical Structure" /></td>
<td>C₁₈H₃₂O₂</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td><img src="image3.png" alt="Chemical Structure" /></td>
<td>C₁₈H₃₂O₂</td>
</tr>
<tr>
<td>Stearic acid</td>
<td><img src="image4.png" alt="Chemical Structure" /></td>
<td>C₁₈H₃₆O₂</td>
</tr>
<tr>
<td>Erucic acid</td>
<td><img src="image5.png" alt="Chemical Structure" /></td>
<td>C₂₂H₄₂O₂</td>
</tr>
<tr>
<td>Eicosenoic acid</td>
<td><img src="image6.png" alt="Chemical Structure" /></td>
<td>C₂₀H₃₈O₂</td>
</tr>
<tr>
<td>Valeric acid</td>
<td><img src="image7.png" alt="Chemical Structure" /></td>
<td>C₂₁H₁₀O₂</td>
</tr>
</tbody>
</table>
I.5. Effluent from Edible oil industries

The refined edible oil manufacturing units generate solid waste (spent earth) and wastewater which are of environmental concern and need proper treatment prior to their disposal (Pandey et al., 2003). In a vegetable oil industry, the effluent mainly comes from the degumming, deacidification and deodorisation steps (Kale et al., 1999). The blow down of the boiler and wash water from de-oiling of the bleaching earth also contribute to the effluent in small amounts.

The wastewater streams generated in an edible oil refinery are mainly from vat house after soap splitting, floor washing, cooling tower, boiler and filter press. The vat house drain carries the major portion of the wastewater, which is acidic in nature. The combined wastewater, generated at the industry under investigation, contained various emulsifiers, biocides, metallic and non-metallic solids, anti-oxidants and other chemical additives. The wastewater contains fatty substances in dispersed and non-dispersed forms respectively and also contains high levels of oil and grease resulting in high COD. Main processes and unit operations generating effluent waste water are shown in the Flow diagram in Figure I.2.

![Flow diagram of a typical wastewater treatment plant](image-url)

**Figure I.2. Flow diagram of a typical wastewater treatment plant**
The major constituents of wastewater of a typical edible oil refinery may be shown in Table I.6.
(data based on Adani Wilmer Ltd.)

Table I.6: Characteristics of wastewater generated at an edible oil refinery

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Influent</th>
<th>Final</th>
<th>Standard for treated effluent for inland water discharge (MoEF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Yellowish</td>
<td>Colorless</td>
<td></td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>35</td>
<td>30.2</td>
<td>Should not exceed 5°C above the receiving water temperature</td>
</tr>
<tr>
<td>pH</td>
<td>2±0.8</td>
<td>7.6±0.4</td>
<td>5.5 to 9.0</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>BDL (below detection limit)</td>
<td>BDL</td>
<td></td>
</tr>
<tr>
<td>Total dissolved solids (mg/L)</td>
<td>4800</td>
<td>2000</td>
<td>2100</td>
</tr>
<tr>
<td>Oil and grease (mg/L)</td>
<td>150±1.0</td>
<td>ND (not detected)</td>
<td>10</td>
</tr>
<tr>
<td>BOD5 (200°C)</td>
<td>359±11.0</td>
<td>10±2.0</td>
<td>30</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>7000±8.0</td>
<td>10.0±5.1</td>
<td>250</td>
</tr>
<tr>
<td>Sulphide</td>
<td>8.4±0.6</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Sulphate</td>
<td>20±0.2</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Phosphate</td>
<td>57.4±0.8</td>
<td>8.2±0.4</td>
<td></td>
</tr>
</tbody>
</table>

Heavy Metals

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>0.01</td>
</tr>
<tr>
<td>Cd</td>
<td>0.005</td>
</tr>
<tr>
<td>Zn</td>
<td>0.2</td>
</tr>
<tr>
<td>Mn</td>
<td>0.04</td>
</tr>
<tr>
<td>Ni</td>
<td>0.25</td>
</tr>
<tr>
<td>Fe</td>
<td>1.4</td>
</tr>
<tr>
<td>Cu</td>
<td>0.03</td>
</tr>
</tbody>
</table>

This wastewater is further treated in the effluent treatment plants before final disposal or reuse.

All values except colour, pH and temperature are in mg/l.

I.6. Effluent Treatment Processes of edible oil industries

In the edible oil industries, the wastewater from cooling tower and boiler sections and solid wastes can be recycled and reused in the process after preliminary treatment. The combined wastewater from other streams is treated in the effluent treatment plant (ETP). The process flow diagram of an effluent

8
treatment plant of an edible oil refinery is shown in Figure I.3. There is primary clarification of the wastewater generating from alkali refining, washing of filters and cleaning to remove oil and grease and BOD. Finally activated sludge processes followed by bioreactors are used to remove the residual BOD and COD before disposal.

Figure I.3: Treatment of wastewater in an Edible Oil Refinery

I.7. Secondary Treatment of lipid-rich wastewater

Lipid is an important component of industrial wastewater to be removed from the liquid effluent as it causes serious water pollution due to its high organic load. Generally, lipid residues are partially
recovered from wastewater by air flotation and the remaining residues are treated by normal wastewater-treatment processes (Lefebvre et al., 1998). The disposal of the floated lipid waste also causes pollution so the necessity of bioremediation of lipid-rich wastes, either aerobically or anaerobically, have been investigated (Borja et al., 1994; Borja and Banks, 1995; Stams and Elferink, 1997; Masse et al., 2001). The secondary treatment for the lipid removal mainly comprises of biological treatment to remove the BOD of the effluent stream through different processes such as, activated sludge process, trickle filters, up-flow anaerobic sludge blanket systems, anaerobic digestion etc.

I.8. Conventional BOD and COD Removal processes

I.8.1. Activated Sludge process

Activated sludge is a process for treating sewage and industrial wastewaters using air and a biological floc composed of bacteria and protozoans. The process involves air or oxygen being introduced into a mixture of primary treated or screened sewage or industrial wastewater combined with organisms to develop a biological floc which reduces the organic content of the sewage. This is an effective and convenient technique of BOD removal from industrial and domestic wastewater. A simplified flow diagram of activated sludge process is shown in figure I.4.

![Figure I.4. Flow diagram of activated sludge process](image)
I.8.2. Demerits of conventional methods

While treating lipid-rich effluent by conventional activated sludge process, there arise some operational problems which significantly reduce the BOD removal efficiency. The presence of fatty molecules in the waste water cause froth formation during circulation of air through the system. Again, in this type of reactor, the lipid molecules getting adhered to the biomass surface will cause washout of active cells with the product stream of the biodigester. Biogas generation from lipid digestion also requires strict anaerobic conditions for the survival of methanogenic bacteria. So, anaerobic digestion of lipid-rich waste in packed bed reactors using attached cellular growth may be attempted instead of the conventional methods of BOD and COD removal.

I.9. Anaerobic Digestion

Anaerobic digestion is a series of complex reactions in which microorganisms break down the high molecular weight biodegradable matters to their lower counterparts in absence of air or oxygen leading to the formation of biogas as an alternate source of energy. A few pioneering works have been reported on the treatment of high-strength wastewater through anaerobic biodegradation in presence of lipids (Young and McCarty, 1969; Ahring BK, 2003; Angelidaki et al; 1992). Anaerobic digestion is a popular pathway for the reduction of BOD values of organic-rich wastewater as reported by several investigators (Hu et al., 2002; Fernandez et al., 2005; Biswas et al., 2006; Palatsi et al., 2011).

As suggested by Cirne et al in 2007, during anaerobic digestion, lipids are enzymatically hydrolyzed to glycerol and free LCFAs (long chain fatty acid) in presence of extracellular lipase secreted by acidogenic bacteria. The further conversion of the hydrolysis products takes place in the intracellular environment whereby glycerol is converted to acetates and LCFAs are converted to acetates (in case of even numbered carbon) and propionate (in case of odd numbered carbon) through β-oxidation pathway (Weng et al., 1976). In case of formation of propionate acetogenic bacteria further convert the propionate molecules to acetates. In the final stage methanogenic bacteria act on acetate to form methane and carbon-di-oxide. Stage wise representation of anaerobic digestion may be shown in Figure I.5.
I.9.1. Different stages of Anaerobic Digestion:

Anaerobic biodegradation of lipid rich wastewater of high BOD value principally consists of four major steps, namely lipolytic, acidogenic, acetogenic and methanogenic.

I.9.1.a. Lipolytic step: lipid molecules in the form of triglycerides are hydrolysed to the corresponding long chain fatty acids (LCFA) catalyzed by the lipase secreted by the microorganisms in anaerobic environment. In this process of fat splitting fatty acid molecules act as fuel molecules, being started as triacylglycerides and broken down to generate energy. The triglycerides of fats and oils are split under proper conditions by the process of hydrolysis to free fatty acids and glycerol as shown in Figure I.6.
Figure I.6: Fat splitting

It is a stage wise reversible process and the reaction is catalyzed by lipolytic enzymes which are secreted by the microorganisms present in the reaction broth. This reaction is carried out at ordinary temperatures as the enzymes are ineffective at high pressure and high temperatures.

I.9.1.b. Acidogenic step: Long chain fatty acids (LCFA) produced through lipid hydrolysis break down through β-oxidation pathway in presence of acidogenic microorganisms. This brings about the oxidation of long-chain fatty acids to shorter chain LCFAs and VFAs alongwith the production of energy, in the form of ATP molecules. The acidogenic bacteria continuously produce the volatile fatty acids causing accumulation of these acids. 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.

I.9.1.b.1. Steps involved in β-oxidation pathway:

• Oxidation by FAD:
The first step is the oxidation of the fatty acid by Acyl-CoA-Dehydrogenase. The enzyme catalyzes the formation of a double bond between C-2 and C-3.
• **Hydration:**
The next step is the hydration of the bond between C-2 and C-3.

• **Oxidation by NAD+:**
The third step is the oxidation of L-3-hydroxyacyl CoA by NAD+. This converts the hydroxyl group into a keto group.

• **Thiolysis:**
The final step is the cleavage of 3-ketoacyl CoA by the thiol group of another molecule of CoA. The thiol is inserted between C-2 and C-3.

Details of β-oxidation pathway is shown in Figure I.7.

![Diagram of β-oxidation pathway](image)

**Figure I.7:** β-oxidation pathway of fatty acid degradation
In case of mustard oil the main substrate for anaerobic digestion is erucic acid (C22:1) which converts to acetic acid in presence of acidogenic bacteria. The mechanism of erucic acid degradation through β-oxidation pathway is shown as follows:

**1.9.1.b.2. Mechanism of erucic acid degradation**

\[
CH_3(CH_2)_7CH = CH - CH_2 - (CH_2)_{10} - C - SCoA
\]

\[
\text{Erucoyl-CoA} \quad \circlearrowleft \quad \beta\text{-oxidation}
\]

\[
5CH_3 - C - S - CoA \quad (5 \text{ cycles})
\]

\[
CH_3(CH_2)_7C = C - CH_2 - C - SCoA
\]

\[
\text{Cis} \Delta^1\text{-Dodecenoyl-CoA}
\]

\[
\text{Enoyl-CoA isomerase}
\]

\[
CH_3(CH_2)_7C = C - CH_2 - C - SCoA
\]

\[
\text{Trans} \Delta^1\text{-Dodecenoyl-CoA}
\]

\[
\text{H}_2\text{O} \quad \text{Enoyl-CoA hydratase}
\]
I.9.1.c. Acetogenic step: In case of even carbon numbered long chain fatty acids (such as erucic acid C22:1) the lower volatile fatty acids (VFA s) are converted to acetic acid in presence of acetogenic bacteria leading to accumulation of the acid. In case of odd carbon numbered fatty acids the end product of acetogenesis will be propanoic acid (CH₃CH₂COOH).

I.9.1.d. Methanogenic step: Methanogenesis is the formation of methane by microbes. This is an important and widespread form of microbial metabolism. In most environments, it is the final step in the decomposition of organic matter. Organisms capable of methanogenesis are called methanogens. These organisms have no nucleus or membrane-bound organelles (they are procaryotes). Methanogens are considered to be a very old group of organisms, being members of the archaeabacteria, also known as archaeca (depending on what taxonomic system is being used).

Methanogenesis is a form of anaerobic respiration. Methanogens do not use oxygen to breathe. In fact, oxygen is a deadly poison to methanogens, and kills all methanogens even at very low concentrations. The terminal electron acceptor in methanogenesis is not oxygen, but carbon. The carbon can occur in a small number of organic compounds, all with low molecular weights. The two best described pathways involve the use of carbon dioxide and acetic acid as terminal electron acceptors.
1.9.2. Reactions involved in Methanogenesis:

**Methanization:** The principal acids produced in the acidogenic step are processed by methanogenic bacteria to produce methane. The reaction that takes place in the process of methane production is called Methanization and is expressed by the following equations (Karki and Dixit, 1984).

\[
\begin{align*}
\text{CH}_3\text{COOH} & \rightarrow \text{CH}_4 + \text{CO}_2 \\
2\text{CH}_3\text{CH}_2\text{OH} + \text{CO}_2 & \rightarrow \text{CH}_4 + 2\text{CH}_3\text{COOH} \\
\text{CO}_2 + 4\text{H}_2 & \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}
\end{align*}
\]

The above equations show that many products, by-products and intermediate products are produced in the process of digestion of inputs in an anaerobic condition before the final product (methane) is produced. The factors which facilitate or inhibit the above process may be listed as follows:

**pH value.** The optimum biogas production is achieved when the pH value of input mixture in the digester is between 6 and 7. The pH in a biogas digester is also a function of the retention time. In the initial period of fermentation, as large amounts of organic acids are produced by acid forming bacteria, the pH inside the digester can decrease to below 5. This inhibits or even stops the digestion or fermentation process. Methanogenic bacteria are very sensitive to pH and do not thrive below a value of 6.5. Later, as the digestion process continues, concentration of \( \text{NH}_4 \) increases due to digestion of nitrogen which can increase the pH value to above 8. Some buffer solution may also be used externally to maintain the pH of the reaction media within a given range. When the methane production level is stabilized, the pH range remains buffered between 7.2 to 8.2.

**Temperature:** The methanogens are inactive at extremely high and low temperatures. The optimum temperature is 35°C. When the ambient temperature goes down to 10°C, gas production virtually stops. Satisfactory gas production takes place in the mesophilic range, between 25 to 30°C.

**Loading rate:** Loading rate is the amount of raw materials fed per unit volume of digester capacity per day. If the plant is overfed, acids will accumulate and methane production will be inhibited. Similarly, if the plant is underfed, the gas production will also be low.

**Retention time.** Retention time (also known as detention time) is the average period that a given quantity of input remains in the digester to be acted upon by the methanogens. In a cow dung plant, the
Retention time is calculated by dividing the total volume of the digester by the volume of inputs added daily.

**Toxicity.** Mineral ions, heavy metals and the detergents are some of the toxic materials that inhibit the normal growth of pathogens in the digester. Small quantity of mineral ions (e.g. sodium, potassium, calcium, magnesium, ammonium and sulphur) also stimulates the growth of bacteria, while very heavy concentration of these ions will have toxic effect. Similarly, heavy metals such as copper, nickel, chromium, zinc, lead, etc. in small quantities are essential for the growth of bacteria but their higher concentration has toxic effects. Likewise, detergents including soap, antibiotics, organic solvents, etc. inhibit the activities of methane producing bacteria and addition of these substances in the digester should be avoided. The inhibiting levels of some of the major ones are given in Table I.7.

**Table I.7: Toxic level of various inhibitors**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Inhibiting concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphate ($SO_4^{2-}$)</td>
<td>5000 ppm</td>
</tr>
<tr>
<td>Sodium Chloride or Common salt (NaCl)</td>
<td>40,000 ppm</td>
</tr>
<tr>
<td>Nitrate (Calculated as N)</td>
<td>0.05 mg/ml</td>
</tr>
<tr>
<td>Copper (Cu++ )</td>
<td>100 mg/l</td>
</tr>
<tr>
<td>Chromium (Cr+++ )</td>
<td>200 mg/l</td>
</tr>
<tr>
<td>Nickel (Ni+++ )</td>
<td>200 - 500 mg/l</td>
</tr>
<tr>
<td>Sodium (Na+)</td>
<td>3,500 - 5,500 mg/l</td>
</tr>
<tr>
<td>Potassium (K+)</td>
<td>2,500 - 4,500 mg/l</td>
</tr>
<tr>
<td>Calcium (Ca+++ )</td>
<td>2,500 - 4,500 mg/l</td>
</tr>
<tr>
<td>Magnesium (Mg++ )</td>
<td>1,000 - 1,500 mg/l</td>
</tr>
<tr>
<td>Manganese (Mn++ )</td>
<td>Above 1,500 mg/l</td>
</tr>
</tbody>
</table>

*Source: The Biogas Technology in China, BRTC, China (1989)*

I.9.3. Advantages and disadvantages of Anaerobic Digestion:

Anaerobic digestion involves a considerable reduction in the organic load of the wastewater. A correctly controlled anaerobic digestion leads to very high rates of purification. Advantages of anaerobic digestion over the aerobic ones are as follows:
1. Anaerobic digestion uses readily available CO₂ as an electron acceptor as its oxygen source. It requires no oxygen, the supply of which adds substantially to the cost of wastewater treatment.

2. Anaerobic digestion produces lower amounts of sludge. This amounts to 3 - 20 times less than aerobic processes. Most of the energy derived from substrate break down is found in the final product, CH₄.

3. Anaerobic digestion produces a useful gas, methane. This gas contains about 90% of the energy, and can be burned on site to provide heat for digesters or to generate electricity. Little energy (3-5%) is wasted as heat. Methane production contributes to the BOD reduction in digested sludge.

4. Energy required for wastewater treatment is reduced.

5. Anaerobic digestion is suitable for high-strength industrial wastes.

6. It is possible to apply high loading rates to the digester.

7. The digested sludge can be transformed to be used as a biofertilizer which is more easily assimilated by the plants.

8. Sustainable management of organic waste can be achieved.

The main disadvantages of anaerobic digestion are as follows:

1. The process requires stringent operating conditions such as complete anaerobic environment, constant reaction pH etc. as the methanogens are very prone to change in reaction environment.
2. It is a slower process than aerobic digestion.
3. It is more sensitive to upsets by toxicants.
4. Start-up of the process requires long periods of time.
5. As regards biodegradation of xenobiotic compounds by co-metabolism, anaerobic processes require relatively high concentrations of primary substrates.

**I.9.4. Different types of Anaerobic Digestion**

There are mainly two types of anaerobic digestion:-
Mesophilic digestion: The digester is heated to 30-35°C and the feedstock remains in the digester typically for 15-30 days. Mesophilic digestion tends to be more robust and tolerant than the thermophilic process but the gas production is less, larger digestion tanks are required and sanitization, if required, is a separate process stage.

Thermophilic digestion: The digester is heated to 55°C and the residence time is typically 12-14 days. Thermophilic digestion system offer higher methane production, faster throughput, better pathogens and virus ‘kill’, but require more expensive technology, greater energy input and a higher degree of operation and monitoring. During this process 30-60% of the digestible solids are converted into biogas.

Mesophilic digestion is the most common approach since it is more reliable and plant management is easier.

I.9.5. Process Microbiology involved in anaerobic digestion of complex wastewater:

Consortia of microorganisms, mostly bacteria, are involved in the transformation of complex high-molecular-weight organic compounds to methane. Furthermore, there are synergistic interactions between the various groups of bacteria implicated in anaerobic digestion of wastes. Although some fungi and protozoa can be found in anaerobic digesters, bacteria are undoubtedly the dominant microorganisms. Large numbers of strict and facultative anaerobic bacteria are involved in the hydrolysis and fermentation of organic compounds. There are four categories of bacteria as described below that are involved in the transformation of complex materials into simple molecules such as methane and carbon dioxide.

Group 1: Hydrolytic Bacteria

Consortia of anaerobic bacteria break down complex organic molecules (proteins, cellulose, lignin, and lipids) into soluble monomer molecules such as amino acids, glucose, fatty acids, and glycerol. The monomers are directly available to the next group of bacteria. Hydrolysis of the complex molecules is catalyzed by extracellular enzymes such as cellulases, proteases, and lipases.
Group 2: Fermentative Acidogenic Bacteria

Acidogenic (i.e., acid-forming) bacteria convert sugars, amino acids, and fatty acids to organic acids (e.g., acetic, propionic, formic, lactic, butyric, or succinic acids), alcohols and ketones (e.g., ethanol, methanol, glycerol, acetone), acetate, CO$_2$, and H$_2$. Acetate is the main product of carbohydrate fermentation. The products formed vary with the type of bacteria as well as with culture conditions (temperature, pH, redox potential).

Group 3: Acetogenic Bacteria

Acetogenic bacteria convert fatty acids (e.g., propionic acid, butyric acid) and alcohols into acetate, hydrogen, and carbon dioxide, which are used by the methanogens. This group requires low hydrogen tensions for fatty acid conversion; and therefore a close monitoring of hydrogen concentrations is necessary. Under relatively high H$_2$ partial pressure, acetate formation is reduced and the substrate is converted to propionic acid, butyric acid and ethanol rather than methane.

Group 4: Methanogens

Anaerobic digestion of organic matter in the environment releases 500-800 million tons of methane per year into the atmosphere and this represents 0.5% of the organic matter derived from photosynthesis. The fastidious methanogenic bacteria occur naturally in deep sediments or in the rumen of herbivores. This group is composed of both gram-positive and gram-negative bacteria with a wide variety of shapes. Methanogenic microorganisms grow slowly in wastewater and their generation times range from 2 days at 35°C to as high as 50 days at 10°C. About two thirds of methane is derived from acetate conversion by methanogens. The other third is the result of carbon dioxide reduction by hydrogen.

Figures I.9 and I.10 show microphotographs of different types of methanogenic bacteria viewed under scanning electron microscope.
Figure I.9: Various types of methanogenic bacteria. The spherically shaped bacteria are of the \textit{methanosarcina} genus; the long, tubular ones are \textit{methanothrix} bacteria, and the short, curved rods are bacteria that catabolize furfural and sulfates. The total length of the broken bar at top left, which serves as a size reference, corresponds to 1 micron.

Source: Production and Utilization of Biogas in Rural Areas of Industrialized and Developing Countries, Schriftenreihe der gtz, No. 97, p. 55

Figure I.10. Sem photographs of methanogenic bacteria
I.9.6. Classification of Microorganisms based on temperature classes:

Microorganisms are classified into ‘temperature classes’ on the basis of the optimum temperature and the temperature span in which the species are able to grow and metabolize (Fig.I.11). The overlapping growth temperature ranges shown in the figure indicate that there is no clear boundary between these classic groups of psychrophilic, mesophilic and thermophilic microorganisms. The bacterial growth rates of methanogenic thermophiles and mesophiles from anaerobic reactors are well determined.

![Figure I.11. Classification of Microorganisms based on temperature classes](image)

I.10. Various types of reactors used for anaerobic digestion process:

I.10.1. Bioreactor types

Numerous reactor designs for bioremediation of lipid rich waste have been reported. This includes batch reactors, sequencing batch reactors, continuously stirred tank reactors, anaerobic contact processes, anaerobic baffled reactors, anaerobic filters, fluidized-bed reactors, gas lift reactors, up flow anaerobic sludge blanket reactors and anaerobic hybrid reactors.
Figure I.12. Continuous stirred tank reactor

The reactor configuration has implications for the ratio of sludge retention time/hydraulic retention time (SRT/HRT) in continuous flow reactors. The loading rates of a process are largely dictated by the biomass retention in the reactor. Maximal sludge retention or biomass retention is desirable for process stability and minimal sludge production. Minimal HRT minimizes the reactor volume and thus reduces capital costs.

Continuously stirred tank reactors (CSTR) are subjected to washout of active biomass (Figure I.12). Biomass retention has been enhanced by employing internal sedimentation systems and cationic flocculants. Anaerobic contact process (ACP) relies on biomass separation and recycling to increase the SRT/HRT.

Figure I.13. Continuously stirred tank reactor (CSTR) and anaerobic contact process (ACP).
I.10.2. Biofilters

Definition

**Biofiltration** is a pollution control technique using living material to capture and biologically degrade process pollutants. A biofilter consists of a packed bed of organic or synthetic material on which microbial films are supported. The main types of biofilters include bioscrubbers, trickling filters, up-flow anaerobic filters, fluidized bed reactors etc. Trickle-bed biofilters are effectively used for controlling the volatile organic compound (VOC) laden exhaust gases, a major cause of air pollution. Biofilm reactors using attached cellular growth on suitable packing matrices may be used to ensure high HRT and to avoid the problem of active biomass washout with the product stream of the reactor.

Fixed film reactors have been used since long for the treatment of wastewater where they have helped reduce the HRT from 30–40 days to a few hours (Kloss, 1991). They help in enhancing the performance of wastewater treatment systems by providing an increased surface area for attached growth of the microbes in the form of a fixed film on an inert medium leading to increased population of microbes in the reactor and their retention in the digester even after the digested slurry flows out (Van der Berg and Kennedy, 1983). Fixed film technique has been used commonly for substrates of low content of solids where filters of very large surface area are used.

I.10.2.1 Anaerobic Filter

Biofilm reactors used for wastewater treatment under anaerobic conditions may be termed as anaerobic filters (AF). In anaerobic filter reactors (AFR) (or packed bed reactors, PBR) biomass is retained as a biofilm on packing material as well as unattached in the packing interstices. AFRs have been operated in horizontal, up flow or down flow modes (Figure I.14). The down flow AFR allows the utilization of gravity and, thus, passive operation. Packing materials used in AFRs include cobbles, polypropylene pall rings, glass beads and alkaline minerals. The main shortcomings of AFRs are the channeling of the flow and clogging of the bed by precipitates.
Figure I.14. Anaerobic filter reactors (AFR) used in horizontal, up flow and down flow modes. Advantages of up flow attached growth anaerobic reactors are high COD loadings, relatively small reactor volumes and operational simplicity. Only a few full-scale applications are in operation today, for chemical and food industry effluents. Two types of Anaerobic upflow filters (AUF) used as pretreatment devices for high-strength wastewater from industries are shown in figures I.15 and I.16.

Figure I.15. Schematic representation of the up flow anaerobic filter

Figure I.16. Schematic representation of the upflow anaerobic sludge blanket reactor
Anaerobic filters having attached cellular growth have been used efficiently for the treatment of lipid rich wastewater. The problem of washing out of the cells with the product stream of the digester can be judiciously tackled using immobilized cells on solid matrices of the filter bed. Lipid molecules also cause operational problems as they adhere to the biomass surface, interrupting the mass transfer operations in the reactor (Pereira et al. 2004). Low up flow velocities of the feed stream are generally maintained to minimize the biomass washout and to ensure high HRT in the filter. This design significantly reduces the lag period of lipid digestion and increases the biodegradation efficiency in terms of BOD and COD removal.

I.10.2.1.a. Backwashing in Anaerobic Filter

Excess biomass accumulation in the filter bed signifies increase in the bio-film thickness with time within the reactor. The loss of contact surface area of the packing materials is then obvious due to increase in the bio-film thickness. This type of operational problems associated with biofilm reactors can be tackled by obtaining suitable backwashing time and using novel regeneration techniques.

I.10.2.1.b. Material of construction of packing materials of AF

Many criteria need to be considered for selection of suitable materials for long life of the fixed film matrix (Young and Song, 1984). The material should be non-biodegradable. The structure of the fixed film matrix should also be mechanically stable. Materials should be easily available in the local market at a reasonable cost. Different materials like nylon sponges, PVC, clay pipes, etc. had been used as support medium for fixed film reactors (Wilkie et al., 1984).

Fixed film reactor packed with sponge nylon as support performed well in terms of specific biogas production rate as compared to conventional reactors. The results showed good digester productivity as well as satisfactory sludge stabilization in fixed film digester (Solicio and Del, 1987; Meier et al., 1993). Residues of earthen containers, wood bagasse, coconut coir etc can also be used as support matrices in the filter bed for their high specific surface area.

I.10.2.2. Fluidized bed reactor

In the fluidized-bed reactor (FBR) (Figure I.17), channeling and clogging are avoided by fluidizing the inert biomass carrier. Fluidization can be carried out either with recycle water or by using a gas stream.
In the latter case the reactor is called a gas lift reactor. Carrier materials used include iron chips, synthetic polymeric granules covered with iron dust, pumice particles, porous glass beads, and carbon dust. The fluidized carrier enables efficient mass transfer and provides a large surface area for biofilm formation.

![Schematic diagram and a photo of a laboratory scale fluidized-bed reactor (FBR).](image)

**Figure L.17 a & b: A schematic diagram and a photo of a laboratory scale fluidized-bed reactor (FBR).**

In up flow anaerobic sludge blanket (UASB) reactors, biomass retention is based on good settling characteristics of granular sludge (Figure L.18). The presence of methanogens in the biomass can enhance granulation. The produced biogas is trapped by a hood located below the water surface and can be periodically burned in a flare. Due to the biomass granulation, no packing or carrier material is needed which reduces the start-up costs of the UASB compared to AFR and FBR. However, extensive biogas production may require extra instrumentation which increases capital costs. Main problems encountered with UASB reactors are poor or slow granulation and the rapid disintegration of the granular sludge under certain conditions.
Figure I.18a & b: A schematic diagram and a photo of a laboratory scale upflow anaerobic sludge blanket reactor (UASB).

The anaerobic hybrid reactor (AHR) is a combination of UASB and AFR, where the granular sludge bed is in the lower section of the reactor and packing material in the upper section (Figure I.19). The packing material improved the separation of solids from the reactor effluent. Another modification of the UASB reactor is an anaerobic baffled reactor (ABR) which is a staged reactor where biomass retention is enhanced by forcing the water through several compartments (Figure I.19).

Figure I.19. Anaerobic hybrid reactor (AHR) and anaerobic baffled reactor (ABR)
I.10.3. Waste to Energy Conversion in AF
Lipid rich wastewater may serve as a potential source of methane through anaerobic digestion and this pathway is attractive from the perspective of transformation of waste to energy in terms of biogas generation. Methane, the main component of biogas, has a high calorific value (approx 39,000kJ/kg) and can be alternatively used as a fuel as a substitute of natural gas. Treatment fat rich liquid effluent in the anaerobic filter, thus, ensures the removal of BOD and COD of the influent stream along with the formation of biogas as a renewable energy source.

I.11. Biogas as a source of renewable energy
Biogas, a promising source of renewable energy could very well substitute for conventional sources of energy (fossil fuel, oil etc.,) which are the main cause of ecological-environmental problems and at the same time are depleting at a faster rate. Despite its numerous advantages, the potential of biogas technology could not be fully harnessed or tapped as certain constraints are also associated with it. Researchers have tried various techniques to enhance biogas production. Application of biotechnology for the treatment of high-strength liquid waste decomposition has opened up a new way of bio-energy generation from this type of waste in terms of methane or biogas.

Biogas is the main product of anaerobic degradation of organic substrates, which is one of the oldest process used for the treatment of industrial wastewater and stabilization of sludges.

I.11.1. Composition of Biogas

The composition of biogas varies depending upon the origin of the anaerobic digestion process. Landfill gas typically has methane concentrations around 50%. Advanced waste treatment technologies can produce biogas with 55–75% CH₄ or higher using in situ purification techniques.

Table I.8: Typical composition of biogas

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical formula</th>
<th>Vol %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane</td>
<td>CH₄</td>
<td>50–75</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>CO₂</td>
<td>25–50</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>N₂</td>
<td>0–10</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>H₂</td>
<td>0–1</td>
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<tr>
<td>Hydrogen sulfide</td>
<td>H₂S</td>
<td>0–3</td>
</tr>
<tr>
<td>Oxygen</td>
<td>O₂</td>
<td>0–2</td>
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</tbody>
</table>
I.12. Energy analysis of typical biogas generator

The bioconversion process of transformation of lipid-rich waste to energy in form of methane is an attractive pathway of wastewater management as it ensures the removal of organic load alongwith the production of biogas. Therefore, for the proper understanding of the process, energy analysis of a typical biogas generator is an absolute necessity. There is not enough literature evidence of this type of analysis in case of treatment of oil-rich feedstock in bioreactors. Therefore, energy analysis of the whole bioconversion process may be accepted as a necessary tool for the process engineers.