Anaerobic Treatment of Whey Permeate Using Upflow Sludge Blanket Bioreactors

Seokhwan Hwang
Utah State University

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ANAEROBIC TREATMENT OF WHEY PERMEATE USING
UPFLOW SLUDGE BLANKET BIOREACTORS

by

Seokhwan Hwang

A thesis submitted in partial fulfillment
of the requirements for the degree
of
MASTER OF SCIENCE
in
Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1993
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I would sincerely like to thank my parents for their love and support throughout my academic year. To my lovely wife, Chaehwa, I can’t express my gratitude in words. Without her support and understanding, I could hardly have done this research.

I am greatly indebted to Dr. Conly L. Hansen for allowing me to work on this research, and for advice, discussion, and patience.

I wish to thank the committee members, Dr. D. K. Stevens and Dr. P. Savello, for their helpful and professional assistance in completion of this study.

Seokhwan Hwang
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<th>Description</th>
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<tr>
<td>( \frac{dS}{dt}_{\text{net}} )</td>
<td>Net rate of change in substrate concentration (kg COD/L/d)</td>
</tr>
<tr>
<td>( \frac{dS}{dt}_u )</td>
<td>The rate of microbial substrate utilization (kg COD/L/d)</td>
</tr>
<tr>
<td>( \frac{dX}{dt}_d )</td>
<td>The rate of decrease in biomass concentration (kg VSS/L/d)</td>
</tr>
<tr>
<td>( \frac{dX}{dt}_g )</td>
<td>Growth rate of microorganism (kg VSS/L/d)</td>
</tr>
<tr>
<td>( \frac{dX}{dt}_{\text{net}} )</td>
<td>Net rate of change in biomass concentration (kg VSS/L/d)</td>
</tr>
<tr>
<td>ALK</td>
<td>Alkalinity</td>
</tr>
<tr>
<td>BOD</td>
<td>Biological Oxygen Demand</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
</tr>
<tr>
<td>CSTR</td>
<td>Completely Stirred Tank Reactor</td>
</tr>
<tr>
<td>F:M</td>
<td>Food to Microorganism ratio</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic Retention Time (day)</td>
</tr>
<tr>
<td>( k )</td>
<td>Maximum specific substrate utilization rate (kg COD/kg VSS/d)</td>
</tr>
<tr>
<td>( k_d )</td>
<td>Specific decay rate of microorganism (a proportionality constant, day(^{-1}))</td>
</tr>
<tr>
<td>( K_L )</td>
<td>Specific organic loading rate at ( q = k/2 ) (kg COD/kg VSS/d)</td>
</tr>
<tr>
<td>( K_{s1} )</td>
<td>Half saturation constant numerically equal to the substrate concentration when ( \mu = \mu_m/2 ) (kg COD/L)</td>
</tr>
<tr>
<td>( K_{s2} )</td>
<td>Half saturation constant numerically equal to the substrate concentration when ( q = k/2 ) (kg COD/L)</td>
</tr>
<tr>
<td>( L_s )</td>
<td>Volumetric loading rate (kg COD/m(^3)/d)</td>
</tr>
<tr>
<td>( L_x )</td>
<td>Specific organic loading rate (kg COD/kg VSS/d)</td>
</tr>
<tr>
<td>( \mu )</td>
<td>Specific growth rate of microorganism (a proportionality constant, day(^{-1}))</td>
</tr>
<tr>
<td>( \mu_m )</td>
<td>Maximum specific growth rate of microorganism (day(^{-1}))</td>
</tr>
<tr>
<td>( P_d )</td>
<td>Daily barometric pressure (atm)</td>
</tr>
<tr>
<td>( P_{\text{STP}} )</td>
<td>Standard pressure (1 atm)</td>
</tr>
<tr>
<td>( Q )</td>
<td>Flow rate (L/d)</td>
</tr>
</tbody>
</table>
\( q \) : Specific substrate utilization rate (a proportionality constant, day\(^{-1}\))

\( \theta_c \) : Solid retention time (day)

\( \theta_{c}^m \) : Minimum solid retention time (day)

\( \text{RBC} \) : Rotating Biological Contactor

\( S \) : Growth-limiting substrate concentration (kg COD/L)

\( \text{SCP} \) : Single Cell Protein

\( S_e \) : Effluent substrate concentration (kg COD/L)

\( S_i \) : Influent substrate concentration (kg COD/L)

\( \text{SRT} \) : Solid Retention Time (day)

\( \tau \) : Hydraulic retention time (day)

\( \text{TDS} \) : Total Dissolved Solid

\( T_r \) : Room temperature (273 + °C)

\( \text{TS} \) : Total Solid

\( \text{TSS} \) : Total Suspended Solids

\( T_{\text{STP}} \) : Standard temperature (273 K)

\( \text{UASB} \) : Upflow Anaerobic Sludge Blanket

\( \text{UF} \) : Ultrafiltration

\( V \) : Reactor volume (L)

\( V_d \) : Daily gas volume produced (L gas/L reactor volume/d)

\( \text{VOA} \) : Volatile Organic Acid

\( \text{VS} \) : Volatile Solid

\( \text{VSS} \) : Volatile Suspended Solids

\( V_{\text{STP}} \) : Biogas volume at standard condition (L gas/L reactor volume/d)

\( X_a \) : Concentration of active microbial population (kg VSS/L)

\( X_e \) : Biomass concentration in the effluent (kg VSS/L)

\( X_T \) : Total active biomass in the system (kg VSS)
Y : Microbial yield coefficient (kg VSS\textsubscript{produced}/kg COD\textsubscript{utilized})
ABSTRACT

Anaerobic Treatment of Whey Permeate Using
Upflow Sludge Blanket Bioreactors

by

Seokhwan Hwang, Master of Science
Utah State University, 1993

Major Professor: Dr. Conly L. Hansen
Department: Nutrition and Food Sciences

Whey permeate was anaerobically digested in laboratory scale upflow anaerobic sludge blanket reactors. Nine hydraulic retention times between 5 and 0.2 days were examined with a fixed influent concentration of 10.6 ± 0.2 g COD/L.

Chemical oxygen demand removal efficiency ranged from 99.0 to 18.9% and maximum production rate of methane gas was 2.67 L/L/day at a hydraulic loading rate of 12.97 kg COD/m³/day. About 70% of the chemical oxygen demand removed was converted to methane.

Both the nonlinear least square method with 95% confidence interval and linear regression were used to evaluate kinetic coefficients. The maximum substrate utilization rate, k, and half saturation coefficient, K_L, were determined to be 1.269 ± 0.163 Kg COD/kg VSS/day and 1.000 ± 0.179 kg COD/kg VSS/day. The yield coefficient, Y, and biomass decay rate coefficient, K_d, were also determined to be 0.160 ± 0.012 kg VSS/kg COD and 0.027 ± 0.004 day⁻¹, respectively.
CHAPTER I
INTRODUCTION

Wastes from food processing and agricultural operations have traditionally presented problems in disposal. Whey is an important by-product of the cheese industry. Whey was initially considered to be a waste product with no value and was mainly used for animal feed because the high organic content of whey led to a severe disposal problem. However, many ways of reusing cheese whey and whey permeate have been suggested because of the increase in the size of cheese production.

Anaerobic biological treatment offers a cost-effective solution for partial treatment of high strength wastewaters like whey permeate prior to discharge to a public wastewater treatment plant or as the first step in a complete treatment sequence.

Numerous anaerobic digester systems have been employed with encouraging results to treat wastewater. The upflow anaerobic sludge blanket (UASB) process has outstanding advantages over other digester types for the effective digestion of whey permeate.

Despite effectiveness and advances of the UASB process, the lack of an adequate kinetic model impedes the ability to achieve optimum performance. Unfortunately, most of the reports about the UASB reactor in the literature do not include kinetic descriptions which are necessary to evaluate the effect of a particular variable or environmental factor.
CHAPTER II
LITERATURE REVIEW

A. Food Processing Waste Management

Food processing waste disposal and treatment are an important part of the overall food production and processing network. Waste products from food processing facilities include bulky solids, airborne pollutants, and wastewater. All of them cause potentially severe pollution problems and are subject to increasing environmental regulation in most countries (Litchfield, 1987).

Generally, wastewater is the most common because food processing operations involve a number of unit operations, such as washing, evaporation, extraction, and filtration. The process wastewaters resulting from these operations normally contain high concentrations of suspended solid and soluble organics such as carbohydrates, proteins, and lipids, which present difficult disposal problems (Whitehead and Revel, 1984). In the United States, the Environmental Protection Agency (EPA) has promulgated regulations on effluent for a variety of food processing industries (Code of Federal Regulations, 1985). Table 1 summarizes pollution characteristics of typical food industry wastes.

Over the past decade, the food processing industry has used available technology to remove major pollutants such as total suspended solids (TSS) and organic materials expressed as biochemical oxygen demand (BOD) or chemical oxygen demand (COD) from the waste stream (Humenik and Overcash, 1984).

The new dimension of food processing waste management has evolved from a disposal approach to one of utilization (Whitehead and Revel, 1984). Many benefits of utilization are available, but there are many areas that can be improved and unknowns resolved.
Table 1. Pollution Characteristics of Selected Food Processing Wastes (Litchfield, 1987)

<table>
<thead>
<tr>
<th>Waste</th>
<th>BOD₅ (mg/L)</th>
<th>TSS (mg/L)</th>
<th>N (mg/L)</th>
<th>Grease (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy</td>
<td>1000-4000</td>
<td>1000-2000</td>
<td>1-13</td>
<td>-</td>
</tr>
<tr>
<td>Fish</td>
<td>500-2500</td>
<td>100-1800</td>
<td>50-300</td>
<td>100-800</td>
</tr>
<tr>
<td>Fruit</td>
<td>1200-4200</td>
<td>2500-6700</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Meat</td>
<td>1000-6500</td>
<td>100-1500</td>
<td>60-150</td>
<td>15-600</td>
</tr>
<tr>
<td>Municipal</td>
<td>100-300</td>
<td>100-500</td>
<td>25-85</td>
<td>0-40</td>
</tr>
<tr>
<td>Poultry</td>
<td>200-1500</td>
<td>75-1100</td>
<td>50-100</td>
<td>100-400</td>
</tr>
<tr>
<td>Vegetable</td>
<td>1000-6800</td>
<td>100-4000</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Under aerobic conditions, microorganisms convert carbohydrates, lipids, and proteins in wastes into microbial biomass and carbon dioxide (CO₂) (Brooks et al., 1977; El-Shawarby et al., 1987).

Under anaerobic conditions, wastes containing those components can be digested to yield methane. Also, ethanol or organic acids can be produced from carbohydrates by the anaerobic microbiological process (Litchfield, 1987; Stryer, 1988; Rawn, 1989).

Key considerations in determining appropriate treatment technology are process running cost, quantity and characteristics of waste, and market value of recovered products.
B. Use of Cheese Whey and Whey Permeate

1. Whey as Nutrient Source

Substances in the environment used by organisms for catabolism and anabolism are called nutrients (Thomas, 1979). The important components are C, H, O, N, P, K, S, Ca, Fe, and Mg (Stanier et al., 1986). Most of those nutrients are available in cheese whey (Table 2), a by-product of cheese manufacture that remains when casein and butter fat are separated as curd from milk. Cheese whey can be divided into two groups according to manufacturing methods. The coagulation of casein with rennet yields sweet whey (pH 4.5-6.7), with high lipid contents. Precipitation of casein by lactic fermentation produces acid whey (pH 3.9-4.5) containing smaller quantities of lactose and proteins (Moulin and Galzy, 1984). Acid whey forms a small fraction of the total whey produced in North America (Kissalita et al., 1987; Kissalita et al., 1989).

Depending on the type of cheese being made, as many as 9 liters of whey are discharged for every kilogram of cheese produced. In 1981, 16.2 million tons of whey were produced in the United States (Chartrain and Zeikus, 1986a), and more than 82 million tons of whey were produced all over the world in 1984 (Zellner et al., 1987). According to Maiorella and Castillo (1984), approximately 18 million tons of sweet cheese whey and 1.7 million tons of acid whey from cottage cheese manufacture are produced each year in the United States. Table 3 summarizes the total whey production rates of five western states in 1991, which collectively produced over 5.1 million tons.

2. Utilization of Whey

Whey was initially considered to be a waste product to be disposed of and was mainly redistributed to milk producers for animal feed (Moulin and Galzy, 1984). The
Table 2. Composition of Different Types of Liquid Whey (g/L) (Moulin and Galzy, 1984)

<table>
<thead>
<tr>
<th></th>
<th>Cow</th>
<th>Ewe</th>
<th>Goat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sweet</td>
<td>Acid</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>5.252</td>
<td>7.333</td>
<td>5.654</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.466</td>
<td>1.251</td>
<td>0.494</td>
</tr>
<tr>
<td>Citric acid</td>
<td>1.298</td>
<td>0.260</td>
<td>1.032</td>
</tr>
<tr>
<td>Dry matter</td>
<td>70.840</td>
<td>65.760</td>
<td>83.840</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.322</td>
<td>7.555</td>
<td>1.763</td>
</tr>
<tr>
<td>Lactose</td>
<td>51.810</td>
<td>45.250</td>
<td>50.980</td>
</tr>
<tr>
<td>Lipid</td>
<td>5.060</td>
<td>0.850</td>
<td>6.460</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.412</td>
<td>0.649</td>
<td>0.545</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.455</td>
<td>1.485</td>
<td>1.281</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.505</td>
<td>0.528</td>
<td>0.616</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>1.448</td>
<td>1.223</td>
<td>2.933</td>
</tr>
</tbody>
</table>
increase in size of cheese plants, the necessity for reduction of pollutant in the effluent, and the need to maximize returns on raw material have encouraged producers to seek new ways of using cheese whey. For example, whey can be used as a food additive either in liquid form or as a dried product (Clark, 1979; Kosikowski, 1979). Whey powder was sold at U.S. $0.32/kg (1983 September level); the cost of drying was about $0.27/kg, not including transportation costs. On this basis, there was a return of U.S. $0.05 for each kilogram of whey powder produced (15 liter of whey are needed to produce 1 kg of whey powder) (Moulin et al., 1983). This process, however, has an undesirable factor in that drying is very energy intensive.

Many processes were developed for the recovery of protein, which constitutes the most valuable part of whey. The processes for protein recovery can be mainly divided into three types, protein precipitation (Moddler and Emmons, 1977; Mathur and Shahani, 1979), ultrafiltration (Fallick, 1969; Forsum, 1974; Yan et al., 1979), and ion-exchange separation (Palmer, 1977). Among these processes, the ultrafiltration technique has allowed the retention of almost all milk proteins (Yan et al., 1979; Moulin et al., 1983). Fallick (1969) proposed this process, and the dairy industry developed it rapidly. In 1984, 35% protein concentrates obtained by ultrafiltration

<table>
<thead>
<tr>
<th>State</th>
<th>Whey produced (tons x 10^6)</th>
</tr>
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<tbody>
<tr>
<td>California</td>
<td>3.36</td>
</tr>
<tr>
<td>Nebraska</td>
<td>0.59</td>
</tr>
<tr>
<td>North Dakota</td>
<td>0.18</td>
</tr>
<tr>
<td>South Dakota</td>
<td>0.69</td>
</tr>
<tr>
<td>Utah</td>
<td>0.32</td>
</tr>
</tbody>
</table>
were sold at $0.76/kg, at a production cost of $0.63/kg. On this basis, protein concentrates made a profit of U.S. $0.13/kg of protein concentrate (60 liters of whey are needed to produce 1 kg of protein concentrate) (Moulin et al., 1983).

3. Utilization of Whey Permeate

All the protein recovery processes mentioned earlier yield a permeate with high contents of lactose, minerals, vitamins, and sometimes lactic acid (Table 4). Whey and ultrafiltration permeate also contain some trace elements and vitamins (Table 5) which make them valuable nutritionally.

The organic matter in cheese whey, however, causes a high COD in the range of 60,000 to 80,000 ppm (Lo and Liao, 1986) and more than 90% of whey COD is due to the lactose components (Kissalita et al., 1987). Whey permeate holds almost 100% of lactose from whey (Chartrain and Zeikus, 1986a; Zellner et al., 1987; Yan et al., 1979), which is still a high pollutant level.

Many solutions have been proposed to reduce the pollution level of whey permeate because of the large amount of production. These solutions mainly rely on converting the lactose to marketable products (Barford et al., 1986; Maiorella and Castillo, 1984; Moulin et al., 1983; Yan et al., 1979; Shay and Wegner, 1986).

One possible use of whey permeate is production of alcohol. *Kluyveromyces fragilis* NRRL Y 2415 produced alcohol of 9.1% (vol/vol) (Mahmoud and Kosikowski, 1982) while Janssens et al. (1984) reported maximum ethanol production as 7.1 g/L/h at hydraulic retention time (HRT) of 0.28 day with the same strain. A maximum butanediol production of 2.3 g/L/h was also achieved at a HRT of 0.06 day (Lee and Maddox, 1986). Operation of this process in full scale, however, usually requires a large amount of permeate, which causes transportation and storage problems.

An intermediate situation is the production of single-cell proteins (SCP) from permeate (Mahmoud and Kosikowski, 1982; Sandhu and Waraich, 1983; Shay and
Table 4. Composition of Cheese Whey and of Whey Permeate (Zellner et al., 1987)

<table>
<thead>
<tr>
<th>Elements</th>
<th>Whey</th>
<th>Whey permeate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mmol/L)</td>
<td>7.0</td>
<td>2.0</td>
</tr>
<tr>
<td>COD (g O2/L)</td>
<td>75.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Lactate (g/L)</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Lactose (g/L)</td>
<td>40.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>38.0</td>
<td>36.0</td>
</tr>
<tr>
<td>Propionate (mmol/L)</td>
<td>5.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Protein content(^a) (%)</td>
<td>0.81</td>
<td>0.075</td>
</tr>
<tr>
<td>Total Nitrogen (g/L)</td>
<td>1.9</td>
<td>0.525</td>
</tr>
<tr>
<td>Total Solid (%)</td>
<td>5.0</td>
<td>4.2</td>
</tr>
</tbody>
</table>

\(^a\): (Total Nitrogen - Ammonia Nitrogen) x 6.38 = Protein content
Table 5. Average Content of the Main Trace Elements and Vitamins in Cheese Whey and Ultrafiltration Permeate (mg/100 g of dry matter) (Moulin et al., 1983)

<table>
<thead>
<tr>
<th>Elements</th>
<th>Whey</th>
<th>Whey Permeate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotin</td>
<td>0.2-0.3</td>
<td>0.1-0.3</td>
</tr>
<tr>
<td>Calcium pantothenate</td>
<td>30-70</td>
<td>50-60</td>
</tr>
<tr>
<td>Cobalamin</td>
<td>0.01-0.05</td>
<td>0.02-0.05</td>
</tr>
<tr>
<td>Pyridoxin</td>
<td>6-10</td>
<td>5-10</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>7-30</td>
<td>15-20</td>
</tr>
<tr>
<td>Thiamin</td>
<td>4-6</td>
<td>5-6</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>30-50</td>
<td>20-40</td>
</tr>
<tr>
<td>Trace elements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>0.5-5.0</td>
<td>1-3</td>
</tr>
<tr>
<td>Iron</td>
<td>1-7</td>
<td>3-11</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.01-0.04</td>
<td>0.5-0.8</td>
</tr>
<tr>
<td>Zinc</td>
<td>5-9</td>
<td>30-33</td>
</tr>
</tbody>
</table>
Wegner, 1986). The yeast cells produced as SCP are intended for two different markets. Human food applications are profitable, but this market is quantitatively limited (Moulin and Galzy, 1984). Animal feed uses are achievable only if production costs are sufficiently low for the product to compete with other comparable protein sources.

Another important area is the enzymatic hydrolysis of lactose. Hydrolyzed whey and milk containing hydrolyzed lactose are readily used in the food industry (Van-Huynh et al., 1986; Miguel and Vassilis, 1986; Baldwin et al., 1986; Chiu and Kosikowski, 1986).

Biological treatment systems, either aerobic or anaerobic, can be used to treat whey and whey permeate. Aerobic treatment may be unsuited to the treatment of very high strength waste, such as whey, due to the energy requirements for aeration and mixing, which lead to high operating costs (Barford et al., 1986). In contrast, anaerobic systems have lower operating costs and produce methane gas (CH$_4$), which can be used as an energy source. In New York State, up to 46% of a cheese manufacturer's energy costs could be cut by using the methane-rich biogas produced from whey (Switzenbaum and Danskin, 1982). Thus, the reduction of treatment costs and a decrease in energy needs would overcome high initial capital costs. Table 6 represents various anaerobic reactor performances treating whey and whey permeate.

C. Anaerobic Process

Energy costs and environmental concerns have increased interest in the direct anaerobic treatment of industrial wastes. Anaerobic waste treatment has several fundamental advantages over aerobic biological treatment process (Table 7).

The major advantage of the anaerobic process is formation of methane (CH$_4$) gas which can be used as another fuel source. Another advantage of this process is the high degree of waste stabilization achieved with little sludge production. About 5-10%
Table 6. The Process Efficiencies of Various Reactors Treating Whey and Whey Permeate

<table>
<thead>
<tr>
<th>Authors</th>
<th>Reactor types</th>
<th>Media</th>
<th>pH(1)</th>
<th>HRT (day)</th>
<th>Loading rate (Kg COD/m³/d)</th>
<th>CH₄ production (L CH₄/L/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callander and Barford (1983)</td>
<td>Semi-stirred flask reactor</td>
<td>Whey</td>
<td>6.8</td>
<td>NA</td>
<td>12.3</td>
<td>51-56(3)</td>
</tr>
<tr>
<td>Haast et al., (1985)</td>
<td>Down-flow fixed bed reactor</td>
<td>Whey permeate</td>
<td>7.4</td>
<td>5.0</td>
<td>2.64</td>
<td>0.286(4)</td>
</tr>
<tr>
<td>Wildenauer and Winter (1985)</td>
<td>Up-flow fixed film loop reactor</td>
<td>Acidic whey</td>
<td>6.7</td>
<td>5.0</td>
<td>14.0</td>
<td>4.42</td>
</tr>
<tr>
<td>Backus et al., (1986)</td>
<td>Anaerobic semi-CSTR</td>
<td>Sweet cheese whey</td>
<td>6.6</td>
<td>30.0</td>
<td>1.57(2)</td>
<td>0.061</td>
</tr>
<tr>
<td>Lo and Liao (1986)</td>
<td>Anaerobic RBC</td>
<td>Whey</td>
<td>6.9</td>
<td>6.0</td>
<td>10.38</td>
<td>2.56</td>
</tr>
</tbody>
</table>

1: lowest pH values  
2: kg VS/m³/d  
3: % CH₄/biogas  
4: m³ CH₄/kg COD converted  
NA: Not Available
Table 7. Benefits and Limitations of Anaerobic Treatment of Wastewaters (Lettinga et al., 1980)

<table>
<thead>
<tr>
<th>Benefits</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Production of methane, which is a useful end product</td>
<td>1) Anaerobic digestion is a sensitive process</td>
</tr>
<tr>
<td>2) Low production of biological solids</td>
<td>2) Relatively long periods of time are required to start up the process as a results of the slow growth rate of anaerobe</td>
</tr>
<tr>
<td>3) Waste biological sludge is a highly stabilized product that can be easily dewatered</td>
<td>3) Anaerobic digestion is basically a pretreatment method: an adequate post-treatment is usually required before the effluent can be discharged</td>
</tr>
<tr>
<td>4) Low nutrient requirements</td>
<td>4) Little practical experience has been gained with the application of the process to the direct treatment of wastewaters</td>
</tr>
<tr>
<td>5) No energy requirement for aeration</td>
<td></td>
</tr>
<tr>
<td>6) Very high loading rates can be applied under favorable conditions</td>
<td></td>
</tr>
<tr>
<td>7) Active anaerobic sludge can be preserved unfed for many months</td>
<td></td>
</tr>
</tbody>
</table>
of the biodegradable organic matter is converted to cell materials under anaerobic condition (Feilden, 1983).

In the aerobic process, the waste is mixed with large quantities of microorganisms and air. Microorganisms use oxygen in the environment to oxidize a portion of organic matter in the waste to carbon dioxide and water (McCarty, 1964). Since these organisms obtain much energy from this oxidation process, their growth is rapid and about 50% of the organic waste is converted into new cells, causing potential disposal problems. This process often requires large amounts of energy to provide sufficient oxygen to the system.

Even though the benefits of the anaerobic process are attractive, the major obstacle to full-scale application is the difficulty of extending the process to a simple operational form.

D. Anaerobic Digestion of Lactose

1. Biochemistry of Lactose Biomethanation

Anaerobic degradation of lactose occurs in three distinct but simultaneous phases (Figure 1).

First, in the hydrolytic phase, multiple fermentation products are formed from lactose yielding lactate, ethanol, formate, CO₂, and acetate. Second, those intermediary metabolites are converted into acetate and H₂-CO₂ in the acetogenic phase. In the last phase, methanogenesis occurs from the methane precursors.

In this ecosystem, lactate is the major intermediary metabolite of lactose fermentation, and acetate is usually the major precursor accounting for about 70 to 80% of the total methane formed (Jerris and McCarty, 1965; Smith and Mah, 1966; Chartrain and Zeikus, 1986a; Kissalita et al., 1987; Schug et al., 1987).
Figure 1. The pathway of anaerobic degradation of lactose (Chartrain and Zeikus, 1986a, 1986b; Schug et al., 1987). (Solid line indicates major pathway from lactose to methane)
2. Microbiology of Lactose Biomethanation

The complete degradation of lactose to methane and carbon dioxide requires the involvement of various microorganisms (Figure 1). Table 8 shows the organization of microorganisms into different trophic groups that perform specific metabolic transformations during the anaerobic degradation of lactose (Schug et al., 1987; Chartrain and Zeikus, 1986b; Kissalita et al., 1987; Kissalita et al., 1989; Bryant, 1979; Wilkie and Colleran, 1986).

The first trophic group is hydrolytic bacteria that degrade lactose into multiple acids and neutral end products. These acids and neutral products are further transformed to methane precursors by acetogenic bacteria. Methanogenic bacteria are the terminal trophic group and produce methane from methane precursors. When sulfate is available, methane production is limited because sulfate-reducing bacteria can outcompete methanogens for acetate and hydrogen (Winfrey and Zeikus, 1977; Kristjansson et al., 1982; Lupton and Zeikus, 1984; Robinson and Tiedge, 1984). All the bacterial trophic groups involved in anaerobic digestion are highly dependent on species metabolic interaction, and inhibition of one group can cause failure of the overall biomethanation process (Sykes and Kirsch, 1972; Zeikus, 1977; Schink and Zeikus, 1982).

In many anaerobic processes, growth rates of hydrolytic and acetogenic bacteria are faster than those of methanogenic bacteria, indicating the methanogenic phase is usually the rate-limiting step.

3. Theoretical Methane Yield

Degradation of 1 mole of lactose theoretically produces 6 moles of methane. Eq. D-1 represents stoichiometry of conversion of lactose to methane and carbon dioxide.
<table>
<thead>
<tr>
<th>Phase</th>
<th>Species</th>
<th>Shape</th>
<th>Gram</th>
<th>Spore</th>
<th>$\mu$ (d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolytic</td>
<td><em>Clostridium butyricum</em></td>
<td>rod</td>
<td>+</td>
<td>o</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>rod</td>
<td>-</td>
<td>x</td>
<td>6.00</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella oxytoca</em></td>
<td>rod</td>
<td>-</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus casei</em></td>
<td>rod</td>
<td>+</td>
<td>x</td>
<td>7.44</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus plantarum</em></td>
<td>rod</td>
<td>+</td>
<td>NA</td>
<td>9.60</td>
</tr>
<tr>
<td></td>
<td><em>Leuconostoc mesenteroides</em></td>
<td>coccus</td>
<td>+</td>
<td>capsule</td>
<td>NA</td>
</tr>
<tr>
<td>Acetogenic</td>
<td><em>Acetobacterium woodii</em></td>
<td>rod</td>
<td>-</td>
<td>x</td>
<td>3.12</td>
</tr>
<tr>
<td></td>
<td><em>Clostridium propionicum</em></td>
<td>rod</td>
<td>+</td>
<td>o</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td><em>Desulfovivrio vulgaris</em></td>
<td>rod</td>
<td>-</td>
<td>x</td>
<td>NA</td>
</tr>
<tr>
<td>Methanogenic</td>
<td><em>Methanobacterium bryantii</em></td>
<td>rod</td>
<td>+</td>
<td>NA</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td><em>Methanobacterium formicicum</em></td>
<td>rod</td>
<td>+</td>
<td>o</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td><em>Methanosarcina barkeri</em></td>
<td>coccus</td>
<td>+</td>
<td>capsule</td>
<td>0.48-0.96</td>
</tr>
<tr>
<td></td>
<td><em>Methanothrix soehngenii</em></td>
<td>filament</td>
<td>+/-</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA : Not Available  
0 : Spore forming  
x : Not spore forming
The following calculation yields the volume of methane produced per unit amount of lactose digested at STP condition:

\[
\frac{6 \text{ mole of CH}_4}{1 \text{ mole of lactose}} \times \frac{1 \text{ mole of lactose}}{342 \text{ g of lactose}} \times \frac{22.4 \text{ L of CH}_4}{1 \text{ mole of CH}_4} = 0.393 \text{ L of CH}_4/\text{g of lactose}
\]

For a 4.0% lactose solution, which is similar to the composition of whey:

\[
\frac{0.393 \text{ L of CH}_4}{1 \text{ g of lactose}} \times \frac{40.0 \text{ g of lactose}}{1 \text{ L of 4\% lactose solution}} = 15.72 \text{ L of CH}_4/\text{L of 4\% lactose solution}
\]

For complete oxidation, 1 mole of lactose requires 12 moles of oxygen (Eq. D-2).

\[
\text{C}_{12}\text{H}_{22}\text{O}_{11} + 12 \text{O}_2 \rightarrow 11 \text{H}_2\text{O} + 12 \text{CO}_2
\]

Further calculations give the amount of oxygen required per unit amount of lactose degraded:

\[
\frac{12 \text{ mole of O}_2}{1 \text{ mole of lactose}} \times \frac{1 \text{ mole of lactose}}{342 \text{ g of lactose}} \times \frac{32 \text{ g of O}_2}{1 \text{ mole of O}_2} = 1.12 \text{ g of O}_2/\text{g of lactose}
\]

For 4.0% lactose solution:

\[
\frac{1.12 \text{ g of O}_2}{\text{g of lactose}} \times \frac{40.0 \text{ g of lactose}}{1 \text{ L of 4\% lactose solution}}
\]
= 44.80 g of O₂/L of 4% lactose solution
= 44.80 g of COD/L of 4% lactose solution

Therefore, theoretical maximum methane yield (CH₄ produced/COD converted) at STP condition is:

\[
\frac{15.72 \text{ L of CH}_4/\text{L of 4\% lactose solution}}{44.80 \text{ g of COD/L of 4\% lactose solution}} = 0.35 \text{ L of CH}_4/\text{g of COD}
\]

4. Theoretical Biomass Yield

The biomass produced per unit amount of lactose degraded can be illustrated with a carbon balance. A general formula of biomass can be expressed as C₅H₇NO₂ (Sykes, 1975). One mole of lactose supplies carbon to produce 2.4 moles of biomass if all the carbon in the lactose is converted to biomass (Eq. D-3).

\[
\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{Nutrient} \rightarrow 2.4 \text{C}_5\text{H}_7\text{NO}_2 + \text{By-product}
\]

Therefore, theoretical maximum biomass yield on the basis of COD converted is obtained by the following calculations:

\[
\frac{2.4 \text{ mole of biomass}}{1 \text{ mole of lactose}} \times \frac{1 \text{ mole of lactose}}{342 \text{ g of lactose}} \times \frac{113 \text{ g of biomass}}{1 \text{ mole of biomass}} = 0.79 \text{ g of biomass/g of lactose}
\]

On the basis of COD

\[
\frac{0.79 \text{ g of biomass/g of lactose}}{1.12 \text{ g of COD/g of lactose}} = 0.71 \text{ g of biomass/g of COD}
\]
E. Upflow Anaerobic Sludge Blanket (UASB) Reactor

1. Reactor Choice

Various types of anaerobic reactors have been used for waste treatment by biological means. These can be broadly classified into two groups, namely the attached growth reactors and the nonattached or the suspended growth reactors. The biomass of the former comprises bacteria attached as films to inert support media while operation of the latter depends on the metabolic activity of microorganisms suspended as flocs or granules in the reactor vessel.

Upflow anaerobic sludge blanket (UASB) (Figure 2) reactor is a recently developed anaerobic process by Lettinga and his co-workers (Lettinga et al., 1980). Because granular sludge in the UASB system has superior settling characteristics (Heertjes and Van Der Meer, 1978), a high solid retention time (SRT) at a high loading rate can be achieved under favorable physical and chemical conditions for sludge granulation (Lettinga et al., 1979; Lettinga et al., 1980; Godwin et al., 1982; Barbosa and Sant’Anna Jr, 1989).

Reasons for the choice of the UASB reactor over other reactors are as follows:

a) The interior biomass retention system means that any separate settling device is not necessary.

b) Little if any mechanical mixing is necessary.

c) Inert media are generally absent from the system. The UASB reactor only requires a simple gas-liquid-solid separator.

Frostell (1981) praised the sludge bed reactor as a system that combined the advantages of a filter process with those of the anaerobic contact process, if adequate solid retention was achieved. Thus, the UASB process distinguishes itself as an economical alternative for the treatment of high strength waste such as whey permeate.
Figure 2. Diagram of upflow anaerobic sludge blanket bioreactor.
The experimental results of UASB processes for various substrates are presented in Table 9.

Like other anaerobic treatments, a major limitation of the UASB process is the considerable time (6-8 weeks using seed sludge) involved in the start of the reactor. High concentration of suspended materials in the waste also adversely affects the UASB process (Lettinga et al., 1980).

2. Reactor Descriptions

The UASB reactor is based on the slow upward movement of waste through dense bed and blanket zones of biologically active sludge. Basically, the reactor consists of three distinct zones: the sludge bed, sludge blanket, and settling/biomass separation zones (Figure 2).

The sludge bed zone is responsible for 80 to 90% of the waste stabilization occurring in the reactor while occupying roughly 30% of the reactor volume (Obayashi and Gorgan, 1985). This main waste stabilization is due to high biomass concentration in the sludge bed. Under favorable conditions for sludge granulation, anaerobic granules with high microbial activities and excellent settling characteristics, up to 3-4 mm in diameter, are formed in the reactor (Lettinga and De Zeeuw, 1980). Lettinga et al. (1980) suggested that the granulation ability of sludge could be improved by the presence of divalent cations like Ca++ and small amounts of suspended materials in the waste.

The sludge bed zone has been described as a well-mixed region (Heertjes and Van Der Meer, 1978; Buijs and Heertjes, 1982) that can be divided into smaller subregions. The first subregion is the area around the influent ports, which is considered to be a completely mixed region. The rest of the sludge bed is considered a transition region between the initial bed zone and the sludge blanket zone. Because the influent enters the reactor at the bottom, the different sludge densities influence the flow
Table 9. Performances of UASB Reactors Treating Different Substrates

<table>
<thead>
<tr>
<th>Authors</th>
<th>Substrate</th>
<th>Substrate concentration (g COD/L)</th>
<th>Temperature (°C)</th>
<th>Loading rate (Kg COD/m³/d)</th>
<th>Removal efficiencies (%)</th>
<th>CH₄ production (L/L/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lettinga et al.,</td>
<td>Methanol</td>
<td>9.2</td>
<td>30</td>
<td>21.6</td>
<td>40-98</td>
<td>0.16-1.82</td>
</tr>
<tr>
<td>(1979)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lettinga et al.,</td>
<td>Sugar beet</td>
<td>4.0-5.2</td>
<td>34</td>
<td>14.0-16.0</td>
<td>87-95</td>
<td>NA</td>
</tr>
<tr>
<td>(1980)</td>
<td>Photato</td>
<td>2.0-5.0</td>
<td>30</td>
<td>15.0-18.0</td>
<td>95</td>
<td>NA</td>
</tr>
<tr>
<td>Frostell</td>
<td>Starch</td>
<td>0.7</td>
<td>35</td>
<td>2.5-10.0</td>
<td>68-87</td>
<td>NA</td>
</tr>
<tr>
<td>(1981)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Godwin et al.,</td>
<td>Acetate</td>
<td>1.0</td>
<td>35</td>
<td>1.6</td>
<td>70</td>
<td>NA</td>
</tr>
<tr>
<td>(1982)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wiegant and Lettinga</td>
<td>Glucose</td>
<td>1.4-14.6</td>
<td>55</td>
<td>16.0-104.0</td>
<td>77-99</td>
<td>0.1-1.25</td>
</tr>
<tr>
<td>(1985)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wu et al</td>
<td>Citrate</td>
<td>36.0</td>
<td>35</td>
<td>22.0</td>
<td>91</td>
<td>7.1</td>
</tr>
<tr>
<td>(1987)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barbosa and</td>
<td>Domestic</td>
<td>0.63</td>
<td>18-28</td>
<td>NA</td>
<td>74</td>
<td>0.46*</td>
</tr>
<tr>
<td>Sant'Anna JR (1989)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA : Not Available

*: \[\frac{L \cdot CH_4/L/day}{Kg-COD \text{ removed}}\]
pattern of the liquid, and by-passing and return flow may occur.

The next zone encountered by the waste stream is the sludge blanket zone, which occupies about 50% of the total reactor volume and contains less sludge concentration than the sludge bed zone (Obayashi and Gogan, 1985). The sludge in the blanket zone has almost uniform particle size and originates from the bed where it is whirled up by the rising gas bubbles. This zone is considered completely mixed because very good mixing conditions exist by rising gas bubbles (Buijs and Heertjes, 1982). The concentration of the sludge in the bed zone and in the blanket zone depends on the properties of the sludge, such as the settling velocity, the particle size distribution, and the density of the sludge particle (Alibhai and Forster, 1986).

A third area is a zone in the settler where the sludge concentration decreases to a minimum. Heertjes and Van Der Meer (1978) pointed out that the fluid flow in the settler was laminar, which might be described as a plug-flow region. In the UASB digester, long solid retention time can be achieved by the gas-liquid-solid separator at the top of the reactor (Heertjes and Van Der Meer, 1978; Lettinga et al., 1981; Wu et al., 1987). Biomass particles become attached to gas bubbles and are carried up with them as they rise through the sludge bed and blanket (Stronach et al., 1986). The main function of the separator is to drive the rising gas and biomass particles in toward the gas collector, where a swirling action occurs, and the biomass settles back down into the reactor, thereby preventing most of the biomass rising with gas bubbles from leaving the reactor.

3. Start-Up Process

According to Lettinga et al. (1980), the start-up of the process is very important with respect to both the biomass activity and formation of sludge granules, which are directly related to settling capability of sludge in the reactor. Some recommendations were suggested:
1. The adaptation of the seed sludge to the new environment is necessary. Therefore, the maximum initial sludge load should be less than 0.1-0.2 kg COD/kg total solids/day.

2. In order to develop sufficient microbial activity, effective decomposition of organic acid present or formed should be achieved before increasing the loading rate of the reactor.

3. Since the methanogenic phase is the rate-limiting step in many anaerobic digestion processes, environmental conditions such as pH and temperature should be favorable for growth of methanogens.

F. Kinetic Model Development

Many kinetic models for biological wastewater treatment have been developed during the last 40 years. They are very useful for the understanding of the treatment process and for the comprehension of applicabilities and limitations. In many cases, development of mathematical models for the process is of great importance to evaluate further developments and operational conditions in the right way. The design of full-scale treatment facilities today should be based on kinetic models derived from experimental and operational data.

Vasicek (1982) pointed out that for fear of system inadequancy, many engineers without a proper kinetic model tended to overdesign wastewater treatment systems.

1. Basic Concepts of Kinetic Models

The change of biomass in a microbial culture undergoing a balanced growth generally follows the first order model. The rate of growth at any time is proportional to the number or mass of microorganisms present in the system at the time as described by the following equation:
\[
\frac{dX_a}{dt} = \mu X_a \tag{F-1}
\]

where \(\frac{dX_a}{dt}\): growth rate of microorganism (mass biomass (VSS)/volume/time)

\(\mu\): specific growth rate of microorganism which is a proportionality constant (time\(^{-1}\))

\(X_a\): concentration of active microbial population (mass biomass (VSS)/volume)

Several models have been developed that indirectly establish a value of \(\mu\). The most widely accepted of these is the Monod equation (Monod, 1949). This equation assumes that the rate of biomass production is limited by the rate of enzyme reactions involving utilization of the substrate compound that is in shortest supply relative to its need. Eq. F-2 shows this relationship.

\[
\mu = \frac{\mu_m S}{K_{s1} + S} \tag{F-2}
\]

where

\(\mu_m\): maximum specific growth rate of microorganism (time\(^{-1}\))

\(S\): residual growth-limiting substrate concentration (mass substrate (COD)/volume)

\(K_{s1}\): half saturation constant numerically equal to the substrate concentration at which \(\mu = \frac{\mu_m}{2}\) (mass substrate (COD)/volume)

Eq. F-2 shows that specific growth rate is a hyperbolic function of the substrate concentration. The Monod equation also indicates that \(\mu\) can have any value between zero and \(\mu_m\), provided that the substrate concentration can be held constant at a given
value of $\mu$. Any system designed for the continuous cultivation of microorganisms meets this condition.

For some increment of time, the change in the substrate concentration is proportional to the concentration of biomass present as described by the following equation:

$$\left(\frac{dS}{dt}\right)_u = q X_a \quad \text{F-3}$$

where

- $\left(\frac{dS}{dt}\right)_u$ : the rate of microbial substrate utilization (mass substrate (COD)/volume/time)
- $q$ : specific substrate utilization rate which is a proportionality constant (time$^{-1}$)

Lawrence and McCarty (1969) presented an equation (F-4) that related the rate of substrate utilization to both the concentration of substrate and of microorganisms, and is as follows:

$$\left(\frac{dS}{dt}\right)_u = \frac{k X_a S}{K_{S2} + S} \quad \text{F-4}$$

where

- $k$ : maximum specific substrate utilization rate (mass substrate (COD)/mass biomass (VSS)/time)
- $S$ : substrate concentration surrounding the microorganisms (mass substrate (COD)/volume)
- $K_{S2}$ : half saturation constant equal to the substrate concentration when $q = k/2$ (mass substrate (COD)/volume)
When applying Eq. F-2 and Eq. F-4 to any microbial ecosystem, substrate concentration surrounding microorganisms is an important consideration for evaluating kinetic parameters. The carbon and energy source, as measured by ultimate biochemical oxygen demand (BOD<sub>u</sub>), chemical oxygen demand (COD), or total organic carbon (TOC), is usually considered to be the growth-limiting substrate in biological wastewater treatment processes.

If all the substrate were converted to biomass, then the rate of biomass production would equal the rate of substrate utilization. However, the rate of biomass production is less than the rate of substrate utilization since catabolism converts some part of substrate into nongrowth factor. Thus:

\[
\left(\frac{dX_a}{dt}\right)_g = Y \left(\frac{dS}{dt}\right)_u
\]

where

\[Y\] : microbial yield coefficient (mass biomass produced (VSS)/mass substrate utilized (COD))

The factor \(Y\) varies depending on the metabolic pathway used in the conversion process. Aerobic processes are more efficient than anaerobic processes with respect to biomass production and have a larger value for \(Y\).

Under certain conditions such as cell lysis, presence of predators, and endogenous metabolism, microorganisms lose the ability to grow or to subdivide. Such morbid microorganisms die, resulting in a decrease in biomass population. To account for this phenomenon, it is assumed that the rate of biomass decrease is proportional to the concentration of biomass in the system.
where

\[
\left(\frac{dX_a}{dt}\right)_d = k_d X_a
\]

\(\left(\frac{dX}{dt}\right)_d\) : rate of decrease in biomass concentration (mass biomass (VSS)/volume/time)

\(k_d\) : specific decay rate of microorganism which is a proportionality constant (time\(^{-1}\))

2. Kinetic Model Development for the UASB Process

The universal assumption of complete mixed type reactors is that the inside characteristics of the reactor are homogeneous at any point, and effluent represents everything the same as the material properties inside the reactors. Therefore, the concentrations of substrate and of microorganism in the effluent are widely used to evaluate kinetic values in many aerobic or anaerobic complete mixed type studies (Lawrence and McCarty, 1969; Christensen and McCarty; 1975, Vasicek, 1982; Paolini and Variali, 1982; Feilden, 1983; Novak, 1984; Chudoba et al., 1989).

As mentioned earlier, the nature of the sludge bed and blanket could be described as a combination of completely mixed region and well mixed region while the flow characteristics in the settling zone could be described as plug flow. However, since rising gas bubbles from the sludge bed and blanket also provide mixing of the settling zone, the settling zone cannot be a perfect plug flow. The assumption that inside characteristics of the completely mixed type reactor are uniform at any point can not be applied to the UASB process. Therefore, effluent characteristics of the substrate and of the microorganisms are unlike other completely mixed reactors and are not proper parameters to represent growth of microorganisms and utilization of substrate in a UASB kinetic study.

Many scientists have emphasized the importance of food to microorganism ratio (F:M ratio), or specific organic loading rate in order to evaluate process performance...
as well as the effluent concentrations (Cook and Kincannon, 1971; Grady and Williams, 1975; Suschka, 1980; Hung, 1984; Kincannon and Stover, 1984). Since anaerobic microorganisms (especially methanogens) are very sensitive to their environmental changes, it is more desirable to consider the amount of substrate per microorganism per unit period than to use the effluent substrate concentration. The specific organic loading rate, \( L_x \), takes into account both the hydraulic retention time and the concentration of the waste per unit biomass and is then defined as:

\[
L_x = \frac{S_i Q}{X_a V} = \frac{S_i}{X_a \tau}
\]

where

- \( L_x \) : specific organic loading rate (mass substrate (COD)/mass biomass (VSS)/time)
- \( S_i \) : influent substrate concentration (mass/volume)
- \( Q \) : flow rate (volume/time)
- \( V \) : reactor volume (volume)
- \( \tau \) : hydraulic retention time (time)

The specific organic loading rate can act as a limiting nutrient because it represents the amount of substrate taken by unit mass of biomass per unit time. Kincannon and Stover (1984) showed that curve-fitting of specific substrate utilization rate as a function of the specific organic loading rate fit a Monod-type relationship.

The relationship between the substrate utilization rate, \( (dS/dt)_u \), and the organic loading rate becomes:

\[
(dS/dt)_u = \frac{k X_a L_x}{K_L + L_x}
\]
where

\((\frac{dS}{dt})_u\) : microbial substrate utilization rate (mass substrate (COD)/volume/time)

\(k\) : maximum specific substrate utilization rate (mass substrate (COD)/mass biomass (VSS)/time)

\(K_L\) : specific organic loading rate at \(q = k/2\) (mass substrate (COD)/mass biomass (VSS)/time)

The specific substrate utilization rate is then defined as:

\[ q = \frac{(dS/dt)_u}{X_a} = \frac{k L_X}{K_L + L_X} \]  \hspace{1cm} F-9

where

\(q\) : specific substrate utilization rate (time\(^{-1}\))

which is similar to the Lawrence and McCarty equation, except that the Lawrence and McCarty equation has the term of substrate concentration surrounding the microorganisms, which is usually expressed as effluent substrate concentration, while Eq. F-9 has the term of rate of substrate uptake per unit biomass.

To evaluate the biokinetic constants, it is necessary to calculate specific substrate utilization rate determined by the following equation (Benefield and Randall, 1980; Novak, 1984):

\[ q = \frac{(S_i - S_e) Q}{X_a V} = \frac{(S_i - S_e)}{X_a \tau} \]  \hspace{1cm} F-10

where

\(S_e\) : effluent substrate concentration (mass substrate (COD)/volume)
Figure 3. Flow diagram of UASB process without recycle.

The flow diagram of the UASB without recycle is shown in Figure 3.

Kinetic models are usually developed by writing material balances describing the mass rate of change in substrate and in biomass. Based on Figure 3, a mass balance on the substrate can be written around the entire system:

\[
\begin{bmatrix}
\text{Net rate of change in the reactor}
\end{bmatrix}
= \begin{bmatrix}
\text{Rate of increase due to the influent}
\end{bmatrix} - \begin{bmatrix}
\text{Rate of decrease due to substrate utilization}
\end{bmatrix} - \begin{bmatrix}
\text{Rate at which substrate leaves the reactor}
\end{bmatrix}
\]

which can be expressed as:

\[
\left(\frac{dS}{dt}\right)_\text{net} V = QS_i - \left(\frac{dS}{dt}\right)_u V - QS_e
\]

where

\[
\left(\frac{dS}{dt}\right)_\text{net} : \text{net rate of change in substrate concentration (mass substrate (COD)/volume/time)}
\]

Steady state implies that:

\[
\left(\frac{dS}{dt}\right)_\text{net} = 0
\]
Thus, at steady state, Eq. F-11 can be written as:

\[
\left( \frac{dS}{dt} \right)_u = \frac{Q}{V} (S_i - S_e) = \frac{(S_i - S_e)}{\tau^*}
\]  

F-13

By substituting from Eq. F-7 and F-8 for substrate utilization rate, \( \left( \frac{dS}{dt} \right)_u \), and specific organic loading rate, \( L_x \), in Eq. F-13 and solving for \( S_e \):

\[
S_e = S_i \left( 1 - \frac{k}{K_L + L_x} \right)
\]  

F-14

Similarly a mass balance for the biomass gives:

\[
\begin{bmatrix}
\text{Net rate of change in the reactor}
\end{bmatrix}
= \begin{bmatrix}
\text{Rate of increase by bacterial growth}
\end{bmatrix}
- \begin{bmatrix}
\text{Rate of decrease by bacterial decay}
\end{bmatrix}
- \begin{bmatrix}
\text{Rate of decrease due to loss in the effluent}
\end{bmatrix}
\]

\[
\left( \frac{dX_a}{dt} \right)_{net} V = (Y \left( \frac{dS}{dt} \right)_u - K_d X_a) V - QX_e
\]  

F-15

where
\[
\begin{align*}
\left( \frac{dX_a}{dt} \right)_{net} & : \text{net rate of change in biomass concentration (mass biomass (VSS)/volume/time)} \\
Y & : \text{yield coefficient (mass of biomass produced (VSS)/mass of substrate removed (COD))} \\
K_d & : \text{decay coefficient (time}^{-1})
\end{align*}
\]

At steady state, which is \( (dX_a/dt)_{net} = 0 \), substituting from Eq. F-7 and F-8 for substrate removal rate, \( \left( \frac{dS}{dt} \right)_u \), in Eq. F-15 and solving for \( X_a \):
\[ X_a = \frac{(S_i Y_k - K_d S_i - K_L X_e)}{2K_d K_L \tau} \pm \frac{\sqrt{(K_L X_e + K_d S_i - S_i Y_k)^2 - 4K_d K_L X_e S_i}}{2K_d K_L \tau} \]  

The following reciprocal form of Eq. F-9 can be used to determine biokinetic parameters \( K_L \) and \( k \) linearly:

\[ \frac{1}{q} = \frac{K_L}{k} \cdot \frac{1}{L_x} + \frac{1}{k} \]  

Lawrence and McCarty (1969) emphasized the importance of the operational parameter called solid retention time (SRT), \( \theta_c \), which is defined as the average time of biomass remaining in the system or:

\[ \theta_c = \frac{X_T}{X_e Q} = \frac{X_a V}{X_e Q} = \frac{X_a \tau}{X_e} \]

where

\( \theta_c \) : solid retention time (time)

\( X_T \) : total active biomass in the system (mass biomass [VSS])

At steady state, Eq. F-15 can be written as:

\[ Y \frac{(dS/dt)}{X_a} = (K_d + \frac{X_e Q}{X_a V}) \]

By combining Eq. F-18 and F-19, the following equation, which facilitates the evaluation of \( Y \) and \( K_d \) linearly, is developed:

\[ \frac{1}{\theta_c} = Y q - K_d \]
When the specific organic loading rate is large enough not to be growth limiting, the value of solid retention time at which washout of biomass occurs is a characteristic parameter of the process. The entire waste stabilization should be maintained above this point or so-called minimum solid retention time, \( \theta_c^m \), to avoid process failure. \( \theta_c^m \) is theoretically calculated from F-9 and F-20 by letting \( L_x \) approach infinity, thus yielding:

\[
\frac{1}{\theta_c^m} = \frac{Y_k}{K_d}
\]

where

\( \theta_c^m \) : minimum solid retention time (time)

Cook and Kincannon (1971) successfully applied the concept of specific organic loading rate for trickling filter performance. This design approach also proved to be reliable for biological towers and rotating contactors with little scatter of the data fitness (Kincannon and Stover, 1984). Because operational characteristics of the biological tower type reactor are similar to UASB reactor (Young and McCarty, 1969; Feilden, 1983; Pedro and Fernando, 1987; Denac and Dunn, 1988), this design approach is believed to be reliable for the kinetics of the UASB reactor.

3. Assumptions

For the purpose of simplicity in kinetic analysis of this study, some assumptions are made as follows:

1. There is no microorganisms in the influent
2. There is no temperature effect in the system
3. There is no nutrient deficiency in the system
(4) All reactions occur in the reactor taking the whole reactor volume as a control volume.

These assumptions do not affect the application of the theory to actual plant evaluation if the conditions are specified.
CHAPTER III

OBJECTIVES

The objectives of this study were

1. To investigate the feasibility of the UASB process for whey permeate treatment.

2. To provide accurate predictive equations which cover the metabolic and biological behavior in the UASB process.
CHAPTER IV
MATERIALS AND METHODS

A. Experimental Materials

1. Bacterial Strain

A mixed culture from a wastewater plant was used in all experimental runs. Anaerobic seed sludge from a municipal wastewater treatment plant at Ogden, Utah, was used as starter culture. The seed sludge is characterized in Table 10.

2. Preparation of Whey Permeate

Dried sweet whey powder, composition shown in Table 11, was obtained from Gossner Food, Inc., Logan, Utah. Dried whey powder was dissolved in water in appropriate proportion (0.057 kg of dry powder/1 L of water) to obtain the solid concentration of typical cheese whey. This solution was used to make whey permeate by ultrafiltration (UF) method.

Ultrafiltration was performed by batch mode using an Abcor HFK-130, single stage, spiral wound, polysulfone membrane with a molecular cut-off of 10,000 daltons and 5 m² of filtering surface. An inlet pressure of 420 kPa (60 psi) and outlet pressure of 210 kPa (30 psi) were used throughout the process. Ultrafiltration was carried out at 50°C until 60% (w/w) of the whey solution was removed as permeate. The whey permeate contained 48.2 ± 5.4 g COD/L.

3. Feed Solution

The whey permeate solution made by UF was diluted with tap water to give a desired influent concentration. A proper amount of concentrated nutrient solution, a mixture of 0.09 mole KH₂PO₄ and 1.00 mole NH₄Cl, was separately added to this Table 10. Characteristics of Seed Sludge
<table>
<thead>
<tr>
<th>TS</th>
<th>TDS</th>
<th>TSS</th>
<th>VSS</th>
<th>VSS/TSS</th>
<th>VOA</th>
<th>ALK</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g/L)</td>
<td>(g/L)</td>
<td>(g/L)</td>
<td>(g/L)</td>
<td>(%)</td>
<td>(mg acetate/L)</td>
<td>(mg CaCO₃/L)</td>
</tr>
<tr>
<td>25.387</td>
<td>0.671</td>
<td>24.715</td>
<td>14.502</td>
<td>56.68</td>
<td>115.8</td>
<td>2917.6</td>
</tr>
</tbody>
</table>

Table 11. Composition of Dry Sweet Whey Powder (%)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>12.00</td>
</tr>
<tr>
<td>Fat</td>
<td>0.50</td>
</tr>
<tr>
<td>Ash</td>
<td>9.40</td>
</tr>
<tr>
<td>Lactose</td>
<td>70.00</td>
</tr>
</tbody>
</table>
dilute whey permeate solution to give a final COD:N:P ratio of 237:5:1. No additional trace elements were supplied (Ying, 1989; also see Table 5).

A proper amount of NaHCO₃, less than 5.0 g/L because of sodium toxicity, was added to the feed solution to keep digester pH above 6.8.

The feed solution was refrigerated (4°C) during feeding. The unused portion of whey permeate solution was kept in the freezer (-20°C) in order to prevent contamination.

4. Upflow Anaerobic Sludge Blanket Reactor

Two identical lab-scale reactors, similar to those used in previous sludge blanket work (Lettinga et al., 1980; Frostell, 1981; Lettinga et al., 1983; West, 1984), were built and modified by Hansen (1987). The use of two reactors provided duplicate data. Each reactor consisted of the following parts:

1. reactor
2. feeding system
3. heating system
4. biogas collection system.

Schematic descriptions of the UASB system used in this research are illustrated with Figures 2 and 4.

Reactors were constructed of transparent plastic material with working volume of 7.2 L. Both reactors contained a biogas-liquid-solid separator in the settling zone and a liquid distributor on the bottom inlet. The liquid distributor was designed to provide efficient contact between biomass and feed solution and to prevent channeling in the sludge bed zone. Sludge sampling channels having an inside diameter of 6.35 mm were spaced along the length of reactors to permit periodic sampling. The reactor top was sealed by a bolted plastic cover. Silicon caulk was used to maintain anaerobic condition. Anaerobic conditions were tested for two days using water before
A : UASB reactors
B : Feed solution containers
C : Electric pumps
D : Acid solution traps
E : Gas collectors
F : Temperature controller
G : Insulated cabinet (35°C)
H : Refrigerator (4°C)

Figure 4. Diagram of overall operating systems.
experimental runs.

Two single-head Masterflex pump (Cole-Palmer Instrument Company, Chicago, IL) units were used for continuous feeding.

Temperature was maintained at 35°C, a mesophilic temperature range, by placing the reactors in a cabinet heated with six 60-watt light bulbs and controlled with a Goldline SP-30 temperature controller (Independent Energy Inc., Chicago, IL).

The volume of biogas produced was measured using a water displacement system (Figure 5). Two identical plastic graduated vessels with maximum capacity of 7 L were used as gas collectors on both reactors. This system maintained pressure within the digesters near atmospheric level with no detectable biogas leaks. Biogas originating from each reactor passed through a water trap and flowed through the top of the collector into biogas storage and measurement.

B. Experimental Procedure

1. Process Variables

To avoid loading shock on both reactors, the loading rates were not increased unless at least 90% of COD removal occurred and volatile organic acids (VOA) concentration in the effluent was below 300 mg acetate/L.

Nine continuous trials over a 13-month period were conducted to examine the feasibility and the kinetic parameters of the UASB process treating whey permeate. Hydraulic retention time at fixed influent concentration, 10.568 ± 0.218 g COD/L, was the only variable to control the process performance. Table 12 shows experimental conditions of all trials.

2. Start-Up Procedures

According to the start-up guidelines mentioned in Chapter II (E-3), loading rate was carefully controlled by slowly increasing COD concentration (Table 13).
Figure 5. Water trap and biogas collection system.
Table 12. Experimental Designs and Conditions

<table>
<thead>
<tr>
<th>Trial No</th>
<th>HRT (days)</th>
<th>Influent COD (g/L)</th>
<th>Temp. (°C)</th>
<th>COD Loading (kg COD/m³/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.0</td>
<td>10.568</td>
<td>35</td>
<td>2.114</td>
</tr>
<tr>
<td>2</td>
<td>4.0</td>
<td>10.056</td>
<td>35</td>
<td>2.514</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>10.125</td>
<td>35</td>
<td>3.375</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>10.374</td>
<td>35</td>
<td>5.187</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>10.120</td>
<td>35</td>
<td>10.120</td>
</tr>
<tr>
<td>6</td>
<td>0.8</td>
<td>10.468</td>
<td>35</td>
<td>13.085</td>
</tr>
<tr>
<td>7</td>
<td>0.5</td>
<td>10.602</td>
<td>35</td>
<td>21.204</td>
</tr>
<tr>
<td>8</td>
<td>0.4</td>
<td>10.781</td>
<td>35</td>
<td>26.953</td>
</tr>
<tr>
<td>9</td>
<td>0.2</td>
<td>10.464</td>
<td>35</td>
<td>52.32</td>
</tr>
</tbody>
</table>

Table 13. Start-Up Schedule

<table>
<thead>
<tr>
<th>Period (week)</th>
<th>HRT (day)</th>
<th>Influent COD (g COD/L)</th>
<th>COD Loading (kg COD/L/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>7</td>
<td>3.5</td>
<td>0.50</td>
</tr>
<tr>
<td>3-4</td>
<td>7</td>
<td>5.0</td>
<td>0.71</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>7.0</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>8.5</td>
<td>1.12</td>
</tr>
<tr>
<td>7-8</td>
<td>7</td>
<td>10.0</td>
<td>1.43</td>
</tr>
</tbody>
</table>
Approximately 20% of seed sludge by reactor volume was used to initiate the process. The initial biomass concentration was 4.501 g VSS/L and influent COD concentration of 3500 mg COD/L at 7 days HRT gave initial specific loading of 0.11 kg COD/kg VSS/day.

Increasing influent COD concentration at fixed HRT (7 days) controlled loading rate of the reactors during the start-up period (Table 13). Feeding was ceased to control reactor performance when process failure occurred.

Thirty milligrams of CaCO₃/L were added to improve settling ability of sludge (Lettinga et al., 1980).

3. Steady State Operation

Data taken during steady state operation were used to estimate reactor performance and kinetic parameters: $k$, $K_L$, $Y$, $K_d$, $\theta_c$. Steady state meant that the given process parameters did not vary by more than $\pm 5.0\%$. The process parameters considered were substrate removal efficiency, biomass concentration, pH, biogas and methane production, and VOA concentration.

4. Sampling Schedules

Process parameters to be sampled and sampling intervals for each experimental trial were

Daily:

- COD of influent and effluent
- pH
- biogas volume
- room temperature and barometric pressure

Twice weekly:

- TSS and VSS in the reactor except sludge bed
- TSS and VSS of effluent
- Alkalinity of effluent
- VOA

Weekly:
- biogas composition analysis

Biweekly:
- TSS and VSS of sludge bed

Once the process reached steady state, all process parameters were sampled daily.

To prevent loss of active biomass by frequent sampling, solid samplings in the sludge bed remained on a biweekly basis during steady-state operation.

C. Analytical Procedures

1. Chemical Oxygen Demand (COD)

   The COD of influent and effluent was measured by ampule method in "Analytical Procedures for Selected Water Quality Parameters" (Adams et al., 1981). Dilutions were made to accommodate high COD concentration of samples.

2. Biomass Concentration

   The most common method of quantifying biomass is the total suspended-solids (TSS) test. When the wastewater contains only soluble organic material, this test should be fairly representative, although it does not distinguish between living and dead cells. The volatile suspended-solids (VSS) test is a better test when the wastewater contains a sizable fraction of suspended inorganics. Neither test will differentiate between biological solids and organic particles originally in the wastewater.

   TSS and VSS concentrations were determined according to the procedures in "Standard Methods" (APHA-AWWA-WPCF, 1980). Fifty milliliters of sample were
taken throughout the experiment. To minimize loss of active biomass by sampling, 20 ml of sludge were used as sample size in the sludge bed zone.

3. Volatile Organic Acid (VOA) and Alkalinity (ALK)

The analysis of VOA and ALK followed the distillation method in "Standard Methods" (APHA-AWWA-WPCF, 1980).

4. Biogas Analysis

The following relationship derived from the ideal gas law standardized biogas volume produced at laboratory conditions to STP conditions.

\[ V_{\text{STP}} = \frac{P_d}{P_{\text{STP}}} \frac{T_{\text{STP}}}{T_r} V_d \]  

where

- \( V_{\text{STP}} \): biogas volume at standard condition (L gas/L reactor volume/day)
- \( P_d \): daily barometric pressure (atm)
- \( P_{\text{STP}} \): standard pressure (1 atm)
- \( T_{\text{STP}} \): standard temperature (273 K)
- \( T_r \): room temperature (273 + °C)
- \( V_d \): daily gas volume produced (L gas/L reactor volume/day)

A Hewlett-Packard gas chromatograph (model 5750) equipped with a molecular sieve (60-80 mesh) column connected to a thermal conductivity detector was used to determine methane contents. The column temperature was maintained at 150°C, and nitrogen was the carrier gas at a flow rate of 30 ml/min.
CHAPTER V
RESULTS AND DISCUSSIONS

A. Start-Up

The total start-up period took 60 days. The acquired data are presented in Appendix 1. \( L_s \) indicates volumetric loading rate, which is defined as:

\[
L_s = \frac{S_i}{\tau}
\]

where

- \( L_s \): volumetric loading rate (kg COD/m\(^3\)/d)
- \( S_i \): influent substrate concentration (g COD/L)
- \( \tau \): hydraulic retention time (7 day)

The initial biomass concentration was 4.501 g VSS/L at influent substrate concentration of 3512 mg COD/L which gave an initial specific organic loading rate, \( L_x \), of 0.111 kg COD/kg VSS/d. Biomass concentration gradually increased up to 6.962 g VSS/L at the end of the start-up period. The \( L_x \) almost doubled to 0.203 kg COD/kg VSS/d with 98.7% of substrate removal seven weeks after start-up.

Substrate removal efficiency, effluent pH, and concentration of volatile organic acids were major parameters that were considered for increasing loading rate. Influent COD concentration was continuously increased once those parameters showed desired values (i.e., COD removal > 80%, effluent pH > 6.8, VOA concentration < 300 mg acetate/L).

Figures 6 and 7 show changes in COD removal efficiency and influent COD concentration and variations in VOA concentration and pH, respectively, during the
Figure 6. The increase of COD concentration and the pH changes during start-up period.

Figure 7. The efficiency of COD removal and the production of VOA during start-up period.
start-up period. COD removal efficiency gradually increased up to 98.5%, and pH was maintained close to 7.0. This indicated that the anaerobic microorganisms successfully accommodated themselves to their environment utilizing substrate. However, temporary process upsets were observed when loading rate was increased.

COD removal efficiency dropped from 78.8% to 63.9% when influent COD concentration increased from 3.488 to 5.101 g COD/L/d. The pH dropped to 6.85 and VOA concentration increased to 401 mg acetate/L. Similar results, but less intense, happened every time influent substrate concentration was increased. Table 14 summarizes the process unstableness under loading shock. Similar loading shock is reported in the literature (Wu et al., 1987; Barbosa and San’t Anna JR, 1989). This might be due to sudden increase of VOA concentration by rapidly growing acidogens that repressed the activity of methanogens.

When the first and second loading shocks occurred, substrate feeding was temporarily ceased for a few days along with addition of 10 - 20% more NaHCO₃ to the reactor to recover reactor performance. Extra addition of buffer was enough for

Table 14. The Changes in the Values of Parameters Indicating Loading Shock (%).

<table>
<thead>
<tr>
<th>Influent substrate</th>
<th>Substrate removal</th>
<th>Effluent pH</th>
<th>VOA concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ 46.2</td>
<td>- 18.9</td>
<td>- 2.1</td>
<td>+ 218.2</td>
</tr>
<tr>
<td>+ 41.0</td>
<td>- 7.0</td>
<td>- 2.8</td>
<td>+ 159.8</td>
</tr>
<tr>
<td>+ 19.7</td>
<td>- 5.3</td>
<td>N.A</td>
<td>+ 120.2</td>
</tr>
<tr>
<td>+ 18.1</td>
<td>- 6.8</td>
<td>- 1.7</td>
<td>NA</td>
</tr>
</tbody>
</table>

+: increase  
-: decrease  
NA: Not Available
the reactor to endure the third and last loading shocks without cessation of feeding. The time for the recovery of reactor performance and of the COD utilization shortened toward the end of the start-up period. This clearly indicated that microbial activity and proper balance between acidogens and methanogens developed with time.

The development of granular sludge was another proof of enhanced microbial activity. Fine granular sludge was formed nearly 40 days after start-up, and the granules became larger throughout the experiment until they had a diameter of 5.1 ± 2.1 mm. The granules, mostly light brown, were retained in the sludge bed region.

No CaCO₃ was added to the reactor after fine granular sludge formed, nor was there any serious process upset after that.

Continuous growth of and formation of granules meant that granules, already formed with the aid of Ca⁺⁺ ion, served as new binding sites for microorganisms. And some cations contained in tap water might also have provided binding sites for naturally negatively charged cells and colloids.

B. Reactor Performance at Steady State

Figures 8 and 9 represent the change of effluent COD concentration and COD removal efficiency at steady state operation, respectively. Influent COD concentration maintained at 10.568 ± 0.218 g COD/L during the experimental period. Figure 8, especially, showed that approximately 60 - 95% of total COD removal occurred in the sludge bed zone.

Higher than 90% of influent COD was continuously removed as short as 0.8 day HRT (Figure 9). Effluent COD concentration increased rapidly at shorter than 0.8 day HRT, which was an indication that biological activity was beginning to decrease. In a mixed culture of acidogens and methanogens, COD reduction is mainly a function of methanogenic activity because methanogens convert intermediate organic materials into final products, CH₄, CO₂, and a small amount of cell mass, while acidogens produce
Figure 8. The COD concentrations at examined HRTs.

Figure 9. The efficiency of COD removal at examined HRTs.
organic acids that still contribute COD concentration. From the graphs it is difficult to specify which microbial genera, acidogens or methanogens, started losing their biological activities first. Methanogens probably lost their activity first in view of their slower growing nature and high sensitivity to environmental changes as compared to acidogens. This hydraulic retention time is very similar to the 0.83 day HRT selected for phase separation of methanogens and acidogens using an anaerobic CSTR for lactose-acidogenesis (Kissalita et al., 1989).

Influent and effluent pH were carefully monitored and controlled using NaHCO₃ as a buffer (Figure 10). Effluent pH could be maintained close to 7.0 while influent pH remained around 7.5 from 5 day HRT to 0.8 day HRT. Approximately 2.5 to 3.5 g of NaHCO₃/L was used to maintain influent pH around 7.5 and effluent pH close to 7.0 during this period. This indicated that methanogenic populations and activities were well balanced with acidogens.

Low VOA concentration, lower than 250 mg acetate/L, and high ALK, higher than 1000 mg CaCO₃/L, in Figure 11 also represented the balanced nature between two microbial genera at these HRT ranges.

Sudden increase of VOA concentration below 0.8 day HRT might have caused the rapid drop in effluent pH (see Figure 10), which was an indication of losing the balance between the two genera. A maximum of 5.0 g NaHCO₃/L was added to the influent to stabilize reactors in the later stage of operation. From 0.4 day HRT to the end, effluent pH continuously dropped until it reached 5.20. During this period, the VOA concentration decreased from more than 2600 mg HAc/L to less than 790 mg HAc/L, corresponding to an increase of ALK from 251 mg CaCO₃/L up to 836 mg CaCO₃/L. This was an indication of loss of acidogenic activity.

Figure 12 represents the change of gas production over the experimental period. Total gas production rate gradually increased and reached a maximum of 4.74 L/L/d at
Figure 10. The pH changes during experiment.

Figure 11. The changes in the ALK and VOA concentrations during experiment.
Figure 12. The rate of gas production at examined HRTs.

Figure 13. The yield of methane at examined HRTs.
0.5 day HRT while maximum rate of methane production showed 2.72 L/L/d at 0.8 day HRT. As mentioned earlier, methanogens clearly started losing their activities near the 0.8 day HRT in terms of COD reduction and CH₄ production.

The rate of total gas and methane production decreased rapidly below 0.4 day HRT down to 0.07 L/L/d and 0.00 L/L/d, respectively. However, a small amount of CO₂ was detected even when methane production had ceased, which might be a result of CO₂ production from NaHCO₃ buffer.

Methane content in the total gas decreased as the experiment went into the later stage. Figure 13 shows that the methane proportion in the total gas and methane yield, expressed as L methane produced/g COD removed at given HRTs, seemed to decrease very rapidly below 0.8 day HRT unlike the methane production rate. Methane composed about 70% of total gas volume in the early stage and nearly 0% at the end of the experiment, which indicated that all methanogens completely lost their activities at 0.2 day HRT. A similar decreasing pattern, from a maximum of 0.265 to 0, was detected in the methane yield. That was another indication of loss of balanced nature between methanogens and acidogens.

One liter of pure CH₄ gas at 25°C can produce 34.54 BTU of energy (Windholz et al., 1983). The maximum yield of CH₄ was 0.26 L of CH₄/g COD removed (Figure 13), which meant that about 8980 BTU of energy were produced for every 1 kg of COD treated in this study.

The anaerobic biological process is similar to a continuous microbial culture and, as such, requires a continuous input of medium that is balanced by a continuous outflow of digested waste and biomass. Hydraulic washout is the most important physical parameter to cause process failure or unstableness resulting in rapid upset of the system without any recovery in many CSTR operations (Suschka, 1980; Paolini
and Variali, 1982; Chudoba et al., 1989). In this UASB system, however, methanogens still showed methane-forming activities even below 0.8 day HRT.

In a biological point of view, microorganisms in the anaerobic processes have a symbiotic relationship, which means both populations benefit as long as both the rates of VOA production and consumption are balanced. If the rates are not balanced, usually at high loading rate, the pH sensitive methanogens are repressed or killed by a detrimental volatile acids concentration produced by more rapidly growing acidogens. This ecosystem is then the same as the amensalism that one population produces a substance which is inhibitory to the other population. Accumulated VOAs are also inhibitory to acidogens, similar to the end product inhibition in many enzyme-catalyzed systems. Furthermore, excess concentrations of substrate or essential nutrients, which reach a limit above which the microbial growth rate decreases, are inhibitory rather than stimulatory because those nutrients may interfere specifically with enzyme systems or membrane components under a very high loading condition. Effects on microbial metabolism may occur at the genetic level of transcription, resulting in catabolic repression. Even the typical level of substrate inhibition by carbohydrates ranges about 100-150 g/L (Stronach et al., 1986); an increased external concentration of substrate may build up an osmotic pressure barrier and partially dehydrate the microbial cell, thus reducing microbial growth rate.

The use of too much sodium could also repress the process. Rinzema et al. (1988) reported that 7,000 mg/L and 12,000 mg/L of sodium resulted in 50% and 100% inhibition of activity of the anaerobe, respectively.

C. Sludge Behavior at Steady State

Figures 14 and 15 represent the changes of total solid concentration and biomass concentration, respectively. The growth in the bed accounted for the general growth patterns in both graphs since the major portion of sludge was retained in the bed zone.
Figure 14. The total solid concentrations in the different phases at assigned HRTs.

Figure 15. The biomass concentrations in the different phases at assigned HRTs.
The TSS concentration in the bed increased as low as 0.4 day HRT and decreased sharply while the TSS concentration in the blanket continuously increased and effluent TSS concentration was steady around $1.109 \pm 0.256$ g/L, respectively. The VSS concentration in the bed, however, started to decrease at 0.8 day HRT while VSS concentrations in the blanket and in the effluent followed a similar pattern.

Under the stressful environment around 0.8 day HRT, especially for methanogens due to the high loading rate and rapid production of VOAs, continuous mineral deposit mainly from tap water would be an explanation of this discrepancy.

More than 95% COD reduction and high methane content in the total gas in the early experimental stage represented a well balanced circumstance between two genera. The concentrations of VOAs produced by acidogens under a low loading rate would not be high enough to repress methanogenic activities.

It was observed that most of the big granules, $5.2 \pm 2.1$ mm diameter, were retained in the sludge bed, but some gas bubbles carried large granules upward.

Figure 16 represents the ratio of VSS to TSS in each zone. Effluent showed the highest VSS/TSS ratio, nearly constant at 70%.

The blanket zone showed a large fluctuation in VSS/TSS ratio, probably because rising gas bubbles carried sludge particles of various sizes from the sludge bed through the blanket where some of the heavier particles continuously settled back due to gravity and the settling device. Some light particles might be floating mainly due to continuous gas production as well as the fluid stream. The sludge bed was maintained around a 40% VSS/TSS ratio through 0.8 day HRT, and then the ratio dropped down to 22%. Total VSS/TSS showed a slightly higher ratio than did the sludge bed zone.

Figure 17 shows the sludge contribution of the bed and blanket to the total sludge concentration. The sludge bed held 67.2-95.1% of overall TSS concentration and held
Figure 16. The portion of biomass in the different phases during experiment.

Figure 17. The sludge contribution of bed and blanket to the total sludge concentration.
54.1-91.1% of overall VSS concentration during the experimental period. The sludge proportion in the bed, however, slowly decreased with time and a rapid drop in both TSS and VSS was observed near the end of the experiment.

The reason for the decreased VSS/TSS ratio might be associated with a loss of methanogens. The acidogens could multiply and grow as low as 0.4 day or even 0.2 day HRT (Chartrain and Zeikus, 1986a; Stronach et al., 1986; Kissalita et al., 1989) while these HRTs are apparently too short for methanogens to keep up with acidogens.

The cell lysis due to osmosis of methanogens by a too high substrate concentration would result in a decrease of total VSS concentration while mineral deposit continued. As biomass concentration in the sludge bed became more dense, especially when the gas production was small (i.e., not enough to provide good mixing condition), the biomass distribution could not be uniform. This dense biomass with little mixing condition would act as a large filter that accelerated mineral deposit. The nonuniform distribution of biomass accelerated the system failure since liquid by-pass and channelization resulted in poor contact between biomass and substrate. Thus, the microbial death, mainly in methanogens, probably accelerated the decrease of the organic proportion in the total solid toward the end of the experiment.

Most of particles in the blanket were colloidal-type sludge and originated from the sludge bed by the action of gas production and of fluid dynamics as well as by cell multiplication.

The sludge concentration in the blanket during the experimental period seemed to be proportional to the gas production rate. This relationship is shown in Figure 18. It was frequently observed that big gas bubbles, trapped in and released from the sludge bed, carried large sludge particles, sometimes larger than 2.0 mm in diameter, when the gas production rate exceeded 3.0 L/L/d. The gas production was not only a factor to distribute sludge particles but the fluid stream carried sludge particles to the
sludge blanket zone. All fluid patterns at each HRT were characterized as laminar flow based on Reynold numbers, described by the following equation (Geankoplis, 1983).

\[ N_{Re} = \frac{\rho v d_i}{\delta} \]

where

\( N_{Re} \): Reynold number
\( \rho \): density of liquid ( = 994.465 kg/m\(^3\))
\( v \): liquid velocity (m/sec)
\( d_i \): inside diameter ( = 0.1016 m)
\( \delta \): viscosity of liquid ( = 719.808 x 10\(^{-6}\) kg/m/sec)

Table 15 represents the Reynold numbers at each HRT. Although all patterns of fluid flow followed laminar motions, pumping feed solution increased about 30% of the...
Table 15. The Patterns of Liquid Flow at Each HRT

<table>
<thead>
<tr>
<th>HRT (day)</th>
<th>Flow rate (L/d)</th>
<th>N_{Re}</th>
<th>Flow pattern*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>1.44</td>
<td>0.000289</td>
<td>Laminar</td>
</tr>
<tr>
<td>4.0</td>
<td>1.80</td>
<td>0.000361</td>
<td>Laminar</td>
</tr>
<tr>
<td>3.0</td>
<td>2.40</td>
<td>0.000481</td>
<td>Laminar</td>
</tr>
<tr>
<td>2.0</td>
<td>3.60</td>
<td>0.000722</td>
<td>Laminar</td>
</tr>
<tr>
<td>1.0</td>
<td>7.20</td>
<td>0.001444</td>
<td>Laminar</td>
</tr>
<tr>
<td>0.8</td>
<td>9.00</td>
<td>0.001804</td>
<td>Laminar</td>
</tr>
<tr>
<td>0.5</td>
<td>14.40</td>
<td>0.002887</td>
<td>Laminar</td>
</tr>
<tr>
<td>0.4</td>
<td>18.00</td>
<td>0.003609</td>
<td>Laminar</td>
</tr>
<tr>
<td>0.2</td>
<td>36.00</td>
<td>0.007217</td>
<td>Laminar</td>
</tr>
</tbody>
</table>

All Reynold numbers are calculated based on the physical properties of water

* : Laminar flow if N_{Re} < 4000
sludge bed volume compared to the nonfeeding condition. Some abnormal sludge concentration below the gas production rate of 1 L/L/d shown in Figure 18 would be a result of the fluid movement associated with the microbial death.

Figure 19 indicated that the total gas production rate did not affect much of the effluent sludge concentration. TSS concentration in the effluent was maintained around 1.0 g/L while TSS concentration in the blanket zone exceeded 15.0 g/L at 4.46 L/L/d of the total gas production rate. Sufficient swirling action around the gas-liquid-biomass separator is necessary for the efficient separation of the sludge particles attached to the gas bubbles. A small amount of sludge particles is carried upward at the low rate of gas production. Even though large amounts of sludge particles are carried upward at a high rate of gas production, many particles are settled back since more vigorous swirling action occurs. Without sufficient gas production, however, we can not expect enough swirling action to separate particles.

When little or no gas is produced, hydraulic shear force produces continuous grinds of granular sludge particles, especially in the bed zone. The finely ground particles, more like colloids, are then carried upward mostly by the hydraulic movement and usually do not settle back. High sludge concentration in the blanket below 0.1 L/L/d of the gas production rate shown in Figures 18 and 19 represents these colloidal particles carried by liquid flow.

Figure 20 shows the effectiveness of the separator in detail. The ratio of solid in the effluent against the blanket zone continuously decreased down to less than 10%, which indicated more than 90% of the sludge in the blanket could be trapped inside the reactor. This indicated that the gas-liquid-solid separator successfully prevented losing biomass particles in the effluent. The high solid ratio at 5 days HRT and slightly increased solid ratio at 0.2 day HRT in Figure 20 showed the colloidal behavior of the fine sludge particles that were not efficiently separated by the separator.
Figure 19. The effect of gas production to the effluent solid concentration.

Figure 20. The portion of sludge lost in the effluent.
D. Evaluation and Verification of Kinetic Coefficients

Both the nonlinear least squares (NLLS) method using a computer program and graphical linear regression were used to determine kinetic coefficients. Equations F-14 and F-16 were used for the NLLS method with 95% confidence interval (C.I.). The computer output and residual plots are presented in Appendix 2 and 3, respectively. The negative sign in the middle of Eq. F-16 was used to predict biomass concentration when the reactor was operating under 0.8 day HRT while a positive sign was used between 5 and 0.8 day HRT. This may be associated with the beginning of repression of the methanogens around 0.8 day HRT. Figures 21 and 22 are graphical representations of Eq. F-17 and F-20, respectively. Equation F-21 was used to calculate minimum solid retention time (SRT). The values for the kinetic coefficients obtained from linear plots and computer program are summarized in Table 16. All four kinetic coefficients determined by linear regression fell into the ranges of corresponding parameters by NLLS method.

Comparisons between observed and predicted values of effluent COD concentration (Eq-14) and amount of biomass (Eq-16) are presented in Figures 23 and 24, respectively.

Data taken at 0.2 day HRT were excluded in estimating kinetic coefficients since those data caused extreme scatters of curve fitting in linear regression and negative discriminants using the NLLS method. This might be because SRT at 0.2 day HRT was below minimum SRT. Washout of all biomass and no substrate removal are usual in many completely mixed type reactors when the reactors are operating under the minimum SRT. While the UASB reactor seemed to be very unstable at 0.2 day HRT in terms of COD removal, formation of CH4, effluent pH, and VOA concentration, it still retained some biomass.

Figure 23 shows that the model predicted that the effluent COD concentration was
Figure 21. The graphical evaluation of $K_L$ and $k$.

Figure 22. The graphical evaluation of $Y$ and $K_d$. 
Table 16. Kinetic Values Obtained in the Study

<table>
<thead>
<tr>
<th>Kinetic parameters (unit)</th>
<th>Linear regression</th>
<th>Non linear regression with 95% C.I</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$ (kg COD&lt;sub&gt;removed&lt;/sub&gt;/kg VSS/day)</td>
<td>1.404</td>
<td>$1.106 &lt; k &lt; 1.432$</td>
</tr>
<tr>
<td>$K_L$ (kg COD/kg VSS/day)</td>
<td>1.147</td>
<td>$0.822 &lt; K_L &lt; 1.179$</td>
</tr>
<tr>
<td>$K_d$ (day&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.022</td>
<td>$0.022 &lt; K_d &lt; 0.031$</td>
</tr>
<tr>
<td>$Y$ (kg VSS&lt;sub&gt;produced&lt;/sub&gt;/kg COD&lt;sub&gt;removed&lt;/sub&gt;)</td>
<td>0.150</td>
<td>$0.148 &lt; Y &lt; 0.173$</td>
</tr>
<tr>
<td>$\theta_c^m$ (day)</td>
<td>5.30</td>
<td>$4.44 &lt; \theta_c^m &lt; 7.56$</td>
</tr>
</tbody>
</table>

almost equal to the influent COD concentration up to about 5 days SRT, which is very close to the calculated minimum SRT. However, a large amount of biomass, approximately 20.0 g VSS/L, was still retained inside the UASB reactor even below the calculated minimum SRT (Figure 24). There could be two possible answers to this biomass retention: (1) it was due to the function of the biomass-liquid separator or (2) this SRT was not low enough for acidogens to be washed out completely. A little COD removal (Figure 9) and VOA production (Figure 11) at 0.2 day HRT corresponding to 3.52 ± 0.28 day SRT indicated the presence of some active acidogens. It is difficult to evaluate the kinetic values of microorganisms responsible for each stage of digestion since a mixed culture was used in this study. However, it is probable that proper control of the methanogenic phase is a key step for successful reactor performance because of the lower growth characteristics of methanogens compared to acidogens (Pohland and Gosh, 1971; Stronach et al., 1986; Kissalita et al., 1989).
Figure 23. Comparison between observed and predicted effluent COD concentration.

Figure 24. Comparison between observed and predicted biomass concentration.
Under the same conditions as this experiment (i.e., 35°C, whey permeate as substrate, and pH above 6.7), the kinetic values displayed in Table 16 can be used to determine physical characteristics like UASB reactor size or proper flow rate of influent and also to predict process performances like effluent concentration or amount of sludge produced per unit time period in a scaled-up process. However, it must be recognized that these are variables that depend on process conditions like temperature or the characteristics of substrate to be treated. The kinetic values may vary if some conditions are different from this experiment.

The high $R^2$ values in Figures 21 and 22 and high correlation in Figures 23 and 24 indicate that the loading model may be used to predict UASB performance for treatment of whey permeate under steady-state conditions.

Figure 25 shows a comparison of the plots between observed and predicted specific substrate utilization rate, $q$, as a function of the specific loading rate, $L_x$, obtained in this experiment. The upper and lower limit values were calculated with the kinetic coefficients from NLLS method with 95% C.I. The observed $q$ values fell into the region between upper and lower limits of the predicted range when the $L_x$ was lower than 1.0 kg COD/kg VSS/day. The discrepancy between predicted and observed $q$ at higher than 1.0 kg COD/kg VSS/d might indicate the existence of inhibition. However, it must be recognized that there was no inhibitor at the beginning, but the inhibition, if it existed, could be due to the biased growth nature between acidogens and methanogens. In that case, acidogens produce VOA much faster than the rate of utilization by methanogens, a process which results in undesirable microhabitat to both acidogens and methanogens by lowering pH. This is biochemically the same as substrate inhibition to methanogens and end product inhibition to acidogens, both of which result in system failure.
Figure 25. Relationship between specific substrate utilization rate and specific organic loading rate.

(1) upper limit of predicted $q$ values
(2) lower limit of predicted $q$ values
After careful evaluation of the data, this research determined that the relationship fits a Monod-type relationship better than it fits first order kinetics. Examination of a batch reactor with a small initial microbial seed showed the specific growth rate or specific substrate utilization rate could just as well be considered as a function of the F:M ratio (Kincannon and Stover, 1984). Thus, the relationship between the specific substrate utilization rate and the specific loading rate could be justified as Eq F-9.

E. Optimum Loading Rate

Because the main purpose of wastewater treatment is to reduce the concentration of pollutant and because the major reduction of pollutant is due to methane production in most anaerobic processes, it could be concluded that a specific organic loading rate of 0.5 kg COD/kg VSS/d was optimum in this study based on methane production, COD removal, and VOA concentration (Figures 26 and 27).
Figure 26. The rate of gas production at different loading rates.

Figure 27. The concentration of VOA and efficiency of COD removal at different loading rates.
CHAPTER VI
CONCLUSIONS

The following conclusions are based on the results of data from two laboratory-scale UASB reactors:

1. The UASB process is useful in the treatment of whey permeate. Over 90% of COD was removed at HRTs as short as 0.8 day with very little sludge produced.

2. Average methane yield (0.25 L CH₄/g COD_removed) at HRTs from 5 to 0.8 day was about 70% of the theoretical maximum value, which means about 70% of the COD removed was converted to methane gas rather than biomass. Approximately 0.05 kg of COD/liter of whey permeate equates to 404 BTU of recoverable energy for every 1 liter of milk that is manufactured into cheese.

3. An HRT of 0.8 day was close to optimum (i.e., for COD removal, CH₄ gas production, and biomass production) for digestion of diluted whey permeate (10.568 ± 0.218 g COD/L).

4. The total organic loading models proved reliable to evaluate UASB performance.

5. Within the range of specific loading rate examined (0.26 - 2.80 kg COD/kg VSS/day), the relationship between the specific organic loading rate and specific substrate utilization rate followed Monod-type kinetics.
CHAPTER VII
RECOMMENDATIONS

On the basis of the results obtained from this study; the following items are suggested for further research:

1. Application of a pilot-scale or full-scale UASB bioreactor to treat whey permeate using the kinetic values evaluated in this study.


3. Investigation of phase-separated anaerobic digestion of high strength organic waste. Since the acidogens and methanogens have their own optimum environment, they will utilize their substrate more efficiently if the biphasic ecosystem can be separated by biological, chemical, or physical means.

4. Investigation of the feasibility of anaerobic cometabolism of other industrial wastes such as chlorinated compounds (trichloroethylene and tetrachloroethylene) with whey or whey permeate.

5. Investigation to shorten the long start-up period, which is one of the biggest disadvantages of the anaerobic process.
REFERENCES


APPENDICES
Appendix 1. Raw data acquired during start-up period

<table>
<thead>
<tr>
<th>Day (day)</th>
<th>Si (g COD/L)</th>
<th>Se (g COD/L)</th>
<th>pH (Si)</th>
<th>pH (Se)</th>
<th>Xa-TSS (g/L)</th>
<th>Xa-VSS (g/L)</th>
<th>VOA (mg HAc/L)</th>
<th>ALK (mg CaCO₃/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.512</td>
<td>1.321</td>
<td>7.5</td>
<td>7.1</td>
<td>7.231</td>
<td>4.501</td>
<td>71.0</td>
<td>1043.0</td>
</tr>
<tr>
<td>3</td>
<td>3.511</td>
<td>1.161</td>
<td>7.5</td>
<td>7.2</td>
<td></td>
<td></td>
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<td></td>
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<td>5</td>
<td>3.451</td>
<td>0.958</td>
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Appendix 2. Nonlinear least squares parameter estimation

Initial parameter values were

\[
k = 1.43 \\
K_L = 1.227 \\
K_d = 0.021 \\
Y = 0.15
\]

After 4 iteration(s), converged parameter estimates are

\[
k = 1.2689 \\
K_L = 1.00065 \\
K_d = 0.026756 \\
Y = 0.16040
\]

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The objective function value is: 2.1191
The number of function calls is: 76
The number of eigenvalue calculation is: 210
The linear theory covariance matrix is:

\[
\begin{array}{cccc}
0.442E-02 & 0.482E-02 & -0.117E-03 & -0.331E-03 \\
0.482E-02 & 0.533E-02 & -0.134E-03 & -0.366E-03 \\
-0.117E-03 & -0.134E-03 & 0.374E-05 & 0.939E-05 \\
-0.331E-03 & -0.366E-03 & 0.939E-05 & 0.255E-04
\end{array}
\]

The linear theory correlation matrix is:

\[
\begin{array}{ccc}
1.000 & 0.9928 & -0.0906 \\
0.9928 & 1.000 & -0.9486 \\
-0.0906 & -0.9486 & 1.000 \\
-0.9486 & -0.9920 & 0.9641 \\
0.9928 & -0.9486 & 1.000 \\
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-0.9486 & -0.9486 & 1.000 \\
-0.9928 & -0.9920 & 0.9641 \\
1.000 & 1.000 & 1.000
\end{array}
\]

95% Confidence Intervals for the Parameters are

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Appendix 3. Residual plots

![Residual plots](image-url)