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Fundamental studies of anaerobic biosorption in wastewater treatment

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Fundamental studies of anaerobic biosorption in wastewater treatment

by

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LIST OF ABBREVIATIONS

AIA	Automatic image analysis
ASBR	Anaerobic sequencing batch reactor
BOD_5	5-day biochemical oxygen demand
BOD_L	Ultimate biochemical oxygen demand
Co	Initial COD concentration in biosorption reactor
Ce	Equilibrium effluent COD concentration
CH_4	Methane
CO_2	Carbon dioxide
COD	Chemical oxygen demand
GC	Gas chromatograph
gm	Grams
H_2	Hydrogen
HRT	Hydraulic retention time
ID	Internal diameter
in	Inches
MLSS	Mixed liquor suspended solids
mg/L	Milligrams per liter
Qe	Uptake of COD by biomass
SRT	Solids retention time
TSS	Total suspended solids
VSS	Volatile suspended solids

1. INTRODUCTION

In the past two decades, there has been a growing interest in anaerobic biotechnology, both in the successful application of the process for industrial wastewater treatment and in the bioconversion of crop-grown biomass to methane. Our fundamental understanding of anaerobic biotechnology has advanced to a new dimension, due to the efforts of numerous dedicated researchers in the United States and Europe.

Anaerobic digestion processes have been successfully used for stabilization of municipal wastewater sludges for over 60 years. Another candidate feedstock for anaerobic treatment was food-processing wastewater, such as the effluent from meat packing operations (Steffen and Bedker, 1961), and sugar beet operations (Speece, 1983). More recently, anaerobic treatment has been applied to chemically treated primary sludges, which contain the chemicals added for phosphorus removal; biological sludges produced by activated sludge or trickling filter processes; and sludge mixtures containing significant industrial waste contributions (Parkin and Owen, 1986). The basic question is no longer whether an industrial wastewater can be anaerobically biodegraded to methane, since most organics are amenable to anaerobic treatment, but rather *at what rate* it is degradable. Also, to what *degree* is it degradable? The answer to these questions are obtained from the improved understanding of the microbial consortium involved. This, together with significant developments in reactor and process design, are laying a strong foundation for the development of efficient and reliable anaerobic biotechnology approaches for treatment of a wide variety of industrial wastewaters.

There are three major categories of treatment processes. They are:

1. Physical/Chemical treatment

2. Aerobic biological treatment

3. Anaerobic biological treatment.

The physical/chemical treatment processes are usually expensive. They often transfer the pollutant from one medium to another, without significant conversion or stabilization. Then operators are faced with further treatment requirements. This increases the cost of the process.

Aerobic biological treatment processes are energy intensive and require high power usage. Some processes require large areas of land. Aerobic processes generate a large quantity of biomass, which has to be treated by anaerobic digestion, thermal reduction or land disposal. The increase in energy costs and sludge disposal costs can make this process unattractive to industrial operations. Compared to the above processes, the anaerobic treatment system provides complete stabilization of wastes with production of a useful end product, methane gas, while requiring less energy and producing a small amount of waste biomass. In recent years, significant progress has been made on the development of high rate anaerobic processes. These high rate systems can successfully handle high organic loading rates with relatively short hydraulic retention times (HRT) and long solids retention times (SRT), e.g. Anaerobic filter, Fluidized bed reactor, Upflow sludge blanket reactor, Anaerobic sequencing batch reactor (ASBR).

The biosorption process has potential for application as a pre-treatment process in anaerobic treatment systems. Biosorption was first utilized for sewage and waste treatment by Ullrich and Smith (1951). Later, this process was successfully used in the wastewater treatment plant at Austin, Texas (Ullrich and Smith, 1957). Subsequently, more research was done on anaerobic biosorption (Mortenson, 1953; Schroepfer and Ziemke, 1959). The process was found suitable for the treatment of relatively cool and dilute wastes without the

application of heat.

In the last decade, the biosorption process has been widely used by different researchers for the removal of hazardous organic pollutants, mainly pesticides and chlorinated hydrocarbons from wastewater, using both live and dead biomass. Biosorption is of current industrial interest because the removal of toxic heavy metals and radionuclides from liquid waste can result in detoxification, and therefore safe environmental discharge. Subsequent treatment of the loaded biomass can enable recovery of valuable metals or further containment of toxic and radioactive materials.

Biosorption is a process that has enormous potential for application in the field of wastewater treatment. Unfortunately, there is a lack of research in this particular area. Further investigations and research should be performed to apply the biosorption process successfully and economically for the treatment of wastewater.

2. SCOPE AND OBJECTIVES

The overall objective of this research is to determine whether anaerobic biomass can be used as an adsorbent for the removal of organic matter from wastewater, and to observe the effect of different variables that affect the treatment process.

The biosorption process was used in the 1950s in a number of treatment operations. But after that time, there has not been much research on the application of this process for wastewater treatment. This research is aimed at determining how the biosorption process can be applied in this area.

This study is an attempt to provide a better understanding of the variables that affect the biosorption process, such as substrate concentration, biomass concentration, temperature, mixing time and biomass particle size. The specific objectives of this research are:

1. Investigate the effect of mixing times.
2. Determine the effect of substrate concentration on biosorption.
3. Observe the effect of temperature.
4. Observe the effect of biomass particle size.
5. Determine the biosorption capacity of the biomass, and formulate appropriate adsorption isotherms.
6. Observe the effect of dilution on biosorption.
7. Determine the effect of biomass concentration on biosorption and removal efficiencies.

These objectives were accomplished by a thorough review of the literature pertaining to this area, followed by detailed experimental investigations in the laboratory. The biosorption experiments were performed as batch experiments. In order to have a constant and reliable source of live biomass, two source reactors were operated as Anaerobic Sequencing Batch

Reactors (ASBR) at 35° C and maintained at equilibrium throughout the investigation. The source reactors were seeded with granular sludge to obtain biomass with good settling characteristics. The Chemical Oxygen Demand (COD) was used as a measure of organic matter in the substrate and biomass. The biomass was quantified in terms of total and volatile suspended solids.

The effect of mixing time was investigated and it was ensured that film diffusion was not a limiting factor in the experiments. At least nine different substrate concentrations were used to understand the effect of each variable and to formulate isotherms. The concentrations ranged from 100 mg/L to 15,000 mg/L as COD. A non-fat dry milk was used as the substrate in all experiments. The biosorption experiments were performed at room temperature with biomass at 35° C, and substrate at different temperatures. Anaerobic conditions were maintained throughout the experiments.

3. LITERATURE REVIEW

3.1. Introduction

Anaerobic treatment of wastewater involves the stabilization of organic matter, with a concurrent reduction in odors, pathogens and the mass of solid organic material that requires further processing. This is accomplished by biological conversion of organics to methane (CH_4) and carbon dioxide (CO_2) in an oxygen-free or anaerobic environment (Parkin and Owen, 1986).

Some of the advantages of anaerobic treatment processes over aerobic processes are (McCarty, 1964a):

1. A high degree of waste stabilization is possible at high organic loads.
2. Low production of waste biological sludge.
3. Low nutrient requirements.
4. No oxygen is required, so treatment rates are not limited by oxygen transfer.
5. Methane gas produced is a good source of fuel.

The anaerobic treatment process also has some disadvantages. The first is the requirement of relatively high temperatures (35° C) for optimal operation. The second one is the slow growth rate of the methane-forming bacteria. This results in several weeks or longer start-up period for the process. However, the advantages normally outweigh the few drawbacks of the anaerobic treatment process.

In this chapter, the history and fundamentals of anaerobic treatment including the process chemistry, microbiology, environmental factors and the kinetics of the process will be reviewed. Adsorption technology as related to water pollution control will then be

discussed, followed by a review of the biosorption process.

3.2. History of Anaerobic Treatment

This section will briefly describe the history and development of the anaerobic treatment process. Some of the commonly used anaerobic systems will be reviewed towards the end. Louis Pasteur was the first scientist to discover anaerobic life, during his research on fermentation processes in 1861 (Dague, 1967). He observed that the bacteria which caused butyric fermentation (genus clostridium) was strictly anaerobic. Exposure to oxygen was toxic to the bacteria. Pasteur concluded that some organisms could grow only in the absence of free oxygen. He introduced the terms "*aerobic*" and "*anaerobic*" to designate, respectiveiy, biological life in the presence and absence of free oxygen (Stainer et al., 1963; Fruton and Simmonds, 1958). Pasteur realized that there was a difference in yield between aerobic and anaerobic processes. Anaerobic conditions of fermentation resulted in less microbial mass in yeast production than under aerobic conditions. Thus fermentation was considered less efficient for yeast production.

The development and use of the first "septic tank" dates back to 1896 at Exeter, England, as reported by Fuller (1912). The septic tank was basically where settling and digestion both took place in the same tank. This was widely used for waste treatment in Europe and the United States until 1906 (Kinnicutt et al., 1919).

In 1906, William O. Travis developed a two-story septic tank in Germany, in which suspended material was separated from wastewater in the first stage by settling. The second stage was a hydrolyzing chamber through which the supernatant was allowed to flow. The Travis tank was modified by Karl Imhoff in 1907 to provide a treatment system, which later became known as the "*Imhoff tank*". The Imhoff tank did not allow the wastewater to flow through the hydrolyzing tank. Instead, the sludge was kept in the hydrolyzing tank for a

long period of time. The Imhoff tank greatly reduced the cost of sludge disposal and rapidly became popular, both in Europe and the United States (Dague, 1967; McCarty, 1981).

The importance of temperature on anaerobic digestion was observed and investigated by a number of researchers in the 1920s. Schaetzle reported that cold weather reduced digestion during studies on separate sludge digestion at Baltimore (Schaetzle, 1924). Rudolfs (1927) investigated the effect of temperature on sludge digestion. He conducted studies at temperatures of 10^{o} , 18^{o}, 24^{o}, 29.5^{o} and 35^{o} C. He observed that gas production increased with increasing temperature. Rudolfs concluded that the total amount of gas produced from a gram of organic matter is not dependent on temperature, but the rate of gas production is temperature dependent.

Babbitt and Schlenz (1929) conducted extensive research to study the effects of heating of sludge in Imhoff tanks and also in separate sludge digesters. They reported that an increase of sludge temperature in Imhoff tanks from 12^{o} C to 24^{o} C resulted in an increase in gas production by a factor of 2.5. They also reported that an increase in temperature from 17^{o} C to 26^{o} C in separate sludge digestion tanks, increased the gas production by a factor of more than 2.5. Previously, separate digesters employing external heating were first used in Wisconsin in 1926. As the advantages of heating on digestion were discovered, the use of this system spread rapidly everywhere.

In the late 1920s, some studies were conducted to obtain a better idea of the microbial chemistry involved in anaerobic sludge digestion. The transformation of carbon and nitrogen compounds in limed and unlimed sludges were investigated by Heukelekian and Rudolfs (1928). They observed an increased reduction in carbon content of limed sludges as compared to unlimed ones. The nitrogen content however, remained the same in both systems.

In another study, Heukelekian (1928) reported the effect of volatile acids on sludge

digestion. He found that without proper buffering, the volatile acids increased quickly and accumulated in the anaerobic reactor. But in limed reactors, these acids were converted to methane and carbon dioxide before they could accumulate.

The effect of a number of acids on digestion was studied by Clark and Adams (1929). They added formic, acetic, butyric, lactic and oxalic acid to digesting sludge. Their results indicated an initial reduction in system pH, followed by a gradual increase in pH as the acids were converted to methane. Different rates of gas production were obtained for different volatile acids.

A number of researchers found that mixing could increase the rate of digestion (Edwards, 1929; Fischer, 1929). They also observed that there was a decrease in pH due to the release of carbon dioxide during mixing. The effect of pH control on sludge digestion was investigated by Clark and Adams (1929). They realized that some quantity of a bicarbonate buffer was necessary to improve the rate of sludge digestion. A system pH of 7.0 caused rapid reactions. The addition of lime was required for sludges with low alkalinity, in order to enhance digestion.

Studies on sludge digestion in the thermophilic temperature range (120^{o} F to 125^{o} F) was reported by Heukelekian in 1931. He observed that reaction rates at the higher temperature could be increased by shaking the sludge or mixing. The concept of an adaptation period for the organisms was also indicated by Heukelekian (1933), from a study on sludge digestion between the thermophilic and non-thermophilic temperature ranges. He suggested that all of the microorganisms responsible for digestion of solids were present at any temperature in the wastewater, but time was required for predomination of specific organisms at a particular temperature.

In 1932, Fair and Moore presented a series of papers on heat and energy relationships in sludge digestion. They observed that gas production was a satisfactory measure of

microbial activity, and a plot of cumulative gas production is an S-shaped or *ogee* curve that could be described mathematically (Fair and Moore, 1932b). Their investigations indicated that "autocatalysis" was the controlling influence in the enzymatic reactions involved in anaerobic digestion (Fair and Moore, 1932c). It was also recognized that factors such as seeding, pH, temperature and mixing affected the rate of sludge digestion.

Fair and Moore (1937) performed an extensive study on the effect of a wide range of temperatures on anaerobic digestion. They concluded that the optimum temperature for thermophilic digestion was 128° F and that for mesophilic digestion was 98° F.

Buswell and Boruff (1932) investigated the relation between gas production and the chemical composition of waste in sludge digestion. They used pure substances to establish a relationship between the chemical composition of the waste material and the quantity of methane and carbon dioxide gas production. They indicated that the lower the oxygen content of the material compared to the carbon and hydrogen content, the lower would be the degree of oxidation, and higher would be the methane content of the gas produced.

Heukelekian and Heinemann (1939a, 1939b, 1939c, 1939d) studied the methane-producing bacteria found in digester solids. The researchers were successful in growing the methane bacteria in synthetic media. They concluded that the optimum temperature for growth of the methane bacteria was 28° C. The growth rate was not affected by increasing temperatures to 35° C, but the rate decreased when temperature was decreased to 20° C. They also observed that the optimum pH for growth of the methane bacteria was 7.0.

The concept and importance of solids retention time as opposed to liquid retention time was reported by Schlenz (1947). He stated that in a two-stage digestion system, it was possible to achieve a solids retention time of 60 days while operating at a liquid retention time of 30 days, by transferring a larger volume of supernatant to the second stage and leaving the sludge in the tank bottom of the first stage. He also suggested the importance of

having a homogeneous optimum temperature of 90° F to 95° F throughout the tank volume.

One of the first reports on the application of anaerobic systems to liquid wastes was by Tatlock (1947). He reported on the treatment of liquid waste from a yeast manufacturing plant by two-stage anaerobic digesters.

The effect of high volatile solids on anaerobic digestion was investigated by Buswell (1947). He reported that when the volatile acids level in an anaerobic digester exceeded 2000 to 3000 mg/l, gas production decreased. The acids increased rapidly and all gas production ceased within 24 to 48 hours. Buswell observed that the level of volatile acids controlled fermentation and addition of alkali to increase the pH was of no use. The rate of substrate addition appeared to be the only way to control increases in volatile acids.

Multi-stage digestion with sludge recirculation was first reported by Rawn and Candell (1950). The research was conducted at the joint disposal plant of the Los Angeles County Sanitation District. There were four tanks in series, with sludge from the last tank recirculated to the first one. They observed that higher loading rates could be handled by the plant due to sludge recirculation.

Buswell and Mueller (1952) investigated the mechanism of methane fermentation. They developed an empirical formula for the calculation of the theoretical quantity of methane and carbon dioxide that could be produced from an organic substrate. They also observed three optimal temperatures of 27°, 37° and 55° C for anaerobic digestion processes.

Two major groups of bacteria involved in anaerobic digestion were reported by Sawyer et al. (1954), while conducting studies on the liming of digesters. They stated that the first major group was the acid forming bacteria. These bacteria were responsible for the production of low molecular weight fatty acids, such as acetic and propionic acids. The second major group was the methane forming bacteria. They utilized the fatty acids and other end-products formed by the acid formers, and produced methane and carbon dioxide.

High concentrations of the volatile acids could lower the pH and inhibit the methane formers, resulting in a *"stuck"* digester. Sawyer et al. investigated the addition of lime to digesters. They found that a stuck digester could be started with the addition of lime as an artificial buffer.

A process for the treatment of packing plant waste was reported by Fullen (1953). The process was used for the treatment of wastewater from the Hormel packing facility in Austin, Minnesota. The raw influent was mixed with an activated anaerobic sludge, then the sludge was separated from the mixture and returned to the digester. Degasification was used to improve solids removal efficiency. About 96% BOD removals were achieved in the process. The detention time for the waste was only 24 hours. This process was later called the *"Anaerobic Contact Process."*

A lot of research was done on the development and improvement of the Anaerobic Contact Process (Schroepfer et al., 1955; Coulter et al., 1957; Schroepfer and Ziemke, 1959a and 1959b). Schroepfer and Ziemke (1959a and 1959b) described the Anaerobic Contact Process as consisting of two parts: a contact portion where the raw waste was intimately mixed with a previously developed anaerobic sludge, and a separation unit where the active sludge particles were separated from the treated liquor and recycled to the contact unit. The anaerobic contact process was similar to the activated sludge process, except that an anaerobic environment was maintained in the former.

Morgan (1954) studied the effects of gas recirculation on anaerobic digestion. He suggested that recirculated digester gas had a catalytic effect on the digestion process. The digesters could be loaded at higher rates and the detention times could be reduced with gas recirculation. This was called accelerated digestion.

The bacteriological and biochemical aspects of anaerobic digestion were investigated by Heukelekian (1958). A number of specific substrates were identified as being utilized by

specific species of the methane producing bacteria. The biochemistry of anaerobic digestion was studied by a number of researchers (Lackey and Hendrickson, 1958; Buswell, 1957; Mylroie and Hungate, 1954). This will be discussed in detail in latter sections of this chapter.

A fundamental study to investigate the effects of individual volatile acids on anaerobic treatment was done by McCarty et al. (1963). They concluded that acetic acid was the most prevalent volatile acid formed from the methane fermentation of carbohydrates, proteins and fats. Acetic and propionic acids were the most important volatile acids that frequently occurred under unbalanced digestion conditions. Other volatile acids, such as formic acid, occurred in low concentrations and were not considered to be significant as indicators of digester imbalance.

Solids retention time (SRT) was stated as the critical factor for the sizing of anaerobic digesters by Dague et al. (1966). He also indicated that the critical solids retention time was temperature dependent. When the temperature of an anaerobic system is decreased, the solids retention time must be increased to obtain equal treatment efficiencies. Dague (1970) reported the results of a study to determine the effect of SRT on methane production, 5-day BOD, COD and volatile solids reduction. He concluded that the minimum SRT at 35^{o} C must be 10 days to avoid system inhibition.

The first major study on attached growth processes was done by Young and McCarty (1969) on the anaerobic filter. The filter was described as a packed bed with upward flow. The media used was quartzite rock and was completely submerged. The laboratory study concluded that the process was very effective for the treatment of soluble wastes. The main advantage of the filter over other anaerobic treatment processes was its ability to retain solids without the need for an external clarifier. This resulted in longer SRTs (solids retention times) and produced minimal excess solids. Another advantage of the filter was its ability to

accept shock loads, provided sufficient buffering capacity was available to maintain a favorable pH.

Results from the first full scale anaerobic filter in the United States was reported by Taylor and Burm (1972). Three filters were operated in series for the treatment of wheat starch wastes. The filter system achieved 60 to 70% COD removals at a hydraulic retention time of 22 hours. A shutdown study demonstrated that the filter had a remarkable ability to recover to maximum efficiency within 24 hours, even after it was shut down for a period of 26 days. Subsequent studies by different researchers have demonstrated the successful application of anaerobic filters for a wide variety of wastes, e.g. pharmaceutical wastes (Dennis and Jennett, 1974), carbohydrate waste (Mosey, 1978), potato processing wastewater (Graham et al., 1980).

Recent laboratory studies have reported on the success of temperature phased anaerobic filters in handling extremely high organic loads (Harris, 1992; Kaiser and Dague, 1992). A synthetic milk was used as the reactor feed. It was observed that a thermophilic filter followed by a mesophilic filter was capable of achieving higher COD removals than was possible with either filter operated individually.

Another high rate anaerobic treatment process is the Upflow Anaerobic Sludge Blanket (UASB) process developed by Lettinga et al. (1980) in the Netherlands. In this process, wastewater flows upward through a sludge blanket composed of biologically formed granules. Adequate upflow velocities are maintained to keep the sludge blanket in suspension. The treated liquid with residual solids are passed into a settling chamber. The settled solids are returned to the sludge blanket. The UASB process is capable of achieving high treatment efficiencies at high loading rates. This type of reactor has been successfully used for the treatment of a variety of wastewaters, both in pilot and full-scale plants, e.g. beet-sugar wastewater (Pette et al., 1980), potato processing wastewater (Hulshoff Pol et

al., 1983).

The Anaerobic Sequencing Batch Reactor (ASBR) was developed by Dague and coworkers at Iowa State University, Ames, Iowa (Dague and Pidaparti, 1991; Habben, 1991; Sung and Dague, 1992). The reactor sequences through four steps: feed, react, settle and decant, all in one reactor. A high degree of anaerobic stabilization is obtained over a range of temperatures. Efficient solids separation and high solids retention are achieved. An important advantage of the ASBR is the formation of granular biomass with high activities which contribute to the high removal efficiencies. Detailed description of this process is given in Chapter 4.

3.3. Fundamentals of Anaerobic Treatment

3.3.1. Process chemistry and microbiology

Anaerobic biological waste treatment is a complex microbiological process involving various types of anaerobic and facultative bacteria. In recent years, a three-stage process has been used to describe the overall treatment (Novaes, 1986; Parkin and Owen, 1986). Although the bacteria are represented by separate groups, it is not possible to separate the metabolism of each group. They are interdependent.

The three stage process is illustrated in Figure 3.1 (Novaes, 1986). The process involves:

1. Adsorption, hydrolysis and fermentation.
2. Hydrogen and acetic acid formation, and
3. Methane formation.

Five groups of bacteria are thought to be involved, each deriving its energy from a limited number of biochemical reactions. They are (Novaes, 1986):

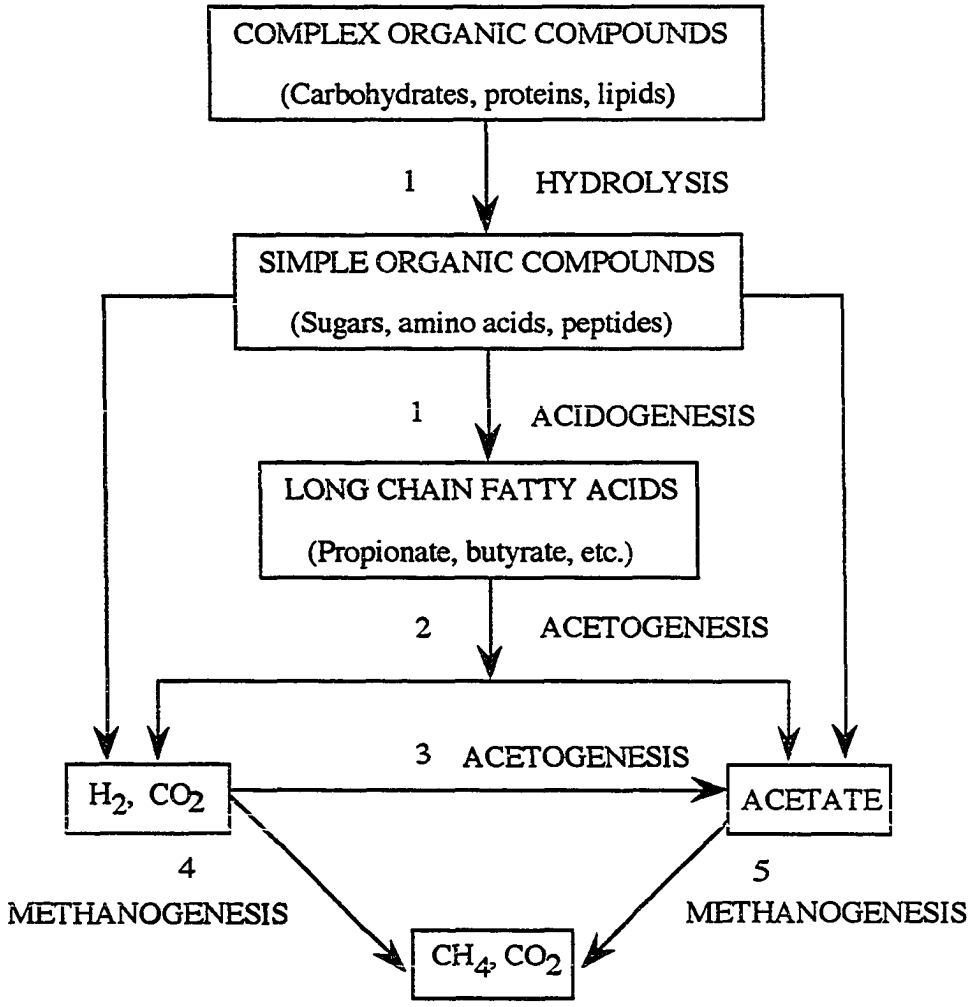


Figure 3.1. Metabolic steps and microbial groups involved in anaerobic digestion (Novaes, 1986): 1) Fermentative bacteria, 2) H_2-producing acetogenic bacteria, 3) H_2-consuming acetogenic or homoacetogenic bacteria, 4) CO_2-reducing methanogenic bacteria, 5) Acetoclastic bacteria

1. **Fermentative bacteria**: This group is responsible for the first two stages of anaerobic digestion, hydrolysis and acidogenesis. Anaerobic species belonging to the family of Streptococcus and Enterobacter, and to the genera of Clostridium, Eubacterium are predominantly found in this group.
2. **Hydrogen producing acetogenic bacteria**: They catabolize sugars, alcohols and organic acids to acetate and carbon dioxide. These include the Syntrophobacter wolinii and Syntrophomonas wolfei.
3. **Homoacetogenic bacteria**: These bacteria use hydrogen and carbon dioxide to produce acetate. They include the Clostridium aceticum, Butyribacterium methylotrophicum.
4. **Carbon dioxide reducing methanogens**: They utilize H_2 and CO_2 to produce methane (CH_4).
5. **Aceticlastic methanogens**: They cleave acetate to form methane and carbon dioxide.

Another group of bacteria often found in association with the methanogens are the sulfate-reducing bacteria. They produce hydrogen, acetate and sulfides which are used by the methanogens.

Adsorption or biosorption of complex organics onto microbial cells are necessary before hydrolysis can occur. Hydrolysis and liquefaction are then accomplished by extracellular, hydrolytic enzymes produced by the bacterial population. The enzymes convert the organic materials to a size and form that will pass through the bacterial cell walls, for use as energy or nutrient sources. The portion of organic matter that can not be hydrolyzed and assimilated by the bacteria are termed non-biodegradable (Parkin and Owen, 1986). After hydrolysis, the organic matter is then fermented to long-chain organic acids, sugars, amino acids and eventually to smaller organic acids, such as propionic, butyric and

valeric acid. This phase is called the *acid forming* or fermentation phase, and involves essentially no stabilization. This phase also produces small amounts of acetic acid, hydrogen and carbon dioxide (McCarty, 1964a).

In the second stage, the organic acids are converted to acetate, carbon dioxide and hydrogen. Hydrogen is produced by the fermentative and hydrogen producing acetogenic bacteria. Acetate is also produced by these groups in addition to the homoacetogenic bacteria.

Waste stabilization occurs in the third and final phase when acetic acid is converted to methane by the methanogenic bacteria. Approximately 72% of the methane formed comes from acetate cleavage, with the remaining 28% resulting from the reduction of carbon dioxide, using hydrogen as the energy source by CO_2-reducing methanogens (McCarty, 1964a; Henze, 1983; Parkin and Owen, 1986).

The major chemical reactions involved in the three stages are given in Table 3.1 (Daniels, 1984). The second step reactions involve the conversion of butyrate, propionate and ethanol formed in the first step, to acetate and hydrogen. These second step reactions can occur even at unfavorable thermodynamic conditions, because the concentration of reactants is kept higher than reaction equilibrium by the action of step-1 organisms, while the step-3 organisms keep the product concentrations lower than that required for equilibrium. The rate limiting step is the conversion of hydrogen to methane by CO_2-reducing methanogens. The hydrogen partial pressure must be maintained at an extremely low level to enable favorable thermodynamic conditions for the conversion of volatile acids and alcohols to acetate. Under standard conditions of 1 atmosphere of hydrogen, the free energy change (ΔG^o) is positive for this conversion and thus precludes it. The free energy change for conversion of propionate to acetate and hydrogen does not become negative until the hydrogen partial pressure decreases below 10^{-4} atmosphere (Speece, 1983; Daniels, 1984).

Table 3.1. Thermodynamics of anaerobic digestion (Daniels, 1984)

Reactions	$\Delta G^{o'}$ (kJ) (standard condition)	$\Delta G'$ (kJ) (in situ condition)[b]
Step 1 organisms [a]		
Glucose => 2 acetate + $2HCO_3^-$ + $4H^+$ + $4H_2$	-206.3	-363.4
Glucose => butyrate + $2HCO_3^-$ + $3H^+$ + $2H_2$	-254.8	-310.9
1.5 Glucose => 2 propionate + acetate + $3H^+$ + CO_2	-465.0	-520.9
Glucose => 2 ethanol + $2CO_2$	-235.0	-265.4
Step 2 organisms		
Butyrate => 2 acetate + H^+ + $2H_2$	+48.1	-29.2
Propionate => acetate + HCO_3^- + H^+ + $3H_2$	+76.1	-8.4
Ethanol => acetate + H^+ + $2H_2$	+9.6	-49.8
Step 3 organisms		
$4H_2$ + CO_2 => CH_4	-135.6	-16.8
Acetate => CH_4 + CO_2	-31.0	-22.7
Overall process		
Glucose => $3CH_4$ + $3CO_2$	-393.1	-383.8

[a] Water left out for brevity

[b] Assume 10^{-5} atm H_2, CO_2 = 0.5 atm, CH_4 = 0.5 atm, HCO_3^- = 60 mM, pH 7.0, propionate = acetate = butyrate = 1 mM, glucose = 10 mM, ethanol = 1 mM, 37° C.

It is therefore obligatory that the hydrogen utilizing methanogens maintain these extremely low hydrogen partial pressures in the system. Otherwise, the higher volatile acids, such as propionic and butyric acids will accumulate and waste stabilization will not occur.

3.3.2. Methanogenic bacteria

Methanogens are often considered the key class of microorganisms in anaerobic biotechnology. They are members of the *Archaebacteria*, a phylogenetically distinct biological grouping. They are obligate anaerobes with relatively slow reproduction rates since less energy is released in the reactions involved in the anaerobic stabilization of organic matter (McCarty, 1964a). This slow growth rate limits the rate at which the process can adjust to changing waste loads, temperatures and other environmental conditions. Two temperature ranges are optimal: 30° to 35° C for mesophilic bacteria and 55° to 65° C for thermophilic bacteria.

The majority of the species use hydrogen and carbon dioxide for both carbon and energy sources. Other substrates include formate, methanol, carbon monoxide, methylamines and acetate. So far, only three types of methanogenic bacteria have been identified that utilize acetate. They are: Methanosarcina sp., Methanothrix soehngenii and Methanococcus mazei. Formate is used by several genera, including the Methanobacterium, Methanogenium, Methanospirillum (Novaes, 1986; Daniels, 1984).

At least two coenzymes or cofactors have been identified that are unique to methanogens. They are, 2-mercaptoethane-sulfonic acid and the nickel containing coenzyme F_{430} (Speece, 1983).

As mentioned before, a solids retention time (SRT) of at least 10 days at 35° C is usually required to prevent washout of the methanogens. There is a variation in growth rate among different species of methanogens. Figure 3.2 illustrates the typical growth kinetics of

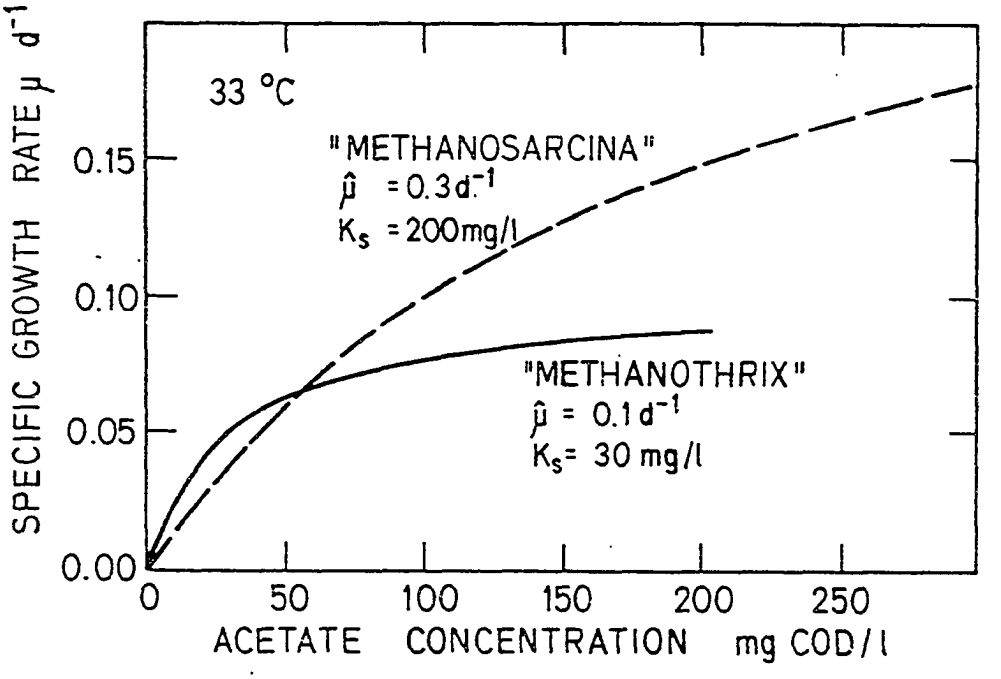


Figure 3.2. Comparison of typical growth kinetics for acetate cleaving methanogens (Gujer and Zehnder, 1983)

two different acetate cleaving methanogens, the Methanosarcina and Methanothrix (Gujer and Zehnder, 1983). At low substrate concentrations the Methanothrix outcompetes the Methanosarcina. But at high substrate concentrations the Methanosarcina will predominate. A reactor operating under such conditions of high substrate levels could have a lower solids retention time without experiencing failure, due to the activity of the Methanosarcina.

Even though the methanogens are the most important and sensitive microbial species in anaerobic treatment, a balance must be maintained between the acid-forming and hydrogen-forming bacteria and the methane-formers, in order to achieve complete conversion of complex organic compounds to methane and carbon dioxide. Table 3.2 (Parkin and Owen, 1986) describes the relative characteristics of the bacteria involved in anaerobic treatment processes.

3.3.3. Environmental requirements and toxicity

Optimum environmental conditions are very important in the design and operation of anaerobic treatment processes. These conditions are usually dictated by the requirements of the methanogens, whose growth rate limits the process of waste stabilization. Several authors have outlined the optimum growth conditions (McCarty, 1964b; Dague, 1968). They are:

1. Optimum temperature: 30° to 38° C for mesophilic bacteria and 50°C to 60° C for thermopholic bacteria.
2. Range of pH between 6.6 to 7.6, with optimum at 7.0.
3. Sufficient nutrients, particularly nitrogen and phosphorus.
4. Absence of toxic materials.
5. Anaerobic conditions.
6. Solids retention time greater than the growth rate of the slowest growing bacteria.

Table 3.2. Relative characteristics of bacteria involved in anaerobic digestion (Parkin and Owen, 1986)

Characteristic	Methane-forming bacteria	Fermentative acid-forming and hydrogen-forming bacteria
Growth rate	Slow	Fast
pH sensitivity	Highly sensitive (optimum: 6.6-7.6)	Low sensitivity (some grow at pH<6.0)
Temperature	Highly sensitive	Moderately sensitive
Sensitivity to toxicants	Highly sensitive	Moderately sensitive
Hydrogen sensitivity	Relatively insensitive	Highly sensitive

pH is one of the most important environmental requirements. Anaerobic systems can operate quite well with a pH varying from 6.6 to 7.6. Beyond these limits, process efficiency decreases greatly. At pH values below 6.2, the acidic conditions produced can be quite toxic to the methanogens. The pH dependency of the growth rate of Methanobrevibacter arboriphilus strain AZ is illustrated in Figure 3.3 (Gujer and Zehnder, 1983). Highest growth rates were obtained at pH 7.0. Growth rates were reduced by as much as four times by a change in the pH by half a unit above or below 7.0.

pH imbalances in anaerobic systems may be corrected by the addition of buffers, such as sodium bicarbonate or potassium bicarbonate. A bicarbonate alkalinity in the range of 2500 to 5000 mg/L (as $CaCO_3$) provides enough buffer capacity, so that a large increase in volatile acids can be handled with a minimum drop in pH (McCarty, 1964b). It should also be remembered that alkalinity is naturally formed by degradation of protein, so reactors treating a high protein waste stream will develop a high alkalinity without the addition of buffers (Parkin and Owen, 1986).

The effect of temperature on reaction rate of a biological process is usually expressed as:

$$K_T = K_{20}\,\Theta^{(T-20)} \qquad (1)$$

where,

K_T = Rate coefficient at T^{o} C

K_{20} = Rate coefficient at 20^{o} C

Θ = Temperature activity coefficient

T = Temperature, ^{o}C

At temperatures below the optimum, the reaction rates will be reduced according to Equation 1. Lower temperatures will have to be compensated by longer SRTs for efficient conversion of waste materials.

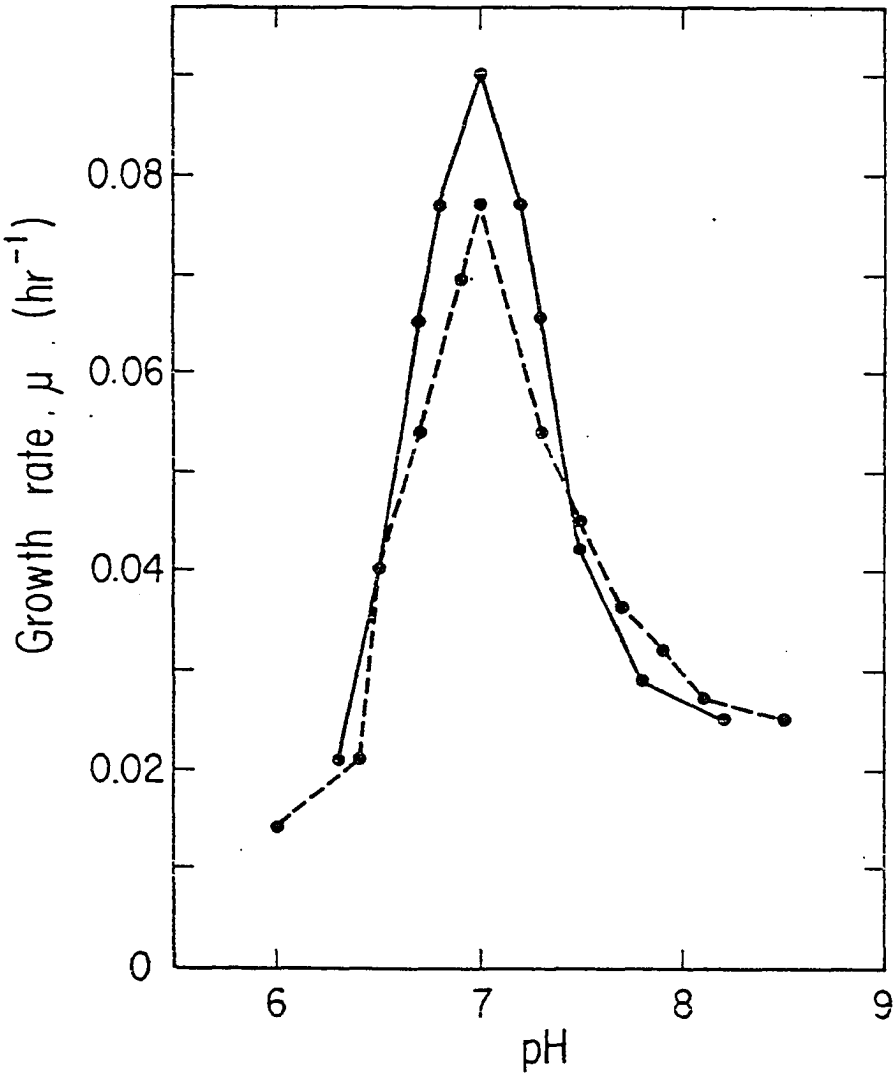


Figure 3.3. pH dependency of *Methanobrevibacter arboriphilus* strain AZ. Dashed line: growth in closed system. Continuous line: growth with constant gas flow (Gujer and Zehnder, 1983)

Nitrogen and phosphorus are the two major nutrients required for microbial growth and reproduction. In addition, iron, cobalt, nickel, sulfur, calcium and some trace metals are necessary for growth of the methanogens. Sulfide is required by methanogens even though it may adversely affect methane production, by precipitating essential trace metals, and it is toxic at concentrations above 100 to 150 mg/L of un-ionized hydrogen sulfide (H_2S) (Speece, 1983). Molybdenum, tungsten and selenium have been reported as necessary trace metals.

The methanogens are commonly considered to be the most sensitive to toxicity of all the microorganisms in the overall consortium for anaerobic conversion of organics to methane. However, acclimation to toxicity and reversibility of toxicity are frequently observed. Whether a substance is toxic to a biological system depends on the nature of the substance, its concentration and acclimation. Changes in the concentration of a toxicant can change the classification of the substance from toxic to biodegradable. Table 3.3 (McCarty, 1964c) and Table 3.4 (Parkin and Owen, 1986) contain a summary of the concentrations of different cations and inorganics, at which they are reported to be stimulatory or inhibitory to anaerobic digestion.

Control of toxicants are vital to the successful operation of an anaerobic treatment process. Some of the possible methods to control toxic materials, according to McCarty (1964c) are:

1. Remove toxic material from waste stream.
2. Dilute below toxic threshold.
3. Form insoluble complex or precipitate.
4. Antagonize toxicity with another material.

Ammonia is produced in anaerobic treatment from the degradation of proteinaceous

Table 3.3. Stimulatory and inhibitory concentrations of alkali and alkaline-earth cations on anaerobic treatment (McCarty, 1964c)

Substance	Concentrations in mg/L Stimulatory	Moderately inhibitory	Strongly inhibitory
Sodium	100 - 200	3500 - 5500	8000
Potassium	200 - 400	2500 - 4500	12000
Calcium	100 - 200	2500 - 4500	8000
Magnesium	75 - 150	1000 - 1500	3000
Ammonia-nitrogen	50 - 1000	1500 - 3000	>3000

Table 3.4. Concentrations of inorganics reported to be inhibitory to anaerobic digestion (Parkin and Owen, 1986)

Substance	Soluble concentration, mg/L	Total concentration, mg/L
Copper	0.5	50 - 70
Chromium VI	3.0	200 - 260
Chromium III	—	180 - 420
Nickel	2.0	30
Zinc	1.0	—

wastes and may be toxic depending on pH, temperature and concentration. Recent research indicates that the toxicity is associated with free ammonia (Parkin and Owen, 1986), which is in equilibrium with ammonium ion according to the following equation:

$$NH_3 + H_2O <=> NH_4^+ + OH^- \quad (2)$$

At a higher pH, more ammonia-nitrogen will be present as free ammonia. At a lower pH, the NH_4^+ ion will predominate. McCarty (1964c) reported that NH_3-N concentrations between 1500 and 3000 mg/L were inhibitory at pH levels above 7.4, and concentrations above 3000 mg/L were toxic regardless of pH. Temperature also has an effect on the relative concentrations of free NH_3-N for a system pH. Higher temperatures result in relatively higher concentrations of free ammonia, lower temperatures have the reverse effect (Parkin and Owen, 1986).

Sulfates and other oxidized compounds of sulfur are reduced to sulfides under anaerobic conditions in a mixed culture of bacteria. Sulfides may exist in soluble or insoluble form, depending on the cations with which they become associated. Sulfides are sometimes useful in removing toxic heavy metals from anaerobic systems by precipitation of heavy metal sulfides (Lawrence and McCarty, 1965). McCarty (1964c) reported that concentrations up to 200 mg/L of soluble sulfides can be tolerated with no significant inhibitory effect on anaerobic treatment.

Hydrogen sulfide may be present in a gaseous or liquid form, or as the HS^- ion as shown below:

$$H_2S_{(gas)} <=> H_2S_{(liquid)} <=> HS^- + H^+$$

At a system pH above 7, the less toxic HS^- ion will predominate, and at a system pH less than 7, the more toxic free soluble H_2S will predominate.

Kugelman and Chin (1971) studied the effect of light metal cations and observed that the addition of antagonistic cations could reduce, and in some cases eliminate the toxicity caused by the light metal cations. Table 3.5 lists the antagonists for each light metal cation, as reported by the authors.

The toxicity of heavy metals depends on the various chemical forms that the metal may assume under anaerobic conditions, and at near neutral pH levels. Soluble species are usually harmful. Some inhibitory concentrations are given in Table 3.4. As mentioned previously, a common method of lowering the heavy metal concentration is to add sulfides in order to form insoluble precipitates. However, one must be careful not to exchange heavy metal toxicity for sulfide toxicity.

Table 3.5. Cations Antagonistic to Light Metal Cation Toxicity (Kugelman and Chin, 1971)

Toxic metal	Antagonistic cations
Na^+	K^+
K^+	Na^+, Ca^{2+}, Mg^{2+}, NH_4^+
Ca^{2+}	Na^+, K^+
Mg^{2+}	Na^+, K^+

3.3.4. Kinetics of anaerobic treatment

An understanding of the kinetics of anaerobic treatment is important in understanding the key factors affecting the process efficiency and stability. There are two basic equations that describe anaerobic biological wastewater treatment adequately for most cases (Andrews and Graef, 1971; Lawrence, 1971; Lawrence and McCarty, 1970).

The first equation is related to growth of microorganisms:

Net growth = Total growth - Biomass lost to endogenous decay

$$(dX/dt) = Y(-dS/dt) - Ke\,X \qquad (3)$$

where, (dX/dt) = Rate of bacterial growth, mass/volume-time

(dS/dt) = Rate of substrate removal, mass/volume-time

X = Bacterial concentration, mass/volume

Y = Bacterial yield, mass bacteria/mass substrate

Ke = Bacterial decay rate, $time^{-1}$

The second equation is used to represent the rate of substrate utilization and is known as the Monod function, which is:

$$-\frac{dS}{dt} = \frac{Km\,S\,X}{Ks + S} \qquad (4)$$

where, S = Substrate concentration, mass/volume

Km = Maximum rate of substrate utilization, mass of substrate utilized per time/ mass bacteria

Ks = Half velocity constant, mass/volume substrate

Equation (4) can also be written as:

$$K = \frac{Km\ S}{Ks+S} \qquad (5)$$

where, $K = (-dS/dt)/X$ = specific substrate removal rate

Figure 3.4 is a graphical representation of the Monod function. At very high substrate concentrations , the Monod function is zero order with respect to substrate concentration and becomes:

$$-(dS/dt) = Km\ X \qquad (6)$$

At very low substrate concentrations, the function is first order with respect to substrate concentration, S:

$$-(dS/dt) = (Km/Ks)\ X\ S \qquad (7)$$

Combining equations (3) and (4) we obtain:

$$\frac{(dX/dt)}{X} = \frac{Y\ Km\ S}{Ks + S} - Ke \qquad (8)$$

Or,

$$\mu = \frac{Y\ Km\ S}{Ks + S} - Ke \qquad (9)$$

where, μ = Specific growth rate of bacteria, $time^{-1}$

Since specific growth rate μ is equal to the inverse of the biological solids retention time (Θ_c), Equation 9 can be written as:

$$\frac{1}{\Theta_c} = \frac{Y\ Km\ S}{Ks + S} - Ke \qquad (10)$$

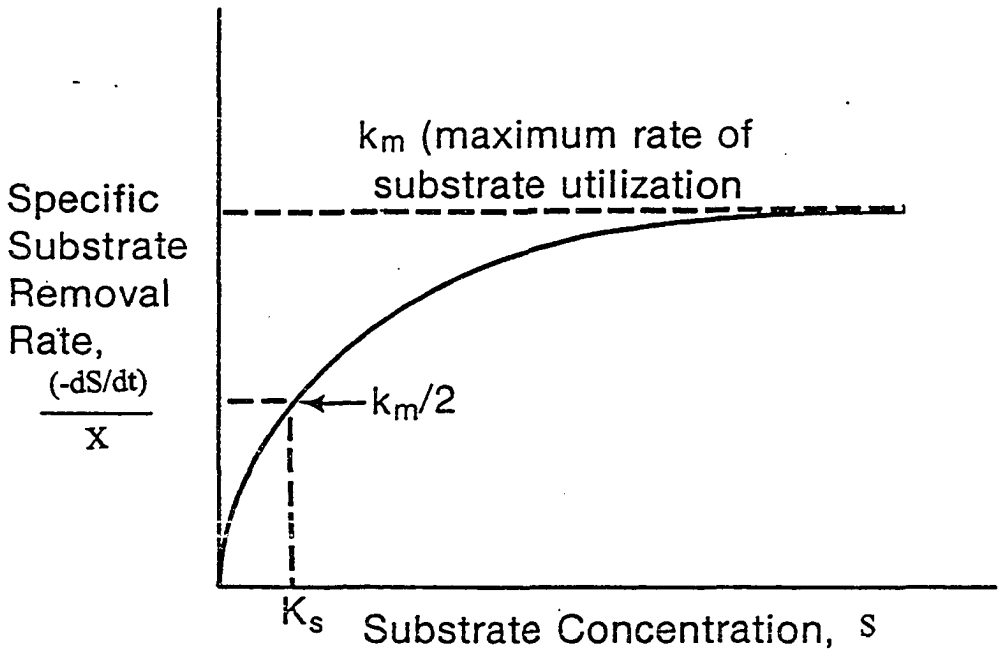


Figure 3.4. Graphical representation of the Monod function

Figure 3.5 (McCarty, 1964a) is a plot of yield or biological growth versus solids retention time for three types of substrates. The two extremes in growth are represented by fatty acid wastes, which produce the lowest yield, to carbohydrates, which produce the highest. Other types of wastes are expected to vary between these two extremes. Figure 3.5 illustrates lower biological cell production at longer SRTs. This is due to endogenous decay of cells resulting in lower net growth. Thus, greater waste stabilization and lower sludge production is obtained at long solids retention times. The system bacterial growth rate and process efficiency can be controlled by controlling the SRT.

The substrate or organic matter destroyed is usually expressed in units of BOD_5 (5 day Biochemical Oxygen Demand) , BOD_L (Ultimate BOD), COD (Chemical Oxygen Demand)or TOC (Total Organic Carbon). The biological solids produced are measured in terms of suspended solids or volatile solids.

Microbiological reactions are autocatalytic. This means that at steady state, the catalyst or enzyme for a biologically mediated reaction will be produced to such an extent that the amount of catalyst present will not limit the rate of a reaction. Rather, the substrate concentration will be rate limiting (Gujer and Zehnder, 1983). Bacterial enzyme kinetics are often described by the Michaelis-Menton equation (Shuler and Kargi, 1992), which is similar to the Monod equation and is given by:

$$\mu = \frac{\mu m\ S}{Ks+S} \qquad (11)$$

where, μ = Specific growth rate or production rate

μm = Maximum specific growth rate

The Michaelis-Menton equation and the Monod equation can be related by the following

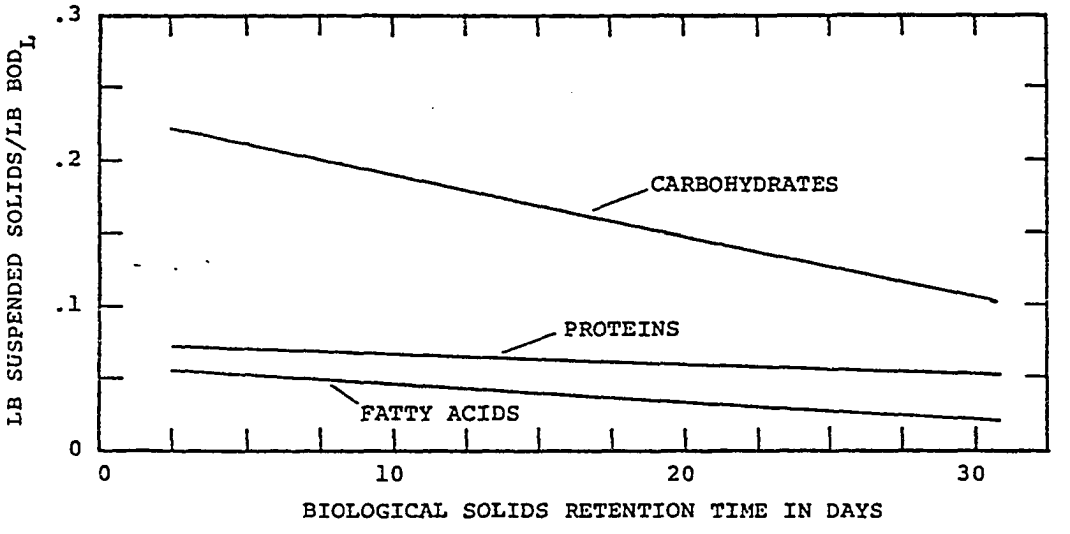


Figure 3.5. Biological solids production resulting from methane fermentation (McCarty, 1964a)

relationship between specific growth rate (μ), yield of biomass (Y) and specific substrate removal rate (K):

$$\mu = KY \tag{12}$$

An understanding of the process kinetics is important for efficient and successful operation of an anaerobic treatment process. The methanogenic bacteria and hydrogen-producing acetogenic bacteria have slower growth rates than other prevalent organisms. The design and operational SRT must be sufficient to allow these critical organisms time to grow and to complete the conversion of complex organics to methane gas.

3.3.5. Granulation process

Granulation is a process whereby the microorganisms tend to adhere to one another as well as to inorganic or organic support particles to form firm, dense granules (Sung and Dague, 1992). The phenomenon of granulation is very important for the retention of biomass in anaerobic reactors.

Hulshoff Pol et al. (1983) outlined some of the factors affecting granulation in anaerobic treatment:

1. Environmental conditions, such as pH, temperature, availability of essential nutrients and composition of wastewater.
2. Type of seed sludge, i.e. with respect to its specific activity, settleability and inert fraction.
3. Process conditions applied during start-up, such as loading rate and amount of seed sludge used.

Hulshoff Pol et al. (1983) observed granulation in Upflow Anaerobic Sludge Blanket

(UASB) reactors. They suggested that the UASB system promoted a selection between the sludge ingredients, such that the voluminous, lighter particles were washed out and the heavier particles were retained. Growth was concentrated at these particles, which ultimately resulted in the formation of distinct granules up to 5 mm in diameter. The researchers obtained two types of granules. An acetate fed reactor produced *filamentous* granules composed of long multicellular filaments of rod-shaped organisms, mostly Methanothrix soehngenii. The granules contained an inert support material originating from digested sewage sludge. Reactors fed with propionate produced smaller, denser granules (rod-type granules), where the organisms grew as short multicellular fragments. Average settling velocity of the granules was about 0.5 m/min. The specific activities at 30° C were 2.3 kg COD/kg VSS-day and 2.2 kg COD/kg VSS-day for the filamentous and rod-type granules.

The major features of the structure of a granule are a core or *medulla* surrounded by a wide peripheral zone or *cortex*. Thin, dense layers encircle the granule and the medulla. The core may contain inert extracellular material. Most of the organisms grow on the surface and in the interstices of the granules, giving a porous surface to the granules. These features were observed in granules grown under both mesophilic (35° C) and thermophilic (55° C) conditions (van Lier et al., 1992). Dolfing (1986) determined by transmission electron microscopy that the granules consisted of a wide variety of bacterial morphotypes, which frequently occurred in micro-colonies throughout the granules. He also observed the presence of a large percentage (20 to 30%) of Methanothrix-like organisms in the granules. The chemical composition of granular biomass was comparable to the chemical composition of bacteria in general. The granules contained about 50% of the total dry weight as protein, 7% carbohydrate, 10 to 20% ash content caused by FeS, and 2% extracellular polysaccharides.

A symbiotic relationship exists between the microbial consortia associated with granular sludge particles that is advantageous in enhancing the rate of biological activity. Nutrient exchange between the species and adaptation to environmental changes are facilitated by the symbiotic association. McCarty and Smith (1986) reported that reactors with granular sludge produced lower hydrogen partial pressures and more rapid hydrogen utilization, than reactors with dispersed sludge, resulting in increased efficiency.

Granulation was observed in Anaerobic Sequencing Batch Reactors (ASBR) by Sung and Dague (1992). The reactors were fed with a synthetic milk substrate. The researchers suggested that the ASBR promotes granulation by imposing a selection pressure during the decant cycle. The decant process tends to wash out poorly settling flocs and selects for heavier, more rapidly settling aggregates. Reactor geometry, hydraulic retention time (HRT) and organic loading rates influenced the size and characteristics of the granules. They obtained settling velocities ranging from 0.98 to 1.2 m/min for the granular sludge.

Granules grown on defined substrates such as ethanol or propionate, with very high specific activities may exhibit mass transfer limitations. However, Dolfing (1986) observed that mass transfer resistance would not be significant in industrial reactors that usually convert a mixture of substrates, which results in relatively low specific activities of the biomass for substrates such as hydrogen, formate and acetate. Furthermore, these latter compounds are intermediates that are produced and consumed in the same granule.

3.4. Adsorption Technology for Water Pollution Control

Adsorption is a process by which a substance accumulates at the interface between two phases (Noll et al., 1992). These phases can be any one of the following: liquid-liquid, liquid-solid, gas-liquid or gas-solid. The molecule that accumulates or adsorbs at the interface is called an *adsorbate*, and the phase on which adsorption occurs is called an

adsorbent.

In case of solid-liquid interface, adsorption is defined as the uptake of molecules by the external or internal surface of solids or by the surface of liquids (Benefield et al., 1982). Adsorption occurs on these surfaces because of attractive forces of the atoms and molecules that constitute the surface.

Adsorption on solid adsorbents has great environmental significance, as it can effectively remove pollutants from both aqueous and gaseous streams. Adsorbents commonly used in water treatment processes include activated carbon, activated alumina, silica gels and adsorbent resins. Research is being done on the use of active or inactive (dead) biomass as an adsorbent, for the removal of pollutants from wastewater (Tsezos and Bell, 1989; Kasan and Baecker, 1989; Bell and Tsezos, 1987; Morper, 1986; Tsezos and Seto, 1986). The phenomenon is termed *biosorption.*

3.4.1. Principles of adsorption

It is useful to distinguish between physical and chemical adsorption, when discussing the fundamentals of adsorption. Physical adsorption occurs when adsorbate molecules are held to the adsorbent by relatively weak van der Waal's forces of attraction or by π-bonding under certain conditions. Physical adsorption is assumed to be multi-layered with each new layer of molecules forming on top of previously adsorbed layers (Benefield et al., 1982). It is not site-specific, is fully reversible, and heat of adsorption is very low.

Chemical adsorption, or *chemisorption* , involves the formation of chemical bonds between the adsorbate and adsorbent molecules. It is an irreversible process with site-specificity. Most adsorption processes in wastewater treatment are neither purely physical nor chemical, but are a combination of the two. It is not always possible to categorize a particular system unequivocally. According to Benefield et al. (1982), such distinction is not

necessary in the analysis and design of adsorption processes.

Removal of substances by adsorption on porous adsorbents involves a number of steps, each of which can affect the rate of removal (Snoeyink, 1990). They are:

1. *Bulk solution transport*: Adsorbates must be transported from bulk solution to the boundary layer of liquid surrounding the adsorbent particle.
2. *Film diffusion*: Adsorbates must be transported by molecular diffusion through the hydrodynamic boundary layer of water surrounding the adsorbent particles.
3. *Pore diffusion*: The adsorbate molecules must be transported through the adsorbent's pores to available adsorption sites.
4. *Adsorption*: The adsorbate molecule must become attached to the surface of the adsorbent. This step is very rapid for physical adsorption, and one of the preceding steps will control the rate of adsorption. If adsorption is accompanied by a chemical reaction, then this may be the rate-limiting step for chemisorption processes.

Many factors that influence the rate and extent of adsorption include mixing, pH, temperature, characteristics of adsorbent, and size and solubility of adsorbate. Film diffusion becomes rate limiting in systems where slow mixing is used, e.g. continuous flow systems at flow rates of 10 gal/min-sqft or less (Weber, 1972). Pore diffusion is usually rate limiting for batch type contacting systems where a high degree of mixing is provided.

3.4.2. Adsorption equilibrium models

Adsorption of molecules can be represented by the reaction:

$$A + B \Longleftrightarrow A.B \qquad (13)$$

Here, A represents the adsorbate, B the adsorbent and A.B the adsorbed compound.

Molecules of solute from solution continue to accumulate on the adsorbent surface until the concentration of solute remaining in solution is in equilibrium with the concentration of solute adsorbed by the adsorbent. At this point, the rate of forward reaction (adsorption) equals the rate of reverse reaction (desorption). Equilibrium is reached and no further accumulation will occur.

One of the most important characteristics of an adsorbent is the quantity of substrate that it can adsorb. The constant temperature equilibrium relationship between the quantity of adsorbate per unit of adsorbent (Qe) and the equilibrium concentration of adsorbate in solution (Ce) is called the *adsorption isotherm* (Snoeyink, 1990). A number of isotherm models are used in the design of water treatment processes. The Freundlich model and the Langmuir model are two of the most common models and will be discussed here.

Langmuir Isotherm: The Langmuir model was originally developed to represent chemisorption on a set of distinct localized adsorption sites. The derivation of the Langmuir adsorption isotherm was based on five implicit assumptions (Langmuir, 1918). They are:

1. Adsorption is confined to a monomolecular layer.
2. The affinity of each binding site for adsorbate molecules is the same.
3. There is no lateral interaction between the adsorbed molecules.
4. Adsorbed gases behave ideally in the vapor phase.
5. Adsorbed molecules do not move around on the surface.

The Langmuir equation is given by:

$$Qe = \frac{Qo\ b\ Ce}{1 + b\ Ce} \qquad (14)$$

where, Qe = Equilibrium surface concentration, mass adsorbate/mass adsorbent

Ce = Equilibrium concentration of adsorbate in solution, mass/volume

Qo = Temperature independent constant

b = Temperature dependent constant

Equation (14) can be linearized as:

$$\frac{1}{Qe} = \frac{1}{Qo} + \frac{1}{Qo\, b\, Ce} \qquad (15)$$

The values of Qo and b can be determined from a plot of 1/Qe versus 1/Ce.

Freundlich Isotherm: Freundlich (1926) developed an empirical equation to describe the adsorption process. His development was based on the following assumptions:

1. No association or dissociation of molecules after they are adsorbed on the surface.
2. Adsorbent has a heterogeneous surface composed of different classes of adsorption sites, with adsorption in each class of site following the Langmuir isotherm.

The Freundlich isotherm is given by:

$$Qe = K(Ce)^{1/n} \qquad (16)$$

where, Qe and Ce are as defined previously

K = Constant related to capacity of adsorbent for the adsorbate

1/n = Constant related to strength of adsorption

The Freundlich equation can be linearized as:

$$\log(Qe) = \log(K) + 1/n \log(Ce) \qquad (17)$$

A plot of log (Qe) versus log (Ce) should yield a straight line for adsorption data which follow the Freundlich theory. The constants K and 1/n can be determined from the intercept and slope of the straight line. Small values of 1/n indicate strong adsorption bonds. As 1/n

becomes very small, the capacity tends to be independent of Ce and the isotherm plot approaches the horizontal; the value of Qe is essentially constant and the isotherm is called *irreversible.* A large value of 1/n indicates weak adsorption bonds, and Qe changes markedly with small changes in Ce. For fixed values of Ce and 1/n, the larger the value of K, the larger is the capacity Qe.

However, the Freundlich equation is unsatisfactory for high coverages. As Ce increases, Qe will increase only until the adsorbent approaches saturation. At saturation Qe becomes a constant and the Freundlich equation no longer applies. In general, a large number of experimental results in the field of van der Waal's adsorption can be expressed by the Freundlich equation in the middle concentration range.

In biosorption studies for wastewater treatment, the following form of the Freundlich equation is used:

$$Qe = \frac{Co - Ce}{M} = K(Ce)^{1/n} \qquad (18)$$

where, Qe = Equilibrium uptake of substrate on biomass, mass substrate/mass biomass

Co = Initial concentration of adsorbate in solution, mass/volume as COD, TOC or BOD

Ce = Equilibrium concentration of adsorbate in solution, mass/volume

M = Amount of biomass, mass/volume as TSS or VSS

K, 1/n = Freundlich parameters.

Another isotherm model commonly used is the BET model developed by Brunauer, Emmett and Teller (1938). In general, the Langmuir and BET equations do not apply as well as the Freundlich equation to mixed solutes or dilute solutions. This is probably due to the heterogeneous nature of the adsorbent surface, interaction between adsorbed molecules and

other factors that were not considered in the Langmuir and BET models. For these reasons, the Freundlich equation is widely applied to wastewater treatment studies.

3.5. Biosorption Process

3.5.1. Introduction

Biosorption may be defined as the removal of metal or metalloid species, compounds and particulates from solution by biological material (Gadd, 1990). This is a general description which takes no account of the mechanistic details involved in the process. Figure 3.6 illustrates a flow diagram for this process (Gadd, 1990).

According to Tsezos and Bell (1989), the uptake or accumulation of chemicals and particulates by microbial biomass is termed *Biosorption.* The mechanism responsible for this accumulation is complex and involves adsorption and/or absorption into various components of the microbial cell.

3.5.2. Biosorption for treatment of wastewater

The biosorption process has been applied for the treatment of wastewater in two ways:
(a) aerobic biosorption
(b) anaerobic biosorption.
Both of these processes will be discussed in some detail.

(a) Aerobic biosorption

In 1951, Ullrich and Smith developed the aerobic biosorption process as an accelerated or high-rate process. It consisted of the following steps:

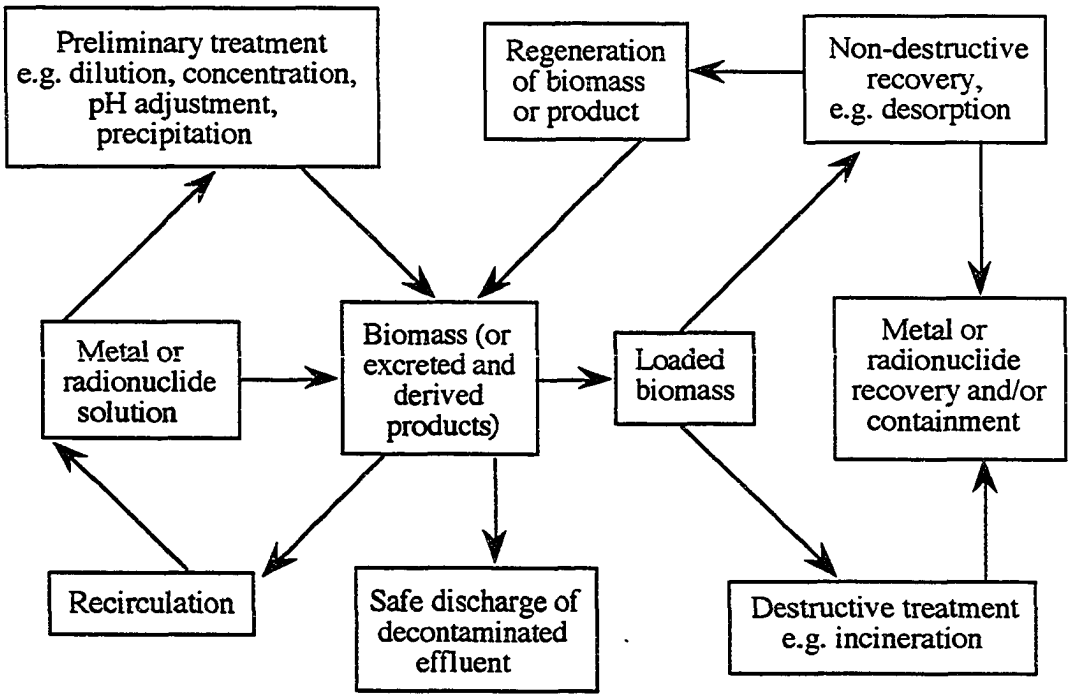


Figure 3.6. Flow diagram of the biosorption process (Gadd, 1990)

(1) Activated sludge was first brought in contact with raw sewage and briefly mixed, either mechanically or with air. The activated sludge adsorbed and absorbed a very high percentage of suspended solids and dissolved pollutants.

(2) The mixed liquor was then delivered to a clarifier and allowed to settle. The clear effluent, low in BOD and suspended solids, was the plant effluent.

(3) The sludge from the clarifier was conducted to an aeration tank, digested aerobically and recycled to the mixing chamber.

The flow diagram for the process is illustrated in Figure 3.7. Data collected from a 15 gpm pilot plant showed BOD and suspended solids reduction in the 90 to 95% range, with an initial mixing of 15 to 30 minutes. Table 3.6 describes the data obtained from the pilot plant at Austin, Texas (Ullrich and Smith, 1951). The process demonstrated a remarkable ability to adjust itself to both high and low flows. The main advantages of the process were the elimination of the primary clarifier, and the requirement of less aeration tank capacity. This process was used successfully in the enlarged and remodeled wastewater treatment plant at Austin, Texas (Ullrich and Smith, 1957; Sawyer, 1960). The remodeled plant gave good BOD and suspended solids removal under widely varying conditions of loading and operation. The problem of sludge bulking was also eliminated.

(b) Anaerobic biosorption

E. N. Mortenson obtained a patent for an anaerobic biosorption process for treating raw wastes in 1953. In the process, anaerobic sludge was mixed with raw waste prior to settling, in order to produce a more concentrated sludge suitable for anaerobic digestion and a supernatant liquid substantially free of suspended solids and low in soluble organic matter. Figure 3.8 illustrates a flow diagram of Mortenson's process. The steps involved are:

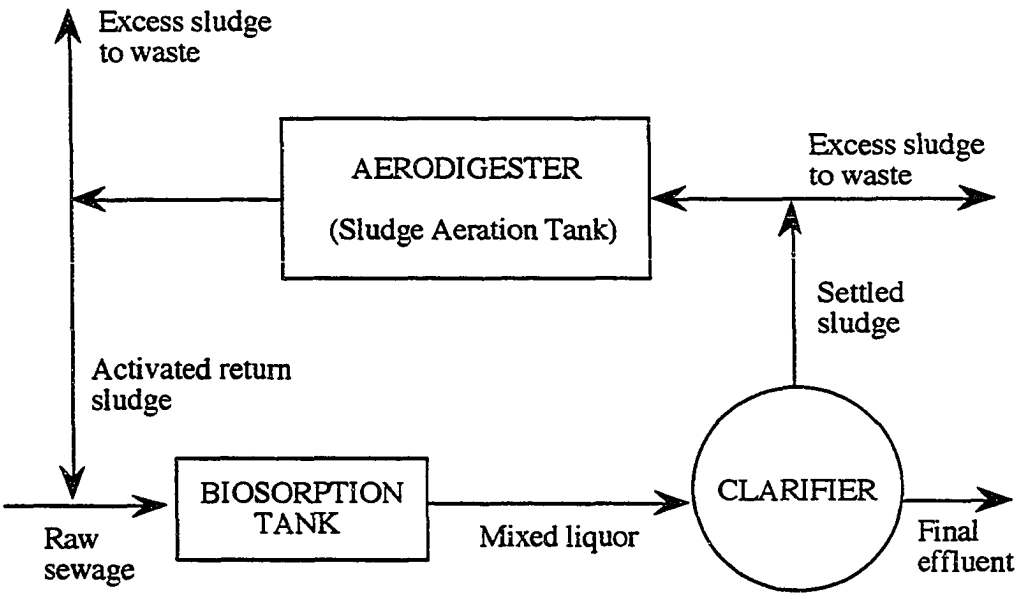


Figure 3.7. Flow diagram of biosorption pilot plant (Ullrich and Smith, 1951)

Table 3.6. Pilot plant data[1] on aerobic biosorption process at Austin, Texas (Ullrich and Smith, 1951)

BOD (ppm)			Suspended Solids (ppm)		
Initial	Final	% Reduced	Initial	Final	% Reduced
200	16.8	91.6	219	9.6	95.6
220	9.6	95.6	190	5.5	97.1
240	19.2	92.0	241	34.0	85.9
260	16.8	93.5	231	5.6	97.6
300	10.8	96.4	228	11.2	95.1
320	20.4	93.6	229	9.0	96.1

[1] Retention periods for 15 gpm raw sewage and 15 gpm return sludge rate were: Biosorption tank 14.3 min.; Clarifier 65 min; Aerodigester 96 min.

(1) Active anaerobic sludge was mixed with raw waste in a flocculation tank, for a period ranging from one-half hour to six hours. Lime was added for pH adjustment.

(2) After flocculation, the material was passed through a degasification tower, to reduce odor problems.

(3) Then the mixture was allowed to settle in a settling tank. The clear supernatant was withdrawn from the top. The settled sludge was delivered to a digester.

(4) About 10 to 15% of the digested sludge was recycled to the flocculation tank, and the remainder was delivered to drying beds.

The main advantage of Mortenson's process was that the flocculation tank or adsorption unit did not have to be heated. This process was suitable for the treatment of relatively cool wastes with low concentrations of suspended solids.

Schroepfer and Ziemke (1959) applied this process to the treatment of synthetic milk waste and obtained about 80% BOD removals, for a sludge age of 5 days. They found that the initial uptake or sorption of organic matter by anaerobic biomass was quite rapid and reached equilibrium in less than one-half hour of reaction time. They called it *the rapid adsorption-sludge regeneration process.*

In their studies on the Anaerobic Sequencing Batch Reactor (ASBR), Sung and Dague (1992) observed that the soluble chemical oxygen demand (COD) was reduced at a very high rate during the first 15 minutes, after the reactor had been fed with a synthetic milk substrate. Bioconversion of substrate was also measured in terms of methane production with a Gas Chromatograph. Figure 3.9 illustrates their results, where methane-COD values were calculated on the basis that 0.35 liters of methane (at STP) were produced for each gram of COD removed. According to Figure 3.9, the removal of COD in the form of methane lagged behind the actual COD removals measured. The authors proposed that this lag may be due to biosorption of substrate, followed by later conversion to methane.

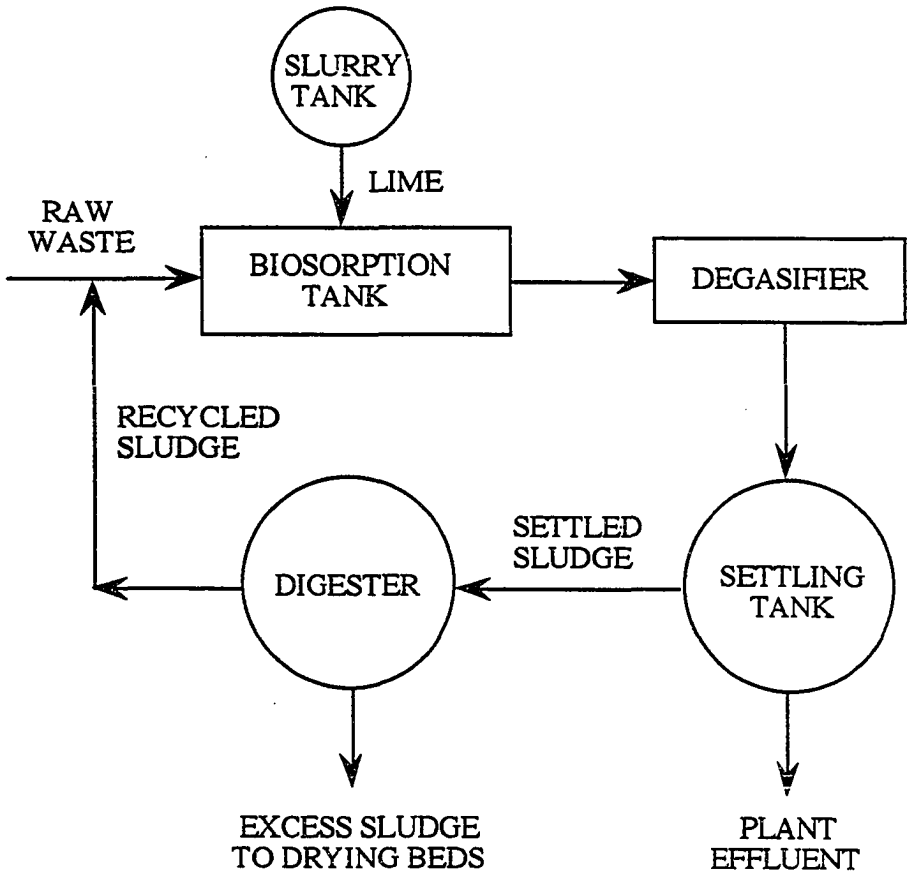


Figure 3.8. Flow diagram of anaerobic biosorption process (Mortenson, 1953)

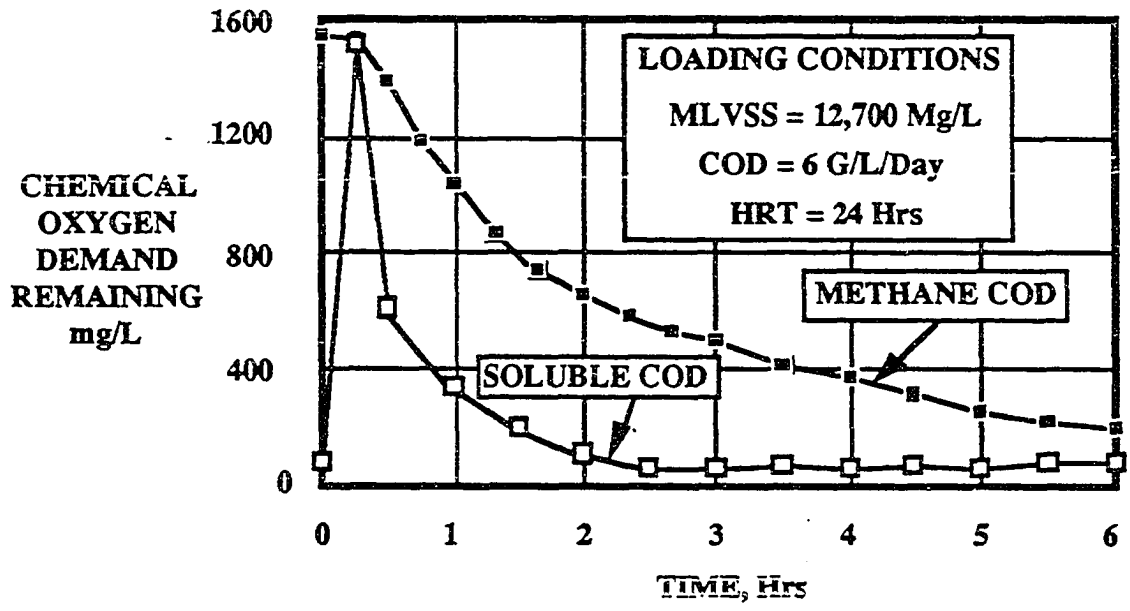


Figure 3.9. COD removals in the Anaerobic Sequencing Batch Reactor (Sung and Dague, 1992)

3.5.3. Removal of hazardous organics

A large amount of research has been done to study the significance of biosorption for the removal of hazardous organic pollutants, namely pesticides and chlorinated hydrocarbons from wastewater. Organic molecules that are not biodegradable, can still be removed from wastewater by microbial biomass via the process of biosorption. Because of the reversibility of biosorption, it is also possible for organic molecules accumulated by the biological solids during a period of time to be desorbed, under appropriate conditions at a later time (Tsezos and Bell, 1988). Biosorption of organics can be accomplished by both live and dead biomass (Tsezos and Bell, 1989; Bell and Tsezos, 1988; Bell and Tsezos, 1987; Tsezos and Seto, 1986). Most of the research work has been done on aerobic biosorption.

Bell and Tsezos (1987) conducted adsorption of organic chemicals, namely lindane, diazinon, malathion, pentachlorophenol and PCB, onto two types of inactive microbial biomass. One biomass type was Rhizopus arrhizus, a fungus grown in the laboratory, and the other biomass was activated sludge obtained from a municipal wastewater treatment plant. Adsorption experiments were conducted by agitating chemical solutions of various concentrations with different quantities of biomass in 250 ml screw top flasks, in a constant temperature room. After a specific period of agitation, the solutions were separated from the biomass by membrane filtration. The Freundlich equation similar to equation 18 (section 3.2) was used to fit the equilibrium data. Figures 3.10 and 3.11 illustrate the adsorption isotherms at 20° C, obtained from the research. The researchers concluded that adsorption by microbial biomass was an important process in the removal of hazardous organic pollutants in biological treatment systems. For compounds that were not readily degraded, the dominant removal mechanism was physical adsorption. The equilibrium adsorptive uptake appeared to be independent of initial concentration and adsorbent concentration.

Further studies were conducted by Bell and Tsezos (1988) to quantify the selectivity of

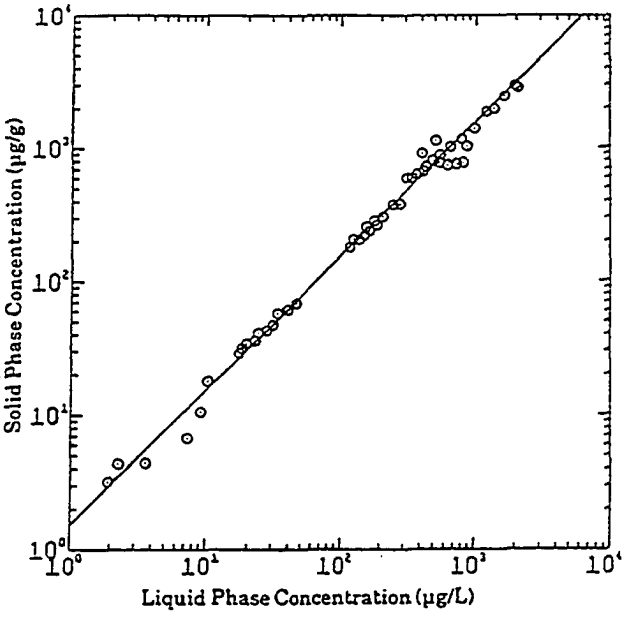


Figure 3.10. Adsorption isotherm of lindane on activated sludge (Bell and Tsezos, 1987)

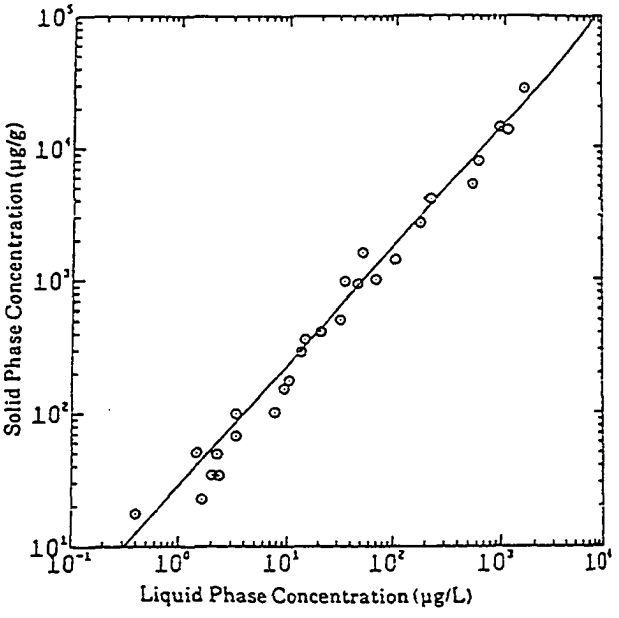


Figure 3.11. Adsorption isotherm of pentachlorophenol on *R. arrhizus* (Bell and Tsezos, (1987)

biosorption through the use of bioconcentration factors. They selected four hazardous organic compounds (lindane, pentachlorophenol, diazinon and malathion), and determined their biosorption isotherms on activated sludge and a fungus (R. arrhizus), from multisolute solutions. Their results demonstrated that competition effects were minimal for the compounds adsorbed and within the range of pollutant concentrations found in wastewater treatment plants.

A comparative study of biosorption and desorption of hazardous organic pollutants by live and dead biomass was conducted by Tsezos and Bell (1989). They concluded that for molecules which were not readily biodegradable, the overall uptake by live biomass appeared to be less than that by dead biomass. For more readily degradable molecules or for strongly adsorbing molecules, the reverse appeared to be true. But the researchers recommended further research before drawing any definite conclusions.

Strong correlations exist between adsorption and some chemical parameters. An inverse relation between solubility in water and adsorption onto biomass exists for a range of organic pollutants (Amy et al., 1988). The octanol/water partition coefficient (K_{ow}) is also an indicator of chemical biosorption potential. The higher the partition coefficient, the more adsorbable the compound. The process is enhanced by the hydrophobicity of the compound. K_{ow} is defined by the following equation:

$$K_{ow} = C_o / C_w \qquad (19)$$

where, K_{ow} = Octanol/water partition coefficient

C_o = Equilibrium concentration of compound in octanol layer

C_w = Equilibrium concentration of compound in water layer

Dobbs et al. (1989) performed investigations to correlate the sorption of toxic organic compounds on primary, mixed-liquor and anaerobically digested solids from municipal

wastewater treatment plants, with octanol/water partition coefficients. They found that the correlations were the same for all three types of wastewater solids, if the partition coefficients were calculated on the basis of organic content of the solids, as measured by weight loss on ignition at 550° to 600° C. The correlations provided a basis for estimating the removal of toxic and hazardous organic compounds from wastewater by the sorption process.

Tsezos and Seto (1986) determined the adsorption isotherms of halogenated aliphatic hydrocarbons (1,1,2-trichloroethane or TCE and 1,1,2,2-tetrachloroethane or TTCE) by various types of inactive microbial biomass. They found that the least water soluble component showed the greatest tendency to be accumulated by the biomass. The biomass exhibited a higher biosorptive uptake capacity for TTCE, which had a lower water solubility, higher octanol/water partition coefficient and a reduced volatility as compared to TCE. The sorption of eight different organic compounds by a green algae, Selenastrum capricornutum, was studied in detail by Casserly et al. (1983), using gas-liquid chromatography. A lot of similar studies can be found in the literature.

3.5.4. Removal of heavy metals and radionuclides by biosorption

Different types of bacteria, algae and fungi are efficient metal biosorbents. This metabolism-independent binding or adsorption of metal and radionuclide species by cell walls, extracellular polysaccharides, pigments or other materials can occur in living or dead cells. It is a very rapid process. Sometimes accumulation can be up to 50% of the dry weight of the biomass (Gadd, 1990).

Morper (1986) conducted experiments to demonstrate the application of anaerobic sludge as a powerful and low cost sorbent for heavy metals. He used an upflow anaerobic sludge bed reactor (Figure 3.12), which consisted of a cylindrical reaction zone with a

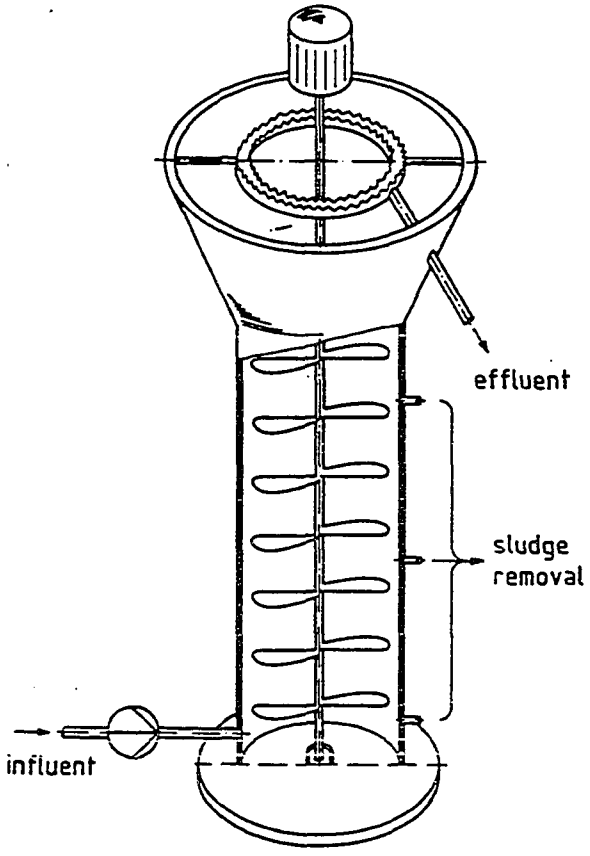


Figure 3.12. Upflow anaerobic sludge bed reactor used for biosorption (Morper, 1986)

conical sedimentation zone at the top. A slow motion multi-blade stirrer was used in the reaction zone. He obtained about 99% removal efficiency for copper, nickel, zinc and mercury. Equally good removals were obtained for silver and gold. About 75% removals were obtained for chromium. Morper concluded that metal removal efficiency and accumulation capacity were best at temperatures above 10^{o} C, and at neutral to slightly alkaline pH.

Toxic metal biosorption studies were performed by Kasan and Baecker (1989), using activated sludge from the coal-gasification effluent of a petrochemical plant. The researchers observed that adsorption of nickel, chromium, copper and iron increased independently of temperature, when applied as single solutions, whereas chromium and iron adsorption diminished at 37^{o} C when applied as mixtures of all metals. They further concluded that copper adsorption was due to both physical and chemical adsorption, while adsorption of zinc, nickel and iron was probably due to physical adsorption alone.

Kiff and Little (1986) conducted biosorption studies of heavy metals by immobilized fungal biomass. They investigated a range of bioreactor configurations and finally performed experiments on glass columns packed evenly with fungal mycelium immobilized on reticulated foam biomass support particles, through which an upward flow of cadmium laden wastewater was pumped at a fixed rate. Their results showed that more than 90% of the cadmium was sorbed onto the biomass within the first 10 minutes. They observed an increase in biosorption with an increase in pH. The maximum accumulation by mycelium was inversely proportional to the biomass concentration.

Biosorption studies of lead and chromium by Penicillium sp. mycelium were successfully conducted by Siegel et al. (1986). Biosorption has been widely used for removal of radionuclides, mainly Uranium and Thorium. Yakubu and Dudeney (1986) utilized immobilized fungus Aspergillus niger pellets fluidized in a compartmentalized

column, through which a uranium solution was pumped semi-continuously in an upward flow motion. They found that at low solution concentrations, about 80% removal of uranium could be achieved.

4. EXPERIMENTAL METHODS

4.1. Introduction

This chapter is divided mainly into two sections. One section describes the design and operation of the source reactor. This reactor was used to cultivate a viable and substantial population of microbial biomass. It was operated as an Anaerobic Sequencing Batch Reactor (U. S. Patent No. 5,185, 079), originally developed by Dr. Richard R. Dague and coworkers at Iowa State University, Ames, Iowa. The second section illustrates the design of the biosorption reactor and describes the experimental procedure for the biosorption experiments, where live anaerobic biomass from the source reactor was used as the adsorbent.

4.2. Source Reactor Design and Operation

The source reactor was used to grow biomass under controlled conditions for experimental purposes. The anaerobic biomass was grown at 35° C in ten-liter reactors operated as ASBRs. With the ASBR it is possible to achieve good bioflocculation and efficient solids separation, which results in a large buildup of biomass in a short period of time (Sung and Dague, 1992).

4.2.1. Design of the ASBR system

The source ASBR was constructed from Plexiglass according to the dimensions shown in Figure 4.1. The total internal volume was 12 liters with an operating volume of 10 liters. A length of tape was calibrated and marked in 0.25 L increments according to reactor volume and attached to the side of the reactor.

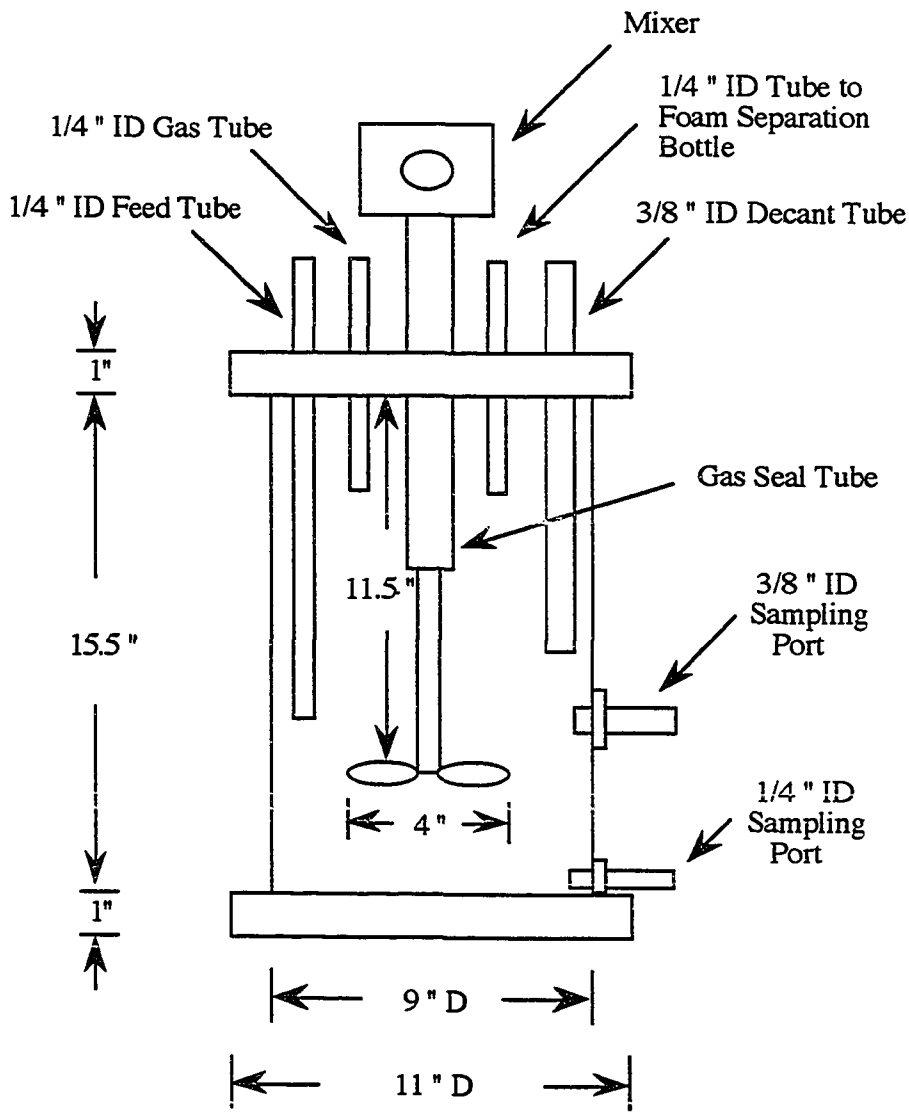


Figure 4.1. Dimensional view of the source reactor (ASBR)

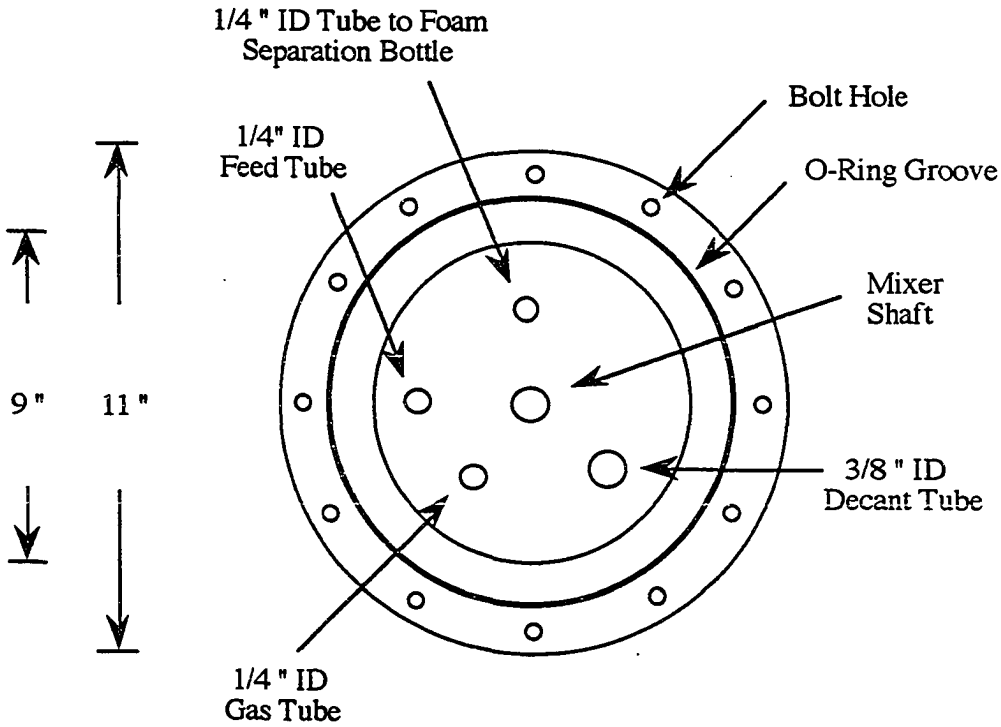
Two sampling ports were provided along the side of the reactor. They were made of 3/8 in ID and 1/4 in ID stainless steel tubes, respectively. The metal tubes were reinforced with 1/2 in thick circular pieces of Plexiglass which were glued to the side of the reactor. All connections were made using Teflon tape and then covered with silicon caulking to prevent leaks.

The upper flange was 11 inches in diameter, with twelve holes drilled at equal intervals to accommodate the 1/4 in bolts used to attach the lid. A groove in the flange held a 10 inch diameter O-ring made of flexible rubber to provide an air-tight seal between the lid and the flange.

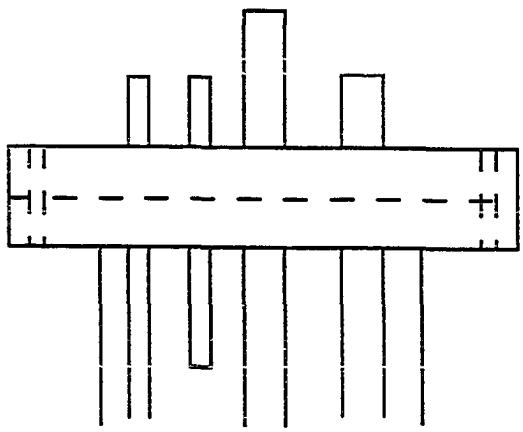
The lid was 11 inches in diameter and made from 1/2 in thick Plexiglass. Similar to the top flange, the lid also contained twelve bolt holes and a groove for the O-ring as illustrated in Figure 4.2. At the center of the lid was a 3/8 in hole for the shaft of the mechanical mixer. Three 1/4 in ID holes were provided on the lid for the feed tube, gas tube and foam separation tube respectively. One 3/8 in ID hole was provided for the decant tube. The feed and decant tubes were designed to be used as adjustable height tubes in the reactor.

Mixing system It was decided to use mechanical mixing in the source reactor. A 1/18 hp variable speed T-Line Laboratory Stirrer was used (manufactured by Cole-Parmer Instrument Company, Chicago, Illinois). Speeds ranging from 75 to 750 to 7500 rpm could be obtained with the triple-shaft stirrer. A three-blade propeller was mounted on the shaft to provide adequate mixing. The mixer was mounted on the top of the lid and the shaft was enclosed in a stainless steel casing up to a depth below the liquid level, in order to maintain anaerobic conditions in the reactor.

Foam separation system An aspirator bottle manufactured by Fisher Scientific



(a) Plan



(b) Sectional Elevation

Figure 4.2. Plan and sectional view of lid for source reactor

Company was used for foam separation and placed along the gas line coming out of the ASBR. Usually after feeding there were periods of rapid gas production, when a foamy mixture of gas and liquid with small amounts of biomass would float to the surface. If enough accumulated to fill the headspace above the liquid, it was carried into the foam separation bottle with exiting gas. In the bottle the gas would slowly bubble out and the settled liquid and biomass could be returned to the reactor through the bottom port.

Gas measuring system The gas produced in the reactor flowed out through the gas tube into the foam separation bottle and then into a gas bag followed by a one-way valve, a water lock, a hydrogen sulfide (H_2S) scrubber, a gas sampler, and a gas meter. Figure 4.3 illustrates the system.

During the decant phase in the ASBR, a vacuum occurred when processed liquid was decanted from the reactor. This disturbed the settled biomass and sometimes caused loss of solids with the effluent. To overcome the problem, a gas bag was used. The bag was an inflatable beach ball purchased from WalMart Stores Inc. in Ames, Iowa. During decanting, the suction caused gas flow from the gas bag into the reactor, keeping the inside of the reactor at atmospheric pressure. Afterwards, when feed was added, the gas was displaced back into the gas bag. The gas bag was followed by a one-way valve and a water lock which provided about 1 in of water head as back pressure. This ensured that the gas bag would be completely full before gas started flowing towards the gas meter. Figures 4.4 and 4.5 illustrate the direction of gas flows during decanting and feeding, respectively.
The water lock consisted of a wide mouth glass bottle fitted with a rubber stopper and partially filled with water. Two 1/4 in glass tubes were inserted through the stopper. One tube extended about an inch into the water and the other reached only to the bottom of the stopper. Gas flowed through the longer tube, bubbled into the water, then flowed out through the other tube into the hydrogen sulfide scrubber.

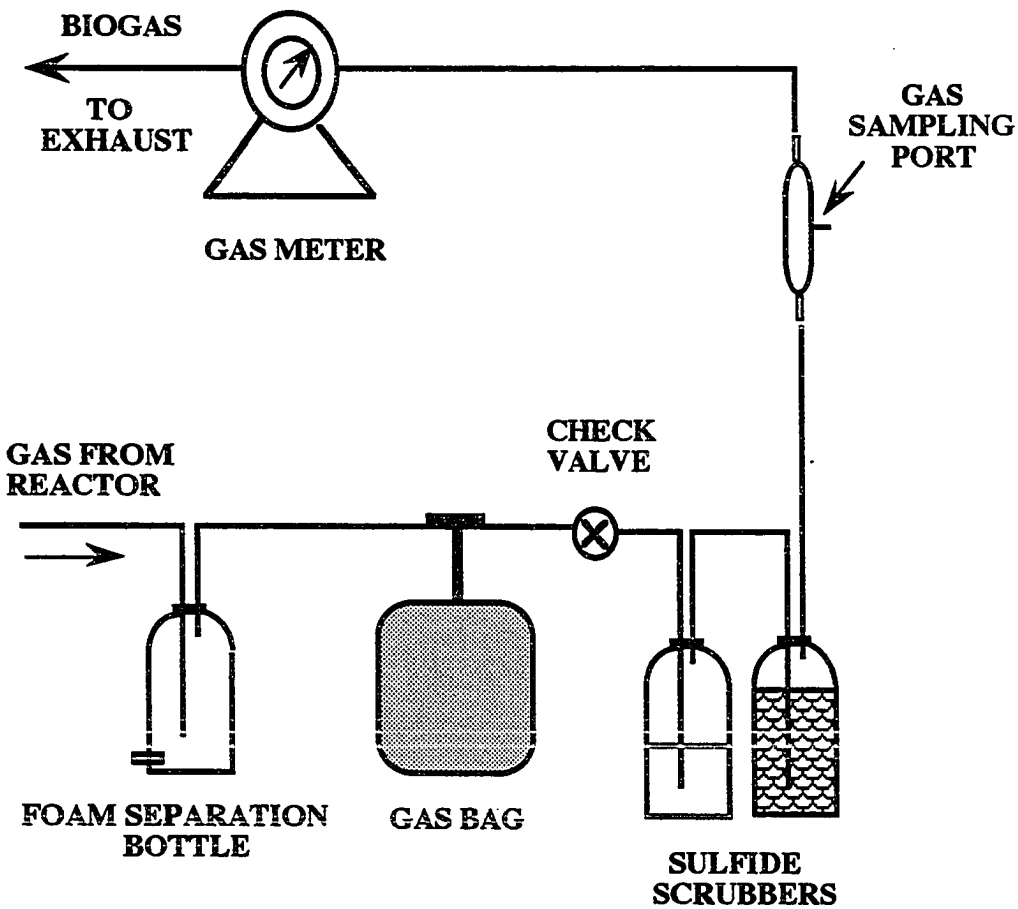


Figure 4.3. Gas measuring system of the ASBR

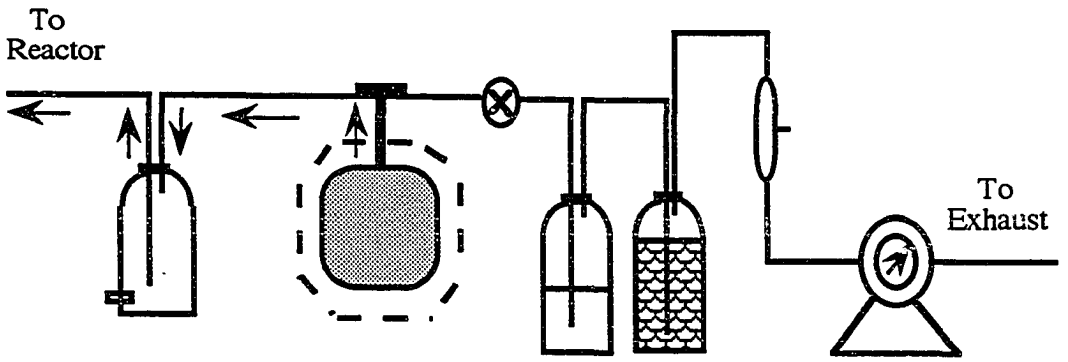


Figure 4.4. Direction of gas flow during decanting

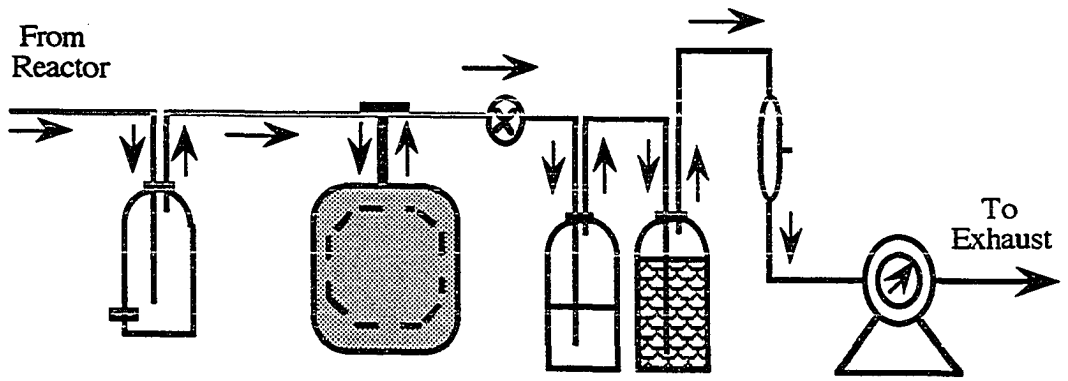


Figure 4.5. Direction of gas flow during all times except decanting

The gas scrubber was filled with steel wool and contained two glass tubes similar to the water lock. The H_2S was removed from the gas stream when it reacted with the iron. Gas flowed from the H_2S scrubber to a gas sampler, which was manufactured by the Iowa State University Glass Blowing Shop in Ames, Iowa. The sampler was fitted with a rubber septum, through which a syringe needle was inserted to remove gas samples for component analysis.

The daily gas production was measured using a Wet Test Gas Meter (Precision Scientific Inc., Chicago, Illinois). The gas meter provided measurements up to 0.001 cubic feet of gas produced. Gas coming out of the gas meter flowed to an exhaust pipe which transported the gas out of the laboratory.

Feed and decant systems A non fat dry milk was used as the feed to the source reactor. The feed was prepared every other day and stored in a refrigerator to prevent degradation. A variable speed Masterflex peristaltic pump, with a size 16 pump head, manufactured by Cole-Parmer Instrument Company, Chicago, Illinois, was used to pump feed from the refrigerator to the reactor. The pump speed was 1 to 100 rpm with a ten turn potentiometer speed controller. The feed line from the refrigerator to the reactor was kept as short as possible, to prevent clogging of degraded milk in the tubing.

The decant pump was the same as the feed pump, except that it was fitted with a size 18 pump head. The adjustable height decant tube in the reactor was fixed at the proper depth below the liquid level, according to the amount that would be decanted. Both the feed and decant pumps were calibrated to obtain the desired flow rates. Figure 4.6 is a schematic of the entire ASBR system.

A ChronTrol Timer was used to control the pumps, which was manufactured by Cole-Parmer Instrument Company, Chicago, Illinois. The Timer was programmed to turn on the mixer, feed and decant pumps for a specified period of time at regular intervals.

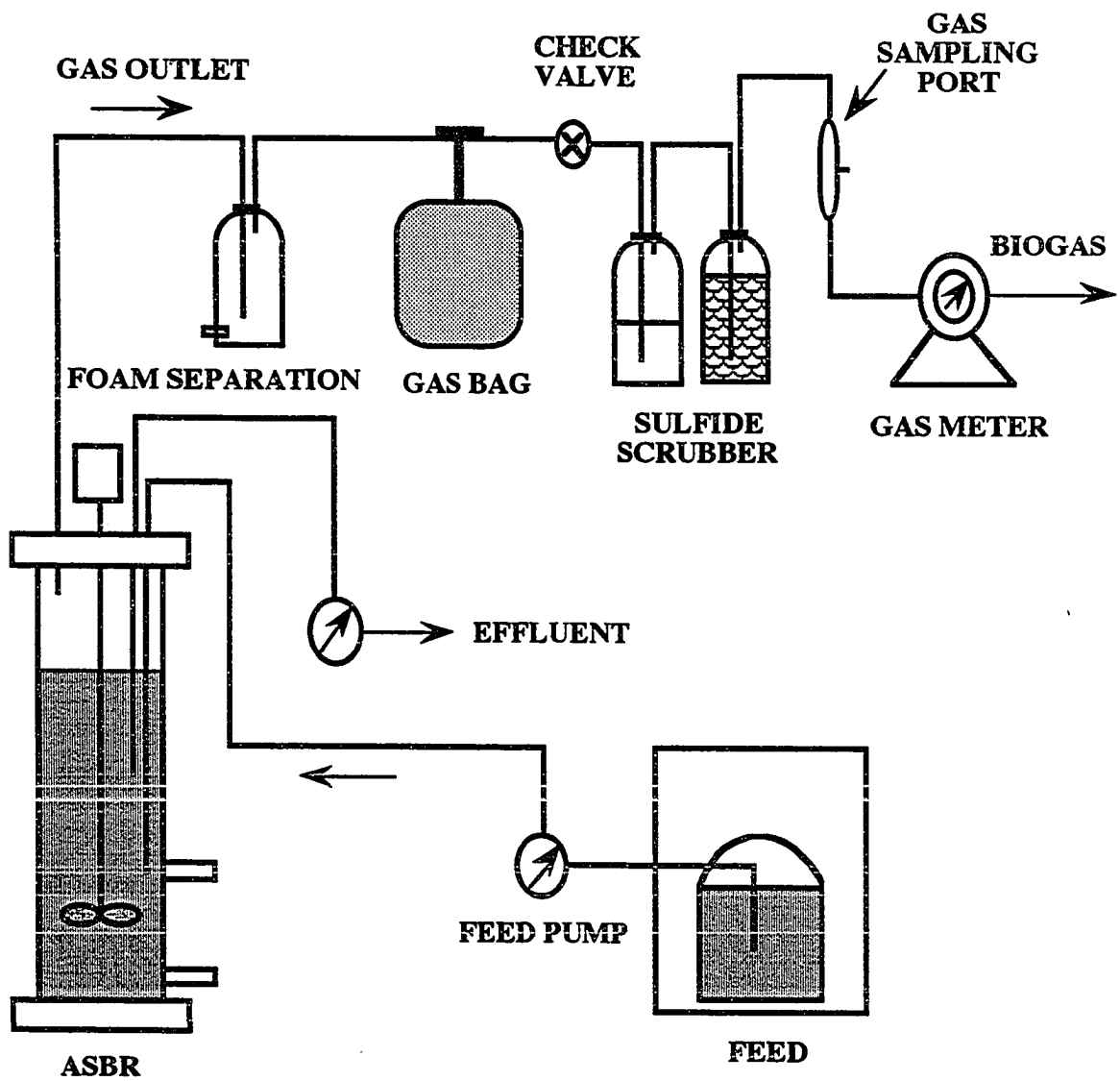


Figure 4.6. Schematic of the source reactor (ASBR) system

Nalgene tubing was used in all the connections throughout the system. In all cases, 1/4 in tubing was used except in the decant system and one side port, where 3/8 in tubing was used. The side ports were closed with Hoffman Screw clamps, manufactured by Fisher Scientific Company. Small polyethylene connectors, also manufactured by Fisher Scientific Company, were used to connect different sections of the tubing.

4.2.2. Principles of the ASBR

The operating principles of the ASBR are illustrated in Figure 4.7. The reactor sequences through four steps. They are:

(i) **Feed:** A specific volume of substrate at a specific strength is fed to the reactor. Feeding is stopped when the operational volume in the reactor is achieved. The reactor contents are usually mixed during feeding.

(ii) **React:** This step is most important in the conversion of substrates to biogas. The reactor contents are mixed intermittently to bring the substrate into close contact with the microorganisms.

(iii) **Settle:** Mixing is shut off and the biomass is allowed to settle, leaving a layer of clear supernatant at the top.

(iv) **Decant:** A specific volume of clear supernatant is decanted from the top. The volume decanted is usually equal to the volume fed in the first step.

These four steps constitute a cycle or sequence. The time for one cycle consisting of the feed, react, settle and decant steps is called the cycle length. The ASBR is a very flexible system. The number of sequences per day may be varied, together with the time required for the various steps. The feeding and decanting times are kept as short as possible. The time required for settling depends on the settling characteristics of the biomass and on the volume

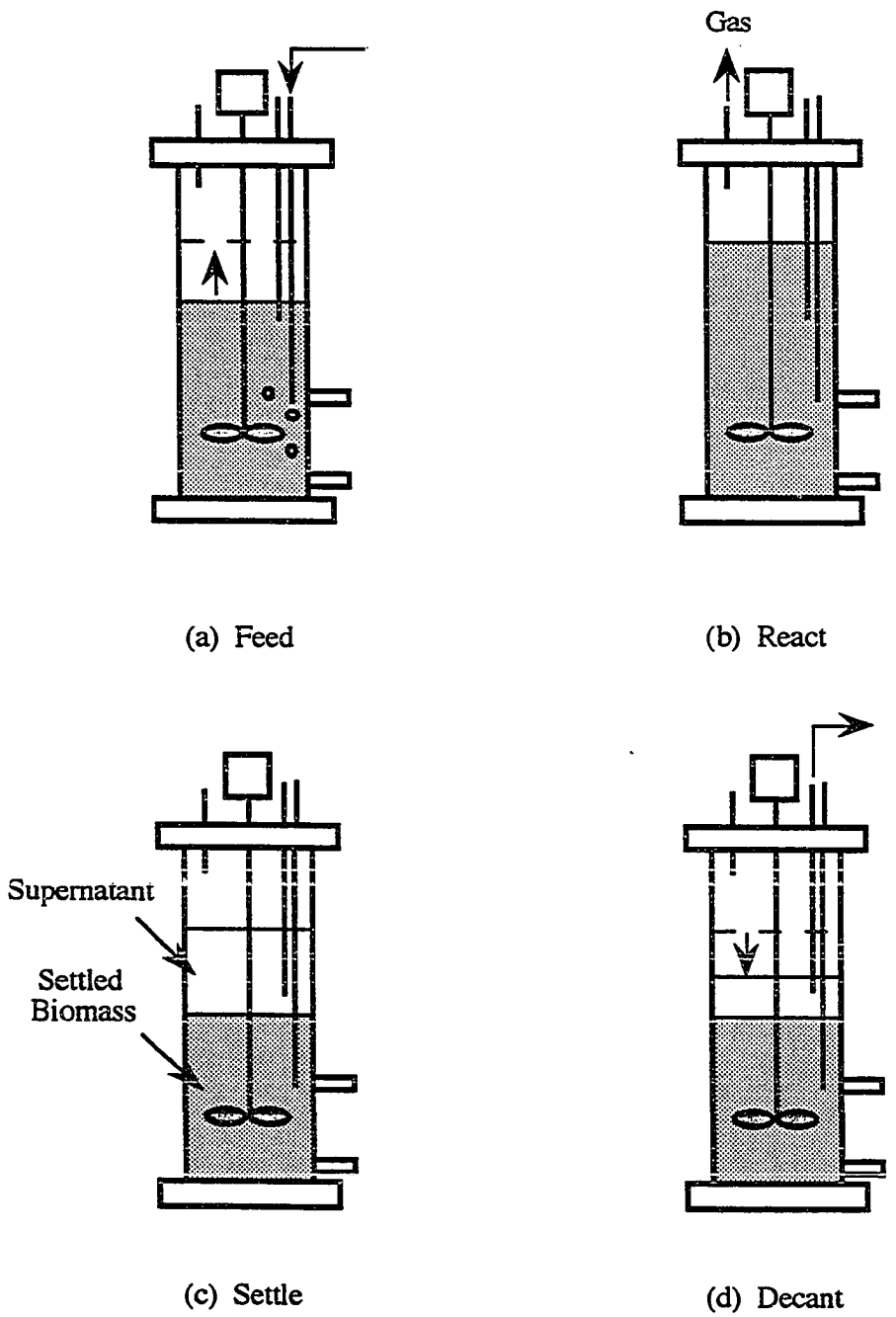


Figure 4.7. Operational steps of the Anaerobic Sequencing Batch Reactor

to be decanted, since that determines how far the biomass must settle to be below the decant level. The time for the react step is the longest, in order to enable the bacteria to assimilate and convert the substrate to methane. Ideally the react step should continue until the F/M ratio is quite low, since a low F/M ratio is associated with improved flocculation and settling. The ASBR is capable of achieving a lower F/M ratio at the end of the react cycle than a similarly loaded CSTR, which was demonstrated by Sung and Dague (1992) according to Figure 4.8.

A number of variables influence efficient operation of the ASBR. The most important variables include the organic loading rate (OLR), the hydraulic retention time (HRT), the mixed liquor suspended solids (MLSS) and the solids retention time (SRT). The ratio of the organic loading rate to the MLSS define the F/M ratio, which is important in achieving efficient solids separation. The loading rate and the HRT are selected by the operator prior to start-up.

4.2.3. Start-up of source reactor

Two source reactors were placed in a 35° C incubator. The pumps and the rest of the equipment were placed on a metal shelf outside the incubator. All connections were made using Nalgene tubing. The reactor was filled to the working level of 10 liters with tap water. Natural gas was flushed through the system and the exhaust line was then clamped shut. The system was left for about 24 hours to see whether the gas bags would deflate, indicating the presence of leaks. Some leaks were observed, which were painstakingly located and sealed, usually with silicone caulking.

The water was drained out of the reactors. Granular seed sludge of two different sizes were obtained from the ASBR's of Shihwu Sung, a coworker at Iowa State University, Ames, Iowa. About 2.5 liters of seed sludge were pumped into each reactor. Then 2.5 liters

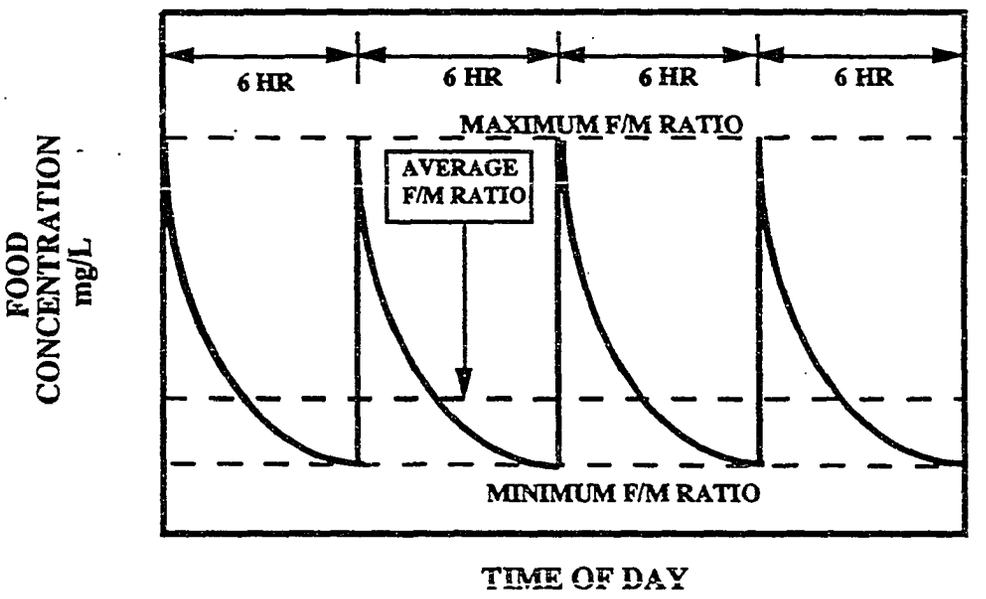


Figure 4.8. Variation in the F/M ratio during ASBR operation (Sung and Dague, 1992)

of non fat dry milk at a strength of 3 gm/L were added, followed by tap water to bring the working volume to 10 liters. The system was flushed for about 10 minutes with natural gas. The reactors were then left for 24 hours to allow the facultative microorganisms present in the seed to remove all available oxygen prior to the first feeding. The operating conditions for the source reactors are given in Table 4.1.

Substrate and nutrients The substrate fed to the source reactors consisted of non fat dry milk (NFDM). It was decided to use this substrate, as it was found by Sung and Dague (1992) to cause and promote granulation in the ASBR . The NFDM is available in a dry powdered form which resists degradation and can be easily measured and diluted to the required strength. The properties of the NFDM are given in Table 4.2. The milk has a consistent COD value of 1.04 gm COD/gm NFDM and has a high protein content which provides nitrogen for cell growth.

To provide the nutrients necessary for microbial growth and reproduction, a trace mineral solution was prepared according to the recipe given in Table 4.3. The trace minerals were added at a rate of 0.1 ml/gm NFDM. This solution was found by previous researchers to be adequate for anaerobic microbial growth (Harris, 1992).

A sodium bicarbonate buffer was added to the reactor feed to keep the pH between 6.8 and 7.2. About 0.25 to 0.50 gm of bicarbonate per gm of NFDM was added, depending on the system pH.

As mentioned previously, the feed was prepared in two, 21-liter plastic carboys every other day and stored in the refrigerator at 4° C to minimize degradation. Each carboy was first partially filled with tap water. The required amount of dry milk powder was measured, and mixed with about one liter of tap water in a kitchen blender, manufactured by Hamilton Beach. The blended milk, sodium bicarbonate and trace mineral solution were added to the plastic carboy and made up to 21 liters with tap water. The feed was then stirred thoroughly

Table 4.1. Operating conditions for the source reactors

System Parameter	Value
Operation temperature	35° C
Organic loading rate	3 gm/L/day as COD
Hydraulic retention time	24 hrs
Number of sequences per day	4
Length of sequence	6 hrs
Total liquid volume	10 L
Volume fed/wasted per sequence	2.5 L
Length of feeding phase	15 - 20 min
Length of decanting phase	10 min
Length of settling phase	20 min

Table 4.2. Properties of the Non Fat Dry Milk (NFDM) substrate

Parameter	Value
Chemical Oxygen Demand, #/# NFDM	1.04
Five-day Biochemical Oxygen Demand, #/# NFDM	0.49
Total Kjeldahl Nitrogen, % as N	5.40
Total Phosphate, % as PO_4	2.20
Lactose, % (1)	51.00
Protein, % (1)	>36.00
Fat, % (1)	<1.00
Ash, % (1)	8.20
Trace Minerals (1):	
Iron, ppm of NFDM	4.60
Nickel, ppm of NFDM	1.00
Cobalt, ppm of NFDM	0.80
Molybdenum, ppm of NFDM	3.00
Zinc, ppm of NFDM	15.00

(1) Source of data is Swiss Valley Farms, Inc., Davenport, Iowa.

Table 4.3. Recipe for trace mineral solution

Chemical	Quantity
$FeCl_2.4H_2O$	35.60 gm/L
$ZnCl_2$	2.08 gm/L
$NiCl_2.6H_2O$	4.05 gm/L
$CoCl_2.6H_2O$	4.04 gm/L
$MnCl_2.4H_2O$	3.61 gm/L

and placed in the refrigerator.

4.2.4. Analytical methods

A number of parameters were measured periodically to monitor and evaluate reactor performance. These include the pH, chemical oxygen demand (COD), suspended solids, gas analysis, volatile acids, alkalinity, and biomass particle size (determined by Automatic Image Analysis). The measurement procedures are described in the following sections.

pH The reactor pH was measured twice weekly, or as often as necessary. A sample of about 30 ml was collected during mixing and analyzed immediately to minimize errors due to carbon dioxide release. The pH was measured using a digital Altex pH meter, model 4500,

fitted with a standard glass membrane -type probe manufactured by Markson Corporation. The probe was calibrated before each measurement with standard buffers of 7.00 and 10.00. The bicarbonate dosage was adjusted according to pH values to suit reactor conditions.

Chemical Oxygen Demand (COD) The COD is a measure of the oxygen equivalent of organic matter than can be chemically oxidized with a strong oxidizer, such as potassium dichromate. The total and soluble CODs were measured both for the reactor contents and the effluent. The total COD was run on the sample without filtration, while the sample for soluble COD was filtered through a 9 cm GFA glass fiber filter paper with a pore size of 1.5 μm, manufactured by Fisher Scientific, PA. A vacuum pump connected to a filter flask and Buchner funnel was used to filter the samples. The tests were run in duplicate for each sample.

The COD was measured according to the procedure outlined in Standard Methods (1985), section 508B, Oxygen Demand (Chemical, closed reflux, titration method). The digestion vessels were 20 x 150 mm culture tubes, which required the following quantities:

Sample	5 ml
Potassium Dichromate	3 ml
Sulfuric acid reagent	7 ml

The maximum COD values that could be measured with this size culture tube was 480 mg O_2/L. Appropriate dilutions were required for samples having higher CODs. The dilutions were prepared in volumetric flasks.

The COD value was calculated according to the following equation:

$$\text{COD as mg } O_2/L = \frac{(A-B) * M * 8000 * D}{\text{ml of sample}} \qquad (20)$$

where, A = milliliters of ferrous ammonium sulfate (FAS) used to titrate blank

B = milliliters of FAS used to titrate sample

M = molarity of FAS titrant

D = dilution factor of the sample

Solids The total and volatile suspended solids in the mixed liquor and effluent were measured according to the procedures in Standard Methods (1985), sections 209C and 209D, respectively, with the following exceptions:

1. The filter papers were not washed prior to use. Instead blanks were used to determine mass changes.
2. A 10 ml sample size was used in all measurements.
3. Only one cycle of drying, cooling, desiccating and weighing was done.

The 9 cm GFA glass fiber filter papers, manufactured by Fisher Scientific, PA, were used for solids analysis. After filtering, the filter papers were placed in disposable aluminum planchet weighing dishes for drying and weighing. Each sample was run in triplicate, with three blanks being run with each set of solids analysis. The following equations were used to determine the total suspended solids (TSS) and volatile suspended solids (VSS).

$$\text{TSS (mg/L)} = \frac{(A-B+C)\,(1000\ \text{mg/gm})\,(1000\ \text{ml/L})}{\text{sample volume (ml)}} \qquad (21)$$

where, A = weight of filter paper + planchet + dried residue (gm)

B = weight of filter paper + planchet (gm)

C = weight loss of blanks after drying (gm)

$$\text{VSS (mg/L)} = \frac{(A-D+C-E)\,(1000\ \text{mg/gm})\,(1000\ \text{ml/L})}{\text{sample volume (ml)}} \qquad (22)$$

where, D = weight of filter + planchet + residue after ignition (gm)

E = weight loss of blanks after ignition (gm)

Gas Gas analyses were performed once or twice a week as necessary. The gas composition was determined using a Gow-Mac 69-350 Gas Chromatograph (GC) which detected the relative amounts of N_2, CH_4 and CO_2. The system was calibrated with a custom gas standard from Union Carbide Industrial Gases, Inc., Indiana, composed of 5% N_2, 70% CH_4, and 25% CO_2. Specifications of the GC configuration for gas analysis is given in Table 4.4.

Samples for gas analysis were removed from the gas sampling port by inserting a 1 ml gas-lock syringe, manufactured by Hamilton Gas-tight No. 1001 TLL, Hamilton Company, Nevada. The syringe was fitted with a 22 gauge side-bore needle, manufactured by Alltech Inc., Deerfield, Illinois. Three samples were withdrawn and expelled to flush out the syringe. The fourth sample was used for analysis. A sample size of 0.9 ml was used throughout the research. Each sample was run in duplicate to determine the percent concentrations of N_2, CH_4 and CO_2 in the biogas.

Gas analyses were found to be an excellent indicator of leaks in the reactor. The level of N_2 gas in a smoothly operating reactor was generally less than 3%. An increase in the level of N_2 in the reactor gas, indicated the inflow of air into the reactor from outside.

Volatile acids Volatile fatty acids concentrations were determined using the procedure outlined in Standard Methods (1985). As the volatile fatty acids (VFA) are water soluble, they can be selectively removed by distillation. Some VFAs have a higher boiling point than water, so a recovery factor of 0.7 was assumed, as recommended in Standard Methods (1985). The procedure was modified slightly because the distillation apparatus operated at a maximum rate of 2.5 ml/min. Instead of using a phenolphthalein indicator solution, a pH

Table 4.4. GC operating parameters for gas analysis

Item	Specification
Column:	
Packing	Chromosorb P
Packing size	80/100 mesh
Temperature	65° C
Carrier gas:	Helium
Flowrate	60 ml/min
Detector:	Thermal conductivity
Temperature	150° C
Injection block temperature	100° C
Sample size	0.9 ml
Data station	Maxima

probe was used to measure the pH.

The samples were collected in 250 ml flasks. When the sample contained a high level of solids, it was allowed to settle before removing the supernatant. A 100 ml sample was prepared for distillation after adding 100 ml of nano pure water and 5 ml of concentrated sulfuric acid. After distillation, the sample was titrated to a pH of 8.3 using a standardized NaOH solution.

The following equation was used to determine the total volatile acids:

$$\text{Volatile acids, as acetic acid (mg/L)} = \frac{A * N * 60{,}000}{f * \text{ml of sample}} \qquad (23)$$

where, A = volume of NaOH solution used to titrate sample (ml)

N = normality of NaOH solution

f = recovery factor (assumed 0.7)

Alkalinity The total alkalinity of the reactor contents was measured according to the procedures in section 403, Standard Methods (1985). A 25 ml sample was taken from the reactor during mixing and titrated immediately to a pH of 4.5, using a standardized acid solution, usually sulfuric acid. The total alkalinity was determined using the following equation:

$$\text{Total alkalinity (mg/L as } CaCO_3) = \frac{A * N * 50{,}000}{\text{ml of sample}} \qquad (24)$$

where, A = volume of standard acid used in titration (ml)

B = normality of standard acid

Biomass particle size The Automatic Image Analysis (AIA) system was used to

determine the particle size distribution of the biomass in the source reactor. The AIA system consisted of an Olympus BH-2 Optical Microscope, manufactured by Olympus Corporation, Lake Success, New York. The optical image of the sample cells from the microscope was picked up by a video camera connected to the Lemont Image Analysis System, manufactured by Lemont Scientific Inc., State College, Pennsylvania. A schematic of the AIA system is illustrated in Figure 4.9.

The AIA system works on the principle of identifying levels of gray in a visual field. It assigns a number "0" to the pure black level and "255" to the pure white level, and divides the continuum between into 255 zones of gray. The AIA system has the ability to take an image and transform it into 512 x 480 pixel digitized image.

A sample of about 30 ml was removed from the reactor during mixing, for particle counting. This sample was then washed a number of times with nano pure water to remove the flocculent particles. A broken tip pipette was used to remove a small amount of the sample and place it on a sampling cell made from Plexiglass. The plan and elevation of the sample collection cell is illustrated in Figure 4.10. The cell was illuminated by a light from above. Under the microscope, the biomass particles or granules appeared pitch black against a white background. The lower and upper grey levels were specified and adjusted to provide such an image. The particles were measured from a number of frames selected at random from the cell. At least ten frames were counted in each cell to obtain a representative measurement. The AIA system counted the number of particles, along with the size and area of the particles. It also provided a statistical analysis of the particles present in each cell. Before each analysis, the microscope-AIA combination was calibrated for magnification using a standard ruler with a precision of 0.1 mm.

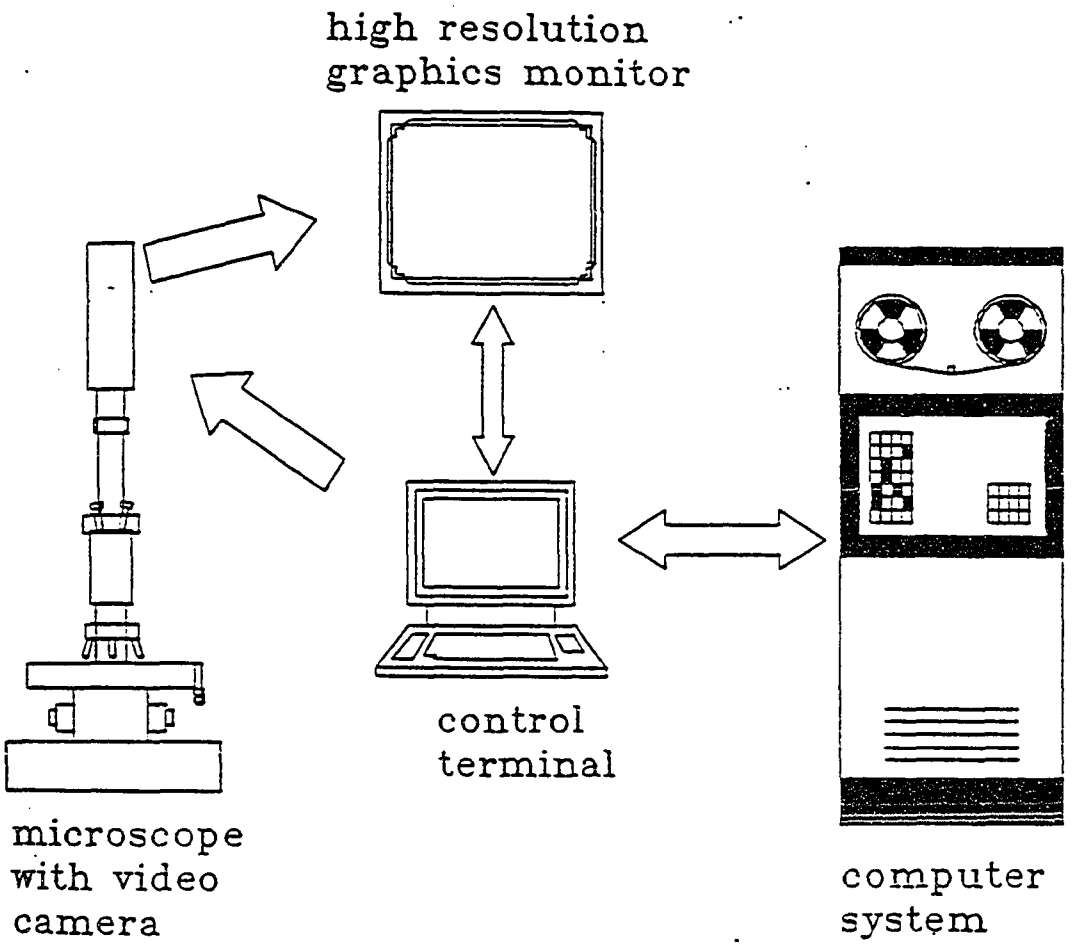
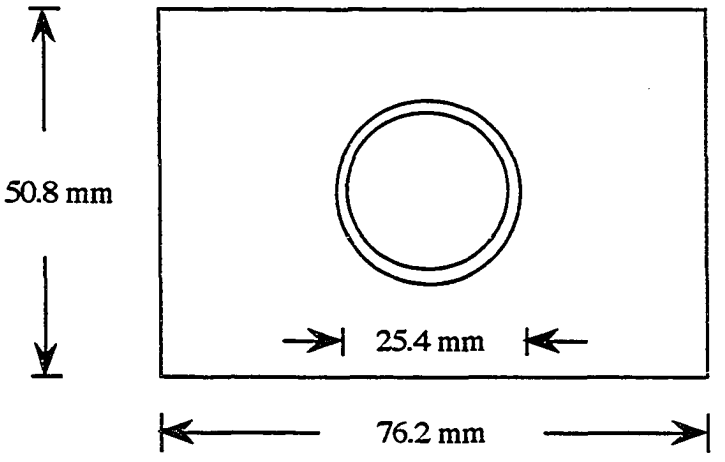
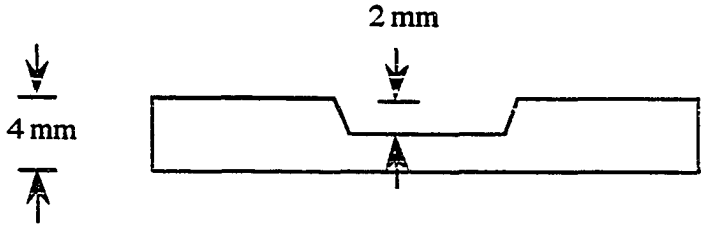


Figure 4.9. Schematic of Automatic Image Analysis (AIA) system



(a) Plan



(b) Elevation

Figure 4.10. Plan and elevation of sample counting cell

4.3. Design of Biosorption Reactor

The biosorption reactor was used to perform the biosorption batch experiments using live anaerobic biomass from the source reactor. The biosorption reactor was made from Plexiglass as illustrated in Figure 4.11. The internal volume was about 3.9 liters, with an internal diameter (ID) of 5.5 in and height of 10 in. A marking tape was calibrated in 0.25 liter increments, according to reactor volume, and attached to the side of the reactor. A 3/8 in ID sampling port was provided near the bottom of the reactor. The port was made from a stainless steel tube reinforced with 1/2 in thick piece of Plexiglass glued to the side of the reactor. A piece of Nalgene tubing was attached to the side port and clamped shut with a Hoffman screw clamp, manufactured by Fisher Scientific, Pennsylvania.

The upper flange was 8 inches in diameter, with 6 holes drilled at equal intervals to accommodate the 1/4 in bolts used to attach the lid. A groove in the flange held a 7 in diameter O-ring, made from flexible rubber, to provide an air-tight seal between the lid and the flange of the reactor.

The details of the lid are illustrated in Figure 4.12. The lid was 8 inches in diameter and made from 1/2 in thick Plexiglass. Similar to the top flange, the lid contained 6 bolt holes and a groove for the O-ring. At the center of the lid was a 3/8 in hole for the shaft of the mechanical mixer. Three 1/4 in ID openings were provided on the lid. A thermometer was inserted through one opening and clamped in a vertical position. The thermometer had a range from -20° C to +110° C, and was manufactured by Fisher Scientific, Pennsylvania. Another opening contained a tube for gas outlet. The third opening contained a stainless steel tube that reached almost to the bottom of the reactor, the height of which could be adjusted as required. This tube was used both for feeding the substrate and for decanting the supernatant with the aid of a variable speed Masterflex pump, manufactured by Cole Parmer Instrument Company, Chicago, Illinois.

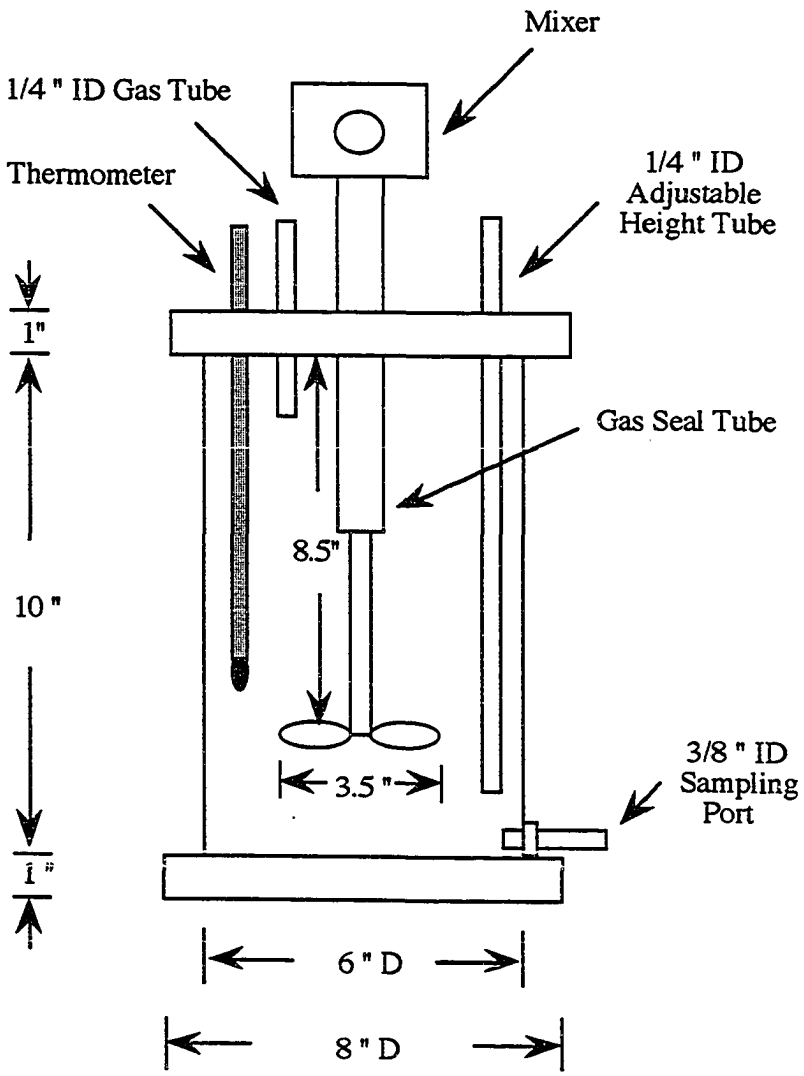
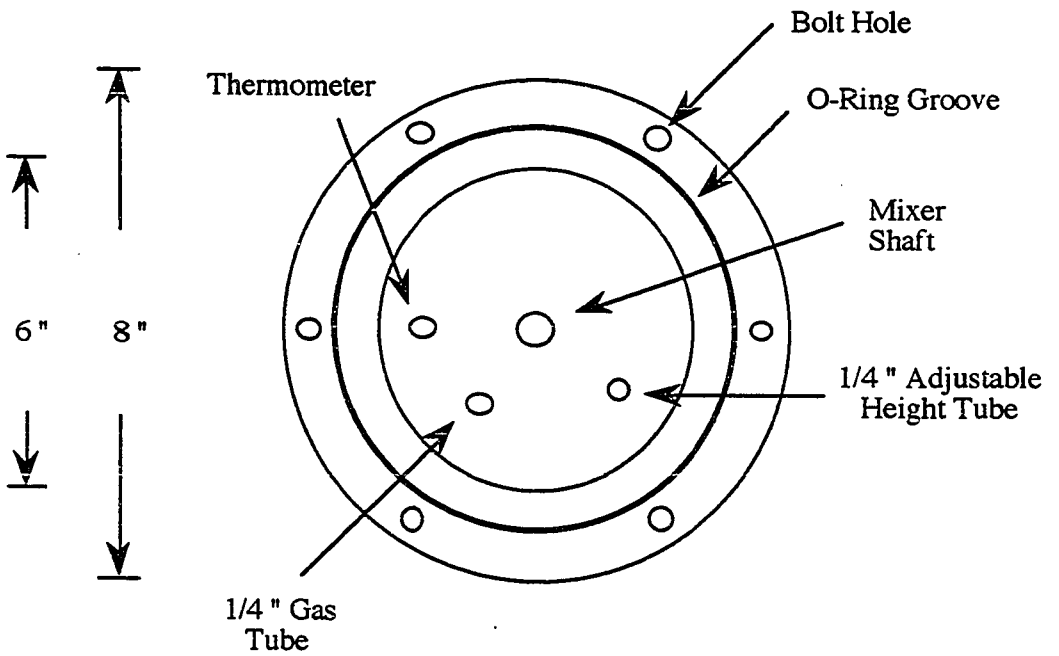
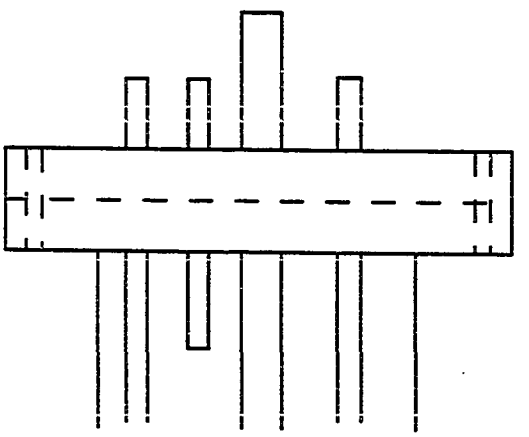


Figure 4.11. Dimensional view of the Biosorption reactor



(a) Plan



(b) Sectional Elevation

Figure 4.12. Plan and section of lid for Biosorption reactor

A T-line Laboratory Stirrer (Cat. No. J-4330-00) manufactured by Cole Parmer Instrument Company, Chicago, Illinois, was used for mixing the reactor contents. The mixer was a dual-shaft variable speed stirrer with a 1/75 hp motor, with speeds ranging from 50 to 750 to 7500 rpm. A three blade propeller with pitched blades (Cat. No. J-4349-00, Cole Parmer Instrument Company, Chicago, Illinois) was mounted on the shaft to provide adequate mixing. The stirrer was mounted on the top of the lid and the shaft was enclosed in a stainless steel casing to a depth below the anticipated liquid level, in order to maintain anaerobic conditions in the reactor during biosorption.

4.4. Experimental Procedure for Biosorption

The substrate was prepared the day before the experiment, so that it was completely homogeneous and at the desired temperature at the time of the experiment. A non-fat dry milk was used as the substrate in all experiments. The properties of the substrate were outlined in Table 4.2. The required amount of milk powder was weighed, put in an appropriate volumetric flask, dissolved in nano pure water by shaking,and made up to the mark with more nano pure water. The flask was then stored at the desired temperature until the experiment.

Prior to the experiment, nitrogen gas was passed through the biosorption reactor for about five minutes to expel existing air and to provide an inert atmosphere inside the reactor. All the outlets were clamped to prevent entry of outside air into the reactor.

When the source reactor (ASBR) was near the end of the react cycle, the mixer was turned on, and the required volume of biomass was drained by gravity from the ASBR to the biosorption reactor through the side port. A 50 ml sample of biomass was also extracted in a beaker for COD and solids analysis.

The biosorption reactor was then placed at room temperature. A tube from the gas outlet was placed in a beaker of water, so that any gas produced would be observed as bubbles in the water. A pump was used to feed the required volume of substrate into the sorption reactor, after a sample of substrate was separated for COD analysis. The mixer was turned on and the reactor contents were mixed vigorously for a specified time. The temperature was noted at the beginning and end of mixing. After mixing, the reactor contents were allowed to settle for a specified time, while the temperature was observed every few minutes. The clear supernatant was decanted from the top by means of a pump, and a sample was separated for COD analysis.

After the experiment, the settled biomass was pumped back into the source reactor. Great care was taken to maintain anaerobic conditions in the biosorption reactor during the entire experiment.

5. EXPERIMENTAL DESIGN

In this section, the different experimental plans that were adopted to investigate the effects of mixing time, substrate concentration, temperature, dilution, biomass particle size and biomass concentration on anaerobic biosorption, will be illustrated.

As mentioned previously, a non-fat dry milk was used as the substrate in the experiments. The biomass was cultivated in two source reactors operated at 35° C. The sorption experiments were carried out at a room temperature of 23° C. The temperature of the substrate was varied as required. Substrate concentrations ranging from 100 mg/L to 8000 mg/L as COD, were used. In one set of experiments, the highest substrate concentration used was 16,000 mg/L COD. At least nine different substrate concentrations were used to determine the effect of each variable.

Figures 5.1 to 5.6 illustrate the experimental plans adopted for investigation of the different variables. An adequate mixing time was selected from the results of the first set of experiments (Figure 5.1) and that was used in all subsequent experiments. A higher mixing time was used in cases where film diffusion was suspected to be a limiting factor at the pre-selected mixing time. A settling period of 15 minutes was found adequate for producing a clear supernatant, and that was used in all experiments. Figure 5.6 illustrates the plan to observe the effect of dilution. Only two dilutions were chosen. Higher dilutions could not be tested due to size limitation of the biosorption reactor.

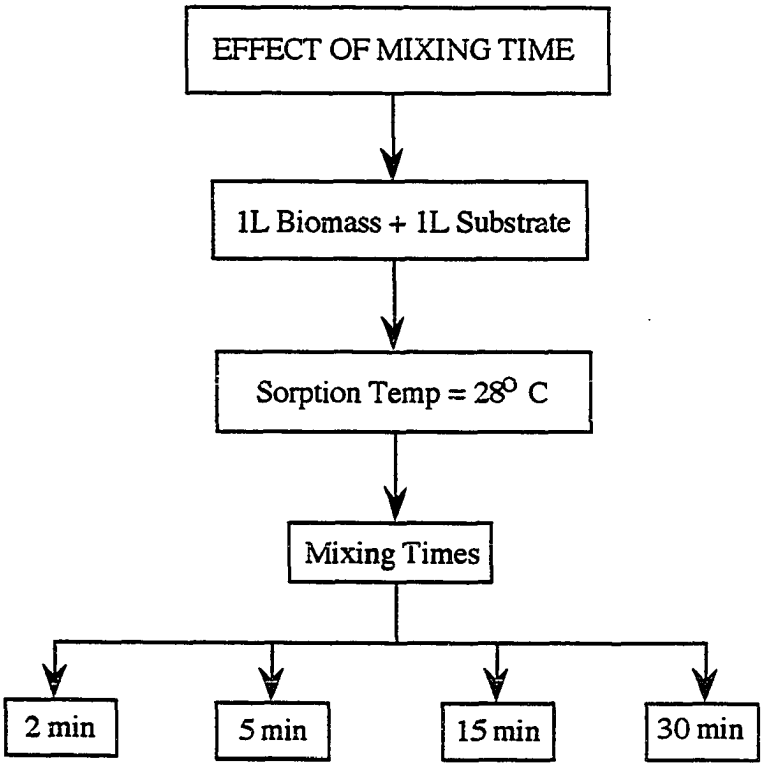


Figure 5.1. Experimental plan to investigate the effect of mixing time on biosorption

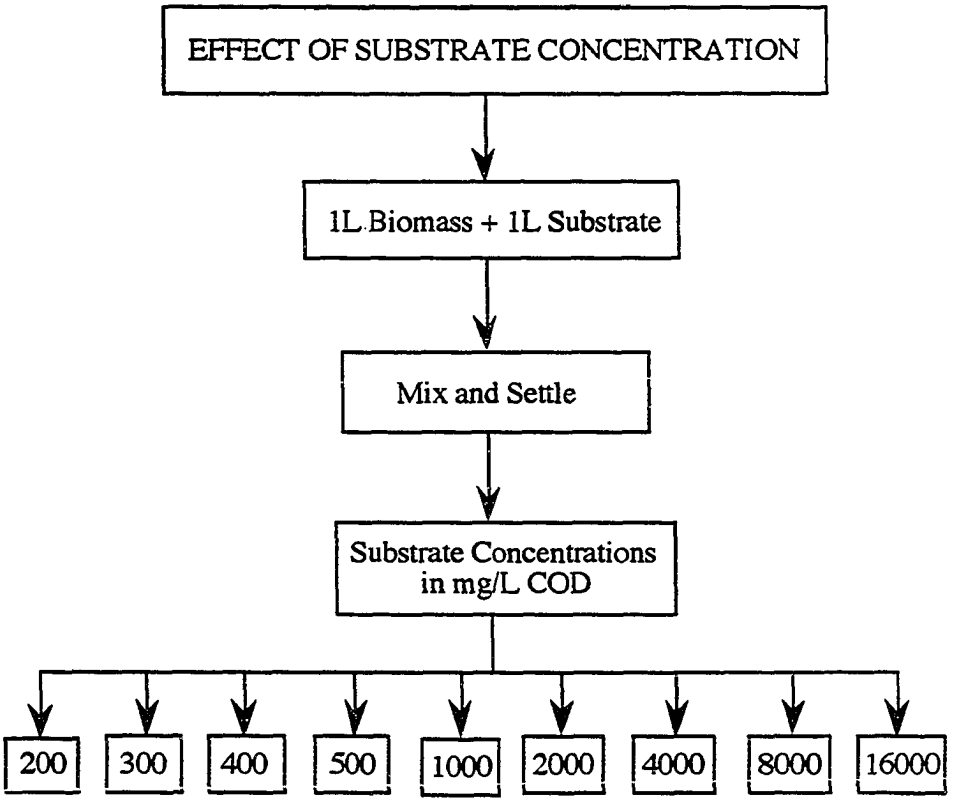


Figure 5.2. Experimental plan to observe the effect of different substrate concentrations on biosorption

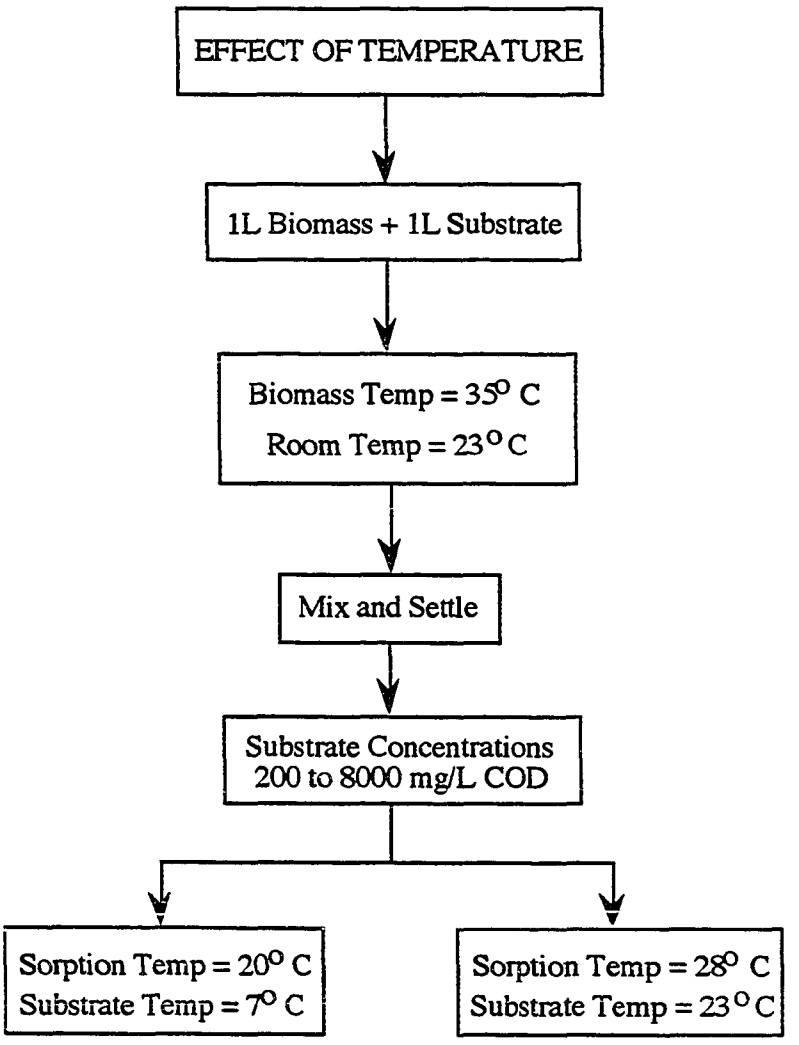


Figure 5.3. Experimental plan to investigate the effect of temperature on biosorption

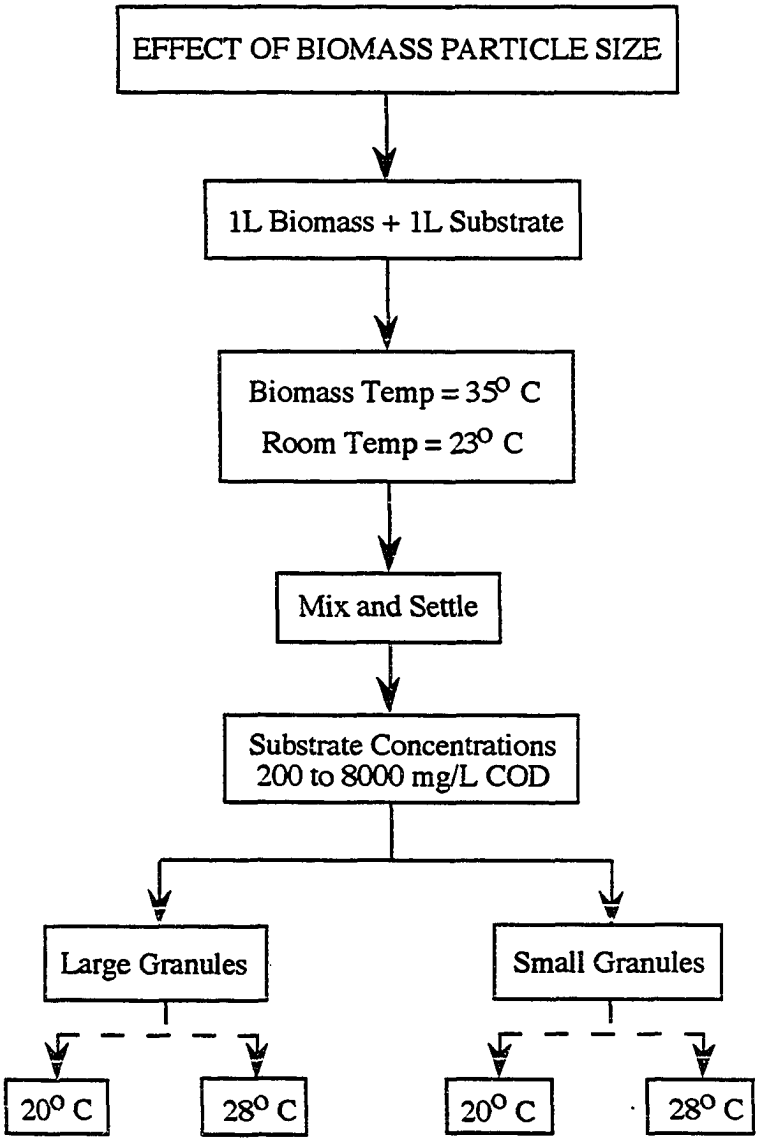


Figure 5.4. Experimental plan to investigate the effect of granular biomass particle size on biosorption

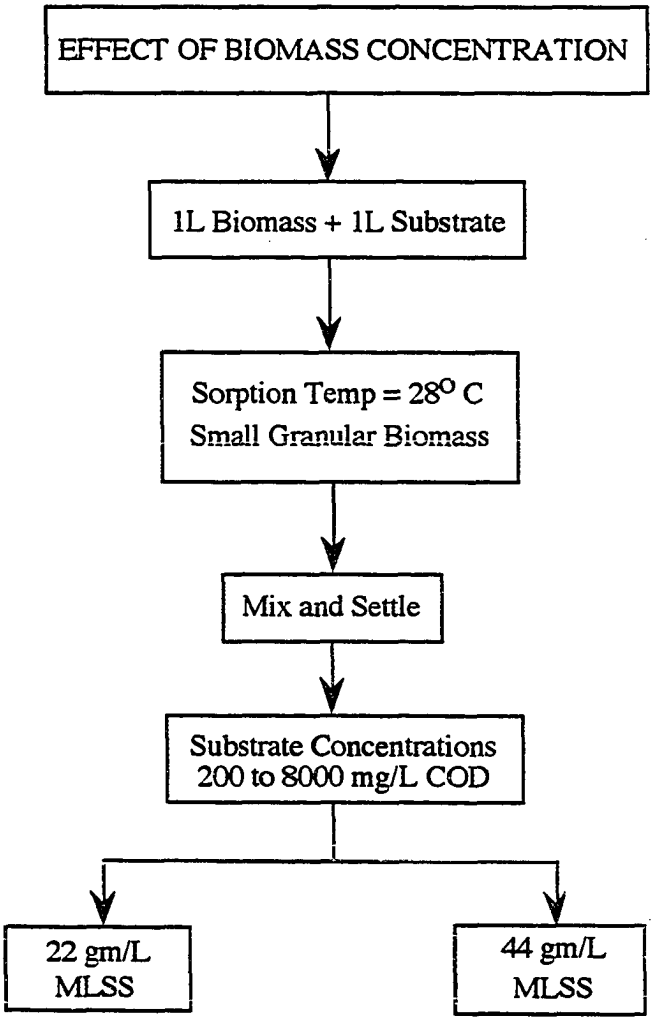


Figure 5.5. Experimental plan to investigate the influence of biomass concentration on biosorption

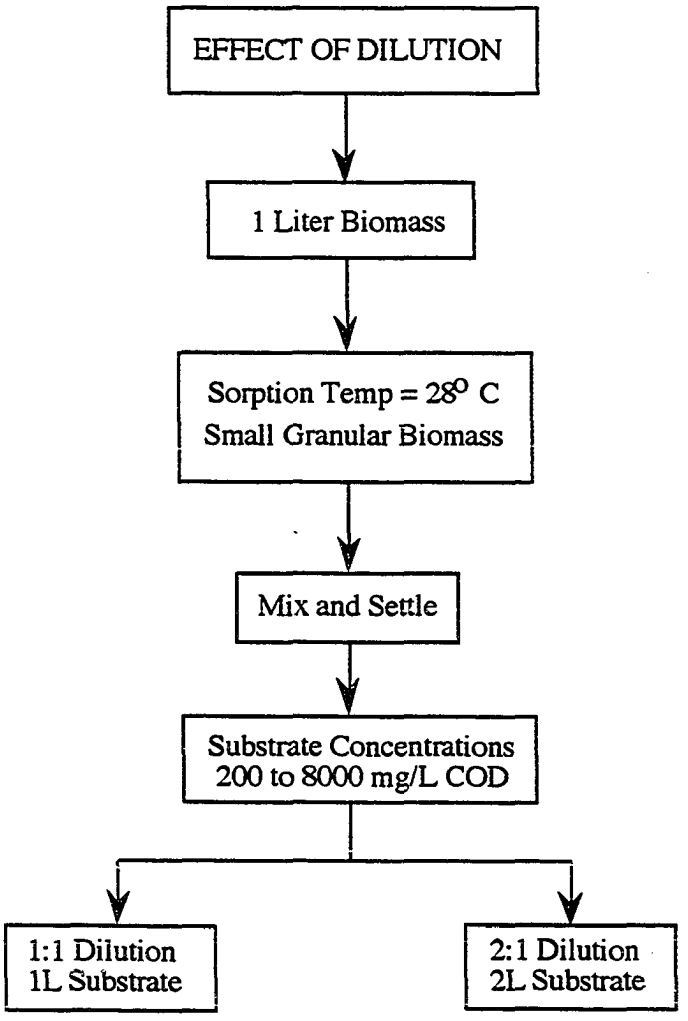


Figure 5.6. Experimental plan to observe the effect of dilution on biosorption

6. RESULTS, DISCUSSION AND CONCLUSIONS

6.1. Results

6.1.1. General

Experimental investigations were conducted to evaluate the different factors affecting anaerobic biosorption, according to the plans outlined in the previous chapter. The results of the biosorption experiments are presented in graphical form. The effects on biosorption of mixing time, biomass particle size, temperature, substrate concentration, dilution and biomass concentration were investigated. The removal of organic matter was measured as the COD of filtered samples. A synthetic milk substrate was used in all experiments. The results are also expressed in terms of isotherms.

6.1.2. Equilibrium achievement

The time required to achieve equilibrium was first investigated. This was done by measuring the COD of filtered samples of the supernatant from the biosorption reactor at regular intervals. The result is illustrated in Figure 6.1, which is a plot of COD versus contact time. From the figure it is evident that biosorption was completed in two minutes. About 22% removal of COD was achieved within the first two minutes. During this short period of time, adsorption of substrate onto the biomass was the primary mechanism for removal of organic matter, since gas production was observed to be negligible. Additional COD removals taking place even after 60 minutes of contact time were not significant.

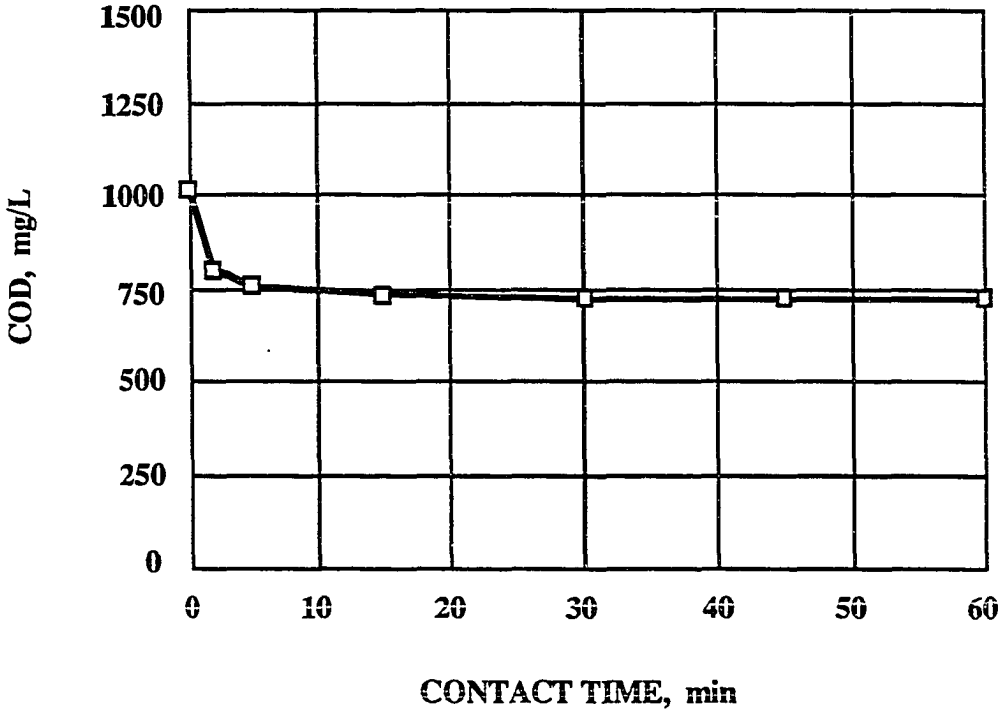


Figure 6.1. Equilibrium achievement in the biosorption reactor

6.1.3. Mixing time

The effect of mixing time was investigated using four different mixing times. The experimental plan is illustrated in Figure 5.1. Equal volumes (one liter of each) of substrate and biomass were used. The sorption temperature was 28° C. The results are illustrated in Figure 6.2. The four curves represent 2, 5, 15 and 30 minutes mixing respectively, at the same intensity and the same initial substrate and biomass concentrations. The mixing intensity was 750 rpm. This corresponded to a velocity gradient (G) of 770 sec^{-1} (calculations are given in Appendix C). The results indicate that two minutes of mixing gave removals almost as good as at longer mixing times. Within the first 15 minutes, there is not much difference between the four mixing times. In all cases, the COD decreased from 1015 mg/L to about 700 mg/L. This initial period of time is when biosorption takes place. Therefore, it was decided that a routine of two minutes mixing at 750 rpm, followed by 15 minutes settling would be adopted in all biosorption experiments.

6.1.4. Granular biomass particle size

Two source reactors were seeded with granular biomass of two different sizes, one large and one small. The particle size distributions of the granular biomass were determined at the time of the sorption experiments, using the Automatic Image Analysis (AIA) system. The procedure for particle measurement with the AIA is described in the Analytical Methods section 4.2.4. The results from the AIA are presented graphically in Figures 6.3 and 6.4 for the small and large granules respectively. The X-axis represents the area-equivalent circular diameter, and the Y-axis represents the percent of total particles with that diameter. In case of the small granules (Figure 6.3), the particles are distributed mainly between the diameter range of 0.39 and 2.66 mm. The large granules (Figure 6.4) are distributed between 0.573 and 3.90 mm diameters. A statistical analysis of the results provided by the AIA are

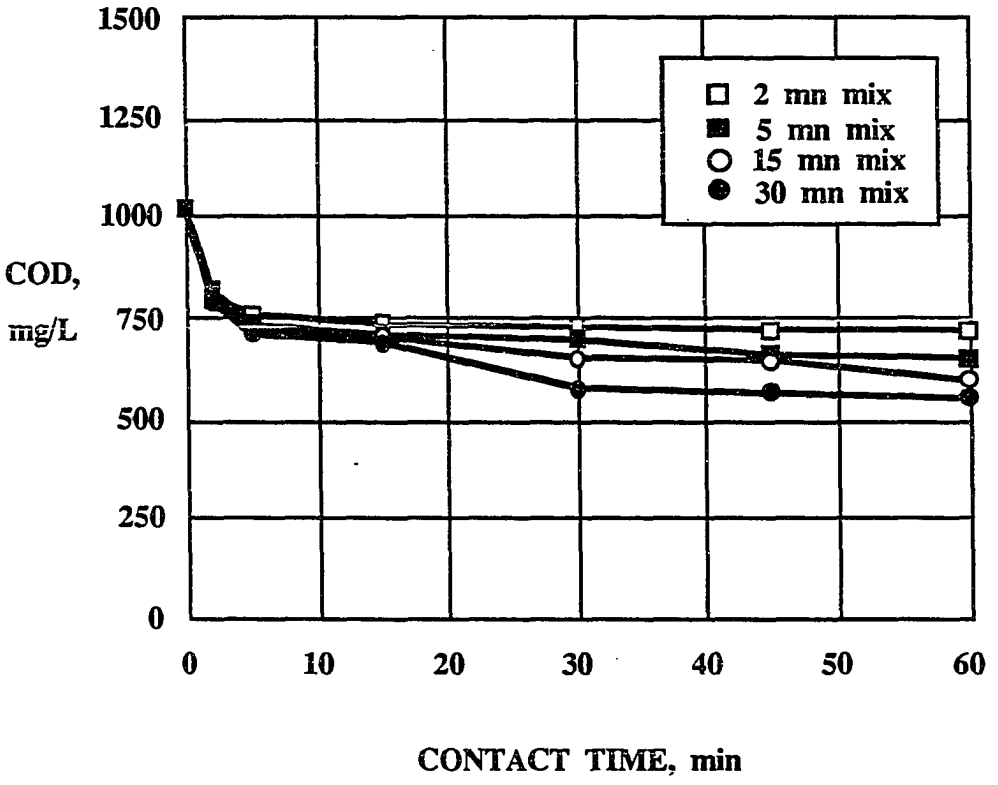


Figure 6.2. Effect of mixing time on soluble COD reduction

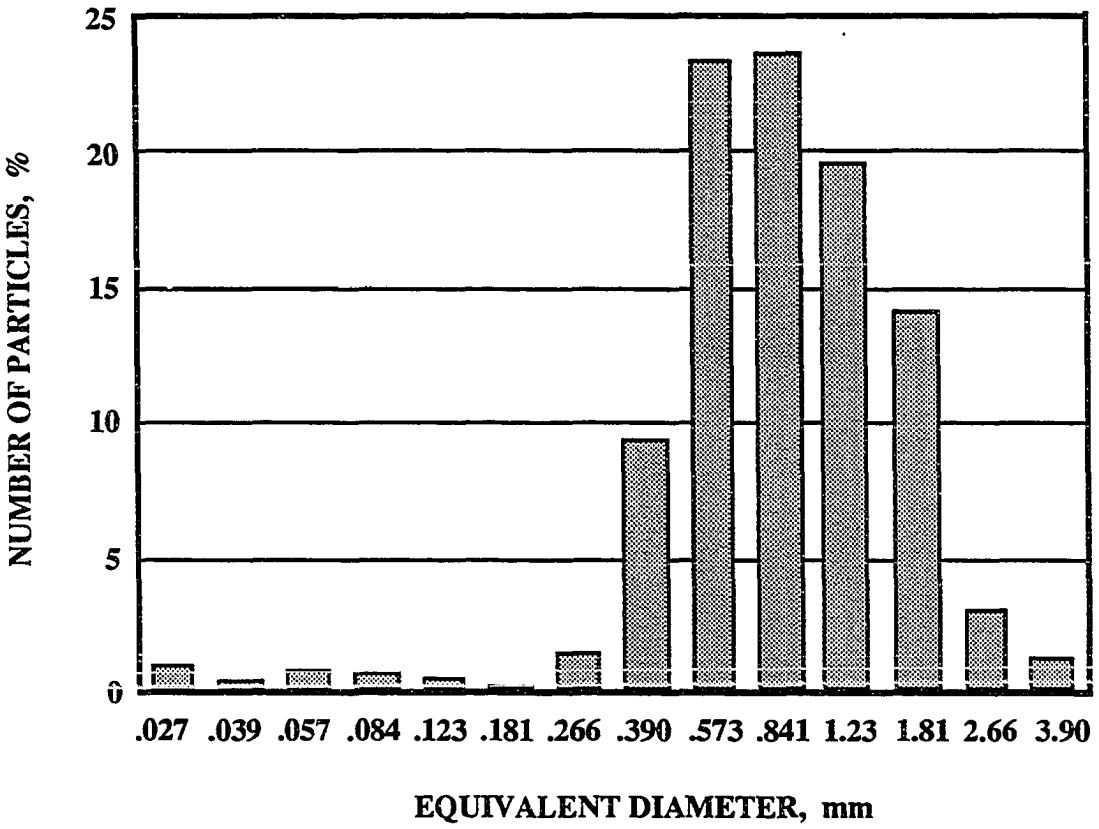


Figure 6.3. Particle size distribution of the small granular biomass

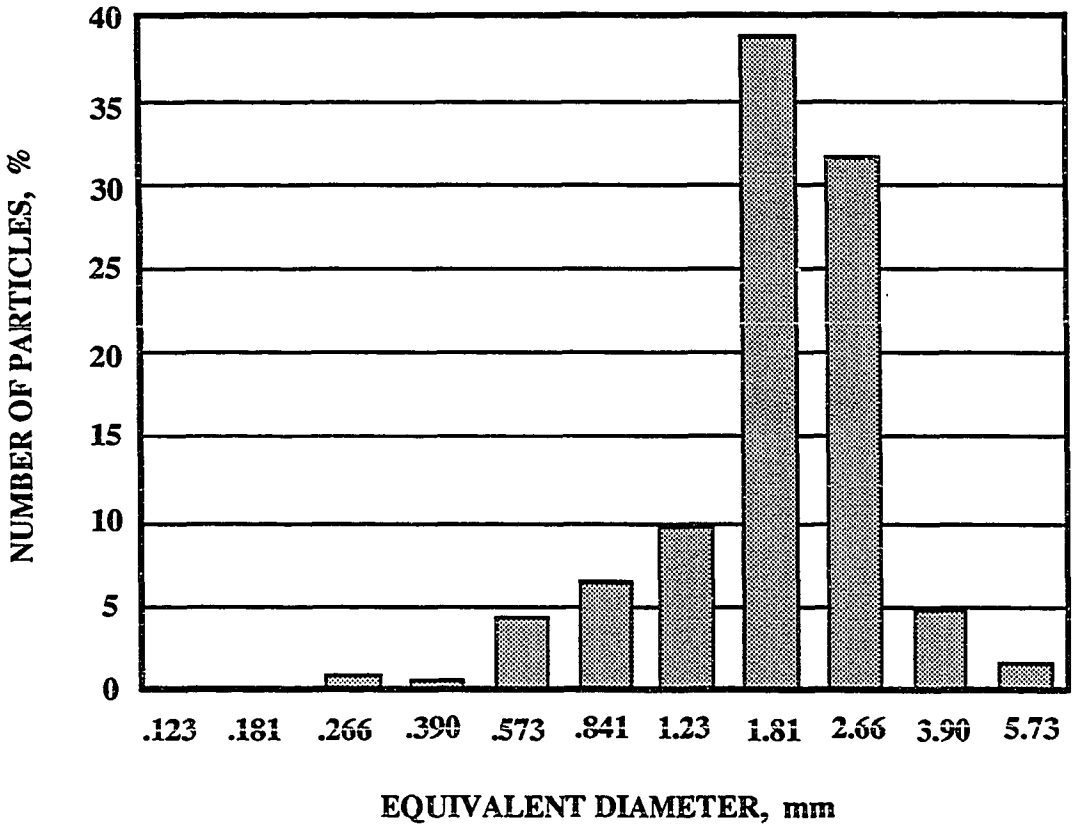


Figure 6.4. Particle size distribution of large granular biomass

Table 6.1. Classification of small and large granules as measured by the Automatic Image Analysis (AIA) system

	Small granules	Large granules
Most probable diameter (mm)	0.841	1.811
Largest equivalent diameter (mm)	3.790	4.960
Smallest equivalent diameter (mm)	0.027	0.033
Median equivalent diameter (mm)	0.821	1.414

presented in Table 6.1.

The experimental plan to investigate the effect of biomass particle size on biosorption is illustrated in Figure 5.4. The difference in biosorption between the small and large granules were characterized by measuring the COD removals obtained at various influent substrate concentrations. The calculation of initial COD concentrations and subsequent removals based on mass balance is demonstrated in Appendix D. Substrate concentrations ranged from COD values as low as 200 mg/L up to 8000 mg/L, and were similar to those outlined in Figure 5.2. The average biomass concentration taken from the source reactor was 22 gm/L total suspended solids. One liter of substrate was mixed with one liter of biomass, mixed intensely for two minutes and allowed to settle for 15 minutes. The filtered COD of the clear

supernatant was measured. Figure 6.5 illustrates the effect of biomass particle size on biosorption at 28° C. The sorption temperature of 28° C resulted from mixing 35° C biomass with 23° C substrate at a room temperature of 23° C. Each point on the graph represents the result of an individual sorption experiment. The curve for the small granules is the result of 10 separate experiments, and that for the large granules is the result of 9 separate experiments. For the small granules, about 38 percent COD removal was obtained at the lower influent concentrations. The removal was reduced to less than 10 percent at 3800 mg/l influent COD. For the large granules, the COD removals varied from 40 percent to near zero at 3800 mg/L initial COD. Overall higher removals were obtained with the small granules than with the large ones. This may be due to the availability of an overall larger surface area in case of the small granular biomass. Saturation occurred around initial COD of 3000 mg/L for the small granules, and at initial COD between 1000 to 2000 mg/L for the large granules.

A similar removal trend was observed with the two types of granules at a sorption temperature of 20° C. This is illustrated in Figure 6.6. The sorption temperature of 20° C resulted from mixing 35° C biomass with 7° C substrate at a room temperature of 23° C. At this lower temperature, there was not much difference in biosorption at the lower substrate concentrations, especially up to an initial COD of 500 mg/L . But at the higher concentrations, the removals were much lower with the large granules than with the small granules. Saturation occurred at initial COD values between 500 and 1000 mg/L for the large granules, and between 2000 to 2800 mg/L for the small granules. For the small granules, COD removals ranged from 38 to about four percent before saturation. For the large granules, COD removals ranged from 33 to one percent indicating lower biosorption.

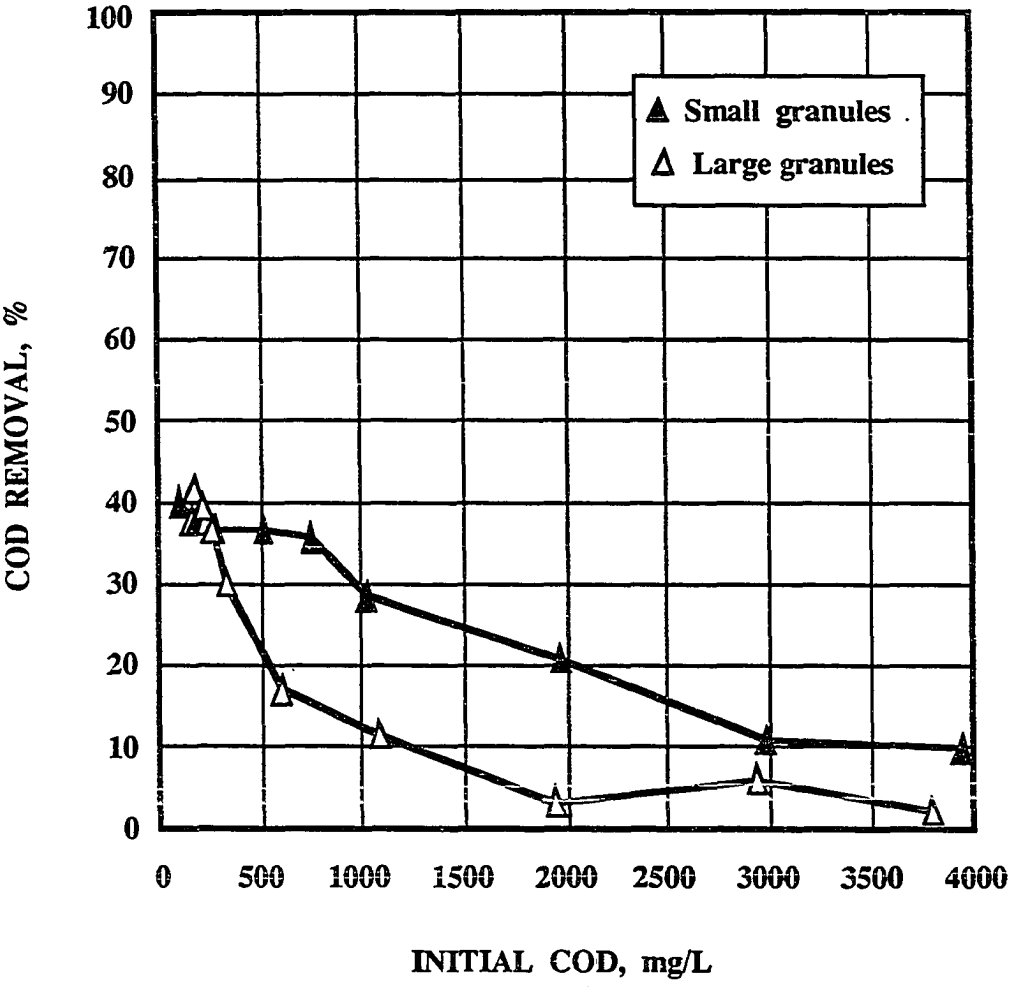


Figure 6.5. Effect of biomass particle size on COD removal by biosorption at 28° C (1: 1 volume, 2 minutes mixing, 15 minutes settling)

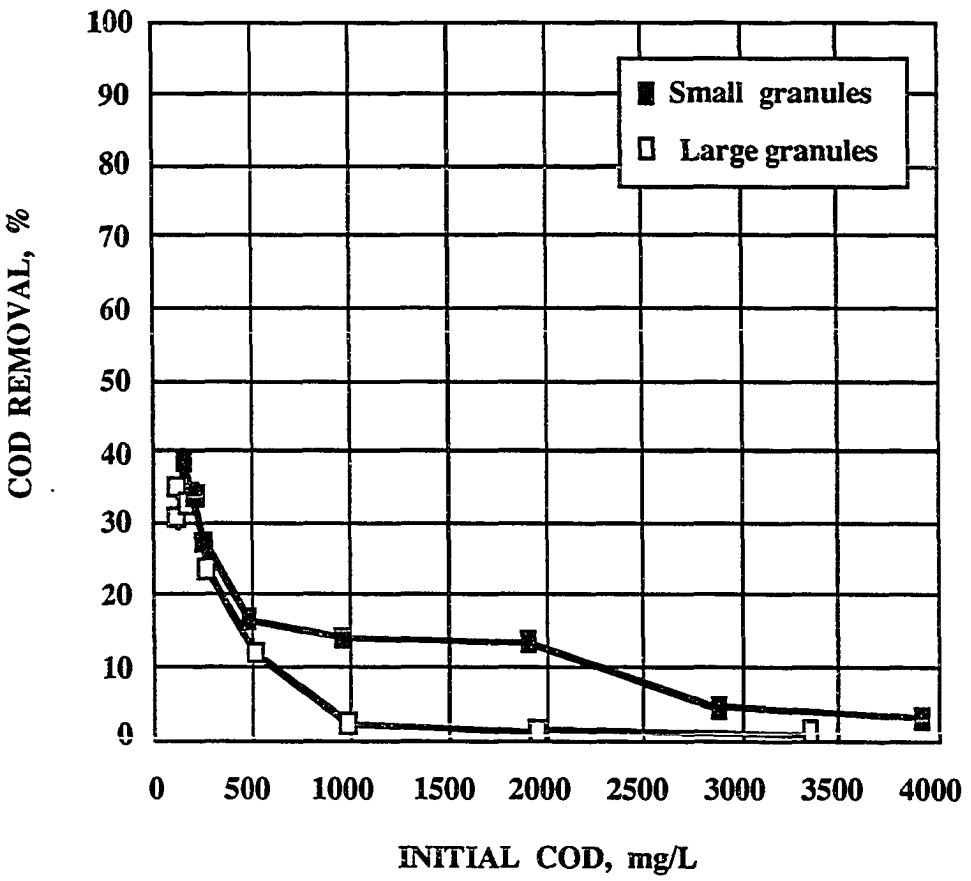


Figure 6.6. Effect of biomass particle size on COD removal by biosorption at 20° C
(1: 1 volume, 2 minutes mixing, 15 minutes settling)

6.1.5. Effect of temperature on biosorption

A major focus of this research was the treatment of low strength, low temperature wastes without the application of heat. Substrates at two different temperatures were utilized for biosorption. The substrate concentrations were similar to those outlined in Figure 5.2. As before, equal volumes of substrate and biomass were used. The sorption temperature was measured at the end of two minutes mixing. This temperature was found to remain constant up to the end of the settling phase. A sorption temperature of 20° C resulted from mixing 7° C substrate with 35° C biomass at a room temperature of 23° C. Similarly, a sorption temperature of 28° C was obtained when 23° C substrate was mixed with 35° C biomass at room temperature. Figure 5.3 illustrates the experimental plan to investigate the effect of temperature on biosorption.

Figure 6.7 is a comparison of the COD removals at 28° C and 20° C for the small granular biomass. At both temperatures, about 38 percent removals were obtained at very low influent substrate concentrations. Overall removals were higher at 28° C than at 20° C. The COD removals ranged from 40 to 10 percent at 28° C. At 20° C, COD removals varied from 38 to four percent. These results are encouraging, since the 20° C sorption temperature represents a substrate temperature of only 7° C. This demonstrates that the biosorption process has high potential for application to the pretreatment of low temperature waste streams without the application of external heat.

The COD removals at the two temperatures for the large granular biomass are illustrated in Figure 6.8. The effect of temperature is not very pronounced with the large granules. The removal was higher at 28° C than that at 20° C. Figure 6.9 represents the sorption isotherms for the large granular biomass at the two temperatures. It is a normal plot of the equilibrium biomass uptake (Qe) versus the equilibrium effluent COD (Ce). The uptake is calculated as mg COD removed per gm suspended solids. The COD removed is the

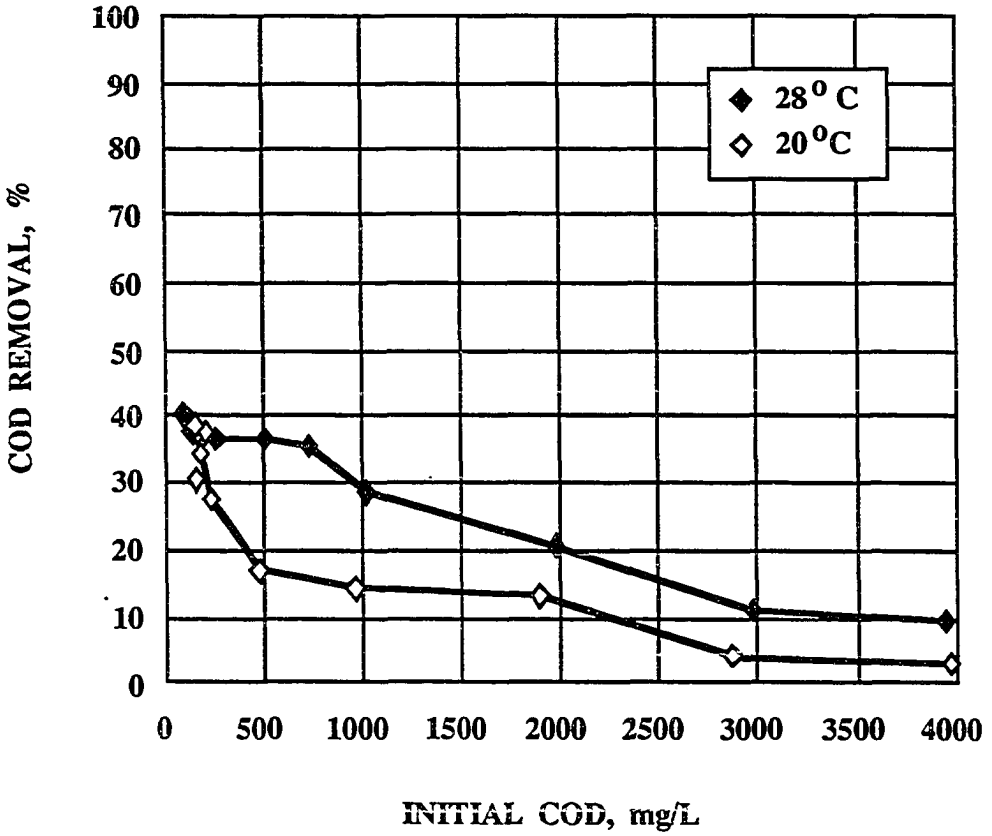


Figure 6.7. Removal of COD by biosorption at two different temperatures by the small granular biomass (1: 1 volume, 2 minutes mixing, 15 minutes settling)

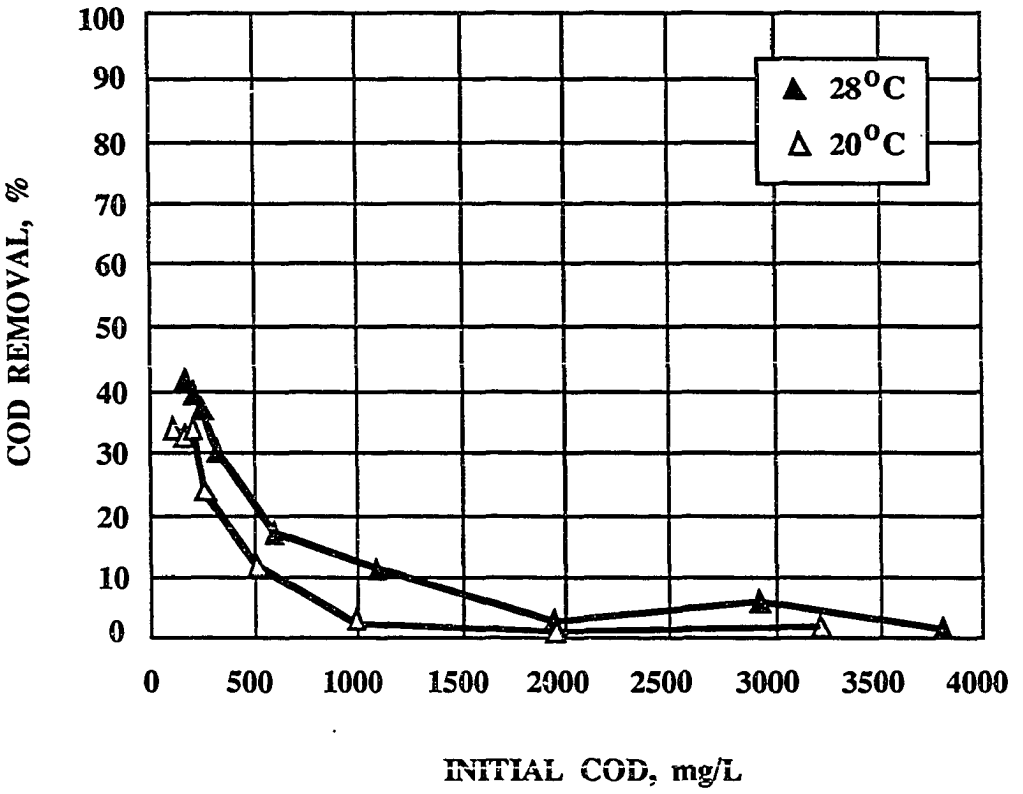


Figure 6.8. Removal of COD by biosorption at two different temperatures by the large granular biomass (1: 1 volume, 2 minutes mixing, 15 minutes settling)

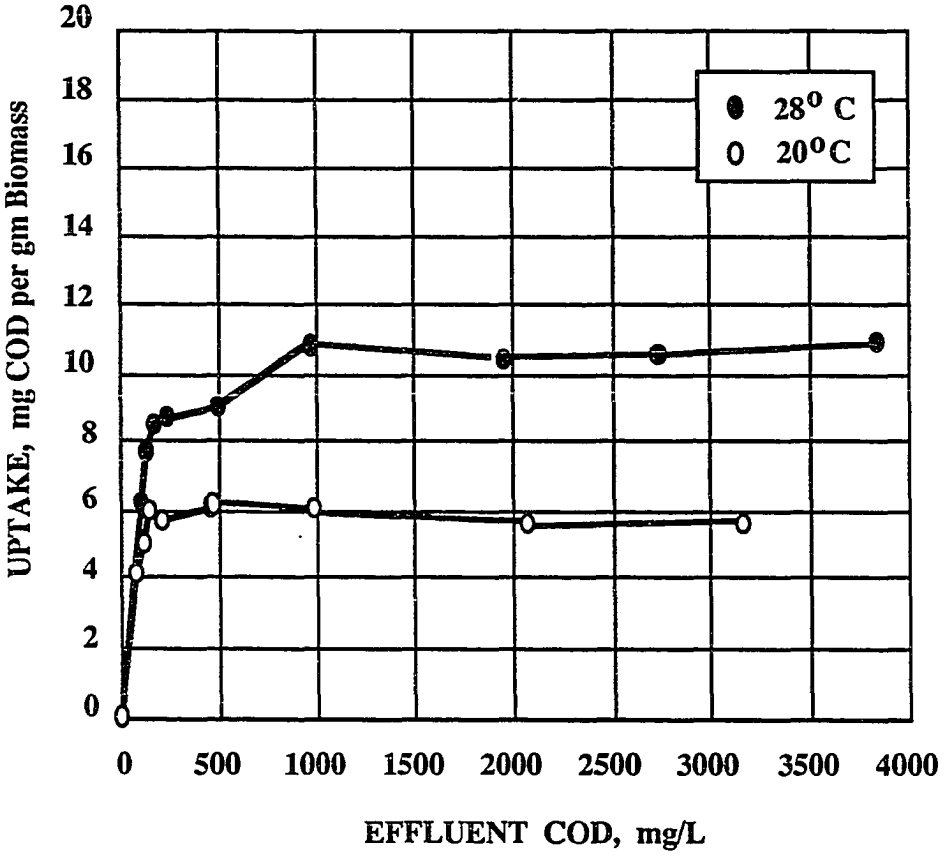


Figure 6.9. Sorption isotherms for large granular biomass at two temperatures (1: 1 volume, 2 minutes mixing, 15 minutes settling)

difference between the initial and effluent COD concentrations. The figure illustrates a marked increase in biosorption with increasing temperature for the large granules. From the graph, maximum uptake at 28° C was 10 mg/gm and at 20° C was 6 mg/gm.

Figure 6.10 illustrates the sorption isotherms for small granular biomass. The uptake at 20° C is similar to that at 28° C, at effluent concentrations below 200 mg/L COD. But at higher concentrations, the uptake at 20° C was much lower than that at 28° C. Since the uptake was similar at lower concentrations, it appeared that film diffusion may be a limiting factor at higher substrate concentrations at 20° C for the small granules. It was decided to test this theory by performing a series of biosorption experiments at 20° C with a mixing time of four minutes, which was twice the time previously used. Figure 6.11 is a comparison of isotherms at the two mixing times and temperatures for the small granules. The increased mixing time did improve the uptake at higher substrate concentrations, indicating that film diffusion was previously limiting biosorption. The maximum uptake at 28° C was 48 mg/gm and at 20° C with four minutes mixing was 36 mg/gm. Previously the uptake was 24 mg/gm at 20° C with two minutes mixing.

6.1.6. Adsorption model

As mentioned previously, one of the most important characteristics of an adsorbent is the quantity of adsorbate that it can accumulate. A number of models that are commonly used to describe adsorption phenomena in wastewater treatment was discussed in the literature review section. Before modeling the biosorption data, a number of graphs were drawn to obtain a general idea of the relationship between influent and effluent substrate concentrations. Figures 6.12 and 6.13 illustrate the relationship between the initial and equilibrium effluent COD concentrations at 28° C for the small and large granular biomass, respectively.

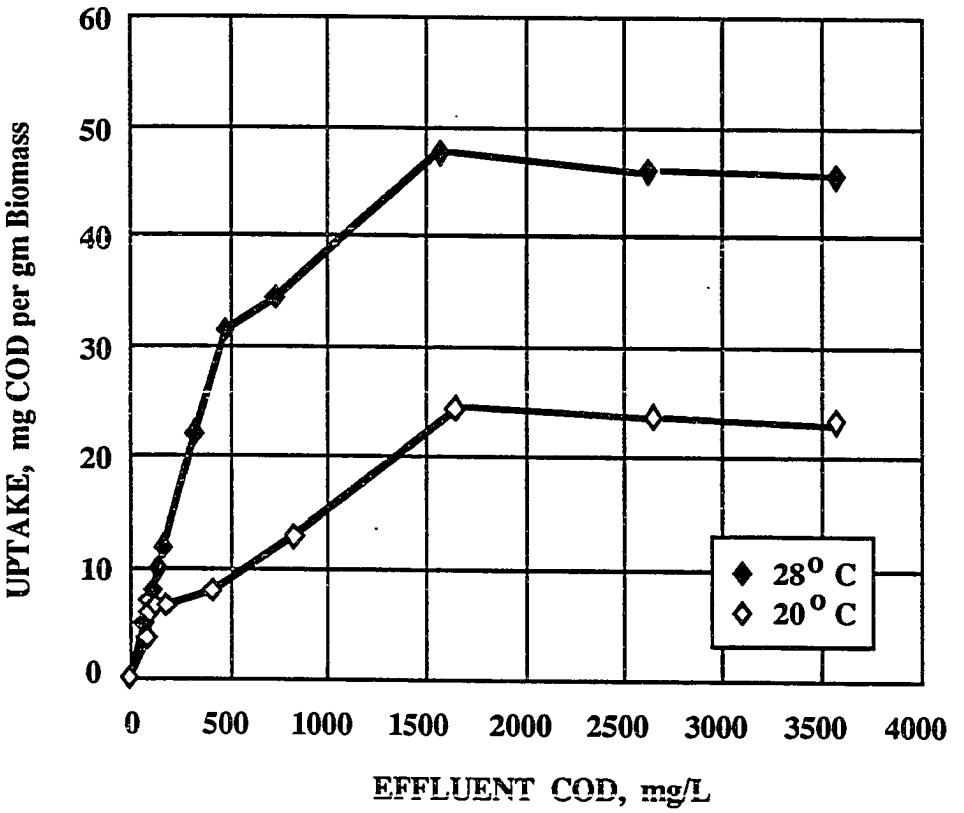


Figure 6.10. Sorption isotherms for small granular biomass at two temperatures (1: 1 volume, 2 minutes mixing, 15 minutes settling)

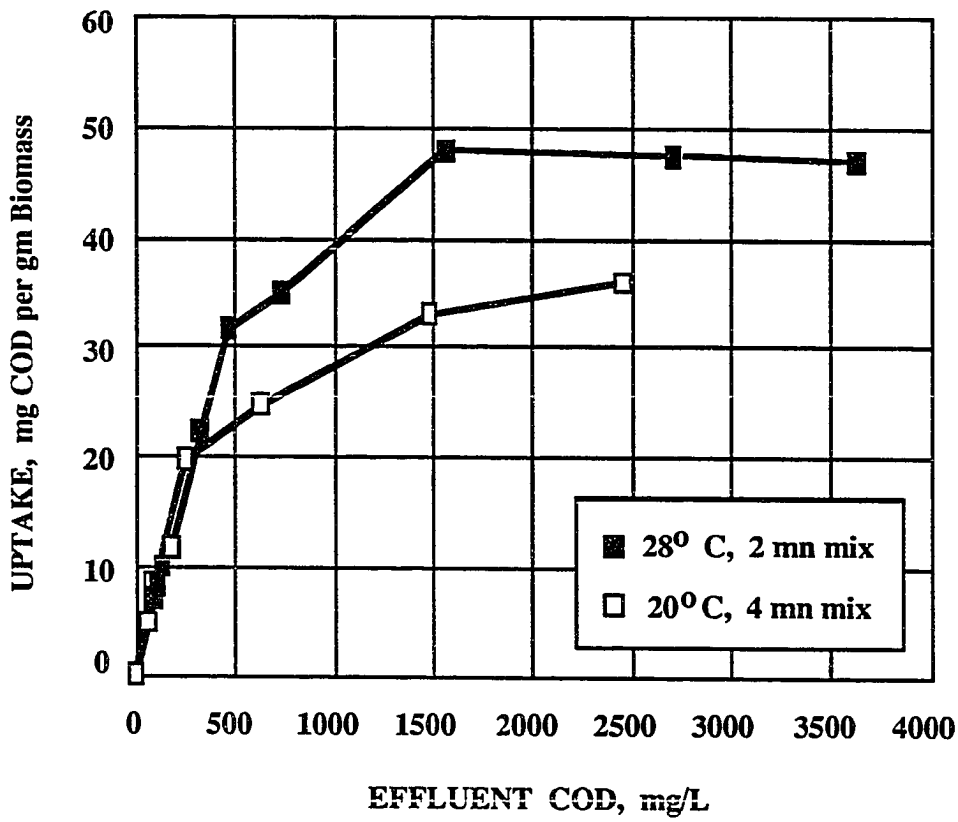


Figure 6.11. Sorption isotherms for small granular biomass at two different mixing times (1:1 volume, 15 minutes settling)

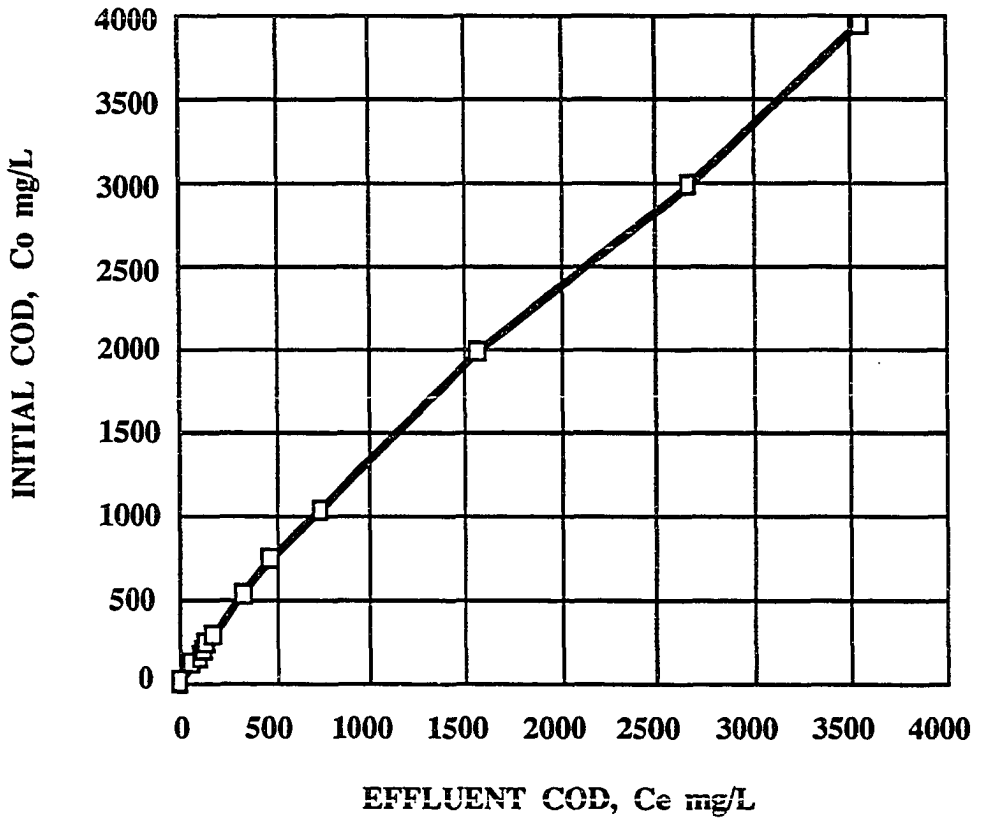


Figure 6.12. Graphical representation of influent versus equilibrium effluent COD concentrations for small granules at 28° C (1:1 volume, 15 minutes settling)

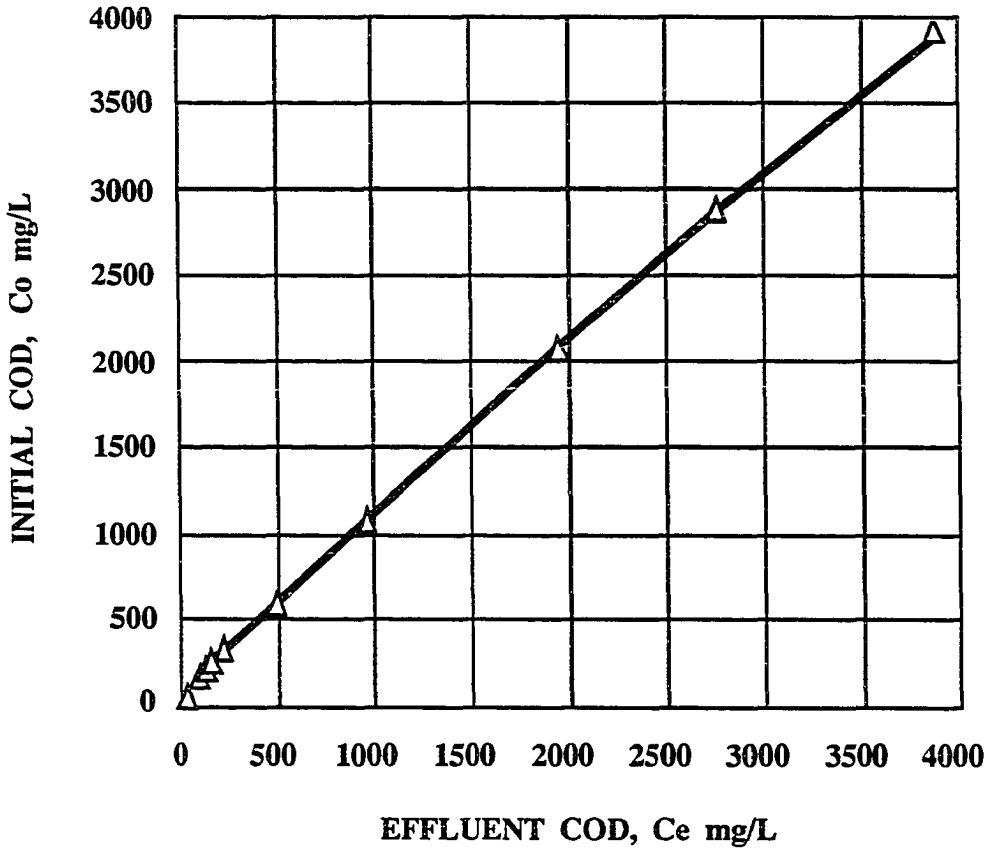


Figure 6.13. Graphical representation of influent versus equilibrium effluent COD concentrations for large granules at 28° C (1:1 volume, 15 minutes settling)

An attempt was made to fit the biosorption data to existing adsorption models. The Freundlich model has been found useful in describing adsorption of organic compounds, especially in biological systems (Benefield et al., 1982). As discussed previously, the Freundlich equation is given by:

$$Qe = K\,(Ce)^{1/n} \qquad (A)$$

where, Qe = Equilibrium uptake of substrate on biomass, mg COD removed/gm biomass = (Co - Ce)/M

Co = Initial soluble COD of substrate, mg/L

Ce = Equilibrium soluble COD of effluent, mg/L

M = Total suspended solids in biomass, gm/L

K = Constant related to capacity of adsorbent for the adsorbate

1/n = Constant related to strength of adsorption

The Freundlich equation can be linearized as:

$$\log (Qe) = \log (K) + 1/n \log (Ce) \qquad (B)$$

A logarithmic plot of Qe versus Ce should yield a straight line for adsorption data that follow the Freundlich theory. This was found to be true for the biosorption data obtained from these experiments. Figure 6.14 illustrates the Freundlich isotherms for the large and small granules at 28° C under identical sorption conditions (one liter of 23° C substrate mixed with one liter of 35° C biomass, two minutes mixing followed by 15 minutes settling at a room temperature of 23° C). There is a marked difference between the two isotherms. The isotherm for the large granules has a flat slope compared to that for the small granules. This indicates a small increase in uptake with increased substrate concentrations for the large granules. Much higher uptake is obtained with the small granules at increased substrate

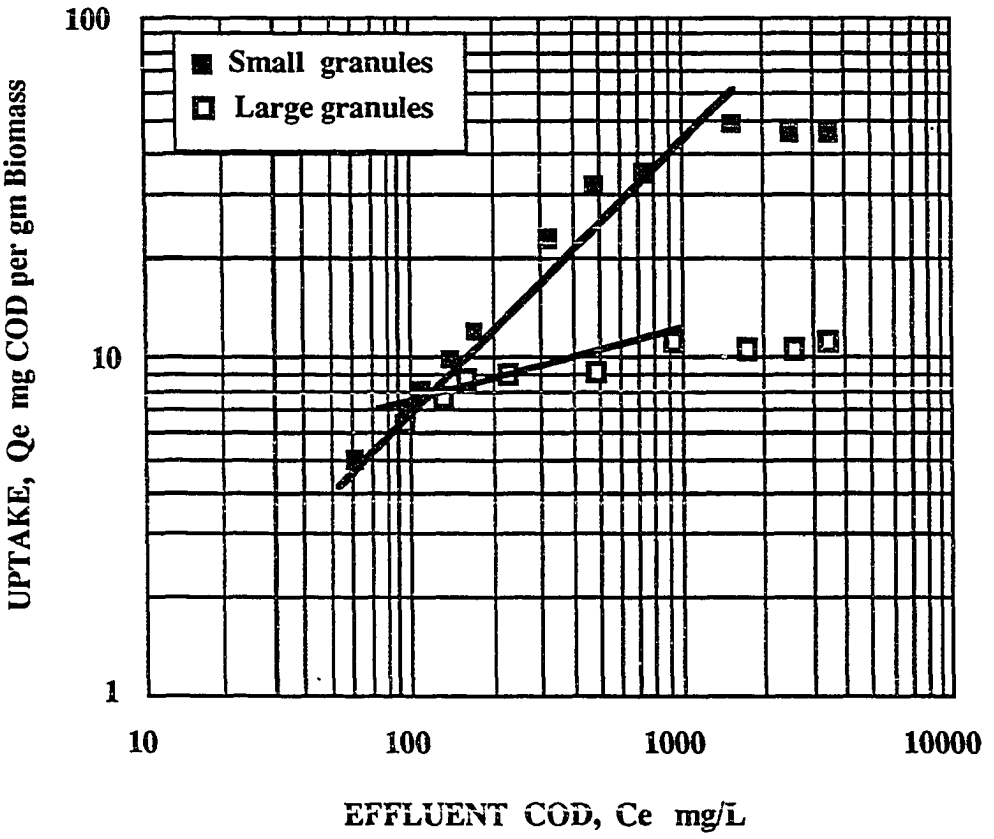


Figure 6.14. Freundlich isotherms at 28° C (1:1 volume, 2 minutes mixing, 15 minutes settling)

concentrations. At effluent concentrations below 100 mg/L COD, the uptake is slightly higher with the large granules. A lower value of 1/n of 0.20 for the large granules as compared to 0.75 for the small granules, indicates stronger adsorption bonds.

Figure 6.15 illustrates the Freundlich isotherms for the two types of granular biomass at 20° C. Sorption conditions involved adding one liter of 7° C substrate to one liter of 35° C biomass, two minutes of mixing followed by 15 minutes settling at a room temperature of 23° C, with average biomass concentrations (from the ASBR) of about 20 gm/L. At a sorption temperature of 20° C, the trend is similar to the one observed at the higher temperature. At the lower substrate concentrations the uptake was similar for both types of biomass. At higher substrate concentrations the uptake decreased for the large granules. It was observed previously that film diffusion was limiting biosorption for the small granules at 20° C (Figure 6.11), so another set of experiments were performed with the small granules at 20° C with twice the mixing time as before. Figure 6.16 illustrates the Freundlich isotherms for the small granules at the two mixing times at 20° C. There is a dramatic improvement due to the increased mixing time. The slopes are identical indicating similar adsorption bonds. But the uptake is increased significantly at all substrate concentrations. The Freundlich parameters obtained at the temperatures for the different types of biomass are presented in Table 6.2. The Freundlich parameters 'K' and '1/n' were obtained from regression analyses of the biosorption data. The regression coefficient is denoted by r^2.

Figures 6.17, 6.18 and 6.19 are the Langmuir isotherms plotted with the biosorption data for the different granules at the two temperatures. A good fit was obtained in most cases. This indicates that chemisorption was probably occurring in the biosorption reactors. Table 6.3 presents the Langmuir constants obtained under different conditions. The Langmuir equation was previously discussed in section 3.4.2.

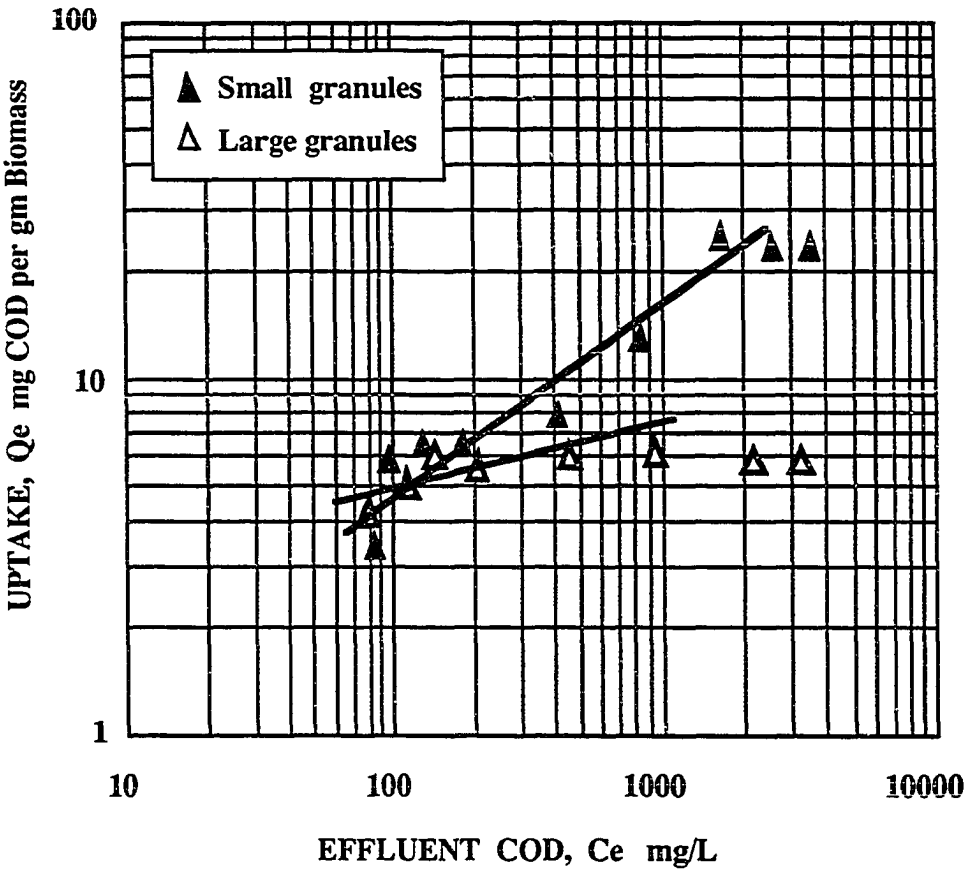


Figure 6.15. Freundlich isotherms at 20° C (1:1 volume, 2 minutes mixing, 15 minutes settling)

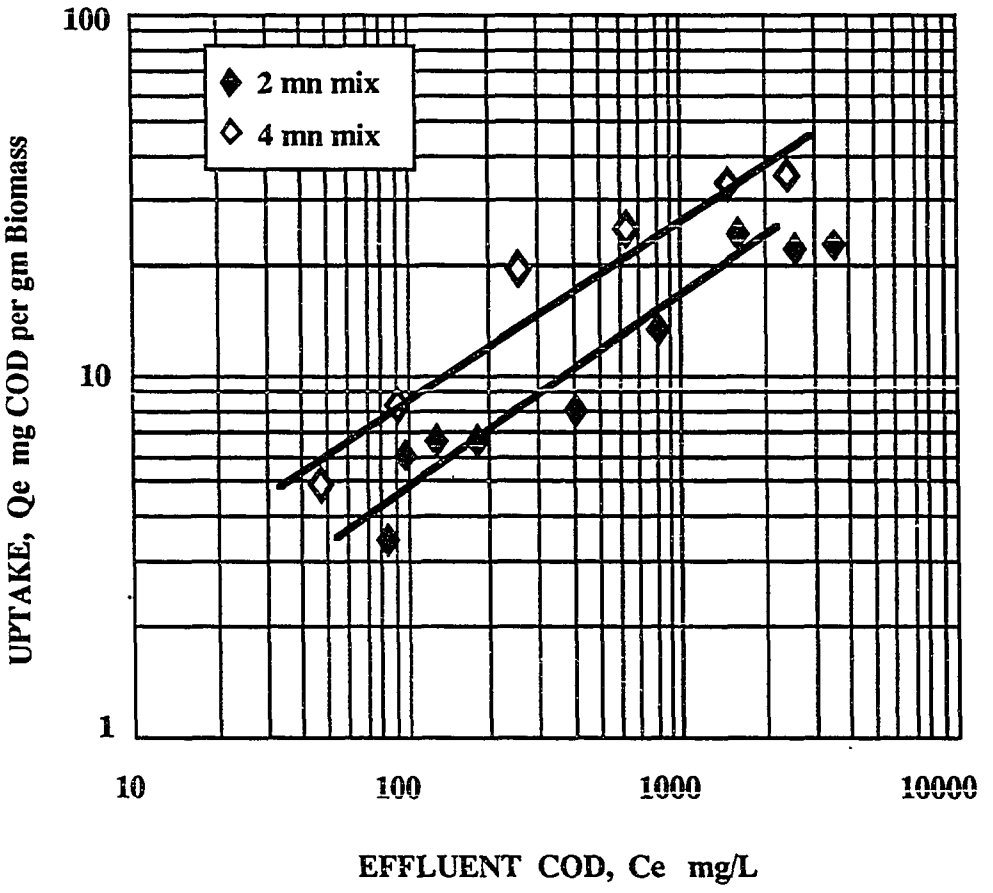


Figure 6.16. Freundlich isotherms at 20° C for the small granules at two different mixing times (1:1 volume, 15 minutes settling)

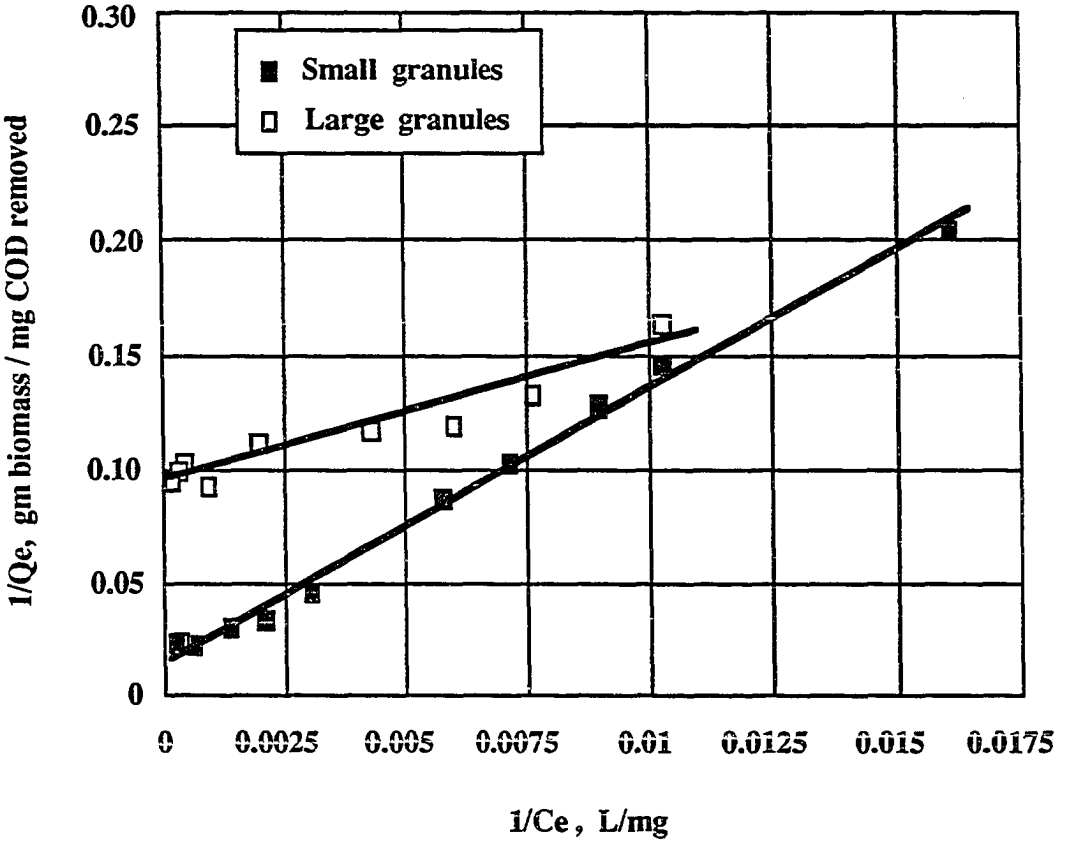


Figure 6.17. Langmuir isotherms at 28° C (1:1 volume, 2 minutes mixing, 15 minutes settling)

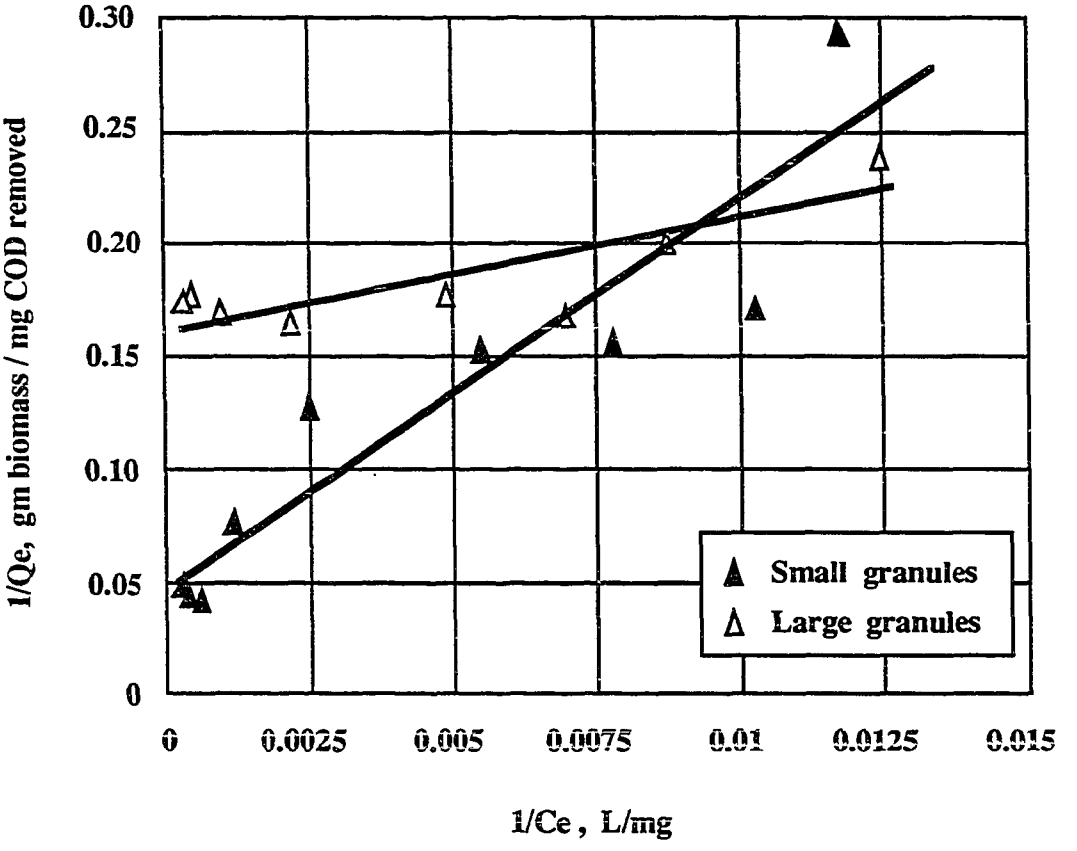


Figure 6.18. Langmuir isotherms at 20° C (1:1 volume, 2 minutes mixing, 15 minutes settling

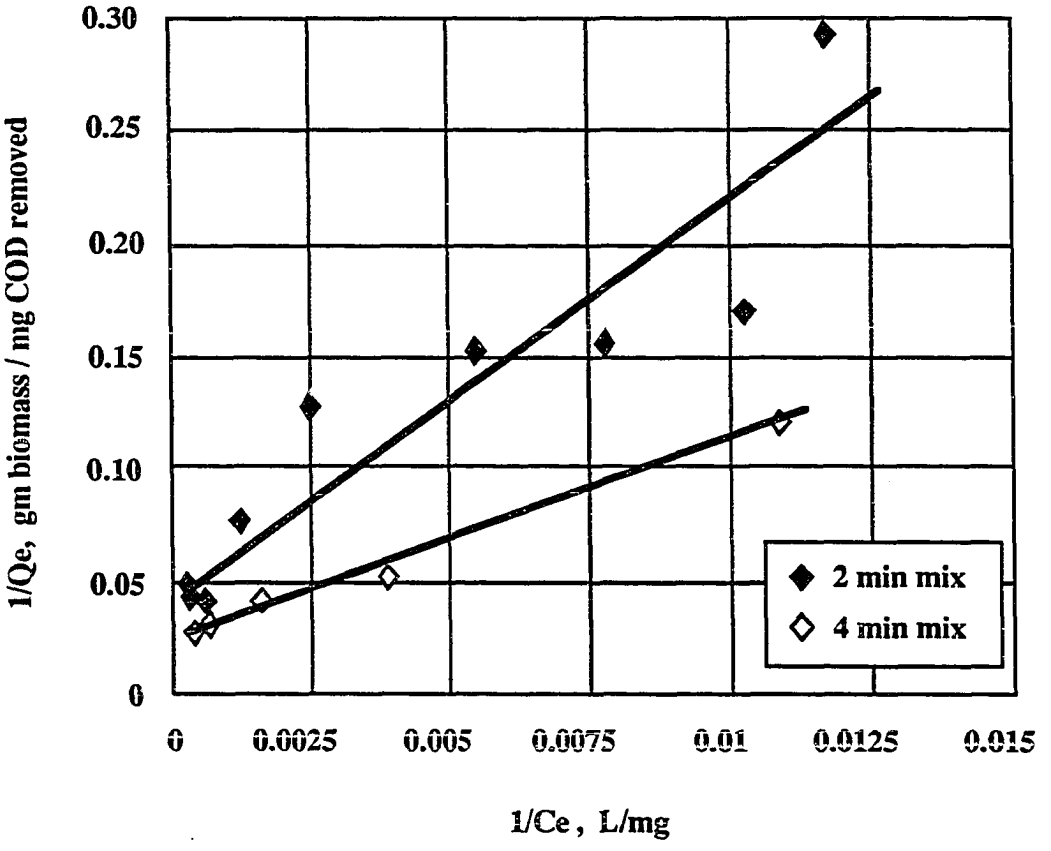


Figure 6.19. Langmuir isotherms at 20° C for the small granules at two different mixing times (1:1 volume, 15 minutes settling)

Table 6.2. Summary of Freundlich parameters for small and large granular biomass at two different temperatures

Granules	Temp °C	Mixing	Data points	K (mg/gm)(L/mg)$^{1/n}$	1/n	r^2
Small	28	2 min	9	0.24	0.75	0.97
Small	20	2 min	7	0.42	0.53	0.90
Small	20	4 min	6	0.86	0.50	0.94
Large	28	2 min	6	2.82	0.20	0.83
Large	20	2 min	5	2.07	0.19	0.64

Table 6.3. Summary of Langmuir constants for small and large granular biomass at two different temperatures

Granules	Temp °C	Mixing	Data points	Q_o (mg/gm)	b (L/mg)	r^2
Small	28	2 min	11	70.76	0.0012	0.99
Small	20	2 min	9	21.22	0.0028	0.86
Small	20	4 min	6	43.07	0.0027	0.99
Large	28	2 min	9	10.80	0.0160	0.91
Large	20	2 min	8	6.18	0.0357	0.66

6.1.7. Biomass concentration

The effect of biomass concentration on biosorption was investigated according to the experimental plan illustrated in Figure 5.5. Two different suspended solids concentrations of the small granular biomass were used. One concentration was almost twice as high as the other. Sorption conditions involved adding one liter of substrate to one liter of biomass, with two minutes of mixing followed by 15 minutes of settling at 28° C. Sorption temperature of 28° C resulted from mixing 35° C biomass with 23° C substrate at a room temperature of 23° C.

Figure 6.20 illustrates the soluble COD removals obtained at various influent substrate concentrations, for two different concentrations of large granular biomass. Higher removals were obtained with 22 gm/L (44 gm/L MLSS) biomass as compared with those obtained at 10 gm/L (20 gm/L MLSS). Soluble COD removals varied from 58 to 13 percent for the 22 gm/l biomass, while 40 to 10 percent removals were obtained with 10 gm/L biomass. This indicates that the uptake can be increased by increasing the biomass concentration, but the increase is not in proportion to the increase in biomass. There are two main reasons for this. The first is, due to the structure of the granules, a large portion of the core of the granules is not available for adsorption. So even though biomass concentration is increased, the number of available sites is not increased proportionately. The second reason is that adsorption follows the Freundlich model. So, as soon as equilibrium is achieved, no further adsorption takes place. Figure 6.21 illustrates the Freundlich isotherm at the two biomass concentrations, under identical sorption conditions at 28° C. All the data points appear to lie on the same straight line. This was expected as the same type of biomass was used for both experimental conditions and they followed the Freundlich theory. The Freundlich parameters corresponding to these data points are: $K = 0.11$ and $1/n = 0.87$ with a correlation coefficient $r^2 = 0.93$.

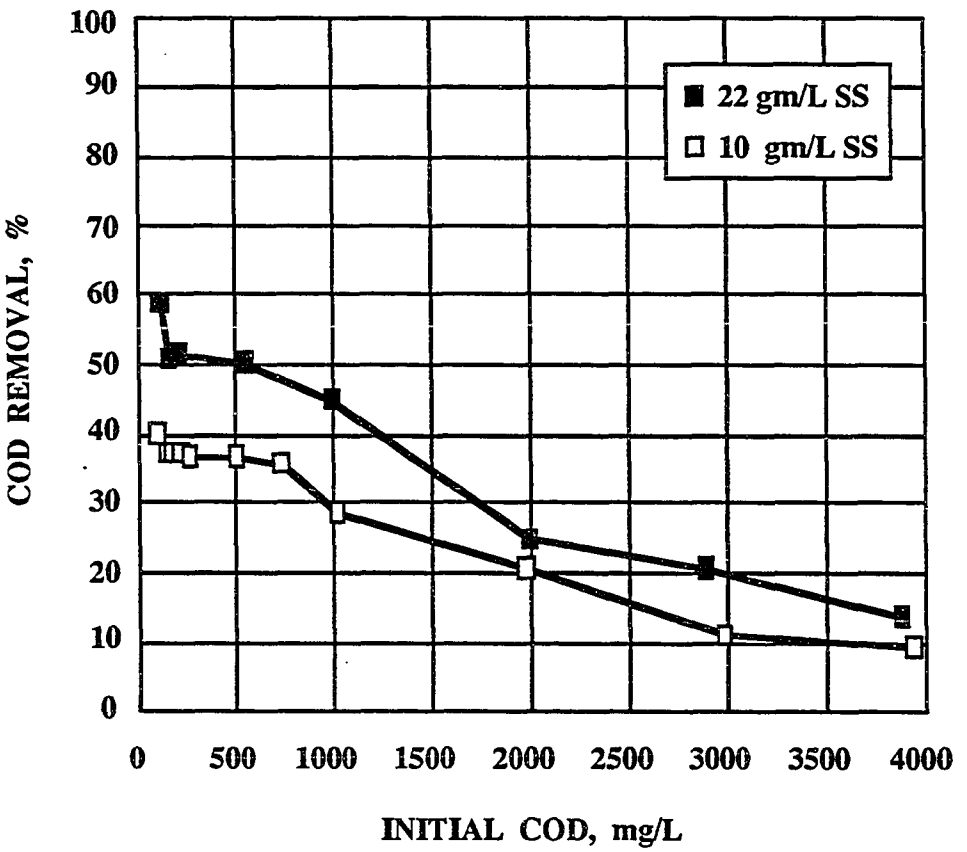


Figure 6.20. Effect of biomass concentration on biosorption for small granular biomass at 28° C (1:1 dilution, 2 minutes mixing, 15 minutes settling, SS = suspended solids)

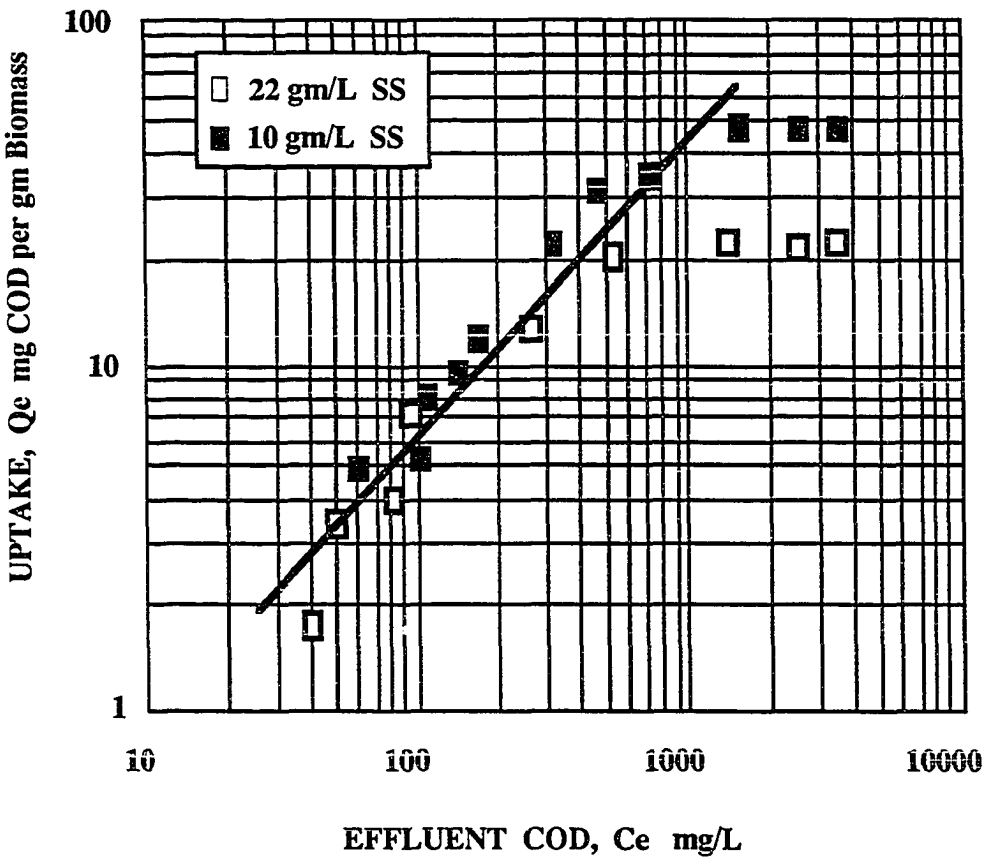


Figure 6.21. Freundlich isotherm for small granular biomass at 28° C at two different suspended solids (SS) concentrations (1:1 dilution, 2 minutes mixing, 15 minutes settling)

6.1.8. Effect of dilution

The experimental plan for investigation of the effect of dilution on biosorption is illustrated in Figure 5.6. One set of experiments consisted of using equal volumes of substrate and biomass (1:1 dilution), namely one liter of each. Another set of experiments was performed using two liters of substrate and one liter of biomass (2:1 dilution). Figure 6.22 illustrates the results of these experiments. The sorption temperature of 28° C resulted from mixing 35° C biomass with 23° C substrate at a room temperature of 23° C. Sorption conditions consisted of two minutes mixing followed by 15 minutes settling with the small granular biomass. From Figure 6.22 it is evident that COD removals are similar at initial COD concentrations below 400 mg/L COD for both conditions. But at higher substrate concentrations, higher COD removals were obtained with the 1:1 dilution. Here also, it seemed that film diffusion might be a limiting factor in biosorption. To overcome this factor, the mixing time was doubled in another set of experiments performed at the 2:1 dilution. These results are illustrated in Figure 6.23. Increasing the mixing time increased the COD removals significantly at the 2:1 dilution. The overall difference in COD removals was not very significant for the two conditions of dilution. This was encouraging from a treatment point of view. Higher volumes of substrate could be treated with similar removal efficiencies, with an increased mixing time.

Figure 6.24 illustrates the Freundlich isotherms for the small granular biomass at 28° C at the two dilution ratios. The two straight lines are similar to one another. The Freundlich parameters corresponding to these data are given in Table 6.4.

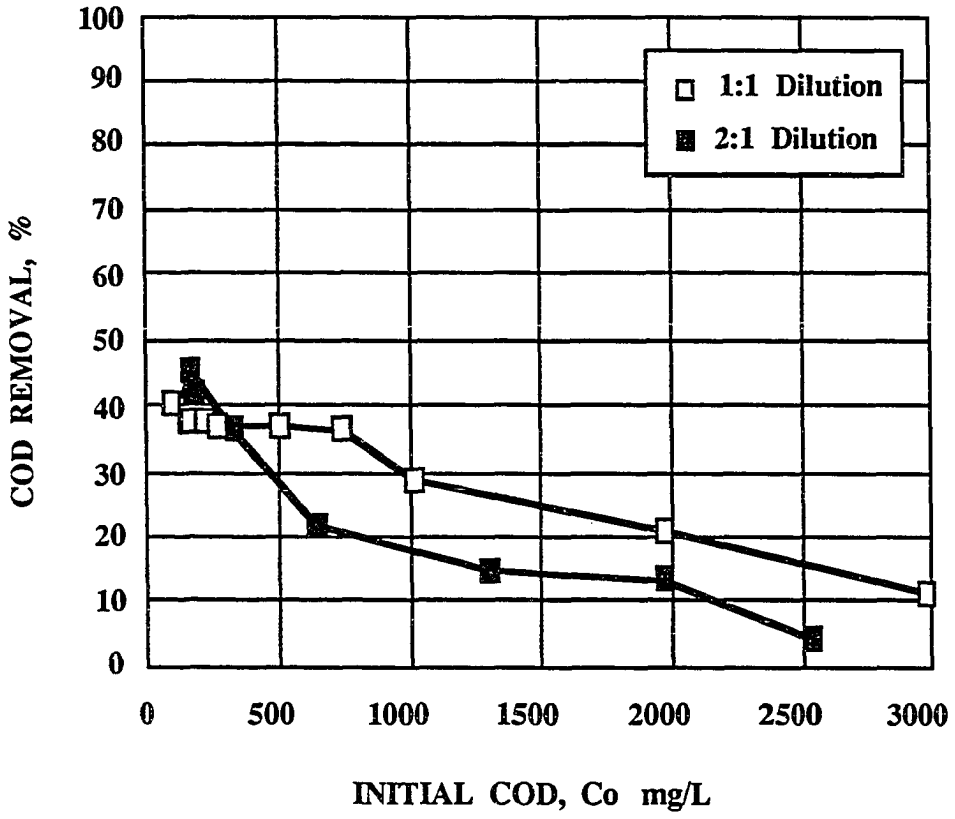


Figure 6.22. Effect of substrate to biomass dilution ratio on biosorption for small granules at 28° C (2 minutes mixing, 15 minutes settling)

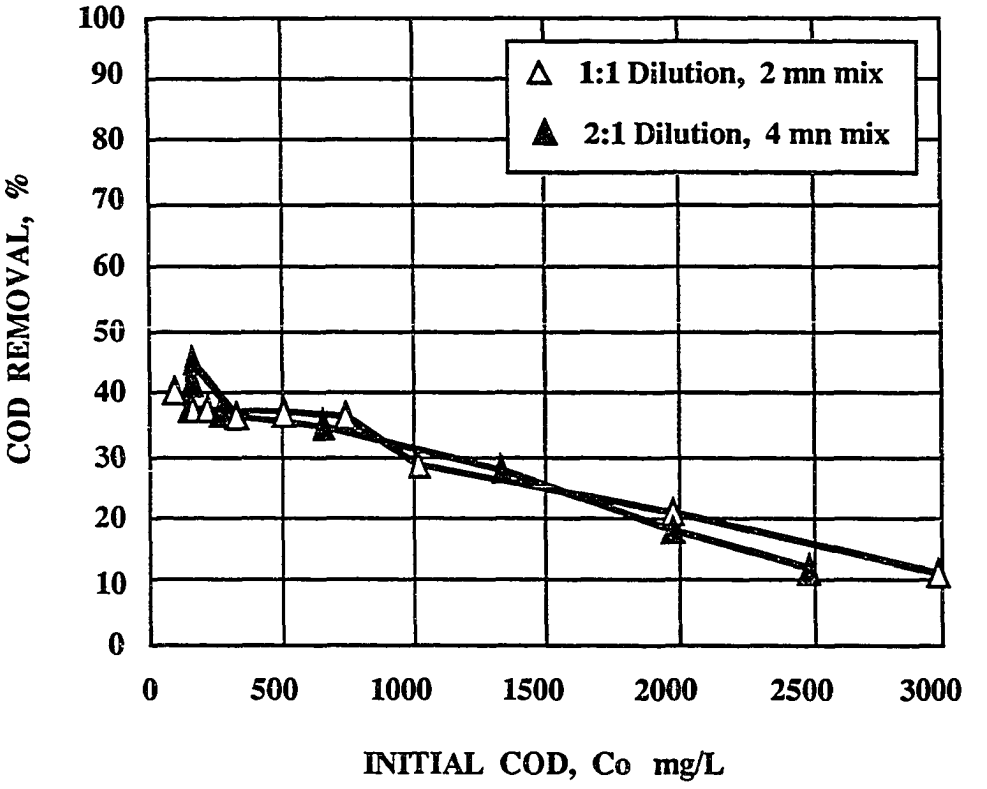


Figure 6.23. Effect of substrate to biomass dilution ratio on biosorption for small granules at two different mixing times (28° C, 15 minutes settling)

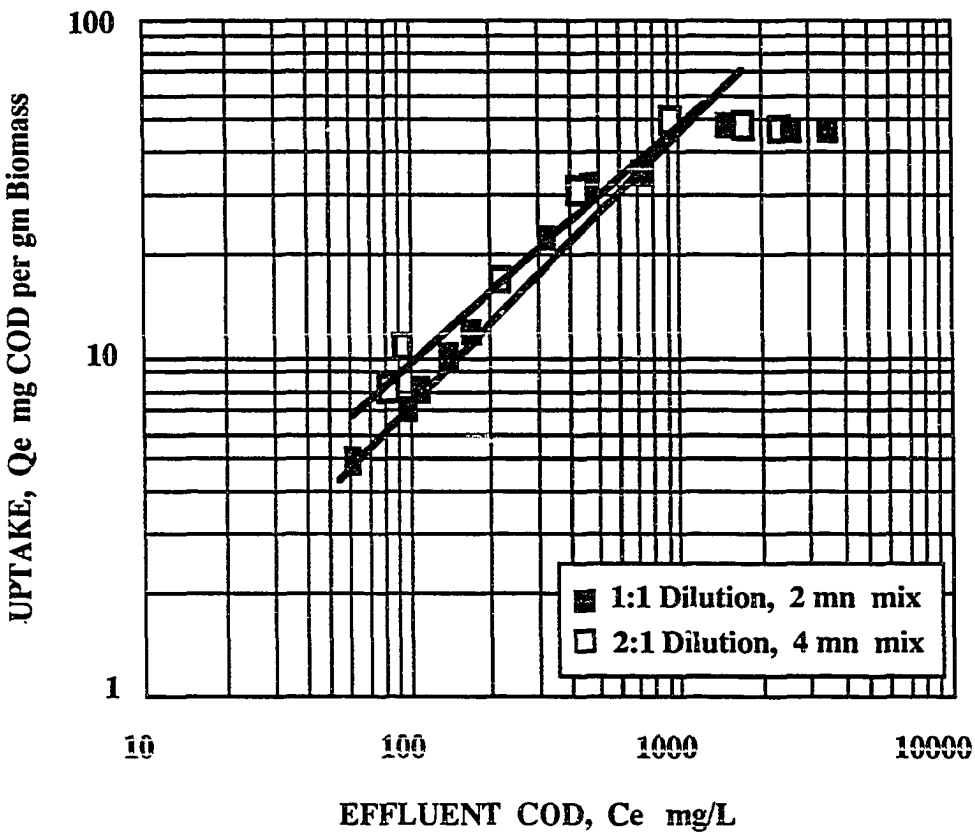


Figure 6.24. Freundlich isotherms for small granular biomass at 28° C for two substrate to biomass dilution ratios

Table 6.4. Freundlich parameters at different dilutions and mixing times for small granules at 28° C

Dilution	Mixing	Data points	K	1/n	r^2
1:1	2 min	9	0.24	0.75	0.97
2:1	4 min	6	0.34	0.73	0.99

6.1.9. Biosorption in a series of reactors

It was decided to investigate the COD removals that could be achieved by biosorption in a series of reactors. Three reactors were used for this purpose. Biosorption was carried out at room temperature of 23° C. The flow diagram for this experiment is illustrated in Figure 6.25. One liter of 35° C small granular biomass was placed in each of the biosorption reactors. One liter of 23° C substrate was introduced into the first reactor, mixed intensely for two minutes and allowed to settle for 15 minutes. One liter of the clear supernatant was then pumped into the second reactor, and at the same time a sample was extracted for analysis. The reactor contents were mixed and allowed to settle as before. One liter of the clear supernatant was pumped into the third reactor. The same mixing and settling routine was followed, and samples were taken for analysis. The results of this experiment are presented in Table 6.5.

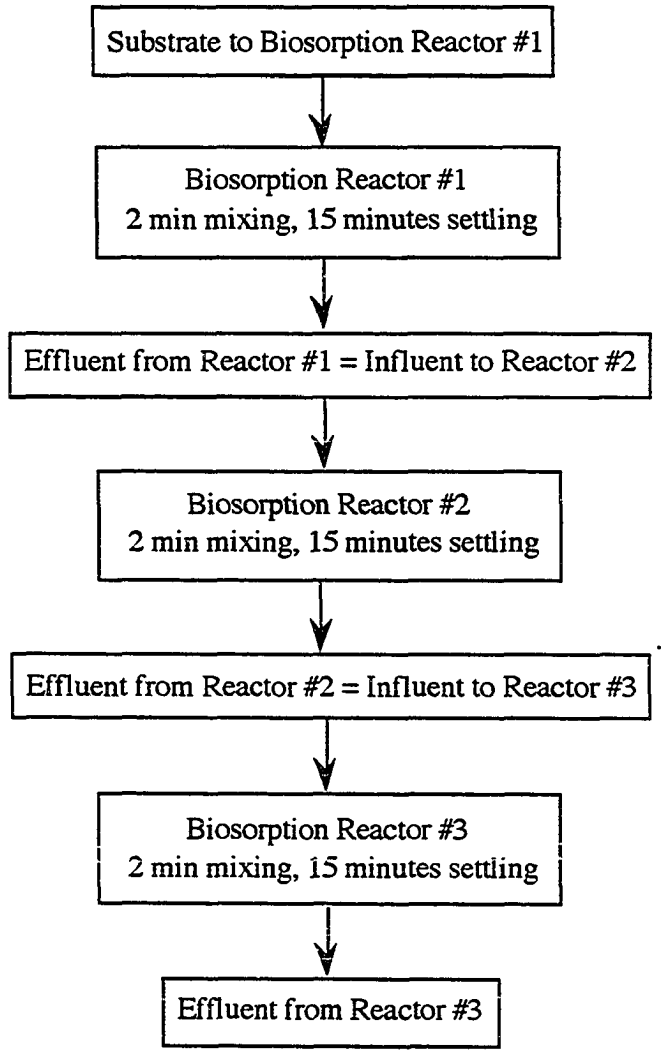


Figure 6.25. Experimental plan for biosorption in a series of three reactors

It is interesting that, when performing biosorption in three consecutive reactors, the removal due to biosorption only, decreases to some extent. One possible reason for this is the changing nature of the influent in the second and third reactor due to dilution with water contained with the biomass. The molecules associated with the biomass water may not be as easily adsorbed on to the biomass as the molecules in the pure milk substrate. As the fraction of biomass water increases in the influent, the removals due to biosorption decreases. In an actual treatment plant, the total COD removals would be calculated as the difference between incoming substrate concentration and effluent concentration. Some water would always be present with live biomass, and dilution due to biomass water would be ignored. For the series of three reactors, the initial substrate concentration is 2036 mg/L COD (which becomes 1018 mg/L after dilution with biomass water), and the effluent from the third reactor is 119 mg/L COD. Therefore, overall COD removal is as high as 94 percent. Even by operating two reactors in series, overall removal of 86 percent can be achieved.

Table 6.5. Results of biosorption in a series of three reactors

Biosorption Reactor	Influent COD mg/L	Effluent COD mg/L	% Removal
1	1018	727	29
2	379	280	26
3	155	119	23

6.2. Discussion of Results

The primary objective of this research was to determine the applicability of active anaerobic biomass as an adsorbent for organic matter. A number of factors were considered important for successful achievement of biosorption. First of all, it was necessary to have a constant healthy source of anaerobic biomass with a very low soluble COD of its own, so that the biomass would provide a negligible contribution to the soluble COD of the substrate. For this reason, the source reactor was operated as an Anaerobic Sequencing Batch Reactor (ASBR). It was possible to achieve almost 98 percent stabilization in the ASBR, thus leaving a background soluble COD in the range of only 20 to 60 mg/L. This made the biomass produced in the ASBR the best choice for a biosorbent. The second criteria was that the biomass should have good settling characteristics. The source reactors were seeded with granular biomass which have excellent settling characteristics. The granules have a porous surface structure, which is advantageous for adsorption. This was another reason for operating the source reactor as an ASBR, particularly since the ASBR has been shown to promote and enhance granulation (Sung and Dague, 1992).

Biosorption is a very rapid process. It usually occurs within the first few minutes after the substrate is brought in contact with the biomass. Initially there is mainly physical adsorption, when the organic matter becomes attached to the surface of the biomass. Afterwards, as time goes on, the process becomes a combination of physical and chemical adsorption, when enzymatic reactions start and the substrate is converted to simpler products and methane and carbon dioxide gas. In order to ensure that the results of these experiments represent only the initial biosorption step, the time frame for the experiments was kept short. During this short period of time, gas production was negligible. This further indicated that

substrate stabilization had not yet begun and biosorption was responsible for removal of organic matter.

The choice of a synthetic non-fat dry milk for a substrate was made due to a number of properties of the milk. The milk was available in a dry powder form which was easily dissolved in water to prepare a highly soluble substrate of the desired strength. The ratio of COD to the weight of the milk powder was convenient for measurements. It was important to have a substrate with constant properties that was easily available.

The success of biosorption was measured as the removal of soluble COD from the substrate, since the COD provides a measure of the organic matter present in the substrate. All the COD values were run in duplicates. Each point on the graphical results are an average of two values. It should also be remembered that each point on a graph represents the result of an individual biosorption experiment (Figures 6.5 to 6.21).

The time required to reach equilibrium in biosorption (reference Figure 6.1) was actually a pseudo-equilibrium stage. Since the COD removals would go on increasing with time due to subsequent stabilization of substrate. But the latter rate was insignificant compared to the initial removal rate or biosorption rate. So, for experimental purposes, it was assumed that equilibrium was achieved within the first 15 minutes of contact time.

A mixing intensity of 750 rpm was used in all experiments. This rpm corresponded to a G value of 770 sec^{-1} at 28° C, and to 697 sec^{-1} at 20° C (calculations are given in Appendix C). The biosorption experiments were performed at constant power since the rpm was the same. A number of different mixing times were tested for biosorption. Adequate mixing was provided to avoid adsorption limitations due to film diffusion. In most experiments, a mixing time of two minutes was used. But sometimes the mixing time was increased to overcome the effects of low temperature (Figures 6.11 and 6.16), or dilution (Figure 6.20) where the volume of substrate was doubled.

From the experimental results it is evident that granular biomass has very good capacity for biosorption. About 35 to 40 percent removal of organic matter was obtained within the first 15 minutes of contact time (Figures 6.5, 6.6 and 6.17). The porous surface structure of the granules contribute to the high rates of adsorption. This indicates that biosorption may be a viable option for preliminary treatment of wastewater.

The experimental data was found to fit the Freundlich model (Figures 6.14, 6.15, 6.21, 6.24). Correlation coefficients obtained were as high as 0.99. In the plots for the sorption isotherms, it is observed that after reaching a maximum uptake value, further uptake remains almost constant with increasing COD concentrations. This suggests that a finite amount of sites are available for adsorption, upon saturation of which no further uptake takes place. This eliminates the possibility of multilayered adsorption. It has been found by previous researchers that the Freundlich equation is useful in describing adsorption of complex organic compounds in biological systems (Benefield et al., 1982; Bell and Tsezos, 1987). The biosorption data also gave a good fit with the Langmuir isotherm model (Figures 6.17, 6.18 and 6.19). This indicated the possible occurrence of chemisorption on the granular biomass.

An interesting phenomenon was the increase in uptake with increasing temperature for both the large and small granules. In case of physical adsorption, the rate of adsorption may increase with increasing temperature, but the uptake is decreased. The opposite was observed in these experiments. This indicates that physical adsorption may not be the primary mechanism for substrate removal, chemisorption may be the dominating mechanism.

An important application of the biosorption process may be in the preliminary treatment of low strength, low temperature wastewater. The main advantage of this process is that the adsorption unit does not have to be heated. Only the source reactor or stabilization unit

requires external heating. The heated biomass serves to increase the temperature of the substrate in the contact tank, thus raising the sorption temperature. The use of equal volumes of substrate and biomass are important for this purpose. Equal volumetric flowrates have been used successfully for treatment of raw sewage in the aerobic biosorption pilot plant at Austin, Texas (Ullrich and Smith, 1951). The results of the present experiments indicated that higher COD removals occurred at higher temperatures. But up to 38 percent removals were obtained with the small granular biomass at the lower sorption temperature of 20° C (Figure 6.7). It should be noticed that the sorption temperature of 20° C corresponds to a substrate temperature of only 7° C. COD removals over 30 percent were also obtained with the large granular biomass at the lower substrate concentrations at the low temperature (Figure 6.8). Although further studies are necessary, this research indicates that anaerobic biosorption may be a feasible option for preliminary treatment of low strength wastewater. For example, a wastewater coming in at 7° C with a COD of 600 mg/L (BOD_5 about 300 mg/L, and which would result in an initial COD of 300 mg/L after volumetric dilution with mixed liquor biomass) would achieve an effluent COD of 200 mg/L (BOD_5 about 100 mg/L), after COD removal without any external application of heat in the anaerobic biosorption process. The effluent from the biosorption unit could then be polished using activated sludge or trickling filter processes. An example calculation for a hypothetical situation is provided in Appendix B which demonstrates that, the heat provided by the biomass is sufficient to raise the temperature of the substrate to the sorption temperature. The biosorption unit does not have to be heated with the help of external sources.

There are two methods in which adsorption by granular biomass can be increased to some extent. This can be done by using a high concentration of anaerobic biomass in the biosorption reactor. Figure 6.20 illustrates the difference in COD removals obtained when the biomass concentration is doubled. Up to 58 percent COD removals were obtained with

22 gm/L suspended solids. But it may not always be possible to achieve such a high concentration of biomass in the source reactor.

The second alternative is to use a series of biosorption reactors, as described in section 6.1.9. By using a series of three reactors, each containing about 10 gm/L suspended solids, it was possible to achieve good COD removals (Table 6.4). Even with two reactors in series, soluble COD removals up to 55 percent were obtained. The use of a series of biosorption reactors appears to be a promising option for wastewater treatment. It should be noticed that total COD removals in an actual treatment plant will always be much greater than those calculated for biosorption. There will always be some water contained with the live biomass which will have a dilutional effect. For example, in the series of reactors the actual COD concentration of the influent substrate is 2036 mg/L. After dilution with the biomass water it becomes 1018 mg/L in the first reactor. Ignoring dilution, the substrate COD decreases from 2036 mg/L to 119 mg/L after the third reactor. Therefore, overall removals were as high as 94%.

6.3. Conclusions

After review and discussion of the experimental results, the following conclusions were drawn:

1. Active anaerobic biomass can be successfully used as an adsorbent for organic matter. Granular biomass is a good choice for an adsorbent due to its porous surface and settling characteristics.

2. Biosorption is affected by the particle size of the granular biomass. Small granular biomass achieved higher COD removals than large granular biomass, especially at higher substrate concentrations and temperatures.

3. Temperature has a significant effect on biosorption. Higher temperature resulted in an increased removal of organic matter in most cases. This was evident with both large and small granular biomass.

4. The biosorption process has potential for application to the pretreatment of low strength, low temperature wastewater streams without the application of external heat. It is possible to achieve about 40 percent removal of organic matter in an unheated biosorption reactor, even when the incoming substrate is at a very low temperature (7^{o} C).

5. Biosorption was observed to follow the Freundlich theory. Adsorption isotherms conformed well to the Freundlich equation.

6. The biosorption data also gave a good fit with the Langmuir isotherm model. This together with temperature effects indicated the possibility of chemisorption.

7. Removal of organic matter can be increased to a significant extent by increasing the concentration of biomass in the biosorption reactor. This was achieved with the small granular biomass by doubling the biomass concentration.

8. Dilution did not appear to have a significant effect on biosorption, at the two dilution ratios tested (substrate volume:biomass volume = 1:1 and 2:1). In most experiments equal volumes of substrate and biomass were used.

9. The efficiency of biosorption can be increased to a great extent by using a series of sorption reactors. High COD removals were obtained with a series of three biosorption reactors.

6.4. Recommendations for Future Research

The biosorption experiments in this research were performed as individual batch experiments. Further research should be done to develop a continuous process for complete treatment of wastewater. The results of this research and the literature pertaining to the aerobic biosorption plant at Austin , Texas (Ullrich and Smith, 1957), would provide some important background information for studies along this avenue.

Future research can be done to determine the time required for a certain quantity of biomass to stabilize the adsorbed substrate, after which the biomass could be used again for biosorption. This information would be important in a continuous treatment process.

Only one type of synthetic waste was used in our research. The biosorption process should be applied to actual waste streams, preferably municipal wastewater. Studies could also be performed with industrial wastewaters. A number of previous studies have focused on the use of inactive or dead biomass as the adsorbent. The problem with dead biomass is that we have to dispose of the adsorbed waste by some other method. Active or live biomass provides a better option, since it initially adsorbs and subsequently stabilizes the waste stream. Good removals of organic matter were obtained with active anaerobic biomass in these experiments. The results also demonstrated the efficiency of the biomass to treat high substrate concentrations, thus indicating a high capacity to withstand shock loads. So further research should be done to apply active biomass successfully for biosorption in a complete treatment process.

Two types of granular biomass were used in these biosorption experiments. It would be interesting to use a flocculent or dispersed type of biomass and compare its performance with the granular biomass.

No microbiological analyses of the granular biomass was performed. Research should

be done to determine the different types of microorganisms associated with the granules and their colony counts. Microscopic analysis of the structure of the granules would provide a better idea of the mechanism of adsorption on the granular surface.

Biosorption experiments can be done using a number of dilutions. The ratio of volume of substrate to volume of biomass can be increased and the removal efficiencies determined at different ratio values. This would be advantageous in treating large volumes of waste with a small amount of biomass. Further research can be done using a series of reactors to enhance the performance.

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APPENDIX A. BIOSORPTION DATA

PARTICLE SIZE DISTRIBUTION OF GRANULAR BIOMASS AS MEASURED BY THE AIA SYSTEM

	% Count	
Equivalent diameter (um)	Small Granules	Large Granules
18	0.00	0.00
27	1.00	0.00
39	0.40	0.08
57	0.90	0.14
84	0.70	0.10
123	0.65	0.23
181	0.30	0.22
266	1.60	0.81
390	9.40	0.60
573	23.30	4.39
841	23.65	6.44
1234	19.60	9.83
1811	14.20	38.85
2658	3.00	31.77
3902	1.30	4.78
5727	0.00	1.75

EFFECT OF MIXING TIMES

Mixing @ 750 rpm

	Soluble COD (mg/L)			
Contact time(mn)	2 mn mix	5 mn mix	15 mn mix	30mn mix
0	1015	1015	1015	1015
2	796	823	790	786
5	756	738	720	716
15	733	715	706	692
30	725	699	651	576
45	722	660	643	568
60	717	652	596	552

SMALL GRANULAR BIOMASS

Sorption temperature = 20° C

Biomass temperature = 35° C

Substrate temperature = 7° C

Mixing = 2 minutes

Volume of substrate = 1 Liter

Volume of biomass = 1 Liter

MLSS = 20.70 gm/L (in source reactor)

Co (mg/L) COD	Ce(mg/L) COD	%Removed	Uptake (mg/gm) (Co-Ce)/M
120	85	29.17	3.38
158	97	38.61	5.89
195	128	34.36	6.47
252	183	27.24	6.62
488	406	16.72	7.87
963	828	14.02	13.04
1906	1652	13.30	24.49
2998	2758	8.00	23.19
4005	3787	5.44	21.06

SMALL GRANULAR BIOMASS

Sorption temperature = 28° C

Biomass temperature = 35° C

Substrate temperature = 23° C

Mixing = 2 minutes

Volume of substrate = 1 Liter

Volume of biomass = 1 Liter

MLSS = 16.88 gm/L

Co (mg/L) COD	Ce(mg/L) COD	%Removed	Uptake (mg/gm) (Co-Ce)/M
104	62	40.10	4.92
155	97	37.42	6.87
178	111	37.46	7.88
222	139	37.39	9.84
271	172	36.53	11.73
515	327	36.50	22.28
744	479	35.62	31.40
1025	733	28.49	34.60
1983	1577	20.47	48.11
3042	2662	12.49	45.03
3941	3569	9.43	44.02

LARGE GRANULAR BIOMASS

Sorption temperature = 28° C

Biomass temperature = 35° C

Substrate temperature = 23° C

Mixing = 2 minutes

Volume of substrate = 1 Liter

Volume of biomass = 1 Liter

MLSS = 22.37 gm/L

Co (mg/L) COD	Ce(mg/L) COD	%Removed	Uptake (mg/gm) (Co-Ce)/M
166	97	41.57	6.17
217	131	39.49	7.65
260	165	36.42	8.45
328	230	29.77	8.72
597	496	16.85	8.99
1088	966	11.21	10.91
2069	1959	5.32	9.84
2881	2766	3.99	10.28
3993	3873	3.01	10.73

LARGE GRANULAR BIOMASS

Sorption temperature = 20° C

Biomass temperature = 35° C

Substrate temperature = 7° C

Mixing = 2 minutes

Volume of substrate = 1 Liter

Volume of biomass = 1 Liter

MLSS = 22.2 gm/L

Co (mg/L) COD	Ce(mg/L) COD	%Removed	Uptake (mg/gm) (Co-Ce)/M
121	80	33.61	4.20
170	114	32.74	5.03
215	143	33.49	6.01
268	205	23.36	5.63
516	455	11.74	6.10
1048	982	6.30	5.95
2167	2104	2.91	5.68
3283	3219	1.95	5.77

SMALL GRANULAR BIOMASS

Sorption temperature = 28° C

Biomass temperature = 35° C

Substrate temperature = 23° C

Mixing = 2 minutes

Volume of substrate = 1 Liter

Volume of biomass = 1 Liter

MLSS = 44 gm/L

Co (mg/L) COD	Ce(mg/L) COD	%Removed	Uptake (mg/gm) (Co-Ce)/M
80	42	47.50	1.73
125	52	58.40	3.32
170	84	50.59	3.91
212	104	50.94	4.91
546	273	49.95	12.39
1002	556	44.48	20.25
1993	1497	24.87	22.52
2904	2300	20.79	27.43
3885	3348	13.81	24.39

EFFECT OF DILUTION (Small granular biomass)

Sorption temperature = 28° C

Biomass temperature = 35° C

Substrate temperature = 23° C

Mixing = 2 minutes @ 750 rpm

Volume of substrate = 2 Liters

Volume of biomass = 1 Liter

MLSS = 22.50 gm/L (in source reactor)

Co (mg/L) COD	Ce(mg/L) COD	%Removed	Uptake (mg/gm) (Co-Ce)/M
145	85	41.38	8.00
174	96	44.93	10.44
342	220	35.74	16.31
649	511	21.26	18.40
1311	1121	14.49	25.33
1980	1726	12.84	33.91
2550	2452	3.83	32.40

EFFECT OF DILUTION (Small granular biomass)

Sorption temperature = 28° C

Biomass temperature = 35° C

Substrate temperature = 23° C

Mixing = 4 minutes @ 750 rpm

Volume of substrate = 2 Liters

Volume of biomass = 1 Liter

MLSS = 22.50 gm/L

Co (mg/L) COD	Ce(mg/L) COD	%Removed	Uptake (mg/gm) (Co-Ce)/M
147	86	41.50	8.13
170	94	44.71	10.13
348	225	35.34	16.40
664	437	34.15	30.22
1335	963	27.87	49.60
1980	1620	18.20	48.04
2553	2195	14.04	47.80

APPENDIX B. HEAT TRANSFER CALCULATIONS

HEAT TRANSFER MEASUREMENTS IN BIOSORPTION REACTOR

The biosorption reactor can be considered to be an "open-type heat exchanger." In an open-type heat exchanger, a hot fluid comes in contact with a cold fluid and leaves as a single stream (Welty et al., 1984). In the sorption reactor, hot biomass is mixed with a cold substrate and the resultant sorption temperature is approximately an average of the individual temperatures. The following is a hypothetical situation where biosorption is used to treat a domestic wastewater flow of 100,000 gpd at influent temperatures ranging from 7° C to 23° C and waste strengths of 500 to 1000 mg/L COD (250 to 500 mg/L BOD_5). The following calculations will demonstrate that the heat from the biomass is sufficient to increase the temperature of the influent wastewater for biosorption. No external heat has to be applied to the sorption reactor.

1. Flow rate of wastewater = 100,000 gal/day

   = (100,000 gal/day) (ft^3 / 7.48 gal)

   = 133,70 ft^3 /day

2. Size of biosorption reactor

   Detention time = 15 min

   Volume of wastewater in 15 min = (13370 ft^3 /day) (day/24 hr) (15 min/60 min/hr)

   = 140 ft^3

   Volume of biomass = volume of wastewater = 140 ft^3

   Total volume = 140 + 140 ft^3 = 280 ft^3

   Diameter = 6 ft Height = 10 ft

Wall area = (3.14) (6) (10) = 188.50 ft^2

Floor area = (3.14) $(6)^2$ / 4 = 28.27 ft^2

Roof area = 28.27 ft^2

CASE 1:

Substrate temperature = 23° C = 73.4° F

Biomass temperature = 35° C = 95° F

Sorption temperature = 28° C = 82.4° F (From laboratory experiments)

Surrounding temperature = 60° F

A. Heat requirement for wastewater is given by

$$q = M \times C_p \times \Delta T$$

where, q = Heat required, BTU/sec

M= Mass flow rate, lb/sec

C_p = Heat capacity, BTU/lb - ° F

ΔT = Temperature difference, ° F

M = 100,000 gal/day

= (100000 gal/day) (day/86400 sec) (8.34 lb/gal)

= 9.65 lb/sec

C_p = 1.0 BTU/lb - ° F (Ref. Metcalf and Eddy, 1991)

Heat required by wastewater, q_w = (9.65 lb/sec) (1.0 BTU/lb - ° F) (82.4- 73.4 ° F)

= 86.85 BTU/sec

= 7.50 MBTU/day

B. Conductive heat losses from biosorption tank is given by

$$q = U \times A \times \Delta T$$

where, q = Conductive heat loss, BTU/hr

U = Overall heat transfer coefficient, BTU/ft^2 - hr - ^{o}F

A = Area of evaluation, ft^2

ΔT = Temperature change, ^{o}F

Assume, biosorption tank is made from concrete and has the following heat transfer coefficients (Ref. Metcalf and Eddy, 1991)

U_{walls} = 0.16 BTU/ft^2 - hr - ^{o}F

U_{roof} = 0.16 BTU/ft^2 - hr - ^{o}F

U_{floor} = 0.15 BTU/ft^2 - hr - ^{o}F

Wall loss, q_1 = (0.16 BTU/ft^2 - hr - ^{o}F) (188.50 ft^2) (82.4 - 60 ^{o}F) (24 hr/day)

= 16214 BTU/day

Floor loss, q_2 = (0.15 BTU/ft^2 - hr - ^{o}F) (28.27 ft^2) (82.4 - 60 ^{o}F) (24 hr/day)

= 2280 BTU/day

Roof loss, q_3 = (0.16 BTU/ft^2 - hr - ^{o}F) (28.27 ft^2) (82.4 - 60 ^{o}F) (24 hr/day)

= 2432 BTU/day

Total loss = $q_1 + q_2 + q_3$ = 20,926 BTU/day = 0.0209 MBTU/day

C. Heat given up by biomass

$$q_b = M \times C_p \times \Delta T$$

= (9.65 lb/sec) (1.0 BTU/lb- ^{o}F) (95 - 82.4 ^{o}F)

= 121.59 BTU/sec

= 10.50 MBTU/day

D. Total heat required = Heat required by wastewater + Conductive heat losses

$$q_r = 7.50 + 0.0209 \text{ MBTU/day} = 7.5209 \text{ MBTU/day}$$

Total heat given up by biomass, $q_b = 10.50$ MBTU/day

Since, $q_r < q_b$

Therefore, Heat given up by biomass is sufficient to heat the incoming wastewater.

External heat is not required to heat the contents of the biosorption tank.

CASE 2:

Substrate temperature = 7° C = 44.6° F

Biomass temperature = 35° C = 95° F

Sorption temperature = 20° C = 68° F (From laboratory experiments)

Surrounding temperature = 60° F

A. Heat requirement for wastewater is given by

$$q = M \times C_p \times \Delta T$$

$$= (9.65 \text{ lb/sec}) (1.0 \text{ BTU/lb-}^{\circ}\text{F}) (68 - 44.6\ ^{\circ}\text{F})$$

$$= 225.81 \text{ BTU/sec}$$

$$= 19.51 \text{ MBTU/day}$$

B. Conductive heat losses from biosorption tank is given by

$$q = U \times A \times \Delta T$$

Wall loss, $q_1 = (0.16 \text{ BTU/ft}^2 - \text{hr} - ^{\circ}\text{F}) (188.50 \text{ ft}^2) (68 - 60\ ^{\circ}\text{F}) (24 \text{ hr/day})$

$$= 5791 \text{ BTU/day}$$

Floor loss, q_2 = (0.15 BTU/ft^2 - hr - ^{o}F) (28.27 ft^2) (68 - 60 ^{o}F) (24 hr/day)

= 814 BTU/day

Roof loss, q_3 = (0.16 BTU/ft^2 - hr - ^{o}F) (28.27 ft^2) (68 - 60 ^{o}F) (24 hr/day)

= 868 BTU/day

Total loss = $q_1 + q_2 + q_3$ = 7473 BTU/day = 0.0075 MBTU/day

C. Heat given up by biomass

$q_b = M \times C_p \times \Delta T$

= (9.65 lb/sec) (1.0 BTU/lb- ^{o}F) (95 - 68 ^{o}F)

= 260.55 BTU/sec

= 22.51 MBTU/day

D. Total heat required = Heat required by wastewater + Conductive heat losses

q_r = 19.51 + 0.0075 = 19.5175 MBTU/day

Total heat gain = Heat given up by biomass

q_b = 22.51 MBTU/day = 22.51 MBTU/day

Since, $q_r < q_b$

Therefore, Heat given up by biomass is sufficient to heat the incoming wastewater.

External heat is not required to heat the contents of the biosorption tank.

APPENDIX C. CALCULATION OF G VALUES

VELOCITY GRADIENTS (G) IN BIOSORPTION REACTOR

Mixing = 750 rpm

Power = 1/750 hp (from manufacturer's specifications)

Volume, V = 2 liters

Case 1: Sorption temperature = 20° C = 68° F

$\mu = 0.687 \times 10^{-3}$ lb_m/ft-sec

$$G = \frac{(P)^{1/2}}{(V\mu)^{1/2}}$$

$$= \frac{[\,(1/750\ hp)\ (550\ ft\text{-}lb_f/\ sec)\ (32.2\ ft\text{-}lb_m/lb_f\text{-}sec^2)\,]^{1/2}}{[\,(2\ L)\ (1/28.3\ ft^3/L)\ (0.687 \times 10^{-3}\ lb_m/ft\text{-}sec)\,]^{1/2}}$$

$$= 697\ sec^{-1}$$

Case 2: Sorption temperature = 28° C = 82.4° F

$\mu = 0.564 \times 10^{-3}$ lb_m/ft-sec

$$G = \frac{(P)^{1/2}}{(V\mu)^{1/2}}$$

$$= \frac{[\,(1/750\ hp)\ (550\ ft\text{-}lb_f/\ sec)\ (32.2\ ft\text{-}lb_m/lb_f\text{-}sec^2)\,]^{1/2}}{[\,(2\ L)\ (1/28.3\ ft^3/L)\ (0.564 \times 10^{-3}\ lb_m/ft\text{-}sec)\,]^{1/2}}$$

$$= 770\ sec^{-1}$$

APPENDIX D. EQUATIONS USED FOR BIOSORPTION

Cs = Substrate concentration, mg/L soluble COD

Cb = Soluble COD concentration of biomass, mg/L

Vs = Volume of substrate, L

Vb = Volume of biomass, L

Co = Initial soluble COD concentration in biosorption reactor, mg/L

Ce = Effluent soluble COD after biosorption, mg/L

Mb = Suspended solids (MLSS) of biomass in source reactor, mg/L

M = Concentration of biomass in biosorption reactor, mg/L

$$Co\ (mg/L) = \frac{Cs * Vs + Cb * Vb}{Vs + Vb}$$

$$\text{Uptake, } Qe\ (mg/gm) = \frac{(Co - Ce) * (Vs + Vb)}{Mb * Vb}$$

$$\text{COD removal } \% = \frac{(Co - Ce) * 100}{Co}$$

$$M\ (mg/L) = \frac{Mb * Vb}{Vb + Vs}$$