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KINETIC STUDY OF ANAEROBIC DIGESTION OF WHEY PERMEATE IN A BATCH REACTOR

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RESUME

Le perméat de lactosérum est un des constituants majeurs des eaux résiduaires produites lors de la transformation du lait et il représente une demande biologique en oxygène (DBO) comprise entre 40 000 et 60 000 ppm. De ce fait, le perméat pose une sévère menace envers l'environnement s'il y est rejeté sans traitement. Les traitements biologiques des déchets organiques deviennent de plus en plus populaires de nos jours puisqu'ils sont plus efficaces et économiques et qu'ils causent très peu de pollution. La conception de tout bioréacteur, en vue d'une opération optimale, requiert une bonne connaissance de la cinétique des biotransformations effectuées. C'était le but de cette étude.

L'étude expérimentale a été menée afin d'évaluer les paramètres cinétiques intrinsèques de la digestion anaérobie du perméat de lactosérum par une population bactérienne mixte. Les essais ont été réalisés dans un réacteur agité de 2 L où le perméat était la seule source de carbone organique. La population mixte provenait d'un digesteur anaérobie de boues produites par une installation aérobie de traitement d'eaux usées municipales et fut, par la suite, acclimatée avant l'inoculation. La demande chimique en oxygène (DCO) du perméat et sa concentration en équivalent-glucose ont été mesurées et étaient respectivement égales à 68 000 et 62 000 mg/L.

Les essais ont été réalisés à diverses concentrations initiales du substrat, c'est-à-dire pour S_o = 6.38, 10.85, 21.02 et 48.77 g/L et dans tous les cas, la température a été maintenue constante à 37°C. Le pH du contenu du réacteur a été mesuré à intervalles d'une heure et ajusté à la valeur de 7.0, si nécessaire. La concentration de la biomasse a été

déterminée par la mesure des poids secs et la concentration du glucose, par la méthode phénol-acide sulfurique.

Les résultats expérimentaux obtenus, concernant la croissance de la biomasse et la biodégradation du substrat, n'ont pu être représentés par les modèles conventionnels cités dans la littérature. Pour chacun des essais, la croissance de la biomasse en fonction du temps a été représentée par une droite. Pour la cinétique de biodégradation du substrat, l'équation proposée a été basée sur le comportement d'une réaction autocatalytique. Les valeurs obtenues pour la constante cinétique k étaient, pour S_o = 6.38, 21.02 et 48.77 g/L, très voisines l'une de l'autre et la valeur moyenne était égale à 0.052 (g biomasse-h/L)$^{-1}$. Par contre, pour S_o = 10.85 g/L, sa valeur était beaucoup plus élevée et égale à 0.096 (g biomasse-h/L)$^{-1}$. Cette anomalie est peut-être due à une vitesse de croissance bactérienne et une production d'enzymes plus élevées d'une ou plusieurs espèces de la population mixte bactérienne pour les autres essais à des concentrations initiales de substrat différentes.

La valeur du coefficient de rendement initial, Y_o, était plus élevée que celle du rendement glogal, $Y_{x/s}$, pour trois des concentrations initiales du substrat. L'exception était pour la concentration initiale égale à 10.85 g/L. Les valeurs numériques de Y_o, pour S_o = 6.38, 10.85, 21.02 et 48.77 g/L, étaient respectivement égales à 0.056, 0.034, 0.065 et 0.050. Celles pour $Y_{x/s}$ étaient respectivement égales, dans le même ordre, à 0.030, 0.054, 0.045 et 0.038.

ABSTRACT

Whey permeate is one of the major constituents of wastewaters produced by transformation of milk and has a biological oxygen demand (BOD) anywhere between 40 000 to 60 000 ppm. Because of its high BOD, permeate poses a severe threat to any clean environment if disposed without treatment. Biological treatments for organic wastes become more popular nowadays as they are more efficient, economic and cause only minimum environmental pollution. The design of any bioreactors to get the optimum operation requires a good knowledge of the kinetics involved. This was the driving force behind this study.

The experimental study was conducted to estimate intrinsic kinetic parameters of anaerobic digestion of whey permeate by a mixed bacterial population. The experiment was conducted in a 2L stirred reactor using permeate as the sole source of organic carbon. The culture was originally obtained from an anaerobic sludge digester treating municipal wastewaters and later acclimatised to permeate before inoculation. The chemical oxygen demand (COD) of the permeate and its glucose-equivalent concentration were found to be, respectively, 68 000 mg/L and 62 000 mg/L.

The experiments were conducted with various initial substrate concentrations S_o = 6.38, 10.85, 21.02 and 48.77 g/L and in all cases, the temperature was kept constant at 37°C. The pH of the reactor contents was checked every hour and adjusted to 7.0 if found different. Biomass concentration was determined by gravimetric method of drying and weighing and glucose concentration by phenol-sulphuric acid method.

The experimental data obtained for biomass growth and substrate utilization did not follow any conventional models cited in the literature. Biomass growth versus time was

approximated by a straight line. For substrate utilization kinetics, the proposed equation was based on the behaviour of an autocatalytic reaction. The values of kinetic constant, k, obtained for S_o = 6.34, 21.02 and 48.77 g/L were very close to one another with an average value of 0.052 (g cells-h/L)$^{-1}$. But the value for S_o 10.85 g/L was much higher and found to be 0.096 (g cells-h/L)$^{-1}$. This increase in k-value may be due to a possibly higher growth rate and enzyme production of any or all species in the mixed bacterial population for the other initial substrate concentrations.

The initial yield coefficient, Y_o , was higher than the overall yield, $Y_{x/s}$, for three of the four initial substrate concentrations. The exception was for S_o = 10.85 g/L. The numerical values of Y_o for S_o = 6.38, 10.85, 21.02 and 48.77 g/L were 0.056, 0.034, 0.065 and 0.050 respectively. Those values of $Y_{x/s}$ were 0.030, 0.054, 0.045 and 0.038 for the S_o's in the same order.

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CHAPTER 1

INTRODUCTION

The study of processes concerning biological systems is one of the most interesting field of bioengineering research. These processes, as often called biochemical processes, differ from chemical processes due to the presence of living organisms in the reacting system. Microorganisms produce their own catalysts (enzymes) to facilitate the transformation of organic substrates to simpler end products. Investigations on these systems are carried out by using typical microbiology techniques and typical chemical engineering methods.

Biological processes have been routinely applied to the treatment of domestic, industrial or agricultural wastewaters. The onset of spiralling energy costs in the early 1970's coupled with an acute awareness of environmental pollution emphasized in the late 1960's provided the springboard for intensive research and development in the field of anaerobic fermentations. The technology born from this considerable effort is the basis from which anaerobic processes have been developed specifically to provide the industrialist with a cost-effective, reliable and flexible method of effluent treatment and energy recovery. Anaerobic digestion process are now being actively applied in a broad spectrum of industrial effluent treatment schemes throughout the world.

A high degree of conversion of available organic carbon

to gaseous end products, low production of biological solids as a result of minimal energy available for microbial growth, and generation of product gases high in recoverable methane content are among some of the advantages of anaerobic stabilization techniques. With increasing cost of energy, this latter advantage becomes particularly significant and the anaerobic stabilization process is receiving renewed attention for energy recovery as well as waste disposal from a wide variety of waste organic materials (Massey and Pohland, 1978).

Worldwide, many industries are looking towards anaerobic biological treatment as an economical method of waste disposal and /or a financially attractive method of recovering a useful and valuable by-product which does not require marketing and which does not compete with or affect the production of the main-line product. Thus, the importance and necessity of microbial growth and substrate utilization kinetic studies become evident and any effort towards this objective will also contribute to the rational design of treatment facilities.

For the anaerobic digestion of wastewaters , many different types of bioreactors such as Sludge Bed, Anaerobic Filter and Fluidised Bed /Expanded Bed reactors are used according to the strength and nature of wastewaters. The design of these bioreactors is very important in order to get the maximum treatment efficiency and maximum desired products.

Improvement of digester design or methane digestion systems, enhancement of yields and rates of methane production cannot proceed successfully without adequate analytical tools and appropriate conditions. In order to design an integral reactor model, besides a model describing the fluid flow and all sorts of other transport phenomena, the kinetics of the conversion of organic wastes has to be known. A lot of attention has been recently devoted to mass, heat, and momentum transfer phenomena in biological systems, but the chemical engineering kinetic aspects also need more investigation in order to complete of the phenomena and to develop relationships useful for designing biological reactors (Bailey and Ollis, 1986, Chapter 9).

Numerous kinetic models describing microbial growth and substrate conversion have been developed by different investigators in the last 50 years. Among them, Monod-type model stands out to be popular. This is mainly because of its wide use by different investigators to describe all sorts of microbial kinetics. It is also applied to describe the kinetics of anaerobic purification; however inhibition effects are not included in this model.

Whey is one of the major constituent of wastewaters, resulting from whole milk during the manufacture of cheese (Tyagi *et al.*, 1991). Whey permeate is the yellowish green

liquid left after the removal of fat and protein from the whey by ultrafiltration. It is important to note that during ultrafiltration, the retention of true protein and fat is close to 99 % or more (Cheryan, 1986). Whey is composed of proteins (β-lactoglobulin and α-lactalbumin), sugar (lactose), minerals, and a small amount of lactic acid in solution (see Table 2.1). About 40 billion kg of whey is produced in the world, half of it is in the U.S.A. alone (Cheryan, 1986). Cheese whey has a biological oxygen demand (BOD) of 40000-60000 ppm and this makes it an environmental hazard. It is estimated that as much as 40-50 % of the whey produced is disposed of as sewage, with the rest being used primarily for animal feed or human food. This explains why ultrafiltration has attracted the attention of cheese producers, since it now affords a mean of simultaneously fractionating, purifying and concentrating the whey thus enhancing its utilization and reducing pollution problem.

The objective of the present study was to evaluate intrinsic kinetic parameters such as maximum specific growth rate, specific substrate utilization rate or otherwise called the kinetic constant k and the initial and overall yield coefficients for the anaerobic bacterial digestion of cheese whey permeate by a mixed bacterial population and compare the data with already existing models. This helps to validate the

adequacy or inadequacy of kinetic models for their capability to predict the overall process in the anaerobic digestion system. The experimental study was conducted in a batch reactor at a constant temperature of 37 °C using whey permeate as the sole source of organic carbon.

CHAPTER 2

LITERATURE REVIEW

2.1 General Introduction to Microbiology

Microbes are generally classified as either eucaryotes or procaryotes on the basis of the complexity of their cellular structure (Gaudy and Gaudy, 1980). Eucaryotic microbes comprise fungi including yeasts, algae and protozoa, whilst procaryotic microbes comprise bacteria and cyanobacteria (formally described as blue-green algae). The eucaryote-procaryote dichotomy has long been accepted as a fundamental concept, but later it has become evident that a third group of microbes, the archaebacteria, exists (Woese, 1982). The most immediately obvious difference between eucaryotic and procaryotic microbes is their size, with the latter, on average, being an order of magnitude smaller than the former. The equivalent spherical diameter of many bacteria is about 1 μm.

However, the fundamental difference between eucaryotes and procaryotes is one of cellular structure and function. The characteristic organelle of eucaryotic cells is the nucleus, which, together with the several other membrane-bounded organelles present, carries out the essential functions of the cell. In procaryotes, these same essential functions are generally performed within the cytoplasmic membrane. The nucleus of eucaryotic cells contains the DNA of the cell and associated proteins that form the chromosomes,

and is separated from the cytoplasm by a double porous membrane. The outer nuclear membrane has a complex internal structure, part of which is covered with ribosomes, ie. protein and RNA containing bodies which are the sites of protein synthesis. In procaryote cells, the DNA molecule is neither complexed with proteins to form a chromosome, nor separated from the cytoplasm by a membrane, but is present as a single, double-stranded covalently joined molecule.

Archaebacteria form a group of microbes that is distinct from both eucaryotic and procaryotic microbes and they are neither related to the true bacteria (eubacteria) nor to eucaryotes. They comprise a small, but diverse, collection of phenotypes, and although they have some unique phenotypic characteristics in common, it is difficult to group them convincingly on this basis. Archaebacteria are recognized primarily by genotypic data, but it also seems probable that they differ from the eucaryotes and procaryotes in significant detail of most of their molecular processes.

The three nutritional requirements that are quantitatively the most important for microbes are a carbon substrate, an energy source and an electron donor. These, together with the electron acceptor, are governed by the enzymes present in the cell. The carbon source available to microbes can be either organic or inorganic. Those microbes that require

organic compounds as their sole or principal carbon substrate are classified as heterotrophs, whilst those that require inorganic carbonaceous compounds as their sole or principal carbon substrate are classified as autotrophs. Microbes that utilize light as their energy source are described as phototrophs and those that obtain energy from the oxidation of either organic or inorganic compounds are described as chemotrophs. Microbes that use organic compounds as their source of electrons are described as organotrophs, whilst those employing inorganic electron sources are called lithotrophs.

The microbes that are classified as phototrophs are, from the eucaryotes, the algae, and from the procaryotes, the cyanobacteria. The vast majority of microbes are chemotrophs, including all the fungi, all the protozoa, all the archaebacteria and most of the true bacteria.

2.2 Biochemistry & Microbiology of Anaerobic Digestion

The anaerobic digestion process is a natural biological process in which a close-knit community of bacteria co-operate to form a stable, self-regulating fermentation that converts waste organic matter into a mixture of CO_2 and CH_4 gases. Its usefulness as a treatment process relies heavily on the sophistication of its microbiology which allows most of the process control to be undertaken directly by the bacteria

themselves. Of particular interest is the way that the bacteria manage to control both the pH value and the redox potential of their own growth medium (Mosey, 1983).

2.2.1 The Mechanism of Anaerobic Digestion

From a kinetic viewpoint, anaerobic treatment may be described as a three-step process involving (a) hydrolysis of complex material, (b) acid production, and (c) methane fermentation. In the first step, complex organics are converted to less complex soluble organic compounds by enzymatic hydrolysis. In the second step, these hydrolysis products are fermented to simple organic compounds, predominantly volatile fatty acids, by a group of facultative and anaerobic bacteria collectively called "acid formers". In the third step, the simple organic compounds are fermented to methane and CO_2 by a group of substrate-specific, strictly anaerobic bacteria called the "methane formers". Thus organic waste materials are converted effectively to bacterial protoplasm and gaseous end products - methane and carbon dioxide.

2.2.2 Types of Bacteria Involved in Anaerobic Digestion

The different groups of bacteria believed to be involved in the apparently simple conversion of glucose into CO_2 and CH_4 are summarized in Figure 2.1. This conversion is :

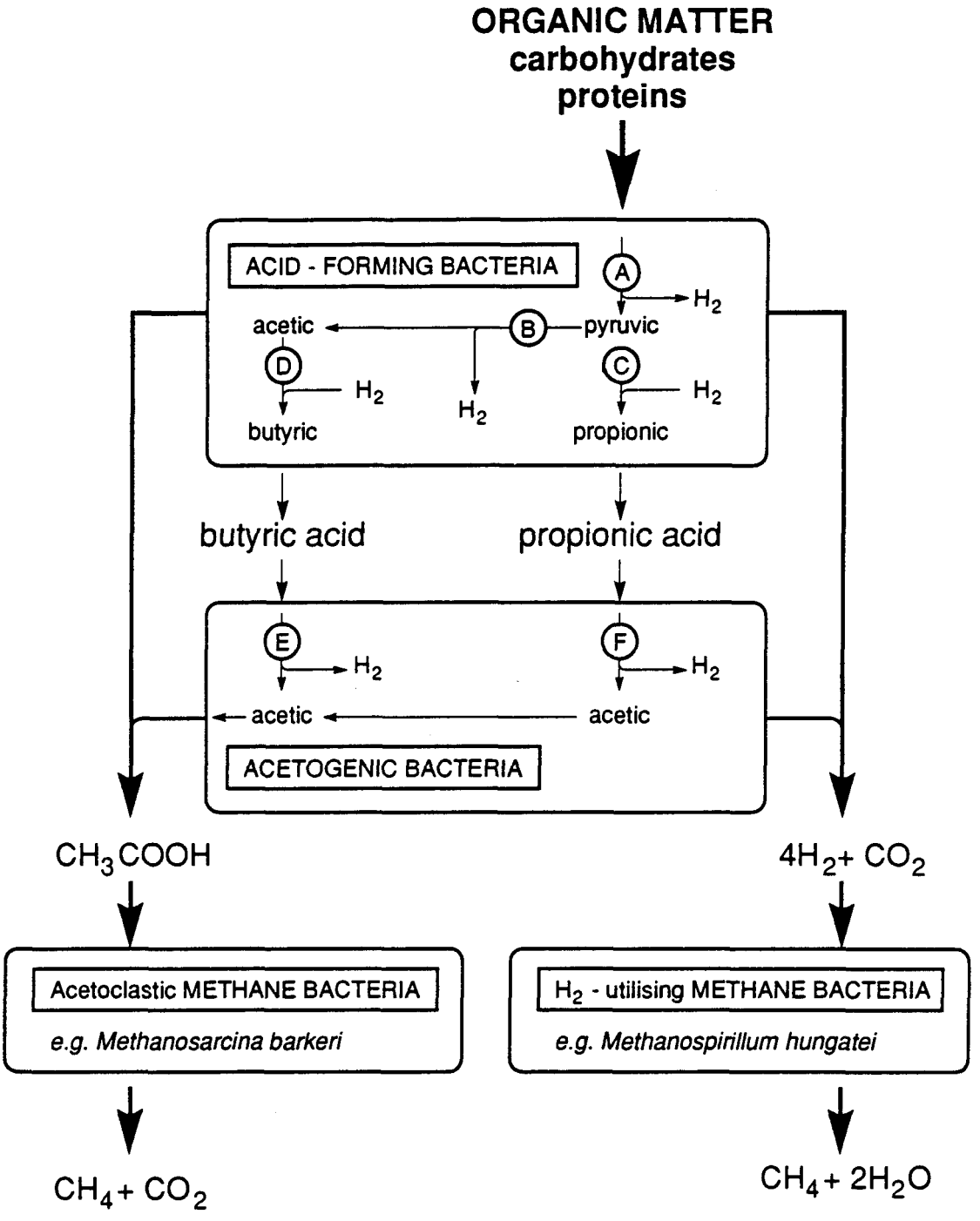

Figure 2.1 The Microbial Ecology of the Anaerobic Digestion Process (Mosey, 1983)

$$C_6H_{12}O_6 \longrightarrow 3\ CO_2 + 3\ CH_4 \qquad (2.1)$$

(i) The Acid-Forming Bacteria: These are fast-growing bacteria (minimum doubling time around 30 minutes) which ferment glucose to produce a mixture of acetic, propionic and butyric acids according to the reactions (Mosey, 1983):

$$C_6H_{12}O_6 + 2\ H_2O \longrightarrow 2\ CH_3.COOH + 2\ CO_2 + 4\ H_2 + 4\ ATP \qquad (2.2)$$

$$C_6H_{12}O_6 \longrightarrow CH_3.CH_2.CH_2.COOH + 2\ CO_2 + 2\ H_2 + 2\ ATP \qquad (2.3)$$

$$C_6H_{12}O_6 + 2\ H_2 \longrightarrow 2\ CH_3.CH_2.COOH + 2\ H_2O + 2\ ATP \qquad (2.4)$$

Their preferred reaction is the first one i.e., the conversion of glucose into acetic acid. It provides the acid-forming bacteria with the biggest energy yield for growth and it provides the acetoclastic methane bacteria with their prime substrate for methane production. Due to these reactions, the formation of butyric and propionic acids are the bacteria's response to acculmulations of hydrogen during surge loads

(ii) The Acetogenic Bacteria: As their name implies, these are the bacteria that convert propionic and butyric acids into acetic acid according to the equations:

$$CH_3.CH_2.COOH + 2\ H_2O \longrightarrow CH_3.COOH + CO_2 + 3\ H_2 \qquad (2.5)$$

$$CH_3.CH_2.CH_2.COOH + 2\ H_2O \longrightarrow 2\ CH_3.COOH + 2\ H_2 \qquad (2.6)$$

Their existence has not yet been demonstrated. It has been deduced by McInerney *et al.* (1971) from the inability of any known methane bacteria to metabolise propionate and butyrate

directly.

Enrichment culture studies of Lawrence and McCarty (1969) indicate that these bacteria grow relatively slowly even under optimum conditions of low concentrations of dissolved H_2, with minimum doubling times of 1.5 - 4.0 days. The reactions that they perform are energetically very difficult (McInerney *et al.*, 1971; Heyes and Hall, 1981) and are easily stopped by accumulations of dissolved hydrogen gas in the growth media.

(iii) The Acetoclastic Methane Bacteria: These are the bacteria that convert acetic acid into a mixture of CO_2 and CH_4 according to the reaction:

$$CH_3.COOH \longrightarrow CH_4 + CO_2 \qquad (2.7)$$

They also grow very slowly with minimum doubling time of 2-3 days, but are believed to be unaffected by the concentrations of dissolved hydrogen gas in the growth media (Mosey, 1983).

They normally control the pH value of fermentation by removal of acetic acid and formation of CO_2. They are responsible for most of the methane produced by the anaerobic digestion process.

(iv) The Hydrogen-Utilising Methane Bacteria: These bacteria are hydrogen-scavengers. They obtain energy for growth from the reaction:

$$4H_2 + CO_2 \longrightarrow CH_4 + 2\ H_2O \qquad (2.8)$$

and in so doing they remove almost all of the hydrogen from

the system. They grow quite quickly with minimum doubling times around 6 hours (Mosey, 1983). They control the redox potential of the digestion process and a great deal more besides.

The traces of hydrogen that they leave behind regulate both the total rate of acid production and the mixture of acids that is produced by the acid-forming bacteria. Hydrogen also controls the rates at which propionic and butyric acids are subsequently converted back into acetic acid. These H_2-utilising methane bacteria regulate the formation of volatile acids and therefore they are the autopilot of the anaerobic digestion process.

2.3 Whey Permeate

2.3.1 Components and Composition

Whey permeate is the yellowish liquid left after the removal of fat and protein from cheese whey, which in turn, is produced from milk during the manufacture of cheese and other dairy products.It is composed of sugar (lactose), minerals and a small amount of lactic acid in solution (Tyagi *et al.*, 1991). There are mainly two types of cheese whey: sweet whey and acid whey. The compositions of whey and whey permeate are shown in Tables 2.1a and 2.1b.

Table 2.1a Major Components of Cheese Whey (Irvine & Hill, 1985)

Component	Sweet Whey (pH 5.9 - 6.7)		Acid Whey (pH 4.4 - 4.8)	
	Fluid(%)	Dried (%)	Fluid (%)	Dried(%)
Protein	0.80	13.10	0.75	12.50
Lactose	4.85	75.00	5.00	67.40
Fat	0.50	0.80	0.04	0.60
Ash	0.50	7.30	0.80	11.80
Lactic acid	0.05	0.20	0.40	4.20
Total solids	6.70	96.40	6.99	96.50

Table 2.1b Components and Composition of Whey Permeate*

Component	Permeate (pH 5.9 - 6.7	
	Fluid(%)	Dried (%)
Lactose	4.85	87.63
Ash	0.50	8.54
Lactic acid	0.05	0.23
Total solids	5.40	96.40

* estimation based on Table 2.1 (a)

In anaerobic biodegradation, whey permeate is digested to produce biogas. And thus the high BOD of the whey is considerably reduced before it is discharged into the effluent stream.

Zellner and Winter (1987) have analyzed and characterized 90% of the bacterial population in a whey digester. Lactobacillus, Eubacterium, Fusobacter and Bacterioides strains were identified along with several methanogenic strains.

2.3.2 Whey Permeate Hydrolysis

The hydrolysis of whey permeate yields glucose and galactose. Procedures that can be followed are acid hydrolysis or enzymatic hydrolysis. The enzymatic hydrolysis of lactose to glucose and galactose may be inhibited by the products of reaction depending upon the microbial culture. Chen *et al.* (1985) studied the enzymatic hydrolysis of lactose to glucose and galactose using Kluyveromyces fragilis (yeast). They found that both products inhibited the hydrolysis. They proposed a kinetic model in which glucose was a non-competitive inhibitor while galactose was considered a competitive inhibitor. A kinetic rate equation based on this multiple inhibition model was obtained and was found to be in good agreement with the experimental results.

2.3.3 Anaerobic Treatability of Whey and Whey Permeate

A study by Kisaalita *et al.* (1990) showed that there was no change in the pathway for lactose degradation in whole whey and whey permeate. It was found that in the acidogenic phase of a two phase anaerobic digester, whole sweet whey

could be fermented as efficiently as whey permeate. However the rate will be lower for whole sweet whey. The anaerobic digestion of whey and or whey permeate has been investigated by a number of researchers. The studies cited in the literature are given in the following paragraphs.

Clark (1988) conducted an experimental study to assess the potential for the anaerobic digestion of whey, to identify any problems that might be encountered in scale-up and to provide design and operational data for the anaerobic digestion of lactic acid casein whey (LACW) and the sweet rennet casein whey (SRCW). One of the main apparent differences between LACW and the SRCW is in the ash content which is much higher in LACW (6.8 g/L) than in sweet whey (4.3-5.2 g/L). He studied the treatability of the wheys in a pilot-scale 700 L Upflow Anaerobic Sludge Blanket (UASB) digester. A stable loading rate of 9 kg COD/m^3-day was achieved for LACW when supplemented with nitrogen (1680 mg total nitrogen/L). For unsupplemented LACW, the maximum loading rate was only 1/3 of the loading rate for supplemented whey. For nitrogen-supplemented SRCW, the corresponding loading rate was 16 kg COD/m^3-day. Nitrogen supplementation allowed higher biocarbonate alkalinity to be maintained, thus providing greater buffering against volatile fatty acid production and possibly also promoted better microbial

nutrition. He obtained COD reductions 91.1 and 96.8 % (average) for LACW and SRCW respectively. The biogas yield averaged 587 and 547 L/kg COD fed for LACW and SRCW respectively.

Higher loading rates for LACW were prevented primarily due to sludge washout. This washout was attributed to the high Ca^{++} concentration causing a higher proportion of dispersed and less flocculent biomass with a reduction in granule size. It has been reported (Lettinga *et al.*, 1980) that Ca^{++} ions have a positive effect on the flocculation and mechanical strength of anaerobic sludges at Ca^{++} levels of 280-480 mg/L. For SRCW, higher loading rates were prevented by high superficial biogas velocities and the resulting turbulence in the top of the digester, which prevented biomass from settling back in the digester and thus led to washout. In a completely mixed full-scale digester (1100 m^3) treating cheese whey without biomass recycle, a loading rate (LR) of 1.6 kg COD/m^3-day was possible. Loading rates up to 3.3 kg COD/m^3-day were achieved in a 25 L completely mixed laboratory system when supplemented with 10% digested swine manure (Adams and Prairie, 1988).

Holder and Sewards (1976) reported a loading rate of 4 kg COD/m^3-day at a COD reduction of 90% in a laboratory-scale anaerobic contact digester. In another contact process

(Sutton, 1986) loading rates up to 8.2 kg COD/m^3-day were achieved in a 38m^3 full-scale demonstration plant treating cheese whey permeate, but ultrafiltration was used to recover biomass which was then recycled.

Barford *et al.* (1986) reported the use of chemical flocculents to enhance biomass accumulation in a laboratory-scale semicontinuous digester treating high strength cheese whey. When it was operated on a mix/settle/fill and draw regime, maximum loading rates of 16.6 kg COD/m^3-day, with more than 98 % soluble COD reduction, were achieved. The same system could be maintained at 16.1 kg COD/m^3-day without flocculent addition. Loading rates from 6.5 to 26 kg COD/m^3-day with a COD removal of 70% were also reported in a two-stage UASB system (12 m^3 acidification followed by 7 m^3 methanogenic reactor) treating dilute whey (Cohen and Borghans, 1986).

van den Berg and Kennedy (1983) treated cheese whey in two laboratory-scale downflow anaerobic filter reactors at loading rates ranging from 5 to 22 kg COD/m^3-day at an HRT as low as 3 days and achieved COD reduction from 97% to 91% respectively. In a further study (Wildenauer and Winter, 1985) cottage cheese whey was treated in a laboratory-scale fixed-film loop reactor at a loading rate of 14 kg COD/m^3-day at an HRT of 5 days and COD reduction of 95% was achieved. Per kg

COD removed, 0.4 m^3 biogas with a methane content of 79% was produced and the steady state gas productivity was 5.6 m^3/m^3 per day. The higher amount of methane than the theoretical value was explained as result of the absorption of CO_2 in the water of the gasometer. A loading rate of 35 kg COD/m^3-day and a soluble COD reduction of 95% was achieved when undiluted sour whey (pH 4.3) was also treated in an upflow fixed-film loop reactor. When whey permeate was substituted for the whole whey, the steady-state loading was reduced by about 50% and addition of nitrogen, phosphate, nickel and tungstate gave no improvements (Winter *et al.*, 1988).

Hickey and Owens (1981), using a 55 L pilot-scale fluidized bed reactor, treated undiluted cheese whey at loading rates from 13.4 to 37.6 kg COD/m^3-day and achieved COD removal efficiencies from 83.6% to 72% respectively. Boening and Larsen (1982), using a fluidised bed, treated dilute unsupplemented lactic permeate at loading rate from 7.7 to 19.5 kg COD/m^3-day and achieved COD removal efficiencies from 90% to 70% respectively.

In an expanded bed reactor, Switzenbaum and Danskin (1981) treated dilute cheese whey supplemented with ammoniun phosphate at a loading rate of 20 kg COD/m^3-day and an HRT of 12 h to achieve 87% COD reduction. In further runs at constant HRT and variable loading rate (8.2-29.1 kg COD/m^3-day), the

efficiency ranged from above 90% to below 60%.

Research has therefore shown that whey has been successfully treated using the main anaerobic digestion technologies currently available. High loading rates and COD reduction efficiencies are obtainable and whey has been treated commercially, but generally as a mixture and hence diluted, with other wastewater.

2.4 Kinetics of Anaerobic Digestion

2.4.1 Microbial Growth

In the anaerobic experimental environment, the living cells consume nutrients and convert substrates to products. At the same time, these cells will grow in numbers at a suitable temperature and pH. A growing cell population interacts with the environment in a complicated way.

Bacteria use glucose not only as a source of energy but also as a source of carbon for the manufacture of new biomass. The empirical equation for the synthesis of biomass from glucose (Mosey, 1981) is given below,

$$5\ C_6H_{12}O_6 + 6\ NH_3 \longrightarrow 6\ C_5H_9O_3N\ (\text{biomass}) + 12\ H_2O \qquad (2.9)$$

from which it may be deduced that 1.15 mg of glucose are required as a carbon source for the production of each milligram (dry wt) of biomass formed.

$$R_a = \frac{1.15}{180} \cdot \frac{dX_a}{dt} \qquad (2.10)$$

where R_g: rate of uptake of glucose for cell synthesis, mMoles/L-day

X_g: concentration of biomass (glucose-fermenters), mg/L

2.4.2 Growth-Cycle Phases in Batch Cultivation

When a microbial culture is grown in a batch mode it passes through several phases of growth (see Fig. 2.2). Initially, when the inoculum is introduced into the system, there is a lag phase when the cells adjust to the new environment. At the end of the lag phase the population of microorganisms is well adjusted to its new environment. Then follows a log phase of growth in which the cell population increases exponentially with time, and the specific growth rate (μ) remains constant. The cells can then multiply rapidly, and cell mass, or the number of living cells, doubles regularly with time. The equations relating to specific growth rate to the biomass concentration is given by

$$\frac{dX}{dt} = \mu X \qquad \text{or} \qquad \frac{1}{X} \cdot \frac{dX}{dt} = \mu \tag{2.11}$$

Integrating the above equation with $X = X_o$ at $t = t_{lag}$ yields,

$$\ln \frac{X}{X_o} = \mu(t-t_{lag}) \text{ or } X = X_o\, e^{\mu(t-t_{lag})} \tag{2.12}$$

where $t > t_{lag}$

From the above equation one can easily deduce the time interval $\bar{t}_d$ required to double the population,

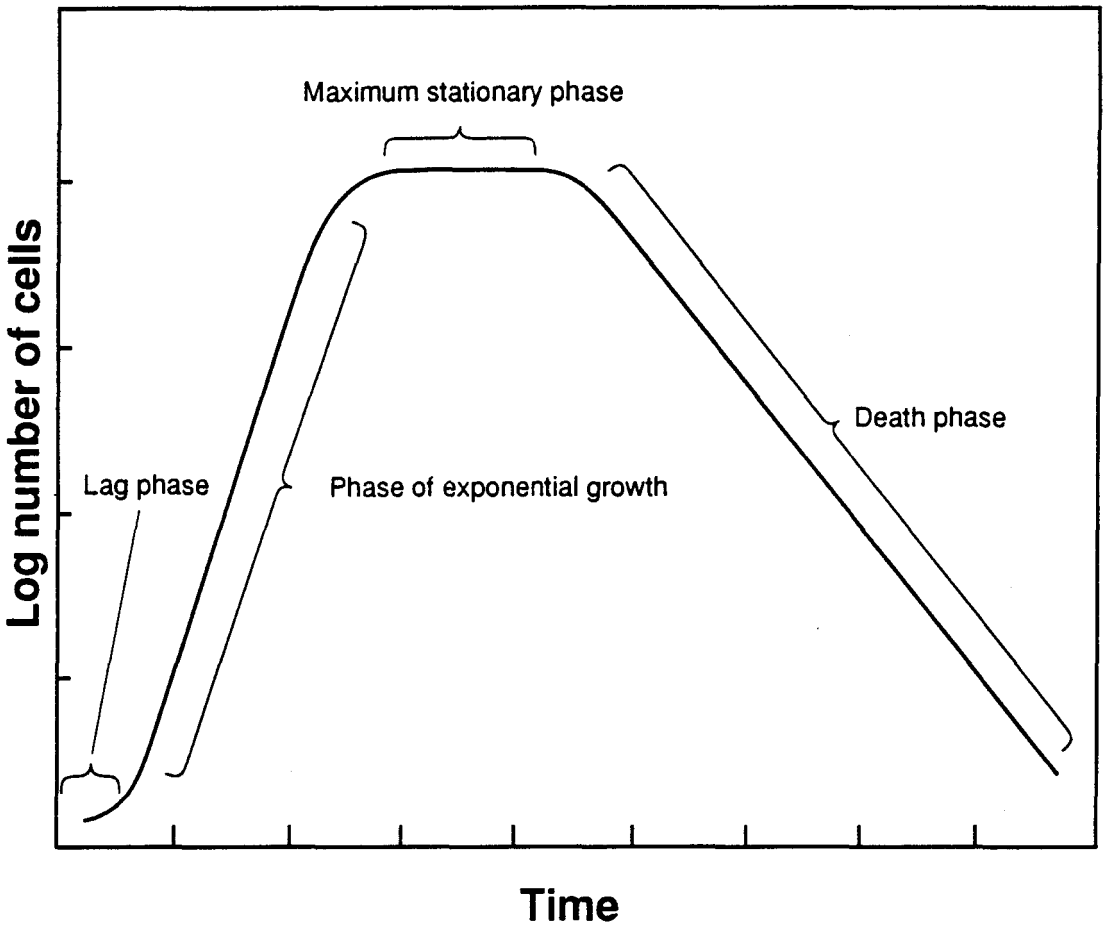

Figure 2.2 Typical Growth Curve for Batch Cell Cultivation (Bailey and Ollis, 1986)

$$\text{i.e.} \quad \bar{t}_d = \frac{\ln 2}{\mu} \qquad (2.13)$$

When the growth limiting substrate is exhausted in the system, the cells go into maintenance mode in the stationary phase. In this phase the cell population is constant. Gradually, the cell population starts to decrease in the death phase due to an accumulation of toxins and/or starvation of nutrients. In each growing culture, there is a maximum specific growth rate (μ_{max}). This is the maximum possible rate of growth per unit biomass with unlimited nutrients in the given environment.

2.4.3 Kinetic Models and their Applications

A kinetic model is a simplified and useful representation of cell population kinetics. With respect to the environment, it is common practice to formulate the growth medium so that all components but one, are present at sufficiently high concentrations that changes in their concentration do not change the overall fermentation rate. Such a system can be modelled by unstructured models. Thus, unstructured models consider a single component as the growth limiting component.

Cellular representations which are multi-components are called structured models. Also, if the average properties of a cell population are considered, an unsegregated model is formed. Consideration of discrete, heterogeneous cells

constitutes a segregated model (Bailey and Ollis, 1986, Chapter 7, p 375-376). The actual situation in a fermentation is a segregated and structured one, but an unsegregated, unstructured approach is easier to use and often a good representation of the system. One simple kinetic model which assumes that the rate of increase of cell mass is a function of cell mass only is Malthas' Law.

$$f(X) = \mu X \qquad (2.14)$$

where μ is constant. This model does not take into account the lag or death phases in a microorganism and assumes unrestricted growth in the cells. Hence this is not at all an useful representation of cell kinetics.

Slater (1985) describes the logistic equation of cell growth which relates the specific growth rate, μ, to the cell mass concentration X, the maximum specific growth rate μ_{max}, and the final population size X_f.

This equation is shown below:

$$\mu = \mu_{max} \left(1 - \frac{X}{X_f}\right) \qquad (2.15)$$

This is an empirical model and has been found to approximate cell growth in a batch culture reasonably well. This equation can also be used to determine the maximum specific growth rate of the biomass by knowing the change in cell population with time.

A semi-empirical equation which has been found very useful in representing cell growth kinetics is the Monod equation (Bailey and Ollis, 1986, Chapter 7, p 383-384). If the concentration of one essential constituent (S) is limiting, the cell growth is given by

$$\mu = \frac{\mu_{max} S}{K_s + S} \qquad (2.16)$$

where μ_{max} is the maximum growth rate achievable when $S >> K_s$ and the concentration of all other nutrients is unchanged. K_s is that value of the limiting nutrient concentration at which the specific growth rate is half its maximum value.

Other related forms of specific growth rate dependence have been proposed which may give better fits to experimental data. Bailey and Ollis (Chapter 7, p 391) list the following models.

$$\mu = \mu_{max}(1 - e^{-S/K_s}) \qquad \text{(Tessier Model)} \qquad (2.17)$$

$$\mu = \mu_{max}[1 + K_s(S)^{-\lambda}]^{-1} \qquad \text{(Moser Model)} \qquad (2.18)$$

$$\mu = \mu_{max}[1 + BX(S)^{-1}]^{-1} \qquad \text{(Contois Model)} \qquad (2.19)$$

The first two examples render algebraic solution of the growth equations much more difficult than the Monod form.

Leudiking and Piret (1959) describe the rate of product formation as a function of rate of biomass formation and the instantaneous biomass concentration:

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \qquad (2.20)$$

where P is the product concentration and α and β are empirical constants. The constant α is related to the growth-associated phase, while the constant β is the maintenance coefficient. This model was applied by the authors for the batch fermentation of lactic acid and was found to approximate closely the experimental results.

Bolle *et al.* (1985) conducted some experiments to study the maximum specific growth rate (μ_{max}) in a batch reactor with different initial concentrations of microorganisms: 8.5, 6.4, 3.4 and 3.0 kg dry wt/m^3. Their experimental set up consisted of a batch reactor of 3L (total volume) capacity, filled with wastewater, nutrients and sodium bicarbonate. The pH was about 7 and the temperature was kept constant at 35°C. The reactor was inoculated with anaerobic sludge. The initial COD was about 5000-5500 ppm. Table 2.2 lists the results of the parameter optimization for the Monod model as well as the Andrews model (see section 2.6.8), together with their rms relative errors.

The overal results of the four experiments to determine the optimal parameter estimations were:

μM_{max} = 12 x 10^{-4} h^{-1} ± 9% and

μA_{max} = 16 x 10^{-4} h^{-1} ± 2%

K_I = 0.0158 g HAc/L ± 2.5%

Table 2.2 Results of the Parameter Optimization for Monod (M) and the Andrews (A) Model (Bolle *et al.*, 1985)

Biomass (kg dry wt/m^3)	M μ_{max} (h^{-1})	ϵ_{rms}	A μ_{max} (h^{-1})	ϵ_{rms}	K_I (g/L)	ϵ_{rms}
8.5	$13x10^{-4}$	9%	$18x10^{-4}$	1%	0.0158	2.5%
6.4	$10x10^{-4}$	10%	$16x10^{-4}$	1%	0.0137	1.0%
3.4	$13x10^{-4}$	8%	$16x10^{-4}$	2%	0.0197	2.0%
3.0	$10x10^{-4}$	8%	$14x10^{-4}$	2%	0.0140	3.0%

From these results, they concluded that with a reliability of over 95%, that Monod Model was inadequate to fit the measurements. On the other hand, the Andrews model fits the data and describes substrate inhibition and reactor failure due to pH changes.

2.4.4 Advantages and Limitations of Different Kinetic Models

The advantages of the Monod-type model are that the kinetic parameters (the microorganism maximum specific growth rate and half-velocity constant) have deterministic connotations which describe the microbial processes and the model is able to predict the conditions for maximum biological activity and when activity will cease (i.e. washout). Disadvantages of the Monod model are that one set of kinetic parameters cannot describe the biological process at short and

long retention times and that the kinetic parameters vary with the influent concentration (Morris, 1976).

To overcome the disadvantages of the Monod model, various forms of the first-order kinetic model have been used. The advantages of these first-order models are that they are simple to use and give good fit of experimental data. Their disadvantages are that they do not predict the conditions for maximum biological activity and system failure (Chen *et al.*, 1980).

2.4.5 Kinetics of Substrate Utilization

In most cases, the utilization of a limiting substrate by a growing cell culture exhibits much the same trends as the biomass (Wallace, 1986). Initially as the microorganisms adjust to their new environment, little or no substrate is consumed and the cell concentration remains more or less constant. Once the "retooling" is over, the cells begin to consume substrate and enters the exponential growth phase. Consequently, the substrate concentration decreases exponentially and reaches a limiting value. At this point, the cell population enters the stationery phase and use the substrate mainly for its endogeneous metabolism. In the exponential phase of substrate utilisation, the specific substrate uptake rate can be represented as,

$$q_s = - \frac{1}{X} \cdot \frac{dS}{dt} \qquad (2.21)$$

At high substrate concentrations, the substrate may inhibit the cell growth and in most cases, this inhibition may prevent the exponential growth of the biomass. Instead, only linear growth and substrate uptake is observed (Shukla *et al.*, 1984). Cell strains which are able to utilize high substrate concentrations and produce high product concentrations are promising from an economic point of view (Converti *et al.*, 1984; Panchal *et al.*, 1982).

Yan *et al.* (1990) investigated the possibilities of treating cheese whey anaerobically . The experiment was carried out over a range of influent concentrations from 4.5 to 38.1 g COD/L at a constant hydraulic retention time of 5 days. A 17.5 L upflow anaerobic sludge blanket reactor was used for their study.

Throughout the experiment the temperature was kept at 33 ± 1 °C. The influent concentration was increased stepwise from 4.56 to 9.93, 17.7, 28.8 and 38.1 g COD/L. There was an operating period of 10 to 15 days for each subsequent increment of influent concentration. The biomass growth was low for the initial low influent concentration phase but increased with higher concentration. Based on their results,

the growth rate was expressed as follows:

$$\frac{dX}{dt} = Y \frac{dS}{dt} - k_d X \qquad (2.22)$$

The sludge growth yield coefficient, Y (g VSS/g COD) and decay rate k_d obtained in their study were 0.058 g VSS/g COD and 0.016 day^{-1} respectively. The specific growth rate (μ) and specific substrate consumption rate (q_s) obtained for different substrate concentrations were given in the Table 2.3.

Table 2.3 The Values of q_s and μ Obtained for Different Loading Rates (Yan *et al.*, 1990)

Influent (g COD/L)	Loading rate g/L-day)	Sludge, X (g VSS)	q_s (g COD/day-g VSS)	μ (day^{-1})
9.93	1.97	107.4	0.263	0.0023
17.7	3.54	91.7	0.548	0.0077
28.7	5.96	114.2	0.714	0.0281
38.1	7.77	164.9	0.654	0.0254

Shieh *et al.* (1985) studied the process kinetics of anaerobic digestion of liquid wastes in a fluidised bed using a homogeneous microbial system. A synthetic wastewater with glucose as the sole carbon and energy source was used as the feed in their investigation. The sludge taken from an anaerobic digester treating animal residues was used as the

seeding material to start up the reactor. The growth support media used was granular activated carbon particles, with a mean diameter of 0.6 mm. All necessary nutrients, trace elements, and alkalinity were provided in excess amounts to ensure that glucose was the only limiting substrate. The temperature and pH were controlled at 35°C and 7.0 ± 0.2 respectively. The experimental data obtained by the authors fit the Michaelis-Menten expression (given below) quite well and found that k = 2.0 day^{-1} and K_S = 154 mg/dm^3

$$q_s = \frac{k\ S_e}{K_s + S_e} \qquad (2.23)$$

where, q_s : specific substrate utilisation rate, kg COD/kg VS-day

k : maximum substrate utilisation rate, day^{-1}

Ks : Michaelis constant, mg/dm^3

S_e: Steady-state effluent substrate concentration, mg COD/dm^3

2.4.6 Yield Coefficient

The overall biomass to substrate yield is defined by the equation,

$$Y_{x/s} = \frac{X-Xo}{So-S} = \frac{\Delta X}{-\Delta S} \qquad (2.24)$$

The usefulness of the yield factor is limited as it includes all the substrate used for cell maintenance, product formation

and cell growth in single variables. Nevertheless, it allows a rough estimation and acts as comparison parameters between cell strains.

Aiba *et al.* (1973) used the following equation to relate the fractions of substrate utilized for cell growth and maintenance:

$$\frac{dS}{dt} = \frac{1}{Y_G} \cdot \frac{dX}{dt} + m\,X \qquad (2.25)$$

where Y_G: Yield factor for cell growth

m: specific rate of substrate uptake for cellular maintenance, h^{-1}

This equation does not account for the substrate converted to products. If significant amount of substrate is utilized for product formation, then the model proposed in Equation 2.25 is only applicable if the product is growth-associated. [The product formation rate is said to be growth-associated when the rate is dependent on the cell growth rate and said to be non-growth associated when it is dependent on the cell concentration, rather than the growth rate]. When significant amount of product is formed, the model should be expanded to include the substrate utilized in the formation of these products (Damino *et al.*, 1985; Shukla *et al.*, 1984).

A substrate balance can then be expressed according to the equation,

$$\frac{dS}{dt} = \frac{1}{Y_o} \cdot \frac{dX}{dt} + \frac{1}{Y_p} \cdot \frac{dP}{dt} + m\,X \qquad (2.26)$$

where P is the product concentration. In their study, Shieh *et al.* (1985) estimated the true biomass yield which was found to be 0.08 mg VSS/mg COD utilized.

2.5 Problem Facing the Single-phase Digestion Process and its Solution

In common homogeneous anaerobic digestion processes a delicate balance exists between initial acidogenesis of the substrate and conversion of the acid products by methanogenic bacteria into methane and carbon dioxide. Especially at high loading rates imbalances between acidogens and methanogens may lead to accumulation of intermediate acid products, thereby exceeding the buffering capacity of the environment, and causing the pH to drop to a level that inhibits methanogenesis (Pohland and Bloodgood, 1963).

As was discussed by Andrews and Graef (1971) and by Kroeker *et al.* (1979) high volatile fatty acid concentrations in combination with a low pH value are particularly detrimental to methanogenic activity through the toxic action of the un-ionized volatile fatty acids. On the other hand, a high cation concentration caused by neutralizing agents added to restore the pH also may inhibit methanogenic activity

(McCarty and McKinney, 1961; Kugelman and Chin, 1971).

One way to solve this problem is to effect the overall digestion process in two separate reactors with the acidogenic phase in the first reactor followed by the methanogenic phase in the other one.

Gosh (1981) studied the kinetics of acid-phase fermentation in anaerobic digestion in a completely mixed reactor with a continuous feed of glucose or sewage sludge. The glucose digester was fed with a mineral salt medium containing glucose as the sole source of carbon and energy. Sewage sludge was used to represent a complex heterogeneous particulate substrate that requires hydrolysis prior to the formation of acetate and higher fatty acids. After obtaining the steady-state performance, enrichment of the acetate formers was effected by operating the digesters at much higher loadings and dilution rates, thereby selecting against the survival of the methane formers.

The terminal end products of acid-phase fermentation by acidogenic and acetogenic bacteria are acetate, higher fatty acids, CO_2 and H_2. The substrate is assimilated by microbes for three primary functions. Part of the substrate is transformed to building blocks of protoplasm thereby resulting in cell growth. A second portion of the substrate is catabolized to derive the energy for protoplasm synthesis and

a third portion is oxidised for maintenance energy. Therefore,

$$dS = U_p dX + U_e dX + mX\,dt \qquad (2.27)$$

where U_p is the mass of substrate assimilated per unit cell mass synthesized. U_e is the mass of substrate assimilated to derive the energy for synthesis per unit cell mass formed, and m is the maintenance coefficient. Pirt (1967) suggested that fermentation products are generated as a consequence of energy metabolism, and originate from substrate fractions catabolized to drive the energy of synthesis and maintenance energy i.e.,

$$\alpha_i = \frac{dP}{U_e dX + mXdt} \qquad (2.28)$$

where α_i is the time yield coefficient for any product i, and P is the concentration of the product in the digester.

To determine the biokinetic constants such as μ_{max}, K_s, m, U_p, and U_e and the product yield constants α_i and α_g, Gosh (1981) derived the following equations from mass balance of organisms, substrate, liquid product and gaseous product around a completely mixed acid-phase digester at steady-state and operated without the recycling of organisms or concentrated substrate.

$$\frac{K_s}{\mu_{max}} \cdot \frac{1}{S} + \frac{1}{\mu_{max}} = \frac{\theta}{\theta} \qquad (2.29)$$

$$(S_i - S)X = (U_p + U_e) + m\theta \qquad (2.30)$$

$$\frac{(S_i - S)}{(P - P_i)} = \alpha_i^{-1} + (X/P)U_p \qquad (2.31)$$

$$\theta G'/(S_i - S) = \alpha_g V - (\alpha_g V U_p)\,[X/(S_i - S)] \qquad (2.32)$$

where P_i: concentration of ith product in the digester

G': observed mass rate of gas production

α_g: true gas yield coefficient

V : digester culture volume

The values of kinetic constants, estimated by analyzing the data (Gosh, 1981) in terms of equations 2.29 to 2.32 are given in Table 2.4.

We can easily see from the data above, that the acid formers grown on glucose exhibited a maximum specific growth rate and order of magnitude larger and a saturation constant three orders of magnitude smaller than those obtained with the sludge substrate. The lower growth rates on sludge could be due to the ratecontrolling nature of the sludge hydrolysis step.

Massey and Pohland (1978) achieved the phase separation by controlling the HRT and recycle to select the particular microbial populations, on the basis of difference in growth rates. Their research has been directed toward providing

Table 2.4 Biokinetic Constants for Acid-Phase Mesophilic Digestion of Sewage Sludge and Glucose Substrates (Gosh, 1981).

Kinetic Constants	Sludge	Glucose
Maximum specific growth rate, μ_{max} (h^{-1})	0.16	1.25
Minimum generation time (h)	4.33	0.56
Saturation constant, K_s (g/L)	26.0 (as Vs)	0.023 (as glucose)
Maintenance coefficient, m(h^{-1})	0.033	0.256
Substrate utilisation coefficients		
Synthesis, U_p	1.12	4.63
Energy metabolism, U_e	1.35	1.16
The biomass yield coefficient, Y	0.40	0.17
Product yield coefficients, α for		
acetic acid	0.28	0.73
propionic acid	0.11	0.19
butyric acid	0.25	0.17
valeric acid	0.25	----
gas	0.071	0.054

confirmation of earlier results, determining the effect of biomass recycle on the operation of both phases and the practicality of accomplishing solids separation and recycle by gravity clarification demonstrating the utility of mathematical models based on bacterial growth kinetics for describing both the acidogenic and methanogenic phases, and

applying the two-phase system to the treatment of both simple and complex soluble-type substrates.

The following equations i.e Equations 2.33 to 2.35 were used (Massey and Pohland, 1978) for the estimation of kinetic parameters in the acid-phase continuous flow reactor.

$$\mu = \frac{1 + R}{\theta} - \frac{RX_R}{\theta X_1} + k_d \qquad (2.33)$$

$$\mu = \frac{\mu_m S_o}{K_s + S_o} \qquad (2.34)$$

$$\frac{S_o - S_1}{\theta} = \frac{\mu X_1}{Y} \qquad (2.35)$$

where

R = fraction of the influent flow rate recycled

X_R = concentration of acid formers in recycle flow

X_1 = concentration of acid formers in effluent

μ = specific growth rate of acid formers

μ_m = maximum specific growth rate of acid formers

θ = hydraulic retention time

S_o = substrate concentration for acid formers in influent

S_1 = substrate concentration for acid formers in effluent

k_d = decay constant for acid formers

K_s = saturation constant

The kinetics parameters for the acid phase were:

Y = 0.31 mg VSS/mg ΔCOD utilised

k_a = 0.065 h^{-1}, μ_m = 2.7 h^{-1} and

K_S = 2583 mg Δ COD/L

(ΔCOD: To permit kinetic analysis, based on the models given in Equation 2.33 to Equation 2.35, the substrate concentration remaining for the acid phase was estimated as the difference in the soluble COD of the effluent and the calculated COD associated with the measured VA concentrations in the effluent. It was assumed that all soluble COD in the effluent, with the exception of that resulting from the volatile acids, would be available as substrate for acid forming bacteria.)

Similar analysis of data obtained from the methane phase indicated maximum specific growth rate of 0.43 day^{-1} and 0.86 day^{-1} and saturation constants of 369 mg HAc/L and 164 mg HBu/L for organisms utilizing acetic acid and butyric acid respectively, with no concentration of methane formers being achieved with recycle (Massey and Pohland, 1978). Lawrence and McCarty (1967) have reported comparative values with μ_{max} of 0.5 day^{-1} and K_s of 207 mg HAc/L at a similar influent substrate concentration.

2.6 Inhibition Kinetics

A change in the chemical activity of one or more chemical

species essential to the cell, a disruption of the permeability barrier of the cell, and a change in activities of enzymes are some effects of the inhibitor. The enzymes or metabolic aggregates within the cell may dissociate, stopping essential pathways. The synthesis of enzymes or the functional activities of the cell may themselves be affected by the inhibitor. The inhibition mechanism may occur in one or more of the several ways:

1) Chemical reaction with one or more components of the cell.
2) Adsorption or complexing with the enzyme, co-enzyme or substrate.
3) Entry of the inhibitor into the reaction sequence.
4) Dissociation of enzyme aggregates.
5) Modifications of the pH, ionic strength, or solvent activity of the medium.
6) Complexing or other interactions with control sites in the cell.

2.6.1 Process Stability and Toxicity

The stability of anaerobic fermentation process depends upon the maintenance of a delicate biochemical balance between the fast growing acid formers and the more fastidious methane formers. Process instability is usually indicated by a rapid increase in the concentration of volatile acids with a

concurrent decrease in methane gas production (Kroeker *et al.*, 1979). The reasons for this process instability include insufficient acclimation of the methane formers to new substrates, overloading and rapid temperature fluctuations. A number of organic and inorganic materials that may be present in the wastewaters play a significant role in process inhibition and toxicity. These include excessive concentrations of volatile acids, ammonia, alkaline earth-metals, salts, heavy metals and sulphides.

Several researchers concluded that volatile acids themselves are toxic to methane bacteria at concentrations above 2000 mg/L, but McCarty and McKinney (1961) concluded that a high volatile acid concentration is the result of unbalanced treatment and not the cause. The debate was resumed by Buswell and Morgan (1962) who reported that propionic rather than acetic acid was toxic to the methane formers. In order to clear up the controversy, McCarty *et al.* (1964) investigated the effects of various volatile acids on methanogenic bacteria and concluded that volatile acids were not toxic to methane bacteria at concentrations that would occur in malfunctioning digesters. Andrews (1969) took an additional step and suggested that digester toxicities were caused by the unionized portion of the volatile acids and that as a result, volatile-acids toxicities were directly related

to mixed liquor pH as well as to the concentration of volatile acids; his work was supported by Brune (1975). Similar ambiguities exist regarding the alleged toxicity of ammonia to the methane formers.

Kroeker *et al.* (1979) studied the process stability in pilotplant treating swine manure and in laboratory digestion of urealaden acetic acid substrate. From the experimental results, they concluded that digester toxicities, although indirectly related to concentration of free ammonia, are more directly related to the concentration of un-ionized volatile acids. Process inhibition by ammonia appeared to be a result of excessive concentrations of free ammonia rather than ammonium ion. Process toxicity was caused by un-ionized volatile acids in a concentration range of 30 to 60 mg/L as acetic acid.

2.6.2 Growth Inhibition

The growth of microorganisms is frequently affected by the presence of inhibitory compounds in the bioreactor. The presence of inhibitory compounds can be either intentional or unintentional. It is the latter that is of particular interest in the context of biotreatment processes. The unintentional presence of inhibitors in microbial growth systems arises from the presence of inhibitory components in the bioreactor feed, the production of either inhibitory

products or intermediates, and the production of inhibitory compounds as a result of cell lysis.

The theory of microbial response to growth inhibitors is based on the kinetics of enzyme inhibition. Essentially two main types of inhibition occur: 1) competitive inhibition, where the inhibitor competes with the growth-limiting substrate for uptake by the microorganism, thus affecting the affinity for the substrate and 2) non-competitive inhibition, where the inhibitor is assumed to react with the microorganism at some site other than that for the uptake of the growth-limiting substrate, and does not affect the affinity for the substrate.

For competitive inhibition, the Monod relationship is modified so that

$$\mu = \frac{\mu_{max} S}{S + \alpha K_s} \quad \text{where } \alpha = \frac{I + K_i}{K_i} \qquad (2.36)$$

and where I is the inhibitor concentration, K_i the inhibition constant and $\alpha > 1$. For non-competitive inhibition, the Monod relationship is modified so that

$$\mu = \frac{\mu_{max} S}{\alpha(S + K_s)} \qquad (2.37)$$

2.6.3 pH Inhibition on Methanogenesis

In general, the anaerobic fermentation process of

methanogenesis is impaired at pH values below 6.0 and above 8.0. Although low pH inhibits methanogenesis the effect is not bactericidal, a fact which was confirmed by Keefer and Urtes (1962) who observed methanogenic bacteria surviving in laboratory reactors for as long as 2 months at pH values below 5. However, a different inhibition mechanism at low and high pH has been observed and was first reported by Clark and Speece (1970). In general, no recovery lag is experienced after pH restoration from values above pH 8.2, whilst a considerable lag in recovery occurs when a low pH exists for several days. However, in the case of a low pH condition existing for 12 hours or less, recovery from inhibition is rapid and complete on correction of the pH within the reactor (Anderson *et al.*, 1982). The pH variation occurs when the effluent is low in nitrogen, strongly alkaline or acidic. Effluents low in nitrogen (i.e. COD:N greater than 100:2) cannot support the formation of ammonium bicarbonate, which is the main source of buffer within the anaerobic environment. Extremely low levels of nitrogen can also cause nutrient deficiency, in which case the faster growing acidogenic bacteria utilize all of the available nitrogen during the formation of short chain volatile fatty acids, thereby leaving insufficient nitrogen to permit the methanogenic bacteria to utilize the acids. Examples of nitrogen-deficient raw

effluents are those produced in the confectionary industry and that from the ultrafiltration of whey permeate in the dairy industry.

Clanton *et al.* (1985) studied the effect of pH and the type of base on the anaerobic digestion, treating raw whey. The average COD of raw whey used was 68700 mg/L. Three pH levels (7.5, 8.0 and 8.5) and two types of base (NaOH and NH_4OH) were used. Digesters, made of vertical tubular acrylic plastic vessels, were loaded at a rate of 35 L/day-m^3 of digester three times a week. They did not find any significant difference among the pH levels for the percent COD removed. But they found that the addition of NaOH resulted in a COD removal of 16.3 ± 1.1 percent and that of NH_4OH in 23.0 ± 9.5 percent. On the other hand, raising the adjusted pH from 7.5 to 8.5 resulted in an approximate doubling of methane production from about 80 to about 140 L/m^3-day

From the results of their study, Clanton *et al.* (1985) came to the following conclusions. In order to maintain a proper digestion process for cheese whey, some form of base or buffer must be added to achieve optimal pH. Any attempt to allow the digestion process to proceed naturally will result in a low percent COD removal and low methane production. Maintaining digester pH more basic has no effect on the percent COD removal but results in increased methane

production.

Zoetemeyer (1982) examined the growth kinetics of an enrichment culture for the anaerobic fermentation of glucose as a function of pH (4.5-7.3) and temperature (20-60°C). He found that the degradation products varied depending on experimental conditions. For pH value of 7 and 30°C, he reported the following values. μ_{max} = 7.2 day^{-1}, Y = 0.1 kg biomass/kg COD and Ks = 22 g COD/m^3. The available data on growth kinetics for fermentative organisms indicate that this reaction does not limit the performance of an anaerobic digester (Gujer and Zehnder, 1983).

Product distribution of anaerobic fermentation in an acid-reactor (first stage of a two phase anaerobic digestion) was studied by Zoetemeyer *et al.* (1979) as a function of pH. The experiment was conducted in a CSTR with 1 % glucose and a mixed culture, cultivated from secondary sludge of an aerobic sewage plant. The temperature was kept at 30°C. They found that the product distribution at 90 % of the maximum growth rate was fairly constant up to a pH value of 6, after which dramatic changes of the main product occured from butyric acid to lactic acid and subsequently to acetic acid, formic acid and ethanol.

The low butyric acid concentration at higher pH values is in good agreement with the low values usually found in single

stage anaerobic reactors. The fact that the degradation of butyric acid by the methanogenic bacteria occurs at a faster rate than acetic acid and much faster than propionic acid results in very low butyric acid concentration and often no detection is possible. An overall maximum specific growth rate was found in the range 5.8 - 6.2 from the experimental data. They (Zoetemeyer *et al.*, 1979) arrived at the following conclusions: the stable operation of the acidogenesis of carbohydrates in a single as well as a two-stage anaerobic process is hardly possible in the pH range of 6 - 8. Running the acid reactor at high dilution rate in the pH range of 5.7 - 6.0 offers a stable and most favourable substrate for the methane reactor. The advantages of the lowest possible reactor volume and the highest butyric acid production are thus ideally combined and makes the acid reactor a suitable tool to control the methane reactor.

2.6.4 Effect of Temperature on Methanogenesis

Temperature is one of the key considerations in the design of anaerobic digestion processes. Lin *et al.* (1987) studied the effect of temperatures on the methanogenesis process in anaerobic digestion, in a chemostat. They used a synthetic substrate containing 50, 25 and 25 % (COD basis) acetic, propionic and n-butyric acids respectively.

The experiments were conducted at temperature intervals of 5°C from 15°C to 50°C. The laboratory-scale digesters used in their study operated continuously for periods from 6 to 16 weeks. The digesters were operated at shorter retention times for higher temperatures and at longer retention times for lower temperatures. At steady state conditions (i.e. when product concentration variations are small, approximately 10%) samples were taken and analyzed. They found that the treatment efficiencies were highest at the optimum digestion temperature of 35°C. Lin *et al.* (1987) also found out that the methane content of the produced gas in the mesophilic digesters (i.e. 35°C) ranged from 62 to 67.5% as against 58 to 61% in the 50°C digesters.

The objectives of their study also included the evaluation of temperature effects on the kinetic constants of biological growth and substrate utilization. The method of least squares was used to determine the line of best fit for the experimental data. The equations used to determine the kinetic constants were shown below and the results are listed in Table 2.5.

$$V = \frac{V_{max} \cdot S}{K_s + S} \qquad (2.38)$$

$$\mu_{max} = Y_g \cdot V_{max} - k_d \qquad (2.39)$$

where V : specific substrate utilization rate, mg COD/mg-day

S : effluent substrate concentration, mg COD/L

K_s : substrate saturation concentration, mg COD/L

V_{max} : maximum specific substrate utilization rate, mg COD/mg -day

Y_g : growth yield of microorganism, mg/mg COD

k_d : endogeneous decay coefficient of microorganism, day^{-1}

μ_{max} : maximum specific growth rate, day^{-1}

Table 2.5 Kinetics Constants (Lin *et al.*, 1987)

Temp. (°C)	V_{max} (mg/ mg-day)	μ_{max} (mg/ mg-day	K_s (mg/L)	Y_g (g/g)	K_d (day^{-1})
50	9.15	0.265	437	0.040	0.101
40	7.63	0.231	738	0.038	0.059
35	17.10	0.414	166	0.030	0.990
30	8.19	0.201	214	0.026	0.012
25	7.36	0.170	233	0.022	0.008
20	5.83	0.166	419	0.031	0.015
15	3.19	0.147	571	0.056	0.032

Also they found that Bacilli are the predominant microbial species in the methanogenesis process using a mixture of volatile fatty acids. The predominance is independent of digestion temperature. Sarcinae and coccoid appear in digestion with short retention times or low

temperatures. As seen from the table, at the mesophilic range (25 to 35°C), the kinetic constant K_s decreases with increasing temperature, but constants V_{max} and Y_g increase with increasing temperature.

2.6.5 Alkali and Alkaline-earth Cation Inhibition

Kugelman and McCarty (1965) have studied the effect of inhibition caused by these cations in acetate-utilising fermenters. They concluded that, on a molar basis, the order of increasing inhibition was Na^+, K^+, Ca^{2+} and Mg^{2+}. The results are shown in Table 2.6

Table 2.6 Cation Concentration Inhibitory to Anaerobic Digestion (Kugelman and McCarty, 1965)

Cation	Moderate Inhibition (mg/L)	Strong Inhibition (mg/L)
Sodium	3500 - 5500	8000
Potassium	2500 - 4500	12000
Calcium	2500 - 4500	8000
Magnesium	1000 - 1500	3000

The toxic limits of heavy metals vary from one digester to another, mainly because of the different levels of sulphides and carbonate, which in turn is precipitated by the metal salts. From laboratory studies of heavy metal toxicity, Mosey and Hughes (1975) found the order of decreasing metal toxicity to be

$$Zn = Cu = Cd > Cr(VI) = Cr(III) >> Fe$$

The most common strategy for controlling heavy metal inhibition is the addition of sulphides or sulphide precursors. Lawrence and McCarty (1965) observed that high concentrations of copper, zinc, nickel and iron can be rendered non-toxic in the anaerobic environment when in the presence of an equivalent concentration of sulphides.

2.6.6 Sulphide Inhibition

Although sulphide can be used effectively to control methanogenesis inhibition which is due to heavy metal toxicity, the sulphide itself is inhibitory due to its role in the production of hydrogen sulphide within the anaerobic digester. In the fermenter the sulphate-reducing bacteria compete with the methanogenic bacteria for hydrogen and other electron donors such as methanol, formate, acetate and propionate. Secondly, inhibition is enhanced by the decline of methanogenic population due to a concentration greater than 200 mg/L as S of soluble sulphides (Lawrence and McCarty, 1965). The conventional solutions for sulphide inhibition are: 1) hydrogen sulphide stripping from the gas and 2) dilution of the feedstock. However these conventional methods do not compensate for the loss of methane due to preferential production of hydrogen sulphide, and the need for expensive tank lining, high corrosion allowances, gas treatment and possibly high burner running costs due to hydrogen sulphide

corrosion.

Research by Biomass International scientists has resulted in the identification of a stable anaerobic microbial association which maximizes the yield of methane in the presence of sulphate. The technique has been found to be successful that free H_2S can no longer be detected in reactors to which it is applied, thus solving all of the conventional problems associated with anaerobic treatment of high sulphate-bearing wastewaters. The technique was proven on a molasses-based distillery effluent containing up to 7500 mg/L sulphate and 62000 mg/L COD (Anderson *et al.*, 1982).

2.6.7 Ammonia Inhibition

Anaerobic fermentation is a reduction process and under such conditions organic nitrogen is converted to ammoniacal nitrogen. Ammonia exist within the digester in equilibrium between two forms, the ammonium ion and free molecular ammonia,

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \qquad (2.40)$$

The ammonia inhibition has been found to be pH dependent by several investigators. Sathananthan (1981) concluded that the effect of pH on inhibition by ammonia was related to the equilibrium concentrations of free ammonia. At a total nitrogen concentration of 7000 mg/L, no inhibition was experienced at an operating pH of 7. However, when the pH was

raised to 7.5, inhibition occurred at a total nitrogen concentration of between 2000 and 3000 mg/L, consistent with the findings of McCarty (1964). From his experimental data, Sathananthan concluded that a free ammonia nitrogen concentration greater than 80 mg/L would cause the onset of inhibition regardless of pH.

Organic and inorganic sources of nitrogen are considered to be valuable raw materials and therefore rarely discharged in significant quantities in the wastewaters.

Two examples of high strength, high nitrogen content wastewaters which do exist in industry are whey, from cheese processing and stillage, from distillery operations. Traditionally, the nitrogen in these high strength effluents has been recovered by feeding the waste directly to pigs or by having the protein extracted by ultrafiltration, as in the case of whey, or evaporation, drying and blending with solid waste material to produce a nitrogen-rich cattle feed as in the case of distillery effluent.

McCarty (1964) stated that ammonia-nitrogen concentrations of 1500 to 3000 mg/L are inhibitory at higher pH values and that when the concentration exceeds 3000 mg/L the ammonium ion itself becomes quite toxic regardless of pH. McCarty and McKinney (1961) reported that pH played a significant role in ammonia toxicity, and they deduced that

when the free ammonia concentration exceeds 150 mg/L severe toxicity will result. Kugelman and Chin (1971) provided an explanation to the wide range of inhibitory ammonia concentrations by conducting experiments to show that in a multiple-cation system, tolerance to potentially toxic cations such as ammonium is produced by acclimation of the microorganisms to the toxic agent and/or by antagonism of other cations to the toxic cation.

2.6.8 Substrate Inhibition

Literature data on microbial kinetics always indicate a strong influence of substrate concentration on microbial growth. At low substrate concentrations, the specific growth rate (μ) is very close to zero, indicating a negligible change in microbial population. When the substrate concentration is increased above a given threshold limit, the specific growth rate is proportional to an increase in the substrate level and approaches a maximum value. A subsequent increase in the substrate concentration will ultimately lead to a decrease in the specific growth rate (Fig. 2.3). This well known phenomenon is termed "substrate inhibition" and it is frequently observed in biological waste treatment, industrial fermentation, and other parts of the biosphere (Mulchandani and Luong, 1989).

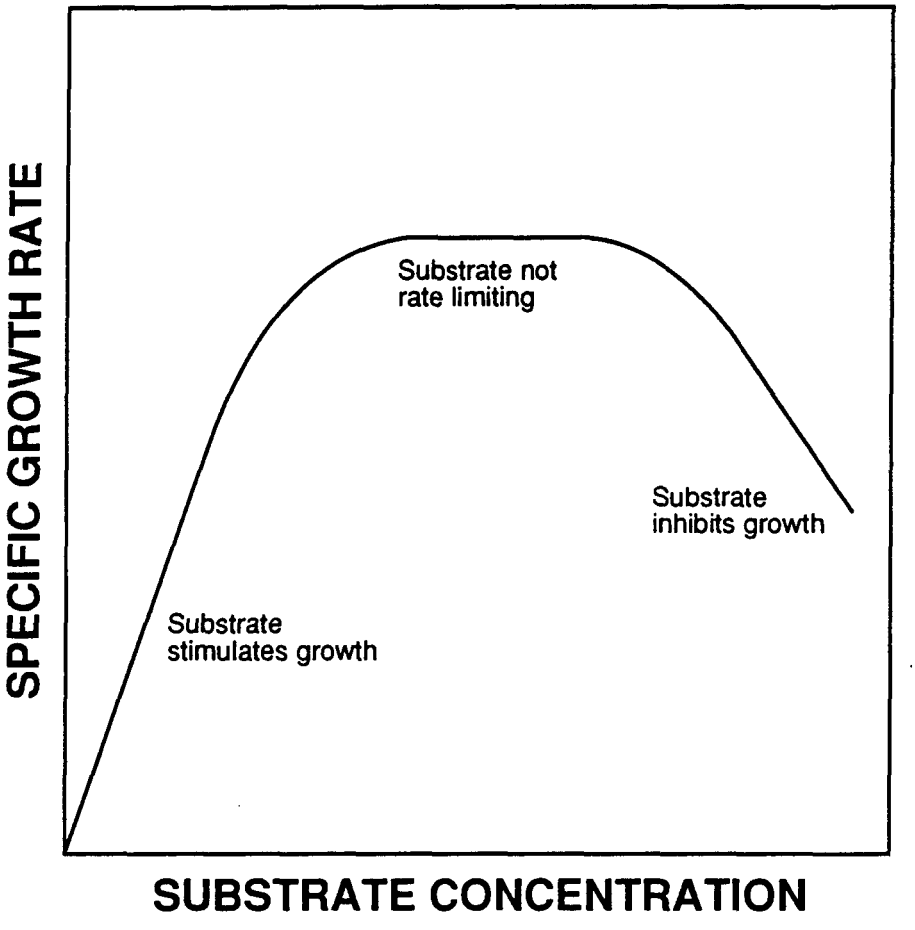

Figure 2.3 Schematic Diagram Showing the Effect of Substrate Concentration on Microbial Specific Growth Rate (Mulchandani and Luong, 1989)

Any modification of the medium physicochemical properties such as osmotic pressure, ionic strength, solvent activity, etc... might alter the cell membrane permeability. At a high substrate level, there is an increase in adsorption or complexation between enzymes, coenzymes, and substrates, which in turn reduces the enzyme activity (Edwards, 1970). From a biological viewpoint, an increase in the substrate concentration could cause an alteration in the cell metabolism such as an overproduction of a molecule by one pathway which results in the feedback inhibition of a second related pathway (Mulchandani and Luong 1989). The best-known example of this phenomenon is the Pasteur effect, which illustrates a change in yeast metabolism from glycolysis to respiration when oxygen (substrate) is highly available.

Edwards (1970) has discussed the influence of high substrate concentration on microbial kinetics. One plausible mechanism for substrate inhibition would be a reduction in the activity of an enzyme by complexing with the excess substrate. Andrews (1968) proposed that substrate inhibition can be represented by the form:

$$\mu = \mu_{max} \frac{S}{K_s + S + S^2/K_i} \qquad (2.41)$$

2.6.9 Product Inhibition

The end products of fermentation such as ethanol,

butanol, acetone, lactic acid etc. inhibits the microbial activity and thereby decrease the product formation rate. The mechanism for inhibition of microbial activity is very complex and still not completely understood. The kinetic models proposed for product inhibition are therefore unstructured and empirical in nature. Aiba, Shoda and Nagatani (Bailey & Ollis, chapter 7, page 392) showed that the product inhibition of anaerobic glucose fermentation could be treated by the form:

$$\mu = \mu_{max} \frac{S}{K_s + S\,K_p} \cdot \frac{K_p}{K_p + P} \qquad (2.42)$$

2.7 Explanatory Remarks

The literature review has been attempted so as to give the reader enough background knowledge to understand and appreciate the work done in this experimental study (see Chapters 3 to 5). It is very important that one know the basic principles of microbiology and biochemistry of anaerobic digestion before he/she undertakes any project on anaerobic digestion. For this purpose, sections 2.1 and 2.2 were introduced in this review. Section 2.3 describes the components, composition and hydrolysis of the whey permeate which is the key component in this study. To compare and contrast the results of this study with those of the previous ones, section 2.4 (kinetics of anaerobic digestion) was introduced. This section deals with microbial growth, various

kinetic models and their applications including the advantages and limitations.

Single-phase digestion usually poses stability problems for the mixed bacterial population and therefore a section, namely 2.5, has been added to look into that aspect and its possible solution. Microorganisms are very sensitive to the environment surrounding them and therefore a section such as 2.6 depicting the inhibition kinetics is a good guideline to pursue any biokenetics as is done in this experimental study.

CHAPTER 3

MATERIALS AND METHODS

3.1 Reactor

All the experiments were conducted in batch in a glass reactor of 2L working volume. The reactor was connected to a mechanical stirrer, biogas sampling tube, pH probe, dropping funnel (for the introduction of alkali) and the nitrogen gas tube. The reactor with its entire assembly was completely air-tight and maintained in a constant temperature bath at 37°C.

3.2 Inoculum

The inoculum was obtained from an anaerobic sludge digester of the municipal wastewater installations of Ville des Deux-Montagnes, Province of Quebec. The culture contained a mixed population of acidogenic and methanogenic bacteria. This inoculum was kept in the fridge at 4°C and maintained anaerobic all the time.

3.3 Whey Permeate

The whey permeate used for the experimental study of kinetics of anaerobic digestion was essentially the left over from the production of cheese from milk products. All the proteins had already been removed by ultrafiltration. The COD of this whey permeate was measured as explained in "Standard Methods" (Greenberg *et al.*, 1985)) and found to be about 68,000 mg/L. The result was accurate within 5% of the average value. The glucose concentration of the raw sample was

determined and found to be 62,000 ± 5 % mg/L.

3.4 Growth medium

The stock solutions used in the preparation of defined medium is given in Table 3.1.

Table 3.1 Stock Solutions Used in Growth Medium

Solution	Components	Concentration in Distilled Water, g/L
Mineral 1	NaCl	50
	$CaCl_2.2H_2O$	10
	NH_4Cl	189.4
	$MgCl_2.6H_2O$	10
Mineral II	$(NH_4)6Mo_7O_{24}.\ 4H_2O$	10
	$ZnSO.7H_2O$	0.1
	H_3BO_3	0.3
	$FeCl_2.4H_2O$	1.5
	$CoCl_2.6H_2O$	10
	$MnCl_2.4H_2O$	0.03
	$NiCl_2.6H_2O$	0.03
	$AlK(SO_4)_2.12H_2O$	0.1
Vitamin B	Nicotinic acid	0.1
	Cyanocobalamine	0.1
	Thiamine	0.05
	p-aminobenzoic acid	0.05
	pyridoxine	0.25
	Pantothenic acid	0.025
Phosphate	KH_2PO_4	50
Resazurin	$C_{12}H_7NO_4$	0.1

The above stock solutions were combined in the proportions given in Table 3.2 and then boiled for 3 minutes. The medium was cooled and kept in the refrigerator at 4° C. This

composition was recommended by Environment Canada for the anaerobic digestion of industrial wastewaters.

Table 3.2 Composition of Growth Medium

Solution	Volume in mL
Mineral I	20
Mineral II	2
Vitamin B	2
Phosphate	20
Rezazurin	30

The initial substrate concentrations (glucose) used in this study were 6.38, 10.85, 21.02 and 48.77 g/L.

3.5 Start-up & Operation

The batch experiments were conducted in the 2.5L (total volume) reactor for different initial concentrations of the substrate, lactose. In all the experiments, substrate concentrations were measured in terms of the glucose equivalents.

3.5.1 Inoculation

Prior to each run (i.e. run 2) the inoculum was acclimatised to substrates with similar concentration for a period of 24 hours. The acclimatising step was indicated as run 1 in the discussion. 400 mL of the sample in run 1 at the end of 24 hours was used as the inoculum for run 2 for the first 3 sets of experiments with initial concentrations 6.38,

10.85, and 21.02 g/L. For the fourth set of experiment (i.e with initial substrate concentration of 48.77 g/L) the sample was inoculated with 400 mL of the sample (at the end of sampling) of run 2 in set 3 (Table 3.3).

Table 3.3 Initial Substrate Concentrations for each Set of Experiments

Experiment	Initial Substrate Concentration, g/L	
	run 1	run 2
set 1	5.44	6.38
set 2	12.35	10.85
set 3	17.81	21.02
set 4	-----	48.77

In each set of runs, the raw whey permeate was diluted withj distilled water to attain the desired initial concentration. After dilution, the whey was heated to about 37° C. 1700 mL of the diluted sample was poured into the reactor maintaining at 37°C. 20 mL of the growth medium was added followed by 1.63 g of sodium bicarbonate (used as buffer). The addition of growth medium resulted in a C/N ratio of 19.7, 8.5, 4.4 and 2.6 for initial substrate concentrations of 48.77, 21.02, 10.85 and 6.38 g/L respectively. Therefore the nitrogen supplements were a little higher in all cases as compared with theoretical value of C/N ratio 30 for anaerobic digestion. Then 400 mL of the inoculum was transferred to the reactor anaerobically by

sparging N_2 gas. The same procedure was adopted for inoculation for all 4 sets of experiments.

3.5.2 Mixing

The mixing of the bioreactor contents was effected by an external mechanical stirrer. The mixing velocity was kept between 180-200 rpm which was found to be good enough to maintain reactor contents in homogeneous state and biomass in suspension. The stirrer shaft was equipped with a mechanical seal to prevent leakage.

3.5.3 Temperature Control

The reactor was kept in a water bath at 37°C. The thermostat was very sensitive and the temperature variation was very small in the range of ±0.1° C during the entire period of operation.

3.5.4 pH Control

The pH of the reactor contents in all 4 sets of experiment was measured at t=0 just after inoculation using the pH meter, Fisher Accumet Model 220. Any value less than 7.0 was brought back to 7.0 ± 0.1 by adding 10 N NaOH drop by drop. Also pH was measured every hour just after sampling and brought back to 7.0 ± 0.1, if necessary, by 10 N NaOH or con. HCl.

3.5.5 Sampling

50 to 60 mL of the sample was taken at time t=0 just

after inoculation and every hour thereafter until the substrate concentration (glucose) was reduced to less than 5% of the initial value using a fast flowing pipette. The reactor was kept purging with N_2 gas while sampling was done. The valve connected to the graduated cylinder for the biogas collection was also kept closed during sampling. Half the volume of the sample taken was removed to an oven maintaining at 96 ± 2° C for biomass determination. The other half was filtered through Watman 5 filter, GF/C filter and finally through 0.2 micron glass filter. All the biomass was retained and a portion of this filtrate was used for the determination of glucose concentration. A measured quantity (15 mL) of the rest of the filtrate was put in the oven at 96 ± 2° C. The temperature was kept below the boiling point to prevent splashing of the contents from the small crucible. Once all the water was evaporated the temperature of the oven was increased to 103 ± 2° C and the sample was allowed to dry for 4 hours. Then it was cooled in a desicator and weighed. Later this sample was taken to a muffle furnace at 550 ± 50° C and ignited for 1.5 hours. This gravimetric analysis was performed for both unfiltered and filtered (filtrate) samples to eliminate any error that may arise due to the presence of any volatile compounds such as glucose, fatty acids etc. other than biomass. Biogas production was almost nil in the initial

sampling period when the converted substrate remained as volatile fatty acids.

3.5.6 Determination of Substrate (glucose) Concentration

The substrate concentration was determined by phenol-sulphuric acid method (Herbert *et al.*, 1971). Concentrated sulphuric acid and 5% (w/v) phenol in water were used as the reagents. The apparatus included a B & L Spectrophotometer, 1 mL pipette, 5 mL fast flowing pipette, vortex-meter, water-bath at 25 - 30 °C and 10 mL B & L test-tubes.

Procedure:

1) Take 1 mL of filtrate sample (passed through the filter of pore size 0.2 micron) and dilute it to different concentrations with distilled water so that at least one of them has a glucose concentration in the range of 20 to 100 µg/mL.
2) Into thick-walled test tubes of 16-20 mm diameter pipette out 1.0 mL each of the diluted samples.
3) To another test tube, pipette out 1 mL distilled water.
4) To all the above test tubes, add 1 mL of 5 % phenol and mix in a vortex meter for 15 seconds.
5) Then from a ast flowing pipette, add 5 mL con. H_2SO_4, directing the stream of acid on to the surface of the liquid and shaking the test tube simultaneously on the vortex meter for another 15 seconds.

6) The tubes are allowed to stand 10 min, shaken and then placed in a water bath at 25 - 30° C for 15-20 minutes.

7) Measure the absorbance of the characteristic yellow colour at 488 nm.

8) Compute the glucose concentration from the standard curve plotted with absorbance vs. known concentration of glucose (see Table 3.4 and Figure 3.1).

3.5.7 Determination of Biomass

a) Principle

The procedure explained in the Standard Methods for Total Solids and Fixed and Volatile Solids (Greenberg *et al.*,1985) was adapted with a slight variation for the biomass determination. In addition to the biomass (viable and non-viable) the sample contains some amount of subtrates such as lactose, glucose and other volatile organic and inorganic matter. Therefore a simple weight difference of the unfiltered sample at 103 ± 2°C and 550 ± 50°C does not give an accurate value of biomass but gives biomass plus other volatile organics such as volatile fatty acids, substrate. To eliminate this error, difference in weight loss of the sample at 103 and 550°C was determined for both unfiltered and filtered (taking the filtrate which contains no biomass) samples. The idea can be expressed as:

Table 3.4 Absorbance vs. Known Values of Glucose Concentration

Glucose (µg/mL)	Absorbance (488 nm)
0	0.000
10	0.075
20	0.190
30	0.290
40	0.390
50	0.470
60	0.630
70	0.690
80	0.750
100	0.950

Non-filtered Sample

Difference in weight between 103 and 550°C, W1	Biomass + Volatile org. matter other than biomass + inorganic matter that may decompose and escape

Filtered Sample

Difference in weight between 103 and 550°C, W2	Organic matter other than biomass + inorganic matter that decomposes and escapes within this temp. range

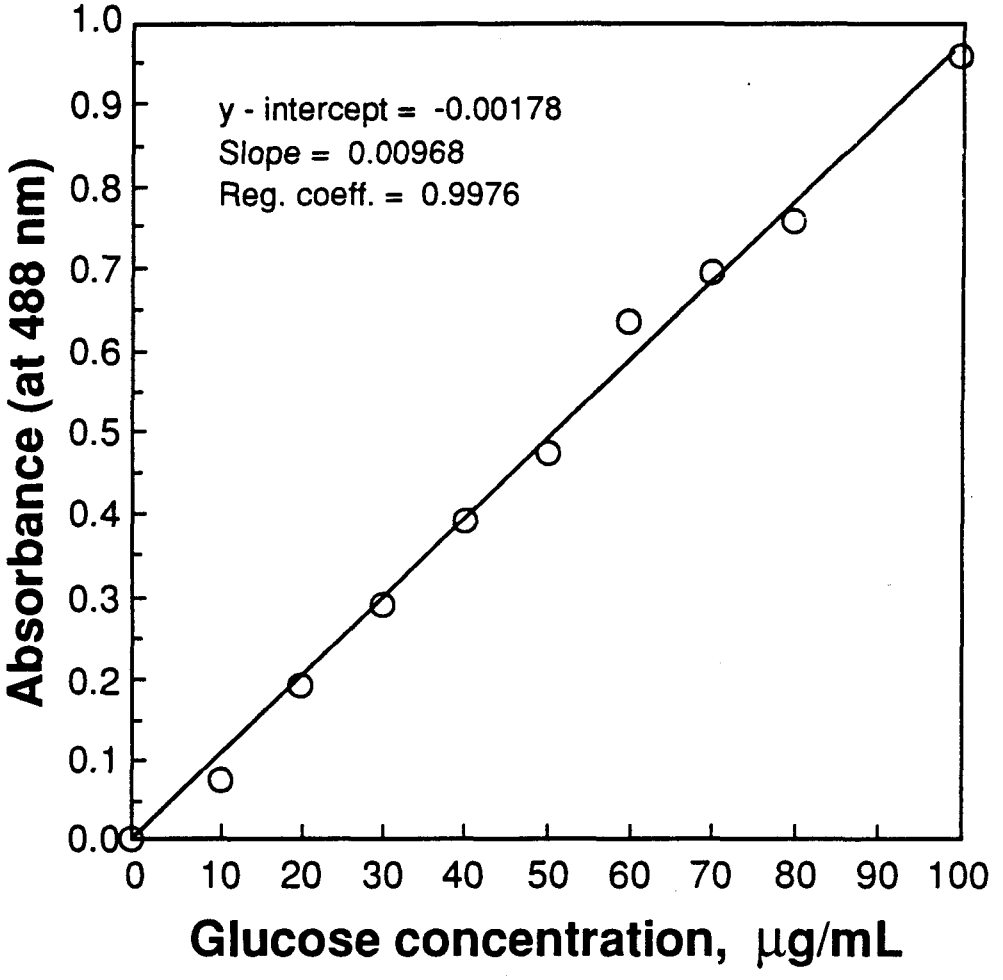

Figure 3.1 Standardisation Curve for Glucose Measurement

$\therefore$ W1-W2 $\equiv$ Dry wt. of biomass (viable+nonviable)

b) Procedure

1) Ignite a clean evaporating dish at 550 ± 50 °C for one hour in a muffle furnace. Cool in desicator, weigh and store in desiccator until ready for use.
2) Pipette out 25 ml well mixed unfiltered sample into a clean pre-weighed evaporating dish.
3) Evaporate the sample in a drying oven at 96 ± 2 °C first to prevent splattering.
4) Dry the evaporated samples for 6 hours in an oven kept at 103 ± 2 °C, cool dish in desicator to balance temperature and weigh. Let this weight be a1 g.
5) Transfer the evaporating dish with the residue, from step 4, to a muffle furnace maintained at 550 °C and ignite it for 2 hours.
6) Cool the dish partially in air until most of the heat has been dissipated and then transfer to the desicator for final cooling.
7) Weigh the dish as soon as it has cooled to balance temperature. Let this weight be a2.
8) Repeat steps 1 through 7 with a measured volume (here it is 15 mL) of corresponding filtrate. Let the weight in step 4 be b1 g and that in step 7 be b2 g.

c) Calculation

Wt. of biomass + other volatile organic matter + inorg. matter that may decompose and escape in 25 mL of unfiltered sample	W1=(a1-a2) g
Wt. of volatile org. matter other than biomass + inorganic matter that may decompose & escape in 15 mL of filtrate	W2=(b1-b2) g
Net Biomass, mg/L	$\{W1-(25/15)W2\}\ 10^6/25$

3.5.8 Determination of Chemical Oxygen Demand (COD)

The COD of the raw permeate and the samples were determined as specified in the "Standard Methods" (Greenberg *et al.*, 1985). The chemicals used for the COD determination included the following reagents: concentrated sulfuric acid containing 5.5 g silver sulfate (Ag_2SO_4) per kg H_2SO_4, 0.25 N potassium dichromate ($K_2Cr_2O_7$), standardised ferrous ammonium sulfate [$Fe(NH_4)_2(SO_4)_2$] and the ferroin indicator. The apparatus consisted of refluxing flasks, condenser for cooling and hot plates.

a) Procedure

1) Pipette out a measured volume (say x mL) of the sample to three of the round bottom boiling flasks.
2) Add (50-x) mL of distilled water to the flasks making the total volume to 50 mL.
3) To the fourth refluxing flask, add 50 mL of distilled water and no sample. This is used as the blank.

4) Add 200 mg of mercuric sulfate ($HgSO_4$) powder to each of the four reflexing flasks. Also put magnetic beads in the flasks.

5) Add 5 mL of H_2SO_4 reagent to each flask and mix it to dissolve $HgSO_4$.

6) To every flask add 25 mL of 0.25 N $K_2Cr_2O_7$ solution and mix.

7) Fix the flasks with the condenser and hot plate stirrer and turn cooling on.

8) Through the open end of the condenser at the top add another 10 ml $HgSO_4$ and then close the open end to prevent any escape of volatile compounds.

9) Turn on both heat at "High" and stirring. Reflux for two hours.

10) After refluxing, wash down condenser with 10 mL distilled water and cool to room temperature.

11) Titrate the excess $K_2Cr_2O_7$ with standardize $Fe(NH_4)_2(SO_4)_2$ using 3 drops of ferroin indicator.

12) End-point is taken at the sharp colour change from the blue-green to reddish brown.

b) Calculation

COD as mg O_2/L = (A-B) x N x 8000/ (volume of sample in mL).

where A = mL ferrous ammonium sulfate used for blank

B = mL ferrous ammonium sulfate used for sample (average of 3 readings provided the volume is the same).

N = Normality of ferrous ammonium sulfate.

c) Standardization of Ferrous Ammonium Sulfate (FAS)

Dissolve 98 g $Fe(NH_4)_2(SO_4)_2 .6H_2O$ in distilled water. Add 20 mL concentrated $HgSO_4$, cool and dilute to 1000 mL. Take 10 mL 0.25 N $K_2Cr_2O_7$ in a conical flask and dilute to about 100 mL. Add 30 mL con. $HgSO_4$ and cool. Then titrate with FAS using 3 drops of ferroin indicator.

Normality of FAS, N = 10 x 0.25/ (volume of FAS used in mL).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Experimental Results

The variations of pH, glucose consumption and biomass formation as a function of time were shown for various sets of runs with different initial substrate concentrations. Table 4.1 shows the above parameters for run1 with non-acclimatized bacteria and run2 with acclimatized bacteria from run1, with initial glucose concentrations 5.44 g/L and 6.38 g/L respectively. Tables 4.2, 4.3, and 4.4 show corresponding values as a function of time in the batch reactor digesting whey permeate with initial substrate concentrations (as glucose equivalents) 12.35 & 10.85 (run1 & run2), 17.81 & 21.02 and 48.77 g/L for sets 2, 3, and 4 respectively. The fourth set of experiment was innoculated with acclimatized bacteria from run2 of set3.

The substrate consumption as a function of time is shown in Figure 4.1 for different initial feed and biomass concentrations. From the figure, it is evident that substrate conversion for S_o = 48.77 g/L followed an elongated s-shape (when taken the mirror image) which is characteristic of the microbial fermentation (Levenspiel, chapter 7, page 198, 1972). Similar were the cases for S_o = 21.02 and 10.85 g/L but with the exception that the initial substrate uptake was higher. This must be due to the comparitively larger biomass

Table 4.1 Variations of Substrate and Biomass Concentrations and pH vs Time for Initial Glucose Concentrations of 5.44 and 6.38 g/L for Run1 and Run2 Respectively in Set1.

Time	Set1, Run1				Set1, Run2			
t(h)	Gluc-ose g/L	Bio-mass g/L	pH meas	pH adj.	Gluc-ose g/L	Bio-mass g/L	pH meas.	pH adj
0	5.44	3.23	7.4	7.0	6.376	3.44	6.8	7.0
1					5.870	3.55	6.1	7.0
2			7.3		4.579		5.6	7.0
3	5.07	3.26	7.45	7.0	3.378	3.49	5.3	7.0
4					0.729	3.61	5.2	7.0
5					0.127		6.1	7.0
6	4.75	3.38	6.8	7.0				
8	3.68	3.83	6.4	7.0				
19	0.05	3.94	5.4	7.0				

meas.: measured, adj.: adjusted to

Table 4.2 Variations of Substrate and Biomass Concentrations and pH vs Time for Initial Glucose Concentrations of 12.35 and 10.85 g/L for Run1 and Run2 Respectively in Set2.

Time	Set 2, Run1				Set 2, Run2			
t(h)	Glu-cose g/L	Bio-mass g/L	pH meas	pH adj.	Glu-cose g/L	Bio-mass g/L	pH meas	pH adj.
0	12.35	3.22	7.3	7.0	10.854	3.41	6.9	7.0
1					8.410		5.7	7.0
2					5.706	3.71	5.4	7.0
3	11.07	2.85	6.8	7.0	1.690	3.90	5.4	7.1
4					0.439	3.81	6.0	7.0
5					0.224	3.82	6.7	7.0
9	8.46	4.32	6.0	7.0				
16	0.17	4.48	5.2	7.0				

meas.: measured, adj.: adjusted to

Table 4.3 Variations of Substrate and Biomass Concentrations and pH vs Time for Initial Glucose Concentrations of 17.81 and 21.02 g/L for Run1 and Run2 Respectively in Set3

Time t(h)	Set3, Run1				Set3, Run2			
	Glu-cose g/L	Bio-mass g/L	pH meas	pH adj.	Glu-cose g/L	Bio-mass g/L	pH meas	pH adj.
0	17.81	2.66	7.0	NA	21.020	2.75	6.6	7.0
1					19.470		5.4	7.0
2					16.637	2.76	5.3	7.0
3					15.515		5.2	7.0
4					11.788		5.4	7.0
5	15.68	2.83	6.6	7.0	9.264	3.39	5.3	7.0
6					7.216		5.5	7.0
7					3.958		5.5	7.0
8					1.180	3.65	5.8	7.1
9	14.17	4.32	5.8	7.0	0.501		6.1	7.0
24	3.17	4.51	5.1	7.0				

meas.: measured, adj.: adjusted to, NA: not adjusted

Table 4.4 Variations of Substrate and Biomass Concentrations and pH vs Time for Initial Glucose Concentration of 48.77 g/L in Set4.

Time	Set 4, one run only (inoculum from run2, set3)			
t (h)	Glucose g/L	Biomass g/L	pH measured	pH adjusted
0	48.772	0.79	6.7	7.0
1	48.239		6.6	7.0
2	47.500		6.4	7.0
3	46.187		6.2	7.0
6	35.024		5.7	7.0
7	30.857	1.59	5.0	7.0
8	27.132		6.3	7.0
9	23.927	2.25	5.6	7.0
10	19.494		5.7	7.0
12	12.465		5.6	7.1
13	8.706	2.32	6.0	7.1
15	6.512		6.4	7.1
17	3.398		6.3	7.0
19	2.164		6.4	7.0

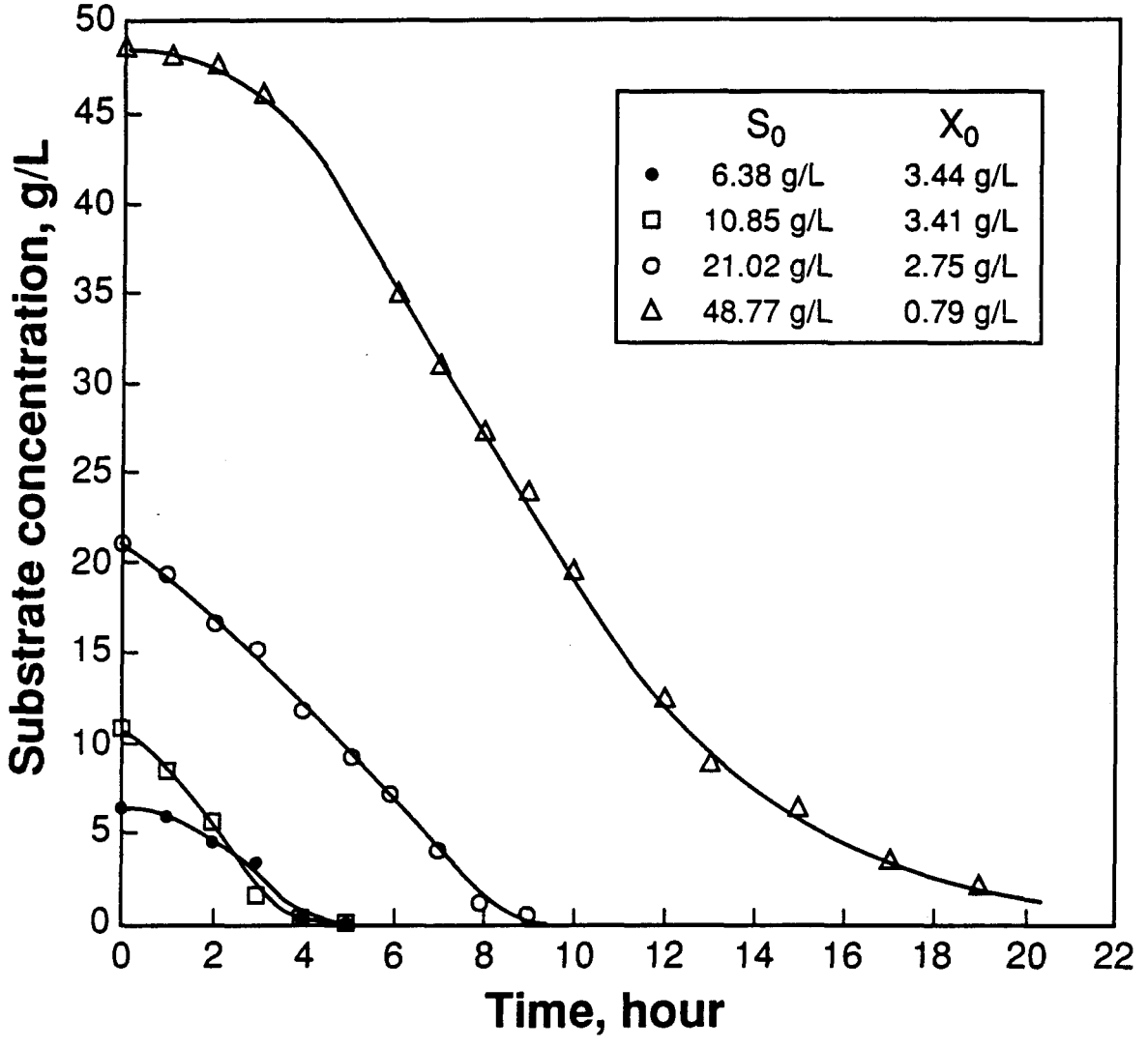

Figure 4.1 Substrate Consumption vs Time for Various Initial Substrate and Biomass Concentrations

concentrations in the latter cases and also perhaps due to the poor acclimation of biomass for the set with S_o = 48.77 g/L. The substrate biodegradation for S_o = 6.38 g/L also showed almost the same behaviour but the maximum rate was attained only after half of the substrate had been converted. A possible explanation for this may be the presence of some inhibitors in the reactor.

The bacterial growth rate was observed for all four sets of experiments. For each S_o, biomass concentration was plotted as a function of time as in Figures 4.2, 4.3, 4.4 and 4.5. Several polynomials and many other models were tried to predict the growth obtained. It was found that the variation of biomass vs time was best approximated by a straight line as shown by Figures 4.2 to 4.5.

4.2 Discussion

The main aim of the study was to evaluate the kinetic parameters, namely specific substrate utilization rate q_s (or the reaction rate constant k) and the specific biomass growth rate μ. The experimental data obtained were compared with various known kinetic models to test the adequacy of these models in explaining the biological system in consideration. The method of least-squares was used to determine the curve fitting with the data.

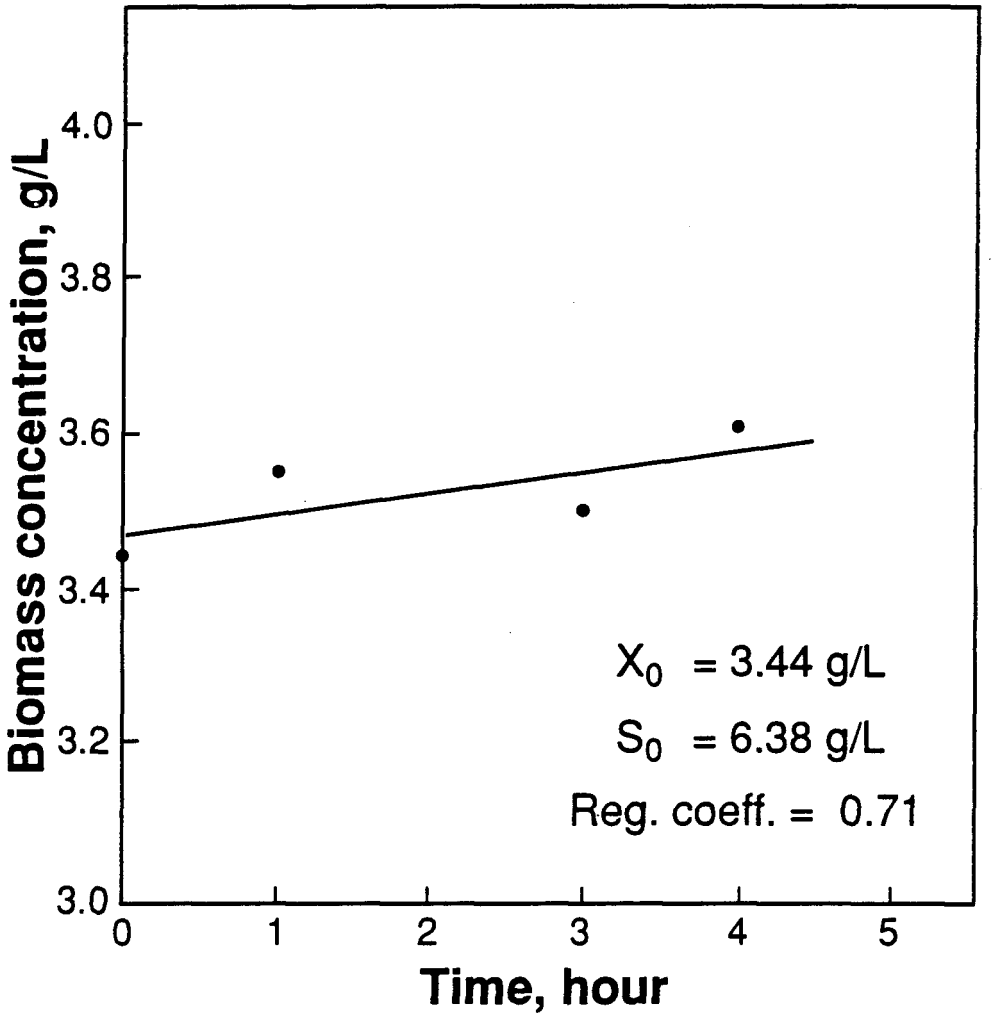

Figure 4.2 Linearised Plot of Biomass Growth vs Time for Initial Glucose Concentration of 6.38 g/L

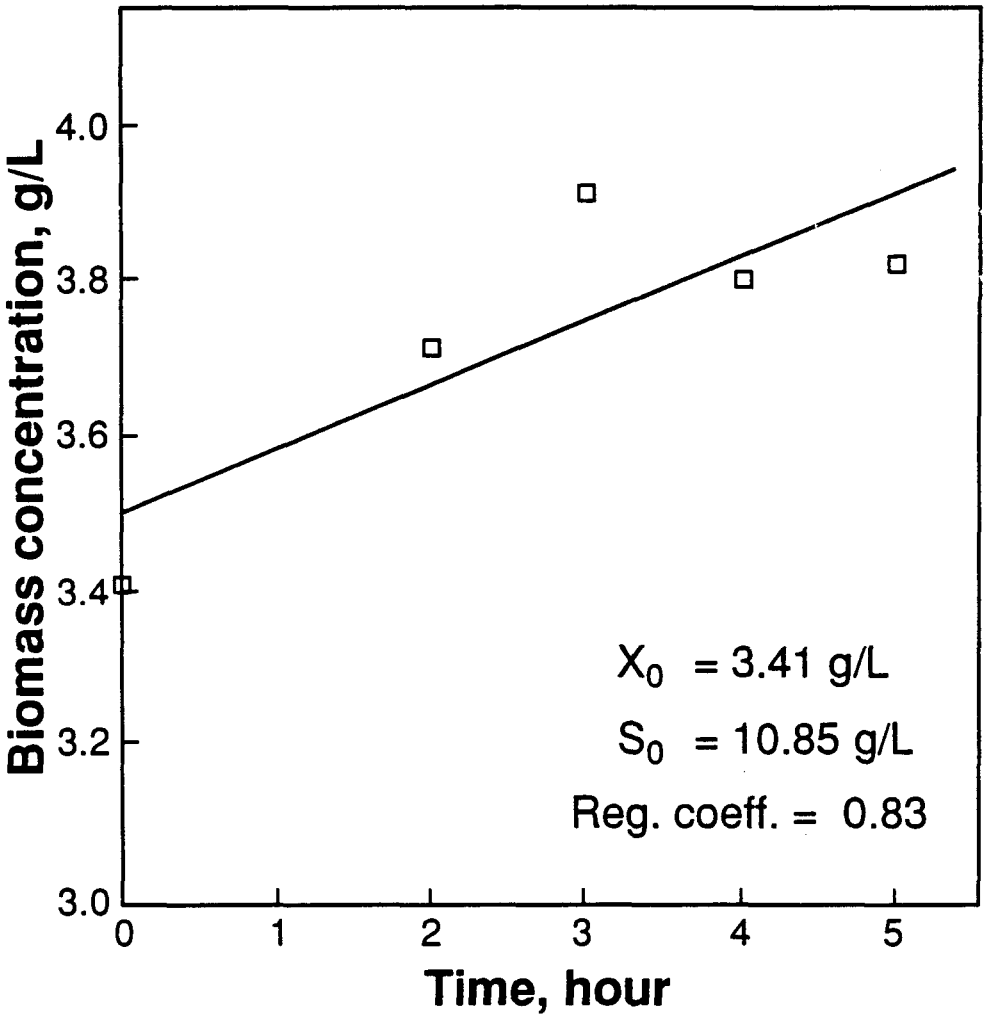

Figure 4.3 Linearised Plot of Biomass Growth vs Time for Initial Glucose Concentration of 10.85 g/L

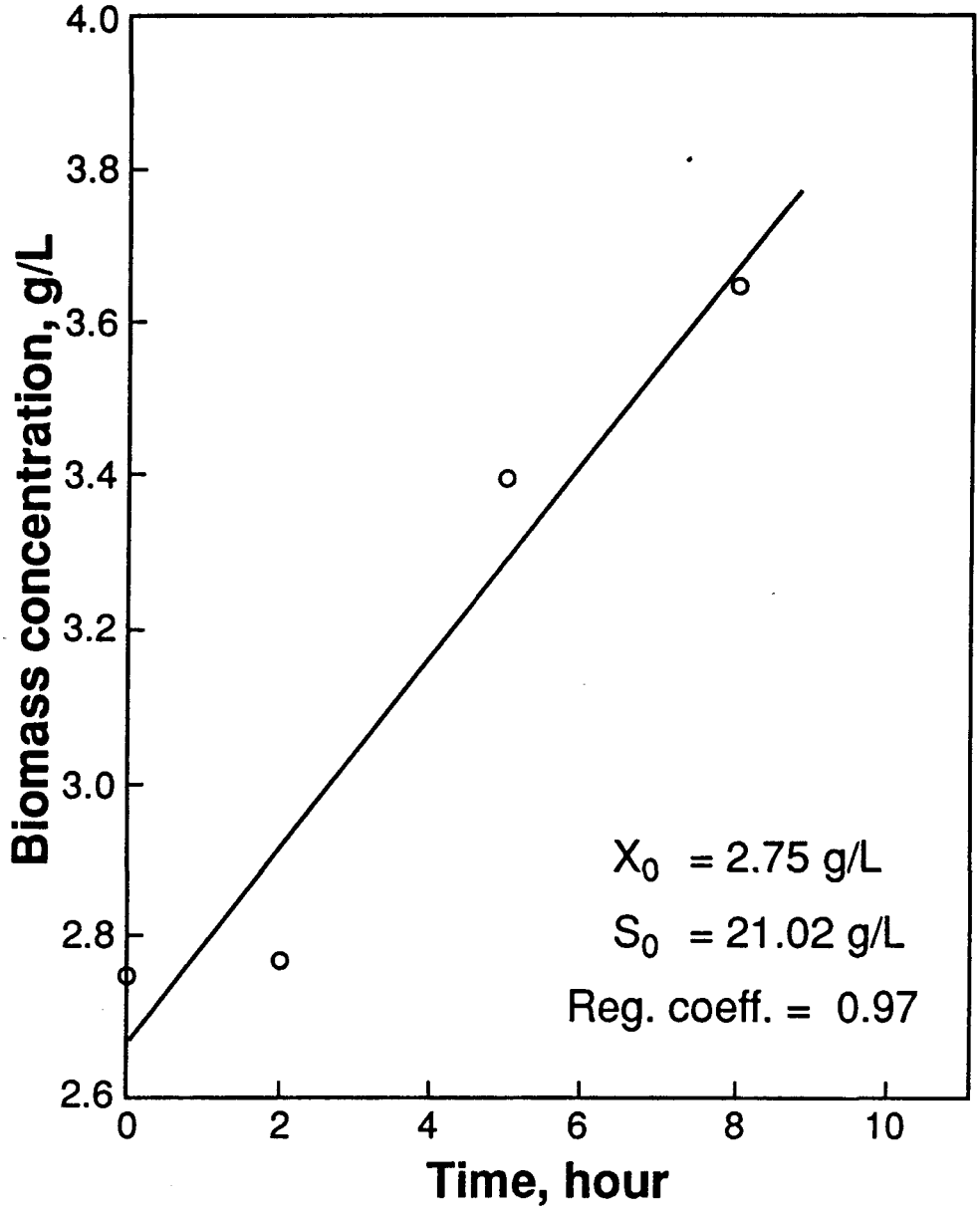

Figure 4.4 Linearised Plot of Biomass Growth vs Time for Initial Glucose Concentration of 21.02 g/L

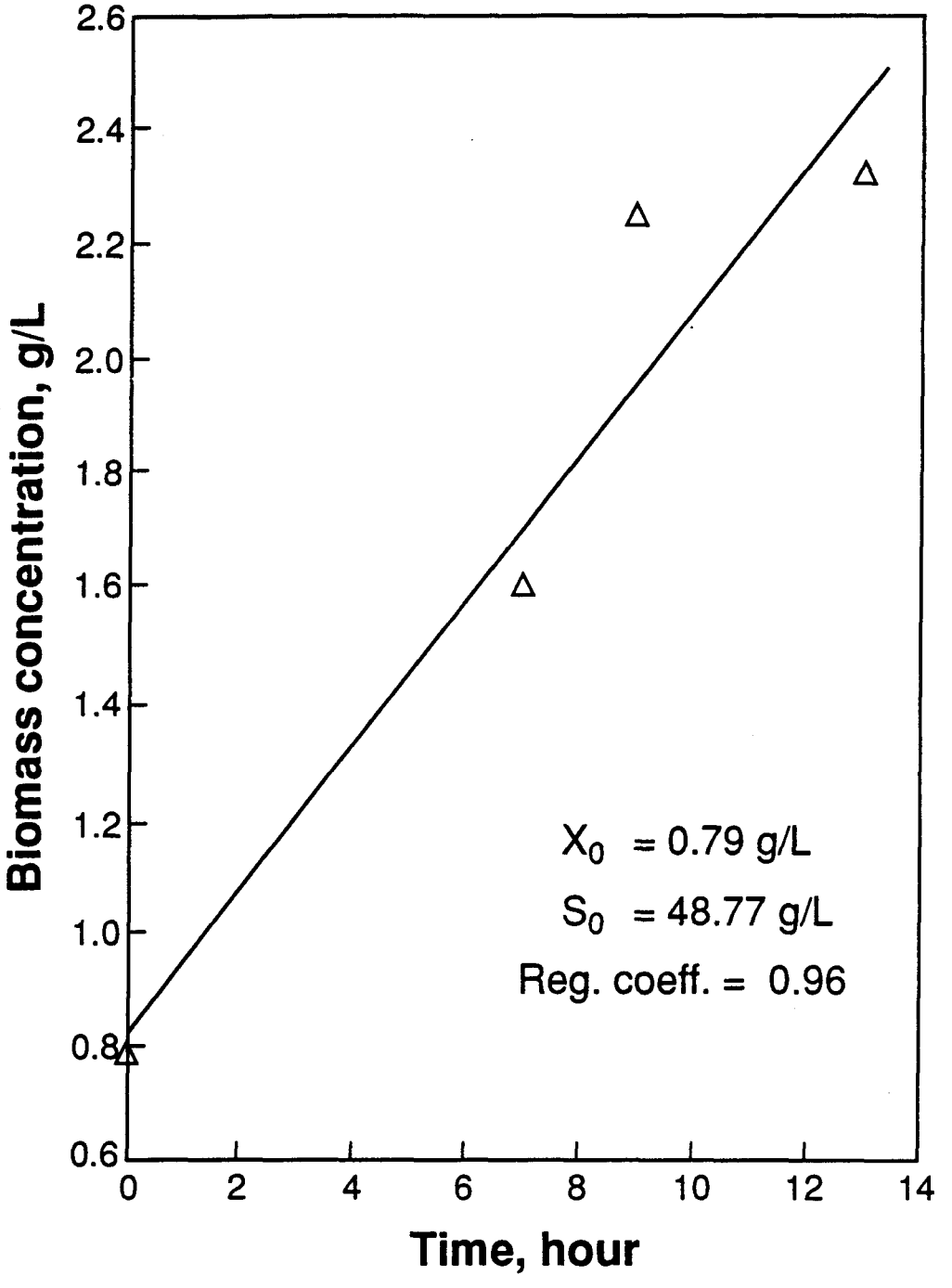

Figure 4.5 Linearised Plot of Biomass Growth vs Time for Initial Glucose Concentration of 48.77 g/L

4.2.1 Biomass Growth Rate

Many models were tested for the biomass growth but none of them gave a good fit. Some of the models tried are given below:

$$\mu = \frac{\mu_m S}{K_1 + S} \qquad \text{(Monod)} \qquad (4.1)$$

$$\mu = \frac{\mu_m (S/X)}{K_2 + (S/X)} \qquad \text{(Contois)} \qquad (4.2)$$

$$\mu = \frac{\mu_m (S/X^2)}{K_3 + (S/X^2)} \qquad (4.3)$$

$$\mu = \frac{\mu_m (S/X^{1/2})}{K_4 + (S/X^{1/2})} \qquad (4.4)$$

$$\mu = \frac{\mu_m S^{\lambda}}{K_5 + S^{\lambda}} \qquad \text{(Moser) (for } \lambda = 0.2 \text{ to } 3.0) \qquad (4.5)$$

$$\mu = \frac{\mu_m (S/X)^{\lambda}}{K_6 + (S/X)^{\lambda}} \qquad (\text{for } \lambda = 0.2 \text{ to } 3.0) \qquad (4.6)$$

$$\mu = \frac{\mu_m (S/X^2)^{\lambda}}{K_7 + (S/X^2)^{\lambda}} \qquad (\text{for } \lambda = 0.5 \text{ to } 2.0) \qquad (4.7)$$

Here, it must be mentionned that the Monod, Contois and Moser models were proposed for the biomass growth of pure cultures. In our case, we have used a complex mixed population of

anaerobic bacteria. The complexity of such populations was described in Chapiter 2. Under some experimental conditions, it is possible that such simple models cannot satisfactorily represent biomass growth evolution versus decreasing substrate concentration.

The best representation of biomass growth was obtained from a linear representation of biomass concentration versus time as shown by Figures 4.2 to 4.5. The linear curve fitting is relatively poor for the run corresponding to S_o = 6.38 g/L with correlation coefficient value of 0.71. For the other runs corresponding to values of S_o of 10.85, 21.02 and 48.77 g/L, the linear curve fitting is better with correlation coefficient values of, respectively, 0.83, 0.97 and 0.96.

4.2.2 Substrate Biodegradation Rate

Several models were tested to see whether any one of them was suitable to predict the behaviour of substrate uptake by a mixed culture of anaerobic bacteria feeding on whey in the batch reactor. None of the models tested gave a satisfactory fit. They were:

$$q_s = \frac{q_{smax} \cdot S}{K_1 + S} \qquad (4.9)$$

$$q_s = \frac{q_{smax} (S/X)}{K_2 + S/X)} \qquad (4.10)$$

$$q_s = \frac{q_{smax}\,(S/X^2)}{K_3 + (S/X^2)} \qquad (4.11)$$

$$t = A_1 \ln\frac{S_o}{S} + A_2\,(S_o - S) \qquad (4.12)$$

(obtained on integration of $\frac{-dS}{dt} = \frac{k'S}{K_4+S}$)

$$t = A_1 \ln\frac{S_o}{S} + A_2(S_o-S) + A_3\,(S_o^2 - S^2) \qquad (4.13)$$

(obtained on integration of $\frac{-dS}{dt} = \frac{k''S}{K_5+S+BS^2}$)

For equations 4.12 and 4.13, the models gave some negative values for the constant A's among other positive values. These models were unaceptable because the constants (i.e. A's) should all be positive.

Still, another rate equation, based on the one proposed by Levenspiel (1972), was tested for the substrate biodegradation. The equation proposed by Levenspiel (1972) is for an autocatalytic reaction written like this:

$$A + R \longrightarrow R + R \qquad (4.14)$$

where A is a reactant and R is a product and also acts as a catalyst for this reaction.

There is a form of similitude between an autocatalytic reaction and a microbial fermentation. So, a microbial fermentation may be written like this:

$$S + X \xrightarrow{k} X + \Delta X + P \qquad (4.15)$$

where S, X and P are respectively substrate, biomass and products concentration and where ΔX represents the increase in biomass concentration.

We are interested only in the initial rate of biodegradation and for the initial stage the product concentration is low and is therefore neglected.

The rate equation can then be written like this:

$$-\frac{dS}{dt} = kSX \qquad (4.16)$$

The relation between S and X is the following:

$$X = X_o + Y\,(S_o - S) \qquad (4.17)$$

where Y is a yield coefficient and S_o and X_o are initial values (at t = 0)

The equation (4.16) can now be written, after substitution in it of the equation (4.17), like this:

$$-\frac{dS}{S\,[X_o + Y\,(S_o - S)\,]} = k\,dt \qquad (4.18)$$

Breaking into partial fractions, considering Y as a constant and integrating equation (4.18) we get:

$$\ln\left[\frac{X/X_o}{S/S_o}\right] = kY\left(\frac{X_o}{Y} + S_o\right)t \qquad (4.19)$$

where k is a kinetic constant including booth biodegradation and growth processes.

To find the kinetic constant k, a graph is plotted with $\ln\left(\frac{X/X_o}{S/S_o}\right)$ vs time. From initial slope of the curve [which is equal to $kY_o(X_o/Y_o + S_o)$], kinetic constant k can be computed. The assumption of low product concentration is also respected by using initial slope values. The initial yield coefficient Y_o rather than the overall yield coefficient $Y_{x/s}$ is used to calculate k.

The points considered in finding the initial slope according to equation (4.19) are shown in table 4.5. The points were fitted for the best straight line in such a way that it also passed through the origin (Figures 4.6 - 4.9). The values in parentheses were not used in the curve fitting.

For the set 4 (i.e. S_o=48.77 g/L), the initial two points were omitted from the curve substrate concentration vs time to account for the lag phase and poor acclimation. Thus, the initial concentration of substrate becomes S'_o=47.50 g/L. The corresponding initial biomass concentration was interpolated at a value of X'_o=1.08 g/L

To estimate the numerical value of k from the slope, one need to know the initial yield coefficient Y_o. The calculated values of Y_o are tabulated in Table 4.6.

Table 4.5 Values of $\left[\frac{X/X_o}{S/S_o}\right]$ and Corresponding Time.

S_o g/L	X_o g/L	t hour	S g/L	X g/L	$\frac{X/X_o}{S/S_o}$	Initial Slope
6.38	3.44	0	6.376	3.44	1.0	0.207
		1	5.870	3.55	1.12	
		3	3.378	3.49	1.92	
		(4)	0.729	3.61	(9.18)	
10.85	3.41	0	10.854	3.41	1.0	0.364
		2	5.706	3.71	2.07	
		(3)	1.690	3.90	(7.35)	
		(4)	0.439	3.81	(27.55)	
		(5)	0.224	3.82	(54.21)	
21.02	2.75	0	21.020	2.75	1.0	0.194
		2	16.637	2.76	1.27	
		5	9.264	3.39	2.80	
		(8)	1.180	3.65	(23.64)	
47.50	1.08	0	47.500	1.08	1.0	0.190
		5	30.857	1.59	2.273	
		7	23.927	2.25	4.136	
		(11)	8.706	2.32	(11.72)	

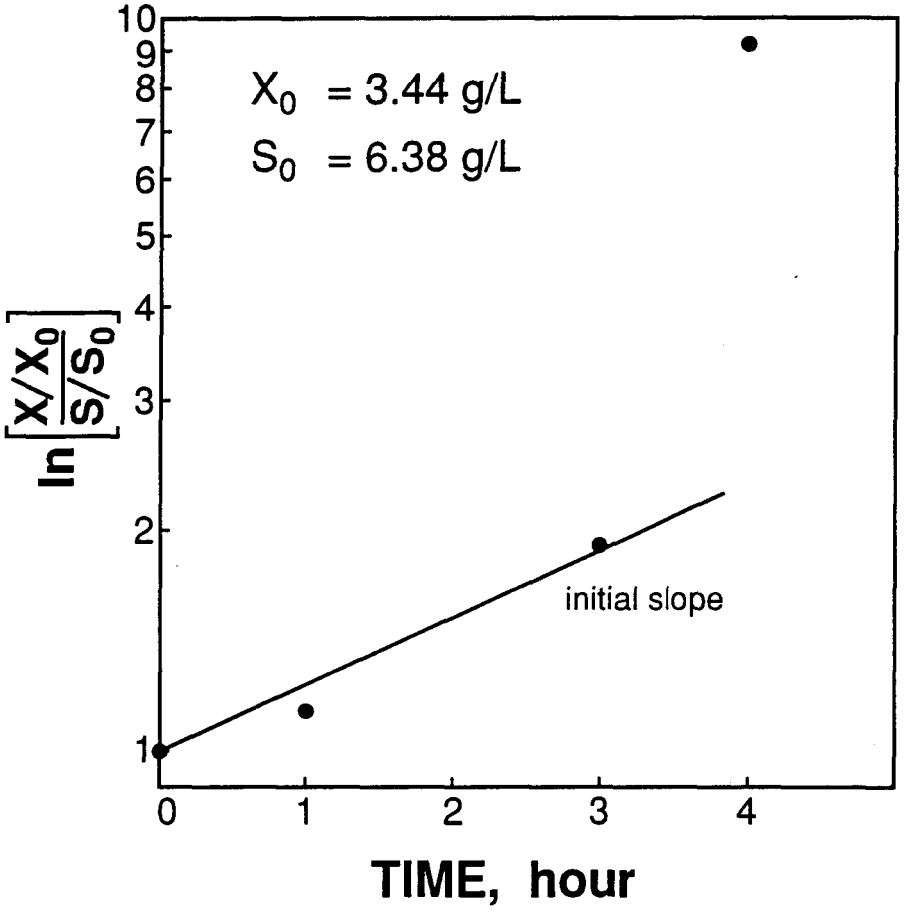

Figure 4.6 Plot of ln $\{(X/X_o)/S/S_o)\}$ vs Time for Initial Glucose Concentration of 6.38 g/L

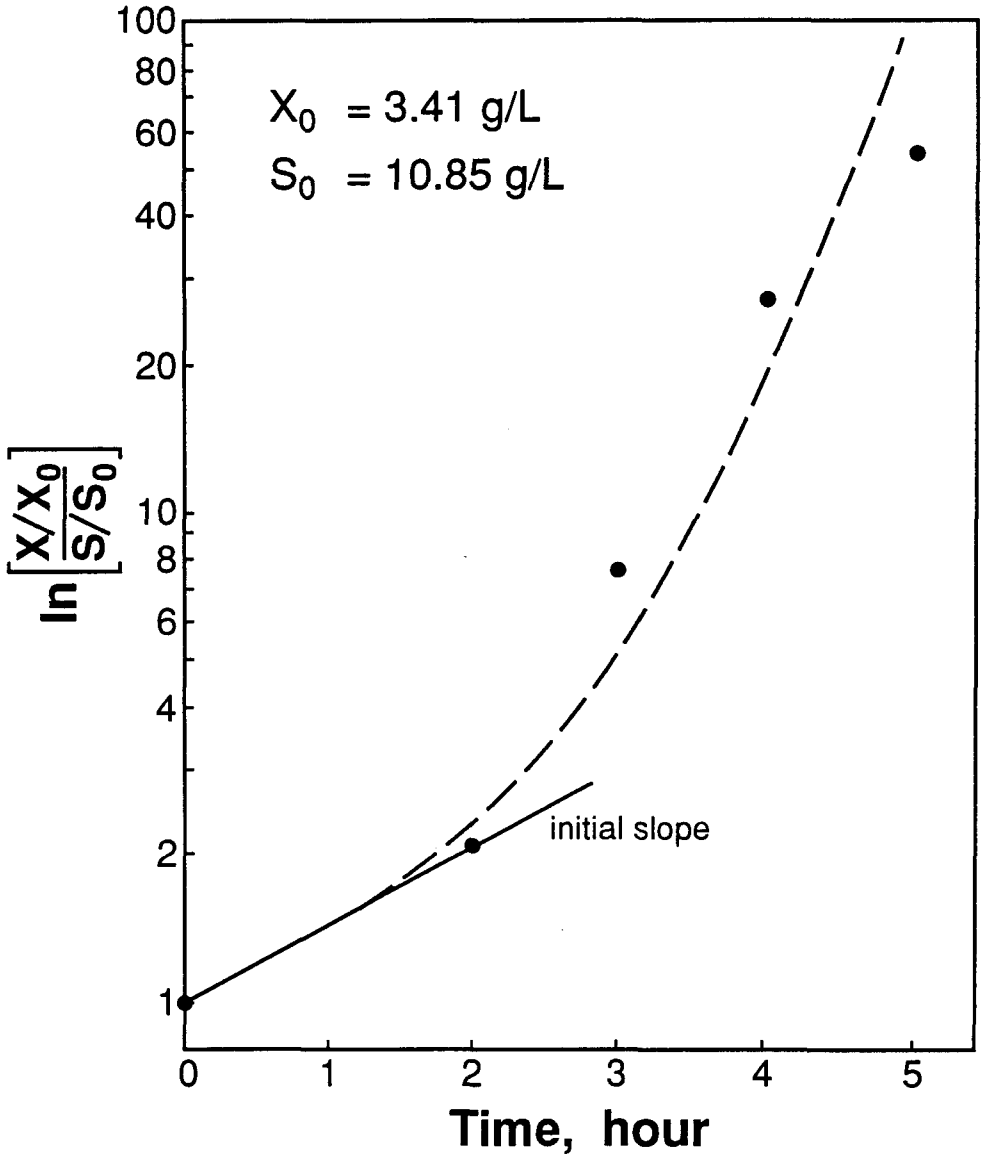

Figure 4.7 Plot of ln $\{(X/X_o)/(S/S_o)\}$ vs Time for Initial Glucose Concentration of 10.85 g/L

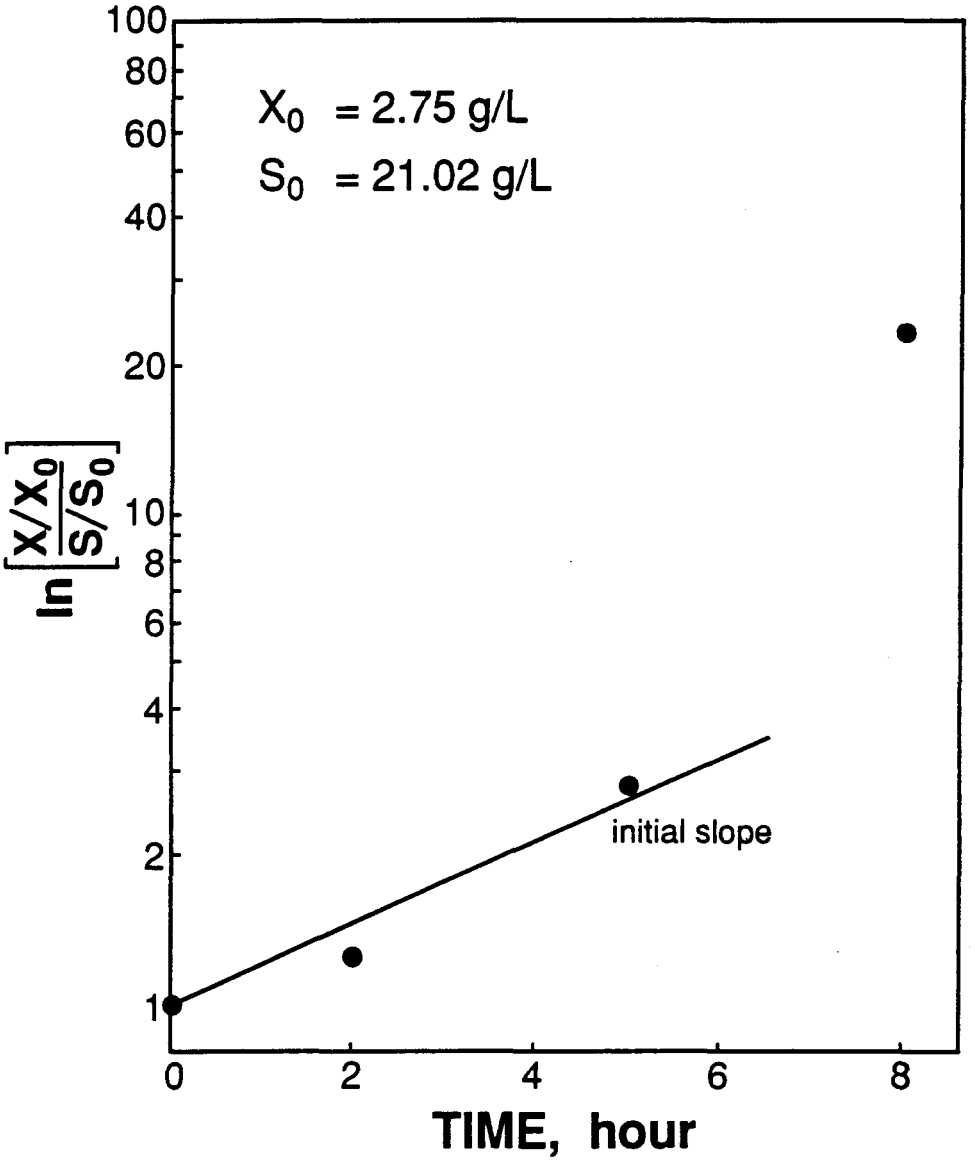

Figure 4.8 Plot of ln $\{(X/X_0)/(S/S_0)\}$ vs Time for Initial Glocose Concentration of 21.02 g/L

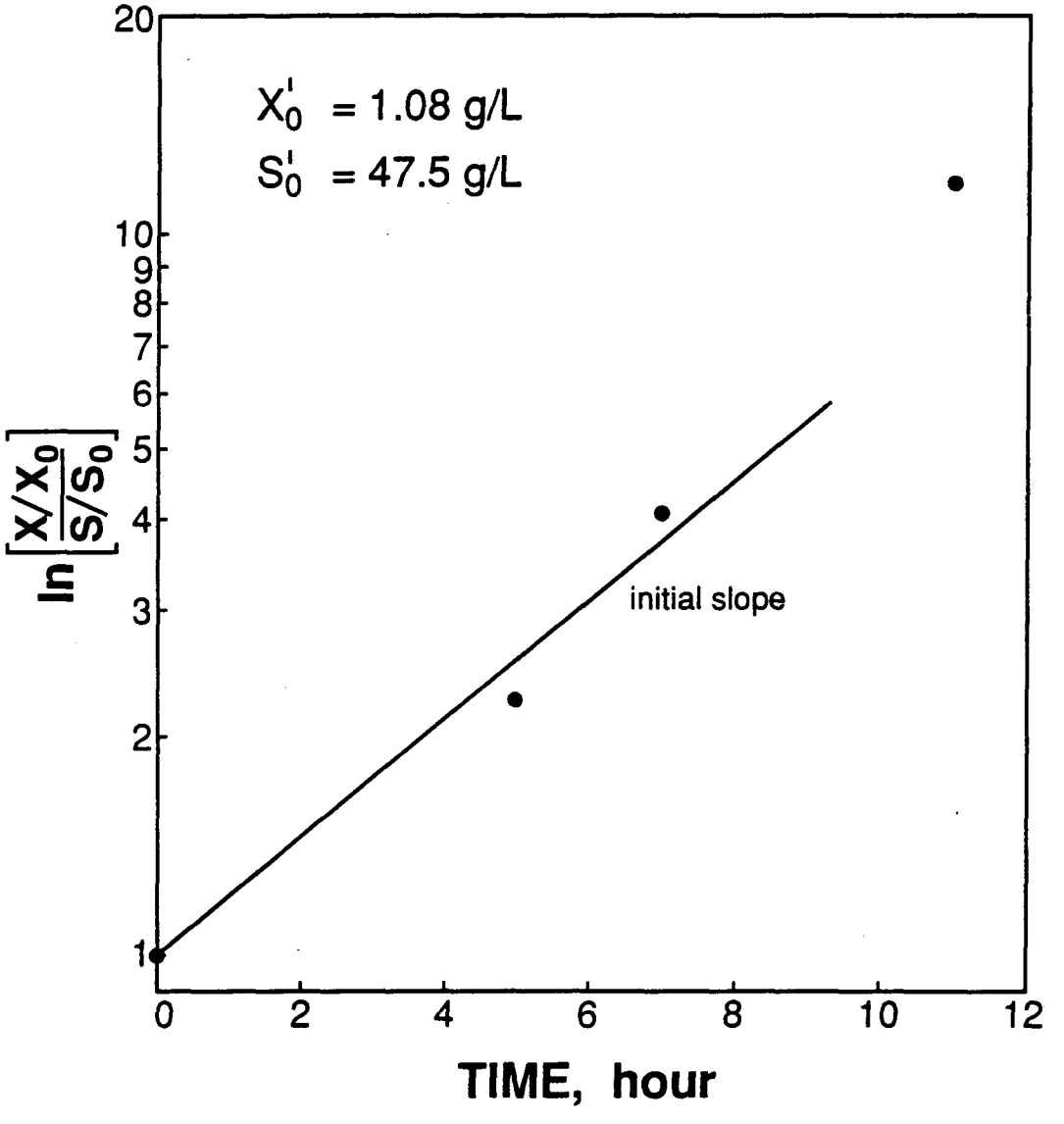

Figure 4.9 Plot of ln $\{(X/X_O)/(S/S_O)\}$ vs Time for Initial Glucose Concentration of 48.77 G/L

Table 4.6 Parameters Used in the Estimation of Kinetic Constant k.

S_o (g/L)	6.38	10.85	21.02	47.50
X_o (g/L)	3.44	3.41	2.75	1.08
$\Delta X/\Delta t$	0.0285	0.0825	0.125	0.126
$-(\Delta S/\Delta t)_o$	0.510	2.444	1.917	2.543
$\frac{\Delta X/\Delta t}{-(\Delta S/\Delta t)_o}=Y_o$	0.056	0.034	0.065	0.050
Initial Slope	0.207	0.364	0.194	0.175
$((X_o/Y_o)+S_o)Y_o$	3.796	3.803	4.107	3.455
k (g cells-h/L)$^{-1}$	0.054	0.096	0.047	0.055

The details about calculation of Y_o are explained hereafter. Since Y_o is the ratio of $(\Delta X/\Delta t)$ over $-(\Delta S/\Delta t)_o$, the values of $(\Delta X/\Delta t)$ were obtained from the slope of the straight line of X vs time (Figures 4.2 to 4.5). To obtain the values of $-(\Delta S/\Delta t)_o$, we have proceeded as follows. For S_o=6.38 and 10.85 g/L, we have calculated the slope of the straight line passing by the first two points of the curve of

S vs time (Figure 4.1), i.e. (0, 6.38) and (1, 5.87), and (0, 10.85) and (1, 8.41) giving, respectively, the values of 0.51 and 2.44 g/L-h. For S_o=21.02 g/L, $-(\Delta S/\Delta t)_o$ was calculated by taking the slope of the straight line which represented the best fit for the points (0, 21.02), (1, 19.47), (2, 16.637) and (3, 15.515) and at the same time passed through the initial point (0, 21.02). The value obtained is 1.917. For S_o=48.77 g/L, the same procedure was adopted as that for S_o=21.02 g/L but with a single exception. In this case, the initial two points were omitted to account for the lag phase and poor acclimation. The value of $-(\Delta S/\Delta t)$. was calculated by taking the slope of the straight line which represented the best fit for the points (2,47.5), (3, 46.187) and (6, 35.024) and at the same time passed through the point (2,47.5); the value obtained is 2.542.

Figures 4.6 to 4.9 represent initial slope of the equation (4.19). In all cases, the value of the initial slope was calculated as the slope of the straight line which represented the best fit for the points used and at the same time passed through the origin of the graph.

The values of k (see table 4.6) obtained for different S_o values were close enough and varied only within a narrow range between 0.047 and 0.055 (g cells-h/L)$^{-1}$ (ignoring the value at S_o=10.85 g/L). The value of k obtained at S_o=10.85 g/L was

higher and was estimated to be 0.096 (g cells-h/L)$^{-1}$. This high value could be due to some error in the initial slope which in turn might come from any experimental error in the determination of biomass and/or substrate concentrations at time t=2 hours. Therefore it was found that the model given by equation (4.19) was the best representation of the anaerobic digestion of the whey permeate in the given controlled environment and when the substrate concentration is approximately greater than the half of the initial substrate concentration.

4.2.3 Yield Coefficients

The yield coefficient $Y_{x/s}$ can be defined as the ratio of weight of biomass formed to unit weight of substrate consumed. In this study, two sets of yield coefficients were estimated for each set of experiments. One was the initial yield represented by Y_o for which $S \geq 0.5\ S_o$ and the other was the overall yield coefficient represented $Y_{x/s}$ where $S \geq 0.2S_o$. As seen from Table 4.7, the initial yield coefficients Y_o were higher than the overall yield coefficients $Y_{x/s}$. Only exception was the yield for S_o=10.85 g/L for which $Y_{x/s}$ was higher than Y_o. Higher value for Y_o is quite normal since the relative proportion of the substrate converted to products other than biomass increases as time passes by.

Table 4.7 Initial and Overall Yield Coefficients for Different Initial Substrate Concentrations

S_o g/L	Y_o	$Y_{x/s}$
6.38	0.056	0.030
10.85	0.034	0.054
21.02	0.065	0.045
48.77	0.050	0.038

At the higher substrate concentrations, the overall yield was found to decrease. This suggested that perhaps methanogens could be the major fraction of the initial culture which might be inhibited and lysed in low pH conditions arised from high level volatile fatty acids. Nevertheless, these values gave a general idea about the yield coefficient of a mixed population digesting whey permeate in anaerobic and other given experimental conditions. As seen from Table 4.7, the yield is very low and the problem of organic waste disposal is very much reduced.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Whey and other similar high-strength organic wastes constitute a severe disposal problem in North America and elsewhere. To treat those type of organic wastes in a bioreactor, a good knowledge of microbial kinetics and reaction stoichiometry is essential. Biological systems are most widely used nowadays because they are more efficient, economic and less hazardous to our environment. In view of this, the present study was directed mainly to determine kinetic constants such as the substrate utilization rate for a mixed bacterial population digesting whey permeate in anaerobic conditions.

The key to optimum design for multiple reactions is proper contacting and proper flow pattern of fluids within the reactor. These requirements are determined by the stoichiometry and observed kinetics (Levenspiel, chapter 7, page 199). The model given by equation (4.19) gave an average value of 0.052 $(g\ cells\text{-}h/L)^{-1}$ for the parameter k, the rate constant for the substrate utilization.

The kinetic parameter developed in this conventional suspended growth rate batch reactor can be used to design large scale bioreactors such as fluidised bed/expanded bed, continuous strirred tank reactor, plug flow reactor, etc...

However one major drawback of this model is that it may not be applicable to describe the specific kinetic behaviour of any other class of microorganisms digesting the same or different substrate in different environmental conditions. Other limitations resulted from the fluctuating pH and elimination of some important initial points from the figure 4.6 to 4.9. pH was found to vary very rapidly for all sets of experiments. Instead of adjusting the pH every hour as done in this study, some provision should have been made to monitor the pH and adjust it on a continuous basis. Also sampling could have been done more frequently, say every 20 minutes, and this would have given a more appropriate value for the rate constant k. Again, for some of the estimated values of substrate concentration, the corresponding values of biomass were not available (happened due to broken crucibles, splashing of sample and some unreal estimated values). Therefore some important points had to be excluded from plotting the graph in Figures 4.6 to 4.9, especially for the cases with S_o = 21.02 and S_o = 48.77 g/L and in turn, affected the value of the rate constant to some extent.

As an extension of this work, it is strongly recommended that the study be repeated for different values of pH and temperature, changing only one variable at a time. Such a study would give an idea about the optimum temperature and pH

for the biotreatment of whey permeate. To best utilize the organic wastes and convert them to potential sources of energy, product formation kinetics should be explored in detail. Finally, the influence of each species of bacteria in the mixed population on the overall biodegradation process including the biogas yield may also be investigated.

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