THE KINETICS OF GAS PRODUCTION IN THE ANAEROBIC DIGESTION OF CELLULOSIC WASTES

By

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ABSTRACT

The rate of substrate utilisation in the anaerobic digestion of cellulosic wastes - pure cellulose, grass, cow dung and horse manure - was studied in continuous stirred digesters, over a range of temperatures and loading rates.

Two litre continuously stirred digesters were operated at temperatures of 28°C, 38°C and 48°C. The digesters were operated at detention times of 10, 17 and 30 days for input substrate concentrations (dry weight) of 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$. The digesters were fed with a mixture of a nutrient rich medium with the substrate every twenty four hours without the addition of sludge in the feed, the mixed culture being acclimatised to the conditions of operation.

The effluents from the digesters were analysed routinely for $_{p}H$, alkalinity, total volatile acid, chemical oxygen demand of the volatile matter, cellulose content and V.S.S. concentration. The gases were analysed, using a gas chromatograph, for methane and carbon dioxide.

The results of the study indicated that the rate limiting step in the anaerobic digestion of insoluble cellulosic wastes was the hydrolysis step. The rate of substrate removal was fitted by a first order rate expression, for the range of conditions studied, of the type

$$R = KS_1$$

Where R = rate of substrate removal per unit digester volume, mass/volume-time.

K = first order rate constant, time $^{-1}$.

S_1 = substrate concentration in digester, mass/volume.

The percentage digestion was found to be independent of the input substrate concentration and the value of K was a function of temperature and followed the Arrhenius expression for the temperature range of 28°C to 48°C.

An attempt to fit a rate expression of the type

$$q = kS_1$$

Where q = rate of substrate removal per unit digester volume per unit cell concentration, time $^{-1}$.

k = first order rate constant, volume/mass-time.

S_1 = substrate concentration in digester, mass/volume.

was not successful; although a linear fit was obtained at one input concentration (i.e. S_o = 15000 mg. lit $^{-1}$ dry weight) the same fit did not follow the data obtained at the higher input concentration (i.e. S_o = 25000 mg. lit $^{-1}$ dry weight), i.e. the rate constant k was found to decrease with increase in the input substrate concentration.

The rate of substrate utilisation was significantly affected by the type of substrate being digested as shown by the values of the rate constants obtained at a temperature of 38^{o}C:

K cellulose	=	0.59 day^{-1}
K grass	=	0.177 day^{-1}
K cow dung	=	0.092 day^{-1}
K horse manure	=	0.123 day^{-1}

For all operating conditions studied the gas produced was found to be proportional to the substrate utilised for all substrates digested.

The maximum amount of gas produced per unit weight of substrate added to the digesters was found from the batch digestion of grass, cow dung and horse manure.

In evaluation of the effects of temperature on the degradation of the substrates it was found that effective digestion occurred at a reduced temperature of 28°C. For the range of temperatures studied (28°C, 38°C and 48°C) the activation energy obtained from a plot of the Arrhenius expression was greatest for the digestion of pure cellulose (12 k cal/mole), and for grass, cow dung and horse manure the corresponding values obtained were 6 k cal/mole, 7.4 k cal/mole and 8.5 k cal/mole.

PREFACE

The work described in this thesis was carried out in the Department of Chemical Engineering, Imperial College of Science and Technology, University of London, between October 1973 and September 1976 under the supervision of Dr. D.L. Pyle.

I would like to express my sincere gratitude to Dr. D.L. Pyle for his valuable help during the course of the work.

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Michael Hadjitofi

CONTENTS

	Page
ABSTRACT	iii
PREFACE	vii
CHAPTER 1: INTRODUCTION	1
CHAPTER 2: LITERATURE SURVEY	9
2.1 THE ANAEROBIC DIGESTION PROCESS	9
2.1.1 Feedstock Constituents	9
2.1.2 Two-Stage Digestion	12
2.1.3 Hydrolysis of the Three Major Components	15
2.1.3.1 Carbohydrates	15
2.1.3.2 Proteins	21
2.1.3.3 Lipids	23
2.1.4 Non-Methanogenic Fermentation of Products arising from the Hydrolysis of the Three Major Components	25
2.1.4.1 Non-methanogenic fermentation of sugars produced from the hydrolysis of carbohydrates	25
2.1.4.2 Non-methanogenic fermentation of products from the hydrolysis of protein and lipid	27
2.1.5 Methanogenic Fermentation	29
2.2 FACTORS AFFECTING THE DIGESTION PROCESS	34
2.2.1 pH	34
2.2.2 Alkalinity and Volatile Acid Concentration	38
2.2.3 Temperature	43
2.2.4 Nutrients	47
2.2.5 Structural and Composition Properties of the Polysaccharides	52
2.3 STOICHIOMETRY	55
2.4 KINETIC ASPECTS OF THE DIGESTION PROCESS	64
2.4.1 Decomposition Rates	64
2.4.2 Reaction Order for the Digestion Process	69
2.4.3 Rate Limiting Step	72

		Page
2.5	INTERMEDIATE TECHNOLOGICAL ASPECTS	75
2.6	PROCESS APPLICATIONS	79
CHAPTER 3:	THEORETICAL CONSIDERATIONS OF THE DIGESTION PROCESS	83
3.1	KINETICS OF BIOLOGICAL REACTIONS	83
3.1.1	History of Biological Reaction Kinetics	83
3.1.2	Biological Kinetics as applied to Waste Treatment Processes	90
3.1.2.1	The Model of Lawrence and McCarty (1970)	91
3.1.2.2	The Model of Eckenfelder (1961) and McKinney (1962)	103
3.1.2.3	Application of the Models to Anaerobic Digestion	104
3.1.2.4	Other Kinetic Formulations used to describe the Anaerobic Digestion of Insoluble Feedstocks	109
3.2	THE EFFECT OF TEMPERATURE ON BIOLOGICAL RATE CONSTANTS	113
3.3	PROPOSALS FOR THIS STUDY	116
CHAPTER 4:	EXPERIMENTAL STUDY OF DIGESTION OF CELLULOSIC WASTES	126
4.1	BATCH DIGESTION	128
4.1.1	Experimental Apparatus	128
4.1.2	Batch Digester Operation	128
4.1.3	Analysis of Batch Digesters	130
4.2	CONTINUOUS DIGESTION	132
4.2.1	Experimental Apparatus	132
4.2.2	Input Feedstock Characteristics	136
4.2.3	Continuous Digester Operation	145
4.2.4	Analysis of Continuous Digesters	151
4.2.5	Calculations for Continuous Digestion	166

			Page
CHAPTER 5:	RESULTS		172
5.1	BATCH DIGESTION		172
5.2	CONTINUOUS DIGESTION		176
	5.2.1	I. Cellulose	179
		II. Grass	203
		III. Cow Dung	203
		IV. Horse Manure	215
	5.2.2	Yield Constant (Y) and Decay Coefficient (b)	222
	5.2.3	Gas Production and Substrate Utilisation	226
	5.2.4	Decomposition Rates	228
CHAPTER 6:	DISCUSSION		234
6.1	REMOVAL RATES OF SUBSTRATES		234
6.2	COMPARISON OF THE DECOMPOSITION RATES OF CELLULOSE, GRASS, COW DUNG AND HORSE MANURE		247
6.3	THE RATE LIMITING STEP IN THE DIGESTION OF CELLULOSE, GRASS, COW DUNG AND HORSE MANURE		253
	6.3.1	Cellulose	253
	6.3.2	Grass, Cow Dung and Horse Manure	
6.4	EFFECT OF TEMPERATURE ON THE REACTION RATE CONSTANTS		256
	6.4.1	Cellulose	256
	6.4.2	Grass, Cow Dung and Horse Manure	258
6.5	ENGINEERING SIGNIFICANCE AND APPLICATION		261

		Page
CHAPTER 7:	CONCLUSIONS	268
APPENDICES		
APPENDIX I:	Stoichiometry of Biological Reactions	275
II:	Particle Size Distribution of Substrates	277
	1. Grass	277
	2. Cow Dung	279
	3. Horse Manure	287
APPENDIX III:	General Characteristics Data	283
REFERENCES		303
LIST OF MAIN SYMBOLS		315

CHAPTER 1

INTRODUCTION

Anaerobic digestion can be defined as a biological process in which organic matter, in the absence of oxygen, is converted to methane, carbon dioxide, cellular material and small quantities of hydrogen, ammonia and hydrogen sulphide. The overall process, starting from insoluble organic material, can be divided into two stages, as shown in Figure 1.1. In the first stage, insoluble organic matter, consisting of carbohydrates (e.g. cellulose), protein, lipids and salts, is hydrolysed to soluble organics such as amino acids, simple sugars and glycerol by extracellular enzymes. The soluble organics are then fermented to simple organic compounds such as alcohols, volatile acids, carbon dioxide, ammonia and gaseous hydrogen. Both anaerobic and facultative anaerobic bacteria are responsible for the hydrolysis and fermentation steps noted above. Together, these two steps can be classified as the non-methane forming (non-methanogenic) stage of the digestion process.

The second stage of the process occurs as the products formed in the non-methanogenic stage are fermented to methane and carbon dioxide by obligate anaerobic bacteria; intracellular enzymes secreted by the bacteria

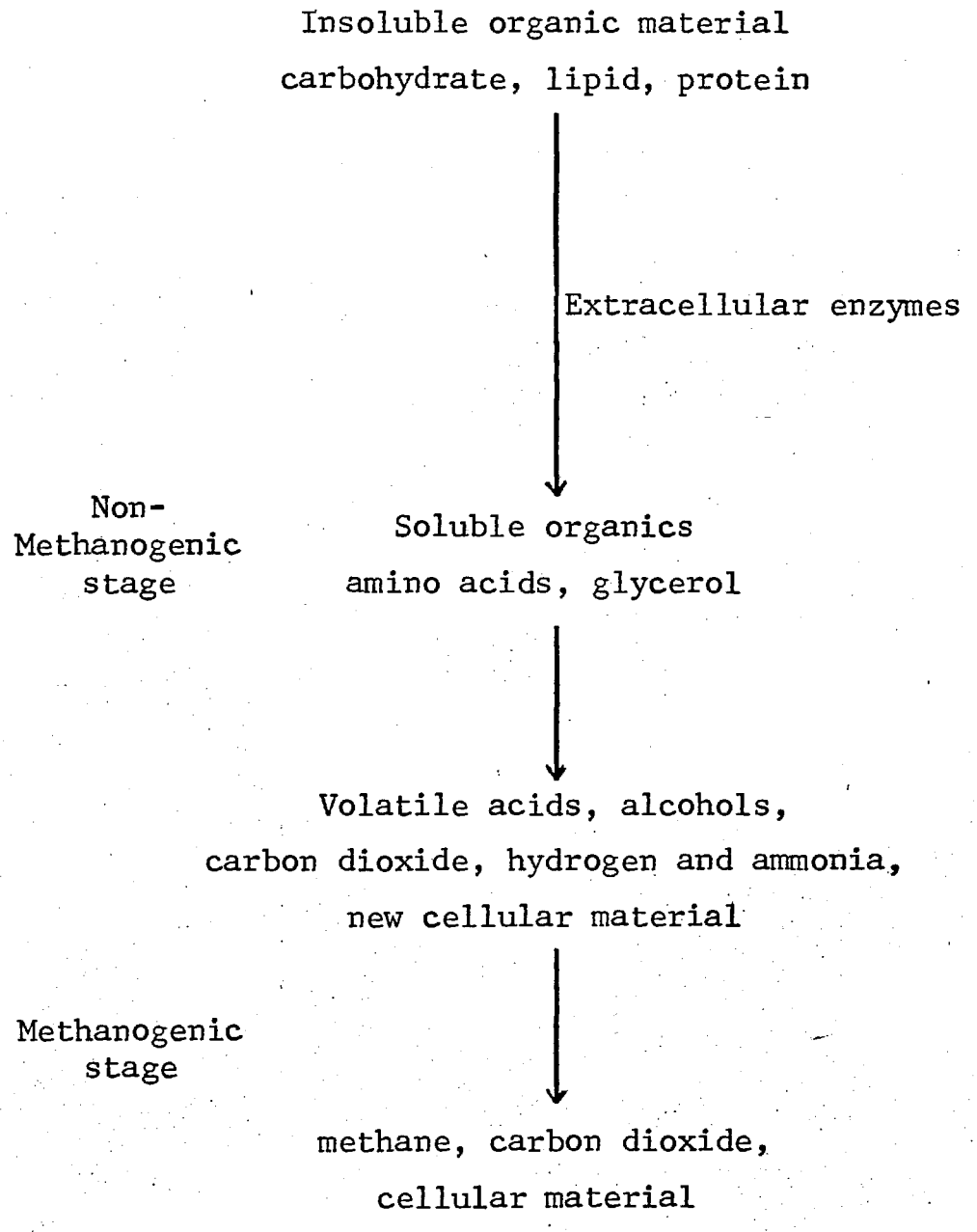


FIGURE 1.1 Degradation of Insoluble Organic Matter by Anaerobic Digestion.

bring about this conversion. The second stage is referred to as the methane forming (methanogenic) stage of the anaerobic digestion process.

The production of methane cannot take place without the occurrence of the non-methanogenic stage and similarly the digestion process cannot proceed further than the non-methanogenic stage without the presence of the methane forming bacteria. In the non-methanogenic and methanogenic stages new cellular material, including the necessary enzymes, is synthesised and so the process may be described as autocatalytic.

Anaerobic digesters, consisting of unheated and unstirred tanks into which the feedstocks were added, have been used in some sewage treatment processes since the early 1900's. In the sewage treatment process digestion is used as a second stage operation for the reduction of the concentrated solids removed from sewage in the primary sedimentation tanks, including the excess biological solids produced from the trickling filters.

Nowadays "high rate" digesters (Sawyer and Schmidt, 1955) are being used for such purposes - the digester is heated to about 35^{o}C and stirred by circulation of the contents through a heat exchanger. The advent of

the "energy crisis" in 1973 brought about an increasing interest in the use of the anaerobic digestion process not only for the disposal of sewage waste effluents but also as a means of energy generation in both developed and less developed countries.

In India, the so-called "gas plants" have been installed for over 16 years (Singh, 1972); since the late 1950's the number of digesters in operation in villages throughout India has risen to some 6,500. The methane produced by the digesters is used to provide energy for electricity generation, heat for cooking purposes, and the effluent is used as a fertiliser.

The increased problems of farm waste disposal arising from the introduction of intensive farming units in the U.K. have resulted in the digestion process being used as a primary treatment for very strong agricultural wastes, such as pig waste.

Klass and Ghosh (1973) considered anaerobic digestion as a serious contender to produce substitute natural gas, from organic wastes such as domestic refuse, to supplement the Natural Gas requirements of the United States.

The potential quantity of feedstocks for digestion

systems in the U.K. or in less developed countries, such as India, is huge. Table 1.1 lists the quantities of manure that had to be disposed of from housed animals in the U.K. in 1972 and Table 1.2 shows estimates of the total quantities of organic wastes produced in the U.K. in 1971.

A 1961 livestock census in India showed the cattle population to be 226 million and Prasad et al. (1974) estimate that each cow produces 7 lbs. of dry dung/day, which amounts to a dry cow dung production exceeding 190 million tons of dry dung/year.

Despite the long history of experimental work on the digestion process there are few kinetic constants found in the literature that would aid digester design for the utilisation of mixed insoluble cellulosic feedstocks; most experimental studies have only supplied quantitative information such as the total gas per unit weight of feed added to the digester, and no comprehensive studies relating loading effects with detention time and temperature for the continuous digestion of cellulosic feeds, without the use of sludge addition, are to be found in the literature.

Along with the shortage of such data, there is an increasing interest in the anaerobic digestion process for the production of methane, using animal or vegetable wastes, in

the U.K. and in less developed countries. It is therefore the purpose of this study to investigate the effects of temperature and loading conditions on the continuous digestion of different cellulosic feedstocks (substrates).

LIVESTOCK	MANURE (million gallons)	NUTRIENTS (thousand tons) N	P	K	BOD_5 (population equivalents in millions)
Pigs	2300	66	17	20	11.2
Cattle	9000	209	25	196	30.7
Poultry	1330	97	33	25	12.4
Total	12,630	372	75	241	54.3

TABLE 1.1 Quantities of Manure to be disposed of from Housed Animals in the U.K., 1975.

(J.R. O'Callaghan, 1975)

COMPONENT	QUANTITY (Tons/year)
Domestic Refuse Total Dry Organic Matter	4,772,000
Wood Wastes includes soft and hard wood harvesting, sawmill wastes, and timber processing wastes	4,170,000
Pulp and Paper Industry Wastes	1,000,000
Waste Paper	6,000,000
Sewage Sludge	2,000,000
Vegetable Wastes	6,000,000
Livestock Wastes:	
Cattle Wastes	14,900,000
Chicken Wastes	1,284,000
Pig Wastes	2,500,000

TABLE 1.2 Quantities of Organic Waste arising in the United Kingdom.

(D.E. Hughes, 1975)

CHAPTER 2

LITERATURE SURVEY

The production of "Bio gas" from the anaerobic digestion of organic material is well documented, with the literature dating back to the latter part of the nineteenth century. Many related topics describing the process have been covered - they include microbiological aspects, kinetic considerations, applications and process developments, biochemical pathways and general principles of digestion technology. Current research in the U.K. emphasises the change in direction towards the process being used for energy generation and not merely for sludge destruction as in the past.

2.1 THE ANAEROBIC DIGESTION PROCESS

2.1.1 Feedstock Constituents

The major components of feedstocks treated in the anaerobic digestion process can be classified into carbohydrates, proteins including (non-protein) nitrogen and lipids. This is the case for municipal, agricultural or sewage sludge feedstocks, although the relative percentage dry weight compositions of the three components may vary in each feedstock, as shown in Table 2.1.

COMPONENT	PERCENTAGE WEIGHT
Domestic Sewage Sludge (dry) (Hobson et al., 1974)	
Hemicellulose	6.15
Cellulose	34.48
Lipids	14.01
Protein	18.98
Ash	34.88
Piggery Waste (dry) (Hobson et al., 1974)	
Protein	20.90
Lipid	7.70
Neutral Detergent Fibre (cellulose, hemicellulose, lignin)	53.80
Acid Detergent Fibre	33.00
Lignin	10.10
Ash	17.60
Average Municipal Waste (Reese et al., 1972)	
Moisture	20.73
Cellulose, sugar, starch	46.63
Lipids (fats, oils, waxes)	4.50
Protein	2.06
Other Organics (plastics)	1.15
Ashes, metal, glass, etc.	24.93

TABLE 2.1 Typical Feedstock Composition.

Plant Material	Hemicellulose	Cellulose	Lignin	Total Lignocellulose
rye grass (early leaf)	15.8	21.3	2.7	39.8
rye grass (seed setting)	25.7	26.7	7.3	59.7
orchard grass (medium maturity)	40.0	32.0	4.7	76.7
rye straw	27.2	34.0	4.7	75.4
Birch wood	25.7	40.0	15.7	81.4
Spruce wood	20.9	46.0	24.1	91.0

TABLE 2.2 Content of Cell Wall Constituents in Lignocellulose of Various Plant Materials (as percentage of Dry Matter).

(Pigden and Heaney, 1969)

Table 2.2 shows the composition of various plant materials. As can be seen from Tables 2.1 and 2.2, lignin forms a considerable portion of plant wastes and agricultural wastes and is not digested under anaerobic conditions, therefore, acting as a fixed solid in the digestion process.

2.1.2 Two-stage Digestion

The composition and structure of the components determines the characteristics of the biomass and the availability of the components to the bacteria and consequently influences the rate at which they are converted to methane and carbon dioxide. Hobson et al. (1974) note on this point that not only will the bacterial population vary in composition with the composition of the feedstock input to digesters but the relative concentrations of the different groups of bacteria would be expected to vary with the concentration of feedstock added.

We have already seen that the digestion process consists of the non-methanogenic and methanogenic stages; Figure 2.1 illustrates the steps occurring in the anaerobic digestion of the three major components for the two stages.

The non-methanogenic stage is subdivided into two steps:

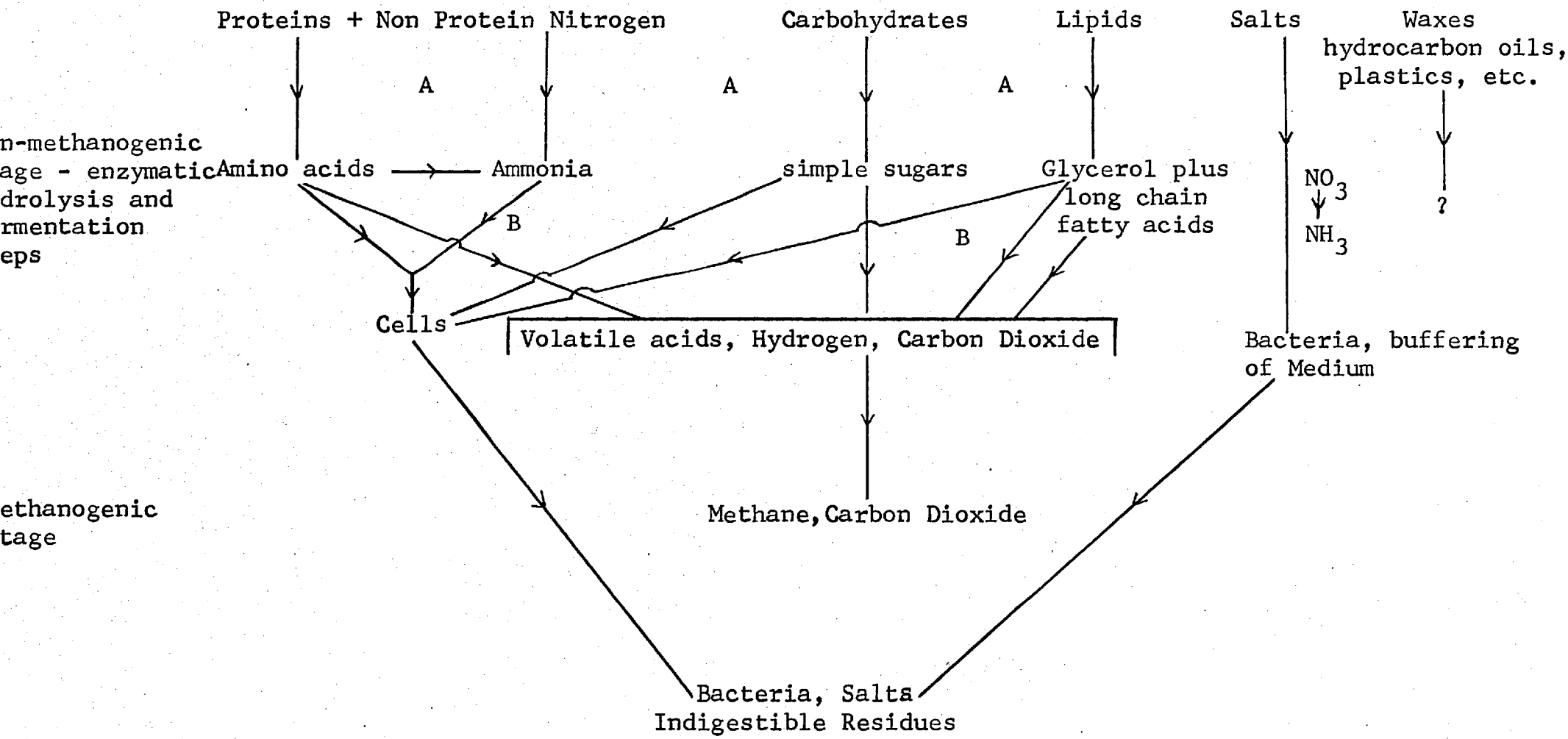


FIGURE 2.1 Digestion Pathways for the Major Feedstock Components.
(Hobson et al., 1974)

1. Hydrolysis Step:

The insoluble organic components of the feedstocks are hydrolysed by enzymes to produce soluble organics. Bareden and Buswell (1933) demonstrated that close association between the substrate and the bacteria is necessary for the hydrolysis step to proceed efficiently. Pollock (1962) concluded that direct absorption of large insoluble molecules into the bacteria cannot occur and metabolism can only occur by extracellular enzymes being made available outside the semipermeable membrane of the bacteria.

This step is shown as A in Figure 2.1.

2. Fermentation Step:

This step is shown as B in Figure 2.1.

The bacteria responsible for the secretion of the extracellular enzymes used for the hydrolysis step are also responsible for the subsequent fermentation step.

The methanogenic stage follows the non-methanogenic stage as shown in Figure 2.1. The feedstock components are continuously transformed into the final end products, methane, carbon dioxide and cells, in balanced digestion.

2.1.3 Hydrolysis of the Three Major Components

2.1.3.1 Carbohydrates:

The carbohydrate content of an average municipal waste can be as high as 50% and the largest constituent of carbohydrates present in vegetable, animal or municipal waste feedstocks is the polysaccharide fraction such as cellulose, hemicellulose; Table 2.1 illustrates this point. The polysaccharides, such as cellulose or starch, have the general chemical formula $(C_6H_{10}O_5)_n$, are known as glycans and are condensation products of at least ten glycosidically linked sugar residues.

Cellulose forms the bulk of cell wall material in higher plants and, as such, is the most widespread and abundant naturally organic polymer. Cellulose contents of various range plants and residues are given in Table 2.3, and Table 2.4 lists the cellulose content of various animal manures.

Cellulose is an insoluble, linear polymer of at least 3000-β-D (1 $\longrightarrow$ 4) linked glucose residues. The enzymatic hydrolysis of cellulose, as described by Reese et al. (1950) depends on at least three extracellular enzymes. The first group of cellulases (C_1) may attack the cellulose

Material	Species	Component	% cellulose content dry weight
Bluegrass	Poa pratensis	pasture - immature	20
		early bloom	28
Corn	Zea mays	stover	40 (average)
Cotton	Gossypium hirsutum	seed hulls	60
		fibre	91
Citrus	Citrus sp.	pulp shredded	62
Rice	Oryza sativa	hulls	42
Soy bean	Glycine max.	hay	31
		hulls	52
Sugarcane	Saccharum officinarum	mature plants	42
		bagasse	48-62
Wheat	Tritticum sp.	straw	50

TABLE 2.3 Cellulose Content of Crop and Range Plants and Residues.

(Stephens and Heichel, 1975)

SOURCE	CELLULOSE - % dry weight
Broiler (caged)	11
Hen (caged)	15
Swine (fattening and growing)	15
Beef cattle (fattening)	17
Dairy cattle (lactating)	25
Sheep (forage fed)	30

TABLE 2.4 Cellulose Content of Animal Manures.
(Stephens and Heichel, 1975)

fibres causing a loss of tensile strength and the production of "reactive cellulose" (hydrocellulose). C_x cellulases perform the subsequent depolymerisation to low molecular weight disaccharides (cellobiose) and oligosaccharides. The third group of enzymes catalyse the hydrolysis of these di- and oligosaccharides to glucose - this group of enzymes are known as β-glucosidases. Subsequent utilisation of the glucose is an intracellular event since glucose is small enough (molecule diameter - 10 Å) to diffuse through the cell wall and semi-permeable membrane which surrounds bacterial cells (bacterium diameter about 10^4 Å). A schematic representation of the hydrolysis step is shown in Figure 2.2A.

There are at least seventy species of bacteria, fungi, actinomycetes and protozoa that have the capacity to split cellulose - genera commonly reported to contain species which split cellulose include Cytophage, Cellulomonas, Streptomyces, Asper gillus, Penicillium and Trichoderma (Higgins and Burns, 1975).

Hemicellulose is a collective and somewhat imprecise term for a group of polysaccharide materials having no structural relationship to cellulose but from which they can be separated by alkaline extraction. These materials

A. Schematic Representation of Cellulose Breakdown by Extracellular Enzymes.

(Chan, 1970)

Native cellulose

↓ C_1 (hypothetical)

hydrated Polyanhydroglucose

↓ C_x's

Cellobiose

↓ β-glucosidase

glucose

$$(C_6H_{10}O_5)_n + nH_2O \xrightarrow[\text{hydrolysis}]{\text{Enzyme}} n(C_6H_{12}O_6)$$

B. Enzymatic Transformation of Xylan .

(Higgins and Burns, 1975)

Xylan $\xrightarrow{\text{xylanase}}$ xylobiose $\xrightarrow{\text{xylobiase}}$ xylose

FIGURE 2.2

are a heterogeneous mixture of linear or highly branched polymers which may contain L-arabinose, D-xylose, D-mannose, D-glucose, D-galactose, 4-O-methyl-D-gluconic acid and D-gluconic acid. They are subdivided into that fraction which precipitates on acidification (hemicellulose A or cellulosans) and the supernatant (hemicellulose B or acidic hemicellulose). Hemicellulose A is predominantly composed of β-D (1 ⟶ 4) linked xylose units - described as xylan, and hemicellulose B is an uronic acid-containing pentose polymer. Plant materials usually contain hemicellulose: cellulose in the ratio of 0.5:1 to 1:1 as shown in Table 2.2.

The enzymatic hydrolysis of xylan bears obvious similarities to cellulose breakdown, as discussed by Higgins and Burns (1975). Xylonases depolymerise xylan to disaccharide (xylobiose) which is subsequently hydrolysed to its monosaccharide components by xylobiase. Figure 2.2B illustrates the transformation. Once more a wide variety of microorganisms are involved in the total degradation of hemicelluloses - as would be expected by the large range of substrates. Genera commonly implicated include Bacillus, Pseudomonas, Streptomyces and Penicillium (Higgins and Burns, 1975).

Next to cellulose and hemicellulose, lignin is

the most abundant constituent of organic waste of plant origin. Most young plants contain less than 10% by dry weight - see Table 2.2. The lignin content of mature wood varies between 20 and 40%; however, lignin is only digestible under aerobic conditions or by acid hydrolysis.

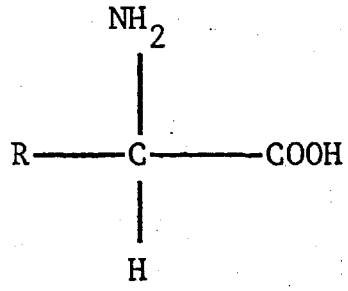
2.1.3.2 Proteins:

The protein component of the feedstock can form as much as 20% dry weight in piggery waste or as low as 6% in the leaves of vegetable wastes (Pfander et al., 1969).

All proteins contain carbon, hydrogen, oxygen, nitrogen and with few exceptions, sulphur. The elementary composition of proteins tells little of their intimate structure since these figures are very similar for most proteins. The approximate values are: Carbon, 45 to 55 per cent; Hydrogen, 6 to 8 per cent; Oxygen, 19 to 25 per cent; Nitrogen, 14 to 20 per cent; and Sulphur, 0 to 4 per cent. The average nitrogen content of proteins is frequently taken as 16 per cent for the estimation of the protein content of organic materials.

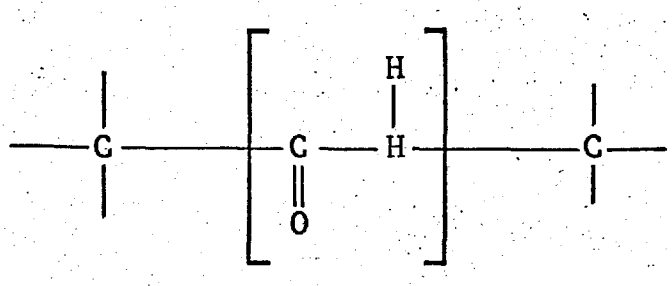
Amino acids are the fundamental structural units of the proteins. The amino acids that have been isolated from proteins are, with two exceptions, primary alpha-amino

acids, i.e. the carboxyl and amino groups are attached to the same carbon atom:



Basic alpha-amino acid

where R may be a hydrogen atom, a single methyl group, a short carbon chain (with or without functional groups such as SH, NH_2 and COOH), or a cyclic structure. The amino acids are joined by peptide bonds (CO-NH). The characteristic peptide bond structure is shown below enclosed in brackets:



Proteins are large complex molecules, containing many amino acids in peptide linkage, with molecular weights ranging from around 6,000 to many millions.

The protein fraction is hydrolysed by extracellular enzymes in the digestion process - the long chain polymers are broken down into small fragments such as peptides and

amino acids by proteases. According to Salle (1961), a pure native protein, when present in a medium as the sole source of carbon and nitrogen, is resistant to attack by even the most proteolytic bacterial species. If, however, a small amount of peptone is added to the medium, multiplication of the organisms and degradation of the protein occur. This indicates that an extracellular enzyme is necessary to convert the indiffusible protein into diffusible fractions. In the absence of an available nitrogen and carbon source, the organisms are unable to multiply and elaborate this necessary enzyme. However, peptone can enter the cell and stimulate the organisms to produce the necessary proteolytic enzyme.

Lackey and Hendrickson (1958) presented the following general scheme for the enzymatic breakdown of proteins:

Protein → proteases → peptones → peptides → amino acids

2.1.3.3 Lipids:

Lipids are present in most material of biological origin, forming as much as 1-10% dry weight of a typical manure (Coe and Turk, 1973) or as high as 14% dry weight for a domestic sewage sludge. Lipids consist of

triglycerides (esters of higher fatty acids with glycerol), phospholipids, cholesterol, waxes and fixed oils. The chain lengths of 16-18 carbon atoms are the most common components of fats of animal and vegetable origin - these include the saturated acids, palmitic ($C_{15}H_{31}COOH$), stearic ($C_{17}H_{35}COOH$) and the unsaturated oleic acid ($C_{17}H_{33}COOH$). In addition fats and fatty acids of bacterial and fungal origin are not uncommon and include acetic (CH_3COOH), propionic (C_2H_5COOH), butyric (C_3H_7COOH), valeric (C_4H_9COOH), lauric ($C_{11}H_{23}COOH$) and a range of branched chain fatty acids (6-methyloctanoic, phthoic).

Enzymatic hydrolysis of lipids yields a mixture of glycerol and fatty acids. Lipase enzymes are responsible for the conversion and are common in plants and animals, and found in aerobic or anaerobic organisms. The typical hydrolysis reaction is shown below:

$$\left.\begin{array}{l} CH_2O.COR_1 \\ CH.O.CO.R_2 \\ CH_2O.CO.R_3 \end{array}\right) + 3H_2O \xrightarrow{\text{lipase}} \begin{array}{l} CH_2OH + R_1\text{-}COOH \\ CHOH + R_2\text{-}COOH \\ CH_2OH + R_3\text{-}COOH \end{array}$$

lipid glycerol fatty acids

R_1COOH, R_2COOH and R_3 COOH represent three molecules of either the same or different fatty acids.

2.1.4 Non-Methanogenic Fermentation of Products arising from the Hydrolysis of the Three Major Components

2.1.4.1 Non-Methanogenic Fermentation of Sugars produced from the Hydrolysis of Carbohydrates

The simple sugars produced from the hydrolysis of polysaccharides are fermented to one or more of the following products: hydrogen, carbon dioxide, ethanol, formic, acetic, propionic, butyric, valeric, caproic, succinic and lactic acids. The conversion of glucose to such products is shown below:

$$\underset{\text{glucose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{lactic}}{2CH_3CHOHCOOH}$$

$$C_6H_{12}O_6 \longrightarrow \underset{\text{butyric}}{CH_3CH_2CH_2COOH} + 2CO_2 + 2H_2$$

$$C_6H_{12}O_6 \longrightarrow \underset{\text{ethanol}}{2CH_3CH_2OH} + 2CO_2$$

The metabolism of glucose has probably been studied in more detail than any other organic substance. There are innumerable reports in the literature of studies on glucose fermentation pathways, intermediates, and end products and on the enzymes involved in these fermentations. There are two principal and well recognised pathways for

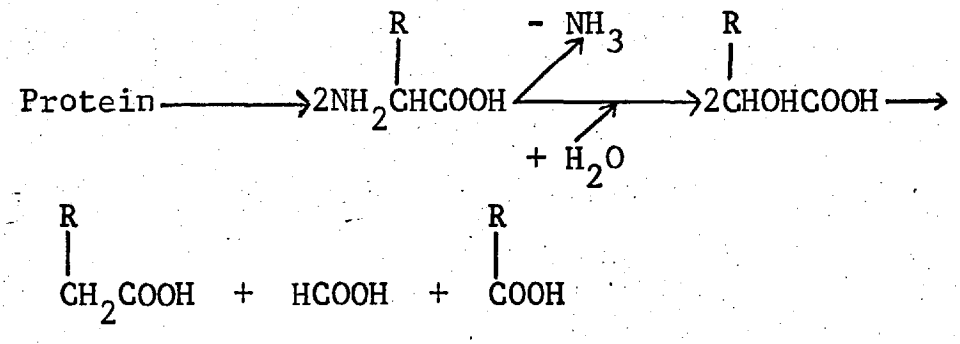
the decomposition of glucose. The best known of these is the Embden-Meyerhof pathway which leads to the formation of pyruvate that can be further metabolised by mixed cultures of facultative and anaerobic organisms to acetic and propionic acids. The second pathway of importance is the phosphogluconate oxidative pathway or the "hexose monophosphate shunt". Both of these pathways have many side reactions which are somewhat dependent on the microbial populations and the environmental conditions involved. Kotzé et al. (1969) have discussed the various pathways in detail.

Hobson et al. (1974) note that the quantity and composition of these intermediate products in the digestion process depend on many factors such as feed composition, pH, temperature, bacterial growth rates and conclude that valeric and caproic acids are rarely found in digesters, as is ethanol and that negligible amounts of hydrogen are present in the gas phase. Hobson et al. (1974) analysed the volatile acid content resulting from piggery waste destruction and showed that 79% was acetic acid, 15% propionic acid and no butyric, but about 6% isovaleric acid was present: lactic, succinic acid and ethanol were not detected in the effluent.

2.1.4.2 Non-Methanogenic Fermentation of Products from the Hydrolysis of Protein and Lipid

Bacteria can decompose the amino acids resulting from the enzymatic breakdown of proteins in many ways, resulting in a great variety of end products depending on the amino acid, the mode of attack of the species and the environmental conditions.

McKinney (1962) stated that the anaerobic degradation of proteins results in the formation of amino acids which are hydrolytically de-aminated to form the equivalent hydroxy acids. The hydroxy acid is in turn reduced to the saturated acid as well as being oxidised to formic acid plus a saturated acid with one less carbon than the initial amino acid. The reactions involved may be expressed as follows:



This must be considered, however, a very simplified scheme of the reactions occurring in a mixed system of common facultative microorganisms.

The glycerol resulting from lipid hydrolysis is metabolised much like carbohydrate - to short chain fatty acids such as acetic acid. The long chain fatty acids are hydrogenated to acetic acid by the Beta-oxidation pathway. The basic scheme of Beta-oxidation for a typical fatty acid in anaerobic waste treatment is outlined as follows:

$$CH_3(CH_2)_{14}CH_2\text{-}CH_2\text{-}COOH \xrightarrow[+2H_2O]{-4H} CH_3(CH_2)_{14}COOH + CH_3COOH$$

This process is repeated $(\frac{n-2}{2})$ times (n = number of carbon atoms in the fatty acid molecule) until the acid is completely broken down.

Because of the known substrate limitations of single pure cultures, Kirsch and Sykes (1971) acknowledge that the digester culture must be highly mixed to accommodate the mixed substrate. Toerien et al. (1967) conclude that obligate anaerobes form the predominant population of bacteria responsible for the non-methanogenic stage, and the presence of hydrolytic bacteria capable of hydrolysing carbohydrates, proteins and lipids have been found to be operative in digesters being fed with mixed substrates. Toerien et al. (1967) and Hobson et al. (1974) report in detail, the various hydrolytic enzymes found in digesters.

2.1.5 Methanogenic Fermentation

It is the production of the gaseous methane end product which actually accomplishes the removal of organic matter, measured in terms of COD from the feedstock. The methanogenic bacterial population is strictly anaerobic and exhibit a high degree of substrate specificity. These organisms couple organic oxidation with carbon dioxide reduction to produce methane.

The substrates used by methanogenic bacteria fall into three distinct categories:

A. The lower fatty acids containing six or less carbon atoms (formic, acetic, propionic, butyric, valeric, caproic).

B. The normal and iso-alcohols containing from one to five carbon atoms (methanol, ethanol, propanol, butanol, pentanol).

C. Three inorganic gases (hydrogen, carbon dioxide and carbon monoxide).

The various compounds fermented by the different methane bacteria are given in Table 2.5. Their extreme sensitivity to oxygen and their slow growth rate have

MICROORGANISM	COMPOUNDS FERMENTED
Methano-bacterium omelianskii	H_2, ethanol, primary and secondary alcohols
Methano-bacterium suboxydans	Butyrate, valerate, caproate
Methano-bacterium Sohngenii	Acetate, butyrate
Methano-bacterium propionicum	Propionate
Methano-bacterium formicicum	H_2, CO_2, formate
Methanococcus mazei	Acetate, butyrate
Methanococcus vanneilii	Formate, hydrogen
Methanosarcina barkerii	Hydrogen, CO, methanol, acetate
Methanosarcina methanica	Acetate, butyrate

TABLE 2.5 Compounds fermented by the Methane Bacteria.

(Andrews et al., 1964)

made isolation and pure culture study extremely difficult. The high degree of substrate specificity often requires the involvement of several species to accomplish the fermentation of simple organic compounds.

A set of typical transformations of the products from the non-methanogenic stage to methane is shown in Figure 2.3. Figure 2.4 illustrates the percentage conversion of waste COD by various routes from the digestion of municipal waste sludges; acetic and propionic acid account for 85% of the methane produced. The biochemistry of short chain fatty acid degradation by methane-forming bacteria has been fairly well established by Stadtman and Barker (1951).

It is important to realise that the successful operation of a digester can only be achieved by the occurrence of both the non-methanogenic and methanogenic stages; if the intermediate products from the non-methanogenic stage are not utilised, then the overall process will cease. Therefore it is important that the non-methanogenic and methanogenic populations are maintained and disturbances, such as changes in temperature or pH, are kept to a minimum, and since a mixed population is a necessity for the digestion process the effects of changes in temperature or pH on the populations are controlled by the interaction of different bacteria growing on different substrates at different times.

$$\underset{\text{hydrogen}}{4H_2} + CO_2 \longrightarrow \underset{\text{methane}}{CH_4} + 2H_2O$$

$$2CH_3CH_2OH + CO_2 \longrightarrow 2CH_3COOH + CH_4$$

ethanol → acetic acid

$$\underset{\text{acetic acid}}{CH_3COOH} \longrightarrow CH_4 + CO_2$$

$$2CH_3(CH_2)_2COOH + 2H_2O + CO_2 \longrightarrow 4CH_3COOH + CH_4$$

butyric acid

FIGURE 2.3 Methane Formation from Fermentation of Products from the Non-Methanogenic Stage.

(Chan, 1970)

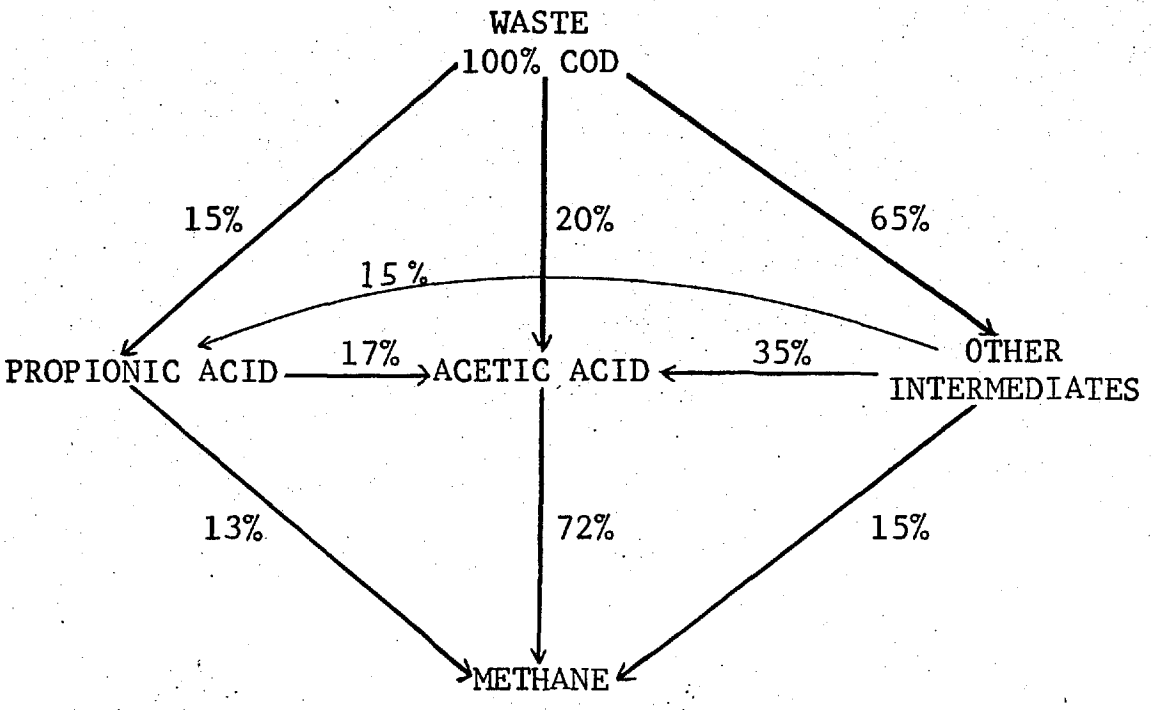


FIGURE 2.4 Percentage Organic Acid Production and Utilisation for the Digestion of Municipal Waste.

(McCarty, 1964)

2.2 FACTORS AFFECTING THE DIGESTION PROCESS

Process stability has been one of the major problems associated with the digestion process. Trevelyan (1975) suggests that the sensitivity of the process to any change in conditions - temperature, pH, loading - constitutes its greatest disadvantage.

Kirsch and Sykes (1971) define the successful digestion treatment as one which proceeds with a minimum of adjustment or control (loading, nutrient supply); given a temperature of 85-100^{o}F, exclusion of air, sufficient nitrogen and phosphorus, trace minerals, a reasonable amount of biodegradable carbon, a pH between 6.6 and 7.5 and the absence of biological inhibitors, the production of methane and the stabilisation of degradable solids proceeding at a steady state level. Some of the factors which can affect the above definition are now discussed.

2.2.1 pH

The pH of the internal environment of all living cells is believed to be approximately 7 (Stanier et al., 1963). Many species can grow well at any value between 6.0 and 9.0 since the membranes of living cells are only slightly permeable to hydrogen and hydroxyl ions (Stanier

et al., 1963); however, the mixed bacterial population in a digester comprises a wide variety of bacterial species and to refer to an optimum pH level has little meaning - the optimum pH is the integral result of the different contributions by the many different reactions taking place.

The effect of pH on the methanogenic bacterial population is shown in Figure 2.5 as found by Heukelekian et al. (1939). Similarly for cellulolytic bacteria Table 2.6 shows the optimum pH for the growth of pure cultures.

Gascoigne and Gascoigne (1960) found that the optimal pH values for cellulose enzyme activity are on the acid side; however, with animal cellulase the optimum pH range is 5.0-5.5 and for bacterial cellulase the range is 5.8-7.0.

Barker (1956) concluded that the pH range of 6.4-7.2 was most effective for methane production and that below pH 6.0 and above pH 8.0 production declined rapidly. Any sudden drop in pH from the equilibrium value, as a result of the accumulation of volatile acids is an indication of a disturbance that requires control. Two factors closely associated with pH are the alkalinity of the system and the volatile acid concentration.

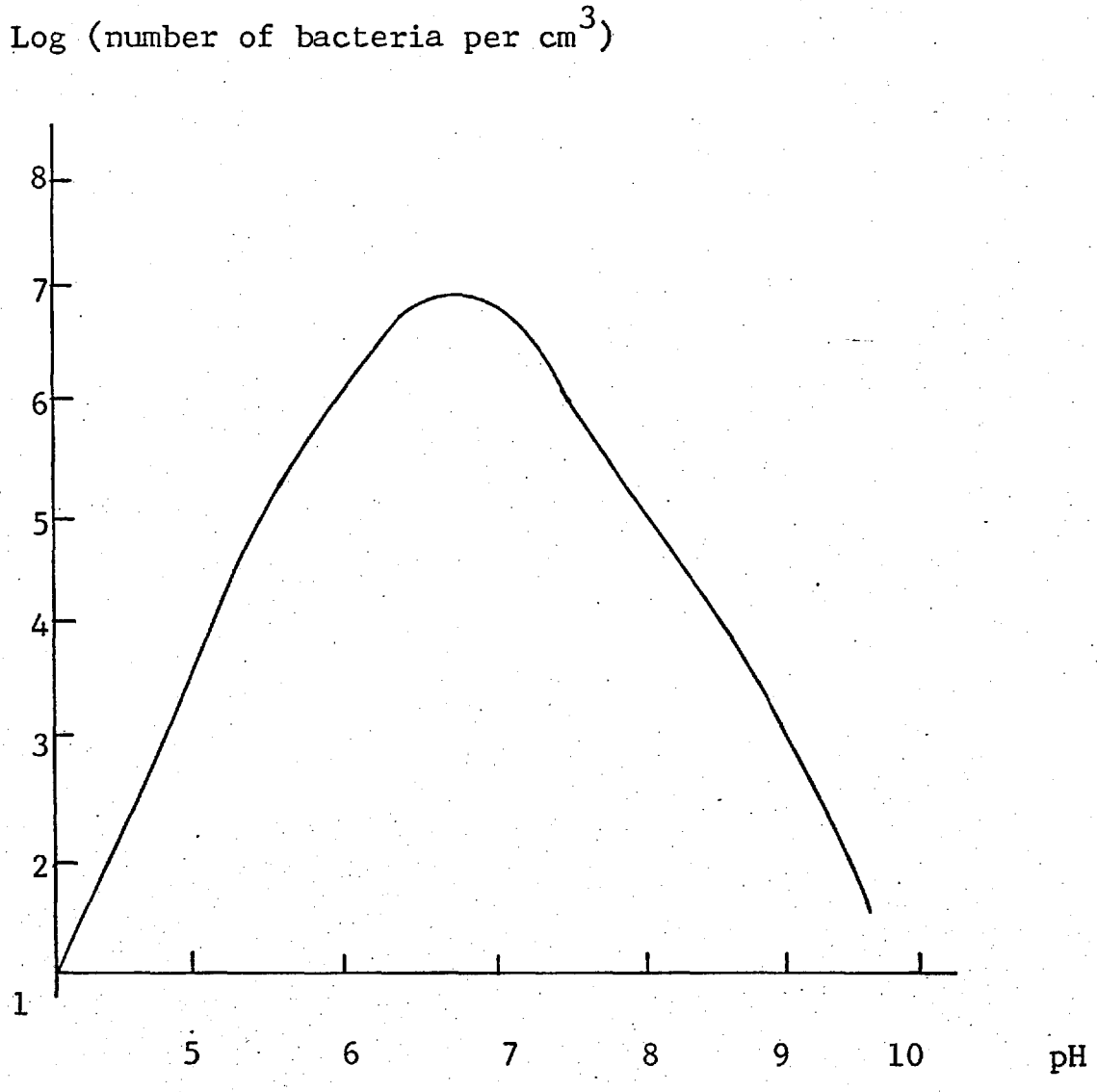


FIGURE 2.5 Effect of pH on Methane Bacteria.
(Heukelekian and Heinemann, 1939)

BACTERIA	pH	
	Range studied	Optimum
Bacillus thermofibrinoclus	3.4 - 11.7	8-8.4
Cellulomonas biazotea	5.2-6.9	6.4
Clostridium cellobioparus	4.0-8.0	5.5
Sorangium compasitium	4.5-9.5	8-8.5
Sporocytophaga cytophaga	2.5-9.5	7.5
Vibrio napi	4.6-7.6	7.6
Vibrio xylitica	5.0-9.2	7.5

TABLE 2.6 pH Optima of Cellulolytic Bacteria. (Siu, 1951)

2.2.2 Alkalinity and Volatile Acid Concentration

The natural buffering system is based on ammonium and bicarbonate ions which usually maintain the pH value near neutrality (Kotzé et al., 1969). Lack of sufficient buffering capacity causes the digestion process to stop since the intermediate products - volatile acids - cause the pH to fall and low pH values primarily inhibit growth of the methanogenic population.

The actual buffering capacity is essentially equal to the bicarbonate alkalinity and is related to the pH by the following expression:

$$pH = pK + \log \frac{(HCO_3^-)}{(CO_2)}$$

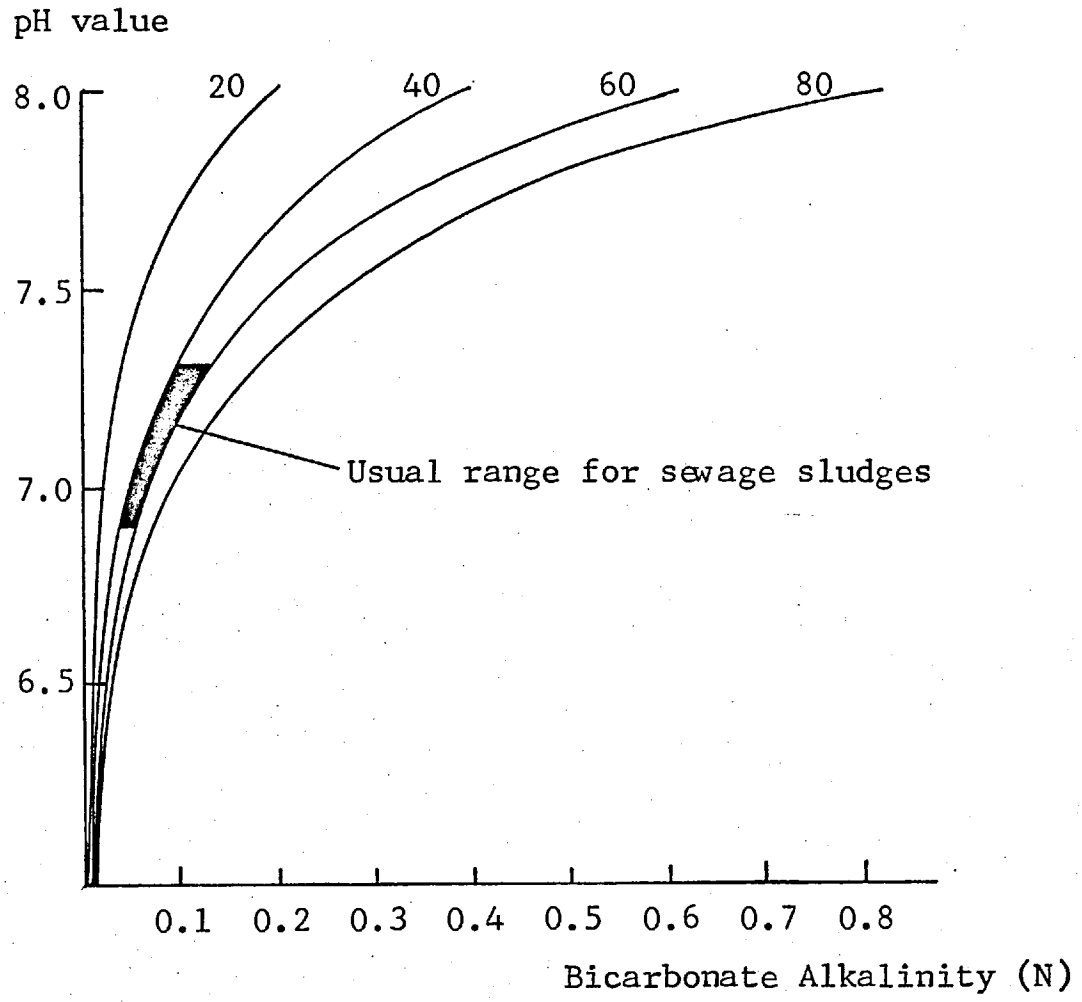
HCO_3^- - concentration of bicarbonate ion in solution, moles lit^{-1};

CO_2 - concentration of dissolved carbon dioxide, moles lit^{-1};

pK - logarithm of the reciprocal of the first dissociation constant of carbonic acid.

A graphical illustration of this equation is shown in Figure 2.6 for a temperature of 35°C, where the pH is plotted versus bicarbonate alkalinity for various gas compositions.

Yenchko (1955), Backmeyer (1955) and Gould (1959)



Percentage carbon dioxide shown against each curve

Assumptions:
1. Gas at atmospheric pressure,
2. Hydrostatic pressure negligible,
3. Gaseous carbon dioxide in equilibrium with dissolved carbon dioxide,
4. Activity coefficients are unity,
5. pK and CO_2 composition are affected by ionic strength of the liquid, but corrections for this are small and may be ignored for practical purposes.

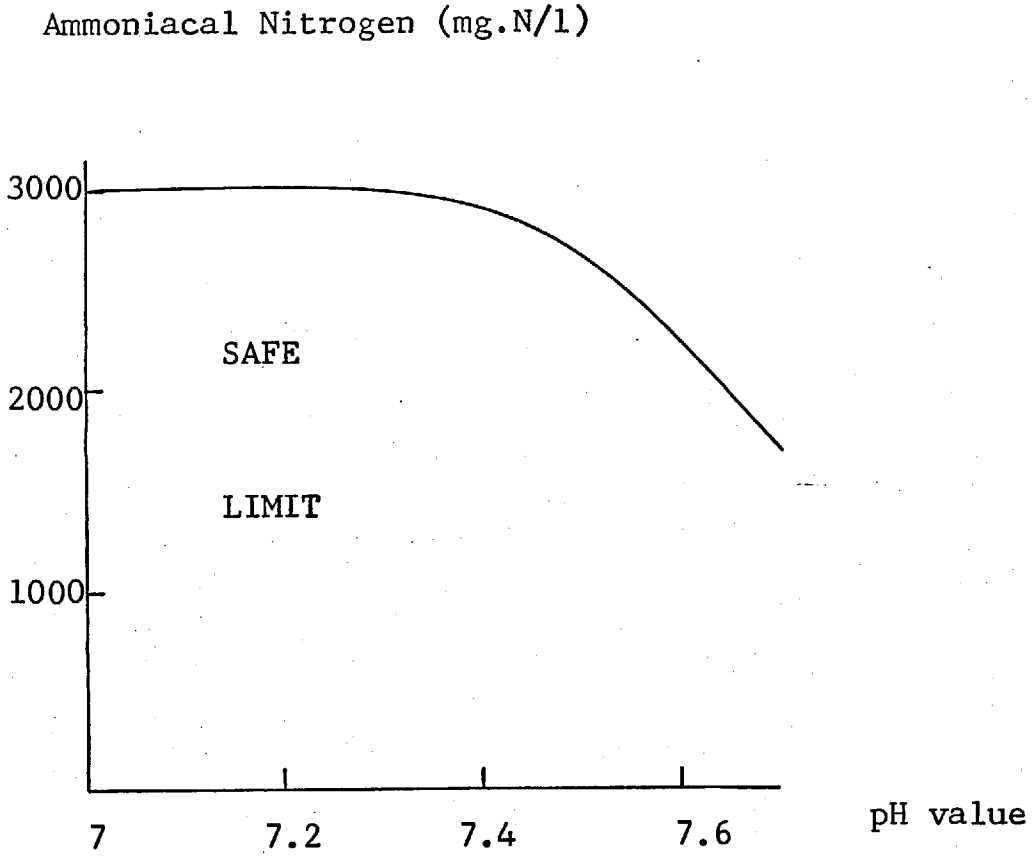
FIGURE 2.6 Relations between pH value and Bicarbonate Alkalinity at 35°C and at various Gas Compositions.

(Mosey, 1974)

considered a total alkalinity of 2000-3500 $mg.l^{-1}$ to be a desirable characteristic of a well established digestion process treating sewage sludge. At low alkalinity values (400-500 $mg.l^{-1}$) a slight increase in the volatile acid concentration causes a large drop in pH, because of the low buffering capacity of the system.

On the other end of the scale it has been shown that excessively high alkalinity is not necessarily desirable since the main buffering substance - ammonium bicarbonate (Simpson, 1960) - remains in solution, and at high pH the ammonium ion $NH_4^+ \xrightarrow{\text{dissociates}} NH_3 + H^+$ and Pfeffer et al. (1967) concluded that digester retardation occurs because of ammonia toxicity. McCarty (1964) stated that if the ammonium nitrogen concentration is between 1500-3000 p.p.m. at pH > 7.4 then free ammonia is inhibitory to cell growth but at greater concentrations then the ammonium ion is toxic regardless of pH. Both Thompson (1962) and Albertson (1961) indicate that ammonia levels of 1800 $mg.l^{-1}$ appear non-toxic, if in balance with alkalinity. The safe limit of the ammoniacal nitrogen concentration against pH has been plotted by Mosey (1974) and is shown in Figure 2.7.

The problem of ammonia toxicity is made worse if thermophilic operating temperatures are used and if the



Maximum Safe Limit

NH_4^+ = 3000 mg.N per lit.

NH_3 = 150 mg.N per lit.

$$NH_3 = \frac{1.13 \times 10^{-9} \left[NH_4^+\right]}{\left[H^+\right]}$$

FIGURE 2.7 Maximum Safe Limit of "Ammoniacal Nitrogen". (Mosey, 1974)

substrate contains a large proportion of protein, since at higher temperatures more complete protein degradation occurs, thus giving rise to greater NH_4^+ and HCO_3^- production.

Backmeyer (1955) maintained that the limiting value of volatile acid concentration should be 300 $mg.l^{-1}$, while Sawyer (1960) stated that for normal digestion the volatile acid concentration should be in the range of 100-200 $mg.l^{-1}$ as acetic acid. McCarty and McKinney (1961) conclude that a rapid increase in volatile acid concentration and subsequent neutralisation, causing decreased activity of the methane bacteria because of the low pH, is not due to volatile acid inhibition but is associated with salt toxicity. Salt toxicity is associated with the type and concentration of the cation portion of alkaline materials used for neutralisation, such as calcium, magnesium, sodium, potassium and ammonium. Therefore, as McCarty and McKinney (1961) note, providing the cation concentration is not inhibiting, then volatile acid concentrations of up to 10,000 $mg.l^{-1}$ can be successfully neutralised with no inhibitory effects. On a molar basis they showed that when the cations were supplied slowly to the digesters over a period of time then the following levels of cation could be tolerated: sodium and potassium 0.2 M, calcium and magnesium 0.175 M,and ammonium 0.15 M. An increase in the volatile

acid concentration away from the steady state level is a warning of pending digester failure.

The addition of sodium bicarbonate is considered by Kirsch and Sykes (1971) to be a better method of dealing with digester acidity rather than the addition of lime because lime reacts with carbon dioxide initially to yield calcium bicarbonate, which is not very soluble and after the bicarbonate alkalinity reaches 500-1000 $mg.l^{-1}$ the addition of more lime reacts to form insoluble calcium carbonate. Sodium bicarbonate can be added to produce alkalinity in the digester of 5000-6000 $mg.l^{-1}$ without producing any adverse or toxic effects, as shown by McCarty (1964).

In the event that the pH value needs to be decreased McCarty and McKinney (1961) state that the addition of hydrochloric acid or calcium chloride reduces the pH sufficiently without the danger of decreasing it much below 7.

2.2.3 Temperature

McGhee (1968) has collated the results of various investigators and found that the following temperature zones exist in the digestion process:

1.	Cryophilic	$< 10^{o}C$
2.	Temperate	$10\text{-}28^{o}C$
3.	Intermediate	$27\text{-}35^{o}C$
4.	Mesophilic	$27.5\text{-}40.5^{o}C$ (optimum at $37^{o}C$)
5.	Thermophilic	$42\text{-}60^{o}C$ (optimum at $52^{o}C$)

Within each temperature range Kirsch and Sykes (1971) suggest that a linear relationship exists between the reciprocal of the temperature and the logarithm of the rate of reaction, but no figures are shown to support this view. From the work of Fair and Moore (1937) the anaerobic decomposition rate increased 2.5 times for every $10^{o}C$ increase in temperature.

Speece and Kem (1970) have shown that the response of gas production from a mesophilic culture, that had been incubated at $35^{o}C$ and then incubated at a range of temperatures for a designated time interval, showed a linear response to temperature - a resumé of their results is shown in Table 2.7. The zero gas production at $20^{o}C$ is incompatible with the results of Fair and Moore (1937) and of McGhee (1968), who found that the digestion of sewage solids is possible at temperatures as low as $5^{o}C$ without requiring the development of a specialised population of cryophilic bacteria. McGhee (1968) concluded the following from his experimental results:

TEMPERATURE ^{o}C	TIME INTERVAL hour	GAS PRODUCTION $cm^3/hr/l$	RELATIVE RATE %
20	20.5	nil	0
25	20.8	24	36
30	25.7	46	68
35	-	68	100
40	5.9	90	132
45	54.0	117	171

TABLE 2.7 Resume of Effect of Short Term Temperature Variations on Methane Gas Production.

(Speece and Kem, 1970)

1. In the temperature range 35°C-10°C the rate of digestion is approximately halved for each 10°C decrease, and

2. Below 10°C the rate of digestion is more than halved for a 10°C decrease.

Nelson et al. (1939) found that cornstalk flour and chopped wheat required half as long to produce 300 cm^3 of gas at 55°C than at 28-30°C in non-stirred batch digesters, although no assimilation periods of the culture to the different temperatures are reported.

Boshoff (1967) found that the reaction velocity constants increased by 14% for digestion of elephant grass in non-stirred batch digesters as the operating temperature was changed from 22°C to 32°C.

At higher temperatures, Cooney and Wise (1975) suggest that the vast number of conflicting reports on the relative advantages and disadvantages of operating digesters at mesophilic or thermophilic temperatures arise from the fact that insufficient effort has been given to the selection and adaptation of the bacterial populations at the higher temperatures.

In addition to the effects of operating digesters at high or low temperatures, Kirsch and Sykes (1971) have pointed out that sudden temperature variations at any set temperature affect digester performance. Fisher and Green (1945) found that during pilot plant scale studies on thermophilic digestion, a drop of 2.7°C or more, during a relatively short period, greatly affected digestion at 46-53°C, while Garber (1954) concluded that, once established, thermophilic digestion (49°C) was quite stable and resistant to upset and temperature changes of 5°C in 48 hours produced no unusual changes, but that prolonged lower temperatures changed the system to mesophilic conditions.

Once more it should be realised that optimum operating temperatures must be chosen in the knowledge that each bacterial species comprising the mixed population will, in most cases, have its own specific optimum temperature. Table 2.8 shows the wide range of optimum temperatures existing for a selection of cellulolytic bacteria.

2.2.4 Nutrients

Essential macronutrients for living organisms include carbon, nitrogen, sulphur and phosphorus.. Saunders and Bloodgood (1965) report a minimum C:N ratio

BACTERIA	RANGE STUDIED °C	OPTIMUM °C
Bacillus cellulosae dissolvens	22-67	37
Bacillus thermobrincolus	30-80	65
Cellulobacillus myxogenes	15-46	37
Cellulomonas biazotea	5-37	28
Microspiraagar-liquefaciens	16-37.5	25
Sorangium compositum	7-42	34-35
Vibrio amylocella	15-42	37
Methane bacteria*	20-35	28

* Heukelekian and Heinemann (1939)

TABLE 2.8 Temperature Optima of Cellulolytic Bacteria.

(Siu, 1951)

of 16:1 necessary for anaerobic digestion and Hill (1939) found that C:N ratios of between 20-52.6:1 caused no deleterious effects on the digestion process. Table 2.9 summarises some of the carbon, nitrogen and phosphorus requirements as found in the literature, for cellulose digestion. From these ratios it can be seen that from 2 to 60 mg of nitrogen and from 1 to 10 mg of phosphorus per gm of cellulose are required for decomposition. According to Helmers et al. (1951), the phosphorus content in bacterial cells is 1.02%, while the nitrogen content is about 8.0% and therefore the phosphorus requirement for bacterial cell synthesis is about $\frac{1}{8}^{th}$ the amount of nitrogen required. The levels of nitrogen and phosphorus required to sustain decomposition vary with the organisms involved but are generally of the same order of magnitude.

The best source of nitrogen for anaerobic decomposition is ammonia and Dubois (1928) showed that adding animal manures and fertiliser materials to a digester operating on cellulose alone increased the nitrogen content to favour cellulose digestion.

Other nutrient requirements include trace metal ions. Table 2.10 shows the optimum concentration range for such ions in the digestion of sewage sludge. Bryant (1973)

REFERENCE	NITROGEN REQUIREMENT	PHOSPHORUS REQUIREMENT
Speece and McCarty 1964	9-20 mg N per gm.COD	C/P 1:7 of N required
Saunders and Bloodgood 1965	C/N 16:1	-
Regan and Jeris 1970	C/N 30-35:1	-
Schroepfer and Ziemki 1959	1 gm/20 gm BOD	1 gm/100 gm BOD
Porges, 1960	C/N 9.4:1	C:P 1:49 $\longrightarrow$ 82

TABLE 2.9 Literature Data on Carbon, Nitrogen and Phosphorus Requirements for Cellulose Digestion.

INORGANIC ION	CONCENTRATION RANGE
Sodium mg/l	100-200
Potassium mg/l	200-400
Calcium mg/l	100-200
Magnesium mg/l	75-150
Sulphur (sulphide in solution) mgS/l	0.1 - 10

TABLE 2.10 Optimum* Concentrations of Inorganic Ions in Sewage Sludge Digestion.
(McCarty, 1964)

* Optimum with respect to cell growth.

has examined the nutritional requirements of the predominant cellulolytic bacteria in the cow rumen and concludes that all the strains studied required one or more B-vitamins for growth, such as biotin and riboflavin; however, Bryant presents no figures on concentration levels required.

For most feedstocks, the C:N ratio can be adjusted by addition of substrates containing a high content of the deficient nutrient, e.g. the addition of wheat straw (C:N ratio about 120:1) to a digester being fed on cow manure (C:N ratio 25:1) will increase the C:N ratio. The micro-nutrients such as B-vitamins and the various inorganic salts are normally present in most animal waste or domestic sewage feedstocks but may be deficient in vegetable wastes.

2.2.5 Structural and Composition Properties of the Polysaccharides

The polysaccharides as they occur in animal and municipal feedstocks, may consist of pure cellulose, as in some pulp wastes, or a complex of lignin, cellulose and hemicellulose with small amounts of pectin. Wood (1970) reports that cellulose, as it occurs in hay, wood and straw, is not an ideal substrate for digestion in that the less crystalline regions are rich in lignin and hemicellulose and these substances have a pronounced effect on the rate

of enzymatic attack on the fibre. The lignin serves to shield or protect the cellulose from bacterial degradation. Hobson et al. (1974) note that in its native form in plant structure, or specialised constituents such as cotton fibres, cellulose is highly polymerised. In addition the fibres may be coated with wax or interlocked with lignin and other polysaccharides, thus shielding the cellulose from bacterial attack.

Prasad et al. (1970) have shown that in the batch digestion of cow dung with vegetable wastes such as sugar-cane, bagasse and groundnut shell, the lignin-cellulose complex is more resilient to decomposition than the ligno-hemicellulose complex; a holocellulose lignin ratio of 2.87:1 resulted in a 5.72% relative decrease in cellulose but with a holocellulose lignin ratio of 3.54:1 the relative decrease in cellulose was 9.41%.

In the enzymatic saccharification of cellulosic wastes Mandels et al. (1974) concluded that substrates such as rice hulls, bagasse, and fibrous cotton were very resistant to saccharification - they obtained less than 5% saccharification at 50^{o}C in 48 hours using the cellulase Trichoderma viride - because of the little available surface, high crystallinity and in the case of rice hulls

and bagasse, high lignin content. They also found rumen fibres to be quite resistant to saccharification - $< 5\%$ saccharification at $50^{o}C$ in 48 hours - but susceptibility increased markedly by pot milling* the fibres - the % saccharification increased to 17% after 48 hours.

* Rolling porcelain mill.

2.3 STOICHIOMETRY

The anaerobic waste treatment process has been shown to be capable of the quantitative conversion of organic matter to methane and carbon dioxide. Buswell and Mueller (1952) have presented the following empirical equation to describe this conversion:

$$C_XH_YO_Z = (\frac{Z}{4}+\frac{Y}{4}-X)H_2O + (\frac{X}{2}-\frac{Y}{8}+\frac{Z}{4})CO_2 + (\frac{X}{2}+\frac{Y}{8}-\frac{Z}{4})CH_4$$

The production of cellular mass is not accounted for by this equation. Trevelyan (1975) acknowledges that: "the assumption of complete decomposition of a substrate to methane, carbon dioxide and water provides useful hints on gas yield and composition, on pH, and on the effects of the composition of a feed to a digester".

More recently the bioconversion of organic materials into products via aerobic or anaerobic treatment processes has been described in terms of stoichiometric equations obtained by considering the oxidation reactions on an electron equivalent basis for the electron donor R_d (feedstock to the digester) electron acceptor R_a (methane in the case of anaerobic digesters) and one for cell synthesis R_c. The overall reaction (R) can then be obtained as follows:

$$R = R_d - f_e R_a - f_s R_c$$

The fraction f_e represents the portion of the electron donor which is coupled with the electron acceptor (the portion used for energy) and f_s represents the portion of the electron donor which is coupled with cell formation. The values of f_e and f_s are functions of the cell yield coefficient a_e. Values of a_e can be estimated from experimental values or from thermodynamic considerations.

McCarty (1971) used classical thermodynamics to indicate the quantity of energy released from a given change from one state to another. Bacteria which can capture more of the energy released by a chemical change will have a higher cell yield. The bacteria obtain the energy they need for growth and maintenance by bringing about inorganic or organic transformations such as the transformation of adenosine triphosphate (A.T.P.) to anedosine diphosphate (A.D.P.). Energy losses result from inefficiencies in the transfer of energy both from substrate to the biological energy carrier A.T.P. and from A.T.P. to the cell for synthesis and maintenance.

Table 2.11 lists the half reactions and overall stoichiometric change for four separate feedstocks to a digester - carbohydrate, protein, grease and wastewater

Component	Half Reactions	Overall Stoichiometric Equation	% CH_4/CO_2 (v/v)	Gas produced lit./gm. of component digested	
				CH_4	CO_2
Wastewater (Sewage Sludge) $C_{10}H_{19}O_3N$	R_c $\frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O = \frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^-$ R_a $\frac{1}{8}CH_4 + \frac{1}{4}H_2O = \frac{1}{8}CO_2 + H^+ + e^-$ R_d $\frac{1}{50}C_{10}H_{19}O_3N + \frac{9}{25}H_2O = \frac{9}{50}CO_2 + \frac{1}{50}NH_4^+ + \frac{1}{50}HCO_3^- + H^+ + e^-$	$C_{10}H_{19}O_3N + 4.7H_2O = 0.195C_5H_7O_2N$ (cells) $+ 5.75CH_4 + 2.48CO_2 + 0.81NH_4^+ + 0.81HCO_3^-$ (nutrient salts)	70 / 30	0.640	0.276
Carbohydrate CH_2O	R_c $\frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O = \frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^-$ R_a $\frac{1}{8}CH_4 + \frac{1}{4}H_2O = \frac{1}{8}CO_2 + H^+ + e^-$ R_d $\frac{1}{4}CH_2O + \frac{1}{4}H_2O = \frac{1}{4}CO_2 + H^+ + e^-$	$CH_2O + 0.0666NH_4^+ + 0.0666HCO_3^-$ (nutrient salts) $= 0.0666C_5H_7O_2N$ (cells) $+ 0.333CH_4 + 0.4CO_2 + 0.266H_2O$	45 / 55	0.248	0.298
Protein $C_{16}H_{24}O_5N_4$	R_c $\frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O = \frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^-$ R_a $\frac{1}{8}CH_4 + \frac{1}{4}H_2O = \frac{1}{8}CO_2 + H^+ + e^-$ R_d $\frac{1}{66}C_{16}H_{24}O_5N_4 + \frac{27}{66}H_2O = \frac{8}{33}CO_2 + \frac{2}{33}NH_4^+ + \frac{31}{33}H^+ + e^-$	$C_{16}H_{24}O_5N_4 + 13H_2O + 0.379HCO_3^- = 7.18CO_2 + 3.6NH_4^+ + 4H^+ + 7.3CH_4 + 0.379C_5H_7O_2N$ (cells)	50.3 / 49.7	0.463	0.456
Grease $C_8H_{16}O$	R_c $\frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O = \frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^-$ R_a $\frac{1}{8}CH_4 + \frac{1}{4}H_2O = \frac{1}{8}CO_2 + H^+ + e^-$ R_c $\frac{1}{46}C_8H_{16}O + \frac{15}{46}H_2O = \frac{4}{23}CO_2 + H^+ + e^-$	$C_8H_{16}O + 3.09H_2O + 0.1035HCO_3^- + 0.1035HN_4^+$ (nutrient salts) $= 2.09CO_2 + 5.49CH_4 + 0.1035C_5H_7O_2N$ (cells)	72 / 27	0.960	0.365

Table 2.11 Stoichiometric Equations and Predicted Yields
(Derived from Stoichiometry of Biological Reactions - Appendix I)

sludge. The stoichiometric equations have been constructed using the relationships given by McCarty (1971). Appendix I describes the method used to develop such equations. Also shown in Table 2.11 is the percentage methane/carbon dioxide ratio (% v/v) produced and the quantity of gas produced per unit weight of the component digested. In comparison to the gas yields obtained using Buswell and Mueller's equation (1952), the yields given in Table 2.11 are slightly lower since Buswell and Mueller's equation assumes that the feedstock is transformed to methane and carbon dioxide with no cell production. As an example, for the digestion of the carbohydrate component, (CH_2O), Buswell's equation predicts a % CH_4/CO_2 ratio of 50/50 with 373 cm^3 methane produced per gm. digested and 373 cm^3 carbon dioxide produced per gm. digested, as opposed to a % CH_4/CO_2 ratio of 45/55, 248 cm^3 CH_4/gm. digested and 298 cm^3 CO_2/gm. digested predicted from the relationships given by McCarty (1971). The equations shown in Table 2.11 provide an estimate of the cell yield and nutrient salts required for the anaerobic digestion of the components. The ammonium salt provides a source of nitrogen and also acts as a buffer as does the bicarbonate salt.

Symons and Buswell (1933) have shown that if water is the only source of oxygen besides that in the

substrate undergoing digestion, the greatest decrease in free energy occurs when carbon dioxide and methane are the end products. Boruff and Buswell (1932) calculated from heats of combustion that 6.7% of the total energy available from the complete oxidation of cellulose is lost to the bacteria by its fermentation to carbon dioxide and water; their calculations show the standard heat of reaction per mole of cellulose digested is about -70,000 Btu/mole (-39,000 cals/mole) or -23,000 Btu/mole of methane formed (-13,000 cals/mole of methane formed).

The major constituents of the digester gas produced are methane and carbon dioxide. For a given digester the percentage of methane produced will be greatest when the system is engaged in active decomposition at steady state levels. As the system becomes more unbalanced the percentage of methane decreases.

Pohland and Bloodgood (1963) reported that less than 7 ft^3/day per pound of volatile solids added (430 cm^3/day - gm V.S. added) was considered to be retarded digestion while greater than 8 ft^3/day per pound of V.S. added (500 cm^3/day - gm V.S. added) was considered normal for sludge digestion. Thompson (1962) indicated 9 ft^3/day per pound of V.S. added (562 cm^3/day - gm V.S. added) for

hog food substrate while Morgan (1954) found only 8.3 ft^3/day per pound V.S. added (518 cm^3/day - gm V.S. added) for sewage sludge digestion in a high rate digester. Sawyer and Roy (1955) reported a value of 16.5-17.1 ft^3/day - lb. V.S. digested also for sludge digestion (1030-1067 cm^3/day - gm V.S. digested). These ranges of values are to be expected since the quantity of gas production is dependent upon the substrate composition. The volume of gas produced from most livestock wastes is somewhat less than for municipal sewage sludge due to the composition differences. Taiganides et al (1963) found a total gas production of 7.8-10.3 ft^3/day - lb. V.S. added (487-643 cm^3/day - gm. V.S. added) with 59% methane from hog waste.

Table 2.12 presents a list of some typical gas yields obtained from batch digestion for cellulosic wastes. Table 2.13 presents a list of some typical gas yields obtained from continuous digestion of various feedstocks.

The ease with which filter paper undergoes digestion relative to cellulosic materials such as seed flax straw, is clearly seen in the results of Nelson et al (1939) from batch digestion results.

For pure feedstocks such as filter paper (almost pure cellulose) the gas production rates and composition

Component	Experimental Conditions	Time of Digestion	Gas Production	% component digested	% CH_4/CO_2 (v/v)	Reference
Cow Manure	100 gm. manure plus sewage sludge, non-stirred, non-acclimated, 95-100°F.	40 days	69.5 cm^3/gm. added	-	68/32	Johnson et al. (1972)
Grass	100 gm. plus sewage sludge, non-stirred, non-acclimated, 95-100°F.	40 days	74.83 cm^3/gm. added	-	76/24	Johnson et al. (1972)
Processed Domestic Garbage - no glass or metal materials	25 gm. plus sewage sludge, non-stirred, non-acclimated, 95-100°F.	14 days	92 cm^3/gm. added	-	78/22	Johnson et al. (1972)
		(a) (b)	(a) (b)		(a) (b)	Nelson et al. (1939)
Filter Paper	Sewage sludge used as a seed.	27 21	674 688	-	49.4/38.5 55.7/40.3	
Cook Liquor	Non-stirred, non-acclimated.	27 21	484 529	-	59.3/32.2 56.8/34.5	
Cornstalk Flour	Ammonium Phosphate added as a source of nitrogen, buffered with lime.	30 15	518 456	-	53.7/38.5 53.5/32.4	
Artichoke-Top Flour		32 14	573 539	-	49.1/38.2 52.6/28.1	
Chopped Corn-stalks	Temperature: 28-30°C (a) and 50-55°C (b).	30 15	360 396	-	53.9/32.4 55.9/35.5	
Chopped Wheat-straw		28 18	323 354	-	55.4/36.0 54.1/29.9	
Ground Wheat-straw		28 18	368 355	-	55.3/36.5 57.3/28.1	
Seed Flax Straw		34 20	317 231	-	58.8/37.4* 55.7/27.1*	

* Small amounts of hydrogen detected.

Table 2.12 Batch Digestion Yields

Component	Experimental Conditions	Gas Production	% CH_4/CO_2 (v/v)	Other Components	Reference
Green Garbage*	76.5% Green Garbage with sludge, non-acclimated, stirred, 30-day detention time, loading rate = 0.077 lb/ft^3 day. Temperature = 37°C	11 ft^3/lb. v.s. dest.-day. 2.4 l.day^{-1}	58/42	74.3% v.s. dest.	SERL Report no. 67-7 (1967)
Green Garbage	100% Garbage. Acclimated over a period of one year, stirred, 30-day detention time, loading rate = 0.154 lb/ft^3 day. Temperature = 37°C	14 ft^3/lb. v.s. dest.-day	60/40	65% v.s. dest.	SERL Report no. 69-1 (1969)
Paper Pulp**	50% Pulp, 50% Sludge, stirred. 4 months acclimation. 30-day detention time, loading rate = 0.077 lb/ft^3/day. Temperature = 37°C.	12.9 ft^3/lb. v.s. dest.-day	65/35	90.3% cellulose dest. 64.7% total solids dest.	SERL Report no. 69-1 (1969)
		ft^3/lb added / ft^3/lb. destroyed		Proportion of Component in Sludge Feed (%) / Cellulose Destroyed (%)	
Green Garbage	Stirred, 30-day detention time, loading rate = 0.077 lb/ft^3.day. Temperature = 37°C.	8.8 / 13.9	54.7/45.3	100 / -	Klein (1972)
Kraft Paper		9.2 / 12.1	66.5/33.5	60 / 92.4	
Newspaper		7.5 / 13.0	69.5/30.5	30 / 41.7	
Garden Debris		7.8 / 11.9	69.5/30.5	50 / 78.5	
Wood		4.3 / 7.7	69.7/30.3	60 / -	
Chicken Manure		5.0 / 17.1	59.8/40.2	100 / -	
Steer Manure		1.4 / 8.7	65.2/34.8	100 / -	
Sewage Sludge		9.7 / 15.2	64.5/35.5	0 / -	

* At a higher percentage input, the volatile acid concentration exceeded 6000 mg.lit^{-1}.

** At 32% sludge, the digestion process failed, C:N ratio was greater than 45:1.

Conversion factor: ft^3/lb. to lit./gm. - multiply by 0.062427
gm/lit. to lb./ft^3. - multiply by 0.062427

Table 2.13 Continuous Digestion Yields

compare favourably with the yields predicted by the equation of Buswell and Mueller (1952). To compare and evaluate the results of many investigations proves a difficult task because of the varying conditions under which the results have been obtained; in some studies intermittently-fed systems were used, some with mixing and others without mixing, other studies have used pH control and others have not, acclimation periods have rarely been mentioned even if they have been followed, and many experiments have been carried out under non-steady state conditions as in batch digesters.

2.4 KINETIC ASPECTS OF THE DIGESTION PROCESS

2.4.1 Decomposition Rates

A summary of the pertinent available information concerning the degree of digestion of carbohydrates, proteins and lipids is given below.

Early studies by Heukelekian (1924) indicated that at 28°C about 73 per cent of the cellulose in sewage sludge disappeared in a limed, batch-type digester after one week and nearly 96 per cent was degraded in three weeks reaction time. Typical decomposition rates of cellulose range from 48-11,400 mg./lit.day as obtained by several investigators. Table 2.14 presents a summary of the rates found in the literature. The differences in the rates are due to the form of cellulose being degraded and the different conditions of digestion.

The degradation of lipids or "grease" in the anaerobic digestion of sewage solids has been demonstrated by a number of investigators. In studies on the digestion of seeded sewage solids, Buswell et al (1930) reported that 90 per cent of the grease content was destroyed in two-stage digestion. Neave and Buswell (1928) stated that sewage grease and soaps were decomposed at temperatures ranging

Authority	System and Culture	Initial Concentration of Cellulose *	Cellulose Material	pH	Digesting Rate*	Remarks
Maki (1954)	Batch, mixed two pure cultures isolated from sewage digester, 38°C, mesophilic.	2,000 mg./l.	Whatman's No. 1 filter paper	6.8	(1) 260 mg./l.-day (2) 660 mg./l.-day	Two experiments were made with different strains.
Heukelekian (1958)	Batch, mixed culture from sewage digester, 25°C., mesophilic.	3,120 mg./l.	Cellulose in sewage sludge	7.4	142 mg./l.-day	pH was maintained with lime.
McBee (1948)	Batch, pure culture, isolated from soil and manure, 55°C.,thermophilic.	(1) 744 mg./l. (2) 2,980 mg./l.	Absorbent cotton	- -	(1) 149 mg./l.-day (2) 426 mg./l.-day	Two experiments were made
Springer (1972)	Batch, mixed culture from fibrous river sludge, 25°C	Approximately 20,000 mg./l.	Cellulose in fibrous river sludge	5.9 to 6.5	48 to 216 mg./l.-day	Range of rates of samples from monthly monitoring.
Stranks (1956)	Batch, mixed culture from rumen fluid, 40°C.	41,200 mg./l.	Whatman no. 2 filter paper		11,400 mg./l.-day	pH maintained with $NaHCO_3$.
Chan (1970)	Continuous, mixed culture from sewage sludge, 35°C	13,744 mg./l.	Particulate milled Kraft - pulp	7 to 7.3	412-1,250 mg./l.-day	Enrichment culture used. Detention time 6-30 days. Type IV model used.**

* per litre digester capacity.

** Table 2.15.

Table 2.14 Summary of Decomposition Rates in the Anaerobic Fermentation of Cellulose

from 22°C to 37°C. The pH in these studies was maintained between 7.2 and 7.6. Heukelekian and Mueller (1958) reported 75 per cent and 89 per cent grease reduction at loadings of 0.41 and 0.88 pounds of volatile solids per cubic foot per day, respectively. The higher grease reduction was accomplished in five days while the lesser percentage reduction, at the lower loading rate, required ten days detention time. These studies were conducted at a temperature range between 32°C and 38°C.

From their observations on two-stage sludge digestion, Buswell et al (1930) concluded that about 64 per cent of the proteinaceous material was destroyed. Sawyer and Roy (1955) reported from high rate digestion studies conducted between 35°C and 40°C that the nitrogen determinations on the digested sludge indicated that protein degradation in a six-day detention digester was 86 per cent of that attained in a twenty-day detention digester.

Table 2.16 presents a compilation of the kinetic parameters found in the literature describing the decomposition rates for a range of substrates - protein, long chain saturated and unsaturated fatty acids, short chain volatile acids, cellulose, domestic waste, elephant grass

Model	Rate of Digestion	Comments
Type I first order with respect to substrate concentration S_1	$R = KS_1$ rate of digestion per unit digester volume	S_1 - substrate concentration in digester, units of $K = time^{-1}$
Type II first order with respect to substrate concentration S_1	$q = k''S_1$ rate of digestion per unit digester volume per unit cell concentration	units of k" lit./mg.day
Type III first order, with substrate concentration defined in terms of gas produced	$R = K_g G_1$ rate of digestion per unit digester volume	G_1 = gas produced/unit weight of substrate added units of $K_g = time^{-1}$
Type IV Monod equation continuous function relating q and S_1	$q = \frac{k'S_1}{K_S+S_1}$ rate of digestion per unit digester volume per unit cell concentration	k' and K_S are constants in Equation units of $k' = time^{-1}$ units of K_S = mass per volume cell growth rate = Y x q where Y = yield constant

TABLE 2.15 Summary of Models used for describing Decomposition Rates in Anaerobic Digestion.

Component	Model Used	Rate Constant	Temperature Effect	Remarks
Protein Ryabov et al (1974)	$R = KS_1$ Type I	$K = 0.0023\ day^{-1}$ at $T = 10^oC$ $K = 0.004\ day^{-1}$ at $T = 20^oC$ $K = 0.0077\ day^{-1}$ at $T = 30^oC$	$E^* = 7.2$ kcal/mole for temperature range 10-20°C $E = 11.8$ kcal/mole for temperature range 20-30°C	Batch digestion, soluble food, input concentration S_o = 200 mg/lit.
Long Chain Saturated Fatty Acids Novak and Carlson (1970) stearic acid $C_{18}H_{36}O_2$ palmitic acid $C_{16}H_{32}O_2$ myristic acid $C_{14}H_{28}O_2$	$q = \frac{k'S_1}{K_s+S_1}$ Type IV	stearic - $k' = 0.77\ day^{-1}$ $K_s = 417\ mg_1/lit.$ palmitic - $k' = 1\ day^{-1}$ $K_s = 143$ mg./lit. myristic - $k' = 0.95\ day^{-1}$ $K_s = 105$ mg./lit.		Continuous digestion, soluble feed, input concentration = 1375 mg./lit. Temperature = 37°C Yield constant = 0.1 mg./mg. C.O.D.
Long Chain Unsaturated Fatty Acids Novak and Carlson (1970) Oleic $C_{18}H_{34}O_2$ Linoleic $C_{17}H_{31}O_2$	$q = \frac{k'S_1}{K_s+S_1}$ Type IV	oleic - $k' = 4.0\ day^{-1}$ $K_s = 3,180$ mg./lit. linoleic - $k' = 5.0\ day^{-1}$ $K_s = 1,816$ mg./lit.		Same conditions as for long chain saturated fatty acids.
Acetic Acid CH_3COOH Lawrence and McCarty (1969)	$q = \frac{k'S_1}{K_s+S_1}$ Type IV	$k' = 4.7\ day^{-1}$, $K_s = 86.9$ mg.COD/lit) $T = 25^oC$ $k' = 4.8\ day^{-1}$, $K_s = 333$ mg.COD/lit.) $T = 30^oC$ $k' = 8.7\ day^{-1}$, $K_s = 154$ mg.COD/lit.) $T = 35^oC$	A plot of log k_s versus 1/T produced a slope of 6980 which corresponds to a value of E = 31 kcal/mole.	Continuous digestion, soluble feed, input concentration = 1568 mg./lit. Yield constant 0.04 mg/mg. C.O.D.
Cellulose $C_6H_{10}O_5$ Chan (1970)	$q = \frac{k'S_1}{K_s+S_1}$ Type IV	$k' = 5.4\ day^{-1}$ $K_s = 7530$ mg.COD/lit.		Continuous digestion, insoluble feed, input cellulose concentration = 13744 mg/lit. Yield constant = 0.104 mg/mg cellulose.
Domestic Waste Pfeffer (1974)	$R = K_gC_1$ Type III	K_g initial day^{-1} / K_g final day^{-1} / T °C: 0.055 / 0.003 / 35 0.084 / 0.043 / 40 0.052 / 0.007 / 45 0.117 / 0.030 / 50 0.623 / 0.042 / 55 0.990 / 0.040 / 60		Continuous digestion, insoluble feed, input feed concentration = 3.33 g./lit.-day + 3% sludge solids.
Elephant Grass Boshoff (1966)	$R = K_gC_1$ Type III with R and s defined in terms of gas production in batch system.	$K_g = 0.052\ day^{-1}$ at 22°C. $K_g = 0.06\ day^{-1}$ at 32°C.		Batch digestion, non-stirred, no acclimation.
Cow Manure Gaddy et al (1974)	$R = K$ Type I	$K = 0.125\ day^{-1}$ at 35°C.		Continuous digestion, liquid cow manure, BOD used to define substrate concentration. Input concentration ranged from 3800 to 15,300 mg.BOD/lit.
Cellulose Springer (1972)	$R = k$ (zero order)	$k = 0.12$ at 10°C $k = 0.23$ at 15°C $k = 0.311$ at 20°C k = mg Gas Pressure/hour/50 cm^3 of sludge fed.	$E = 16.5$ kcal./mole	Batch digestion, input concentration = 20,000 mg./lit., stirred.

* Activation Energy obtained from Arrhenius Equation.

Table 2.16 Compilation of Kinetic Rate data Describing Decomposition Rates for Various Feedstocks.

and liquid cow manure. A tabulated description of the models used by the investigators noted in Table 2.16 is given in Table 2.15.

2.4.2 Reaction Order for the digestion process

In order to define a reaction order for a chemical or biochemical transformation of reactants to products requires a knowledge of the reaction mechanism and of the governing rate equation. Now, since the rate limiting step for the overall conversion of the feedstock to methane is in question (refer to Section 2.4.3), then it is to be expected that the reaction order for the digestion process is also in question and subject to similar influences which have given rise to conflicting reports regarding the definition of the rate limiting step, namely, the experimental conditions, feedstock concentration and composition and the interpretation given to experimental results.

The analyses found in the literature show that the order can be interpreted as being zero, or more commonly first order, with respect to substrate concentration, with the rate of reaction being defined in terms of unit digester volume or unit digester volume per unit cell mass as shown by the examples given below, for a range of substrates.

Sawyer (1955) conducted laboratory scale high rate digestion experiments to study the relationship between the concentration of solids in sewage sludge fed to digestion units and detention time. At detention times of 11 and 14 days the results showed that the volatile solids destruction was essentially independent of solids loading and a direct function of detention time. Rankin (1948) was the first to recognise this fact. The results of Sawyer fit a first order rate expression of Type I shown in Table 2.15, The results processed by Gaddy et al (1974) also follow a first order rate expression of Type I for the continuous digestion of liquid cow manure, using COD as a measure of substrate concentration, as do the results of Hindin and Dunstan (1959) and Pfeffer et al (1967) for the continuous digestion of sewage sludge.

W.H. Boshoff (1967) calculated first order reaction velocity constants for gas production from the batch digestion of elephant grass. He assumed at any particular time the rate of gas production is a function of the gas yet to be produced (i.e. of the substrate remaining).

As shown in Table 2.16, various investigators have made use of the Monod expression (refer to Section 3.1) to describe the kinetics of the anaerobic digestion of soluble substrates. The model is a continuous function which approaches a first order reaction at low substrate concen-

trations and approaches zero order at high substrate concentrations. Chan (1970) has also applied the model to describe the decomposition rate of cellulose in a continuous stirred tank digester under anaerobic conditions, although, for a detention time range of 10-30 days the function closely follows first order kinetics (cellulose determination was used by Chan to define the limiting substrate concentration).

From work on the enzymatic hydrolysis of cellulose to produce glucose first order rate constants have been obtained (Ghose and Das, 1971) for the initial enzymatic saccharification of cellulose and the Michaelis Menten equation has also been used to describe variations in the initial reaction velocity with cellulose concentration (Ghose and Das, 1971) - in this case inhibition terms to account for collobiose and glucose production are included in the Michaelis Menten equation. The above authors conclude that it is essential to know more about the biological meaning of cellulose degradation in consecutive or simultaneous steps before a better evaluation of the kinetics can be possible. The use of Monod or first order kinetic equations must be understood in relation to the digestion system under consideration.

2.4.3 Rate limiting step

Since the overall conversion of insoluble feedstocks to methane in the digestion process involves two stages which can be subdivided into at least three biochemical steps, then there exists the possibility that one of the steps is slower than either of the remaining steps. Environmental factors such as pH, temperature, or stirring can limit the conversion and mask the true biochemical limiting step as discussed in Section 2.2; however, for normal high rate digestion such factors should not limit the stepwise degradation. Under these conditions the slowest of the biochemical steps will limit the overall process.

For the digestion of soluble feedstocks such as glucose or tryptone in which the conversion to methane does not involve the initial hydrolysis step of the digestion process, it has been shown by Pohland and Ghosh (1971) and Andrews et al (1964) that the slowest of the biochemical steps is the fermentation of the volatile acids to methane, i.e. the methanogenic stage. From the work of Pohland and Ghosh it was shown that at a detention time of 23.6 hours the percentage glucose removal was 99.7% for an influent glucose concentration of 931 mg./lit. but the

volatile acid concentration was greater than 300 mgl^{-1} acetic acid indicating volatile acid accumulation.

With regard to the digestion of insoluble feedstocks, such as cellulose, the digestion process incorporates all three steps previously described. Chan (1970) concluded that the rate limiting step for the overall removal of cellulose to methane in continuous anaerobic digestion is the initial hydrolysis step. Chan found from his experimental results that a change in the detention time from 30 days to 6 days produced no significant increase in the production of the intermediate volatile acid products and yet the cellulose concentration in the digester increased with decrease in detention time. Springer (1972) supported Chan's conclusions for the batch digestion of cellulose.

Pfeffer (1968) suggested the possibility that the rate limiting step could be controlled by the methanogenic stage at low detention times ($<$ 10 days) and by the hydrolysis of organic solids at longer detention times ($>$ 10 days) in the digestion of primary activated sludge. Such a waste would contain the more easily digestible soluble feedstocks and a proportion of insoluble feedstocks, such as cellulose. Therefore the possibility of the rate

limiting step being a function of detention time appears to be reasonable in view of the results obtained for soluble feeds (Pohland and Ghosh, 1971) and the results for insoluble feeds (Chan, 1970), in that at low detention times the volatile acid concentration would build up because of the relatively fast digestion of the easily digestible substrates and at longer detention times the slower digesting substrates would still be present, such as cellulose.

2.5 INTERMEDIATE TECHNOLOGICAL ASPECTS

In India today one in every five villages is without any electrical power and the only sources of energy available to most of the villages are the non-commercial sources such as animal wastes and firewood.

M.A. Sathianathan (1975) presents four reasons why the possibility of electrifying the villages seems beyond attainment in the reasonable future:

1. Inadequacy of power generation;

2. Scarcity of materials for transformers, wires and other materials needed for transmission;

3. Inadequacy of transmission lines, and

4. Lack of sufficient return of the investment for rural electrification schemes to make them self-sustaining.

He suggests that methane generation from cow dung produces a valuable fuel that can be used for cooking, lighting, refrigeration, operating motors and pumps. The method also provides a sanitary treatment of organic wastes such as night soil, where the long detention times destroy

most pathogenic organisms and parasites and the sludge produced can be used as a fertiliser. This last point is of particular importance to less-developed countries - India is barely able to meet half of its fertiliser needs. Because of the energy crisis fertiliser costs have soared (in 1971 Urea = $46/ton, in 1975 Urea = $300/ton on the world market), and therefore fertiliser production from digestion units is as important as the methane produced.

The costs and benefits of methane production in less developed countries have been considered by D.L. Pyle (1974). The benefits of the process accrue if the waste material is producing a sufficient quantity of gas with a net heating value greater than that of the raw material. Table 2.17 lists the more important costs and benefits in relation to a less developed country.

From a consideration of the gas production from cow dung and the present needs of a typical village in India, Prasad et al (1974) conclude that bio gas plants can make a substantial contribution to the energy needs of Indian villages. However, they stress the necessity of examining the characteristics of each particular village, the amount of dung which can be actually collected, the availability of water, the distribution of houses and land,

COSTS	Comments
1. Capital	No foreign component. Costs lowered by use of the indigenous material. Effects of scale may not be so high as in U.K. Low per unit output: may have positive distributional effect.
2. Inputs:	
Dung, vegetable wastes	There needs to be a clear advantage in "fuel out" over "fuel in". Local resources: but real cost?
Water	Often expensive - can it be recycled?
Heat	Another cost to system.
Labour	One man's cost is another's benefit: employment generated will depend on scale.
BENEFITS	
1. Material:	
Gas	Calorific value about 650 B.T.U./ft^3.
Slurry	Positive fertiliser value (1-2% N dry basis).
2. Indirect	Simple technology; "soft" operating conditions. Local manufacture is possible. Convenience. Village hygiene/health improved on larger scale.

TABLE 2.17 Costs and Benefits of Methane Generation in Less Developed Countries.

(D.L. Pyle, 1974)

and then considering to what extent the bio gas plants can supply the energy needs of the particular village.

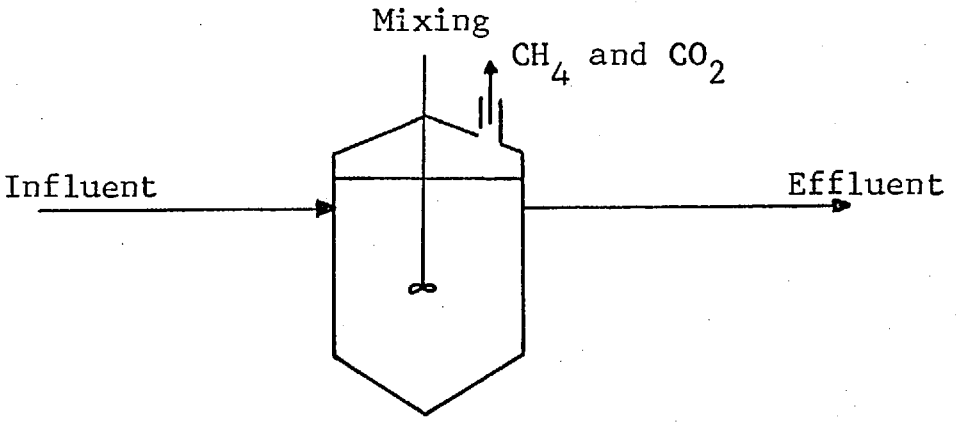
The process is being used in many less developed countries - besides the large scale adoption of the process in India, there are 30,000 digesters in operation in South Korea, and reports of the process being used in various countries, e.g. the Fiji Islands. The feedstocks in such countries range from animal wastes, such as pig waste and cow dung, to vegetable matter and night soil.

Despite the use of the digestion process in less developed countries, the progress of the system is being hampered by the problems so far encountered - lack of interest amongst the potential users of the system, the availability of capital to invest in the necessary plant equipment, the location of the digesters and distribution of the gas produced, heating and stirring problems - stirred digesters are not usually used in less developed countries and the only significant mixing occurs during the loading of the digesters.

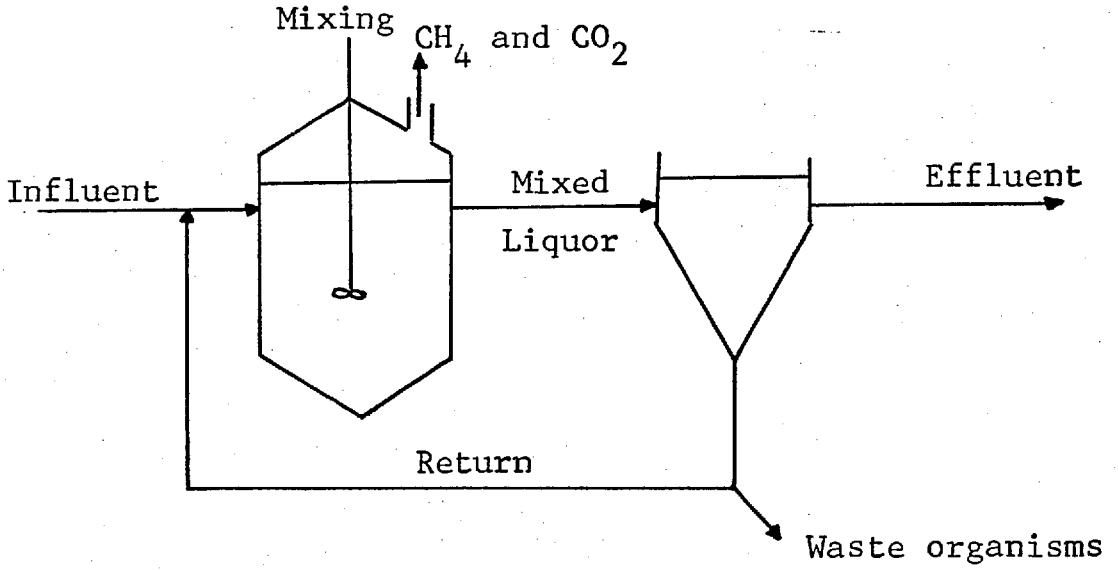
2.6 PROCESS APPLICATIONS

There are three different processes currently used in the anaerobic treatment of wastes in industrialised countries. The first is a completely mixed, semi-continuous flow system commonly called the conventional process. This process is a modification of the basic anaerobic process which has been used in the treatment of sewage sludges for many years. The second is a continuous flow process employing solids separation prior to effluent discharge and the subsequent return of all or a portion of these solids to the digester. This system is called the anaerobic contact process.

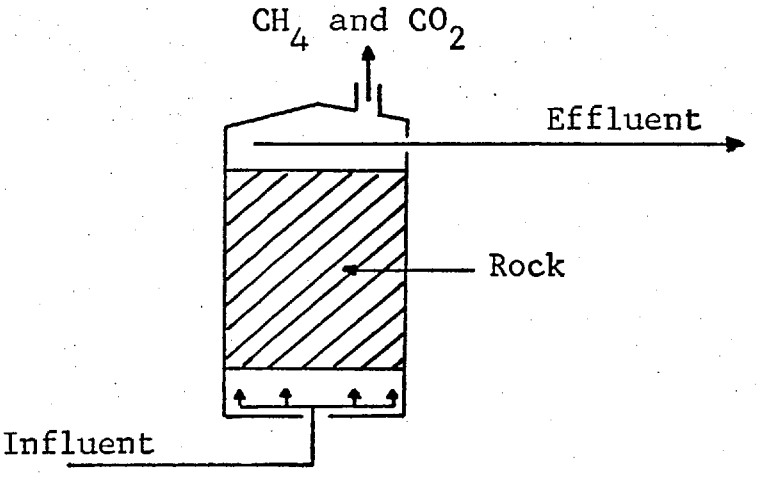
While the anaerobic contact process has been used at ambient temperatures in the treatment of cooler wastes by anaerobic cultures, the third process - the anaerobic filter - offers promise as a means of removing the soluble C.O.D. from waste streams. The anaerobic filter is an upflow rock filter wherein wastes are introduced at the base of a rock support system for an anaerobic culture. The anaerobic filter process is particularly suitable for the treatment of soluble or colloidal wastes such as potato wastes. McCarty (1974) and Hobson et al (1974) discuss the different digester types in more detail. Schematic diagrams of the three basic anaerobic processes are shown in Figure 2.8.



Conventional Process



Anaerobic Activated Sludge Process



Anaerobic Filter Process

FIGURE 2.8 Schematic Diagrams of Three Basic Anaerobic Processes.

In India the "Gobar Gas Plant" has been widely adopted as a standard design to meet the requirements of the village digester. The gas plant consists of two parts:

1. Digester. The digester body is built below ground and is formed in concrete. The depth of the well is about 3.5 metres and the diameter varies between 1.35 metres to 6 metres depending upon the quantity of materials to be fed in. Figure 2.9 illustrates the set up of a Gobar Gas Plant. The digester has a partition wall in the middle dividing it into two semi-circular compartments. Cement pipes serve as the inlet and outlet to the digester.

2. Gas Holder. This is a drum constructed of mild steel sheets. The drum collects the gas which bubbles out from the cattle dung slurry put into the digester. The drum rises as the gas is collected in the gas holder drum. The gas so accumulated flows through the pipe provided at the top, whenever the top is opened.

Singh (1972) and the Khadi and Village Industries Commission Report (1975) discuss the various plant designs in detail.

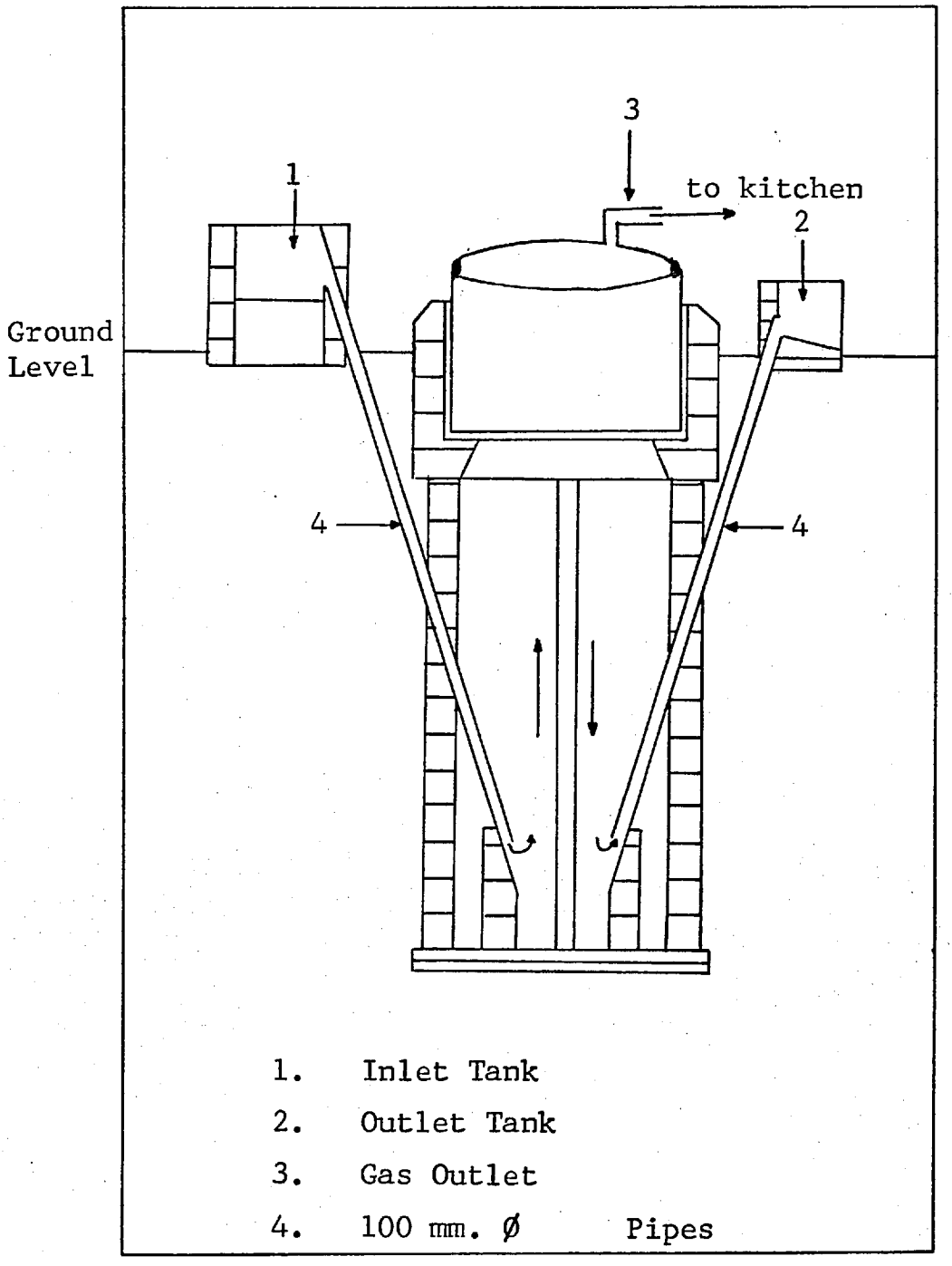


FIGURE 2.9 Gobar Gas Plant.

CHAPTER 3

THEORETICAL CONSIDERATIONS OF THE DIGESTION PROCESS

3.1 KINETICS OF BIOLOGICAL REACTIONS

3.1.1 History of Biological Reaction Kinetics

The kinetics of bacterial growth have given rise to a considerable volume of literature, particularly with reference to continuous culture, in which field Monod's (1950) formulation of growth has been extensively applied. This formulation shows that the cell growth rate is dependent on substrate concentration according to the expression:

$$\mu = \frac{\mu_{max.} S}{K_S + S} \qquad \text{Equation 3.1}$$

where: μ = specific growth rate [the rate of change of organism concentration $\frac{dX}{dt}$, per unit of organism concentration X, i.e. $\mu = (\frac{1}{X})(\frac{dX}{dt})$].

μ_{max} = the maximum specific growth rate.

S = limiting substrate concentration.

K_S = saturation constant - being that concentration of limiting substrate at which the specific growth rate is half the maximum specific growth rate.

The Monod equation applies for single cellular organisms undergoing binary fission where

the growth can be expressed in terms of a growth rate constant and the cell concentration:

$$\frac{dX}{dt} = \mu X \qquad \text{Equation 3.2}$$

This is termed exponential growth. The Monod equation predicts no lag phase nor can it predict a phase of decline; basically the model predicts a phase of exponential growth followed by a stationary phase when the substrate is exhausted. Equations 3.1 and 3.2 were originally formulated to apply to the growth of a pure culture on a limiting substrate concentration, but have been applied to describe the growth of mixed (heterogeneous) cultures (Gaudy and Gaudy, 1972).

Monod also showed that there is a simple relationship between cell growth and substrate utilisation. This is shown in its simplest form in growth media containing a single organic substrate (e.g. glucose, ammonia and salts); under these conditions the growth rate is a constant fraction Y, of the substrate utilisation rate ($\frac{ds}{dt}$):

$$\frac{dX}{dt} = -Y\frac{dS}{dt} \qquad \text{Equation 3.3}$$

where Y is the yield constant.

The equation presented by Monod is an empirical expression and was chosen because he considered that the character of cell growth on a single limiting substrate

suggested that it might be fitted by an equation of a form similar to that for simple enzyme kinetics with the cell growth rate μ following a Briggs-Haldane (Michaelis Menten) form.

Herbert et al (1956) carried out a theoretical and experimental study of the continuous culture of Aerobacter Cloacae using glycerol as the sole carbon source and concluded:

> "It might well be objected that even under ideal conditions the growth behaviour of bacteria cannot be completely represented by such simple equations..." (such as those given above) "... qualitatively it [Monod equation] provides a general picture of the behaviour of a continuous or batch culture, and quantitative agreement is good over most of the range".

For continuous culture Herbert et al (1956) noted the following quantitative deviations:

1. The net rate of increase of concentration of organisms in continuous culture is given by:

$$\text{increase} = \text{growth} - \text{output}$$

$$\frac{dX_1}{dt} = \mu X_1 - DX_1 \qquad \text{Equation 3.4}$$

where: D = dilution rate (the number of complete volume-changes/time).

X_1 = cell concentration in reactor.

When a steady state exists $\frac{dX_1}{dt} = 0$, and Equation 3.4 reduces to $\mu = D$. Therefore according to this equation when the dilution rate exceeds the maximum possible growth rate, complete "wash-out" should occur and no steady state should be possible. However, Herbert et al found that steady state conditions did prevail with $D > \mu_{max}$, thus meaning that the wash-out rate is less than that predicted. It was suggested that the actual wash-out rate in the culture vessel is effectively less than that predicted because of imperfect mixing. Herbert et al also noted that wall cell growth can cause similar effects to those of imperfect mixing. Together the effects of imperfect mixing and wall cell growth were described as "apparatus effects".

2. At high flow rates ($D = 1.2\ hr^{-1}$) the yield constant was found to decrease. No explanation for this effect is given by Herbert et al, although they suggested at the time of the investigation that Equation 3.3 may need to be modified.

Several modifications of the above equations have been proposed (e.g. Powell, 1967) in an attempt to improve the accuracy of prediction for continuous culture. Herbert (1958) accounted for the fact that a constant yield does not fit experimental data by adding a maintenance term to

the yield equation (3.3) - this term accounts for substrate not consumed for growth. According to Herbert's model of maintenance, the organism uses up part of its own biomass in order to maintain its viability and ability to function.

James (1961) and Moser (1958) have reviewed some of the objections, on a theoretical basis, to the Monod equation, and Kirsch and Sykes (1971) have reviewed some of the modifications that have been applied to account for the deviations in continuous culture.

The Monod equation has also been applied to heterogeneous populations utilising a single limiting nutrient. As would be expected, deviations of experimental data from the model have been noted, for example:

(i) Gaudy and Gaudy (1972) found that the growth "constants" Y, μ_{max} and K_S which are used in pure culture studies, can be employed for heterogeneous populations, but that the constants may not be constant (the yield constant can vary as much as 50%; Gaudy and Ramanathan, 1971) and therefore a range of values should be used. The variations were explained by shifts in the predominant population when operating at different steady state conditions.

(ii) Grady et al (1972) concluded from work on pure

and heterogeneous cultures, with glucose as the sole carbon source, the following:

(a) For pure cultures the hypothesis that the concentration of growth limiting substrate in a chemostat is controlled by the growth rate alone and is independent of the concentration of substrate entering the reactor (as predicted by the use of the Monod equation for continuous culture studies) depends on the measurement employed for growth-limiting substrate. When the concentration of glucose was measured directly, the hypothesis was found to be true within the limits of the study. However, if the chemical oxygen demand (COD) test was used as the measure of growth limiting substrate the hypothesis was found to be false.

(b) When heterogeneous populations were employed the hypothesis was false regardless of the technique used to measure the concentration of growth limiting substrate.

(iii) The growth rate has been found to be controlled by a wide variety of substances acting as essential nutrients. These have been summarised by James (1961) as: (i) required amino acids such as tryptophane, arginine,

(ii) the energy source, lactose, glucose, maltose, etc., (iii) the nitrogen source, ammonia, (iv) phosphate, and (v) sulphate.

The use of multicomponent substrates with heterogeneous cultures has also given rise to deviations from the Monod equation. For example, Grady and Williams (1975) have noted that the influent substrate concentration (measured in terms of COD) had a significant effect upon the steady state concentration of the substrate in the reactor - an increase in the influent substrate concentration fed to a C.S.T.R. caused the steady state effluent substrate concentration to increase. The use of the Monod equation for continuous culture studies predicts that the effluent substrate concentration is independent of the influent substrate concentration.

Tischler and Eckenfelder (1969) and Jones (1973) found that the overall removal of mixtures of organic compounds by an activated sludge acclimated to the compounds is better described by first order kinetics than by the Monod equation.

The constants Y, μ_{max} and K_S are dependent on the composition of the substrate mixture, thus each component

of the mixture has its own unique value of K_S and each organism in the heterogeneous culture is defined by a particular Y and μ_{max}, hence steady state changes in continuous culture which cause population shifts will result in different values for the constants (Jones, 1973).

It should be emphasized that the above work relating cell growth to substrate utilisation using the Monod equation, whether applied to heterogeneous or pure cultures utilising single or multicomponent substrates, is only strictly true for soluble substrates.

3.1.2 Biological Kinetics as applied to Waste Treatment Processes

The successful treatment of wastes by biological means depends on the versatility of bacterial metabolism and the ability of the microorganisms to carry out their metabolic processes in an aqueous environment at normal temperatures and pressures, at or near neutral pH. Since bacterial species are predominantly responsible for degradation of polluting matter it is reasonable to suppose that any attempt to describe the kinetics of the process must involve consideration of the dynamics of cell growth and substrate utilisation. Over the past several years, a number of mathematical models have been applied to define

the kinetics of substrate utilisation for general biological treatment processes - anaerobic or aerobic digestion systems. The most commonly used of these are those of Eckenfelder (1961), McKinney (1962) and of Lawrence and McCarty (1970). The Eckenfelder and McKinney model is essentially derived from first order substrate removal kinetics, while the Lawrence and McCarty model is based on the Monod model.

3.1.2.1 The model of Lawrence and McCarty (1970):

Consider first the model of Lawrence and McCarty (1970).

Many of the commonly used biological waste treatment processes employ bacteria as the primary organisms. While the overall reactions carried out may be quite different, e.g. anaerobic versus aerobic conditions, the process of microbial growth and energy utilization are similar. For this reason Lawrence and McCarty (1970) found that it is possible to develop general relationships which are applicable to a wide variety of bacterial mediated treatment processes. They developed a "unifying parameter" termed biological solids retention time, θ_c, and obtained its relation with microbial growth, substrate assimilation, and process efficiency for both aerobic and anaerobic biological processes employing suspensions of microorganisms.

The Basic Equations are:

1. Microbial growth and substrate utilisation:

The relationship between biological growth and substrate utilisation is formulated in two basic equations. The first describes the relationship between net rate of growth of microorganisms and rate of substrate utilisation:

$$\frac{dX_1}{dt} = Yr - bX_1 \qquad \text{Equation 3.5}$$

where: $\frac{dX_1}{dt}$ = net growth rate of microorganisms per unit volume of reactor, mass per volume-time;

Y = growth yield coefficient, mass per mass;

r = rate of microbial substrate utilisation per unit volume, mass per volume-time;

b = microorganism decay coefficient, $time^{-1}$;

X_1 = microbial mass concentration, mass per volume.

This equation was developed empirically from waste treatment studies (Weston and Eckenfelder, 1955).

The second basic equation relates the rate of substrate utilisation both to concentration of microorganisms in the reactor and to the concentration of substrate surrounding the organisms. The equation, in a slightly different form than the Monod equation, is:

$$r = \frac{k'S_1X_1}{K_S + S_1} \qquad \text{Equation 3.6}$$

(c.f. Equation 3.1, where $\mu = Y(\frac{r}{X_1})$)

where: k' = maximum rate of substrate utilisation per unit weight of microorganisms (occurring at high waste concentration), time^{-1};

S_1 = concentration of substrate surrounding the microorganisms, mass per volume;

K_S = half velocity coefficient, equal to the substrate concentration when $r/X_1 = \frac{1}{2}(k')$, mass per volume;

$\frac{r}{X_1}$ = rate of microbial substrate utilisation per unit volume per unit cell mass, time^{-1}, termed as q.

This equation shows that the functional relationship between the substrate utilisation rate and substrate concentration is continuous over the total range of substrate concentration. This is shown in Figure 3.1. In the two extreme cases,

(i) when S_1 is very high ($S_1 \gg K_S$),

and (ii) when S_1 is very low ($S_1 \ll K_S$),

Equation 3.6 can be approximated by the following discontinuous functions:

$$q = k' \qquad \text{for } S_1 \gg K_S \qquad \text{Equation 3.7}$$

This is a zero order reaction with respect to substrate concentration: and

$$q = k'' S_1 \qquad \text{for } S_1 \ll K_S \qquad \text{Equation 3.8}$$

where: $k'' = \frac{k'}{K_S}$

This is a first order reaction with respect to substrate concentration. Lawrence and McCarty (1970) have discussed the use of the continuous or discontinuous functions of the model and conclude:

"While the merits of the two models have been debated, it should be emphasised that both are empirical and a choice between the two should properly be based more upon convenience and ability to furnish a satisfactory solution than upon any fundamental considerations".

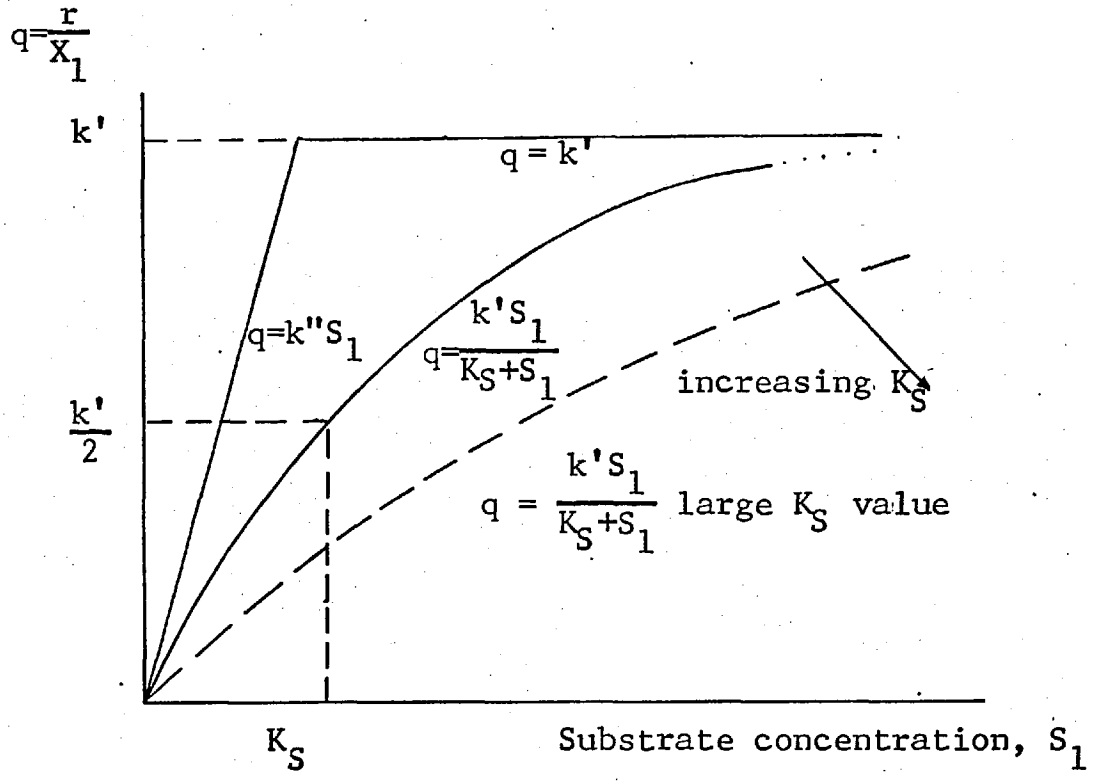


FIGURE 3.1 Schematic Diagram showing continuous and discontinuous functions of Equation 3.6.

When applying the model to continuous culture systems the following restrictions are necessary:

(i) Microbial growth is limited by the availability of the limiting substrate. All other growth requirements, i.e. inorganic nutrients and trace organic growth factors, are present in excess amounts.

(ii) The equations given (Equations 3.5 and 3.6) were developed for a single soluble substrate and pure culture system, although they have been successfully applied to systems using complex mixtures of soluble substances and mixed biological populations (Pearson, 1968).

(iii) The concentration of metabolic end products must be kept sufficiently dilute to prevent them from exerting a toxic or retarding effect on the metabolic activity.

The Model as applied to Continuous Culture

Equations describing the steady state conditions in continuous culture systems can be developed from the appropriate material balances.

Consider a completely mixed reactor with no cell recycle, as shown in Figure 3.2.

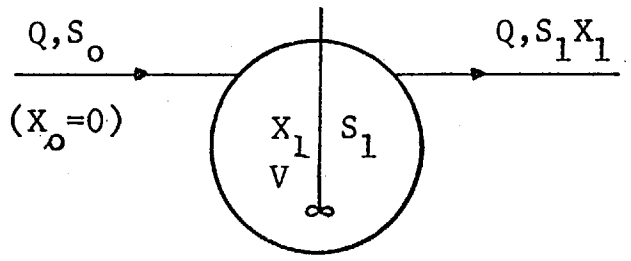


FIGURE 3.2 Single stage stirred fermentor.

Q = hydraulic flowrate, volume/time;

V = reactor volume;

θ = detention time = $\frac{V}{Q}$, time;

S_o = input substrate concentration, mass per volume;

S_1 = output substrate concentration, mass per volume;

X_1 = microbial mass concentration, mass per volume;

X_o = input microbial mass concentration assumed to be zero, mass per volume.

A material balance for the net rate of change of microbial mass in the system is written as:

$$V\left(\frac{dX_1}{dt}\right) = (Yr - bX_1)V - QX_1 \qquad \text{Equation 3.9}$$

(growth rate; washout rate)

$\left(\frac{dX_1}{dt}\right)$ = net rate of change of microbial mass in the reactor.

At steady state conditions, $\left(\frac{dX_1}{dt}\right) = 0$, then

$$\frac{1}{\theta} = \frac{Yr}{X_1} - b \qquad \text{Equation 3.10}$$

The biological solids detention time is defined as θ_c:

$$\theta_c = \frac{X_T}{\left(\frac{\Delta X}{\Delta t}\right)_T} \qquad \text{Equation 3.11}$$

X_T = total active microbial mass in treatment system, mass;

$(\frac{\Delta X}{\Delta t})_T$ = total quantity of active microbial mass withdrawn per unit time, mass per time.

Therefore for no recycle:

$$\theta_c = \frac{X_1 V}{X_1 Q} = \theta \qquad \text{Equation 3.12}$$

From equations 3.6, 3.10 and 3.12 the output substrate concentration S_1 is

$$S_1 = \frac{K_S[1 + b(\theta_c)]}{\theta_c(Yk'-b)-1} \qquad \text{Equation 3.13}$$

Similarly a substrate mass balance on the reactor gives:

$$V\frac{dS_1}{dt} = QS_o - Vr - QS_1 \qquad \text{Equation 3.14}$$

so that at steady state $\frac{dS_1}{dt} = 0$,

$$r = \frac{S_o - S_1}{\theta_c} \qquad \text{Equation 3.15}$$

and since from Equation 3.10

$$r = \frac{X_1}{Y}(\frac{1}{\theta_c} + b)$$

then substitution of this equation into Equation 3.15 gives:

$$X_1 = \frac{Y(S_o - S_1)}{1 + b\theta_c} \qquad \text{Equation 3.16}$$

As θ_c is reduced S_1 increases until it equals S_o and the process fails. Thus θ_c^m is the minimum biological solids detention time at which $S_1 = S_o$, θ_c^m can be found by substituting $S_o = S_1$ into Equation 3.13:

$$(\theta_c^m)^{-1} = \frac{Yk'S_o}{K_S + S_o} - b \qquad \text{Equation 3.17}$$

In the limiting case when $S_o \gg K_S$ then

$$(\theta_c^m)_{Lim.} = \frac{1}{Yk'-b} \qquad \text{Equation 3.18}$$

Similar equations can be developed for a completely mixed reactor with cell recycle. A schematic representation of the system is shown below:

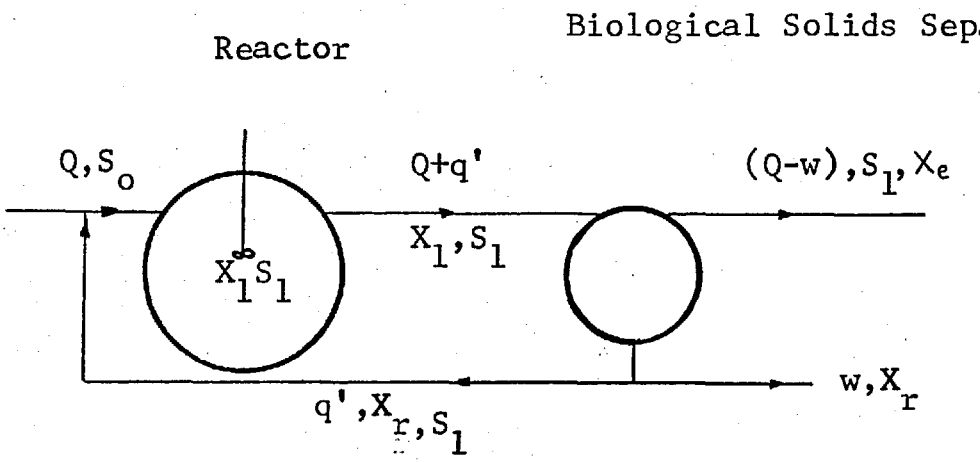


FIGURE 3.3 Single stage stirred fermentor with cell recycle.

in which: q' = flow rate at which solids separator underflow is recycled to the reactor, volume per time,

and w = flow rate of the fraction of the biological solids separator underflow which is wasted from the system, volume per time,

X_r = biological solids concentration in the underflow from the solids separator, mass per volume,

X_e = biological solids concentration in the clarified overflow from the solids separator, mass per volume.

The completely mixed reactor employing post reactor separation of the mixed liquor suspended solids and continuous recycle of a fraction of the fraction of the separated solids back to the reactor is shown in Figure 3.3. The completely mixed aerobic and anaerobic activated sludge processes belong in this category. The development of the model for a recycle system is based on the following assumptions:

1. All waste utilisation occurs in the biological reactor, and

2. The total biological mass in the system is equal to the biological mass in the reactor, i.e. the separator volume is small and recycle is continuous.

For this system θ_c, as defined by Equation 3.11, is

$$\theta_c = \frac{VX_1}{wX_r+(Q-w)X_e} \qquad \text{Equation 3.19}$$

A material balance for microbial mass around the entire treatment system yields the same relationship between θ_c and S_1 as given by Equation 3.13, for a system without recycle.

A material balance for microbial mass around the biological reactor alone gives:

$$\frac{1}{\theta_c} = \frac{Q}{V}\left[1 + r'_v - \frac{r'_v X_r}{X_1}\right] \qquad \text{Equation 3.20}$$

in which $r'_v = \frac{q'}{Q}$, the volumetric recycle ratio.

Biological solids detention time θ_c is related to the performance of continuous biological processes employing suspensions of microorganisms in a fundamental way, as Lawrence and McCarty (1970) have considered the modelling treatment. Relationships between θ_c and other process parameters were developed by Lawrence and McCarty (1970) for three commonly used biological process systems employing either aerobic or anaerobic conditions. The relationships between θ_c and the process parameters are shown in Table 3.1.

The steady state relationships between solids detention time, effluent waste concentration and specific treatment efficiency E $\left[E = 100 \times \left(\frac{S_o - S_1}{S_o}\right)\right]$ are shown in Figures 3.4 and 3.5.

Since the efficiency of waste removal is greatly decreased at values near $\theta_c^m (S.R.T_m)$, waste treatment systems should be operated at SRT considerably longer than the SRT_m. K_S is of considerable importance at these longer SRT values. For a given value of k', the SRT necessary to maintain a constant effluent substrate concentration and waste removal efficiency is directly proportional to the magnitude of K_S. Thus the lower the value of K_S, the shorter the SRT may be made without sacrificing a given level of effluent quality.

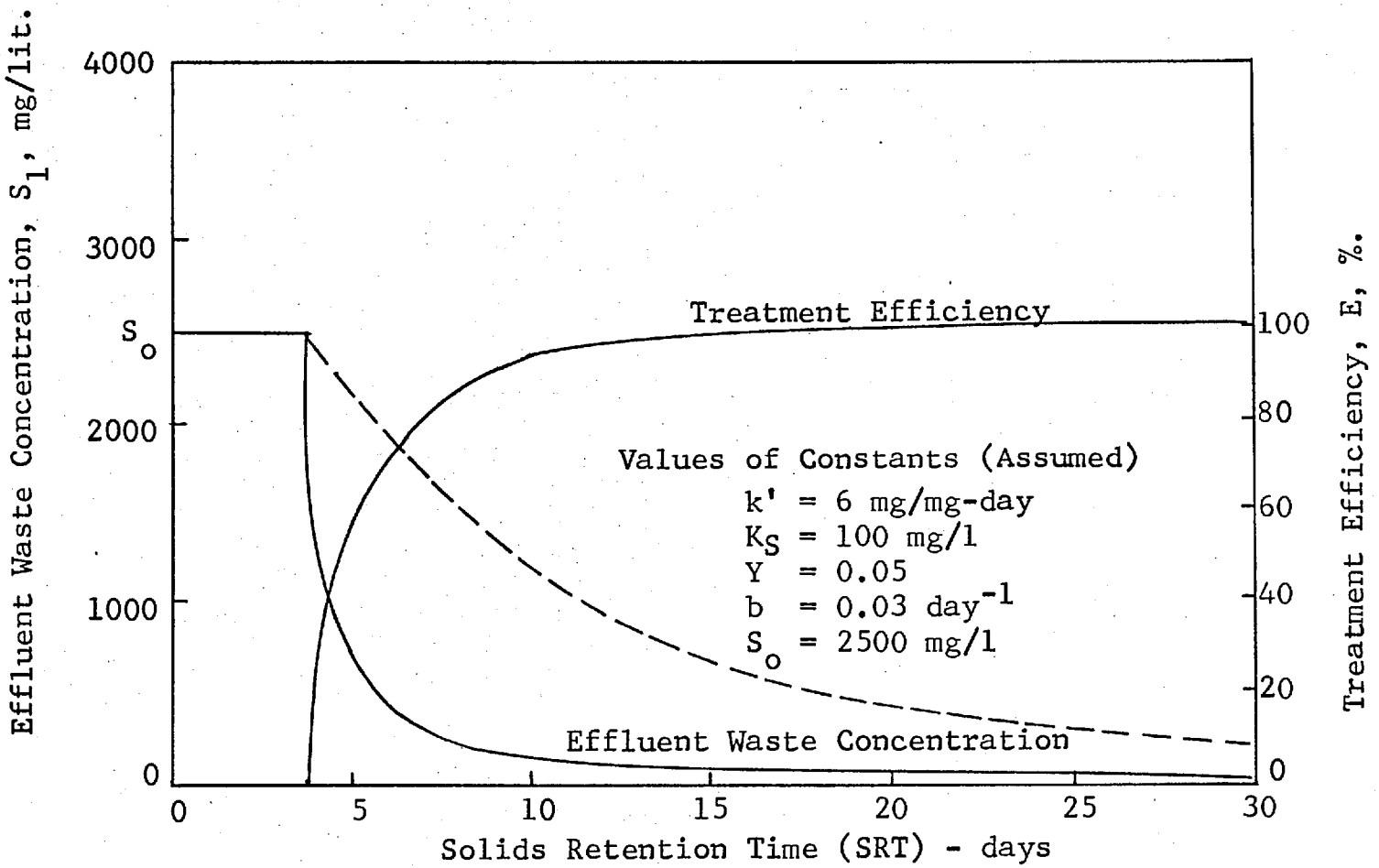


FIGURE 3.4 Steady State Relationships between Solids Retention Time, Effluent Waste Concentration and Treatment Efficiency in the Continuous Flow Biological Treatment Model of Lawrence and McCarty (1970).

——————— large K_S value

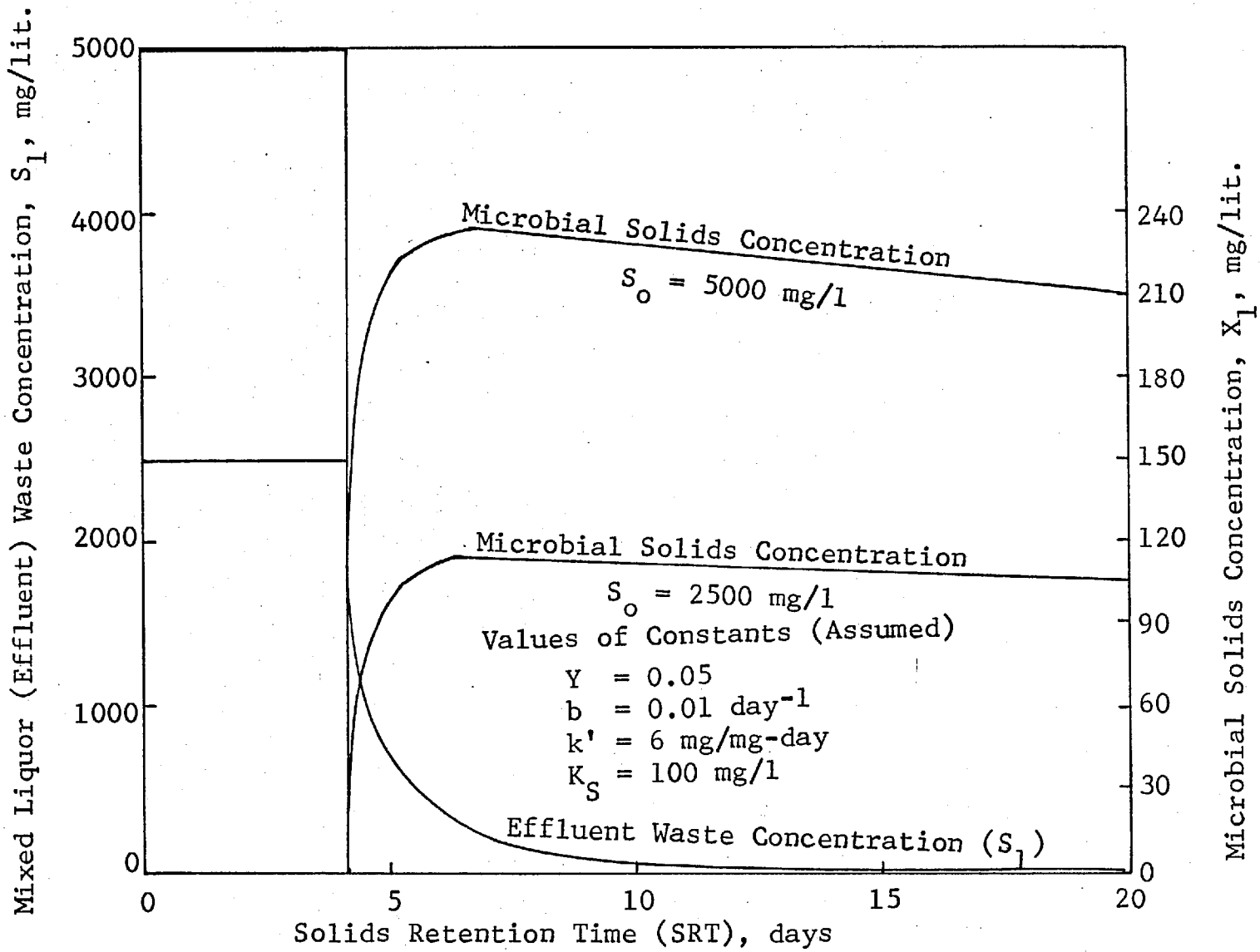


FIGURE 3.5 Steady State Relationships between solids retention time and mixed liquor (effluent) microbial solids and waste concentrations for the continuous flow biological treatment model of Lawrence and McCarty (1970).

High K_S values have the reverse effect and cause the increased sensitivity of S_1 to changes in SRT. Referring to Figure 3.4, high K_S values produce the effect shown by the dashed line.

3.1.2.2 The model of Eckenfelder (1961) and McKinney (1962)

The work of Garret and Sawyer (1952) laid the foundation for the use of another type of relationship between substrate concentration and growth rate. Garret and Sawyer plotted soluble BOD remaining against dilution rate for continuous flow experiments, using three different detention times. Although there was some scatter in the data for replicate experiments, they could be fitted to a straight line, yielding a linear relationship between remaining substrate and growth rate, as shown in Figure 3.1 (Equation 3.8). While the authors felt that such a discontinuous function could be used for practical purposes, their concept was adopted as theory by other workers, namely Eckenfelder and McKinney, in design procedures for biological waste treatment systems. Both workers have used first order substrate removal relationships which are equivalent to the limiting case of the Monod equation where $S_1 \ll K_S$ and $q = k'' S_1$.

The formulation of the Eckenfelder model is identical to that given for the Lawrence and McCarty model with the following exception:- The rate of microbial substrate utilisation per unit digester volume is $r = k''X_1S_1$ (Equation 3.21) instead of that given in Equation 3.6 in the Lawrence and McCarty model. Solving the material balances for substrate and cellular matter for the case of no-recycle and recycle results in equations for S_1 and X_1, as given in Table 3.1.

3.1.2.3 Application of the Models to Anaerobic Digestion

(i) Soluble Feedstocks:

Since anaerobic digestion is a complex multi-stage process in which the various steps are brought about by physiologically different groups of bacteria then kinetic studies have to be considered in two ways:

(a) Studying the overall process, and

(b) Considering each step separately for the process - the rate limiting step approach.

For the overall process approach, all the various steps are allowed to take place simultaneously in the same

Characteristic	Lawrence and McCarty (1970)		Eckenfelder (1961)	
	No recycle	Recycle	No recycle	Recycle
Effluent substrate concentration, S_1	$S_1 = \frac{K_S[1+b(\theta_c)]}{\theta_c(Yk'-b)-1}$	$S_1 = \frac{K_S[1+b(\theta_c)]}{\theta_c(Yk'-b)-1}$	$S_1 = \frac{S_o}{1+k''X_1\theta}$	$S_1 = \frac{S_o}{1+k''X_1\theta}$
Cell concentration, X_1	$X_1 = \frac{Y(S_o - S_1)}{1+b\theta_c}$	$X_1 = \frac{Y(S_o-S_1)}{1+b\theta_c}\frac{\theta_c}{\theta}$	$X_1 = \frac{Y(S_o - S_1)}{1+b\theta}$	$X_1 = \frac{Y(S_o-S_1)}{1+b\theta_c}\frac{\theta_c}{\theta}$
Hydraulic Detention Time, V/Q	$\theta = \theta_c$	$\theta = \theta_c(1+r_v'-r_v'\frac{X_r}{X_1})$	$\theta = \theta_c$	$\theta = \theta_c(1+r_v'-r_v'\frac{X_r}{X_1})$
Limiting minimum biological solids detention time $[\theta_c^m]_{lim.}$ $(S_o \gg K_S)$	$[\theta_c^m]_{lim} = (Yk'-b)^{-1}$	$[\theta_c^m]_{lim} = (Yk'-b)^{-1}$	-	-

TABLE 3.1 Relationships obtained for substrate and cell concentration for a complete-mix recycle and non-recycle system for Biological Waste Treatment systems.

reactor. If properly conducted, kinetic studies on such systems should therefore determine the kinetic characteristics of the rate-limiting step. Eckenfelder (1970) has used the Eckenfelder model to describe the digestion of sewage sludge over a range of detention times (0.75 to 22.5 days) using COD to define substrate concentration. The model was applied to the overall digestion process.

For the digestion of soluble feeds such as glucose, acetic acid, etc., the methane-producing stage (methanogenic stage) has been considered to be the rate limiting step. On this basis various workers in the digestion field (Andrews et al, 1964; Agardy et al, 1963; Lawrence and McCarty, 1969; and O'Rourke, 1968) have applied the Monod formulation (referred to by various workers as being analogous to the Michaelis Menten equation used to study enzyme kinetics) to the process.

(ii) Insoluble Feedstocks:

There have been very few kinetic studies in the digestion field, that have made use of the Lawrence and McCarty model or the Eckenfelder model for insoluble feeds. One of the more extensive studies is that of Chan (1970). In his study on the hydrolysis rate of pure cellulose in the anaerobic fermentation process it was assumed that the

rate limiting step in the overall cellulose fermentation is the hydrolysis step and the Monod growth equation was chosen to relate organism growth to substrate concentration.

The following assumptions were made by Chan for the continuous flow fermentation of cellulose:

(i) The yield coefficient Y, and the specific organism decay rate b, are constant over the range of detention times studied.

(ii) A constant proportion of the organisms in the vessel is viable.

(iii) The cellulose concentration supplied serves as the growth limiting nutrient.

(iv) No active organisms are present in the influent stream.

The analysis of Chan is given below, and is identical to the analysis given by Lawrence and McCarty, with slight differences in nomenclature.

For the configuration shown in Figure 3.2, a material balance for the cells yields:

$$V\frac{dX_1}{dt} = \mu X_1 V - bX_1 V - X_1 Q \qquad \text{Equation 3.22}$$

μ = specific growth rate, time^{-1},

$(= Y\left[\frac{r}{X_1}\right]$, Equation 3.9 in Lawrence and McCarty formulation),

b = specific cell decay rate, time^{-1},

θ = V/Q and at steady state $dX_1/dt = 0$, then

$$\frac{1}{\theta} = \mu - b \qquad \text{Equation 3.23}$$

Now since $\mu = Y(\frac{r}{X_1})$ and $q = \frac{r}{X_1}$, then Equation 3.23 becomes

$$\frac{1}{\theta} = Yq - b \qquad \text{Equation 3.24}$$

Similarly for the substrate mass balance,

$$V\frac{dS_1}{dt} = QS_o - X_1Vq - QS_1 \qquad \text{Equation 3.25}$$

(c.f. to Equation 3.14 in Lawrence and McCarty formulation)

At steady state $\frac{dS_1}{dt} = 0$ and $\frac{V}{Q} = \theta$, Equation 3.25 becomes

$$q = \frac{S_o - S_1}{X_1\theta} \qquad \text{Equation 3.26}$$

Equation 3.26 is identical to Equation 3.15 in the Lawrence and McCarty model.

From Equations 3.6 and 3.26 the following expression is obtained:

$$\frac{r}{X_1} = \frac{k'S_1}{K_S+S_1} = q = \frac{S_o - S_1}{X_1\theta} \qquad \text{Equation 3.27}$$

The maximum rate of substrate utilisation per unit weight of microorganism is denoted by q_{max} in Chan's notation, instead of k'.

It is now a relatively simple matter to determine the values of the constants by experiment. For the digestion of cellulose at a given input feed concentration, over a range of detention times, the value of q ($= \frac{S_o - S_1}{X_1 \theta}$) can be experimentally determined and a plot of q versus S_1 should yield a curve described by $q = \frac{q_{max} S_1}{K_S + S_1}$ if the data fits the Lawrence and McCarty model. The constants q_{max} and K_S can therefore be found, similarly for the yield constant Y which can easily be found from a plot of Equation 3.24.

3.1.2.4 Other Kinetic Formulations used to describe the Anaerobic Digestion of Insoluble Feedstocks

Pfeffer (1974) studied the anaerobic fermentation of refuse with sludge solids and found that it was not possible to determine the mass of organisms or account for a rate limiting substrate and therefore, after analysing the data, concluded that the best approach was to establish an overall rate constant based on total gas production. The gas data was more indicative of the degree of stabilisation than any other measurable parameter in his system.

The input substrate concentration was assumed to be the gas yield (G_o) defined as the maximum gas produced per unit weight of volatile solids added to the system. Pfeffer used a standard first order rate expression of the

type:

$$R = \frac{dS_1}{dt} = KS_1 \qquad \text{Equation 3.28}$$

R = rate of substrate removal per unit digester volume,

K = first order rate constant, $time^{-1}$.

For a completely mixed reactor with no recycle, a mass balance for the substrate gives:

$$V\frac{dS_1}{dt} = QS_o - QS_1 - KS_1V \qquad \text{Equation 3.29}$$

Now defining the substrate concentration in terms of gas production

$S_o = G_o$

$S_1 = G_1$ = potential gas production of output substrate per unit weight of volatile solids added to the system.

Since G_o is known and the actual gas produced per unit weight of volatile solids added (G_o-G_1) is also known, then G_1 can be found by difference.

Substituting the appropriate gas production terms into Equation 3.29, then

$$V\frac{dG_1}{dt} = QG_o - QG_1 - K_gG_1V \qquad \text{Equation 3.30}$$

At steady state $\frac{dG_1}{dt} = 0$ and $\frac{Q}{V} = \frac{1}{\theta}$, then

$$\frac{G_o-G_1}{G_1} = K_g\theta \qquad \text{Equation 3.31}$$

K_g = first order rate constant based on gas production per unit weight of volatile solids added, $time^{-1}$.

This approach is easily applied and overcomes the problems of substrate analysis for such a complex waste. The initial value of G_o can be found from batch digestion of the substrate and it is only necessary to record the actual gas produced per unit weight of substrate added, (G_o-G_1), for various detention times so as the rate constant can be determined. Pfeffer obtained rate constants for a range of temperatures (35^oC to 60^oC) for continuous digestion over 4-30 days detention time.

If gas production and substrate utilisation occur simultaneously, i.e. no accumulation of intermediate products (volatile acids) occurs, then the rate constant arising from Pfeffer's analysis would be identical to that given by Equation 3.28 using substrate concentration terms such as COD where $K_g = K$.

Boshoff (1967) used a similar approach to describe the batch digestion of elephant grass and determined first order velocity rate constants.

Gaddy et al (1974) have also applied a first order rate equation, for continuous digestion, of the type,

$$R = \frac{\Delta C_S}{\theta} = KS_1 \qquad \text{Equation 3.32}$$

ΔC_S = difference in the input and output substrate concentrations,

θ = detention time,

K = first order rate constant ($time^{-1}$).

to describe the continuous digestion of liquid cow dung.

Gaddy et al studied the digestion of cow dung for 10 to 26.3 days detention time for input concentrations ranging from 3800 $mg.lit^{-1}$ BOD_5 to 15300 $mg.lit^{-1}$ BOD_5.

The first order rate relationships obtained by Pfeffer, Boshoff and Gaddy show that for a given detention time in a continuous digester the percentage digestion is independent of the input substrate concentration, i.e. the same percentage digestion would be obtained for an input concentration of 10000 $mg.lit^{-1}$ COD at a 10 day detention time as that obtained for an input concentration of 20000 $mg.lit^{-1}$ COD at the same detention time. The analysis of Lawrence and McCarty shows that the output substrate concentration is independent of the input substrate concentration (refer to Equation 3.13), i.e. the percentage digestion increases with increase in input concentration, assuming no limitations such as nutrient deficiency are imposed on the system.

3.2 THE EFFECT OF TEMPERATURE ON BIOLOGICAL RATE CONSTANTS

In interpreting the influence of temperature on biological rate constants, it is necessary to consider the studies of Van't Hoff and Arrhenius. Their work forms the starting point for all modern theories of the temperature dependence of rate constants.

A quantitative relationship shows the influence of temperature on the rate of a given reaction:

$$\frac{d(\ln K_T)}{dT} = \frac{\Delta E}{RT^2} \qquad \text{Equation 3.33}$$

where: E = activation energy,
R = gas constant (1.99 cal per ^{o}C),
T = absolute temperature, ^{o}K,
K_T = the reaction rate constant.

Integration of Equation 3.33 yields

$$\ln K_T = C - \frac{\Delta E}{RT} \qquad \text{Equation 3.34}$$

This expression obeys the equation of a straight line when the natural logarithm of K_T is plotted against the reciprocal of the absolute temperature. The energy of activation may be thought of as the energy required to impart sufficient energy to some constant fraction of the molecules per unit of time in order for effective reactions to take place at a given temperature.

This relationship is generally applicable to enzymatic reactions and physiological phenomena (Fruton and Simmonds, 1958).

Ingraham (1958) compared the effect of incubation temperatures on the rates of growth of a mesophilic strain of Escherichia Coli and a psychrophilic pseudomonad. Over most of the range of temperatures Ingraham showed that the Arrhenius expression is followed. In their evaluation of the kinetics of methane fermentation of volatile acids Lawrence and McCarty (1969) showed that the temperature coefficient described as the "half velocity" constant for acetate fermentation obeys the temperature relationship of Arrhenius from 25°C to 35°C. Their data further indicated that the biological growth constant (Y) is not appreciably affected by temperature within the range studied.

Fair and Moore (1934) found that the effects of temperature on the rate of digestion agree well with the law of Arrhenius between 10°C and 28°C and between 42°C to 55°C, but found considerable deviation from the straight line beyond the ranges given above. There have been a number of investigations on the effect of temperature on anaerobic treatment at 30°C and above using feedstocks such as sewage sludge or domestic refuse digested with sludge

solids; however, a general lack of studies on the effects of temperatures below or above 35°C on the kinetics of anaerobic digestion of insoluble feeds, such as cellulose and grass, is apparent in the literature.

3.3 PROPOSALS FOR THIS STUDY

Continuous digestion is to be used in this study since operation at steady state conditions allows for better and easier control over the digestion process; thus the quality of the mixed culture, build up of toxins can be controlled and analysis of the effluent can be carried out over long periods without affecting the quantity or quality of the digester contents.

The simplest and most common type of continuous digester operation is conducted in a stirred digester into which the substrate and nutrient solution is fed and from which the spent substrate is continuously removed at the same volumetric flow rate. The mixing must be adequate such that the substrate is uniformly suspended in the digester and the effluent stream has the same chemical composition as the digester contents.

It was convenient in this study to add substrate and remove the effluent from the digesters once per day because of the low flows required and also the particulate substrate could not be automatically fed. The effect on the performance of a once per day fed continuous digester has been estimated by Chan (1970) by considering the effects on cell growth for a cellulose-fed digester. Chan concluded that when the dilution rate has a small value (0.0333-0.167

in Chan's work) then for a once per day fed digester the cell growth rate is essentially equal to a true continuously fed digester. In this study the range of dilution rates is 0.0333 to 0.1 and since this falls well within the range considered by Chan, then the effect on performance of once per day feeding is not significant.

A substrate material balance for a single stage CSTR without recycle, as shown in Figure 3.2, yields

$$V\frac{dS_1}{dt} = S_oQ - S_1Q - X_1Vq \qquad \text{Equation 3.35}$$

where: Q = liquid flowrate, volume per time,

V = digester volume,

S_o = input substrate concentration, mass per volume,

S_1 = output substrate concentration, mass per volume,

q = rate of substrate utilisation per unit digester volume per unit cell concentration, $time^{-1}$,

$\frac{V}{Q} = \theta$ = detention time.

At steady state $\frac{dS_1}{dt} = 0$, Equation 3.35 becomes

$$q = \frac{S_o - S_1}{X_1\theta} \qquad \text{Equation 3.36}$$

Similarly the rate of substrate utilisation can be expressed in terms of the rate of substrate removal per unit digester volume (R), in which case:

$$R = \frac{S_o - S_1}{\theta} \qquad \text{Equation 3.37}$$

as obtained from the above mass balance using R instead of q.

The bacterial cell accumulation in the digester is the result of the following activities:

1. Increase in cells attributable to the influent, assumed to be zero.

2. Increase in cells due to cell growth.

3. Decrease in cells due to endogenous respiration.

The following limitations are noted before a material balance for the cells is made:

1. The yield constant Y, and the endogenous respiration rate are constant over the range of detention times studied.

2. A constant proportion of the cells in the digester is viable.

3. All nutrients required for growth are present in excess.

The term for the endogenous respiration rate is included in the mass balance for the cells because a loss of cell material takes place due to a series of processes such as endogenous respiration, cell lysis - the term b

accounts for the utilisation of cellular material to furnish energy for the replacement of protoplasm. The term b is sometimes referred to as the specific organism decay rate. The cell material balance is as follows:

$$V\frac{dX_1}{dt} = QX_o + Y(S_o-S_1)Q - X_1bV - QX_1 \qquad \text{Equation 3.38}$$

where: Q = hydraulic flowrate, volume/time,

Y = yield constant relating the cell production to the change in substrate concentration, $(\frac{X_1}{S_o-S_1})$,

b = fraction of cells lost due to endogenous respiration, time^{-1},

If $X_o = 0$ and at steady state $\frac{dX_1}{dt} = 0$, then $Y(S_o-S_1)Q = X_1(bV+Q)$ and

$$X_1 = \frac{Y(S_o-S_1)}{1+b\theta} \qquad \text{Equation 3.39}$$

or

$$\frac{S_o-S_1}{X_1} = \frac{1}{Y} + \frac{b}{Y}\theta \qquad \text{Equation 3.40}$$

Equation 3.40 is identical to that found previously (see Table 3.1) the form of which is not dependent on the use of any particular model.

Andrews et al (1963) have discussed the possibility of the yield constant and decay coefficient not being constant when using V.S.S. content for cell concentration on systems covering a wide range of detention times (4 hours to 20 days). In this study detention times of

10-30 days are used and Andrews et al considers this as a limited range over which the constants should not vary; however, no data is published on this point.

Postgate and Hunter (1962) have shown that loss of organisms by death can be significant at low growth rates. Their work was done with continuous cultures of Aerobacter aerogenes using carbon, nitrogen, phosphorus and sulphur as the growth limiting nutrients. Steady states were obtained in which constant proportions of the organisms were dead and as the cell detention time was increased the percentage of viable organisms decreased. They found that the percentage viable organisms decreased nearly 50 percent as the cell detention time increased by a factor of about eight (33.9 hours to 263 hours). The range used in this study is much less than that used by Postgate and Hunter and the decrease in percentage viable organisms over the range in this study would be expected to be considerably lower than 50 percent. However, no results are available in the literature for the case of mixed insoluble substrates or heterogeneous cultures, as used in this study, and this factor needs to be considered when discussing the experimental results.

The insoluble substrates used in this study are cellulose, grass, cow dung and horse manure. In view of the conclusions of Pfeffer (1968) and Chan (1970), the rate limiting step is assumed to be the hydrolysis of the initial insoluble feed solids and therefore the measurement of substrate concentration (S_1) is the concentration of the undigested solids in the effluent. For the cellulose digesters the substrate concentration is easily determined as cellulose; however, for the digesters fed on grass, cow dung and horse manure, since no specific determination for substrate concentration is possible (refer to 4.2.4), then the substrate concentration is to be measured as the Chemical Oxygen Demand (COD) of the volatile matter.

The cell concentration term (X_1) is to be measured as the V.S.S. for the cellulose digesters and as the V.S.S. content of less than 25 μm diameter in the supernatant liquor for grass, cow dung and horse manure. The parameters, substrate concentration and cell concentration and their measurement are discussed in 4.2.4.

The three operating temperatures used in this study are 28°C, 38°C and 48°C - these values are convenient and have often been quoted as covering the intermediate to thermophilic range of temperatures (McGhee, 1968).

The substrates have been digested in a nutrient rich medium to ensure active cell growth (Speece and McCarty, 1964) and input concentrations of 15000 $mg.lit^{-1}$ and 25000 $mg.lit^{-1}$ (dry weight) have been employed. Detention times of 30, 17 and 10 days have been used with sufficient time given when changing the steady state conditions (refer to 4.2.3), detention times of less than 10 days were not possible in this study because of the limitations of time and of the apparatus.

A mixed culture obtained from a local sewage works (anaerobic digester supernatant) has been used as the source of bacteria and acclimation effects (loading and temperature) have been minimised by careful control over digester loading (refer to 4.2.3).

Since the composition of grass, cow dung and horse manure could not be determined accurately enough, it was necessary to carry out batch experiments to obtain an estimate of the potential gas production per unit weight of substrate. This is a useful figure for comparing the different substrates in terms of user requirements.

The Model to be used

The difficulties in determining and analysing for the substrate and cell concentration in the digesters led Gates et al (1967) to conclude the following concerning the use of kinetic models based on COD and V.S.S.

"It would be illogical to attempt to rationalise the results in terms of describing specific (individual) bacterial components of the anaerobic process, it is mandatory that the resulting experimentally determined values be considered and treated as overall process kinetic constants".

It is therefore understood that the resulting kinetic constants apply for a mixed culture and mixed substrate system; changes in the individual components in the culture or substrate mixture can cause deviations between different systems. Jones (1973) points out that attempts to provide models for the design and operation of waste treatment plants (covering a range of treatment processes) have been many and confusing and zero, first or second order rate equations have been used by investigators claiming that their particular model offers a "unified basis" for design of biological treatment plant.

Because of the lack of experimental studies on the digestion of mixed cellulosic feedstocks, the approach

adopted in this study is to examine experimentally the effect of the operating conditions outlined above on:

(i) q, the rate of substrate utilisation per unit digester volume per unit cell mass, time^{-1}. For a continuous digester with no recycle $q = \frac{S_o - S_1}{X_1 \theta}$, as given by Equation 3.36.

A plot of q versus S_1 will yield the functional form of the relationship between the two parameters. The model of Lawrence and McCarty is followed if $q = \frac{k' S_1}{K_S + S_1}$ (Equation 3.27), whilst if the model of Eckenfelder is followed then $q = k'' S_1$ (Equation 3.21 where $q = \frac{r}{X}$), where k', K_S and K'' are all constants, as previously defined.

The values of S_o, S_1, X_1 and θ are all found experimentally, hence q can be found.

(ii) R, the rate of substrate utilisation per unit digester volume, mass/volume-time. For a continuous digester with no recycle $R = \frac{S_o - S_1}{\theta}$, as given by Equation 3.37.

A plot of R versus S_1 will yield the functional form of the relationship between the two parameters. The first order model of Pfeffer or Gaddy et al is followed if $R = KS_1$ (Equation 3.28), i.e. the percentage

digestion $\left[100 \times \frac{S_o - S_1}{S_o}\right]$ is independent of the input substrate concentration.

The model of Lawrence and McCarty, or Eckenfelder, is followed if steady state values of S_1 are independent of the input concentration S_o, and a plot of R versus S_1 yields a series of curves, as predicted by Equations 3.6 or 3.21, for different input substrate concentrations.

The values of S_o, S_1 and θ are all determined by experiment, hence R can be found.

The yield constant Y and decay coefficient b are found from a plot of Equation 3.40 - $\frac{S_o - S_1}{X_1}$ versus θ, all values being experimentally determined.

In processes connected with growth, the reactions leading to product formation are proportional and proceed parallel to cell growth, since they supply the energy required for growth (Gaden, 1956). It is apparent in the kinetic models given above that for continuous flow fermentation the removal of the substrate is proportional to the production of cells (constant of proportionality = yield constant), i.e. the process is growth associated, the use of Equation 3.40 can illustrate this point.

Similarly if the removal of substrate and gas production are growth associated, then from Equation 3.40, since substrate removal and cell production are growth associated, i.e. q is proportional to $\frac{1}{\theta}$, then the total gas production should also be proportional to $\frac{1}{\theta}$. Therefore if a plot of q and total gas production G_T, determined experimentally, yields two curves which are of the same general form, it can be concluded that substrate removal and gas production are growth associated processes.

It is proposed to study whether the rate constants obtained follow the temperature relationship developed by Arrhenius and Van't Hoff and presented earlier as Equation 3.34:

$$\ln K_T = C - \frac{\Delta E}{RT}$$

This relationship shows that the plot of the logarithm of the biological rate constant versus the reciprocal of the absolute temperature of the reaction produces a straight line. The biological rate constants are those determined from plots of q or R versus S_1, as previously outlined.

CHAPTER 4

EXPERIMENTAL STUDY OF DIGESTION OF CELLULOSIC WASTES

The experimental work was separated into two sections:

1. Batch Digestion:

To determine the quantity of gas produced from the digestion of grass, horse manure and cow dung at operating temperatures of 28°C, 38°C and 48°C.

2. Continuous Digestion:

To determine the effect of temperature and loading rate on solids utilisation for the anaerobic digestion of cellulose, grass, cow dung and horse manure in a semi-continuous digester system.

METHODS AND PROCEDURES

4.1 BATCH DIGESTION

4.1.1 Experimental Apparatus

Plate 4.1 shows the type of batch digester used. The system consisted of the following major components:

1. Digesters: One or two litre capacity conical flasks fitted with rubber stoppers were used as digesters. The digesters were fitted with inlet and outlet ports for sampling and addition of materials.

2. Gas Collectors: Fermentation gases were collected over an acidified brine solution (3.13% H_2SO_4, 10% NaCl) contained in graduated glass cylinders.

The digesters were mounted on hot plates and magnetically stirred; the temperature of each unit could be controlled separately.

4.1.2 Batch Digester Operation

The substrates used - grass, cow dung and horse manure - were collected and pretreated as described in Section 4.2.2(2). The substrate characteristics are given in Table 4.4.

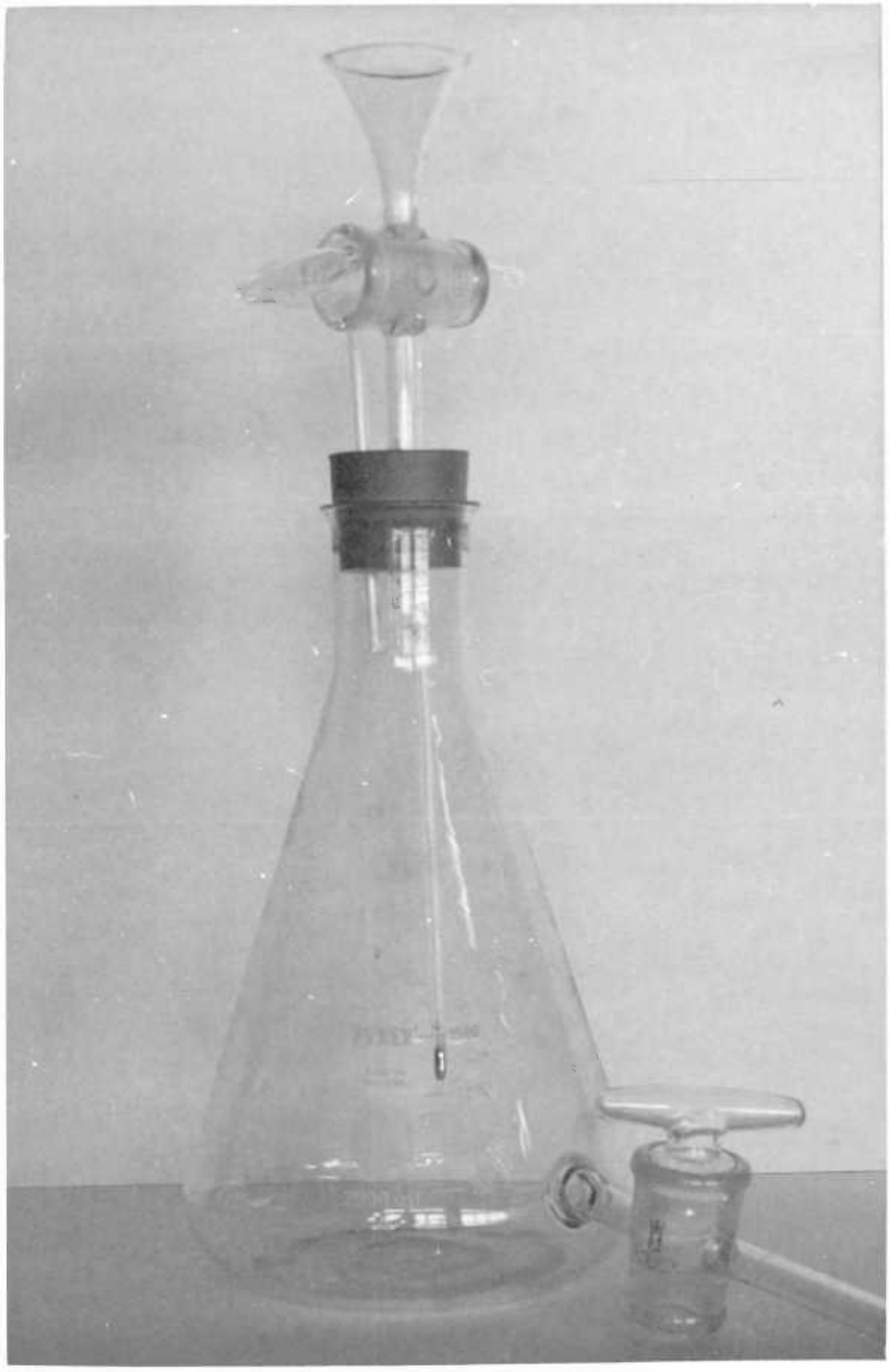


Plate 4.1 Batch Digester

The batch digesters were seeded with the mixed culture obtained from the continuously operated digesters - the effluent was diluted 10:1 with tap water and the liquid medium defined in Table 4.6, all residual solids were removed from the effluent before being used as seed material.

The batch digester operating conditions are shown in Table 4.0.

4.1.3 Analysis of Batch Digesters

For the batch digesters the following parameters were recorded:

a) Gas production - daily.

b) Gas composition - once every two days.

c) pH - this was checked once every three or four days for the batch digesters.

d) When gas production ceased the volatile solids, cellulose content, pH and alkalinity were recorded. When gas production fell below 10 cm^3 day^{-1} for five days or more the process was considered to be complete, and the cumulative gas production per gm. of substrate added for the period of digestion was calculated and designated G_o.

	T = 28°C	T = 38°C	T = 48°C
Substrate: GRASS			
weight added, gm.	10.5	10.5	10.5
volume of seed used, cm^3	700	700	700
concentration, mg/lit.	15,000	15,000	15,000
Substrate: COW DUNG			
weight added, gm.	8.6	7.95	7.95
volume of seed used, cm^3	700	645	645
concentration, mg/lit.	12,333	12,333	12,333
Substrate: HORSE MANURE			
weight added, gm.	8.5	8.5	8.5
volume of seed used, cm^3	580	580	580
concentration, mg/lit.	15,000	15,000	15,000

TABLE 4.0 Batch Digester Operating Conditions.

4.2 CONTINUOUS DIGESTION

4.2.1 Experimental Apparatus

Plate 4.2 shows the digesters employed for continuous digestion.

The system consisted of the following major components:

1. Digesters: 2 litre capacity flat-bottomed culture vessels fitted with mutli-socket (5 sockets)/flat flanges served as digesters. Each digester was fitted with an inlet and outlet port, stirrer gland with screw cap, a gas sampling port and a gas outlet pipe.

2. Gas Collectors: Fermentation gases were collected over an acidified brine (3.13% H_2SO_4 and 10% NaCl) solution in inverted graduated glass cylinders.

Constant temperature was achieved by the use of thermostatically controlled water baths. Three such baths were used and six digesters were positioned in each bath.

Each digester was stirred by either individual handilab stirrers linked by V-belts on a pulley system or by a centrally positioned Parvalux motor linked by V-belts

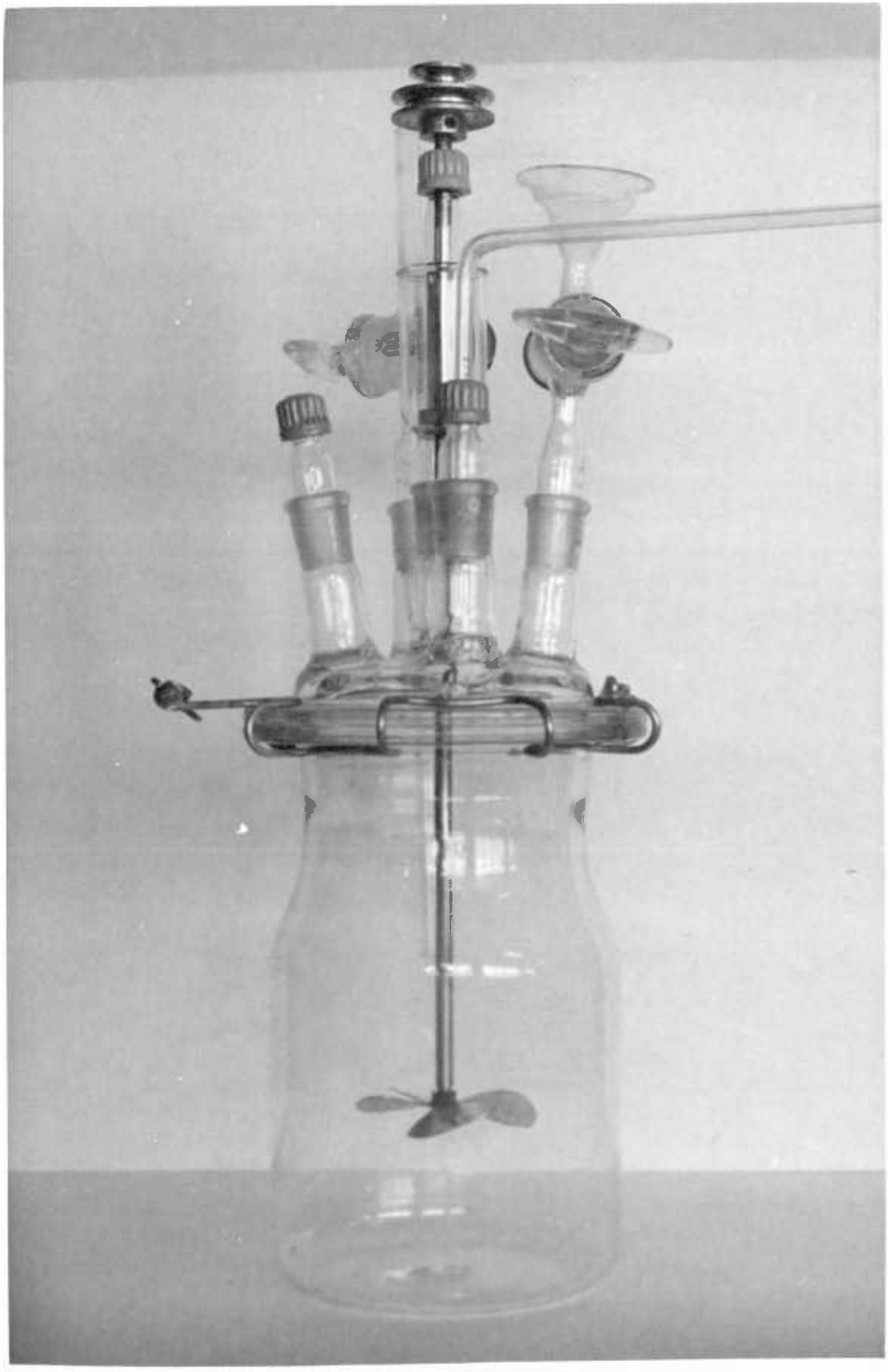


Plate 4.2 Continuous Digester

on a pulley system to one or more digesters.

In all, nineteen continuously stirred digesters were used in the investigation.

During the course of the experimentation period, a 50 litre capacity continuous stirred digester was constructed. It was hoped to digest the cellulosic feedstocks - grass, cow dung and horse manure - in the digester; however, the quantity of feedstock required by the digester was excessive and so this digester was fed on pure cellulose which was commercially available at the time of the experiments.

Plate 4.3 shows the digester set-up. The system consisted of the following major components:

1. Digester: A 50 lit. capacity Q.V.F. round-bottomed vessel fitted with inlet and outlet ports. The digester was stirred by a four-bladed glass stirrer. At low stirrer speeds (50 r.p.m.) the stirrer proved very efficient in preventing cellulose from settling at the bottom of the digester. The stirrer was driven by an externally mounted Parvalux motor.

2. Gas Collection: Fermentation gases were collected over an acidified brine solution in a 15 lit. Mariotte-type gas reservoir.

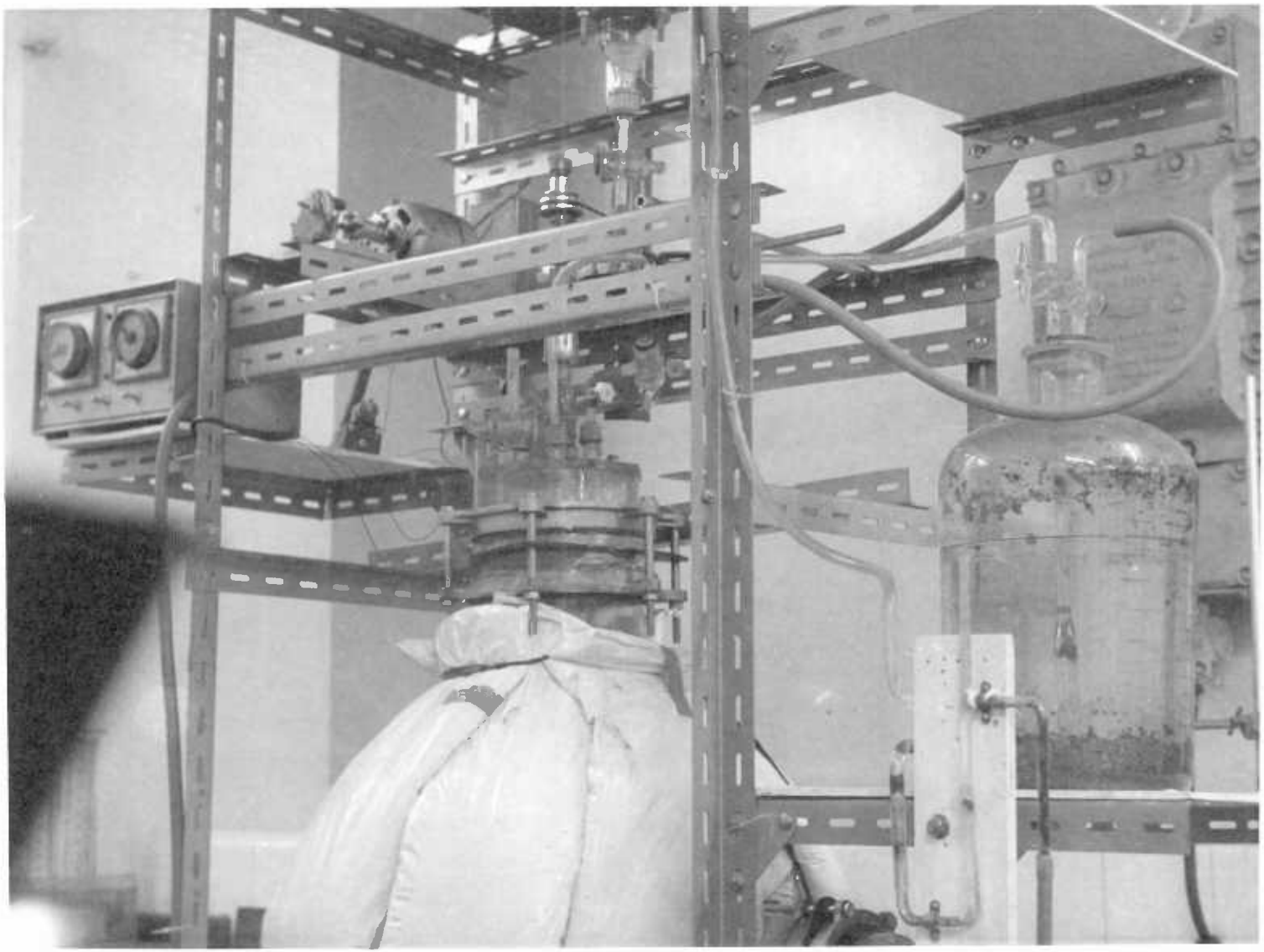


Plate 4.3 50 litre capacity Continuous Digester

The digester was heated using an externally mounted heating tape covering the entire surface of the digester; the heating tape was then lagged with asbestos matting and covered with a 1 inch thick insulating blanket.

The digester was also fitted with an automatic feeding and sampling device operated by a time switch, but this facility was not used because of difficulties in pumping the influent into the digester.

4.2.2 Input Feedstock Characteristics

1. Cellulose

The cellulose used was obtained from B.D.H. The characteristics of the substrate are shown in Table 4.1. The cellulose was added to a soluble synthetic medium to formulate a suitable and reproducible substrate supply and to provide a source of nitrogen. The soluble liquid medium used was the same as that used by Chan (1970) on investigating the hydrolysis rate of cellulose in anaerobic digestion. The composition of the liquid medium is shown in Table 4.2 and its characteristics are given in Table 4.3.

CHARACTERISTIC	VALUE
Type	chromedia CF.11 crystalline powder
Purity ash Fe	0.01% by weight 0.0005% by weight
Mean Particle Size	15 - 40 μm
Molecular Weight	162
Carbon Content per gm. of cellulose	0.444 gm/gm of cellulose
Pretreatment	samples dried in oven at 70°C for one day

TABLE 4.1 Characteristics of Cellulose.

Table 4.2 Liquid Medium Composition used for daily feeding of Cellulose to Digesters.

Item	Concentration
Inorganics	
NaCl	1000 mg./litre
$(NH_4)_2$ HPO_4	1000 mg. /litre
K_2 HPO_4	500 "
Mg SO_4	100 "
Ca Cl_2	100 "
Na HCO_3	2,500 "
Organics	
Yeast Extract	1000 "
Tap Water	1000 cm^3/lit.

ITEM	CONCENTRATION $mg.l^{-1}$
Total solids	5,320
Volatile solids	1,675
Volatile suspended solids	negligible
pH	7.45
Alkalinity	1,995
Total Nitrogen	338
Total Volatile Acids	35
Total Carbon	810

TABLE 4.3 Liquid Medium Characteristics used for daily feeding of Cellulose to Digesters.

2. Grass, Cow Dung and Horse Manure

Collection and Pretreatment

A. GRASS

Grass clippings were collected from the College grounds in one batch. The composition of grass is known to change with maturity (Pidgen and Heaney, 1969) and therefore it was necessary to collect sufficient supplies to last for the entire experimentation period. The clippings were collected immediately after the lawns were mown and then dried in the sun for about ½ week period. The clippings were then milled several times to produce the size distribution shown in Appendix II. In order to reduce the bacterial activity of the clippings the substrate was further dried in an oven at 70°C for ½ day (small quantities at a time). Finally the grass was stored in P.V.C. buckets covered with aluminium foil. For long storage periods the clippings were kept under refrigeration.

B. COW DUNG

Cow dung was collected from a local farmer. Only dry dung was collected.

The dung was initially ground by hand and then dried in the sun for up to one week. It was subsequently

ground in a mill to the size distribution shown in Appendix II. Finally the dung was dried in an oven at 125°C for up to a day and then stored.

C. HORSE MANURE

The manure was collected from stables close to the College. The material was dry when received and contained considerable quantities of bedding plus manure. As with the grass and cow dung, the manure was dried in the sun and ground to produce the size distribution as shown in Appendix II. The manure was then dried in an oven at 125°C and then stored.

Both horse manure and cow dung contained quantities of grass clippings obtained during the collection procedure.

The measured characteristics of grass, cow dung and horse manure are given in Table 4.4.

All three substrates were added to a liquid medium of composition shown in Table 4.5 before being fed to the digesters. The liquid medium provided the necessary nutrients for cell growth (Speece and McCarty, 1964 and Chan, 1970). Table 4.6 gives the characteristics of the liquid medium used.

CHARACTERISTIC	GRASS	COW DUNG	HORSE MANURE
Volatile Solids, % w/w	83.5	64	62.7
Fixed Solids, % w/w	16.5	36	37.3
Cellulose content, % w/w	37.5	31.2	30.7
CHN Analysis: C, % w/w (Volatile matter)	40.9	42.0	40.8
H, % w/w	5.7	5.5	4.3
N, % w/w	1.36	2.35	2.4
Protein, % w/w	10.0	12.2	12.6
Lignin Content, % w/w	13.1	27.1	25.5
Water Soluble Component, % w/w	1.5	0.3	0.2
Ether Soluble Component, % w/w	3.5	5.1	3.9

TABLE 4.4 Characteristics of Grass, Cow Dung and Horse Manure.

Table 4.5 Liquid Medium Composition used for daily feeding of Grass, Cow Dung and Horse Manure to Digesters.

Item	Concentration
Inorganics	
NaCl	1000 mg./litre
K_2HPO_4	500 "
$MgSO_4$	100 "
$CaCl_2$	100 "
$NaHCO_3$	2500 "
Tap Water	1000 cm^3/lit.

ITEM	CONCENTRATION, $mg.l^{-1}$
Total solids	4630
Volatile solids	1585
Volatile suspended solids	negligible
pH	7.3
Alkalinity	1860
Total nitrogen	105
Total volatile acids	35
Total carbon	810

TABLE 4.6 Liquid Medium Characteristics used for daily feeding of Grass, Cow Dung and Horse Manure to Digesters.

4.2.3 Continuous Digester Operation

Operating Conditions

The operating conditions for the digesters being fed on cellulose, grass, cow dung and horse manure are shown in Tables 4.7 and 4.8. The digestion of cellulose at an input concentration of 25,000 $mg.lit^{-1}$ at $38^{o}C$ was carried out in the 50 lit. digester. All other conditions shown apply to the 2 lit. digesters.

Operating Procedure

All continuous digesters fed on cellulose, grass, cow dung and horse manure were operated in exactly the same way. For example, considering the digesters fed on grass, the procedure stated below was adopted for loading the digesters over the conditions shown in Table 4.8.

Six digesters, two at each of the three operating temperatures, were initially filled with 1.7 litres of anaerobic digester supernatant obtained from a local sewage works digester. The digesters were simultaneously acclimated to the desired temperature by increasing the temperature by approximately $3^{o}C$ per week.

The detention time was initially set at 30 days

Conditions	T = 28°C			T = 38°C					
	S_o = 15000 mglit^{-1}			S_o = 15000 mglit^{-1}			S_o = 25000 mglit^{-1}*		
Detention Time, days	30	17	10	30	17	10	30	17	10
Working Volume, lit.	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Dry weight of feed added, gm.	0.9	1.5	2.55	0.9	1.5	2.55	25	44.1	75
Daily feed volume, cm^3.	60	100	170	60	100	170	1000	1764	3000
Loading rate: lb VS/ft^3 day	0.032	0.054	0.092	0.032	0.054	0.092	0.051	0.09	0.15
gm VS/lit.day	0.53	0.88	1.5	0.52	0.88	1.5	0.833	1.47	2.5
% volatile solids in feed	1.5	1.5	1.5	1.5	1.5	1.5	2.5	2.5	2.5
Carbon/nitrogen ratio	22:1	22:1	22:1	22:1	22:1	22:1	22:1	22:1	22:1
Nitrogen/phosphorus ratio	1.14:1	1.14:1	1.14:1	1.14:1	1.14:1	1.14:1	1.14:1	1.14:1	1.14:1

S_o - Input concentration of dry cellulose in liquid medium fed.
loading rate - weight of cellulose added per unit volume of digester volume.
* - 50 litre capacity digester.

TABLE 4.7 Operating Conditions for Digesters being fed on Cellulose.

Conditions	T = 28°C						T = 38°C						T = 48°C					
	S_o = 15000 gml^{-1}			S_o = 25000 mgl^{-1}			S_o = 15000 mgl^{-1}			S_o = 25000 mgl^{-1}			S_o = 15000 mgl^{-1}			S_o = 25000 mgl^{-1}		
Detention Time, days	30	17	10	30	17	10	30	17	10	30	17	10	30	17	10	30	17	10
Working Volume, litres	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Dry weight of feed added, gm.	0.9	1.5	2.55	1.5	2.5	4.25	0.9	1.5	2.55	1.5	2.5	4.25	0.9	1.5	2.55	1.5	2.5	4.25
Daily food volume, cm^3.	60	100	170	60	100	170	60	100	170	60	100	170	60	100	170	60	100	170
Loading Rate, lb vs/ft^3.day	0.028	0.046	0.08	0.046	0.079	0.13	0.028	0.046	0.08	0.046	0.077	0.13	0.028	0.046	0.08	0.046	0.077	0.13
gm vs/ft^3.day	0.44	0.737	1.25	0.737	1.23	2.08	0.44	0.737	1.25	0.737	1.23	2.08	0.44	0.737	1.25	0.737	1.23	2.08
% Volatile solids in feed	1.2	1.2	1.2	2	2	2	1.2	1.2	1.2	2	2	2	1.2	1.2	1.2	2	2	2
Carbon/nitrogen ratio - grass	20.2.1	20.2:1	20.2:1	20.2:1	20.2:1	20.2:1	20.2:1	20.2:1	20.2:1	20.2:1	20.2:1	20.2:1	20.2:1	20.2:1	20.2:1	20.2:1	20.2:1	20.2:1
Nitrogen/phosphorus ratio - grass	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1	1.29:1
Carbon/nitrogen ratio - cow dung	18:1	18:1	* 18:1	18:1	18:1	* 18:1	18:1	18:1	18:1	18:1	18:1	18:1	18:1	18:1	18:1	18:1	18:1	18:1
Nitrogen/phosphorus ratio - cow dung	1.4:1	1.4:1	* 1.4:1	1.4:1	1.4:1	* 1.4:1	1.4:1	1.4:1	1.4:1	1.4:1	1.4:1	1.4:1	1.4:1	1.4:1	1.4:1	1.4:1	1.4:1	1.4:1
Carbon/nitrogen ratio - horse manure	17.4:1	17.4:1	17.4:1	* 17.4:1	* 17.4:1	* 17.4:1	17.4:1	17.4:1	17.4:1	* 17.4:1	* 17.4:1	* 17.4:1	17.4:1	17.4:1	17.4:1	* 17.4:1	* 17.4:1	* 17.4:1
Nitrogen/phosphorus ratio - horse manure	1.31:1	1.31:1	1.31:1	* 1.31:1	* 1.31:1	* 1.31:1	1.31:1	1.31:1	1.31:1	* 1.31:1	* 1.31:1	* 1.31:1	1.31:1	1.31:1	1.31:1	* 1.31:1	* 1.31:1	* 1.31:1

S_o = input concentration of dry substrate in liquid medium feed.

* = no results obtained at these operating conditions.

Table 4.8 Operating Conditions for Digesters being fed on Grass, Cow Dung and Horse Manure.

for all of the six digesters which were fed with a feedstock concentration of 15,000 $mg.l^{-1}$ (dry weight of substrate). This initial acclimation period lasted for 2 detention times. Following this period one digester at each of the operating temperatures was fed with a feedstock concentration of 25,000 $mg.l^{-1}$. The change to a higher feed concentration was accomplished gradually over a 2 week period.

The six digesters were then operated at the 30 day detention time for five complete detention time periods, after which the digestion conditions such as substrate utilisation, gas production, V.S.S. concentration, were determined as shown in the Analysis section. The analysis period took five weeks with material balances being performed on a weekly basis.

The detention time, for all six digesters, was changed to seventeen days over a 10 day period. The digesters were once again operated at this detention time for five detention time periods, after which the analysis period was followed.

Finally, the detention time was changed to 10 days over a 10 day period. The digesters were operated at this detention time for a total of seven detention time periods, after which the analysis period was followed.

The criterion of steady state operation was

established by observing that digestion conditions - gas production, COD solids utilisation and V.S.S. concentration - established values with a maximum coefficient of variation of 20% (Chan, 1970).

The total operating period for the digesters fed on grass clippings was approximately 60 weeks.

Table 4.9 shows the operating schedule for digesters being fed on grass with feed concentrations of 15000 $mg.l^{-1}$ and 25000 $mg.l^{-1}$. The operating schedule was formulated so as to ensure minimum effects due to population shifts and therefore create similar operating conditions in each digester.

The liquid medium feed was prepared at least once every three days and stored under refrigeration to minimise bacterial degradation. Each day the required amount of medium was measured in a graduated cylinder and warmed to the required temperature. The substrates were added to the liquid medium and gravity fed to the digesters after an equal volume of effluent had been removed from the digesters by suction.

The anaerobic digester supernatant used was treated as follows: (i) the residual solids were allowed to settle after being collected from the sewage works; (ii) the supernatant was decanted through a BS410 90 μm sieve; (iii) prior to being used the liquid was stored at $2^{o}C$ in a refrigerator.

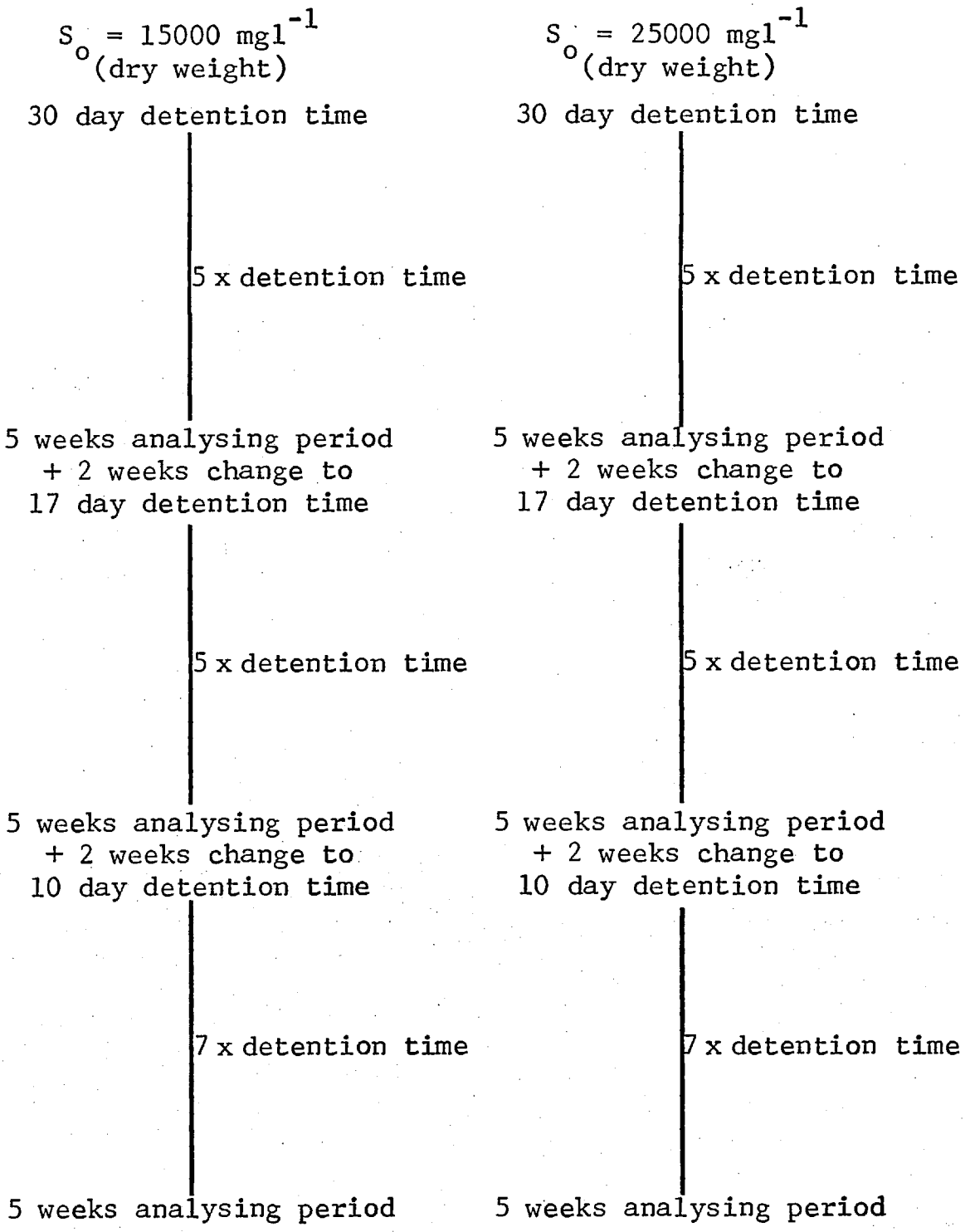


TABLE 4.9 Operating Schedule for Digesters fed on Grass Clippings.

The 50 litre digester was operated on a cellulose feedstock of concentration 25000 mgl^{-1} at 38°C. The operating conditions are shown in Table 4.7. The operating schedule was identical to that used in the 2 litre continuous digesters.

4.2.4 Analysis of Continuous Digesters

The sample analysing procedure for the continuous digesters is shown in Figure 4.1.

Each variable recorded fitted into the following categories:

a) digester performance and control: pH, alkalinity, total and volatile solids, total volatile acids, gas production, cellulose content, COD.

b) cell concentration parameters: volatile suspended solids.

c) material balance: total carbon in fermentation gases, input carbon, output carbon in solid and liquid components.

Gas Composition

Gas solid chromatography was selected as a rapid

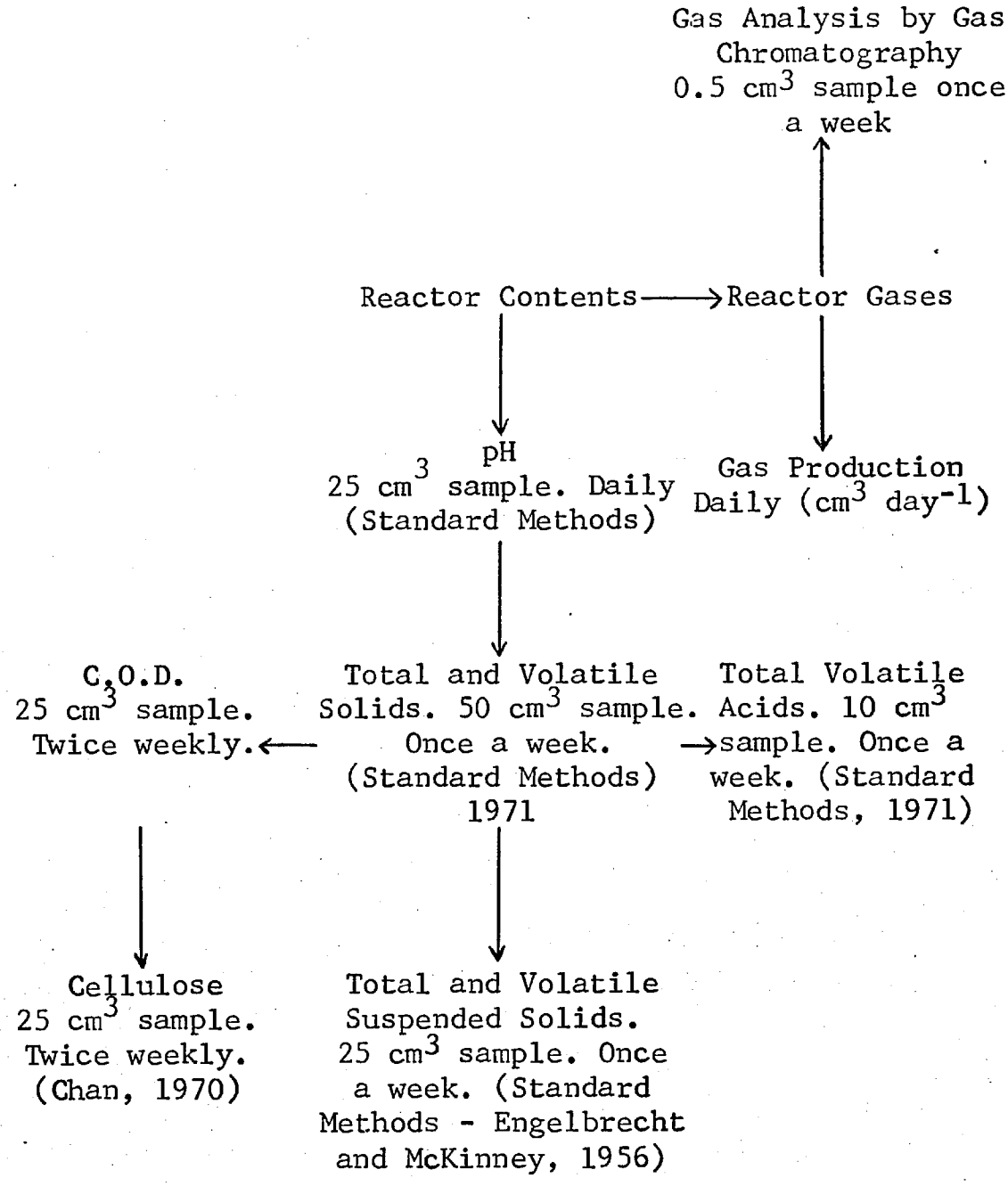


FIGURE 4.1 Analysis of Digester Contents and Gases.

and accurate means of gas analysis (Grune and Ghueh, 1962 and J.J. Van Huyssten, 1967).

The thermal conductivity detector was used along with a one column system to achieve full resolution and detection of carbon dioxide, oxygen and nitrogen mixture (recorded as air in this equipment) and methane. A 10 feet stainless stell column packed with Popapak Q (mesh size 100-120 μm) was used to achieve the separation of the gases.

Each of the gases had a characteristic resolving time which was determined by introducing pure samples of each gas into the chromatograph. The peak areas were determined by automatic area integration, and multiplied by the appropriate calibration factor to give the percent composition by volume for each component. The calibration curves were prepared and checked periodically by analysing gas samples of known composition prepared from pure bottled gases.

The components of a gas sample are represented qualitatively by the position of a peak on the time scale and quantitatively by the area of the peak above the base line. Hamilton gas-tight syringes were used to inject 0.5 cm^3-1 cm^3 gas samples in the detector column. For each recording three samples were injected, and the % C_v between

any three samples was never greater than 2%.

The statistical accuracy of the analysis was determined by analysing ten replicate samples containing carbon dioxide, oxygen, nitrogen and methane. The coefficients of variation ranged from a minimum of 0.5 to a maximum of 1.85.

Cellulose Content:

The method for cellulose determination was that developed by Chan (1970).

A 25 cm^3 aliquot of sample was diluted to 100 cm^3 with distilled water. To this was added 0.6 cm^3 of 50% NaOH w/w and 2.5 cm^3 of 1:8 dilution antifoam followed by refluxing for 30 minutes to decompose the proteins. The excess NaOH was washed out by centrifugation with distilled water and decantation. The sample was then held at 70°C in a water bath and 1.25 gm. of sodium chlorite and 0.5 cm^3 glacial acetic acid were added to hydrolyse the non-organic components and the sample was kept at 70°C for 5 minutes. The sample was then filtered through a fritted glass filter crucible, washed with distilled water until chloride free (test with 1 drop of $AgNO_3$ and 1 drop of 1% HNO_3) and then washed with 80% ethanol. The crucibles were dried and weighed, ignited, cooled and reweighed. The weight difference

between the two weights was cellulose.

The sodium chlorite and acetic acid dissolve the lignin and the holocellulose (residue of hemicellulose and cellulose) is extracted by reflux with 0.6 cm^3 sodium hydroxide. The residue is nearly pure cellulose; it normally contains trace amounts of hemicelluloses.

The accuracy and precision of the procedure for pure cellulose was determined by analysing five replicate samples. The average % cellulose recovery was 93.4% with a coefficient of variation (C_v) of 4.2%.

Using ten replicate samples each of grass, cow dung and horse manure the following C_v values were obtained:

1. Grass $C_v = 8.9\%$
2. Cow dung $C_v = 6.3\%$
3. Horse manure $C_v = 7.6\%$

The larger C_v values for grass, cow dung and horse manure were partially due to experimental error and also due to interference by the fixed solids - this was apparent because the resulting cellulose had a brown tinge instead of the usual white appearance. To reduce this error the time of the solubilising stage was increased and the concentrations of acetic acid and sodium chlorite were doubled (Klein, 1972).

Measurement of Substrate Concentration and Cell Concentration:

Two important parameters required in the analysis of the digestion process are:

I. Substrate Concentration:

This is the concentration of substrate responsible for the production of intermediate digestion products which are further digested to methane and carbon dioxide. It is assumed in this study that the initial hydrolysis step is the rate limiting step in the digestion process and therefore the substrate concentration is taken as being equal to the concentration of the digestible solids.

II. Cell Concentration:

This is the resulting biomass concentration produced by the utilisation of the substrate concentration.

1. Substrate Concentration

A. Cellulose:

For the digesters being fed on pure cellulose the measurement of substrate concentration was determined by using a specific analysis for cellulose - refer to cellulose content determination.

B. Grass, Cow Dung and Horse Manure:

The substrates used in this study - grass, cow dung and horse manure - contain a variety of compounds capable of being digested to methane and carbon dioxide via the intermediate products formed and therefore a non-specific measure of the concentration of organic substrate in the digester was used. Two such determinations are:

1. Chemical Oxygen Demand, C.O.D.
2. Biological Oxygen Demand, B.O.D.

The measurement of C.O.D. is recommended to the B.O.D. measurement because the latter analysis often gives non-reproducible results due to the varying degrees of oxidation during the test. B.O.D. values also vary because of the oxidation of ammonia to nitrate that may take place during the test. The B.O.D. test is also very time consuming and is affected by seed acclimation.

The C.O.D. is a measure of the oxygen equivalent of the organic fraction in the sample which is susceptible to permanganate or dichromate oxidation in acid solution.

The C.O.D. analysis suffers from the following disadvantages:

1. The analysis measures almost all the organic matter present without regard for its availability to the microorganisms - many organic compounds are not biologically oxidizable.

2. The C.O.D. analysis cannot differentiate between one constituent and another and is therefore a bulk determination of the organic components.

Gaudy and Gaudy (1972) have assessed the use of the C.O.D. as a measure of substrate concentration and conclude that in studies using complex wastes, the constituents of which are not known, only a measurement of C.O.D. can be employed. Even in laboratory experiments using a defined medium, excretion of partially oxidised products of metabolism often occurs (Krishnan and Gaudy, 1965) and therefore measurements based on specific analyses for the original substrate result in false estimates of purification in such cases and Gaudy and Gaudy (1972) therefore recommend the use of C.O.D. for substrate concentration even though more specific analyses may be made.

The method of C.O.D. Measurement:

The C.O.D. of the volatile matter for the effluent samples was found by:

1. Filtering the effluent sample through a 25 μm. filter, discarding the filtrate, drying and weigh ing and analysing for:

A. The volatile matter content according to Standard Methods (1971).

and B. Determining the COD of the volatile matter using the CHN analyser (as used for the carbon analysis). Since the number of samples was very large and the COD determination according to Standard Methods (the dichromate refluxing technique) requires a considerable amount of time and effort (two hours per sample are required for the refluxing stage), it was decided to use the carbon analyser. The analyser is specifically designed for the determination of the CHN content in organic samples and is a fully automatic instrument except for the weigh ing of the samples. Assuming a complete oxidation of the CHN components in the volatile matter the COD per unit weight of substrate (volatile matter) oxidised is found (analyser sample size is 1 to 10 mg.).

II. Cell Concentration

An accurate determination of the active cell mass for a system digesting insoluble materials is a difficult task. Various methods have been used to evaluate the organism concentration, such as the following:

1. Dry weight of suspended matter. This has been used by Thimann (1955) and most microbiologists. Volatile suspended matter has also been used for anaerobic fermentation systems by Stewart (1958), Andrews et al (1964), Toerien et al (1967) and Gates et al (1967).

2. Measurement of elementary cellular constituents such as carbon, nitrogen and phosphorus by Lawrence and McCarty (1969).

3. Employment of scattered or transmitted light as a means of measuring bacterial densities by Contois (1959).

4. Organism concentration expressed in terms of the number of living cells per unit volume either by direct microscopic count or estimated by a viable cell plate count.

All of the above methods have been applied to

systems being fed essentially with soluble substrates such as glucose - in such systems it is an easy task to differentiate between substrate and cell concentration. However, the substrates used in this study consist mainly of insoluble material plus small quantities of soluble matter. Such feedstocks contain carbon and nitrogen and on digestion form a variety of C/N compounds which interfere with specific analyses such as light transmission or C/N analysis for cell concentration.

A total viable cell determination (T.V.C.) would require extensive analysis and expertise if a meaningful determination of the mixed culture viability is to be obtained, and such a determination was not possible in this study.

Toerien et al (1967) and Agardy et al (1963) observed that in an anaerobic fermentation system, volatile suspended solids concentration has a good correlation with bacterial cell count and D.N.A. concentration. In his study of the anaerobic digestion of cellulose, Chan (1970) selected the volatile suspended solids determination as the primary method for estimating cell concentration. Chan measured the V.S.S. by subtracting the cellulose concentration (determined separately) in a given sample from the

total volatile suspended solids concentration (includes cell concentration and cellulose concentration) from the sample.

The V.S.S. measurement is a mass measurement and includes both living and dead organisms. The measurement takes no account of the activity of the organisms and it is therefore possible to have identical weights of V.S.S. with widely different activities. The V.S.S. measurement also includes organic matter stored in the cell or adsorbed on the surface of the cell. Besides these drawbacks, Gaudy and Gaudy (1972) consider that gravimetric measurement has the widest applicability for cell concentration determination and is also a very accurate method.

On this basis and for being a quick and reliable method, the V.S.S. determination was used for the cell concentration. The method used was that given in Standard Methods (1971) with a modification to account for the presence of insoluble substrate.

Method of V.S.S. Measurement

A. Cellulose:

The output samples from the digesters fed on cellulose contained undigested cellulose, soluble products and volatile suspended solids (cells). Since the cellulose

concentration was easily determined, then the volatile suspended solids could be found by analysing for total volatile suspended solids (includes cellulose and cell concentration) and then subtracting the cellulose concentration in order to give the V.S.S. of the cells only. The V.S.S. determination was carried out according to Standard Methods (1971) using a 25 cm^3 sample. Fifteen consecutive determinations of V.S.S. on the same sample yielded a % C_v of 1.5%.

B. Grass, Cow Dung and Horse Manure:

Since the samples contained insoluble material - undigested volatile solids and fixed solids - it was necessary to remove these before analysing for V.S.S. Therefore the following procedure was adopted:

1. The sample was vacuum filtered through a 25 μm filter to remove solids other than cells (bacterial cell size about 1 μm).

2. A 25 cm^3 sample of the filtrate from 1 was then filtered through a 0.45 μm (4.25 cm. dia.) glass fibre filtre pad, dried at 105°C for 2 hours and then ignited at 600°C for 30 minutes. The difference in weight gave the V.S.S. concentration.

Engelbrecht and McKinney (1956) obtained an average % C_v of 1.12% for the V.S.S. determination using the filter pad (0.45 μm) technique.

Since the solids produced by stage 1 above removed a portion of the cells (attached to the solids) it was necessary to determine this quantity. The solid sample was therefore added to 25 cm^3 distilled water and homogenised using an ultrasonic cleaning tank. To aid deflocculation of the cells from the solids sodium tripolyphosphate solution (5 $mg.lit^{-1}$) was added as a deflocculant. Following deflocculation the sample was diluted (1:20) with distilled water and a helber bacteria counting chamber was used for counting the number of cells.

A total count was also carried out on the filtrate sample used for the V.S.S. determination. This procedure was followed for thirty samples from digesters being fed on different substrates at different steady state conditions and for all cases the number of cells counted from the homogenised solids accounted for less than 3% of the total count on the filtrate sample.

Carbon Content of Solid and Liquid Components

A Carlo Ebba elemental analyser model 1102 was

used for carbon analysis of the solid and liquid samples. A 5 cm^3 sample of the digester effluent was vacuum filtered through a glass fibre prefilter (0.45 μm, 4.25 cm^3 dia. GF/C). The solid sample was then dried at 105°C and weighed before being mixed and ground to a powder. Three samples were then weighed out and analysed for carbon. The % C_v between any three samples was less than 4.5%. With all samples analysed a standard (containing a known quantity of carbon) was used and the analyses were corrected to the standard determination. The carbon content of the sample was designated C_s.

The filtrate from above was used for carbon determination of the liquid sample. After weighing the liquid sample three portions were weighed out (volume < 10 μl) and then injected into the carbon analyser. The samples were subject to evaporation and to loss of carbon dioxide; however, the % C_v between any three replicate samples was less than 3%. The carbon content of the filtrate was designated C_f.

4.2.5 Calculations for Continuous Digestion

Material balances were made for each detention time studied by tabulating the inputs and outputs to each digester over a one week period. This period is referred to as the balance period, five such balance periods were made for each steady state at a particular operating condition.

The following parameters have been determined for all digesters fed on cellulose.

1. The input cellulose concentration, dry weight, (S_o), experimentally known (mg. lit^{-1}).

2. The output cellulose concentration, (S_1), experimentally determined by cellulose analysis (mg. lit^{-1}).

3. The percentage digestion as defined by

$$\% \text{ dig} = \left(\frac{S_o - S_1}{S_o}\right) \times 100$$

4. V.S.S. concentration (X_1) - experimentally determined (mg. lit^{-1}).

5. The rate of digestion per unit cell concentration (X_1) per unit digester volume, designated q,

$$q = \frac{S_o - S_1}{X_1 \theta} \quad (day^{-1})$$

6. The rate of digestion per unit digester volume, designated R,

$$R = \frac{S_o - S_1}{\theta} \quad (\text{mg. lit}^{-1}\ \text{day}^{-1})$$

7. The yield constant Y, calculated as $\frac{X_1}{S_o - S_1}$.

8. The total gas production G_T, corrected to dry conditions at 0^oC and 760 mm Hg ($cm^3\ day^{-1}$).

9. The percentage methane content (% CH_4) of the gas produced, experimentally determined.

10. The carbon content of the cellulose and liquid medium input, C_i (mg. day^{-1}).

11. The carbon content of the gas produced, for methane and carbon dioxide, C_g (mg. day^{-1}). Since the percentage methane content is known then C_g is determined.

12. The carbon content of the filtrate, experimentally determined, C_f (mg. day^{-1})

13. The carbon content of the solids after filtration experimentally determined, C_s (mg. day^{-1}).

14. The percentage carbon recovery, as defined by

$$\%\ C_{rec} = \frac{(C_g + C_f + C_s)}{C_i} \times 100$$

The results obtained for the above parameters are given in Appendix III, tables III.1, III.2 and III.3 for the digestion of cellulose. At each detention time the results obtained for five balance periods and the average and coefficient of variation (% C_v) for the results are shown in the tables.

For the range of temperatures and loading rates studied the average conditions - $_pH$, alkalinity, total volatile acid concentration, output cellulose concentration (S_o), total gas production G_T, and the percentage methane content of the gas produced - are plotted versus detention time in graphs 5.1 and 5.2 for the digestion of cellulose.

The following parameters have been determined for all digesters fed on grass, cow dung and horse manure.

1. The input COD of the volatile matter (COD_{vm}) concentration, (S_o), experimentally determined (mg. lit $^{-1}$).

2. The output COD_{vm} concentration, (S_1), experimentally determined (mg. lit $^{-1}$).

3. The percentage digestion, as defined by

$$\% \text{ dig} = \frac{(S_o - S_1)}{S_o} \times 100$$

4. V.S.S. concentration (X_1), experimentally determined (mg. lit^{-1}).

5. The rate of digestion per unit cell concentration (X_1) per unit digester volume, designated q,

$$q = \frac{S_o - S_1}{X_1 \theta} \quad (day^{-1}).$$

6. The rate of digestion per unit digester volume designated R,

$$R = \frac{S_o - S_1}{\theta} \quad (mg.\ lit^{-1}\ day^{-1}).$$

7. The yield constant Y, calculated as $\frac{X_1}{S_o - S_1}$.

8. The total gas production G_T, corrected to dry conditions at 0^oC and 760 mm H_g ($cm^3\ day^{-1}$).

9. The percentage methane content (% CH_4) of the gas produced, experimentally determined.

10. The carbon content of the substrate and liquid medium input feed, designated C_i (mg. day^{-1}).

11. The carbon content of the gas produced, for methane and carbon dioxide, designated C_g. Since the percentage methane content is known then C_g is determined (mg. day^{-1}).

12. The carbon content of the filtrate, experimentally determined, designated C_f (mg. day $^{-1}$).

13. The carbon content of the solids after filtration, experimentally determined, C_s (mg. day $^{-1}$).

14. The percentage carbon recovery as given by,

$$\% C_{rec} = \frac{(C_g + C_f + C_s)}{C_i} \times 100$$

15. The percentage cellulose digestion, experimentally determined and defined by

$$\% C_e \text{ dig} = \left[\frac{(\text{cellulose concentration})_{input} - (\text{cellulose concentration})_{output}}{(\text{cellulose concentration})_{input}}\right] \times 10$$

The results obtained for the above parameters, for the digestion of grass are given in Appendix III, tables III.4 to III.9 (inclusive), similarly for cow dung the results are given in Appendix III, tables III.10 to III.15 (inclusive), and for horse manure the results are given in Appendix III, tables III.16 to III.18 (inclusive). For the range of temperatures and loading rates studied the average conditions - pH, alkalinity, total volatile acid concentration, output substrate concentration, total gas

production and methane content of gas produced - are plotted in graphs 5.6, 5.7 and 5.8. versus detention time for the digestion of grass. Similarly for cow dung the average conditions are plotted in graphs 5.13, 5.14 and 5.15 and for horse manure the average conditions are shown in graph 5.20.

CHAPTER 5

RESULTS

5.1 Batch Digestion

For the operating conditions shown in table 4.0, the experimental results obtained from the batch digestion of grass, cow dung and horse manure are given in tables 5.1, 5.2 and 5.3 respectively.

The gas produced was collected for the period given in the tables after which the gas production fell below 10 cm^3/day for more than five days.

The percentage volatile matter digestion given in the tables is calculated from the solid fraction of the remaining digester contents. The digester contents were filtered through a 25 μm. filter before analysing for the volatile matter.

The maximum methane gas produced /gm of substrate added is not significantly affected by temperature , as shown in tables 5.1 to 5.3 for the digestion of grass, cow dung or horse manure.

	Temperature = 28°C	Temperature = 38°C	Temperature = 48°C
% Volatile Matter Digested	88.7	89.8	90.8
% Cellulose Digested	90.1	89.1	93.2
% CH_4 (average)	59	57.5	58.5
Methane produced/gm. grass added (cm^3/gm. added)	236	248	236
Gas produced/gm. V.M. digested (cm^3/gm. dig).	552.5	575.2	532.1
Time of digestion (days)	52	45	38

Average of gas produced/gm. substrate added G_o = 411.5 cm^3 STP

Table 5.1 Batch Digestion of Grass

	Temperature = 28°C	Temperature = 38°C	Temperature = 48°C
% Volatile Matter Digested	82.4	87.5	90.0
% Cellulose Digested	82.1	89.8	87.6
% CH_4 (average)	57.5	58	58.0
Methane produced/gm. dung added (cm^3/gm. added)	163	171	170
Gas produced/gm. V.M. digested	537.5	526.5	508.9
Time of Digestion (Days)	49	42	37

Average of gas produced/gm. substrate added G_o = 290.4 cm^3 STP

Table 5.2 Batch Digestion of Cow Dung

	Temperature = 28°C	Temperature = 38°C	Temperature = 48°C
% Volatile Matter Digested	91.0	92.0	93.9
% Cellulose Digested	85.8	89.6	86.2
% CH_4 (average)	57	57.5	58
Methane produced/gm. manure added (cm^3/gm. added)	159	168	171
Gas produced/gm. V.M. digested (cm^3/gm. dig)	488.9	506.5	500.7
Time of Digestion (days)	49	43	35

Average of gas produced/gm. substrate added G_o = 288.6 cm^3 S.T.P.

Table 5.3 Batch Digestion of Horse Manure

5.2 Continuous Digestion

The treatment of the results for the continuous digestion of cellulose, grass, cow dung and horse manure is separated into four sections:

5.2.1 Plots of State Variables and Rate data.

(i) The state variables (average conditions) obtained for steady state operation - $_{P}H$, alkalinity, total volatile acid concentration, output substrate concentration (S_1), total gas production G_T, and the percentage methane content of the gas produced (% CH_4) are plotted versus detention time.

(ii) The rate data is plotted in the form of q versus S_1, where $q = \frac{S_o - S_1}{X_1 \theta}$, as outlined in 3.3, Equation 3.36, all parameters - S_o, S_1 and X_1 - being experimentally determined for steady state digester operation.

(iii) The rate data is also plotted as R versus S_1, where $R = \frac{S_o - S_1}{\theta}$, as outlined in 3.3, Equation 3.37, all parameters being experimentally determined for steady state digester operation.

(iv) The rate constants (best fit) obtained from the above plots are plotted according to the Arrhenius equation, as outlined in 3.3, equation 3.34.

The kinetic constants obtained from the above analysis are presented in the form of a summary table and the above treatment is repeated for each substrate.

5.2.2 A tabulated summary of the yield constant Y, (mass of cells produced per mass of substrate utilised) and the decay coefficient b, (accounts for decrease in cell concentration due to utilisation by a cell of its own substance and includes the decrease in cellular mass due to cellular death and subsequent lysis, also termed as the specific organism decay rate) is given for all substrates studied, as outlined in 3.3, equation 3.40.

5.2.3. A graphical plot of $\frac{1}{\theta}$ versus q and G_T, as outlined in 3.3, Equation 3.40. This plot is an indication of whether substrate utilisation and gas production are growth associated processes, i.e. whether gas is produced at the expense of substrate beingutilised with no intermediate product accumulation (volatile acids) or side reactions affecting the overall conversion yield.

5.2.4 A graphical plot of the percentage digestion versus detention time is given for all four substrates at the different operating temperatures. The percentage cellulose digestion occurring in the digestion of grass, cow dung and horse manure is shown on the same plot as the percentage digestion for the digesters fed on cellulose.

5.2.1.

I. Cellulose

The average conditions, p^H, alkalinity, total volatile acid concentration, etc., are shown in graphs 5.1 and 5.2 for digesters fed on cellulose.

Graph 5.1 shows the conditions obtained in the digestion of cellulose at an input concentration of 15000 mg. lit^{-1} (dry weight) at temperatures of 28°C and 38°C.

Graph 5.2 shows the conditions obtained in the digestion of cellulose at an input concentration of 25000 mg. lit^{-1} (dry weight) at a temperature of 38°C. (The results shown in this graph were obtained from the 50 lit. digester).

From the conditions shown in graphs 5.1 and 5.2, the digestion of the substrate is proceeding under "normal conditions" as previously defined in 2.2, as is true for the digestion of grass, cow dung and horse manure for the range of conditions studied.

Graph 5.3 is a plot of q versus S_1, for the conditions given below:

The graphs shown in graph 5.3 were plotted from the fifteen data points given in Appendix III (Tables III.1,

III.2 and III.3), five points at each detention time, for the different operating conditions.

Graph 5.3.4 is a plot of the results of Chan (1970).

Graph 5.3.1 S_o = 15000 mg. lit^{-1} (dry weight), temperature = 28°C. The results are correlated as $q = kS_1$, the gradient (k) of the best straight line and the correlation coefficient (r) are shown in the graph.

Graph 5.3.2 S_o = 15000 mg. lit^{-1} (dry weight), temperature = 38°C. The results are correlated as $q = kS_1$, the gradient (k) of the best straight line and the correlation coefficient (r) are shown in the graph.

Graph 5.3.3 S_o = 25000 mg. lit^{-1} (dry weight), temperature = 38°C. The results are correlated as $q = kS_1$, the gradient (k) of the best straight line and the correlation coefficient (r) are shown in the graph.

Graph 5.3.4. The results of Chan (1970), S_o = 13744 mg. lit^{-1} (dry weight), temperature = 35°C, are shown for

(i) The results correlated as $q = k S_1$,

(ii) The results correlated as $q = \frac{q \text{ max } S_1}{K_s + S_1}$, this is the correlation adopted by Chan.

Only fifteen of Chan's original 40 data points are shown. For the correlation $q = kS_1$ the fit is satisfactory for the results of 10 - 30 days detention time, the gradient (k) of the best straight line and the correlation coefficient(r) for this fit are shown in the graph.

For the correlation of $q = \frac{q_{max} \; S_1}{K_s + S_1}$, the fit is valid for the results of 6 - 30 days detention time, the correlation coefficent(r) for this fit is shown in the graph.

It can be seen that the linear fit $q = ks_1$ deviates considerably from the fit of Chan for detention times of less than 10 days.

From the linear regression it is clear that a significant correlation exists between q and S_1 for each of the different operating conditions as shown in graphs 5.3.1 5.3.2 and 5.3.3, and also in graph 5.3.4 for the results of Chan at detention times of 10 - 30 days. It is seen from the gradients of graph 5.3.2 and graph 5.3.3 that the output cellulose concentration is dependent on the input cellulose concentration, a result not predicted by the use of the Monod equation (Lawrence and McCarty model) as used by Chan, which predicts that the output substrate concentration should be independent of the input substrate concentration.

Graph 5.4 is a plot of R versus S_1, for the conditions given below:

The graphs shown in graph 5.4 are plotted from the fifteen data points given in Appendix III (Tables III.1, III.2 and III.3) five points at each detention time, for the different operating conditions. Graph 5.4.3 is a plot of the results of Chan (1970).

Graph 5.4.1. S_o = 15000 mg. lit $^{-1}$ (dry weight), temperature = 28^{o}C. The results are correlated as $R = KS_1$, the gradient (K) of the best straight line and the correlation coefficient (r) are shown in the graph.

Graph 5.4.2. S_o = 15000 mg. lit $^{-1}$ (· data points) and 25000 mg. lit $^{-1}$ (• data points) (dry weight), temperature = 38^{o}C. The data obtained at the two input cellulose concentrations has been shown on the same graph, because it was found than when correlating the results according to $R = KS_1$, the difference in the values of the gradients for the fits at the two input cellulose concentrations was insignificant at a 95% confidence level using the 't' test. Therefore a single best straight line is shown in the graph with the gradient (K) and the correlation coefficient (r).

Graph 5.4.3. The results of Chan (1970), S_o = 13744 mg. lit $^{-1}$ (dry weight), temperature = 35^{o}C. The results are correlated as $R = KS_1$ for 10-30 days detention time, the gradient (K) of the best straight line and the correlation coefficient (r) are shown in the graph.

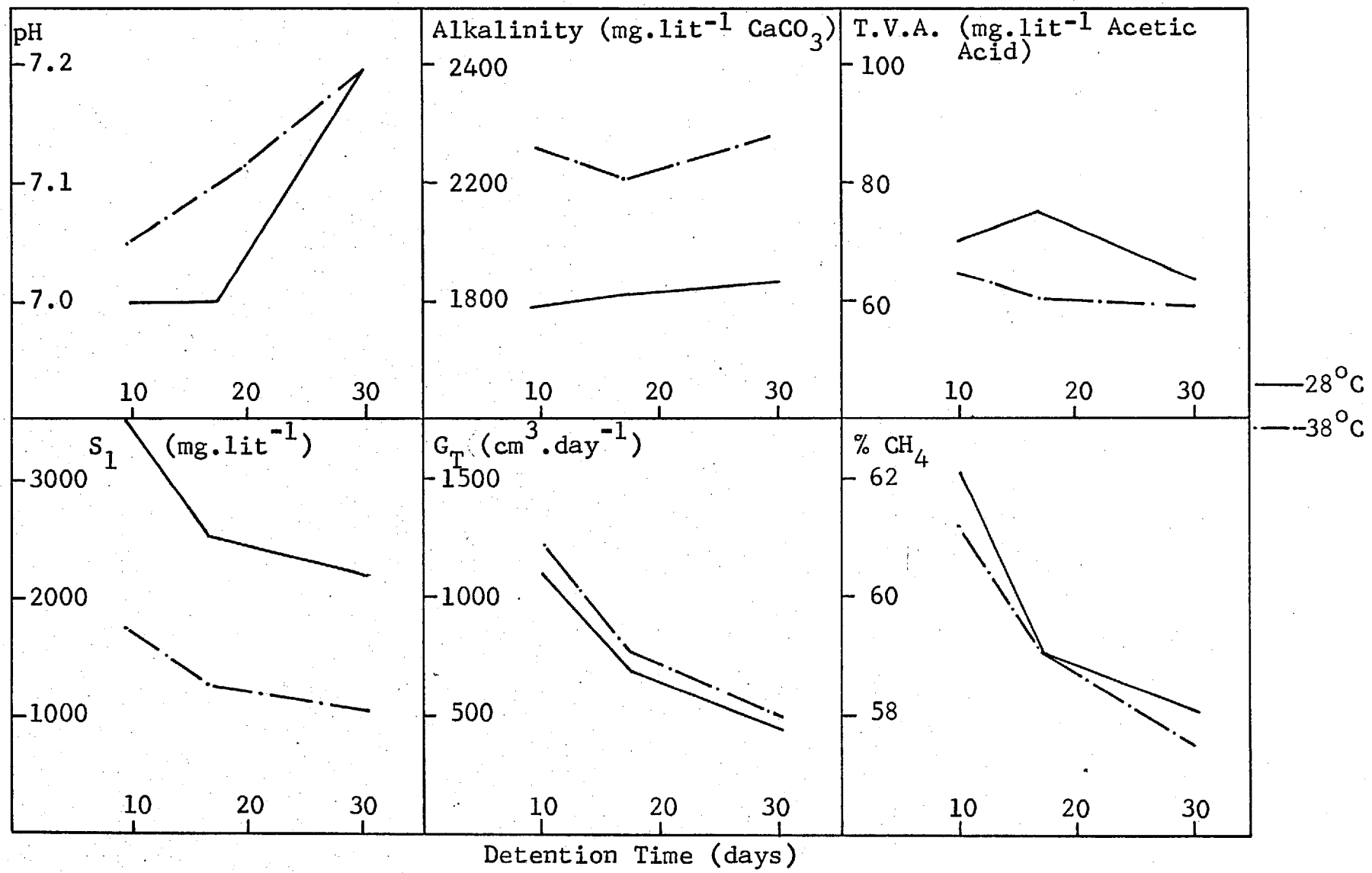
From the linear regression it is clear that a significant correlation exists between R and S_1, for the different operating conditions. It is seen from graph 5.4.2 that the percentage digestion was independent of the input cellulose concentration.

Graph 5.5 is a plot of $\log_{10}$ (rate constant) versus $\frac{1}{T}$ for the following conditions:

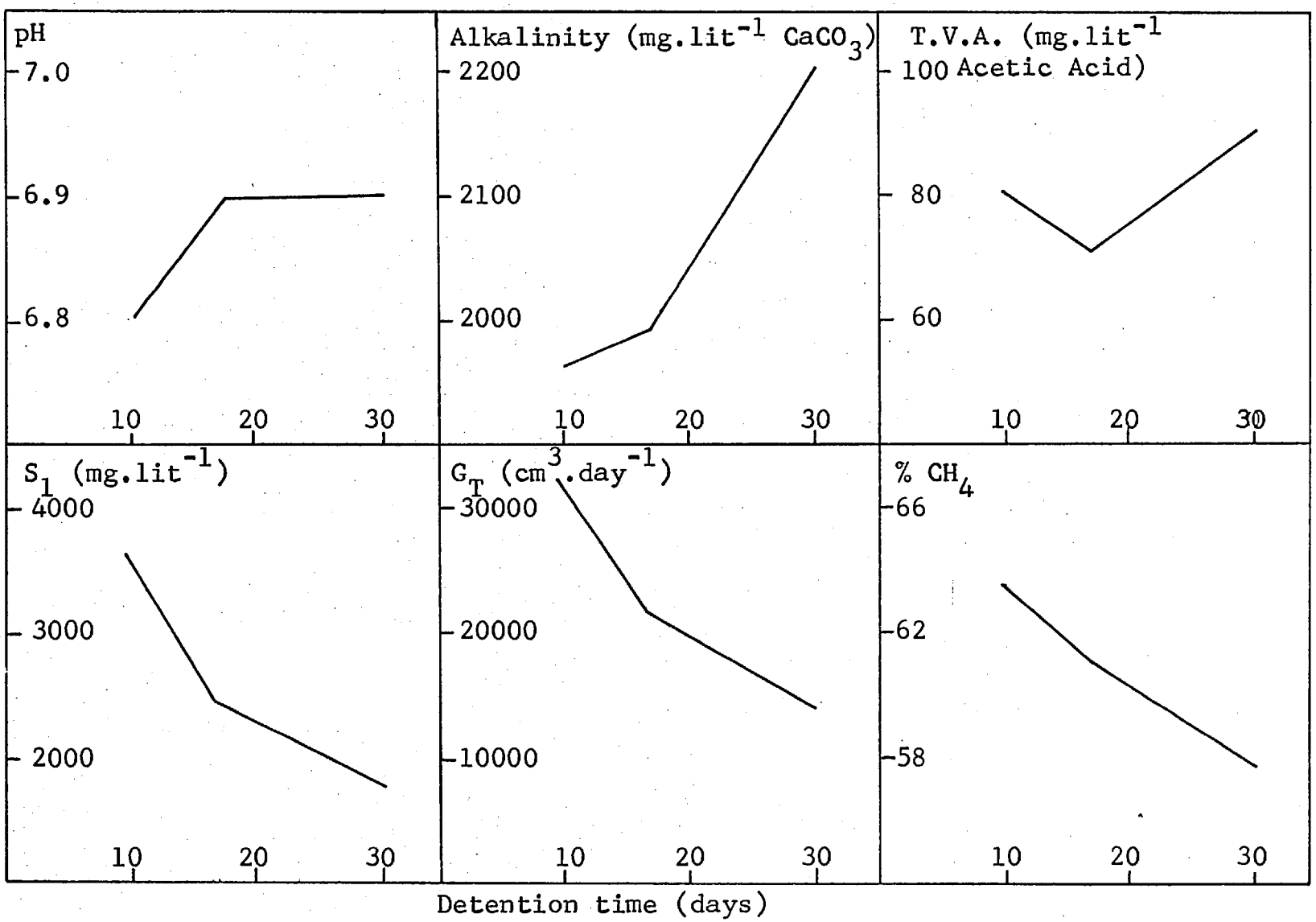
Graph 5.5.1. S_o = 15000 mg. lit $^{-1}$ (dry weight), temperature = 28^{o}C and 38^{o}C, for the values of k obtained from graphs 5.3.1 and 5.3.2. The slope of the plot is shown in the graph

Graph 5.5.2. S_o = 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$ (dry weight), temperature of 28^{o}C and 38^{o}C, for the values of K obtained from graphs 5.4.1. and 5.4.2. The slope of the plot is shown in the graph.

Table 5.4 is a summary of the constants obtained from graphs 5.2, 5.3, 5.4 and 5.5 for the digestion of cellulose.



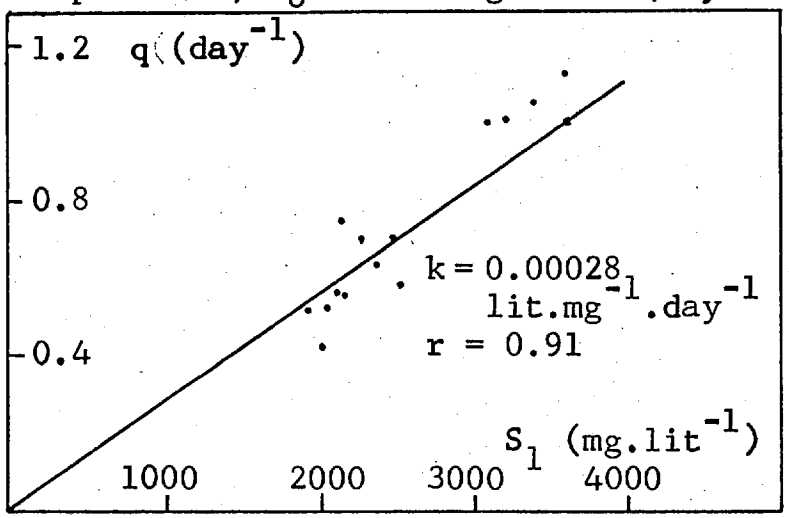
GRAPH 5.1 Average conditions plotted for the digestion of cellulose, S_1 = 15000 mg.lit^{-1} (dry weight).



GRAPH 5.2 Average conditions plotted for the digestion of cellulose, S_o = 25000 mg.lit^{-1} (dry weight, Temperature = 38°C (50 litre digester).

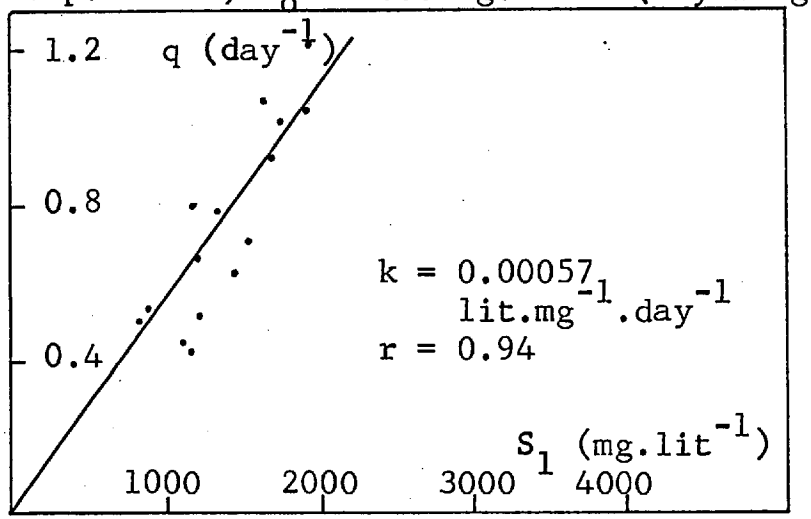
GRAPH 5.3.1

Temp. = 28°C, S_o = 15000 mg.lit^{-1} (dry weight)



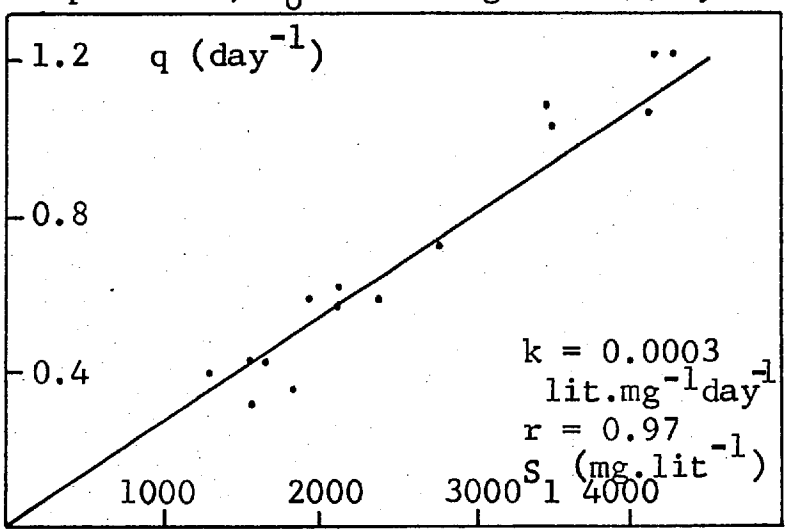
GRAPH 5.3.2

Temp. = 38°C, S_o = 15000 mg.lit^{-1} (dry weight)



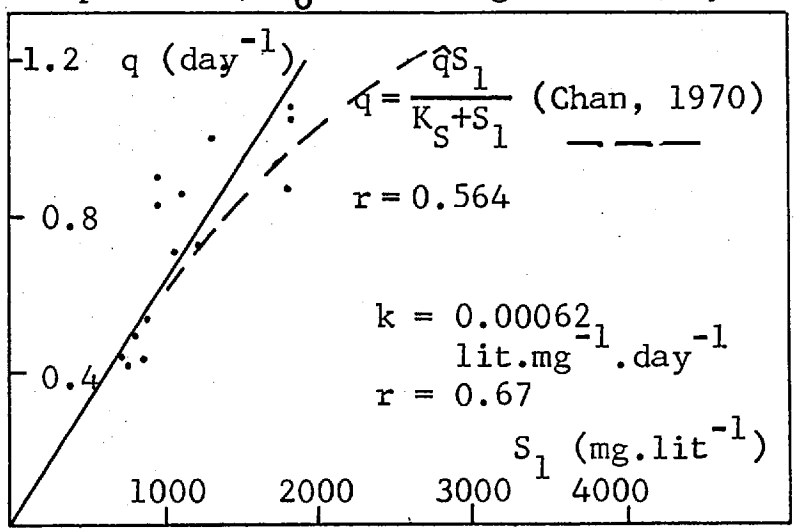
GRAPH 5.3.3

Temp. = 38°C, S_o = 25000 mg.lit^{-1} (dry weight)



GRAPH 5.3.4

Temp. = 35°C, S_o = 13744 mg.lit^{-1} (dry weight)

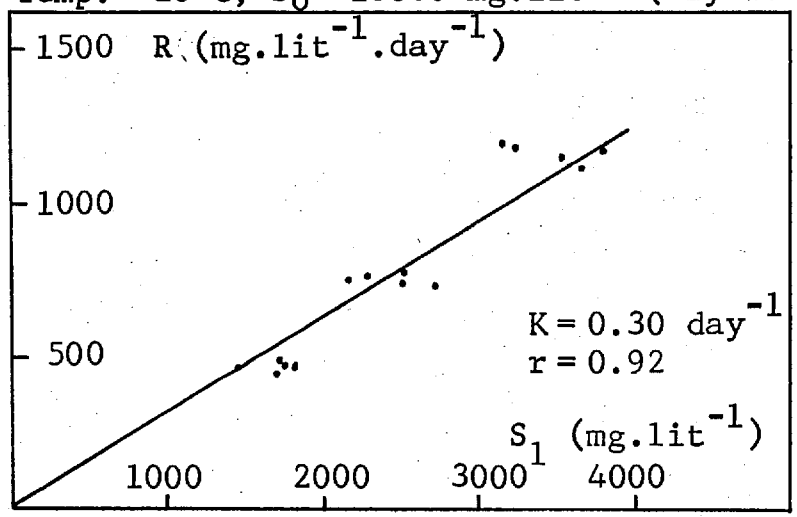


The results of Chan, 1970.

GRAPH 5.3 A plot of q versus S_1 for the digestion of cellulose.

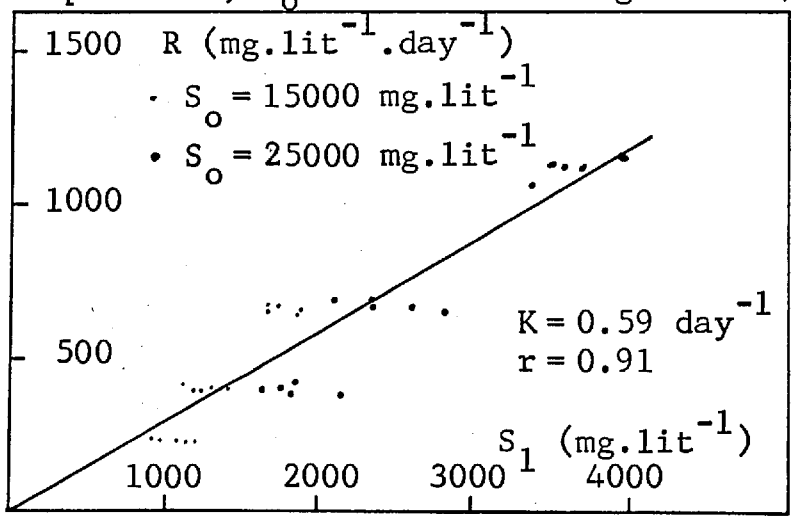
GRAPH 5.4.1

Temp. = 28°C, S_o = 15000 mg.lit^{-1} (dry weight)



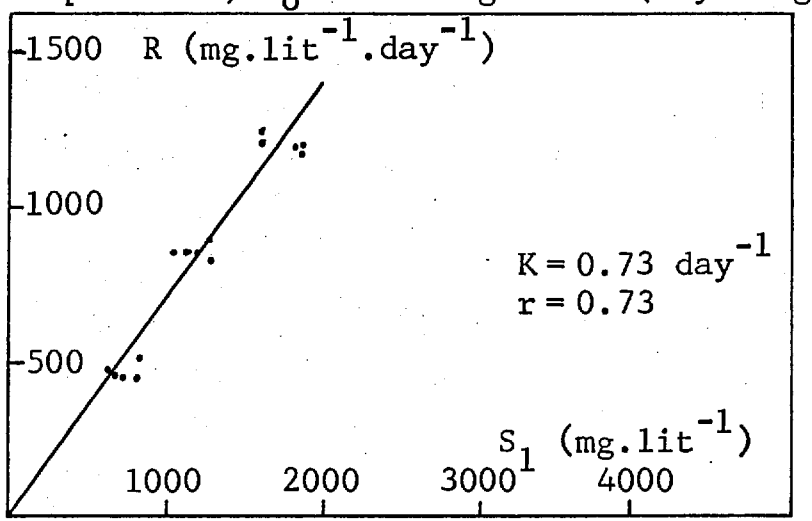
GRAPH 5.4.2

Temp. = 38°C, S_o=15000 & 25000 mg.lit^{-1} (dry weight)



GRAPH 5.4.3 The Results of Chan, 1970.

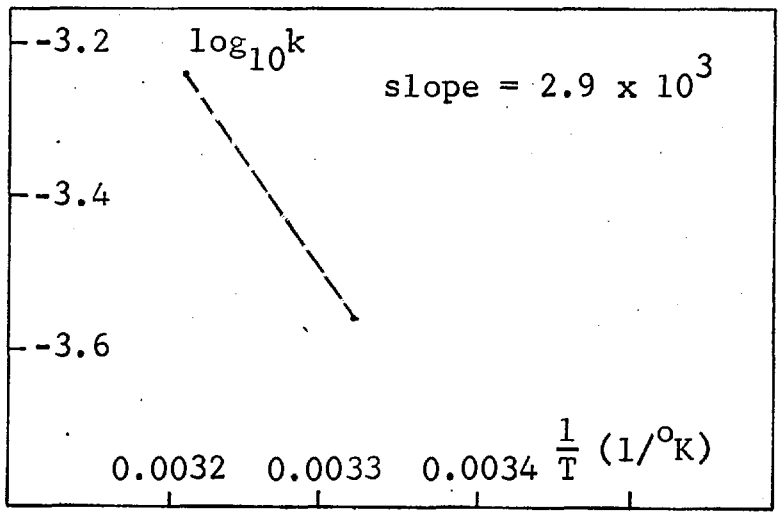
Temp. = 35°C, S_o = 13744 mg.lit^{-1} (dry weight)



GRAPH 5.4 A plot of R versus S_1 for the digestion of cellulose.

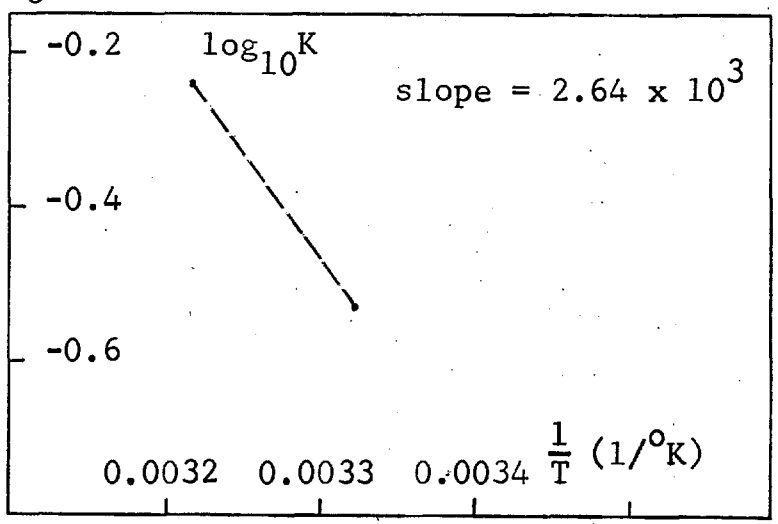
GRAPH 5.5.1

Values of k at 28°C and 38°C,
S_o = 15000 mg.lit^{-1} (dry weight).



GRAPH 5.5.2

Values of K at 28°C and 38°C,
S_o = 15000 mg.lit^{-1} (dry weight).



GRAPH 5.5 The Effect of Temperature on the values of the rate constants: Arrhenius plot for cellulose.

Kinetic Constant	Temperature = 28°C	Temperature = 38°C		The results of Chan - Temperature = 35°C	Slope of Arrhenius Plot: (cals./mode deg K.)
	S_o = 15000 mg. lit^{-1} (dry weight)	S_o = 15000 mg. lit^{-1} (dry weight)	S_o = 25000 mg. lit^{-1} (dry weight)	S_o = 13744 mg. lit^{-1} (dry weight)	
k ($k = \frac{q}{S_1}$ lit. mg.$^{-1}$ day^{-1})	0.00028	0.00057	0.00030	0.00062	
n	1.5	15	015	40	2.9×10^{3}**
r	0.91	0.94	0.97	0.67	
p	0.01	0.01	0.01	0.01	
K ($K = \frac{R}{S_1}$ day^{-1})	0.30	0.59	0.59*	0.73	
n	15	30	30	40	2.64×10^{3}***
r	0.92	0.91	0.91	0.73	
p	0.01	0.01	0.01	0.01	

n = number of data points
r = correlation coefficient
p = level of significance
* = as for S_o = 1500 mg. lit^{-1}
** = for k values at 28°C and 38°C, S_o = 15000mg. lit^{-1}
*** = for K values at 28°C and 38°C S_o = 15000 and 25000 mg. lit^{-1}.

Table 5.4 Summary of Kinetic Rate Constants for the Digestion of Cellulose

II . Grass

The average conditions are shown in graphs 5.6, 5.7 and 5.8 for digesters fed on grass.

Graph 5.6 shows the conditions obtained in the digestion of grass at input concentrations of 15000 mg. lit $^{-1}$ dry weight [18875 mg. lit $^{-1}$ $COD_{v.m}$] and 25000 mg. lit $^{-1}$ dry weight [31458 mg. lit $^{-1}$ $COD_{v.m}$], at a temperature of 28^{o}C.

Graph 5.7 shows the conditions obtained in the digestion of grass at input concentrations of 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$ (dry weight) at a temperature of 38^{o}C.

Graph 5.8 shows the conditions obtained in the digestion of grass at input concentrations of 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$ (dry weight) at a temperature of 48^{o}C.

Graph 5.9 is a plot of q versus S_1, for the following conditions:

The graphs are plotted using the fifteen data points, five points at each detention time for the different operating conditions, all data being given in Appendix III (Tables III.4 to III.9 inclusive).

Graph 5.9.1 Input grass concentration of 15000 mg. lit $^{-1}$ (dry weight), temperature of 28^{o}C. The results are correlated as $q = kS_1$, the gradient (k) of the best straight line and the correlation coefficient (r) are shown in the graph.

Graph 5.9.2. S_o = 15000 mg. lit $^{-1}$ (dry weight), temperature = 38^{o}C. The results are correlated as $q = kS_1$, the gradient (k) of the best straight line and the correlation coefficient (r) are shown in the graph.

Graph 5.9.3. S_o = 15000 mg. lit $^{-1}$ (dry weight), temperature = 48^{o}C. The results are correlated as $q = kS_1$, the gradient (k) of the best straight line and the correlation coefficient (r) are shown in the graph.

Graph 5.10 is a plot of q versus S_1, as graph 5.9, for the input concentration S_o = 25000 mg. lit $^{-1}$ (dry weight), and the graphs are for:

Graph 5.10.1	Temperature = 28^{o}C.
Graph 5.10.2	Temperature = 38^{o}C.
Graph 5.10.3	Temperature = 48^{o}C.

For all three graphs the results are correlated as $q = kS_1$, the gradient (k) of the best straight line and the correlation coefficient (r) are shown for each plot.

It is seen from the plots shown in graphs 5.9 and 5.10 that the output concentration ($COD_{v.m}$) is dependent on the input concentration for each of the three temperatures studied.

Graph 5.11 is a plot of R versus S_1 for the following conditions:

The graphs are plotted using the fifteen data points, five points at each of the three detention times studied - 10, 17 and 30 days, for the different operating conditions, all data being given in Appendix III (Tables III.4 to III.9 inclusive).

Graph 5.11.1 S_o = 15000 mg. lit $^{-1}$ (· data points) and 25000 mg. lit $^{-1}$ dry weight (• data points), at a temperature = 28°C. The data obtained at the two input concentrations has been shown on the same graph because it was found that when correlating the results according to $R = KS_1$ the difference in the values of the gradients for the fits at the two input concentrations was insignificant at a 90% confidence level using the 't' test. Therefore a

single best straight line is shown in the graph with the gradient K and the correlation coefficient (r).

Graphs 5.11.2 and 5.11.3 are for temperatures of 38°C and 48°C respectively. Once more the data is correlated as a single plot of $R = KS_1$ for S_o = 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$ (dry weight). The difference in the values of the gradients for the fits at the two input concentrations was not significant at a 95% confidence level using the 't' test.

The plots of Graph 5.11 show that the percentage digestion is independent of the input concentration for the three operating temperatures.

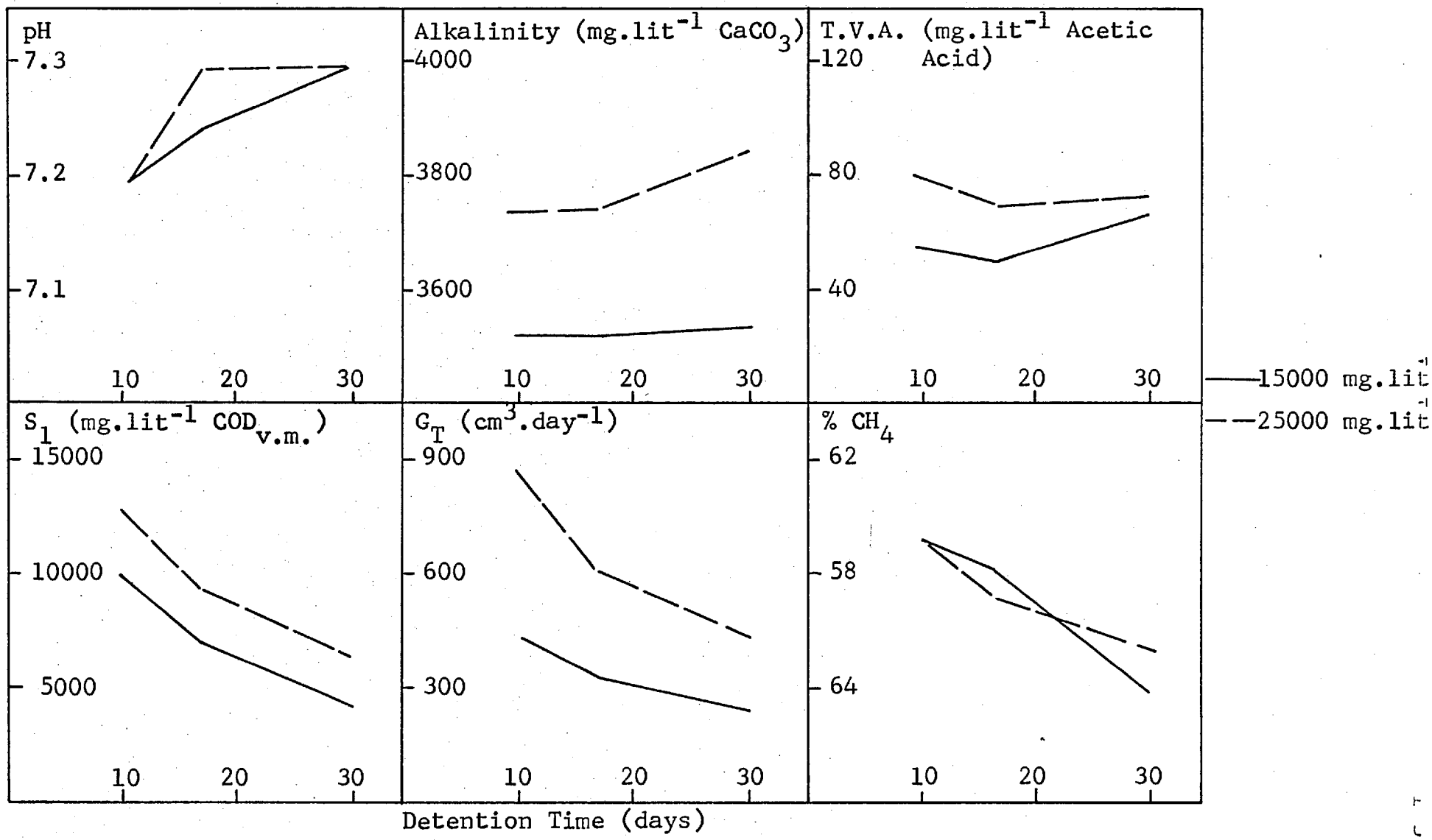
Graph 5.12 is a plot of $\log_{10}$ (rate constant) versus $\frac{1}{T}$ for the following conditions:

Graph 5.12.1 S_o = 15000 mg. lit $^{-1}$ (dry weight) at temperatures of 28°C, 38°C and 48°C for the values of k obtained from graphs 5.9.1, 5.9.2 and 5.9.3.

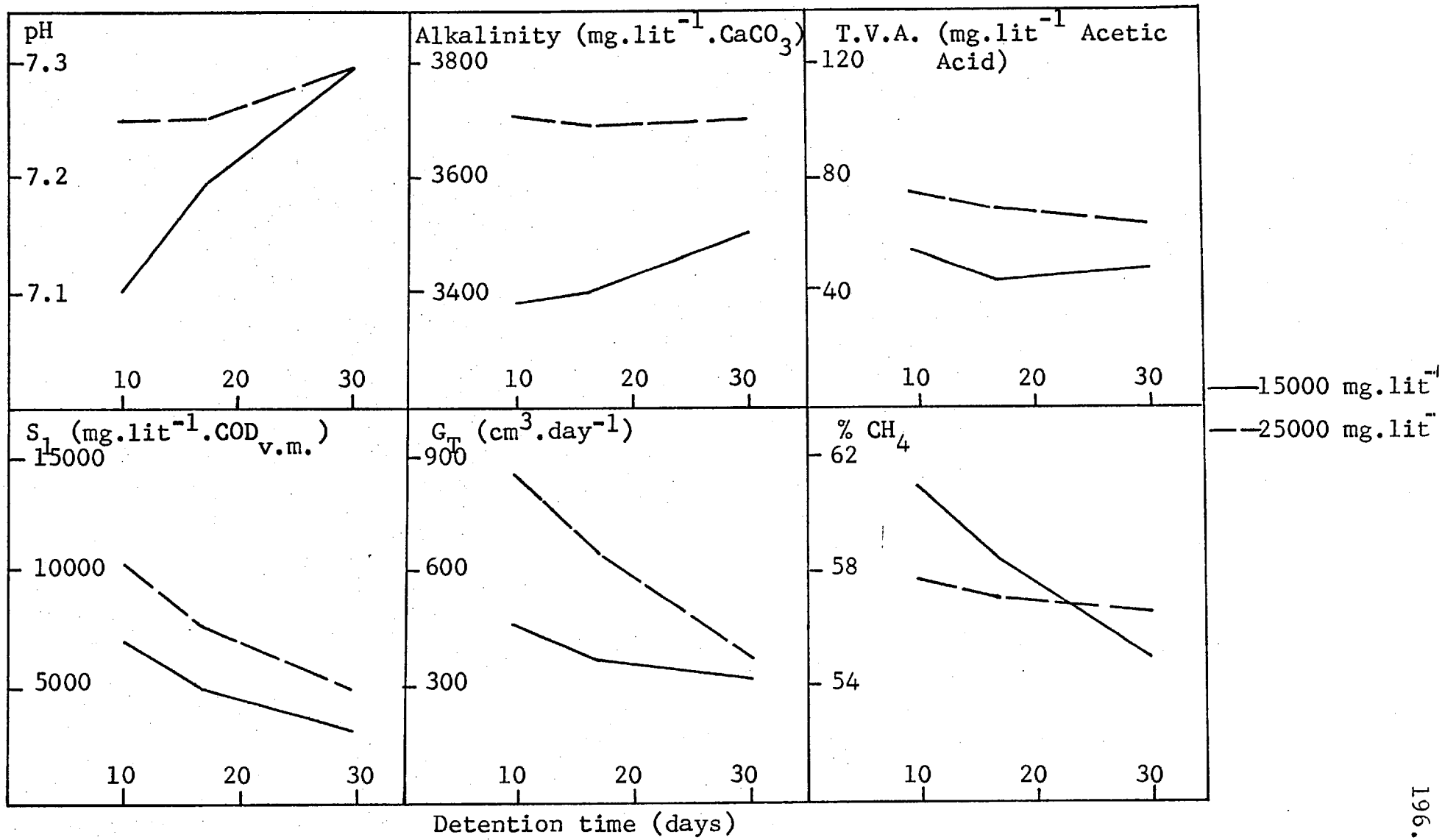
Graph 5.12.2 S_o = 25000 mg. lit $^{-1}$ (dry weight) at temperatures of 28°C, 38°C and 48°C for the values of k obtained from graphs 5.10.1, 5.10.2 and 5.10.3.

Graph 5.12.3 S_o = 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$ (dry weight) at temperatures of 28°C, 38°C and 48°C for the values of K obtained from graphs 5.11.1, 5.11.2 and 5.11.3.

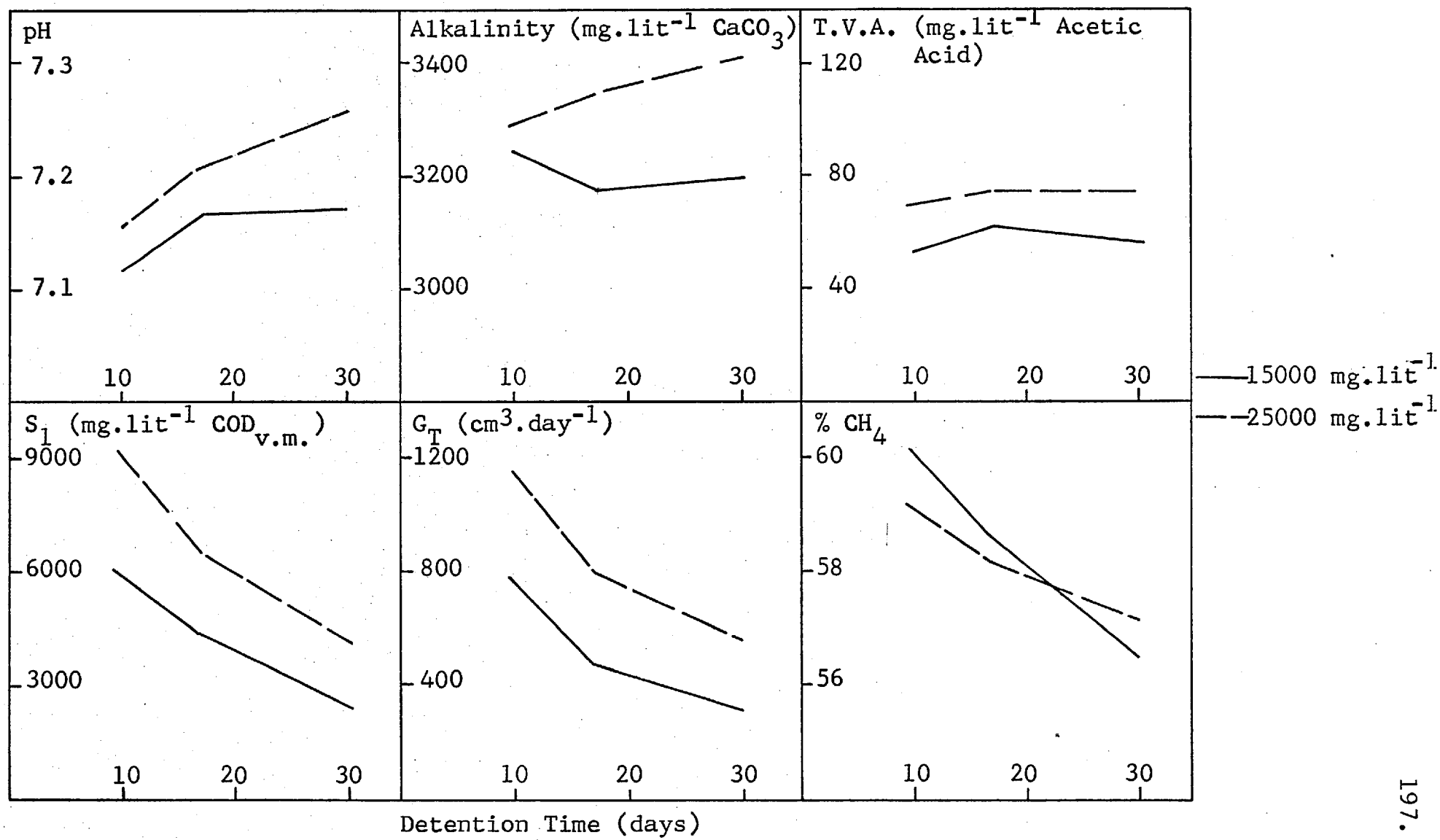
Table 5.5 is a summary of the constants obtained from graphs 5.9, 5.10, 5.11 and 5.12.



GRAPH 5.6 Average Conditions plotted for the digestion of Grass at an input concentration of 15000 and 25000 mg.lit^{-1} (dry weight), Temperature = 28°C.



GRAPH 5.7 · Average conditions plotted for the digestion of grass at an input concentration of 15000 mg.lit^{-1} and 25000 mg.lit^{-1} (dry weight), temperature = 38°C.



GRAPH 5.8 Average Conditions plotted for the digestion of Grass at an input concentration of 15000 and 25000 mg.lit^{-1} (dry weight), temperature = 48^{o}C.

TABLE 5.9.1

Temperature = 28°C

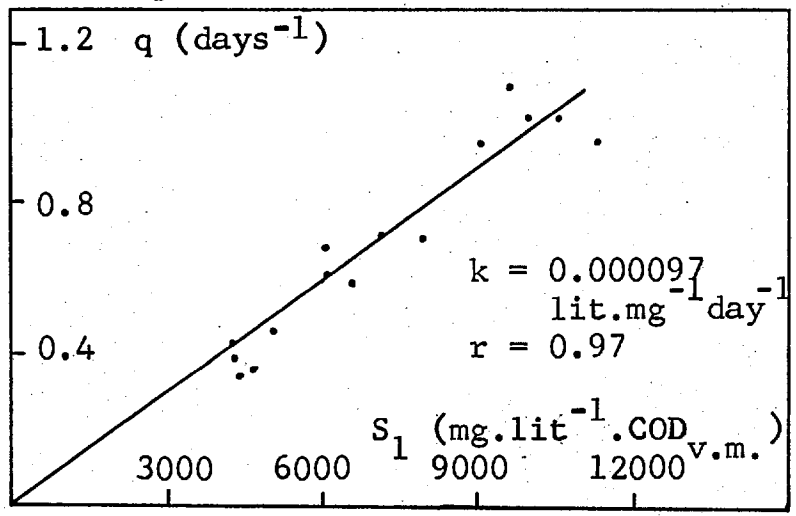


TABLE 5.9.2

Temperature = 38°C

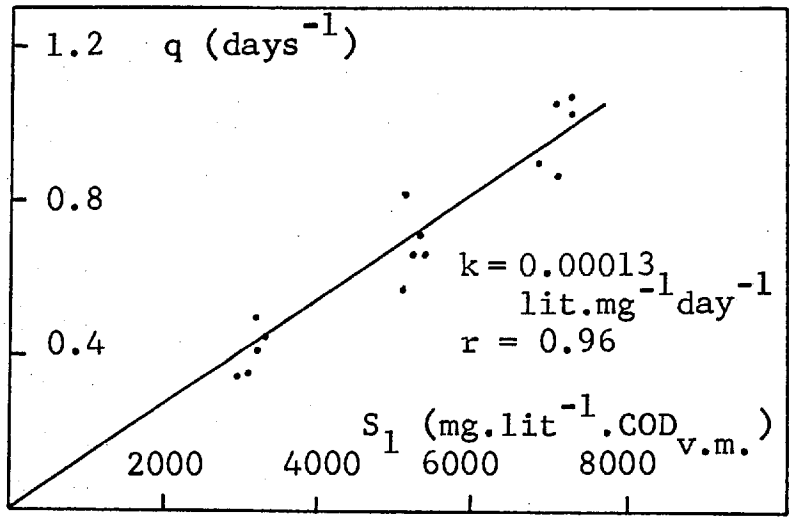
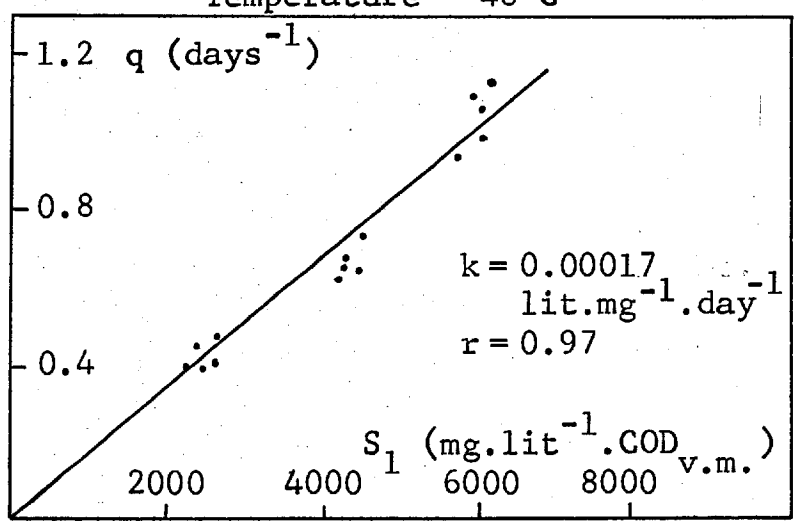


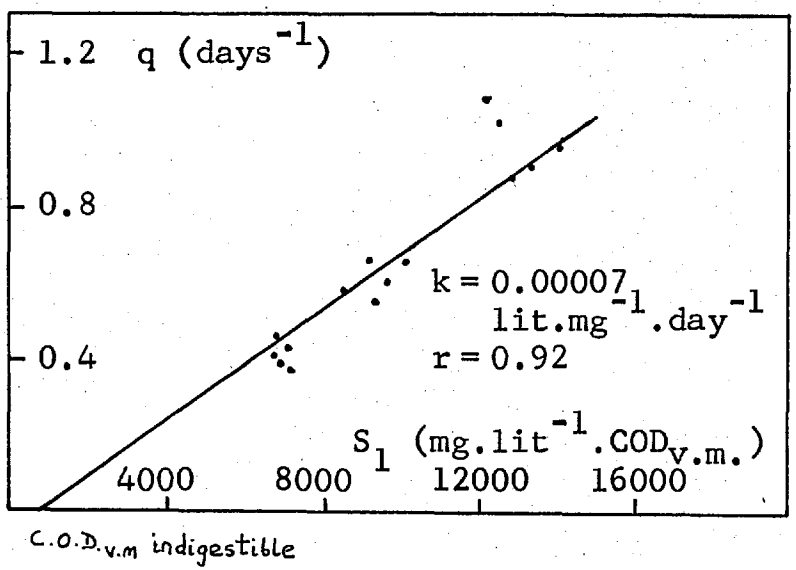
TABLE 5.9.3

Temperature = 48°C

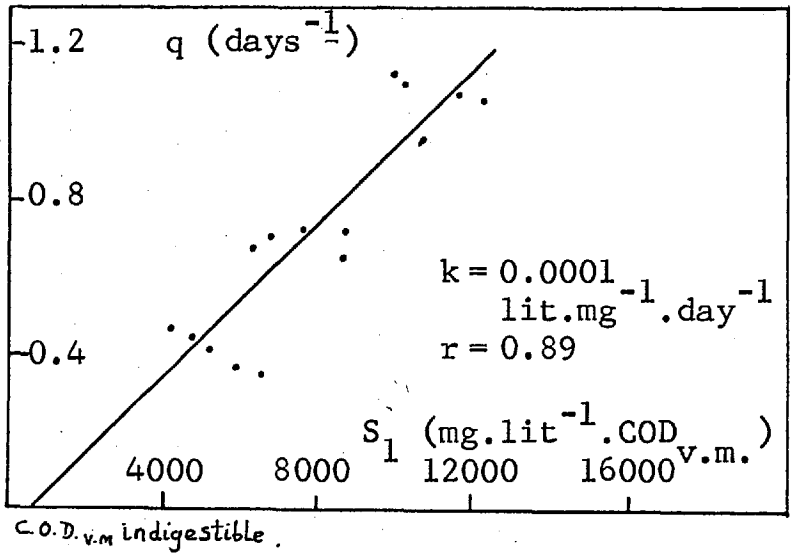


GRAPH 5.9 A plot of q versus S_1 for the digestion of grass (S_o = 15000 mg.lit^{-1} dry weight)

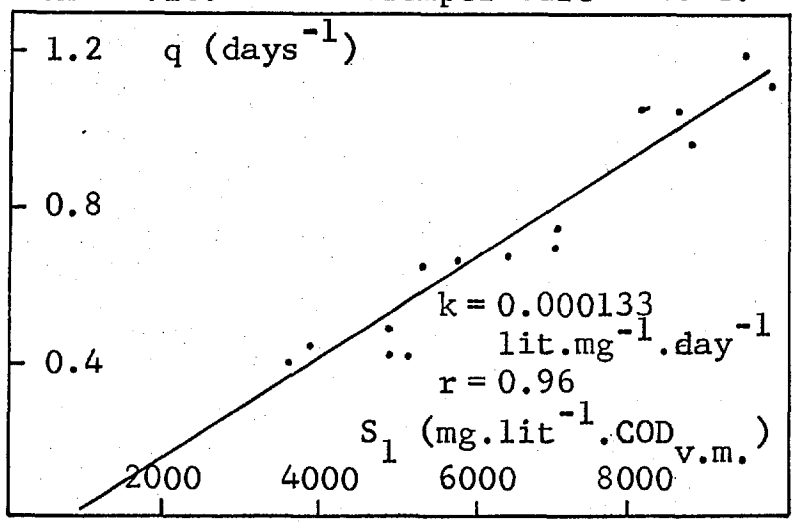
GRAPH 5.10.1 Temperature = 28°C.



GRAPH 5.10.2 Temperature = 38°C.

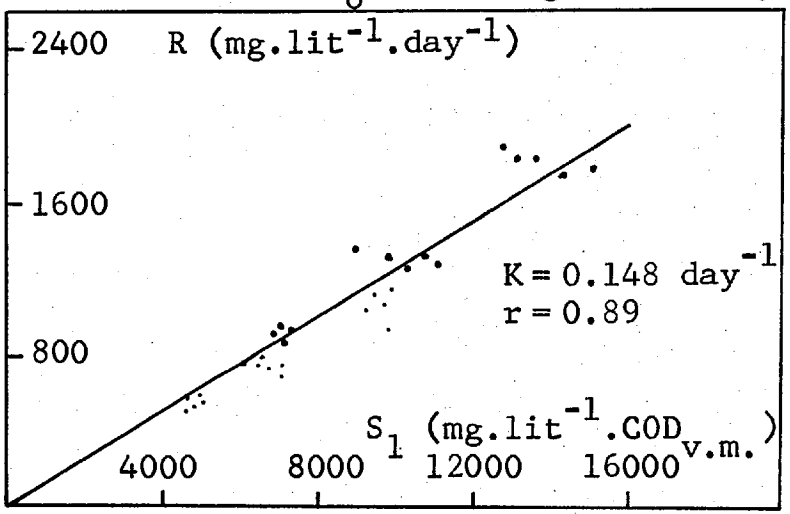


GRAPH 5.10.3 Temperature = 48°C.

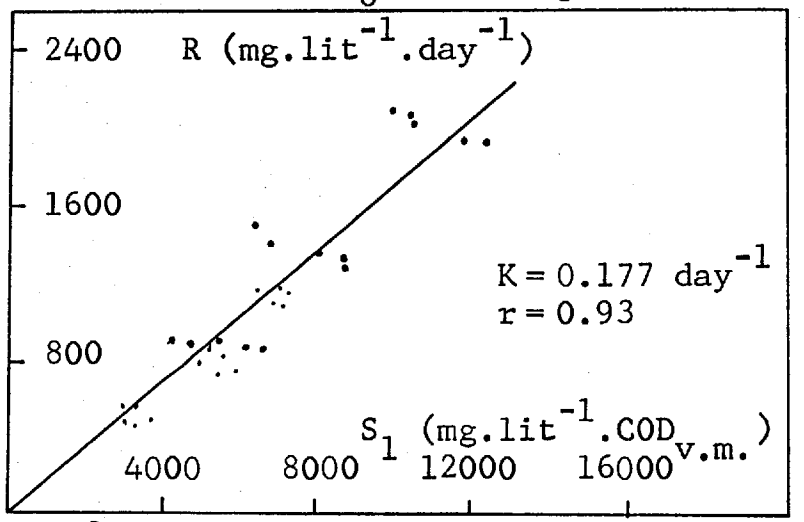


GRAPH 5.10 A plot of q versus S_1 for the digestion of grass (S_o = 25000 mg.lit^{-1} dry weight).

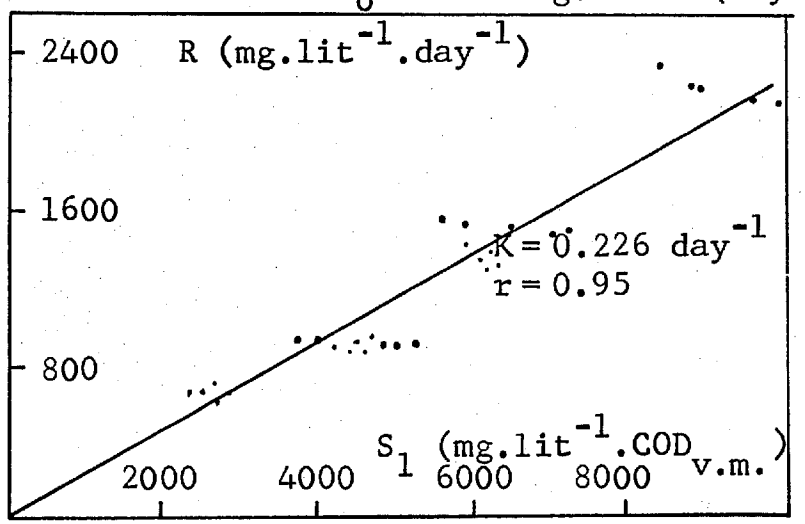
GRAPH 5.11.1 Temperature = 28°C,
· S_o = 15000 mg.lit^{-1} (dry weight),
• S_o = 25000 mg.lit^{-1} (dry weight).



GRAPH 5.11.2 Temperature = 38°C,
· S_o = 15000 mg.lit^{-1} (dry weight),
• S_o = 25000 mg.lit^{-1} (dry weight).

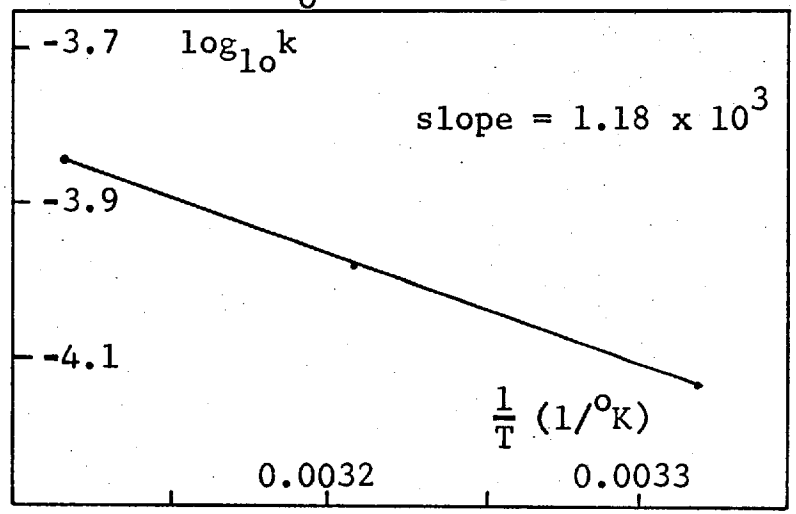


GRAPH 5.11.3 Temperature = 48°C,
· S_o = 15000 mg.lit^{-1} (dry weight),
• S_o = 25000 mg.lit^{-1} (dry weight).

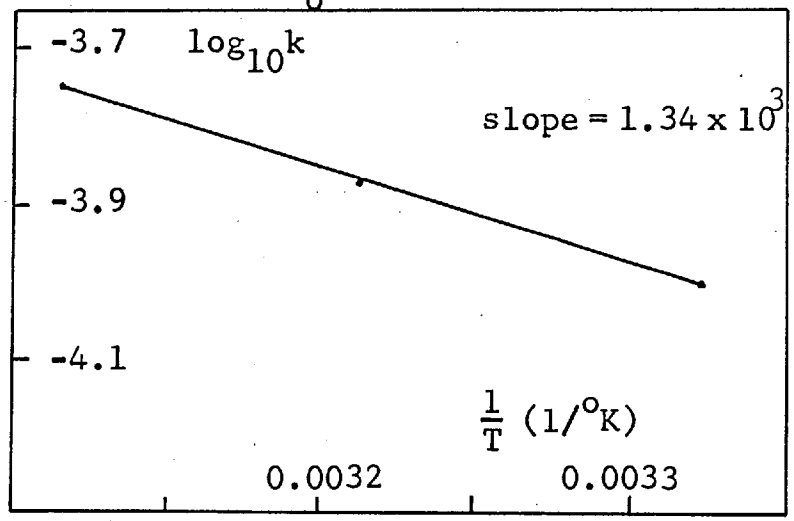


GRAPH 5.11 A plot of R versus S_1 for the digestion of grass.

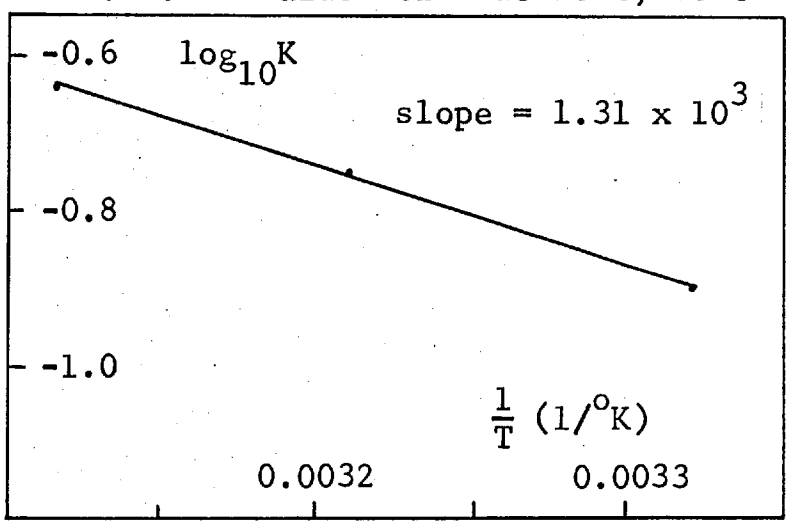
GRAPH 5.12.1 Values of k at 28°C, 38°C & 48°C, S_o = 15000 mg.lit^{-1} (dry weight).



GRAPH 5.12.2 Values of k at 28°C, 38°C & 48°C, S_o = 25000 mg.lit^{-1} (dry weight).



GRAPH 5.12.3 Values of K at 28°C, 38°C and 48°C.



GRAPH 5.12 The effect of temperature on the values of the rate constants; Arrhenius plot for Grass.

Kinetic Constant	Temperature = 28°C		Temperature = 38°C		Temperature = 48°C		Slope of Arrhenius plot cals/mole degK
	S_o=15000 mg.lit^{-1}* (dry weight)	S_o=25000 mg.lit^{-1}** (dry weight)	S_o=15000 mg.lit^{-1} (dry weight)	S_o=25000 mg.lit^{-1} (dry weight)	S_o=15000 mg.lit^{-1} (dry weight)	S_o=25000 mg.lit^{-1} (dry weight)	
k($k=\frac{q}{S_1}$ lit.mg^{-1}day^{-1})	0.000097	0.00007	0.00013	0.00010	0.00017	0.000133	
n	15	15	15	15	15	15	(1) 1.18×10^3
r	0.97	0.92	0.96	0.89	0.97	0.96	(2) 1.34×10^3
P	0.01	0.01	0.01	0.01	0.01	0.01	
K ($K=\frac{R}{S_1}$ day^{-1})	0.121	0.121***	0.177	0.177***	0.226	0.226***	
n	30	30	30	30	30	30	(3) 1.31×10^3
r	0.89	0.89	0.93	0.93	0.95	0.95	
P	0.01	0.01	0.01	0.01	0.01	0.01	

n = number of data points
r = correlation coefficient
P = level of significance
*** - As for S_o =15000 mg. lit.$^{-1}$
* - S_o = 18875 mg. lit^{-1} $COD_{v.m.}$
** - S_o = 31458 mg. lit^{-1} $COD_{v.m.}$

1 for k values at 28°C, 38°C and 48°C, S_o = 15000 mg. lit^{-1}
2 for k values at 28°C, 38°C and 48°C, S_o = 25000 mg. lit^{-1}
3 for K values at 28°C, 38°C and 48°C, S_o = 15000 mg. lit^{-1} and 25000 mg. lit^{-1}

Table 5.5 Summary of Kinetic Rate Constants for the Digestion of Grass

III. COW DUNG

The average conditions are shown in Graphs 5.13, 5.14 and 5.15 for digesters fed on cow dung.

Graph 5.13 shows the conditions obtained in the digestion of cow dung at input concentrations of 15000 mg. lit $^{-1}$ dry weight [14652 mg. lit $^{-1}$ C.O.D.$_{v.m.}$] and 25000 mg. lit $^{-1}$ dry weight [24420 mg. lit $^{-1}$ C.O.D.$_{v.m.}$] at a temperature of 28^{o}C. No results have been obtained for the digestion of cow dung at 28^{o}C for input concentrations of 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$ (dry weight) for 10 days detention time; steady state conditions could not be obtained and subsequent analysis revealed the unexplained presence of 4 gm. lit $^{-1}$ mercury in the two digesters.

Graph 5.14 shows the conditions obtained in the digestion of cow dung at input concentrations of 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$, at a temperature of 38^{o}C.

Graph 5.15 shows the conditions obtained in the digestion of cow dung at input concentrations of 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$, at a temperature of 48^{o}C.

Graph 5.16 is a plot of q versus S_1 for the following conditions:

Graph 5.16.1, S_o = 15000 mg. lit $^{-1}$, Temperature of 28^{o}C (10 data points)

Graph 5.16.2, S_o = 15000 mg. lit $^{-1}$, Temperature of 38^{o}C (15 data points)

Graph 5.16.3, S_o = 15000 mg. lit $^{-1}$, Temperature of 48^{o}C (15 data points)

The results for each graph are correlated as $q = kS_1$, the gradient (k) of the best straight line and the correlation coefficient (r) are shown in the graphs.

Similarly Graph 5.17 is a plot of q versus S_1 for

Graph 5.17.1 S_o = 25000 mg. lit $^{-1}$, Temperature of 28^{o}C (10 data points)

Graph 5.17.2 S_o = 25000 mg. lit $^{-1}$, Temperature of 38^{o}C (15 data points)

Graph 5.17.3 S_o = 25000 mg. lit $^{-1}$, Temperature of 48^{o}C (15 data points)

The results for each graph are correlated as $q = kS_1$, the gradient (k) of the best straight line and the correlation coefficient (r) are shown in the graphs.

Once more it is seen from graphs 5.16 and 5.17 that the output concentration ($C.O.D_{v.m.}$) is dependent on the input concentration for each of the three temperatures.

The data used to plot Graphs 5.16 and 5.17 is given in the Appendix III (Tables III.10 to III.16 inclusive)

Graph 5.18 is a plot of R versus S_1 for the following conditions:

Graph 5.18.1. S_o = 15000 mg. lit $^{-1}$ (· data points) and 25000 mg. lit $^{-1}$ dry weight (• data points) at a temperature = 28°C (10 data points).

Graph 5.18.2 S_o = 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$ at a temperature = 38°C (15 data points).

Graph 5.18.3 S_o = 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$ at a temperature = 48°C (15 data points).

Once more the data is correlated as a single plot of $R = KS_1$ for S_o = 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$ at all three temperatures studied. The difference in the values of the gradients for the fits at the two input concentrations was not significant at a 95% confidence level using the 't' test.

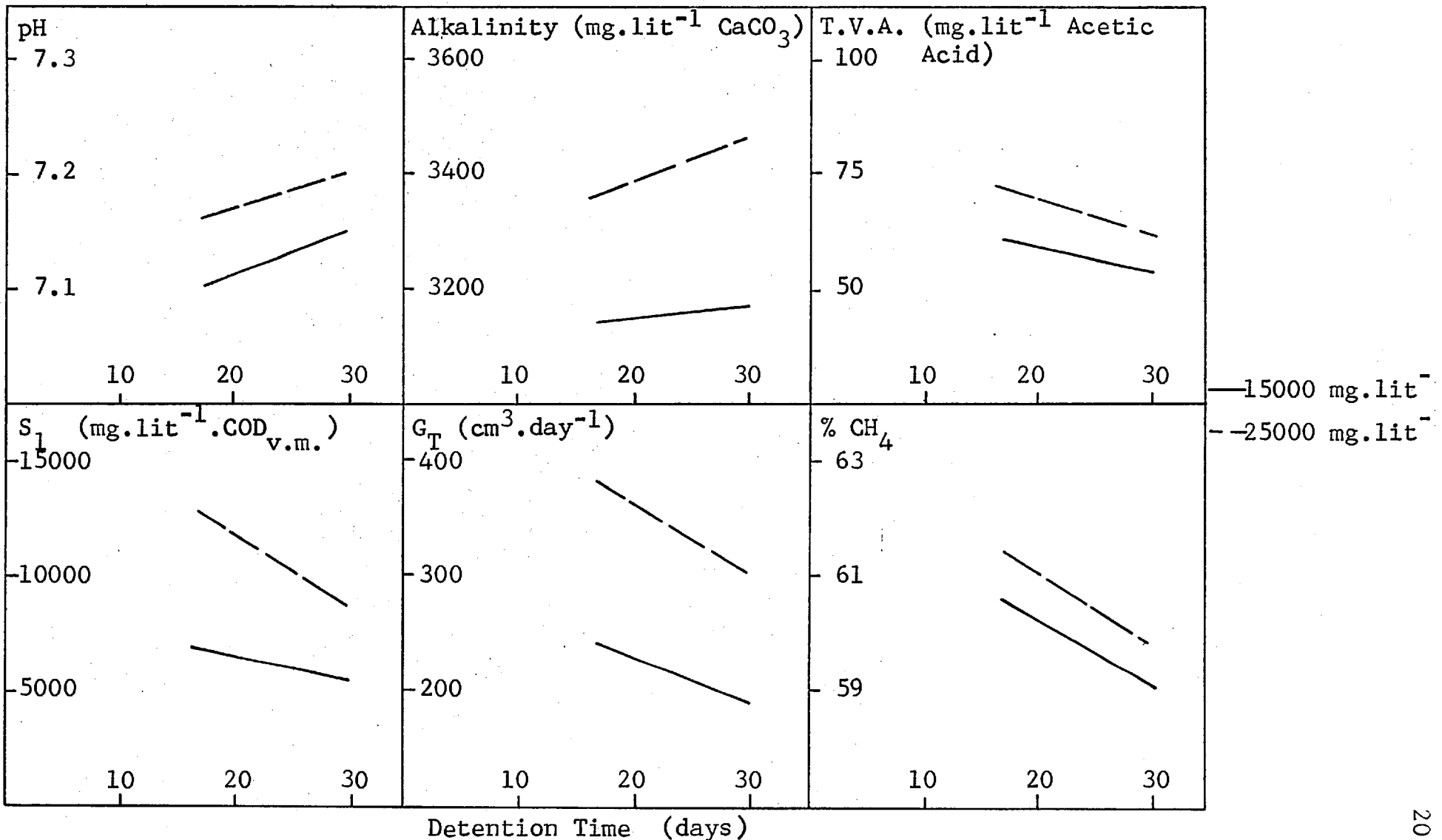
The percentage digestion of cow dung is seen to be independent of the input concentration from the plots in Graph 5.18.

Graph 5.19 is a plot of $\log_{10}$ (rate constant) versus $\frac{1}{T}$ for the following conditions:

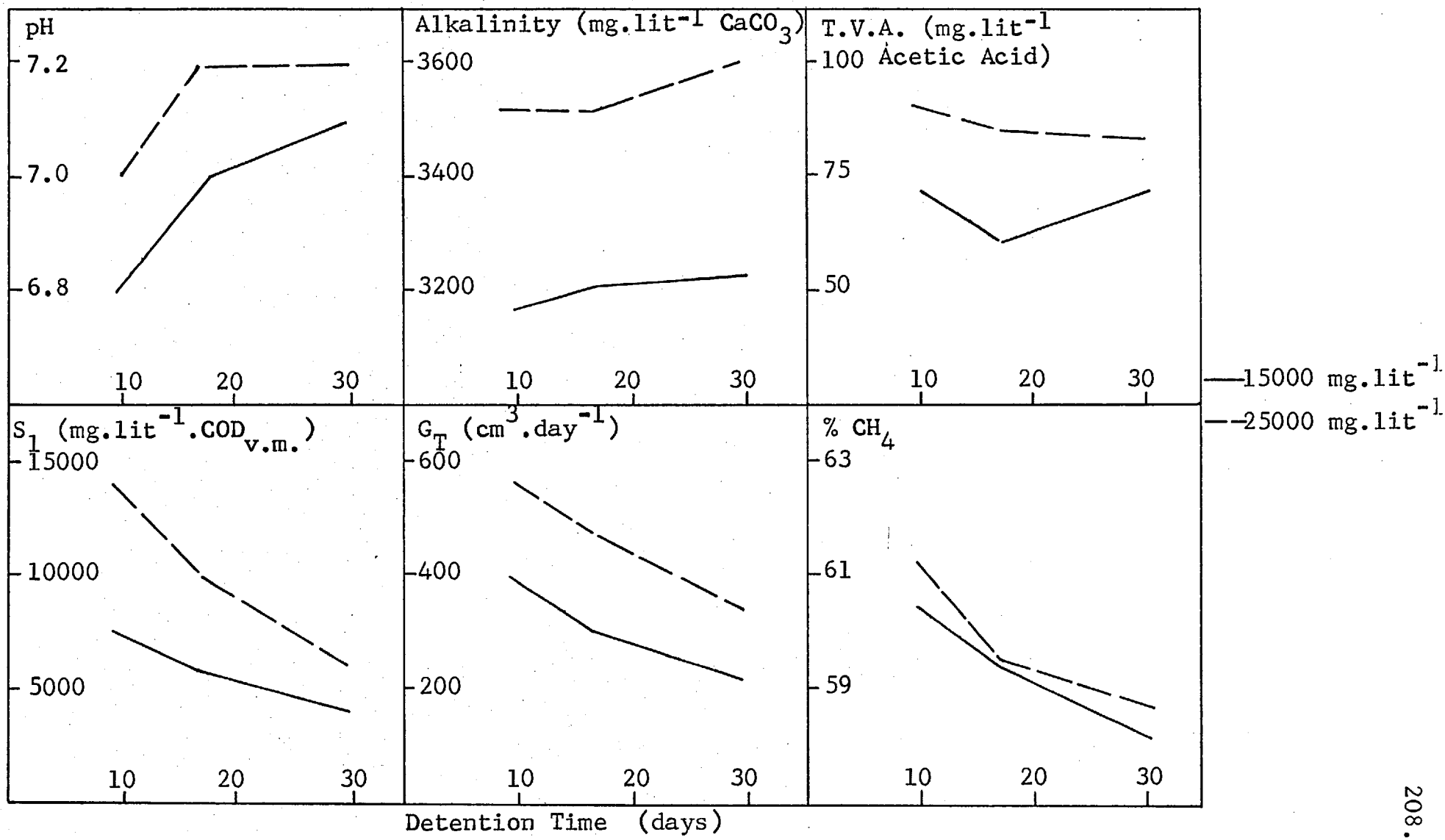
Graph 5.19.1 S_o = 15000 mg. lit^{-1} (dry weight) at temperatures of 28°C, 38°C and 48°C for the values of k obtained from Graphs 5.17.1, 5.17.2 and 5.17.3.

Graph 5.19.2 S_o = 15000 mg. lit^{-1} and 25000 mg. lit^{-1} (dry weight) at temperatures of 28°C, 38°C and 48°C for the values of K obtained from Graphs 5.18.1, 5.18.2 and 5.18.3.

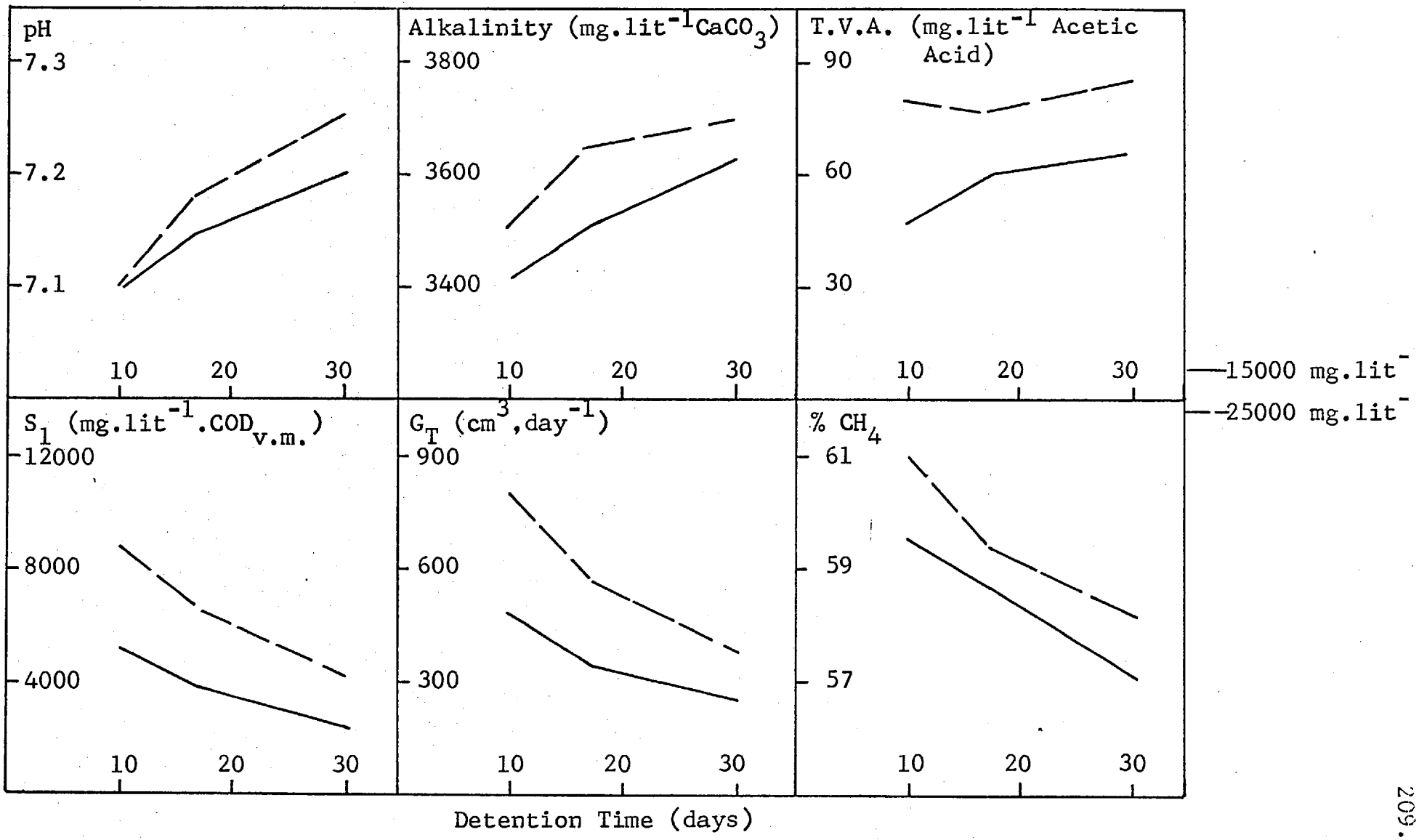
Table 5.6 is a summary of the constants obtained from graphs 5.16, 5.17, 5.18 and 5.19.



GRAPH 5.13 Average conditions plotted for the digestion of Cow Dung at input concentrations of 15000 mg.lit^{-1} and 25000 mg.lit^{-1} (dry weight), temperature = 28°C.

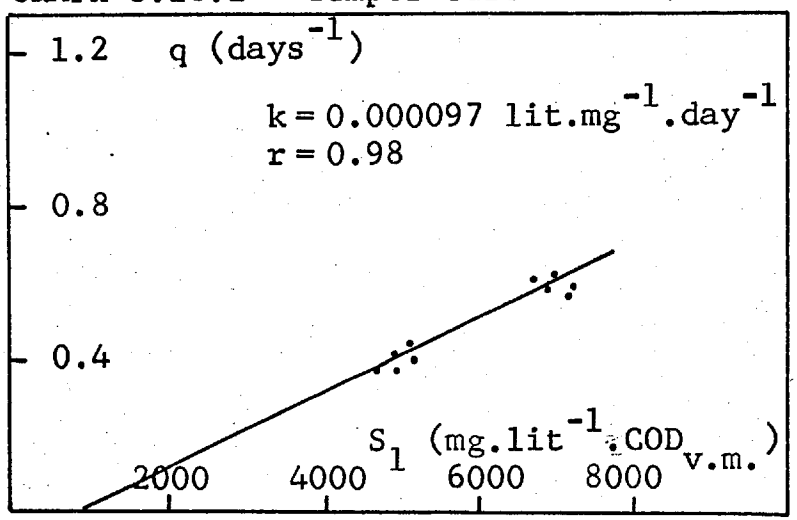


GRAPH 5.14 Average conditions plotted for the digestion of Cow Dung at an input concentration of 15000 mg.lit^{-1} and 25000 mg.lit^{-1} (dry weight), temp. = 38°C.

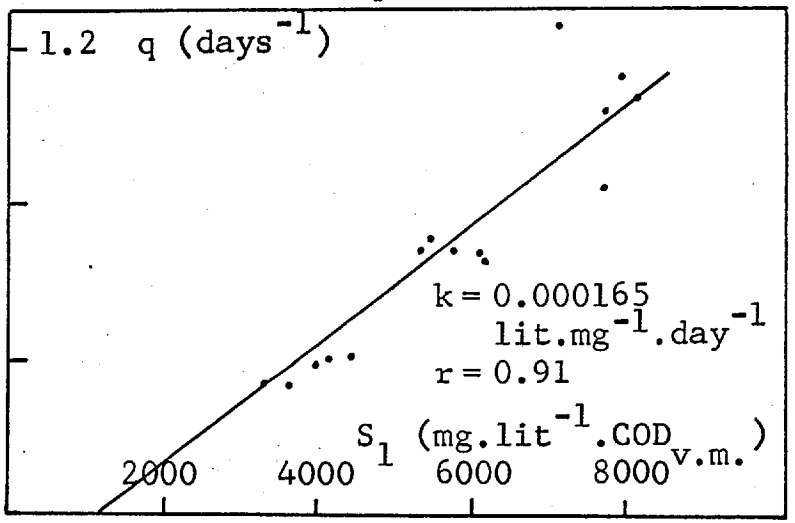


GRAPH 5.15 Average conditions plotted for the digestion of Cow Dung at an input concentration of 15000 mg.lit^{-1} and 25000 mg.lit^{-1} (dry weight), temperature = 48°C.

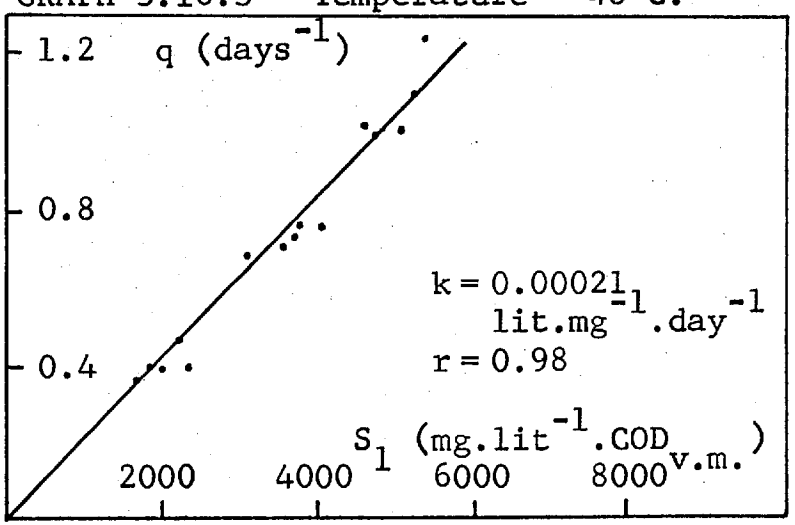
GRAPH 5.16.1 Temperature = 28°C.



GRAPH 5.16.2 Temperature = 38°C.

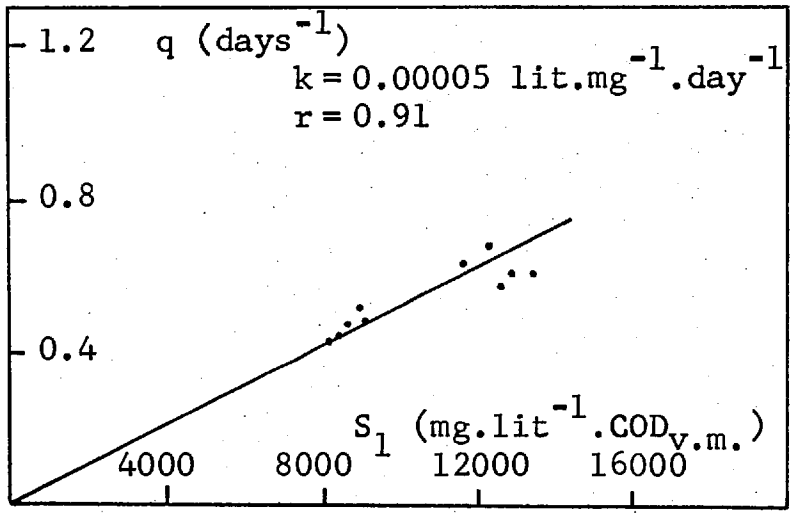


GRAPH 5.16.3 Temperature = 48°C.

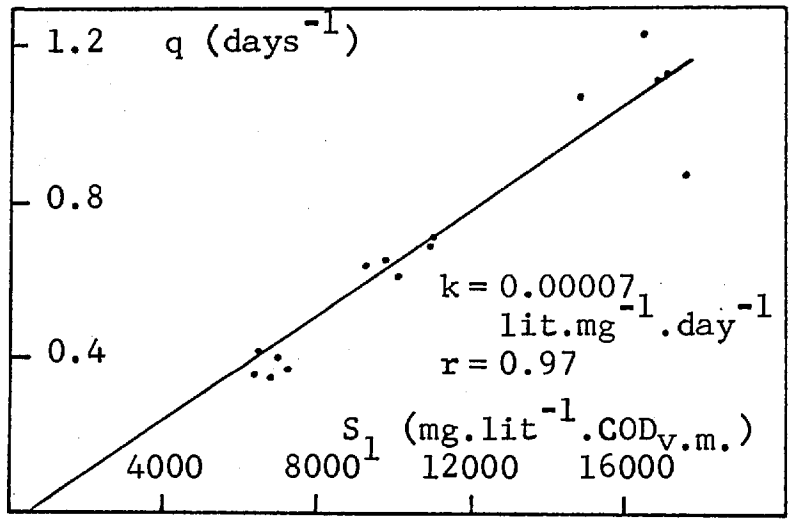


GRAPH 5.16 A plot of q versus S_1 for the digestion of Cow Dung (S_o = 15000 mg.lit^{-1}. dry weight).

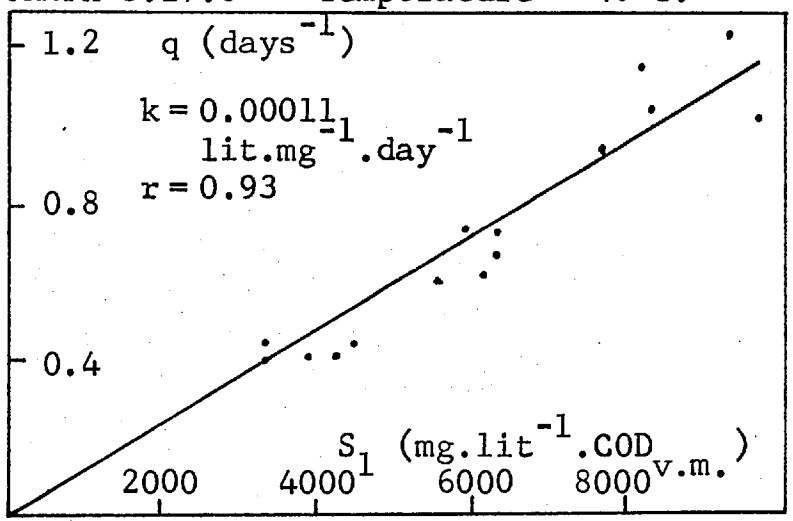
GRAPH 5.17.1 Temperature = 28°C.



GRAPH 5.17.2 Temperature = 38°C.

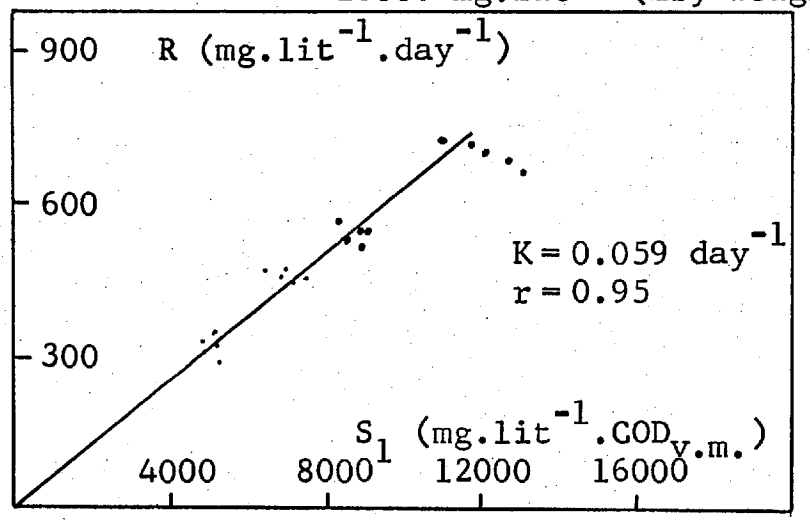


GRAPH 5.17.3 Temperature = 48°C.

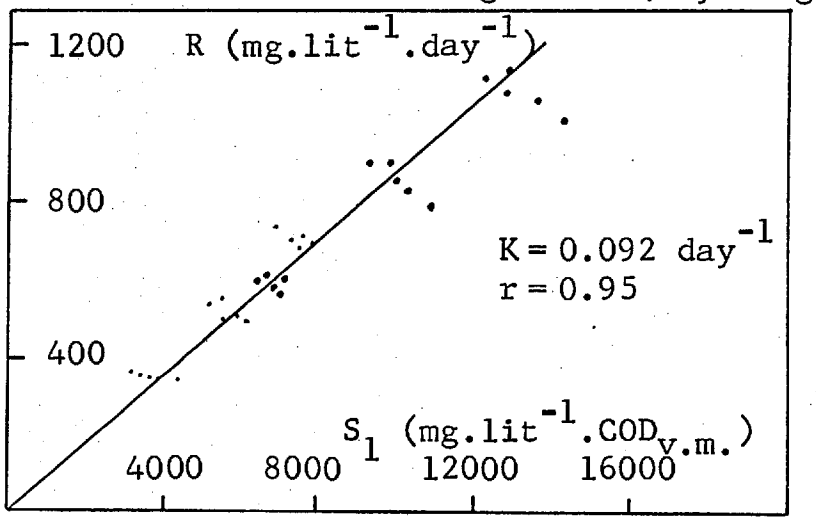


GRAPH 5.17 A plot of q versus S_1 for the digestion of Cow Dung (S_o = 25000 mg.lit^{-1}. dry weight).

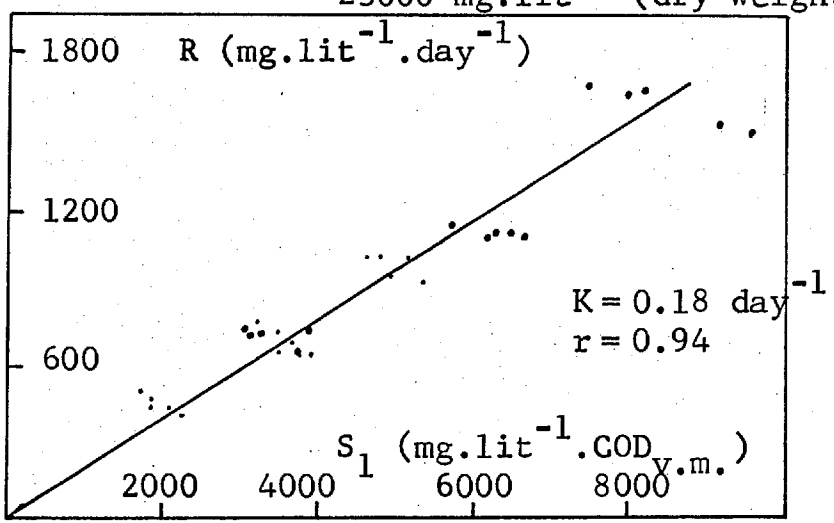
GRAPH 5.18.1 Temperature = 28°C,
· 15000 mg.lit^{-1} (dry weight),
• 25000 mg.lit^{-1} (dry weight).



GRAPH 5.18.2 Temperature = 38°C,
· 15000 mg.lit^{-1} (dry weight),
• 25000 mg.lit^{-1} (dry weight).

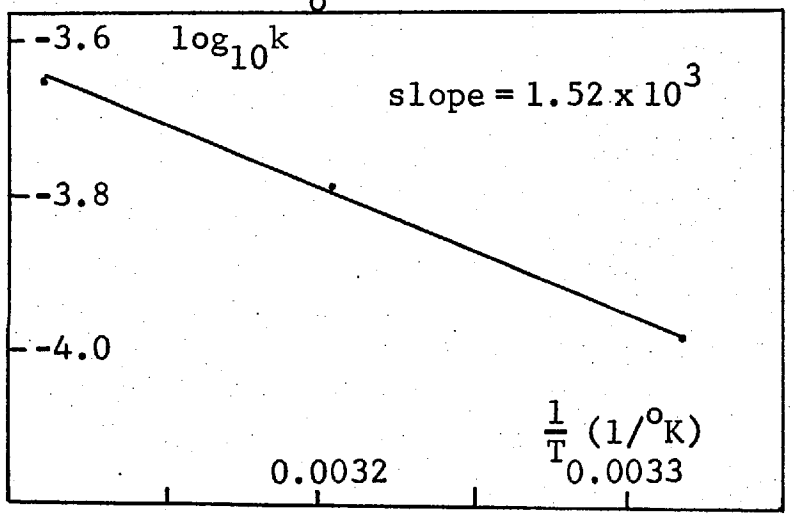


GRAPH 5.18.3 Temperature = 48°C,
· 15000 mg.lit^{-1} (dry weight),
• 25000 mg.lit^{-1} (dry weight).

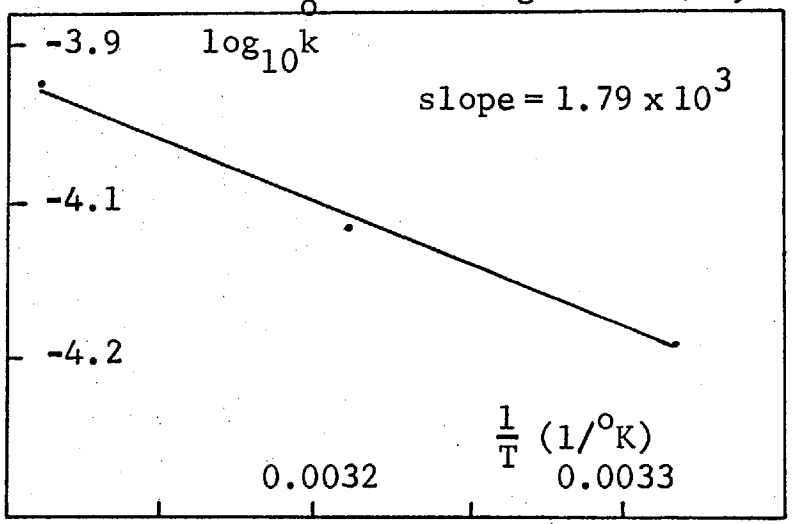


GRAPH 5.18 A plot of R versus S_1 for the digestion of Cow Dung.

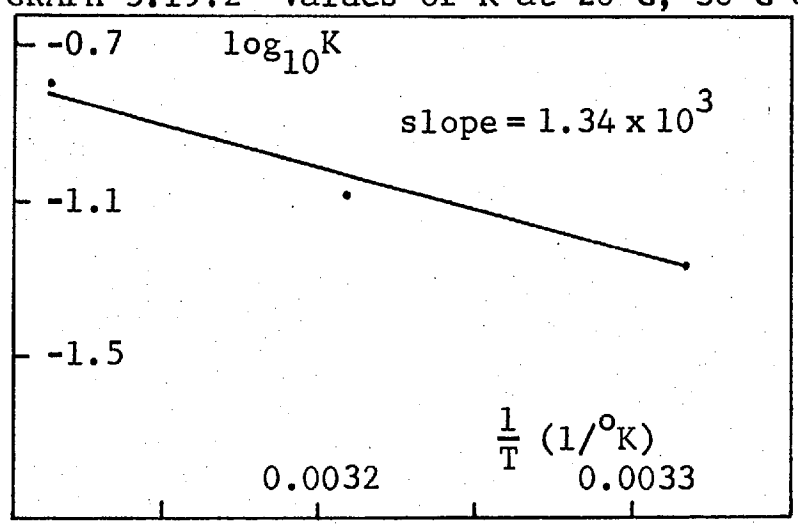
GRAPH 5.19.1 Values of k at 28°C, 38°C and 48°C. S_o = 15000 mg.lit^{-1} (dry weight).



GRAPH 5.19.2 Values of k at 28°C, 38°C and 48°C. S_o = 25000 mg.lit^{-1} (dry weight).



GRAPH 5.19.2 Values of K at 28°C, 38°C and 48°C.



GRAPH 5.19 The effect of Temperature on the values of the rate constants: Arrhenius plot for Cow Dung.

Kinetic Constant	Temperature = 28°C		Temperature = 38°C		Temperature = 48°C		Slope of Arrhenius plot (cals/mole deg.K)
	S_o=15000 mg.lit^{-1}* (dry weight)	S_o=25000 mg.lit^{-1}* (dry weight)	S_o=15000 mg.lit^{-1} (dry weight)	S_o=25000 mg.lit^{-1} (dry weight)	S_o=15000 mg.lit^{-1} (dry weight)	S_o=25000 mg.lit^{-1} (dry weight)	
k($k = \frac{q}{S_1}$ lit.mg.$^{-1}$ day^{-1})	0.000097	0.00005	0.000165	0.00007	0.00021	0.00011	
n	10	10	15	15	15	15	(1) 1.52×10^3
r	0.98	0.91	0.91	0.97	0.98	0.93	(2) 1.79×10^3
p	0.01	0.01	0.01	0.01	0.01	0.01	
K ($K = \frac{R}{S_1}$ day^{-1})	0.059	0.059***	0.092	0.092***	0.18	0.18***	
n	20	20	30	30	30	30	(3) 1.34×10^3
r	0.95	0.95	0.95	0.95	0.94	0.94	
p	0.01	0.01	0.01	0.01	0.01	0.01	

n = number of data points
r = correlation coefficient
p = level of significance
* S_o = 14652 mg. lit^{-1} $COD_{v.m.}$
** S_o = 24420 mg. lit^{-1} $COD_{v.m.}$
*** As for S_o = 15000 mg. lit^{-1}

1. for k values at 28°C, 38°C, and 48°C, S_o = 15000 mg. lit^{-1} (dry weight)
2 for k values at 28°C, 38°C, and 48°C, S_o = 25000 mg. lit^{-1} (dry weight)
3 for K values at 28°C, 38°C, and 48°C, S_o = 15000 and 25000 mg. lit^{-1} (dry weight)

Table 5.6 Summary of Kinetic Rate Constants for the Digestion of Cow Dung

IV. HORSE MANURE

The average conditions are shown in Graph 5.20 in the digestion of horse manure at an input concentration of 15000 mg. lit $^{-1}$ dry weight [12508 mg. lit $^{-1}$ C.O.D.$_{v.m.}$] and temperatures of 28°C, 38°C and 48°C.

Graph 5.21 is a plot of q versus S_1 for the following conditions:

Graph 5.21.1 S_o = 15000 mg. lit^{-1}, Temperature of 28°C (15 data points)

Graph 5.21.2 S_o = 15000 mg. lit^{-1}, Temperature of 38°C (15 data points)

Graph 5.21.3 S_o = 15000 mg. lit^{-1}, Temperature of 48°C (15 data points)

The results for each group are correlated as $q = kS_1$, the gradient (k) of the best straight line and the correlation coefficient (r) are shown in the graphs.

Graph 5.22 is a plot of R versus S_1 for the following conditions:

Graph 5.22.1 S_o = 15000 mg. lit^{-1}, Temperature of 28°C (15 data points)

Graph 5.22.2 S_o = 15000 mg. lit^{-1}, Temperature of 38°C (15 data points)

Graph 5.22.3 S_o = 15000 mg. lit^{-1}, Temperature of 48°C (15 data points)

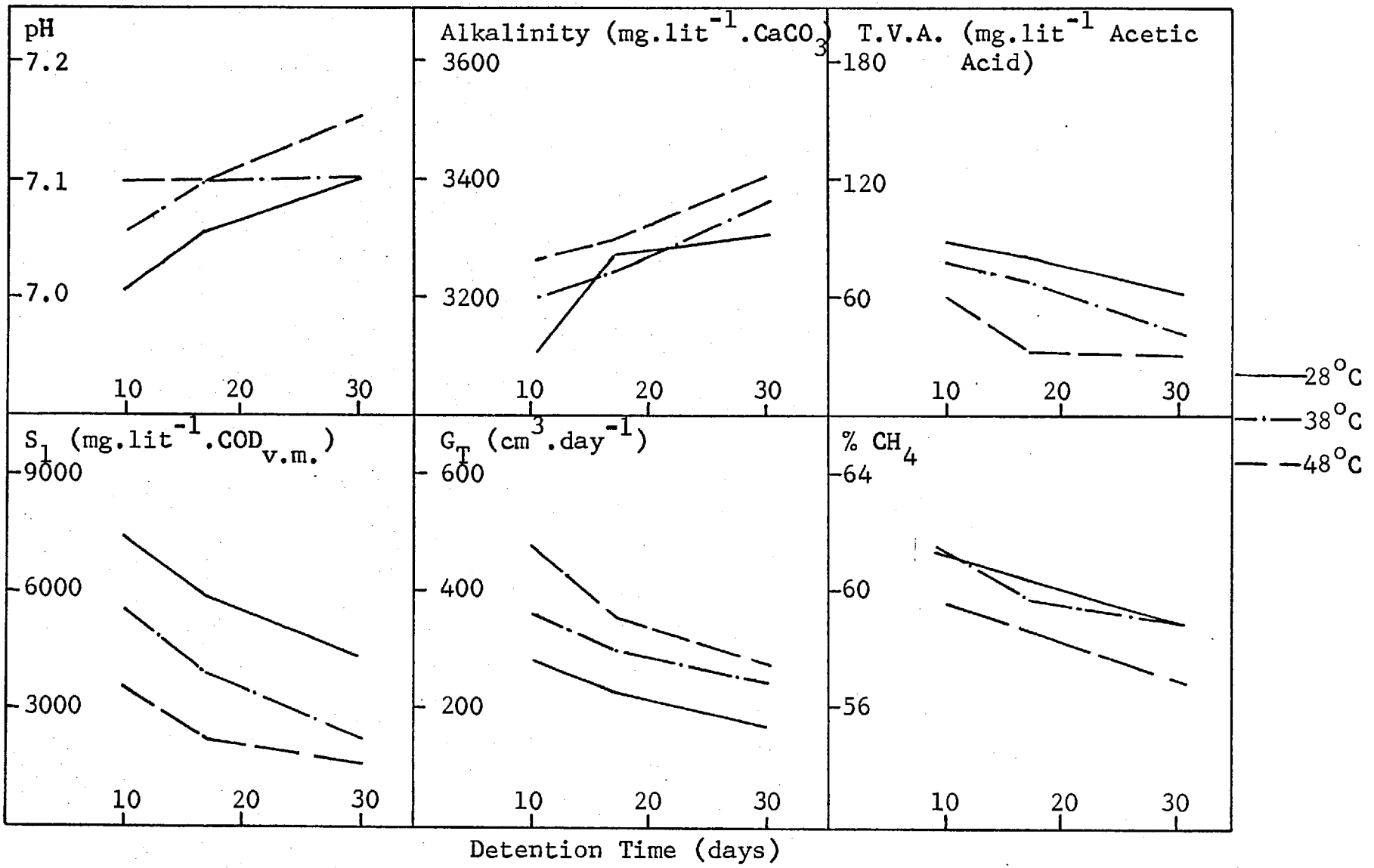
The results for each graph are correlated as $R = KS_1$, the gradient (K) of the best straight line and the correlation coefficient (r) are shown in the graphs. The data used to plot Graphs 5.21 and 5.22 is given in the Appendix III (Tables III.16 to III.18).

Graph 5.23 is a plot of $\log_{10}$ (rate constant) versus $\frac{1}{T}$ for the following conditions:

Graph 5.23.1 S_o = 15000 mg. lit $^{-1}$ at temperatures of 28°C, 38°C and 48°C for the values of k obtained from Graphs 5.21.1, 5.21.2 and 5.21.3.

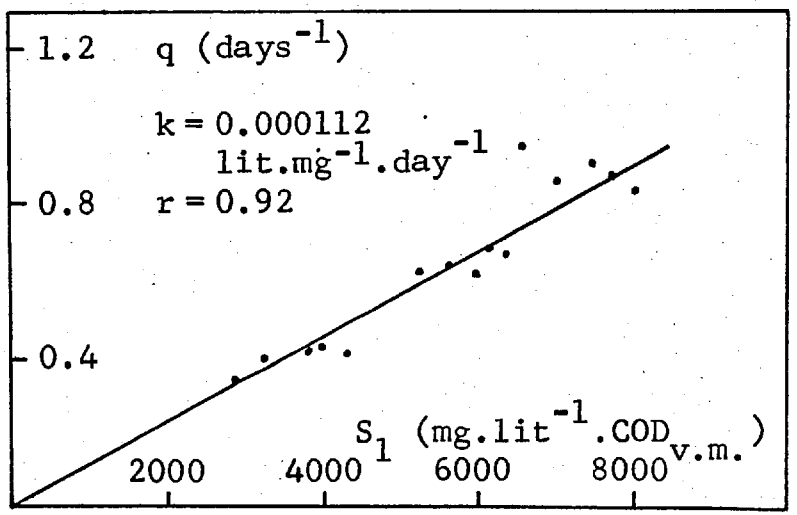
Graph 5.23.2 S_o = 15000 mg. lit $^{-1}$ at temperatures of 28°C, 38°C and 48°C for the values of K obtained from Graphs 5.22.1, 5.22.2 and 5.22.3.

Table 5.7 is a summary of the constants obtained from Graphs 5.21, 5.22 and 5.23.

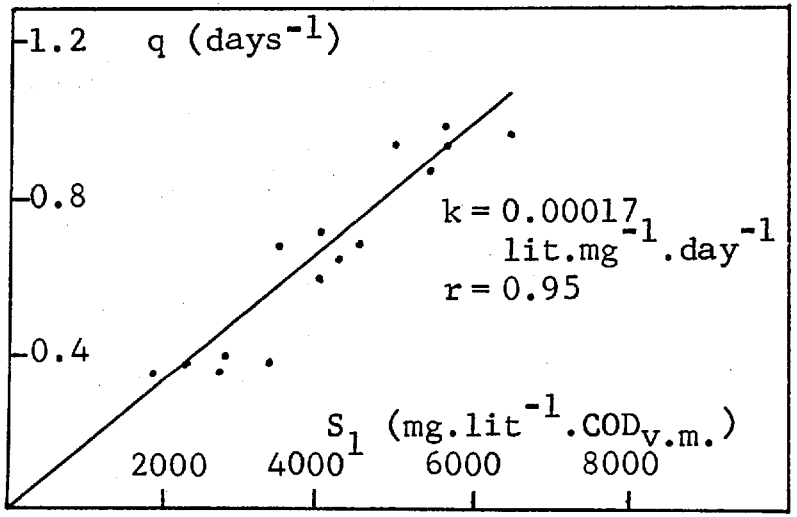


GRAPH 5.20 Average conditions plotted for the digestion of Horse Manure at an input concentration of 15000 mg.lit^{-1} (dry weight), temperature = 28°C, 38°C, 48°C.

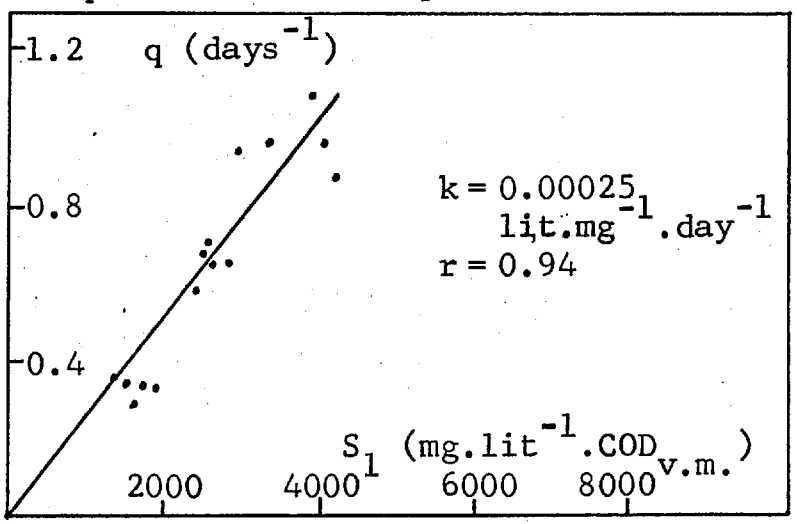
Graph 5.21.1 Temperature = 28°C.



Graph 5.21.2 Temperature = 38°C.

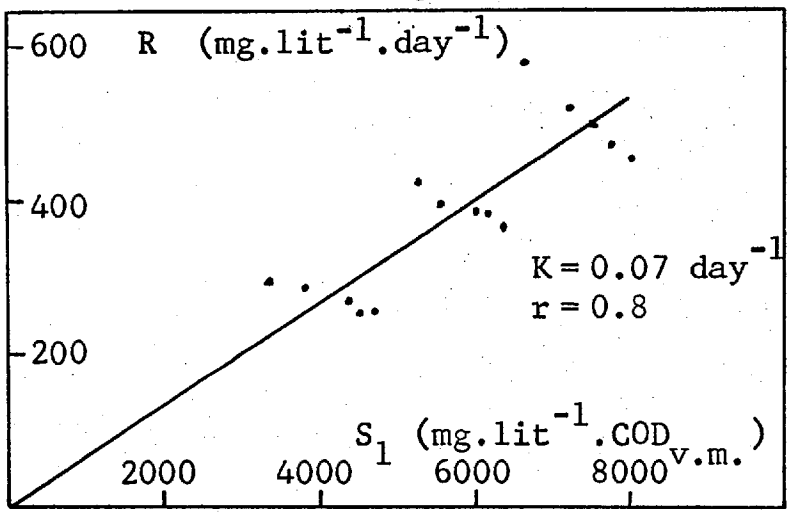


Graph 5.21.3 Temperature = 48°C.

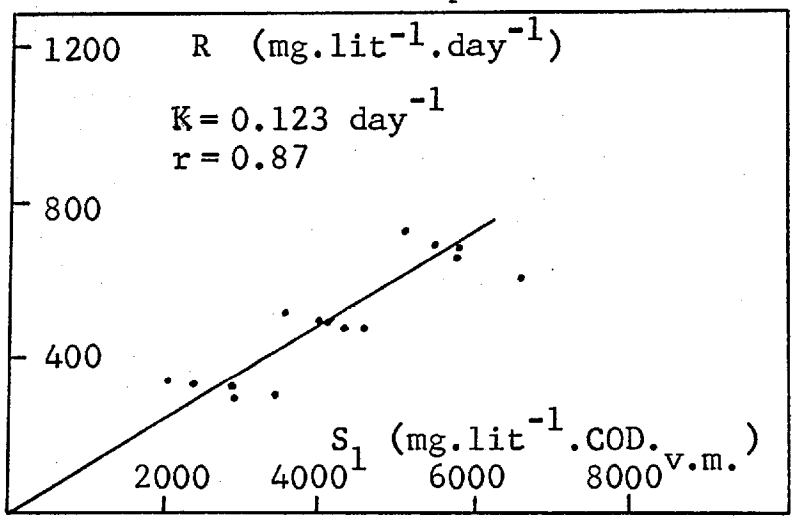


GRAPH 5.21 A plot of q versus S_1 for the digestion of Horse Manure (S_o = 15000 mg.lit^{-1} dry weight).

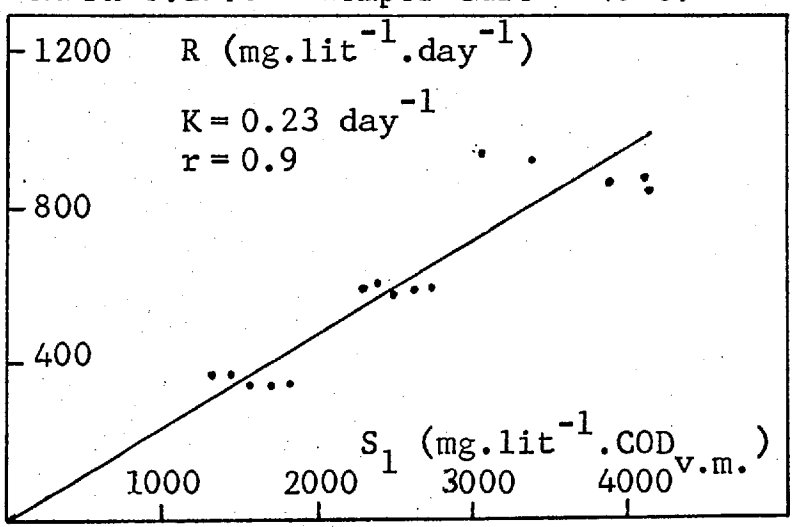
GRAPH 5.22.1 Temperature = 28°C.



GRAPH 5.22.2 Temperature = 38°C.

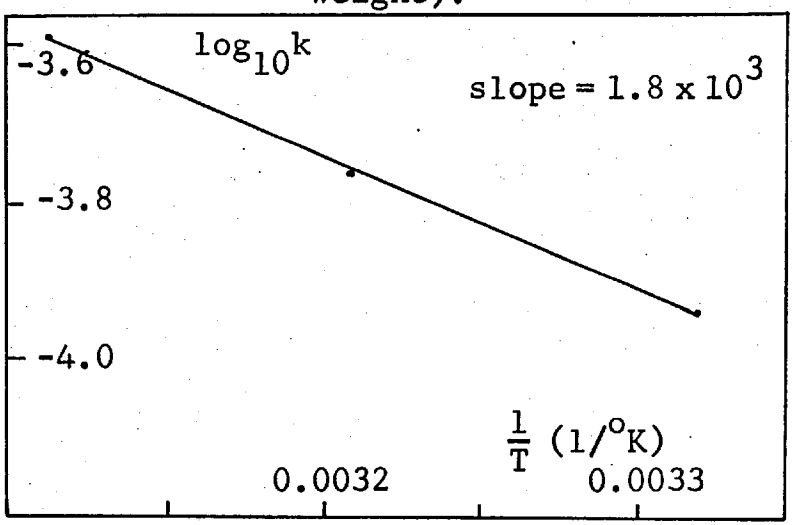


GRAPH 5.22.3 Temperature = 48°C.

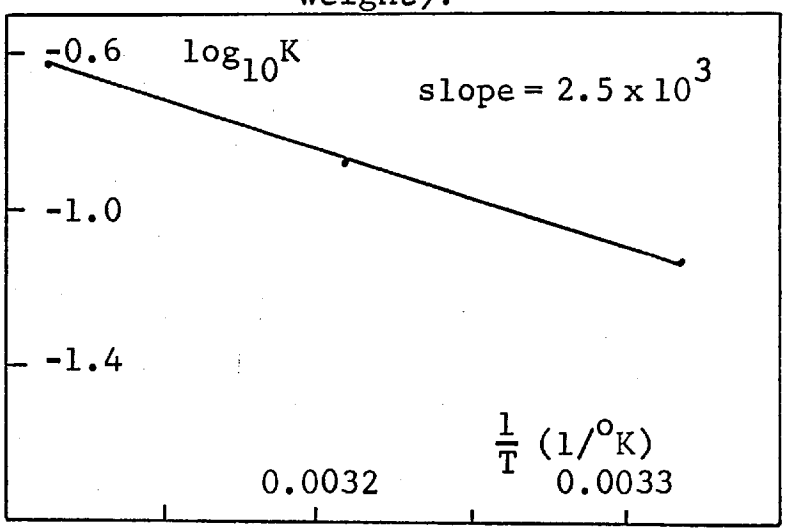


GRAPH 5.22 A plot of R versus S_1 for the digestion of Horse Manure (S_o = 15000 mg.lit^{-1} dry weight).

GRAPH 5.23.1 Values of k at 28°C, 38°C and 48°C (S_o = 15000 mg.lit^{-1} dry weight).



GRAPH 5.23.2 Values of K at 28°C, 38°C and 48°C (S_o = 15000 mg.lit^{-1} dry weight).



GRAPH 5.23 The Effect of Temperature on the values of the rate constants: Arrhenius plot for Horse Manure.

Kinetic Constant	Temperature = 28°C	Temperature = 38°C	Temperature = 48°C	Slope of Arrhenius plot (cals/mole deg K.)
	S_o = 15000 mg. lit^{-1}* (dry weight)	S_o = 15000 mg. lit^{-1} (dry weight)	S_o = 15000 mg. lit^{-1} (dry weight)	
k ($k = \frac{q}{S_1}$ lit. mg.$^{-1}$day^{-1})	0.000112	0.00017	0.00025	
n	15	0.15	15	(1) 1.8×10^3
r	0.92	0.95	0.94	
p	0.01	0.01	0.01	
K ($K = \frac{R}{S_1}$ day^{-1})	0.07	0.123	0.23	
n	15	15	15	(2) 2.5×10^3
r	0.08	0.87	0.9	
p	0.01	0.01	0.01	

n = number of data points
r = correlation coefficient
p = level of significance
* S_o = 12 508 mg. lit^{-1} $COD_{v.m.}$

1. for k values at 28°C, 38°C, 48°C, S_o = 15000 mg. lit^{-1}
2. for K values at 28°C, 38°C, 48°C, S_o = 15000 mg. lit^{-1}

TABLE 5.7 SUMMARY OF KINETIC RATE CONSTANTS FOR THE DIGESTION OF HORSE MANURE

5.2.2. Yield Constant (Y) and Decay Coefficient (b).

At each of the operating temperatures the yield constant (Y) and decay coefficient (b) have been determined from a plot of $\frac{X_1}{S_o - S_1}$ versus θ (Equation 3.40) for each of the substrates. The values of $\frac{X_1}{S_o - S_1}$ and θ for the different operating temperatures are given in Appendix III for each of the substrates. Table 5.8 is a summary of the values of Y and b obtained from the different plots.

Agardy et al (1963) and Stewart (1958) measured yields of 0.104 and 0.18 mg. VSS/mg. COD respectively in the anaerobic fermentation of complex substrates (dextrose, peptone and beef extract).

When a fatty acid is used as substrate for an enriched culture, Lawrence and McCarty (1969) obtained a much lower value of Y 0.041-0.047 mg. VSS/mg. COD. Shea et al (1968) using hydrogen/CO_2 as the substrate for an enriched culture obtained Y = 0.043. The yield constants for complex substrates therefore appear to be larger than for simple substrates.

The values for Y in this study are not significantly affected by temperature for the range of 28°C to 48°C. Novak (1974) considered the yield constant to be independent

Table 5.8 A summary of the Yield Constant and decay coefficient for the substrates digested at 28°C, 38°C and 48°C.

Component	Temperature	Y $\frac{\text{mg. V.S.S. Produced}}{\text{mg. substrate digested}}$	b Decay Rate day^{-1}	Correlation Coefficient r	Level of Signifiance p
Cellulose	28°C	0.105 $\frac{\text{mg. V.S.S}}{\text{mg. cellulose digested}}$	0.010	0.78	0.01
	38°C	0.101 " "	0.006	0.71	0.01
Cellulose (Chan's Results)	35°C	0.104 " "	0.005	0.901	0.001
Grass	28°C	0.116 $\frac{\text{mg. V.S.S.}}{\text{mg.COD}_{\text{v.m}} \text{ digested}}$	0.013	0.75	0.01
	38°C	0.104	0.0095	0.80	0.01
	48°C	0.109	0.01	0.71	0.01
Cow Dung	28°C	0.139	0.020	0.86	0.01
	38°	0.096	0.004	0.74	0.01
	48°	0.096	0.005	0.77	0.01
Horse Manure	28°C	0.12	0.013	0.79	0.01
	38°C	0.15	0.03	0.69	0.01
	48°C	0.104	0.005	0.80	0.01

of temperature for a range of 10-40°C. The yield constant for cellulose found in this study is in close agreement with that obtained by Chan (1970) as is also the decay coefficient.

The lower values of the decay coefficient for the digestion of cellulose show that at low detention times (10 days) the death coefficient is a negligible term (i.e. loss of cells due to death decay is approximately 8% of total cell production at θ = 10 days), but becomes significant at long detention times (i.e. loss of cells due to death decay is approximately 24% of total cell production at θ = 30 days).

Helmers, as reported by Ludzak (1961) concluded that the yield constant was independent of temperature. However calculations based on Garret and Sawyer's (1952) data, as reported by Ludzak gave yield constants of 0.53, 0.44 and 0.38 at temperatures of 10°C, 20°C and 30°C for the continuous activated sludge process. No previous yield data is available in the literature for the digestion of insoluble substrates such as grass, cow dung or horse manure.

In this study a narrow range of dilution rates has been used (0.0333 to 0.1 day $^{-1}$) and since the decay coefficient, b, is low for all substrates, then the viable fraction of organisms in the cell mass determination would not significantly change over such a narrow range of dilution rates (Grady and Roper 1974) and it is doubtful whether the introduction of sophisticated parameters to account for the change in cell viability with detention time is warranted because of the inherent difficulties in parameter evaluation in a study of this kind.

Since the substrate removal rate q at each detention time was computed using V.S.S. as a measure of the cell concentration, then computation of q using a viable cell concentration term would result in a higher value at the same detention time. However, since the viable cell content is considered as a constant proportion of the V.S.S. determination over the range of detention times studied, then the above factor would not alter the value of the rate constants obtained from the correlation of $q = kS_1$ as used in this study.

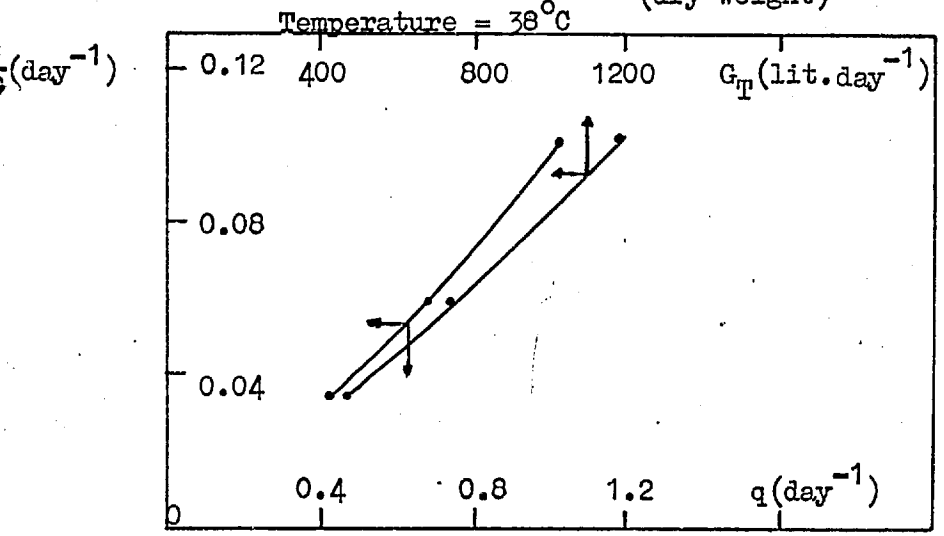
5.2.3 Gas Production and Substrate Utilisation

For the digestion of the four substrates over the range of operating conditions it is clear that from plots of the total volatile acid concentration versus detention time no significant increase in the volatile acid concentration occurred over the range of detention times. Since the volatile acid concentration is the main intermediate in the digestion of the insoluble substrate to methane then providing substrate is only removed in the digester via the anaerobic digestion process the gas produced should be proportional to the solids utilised.

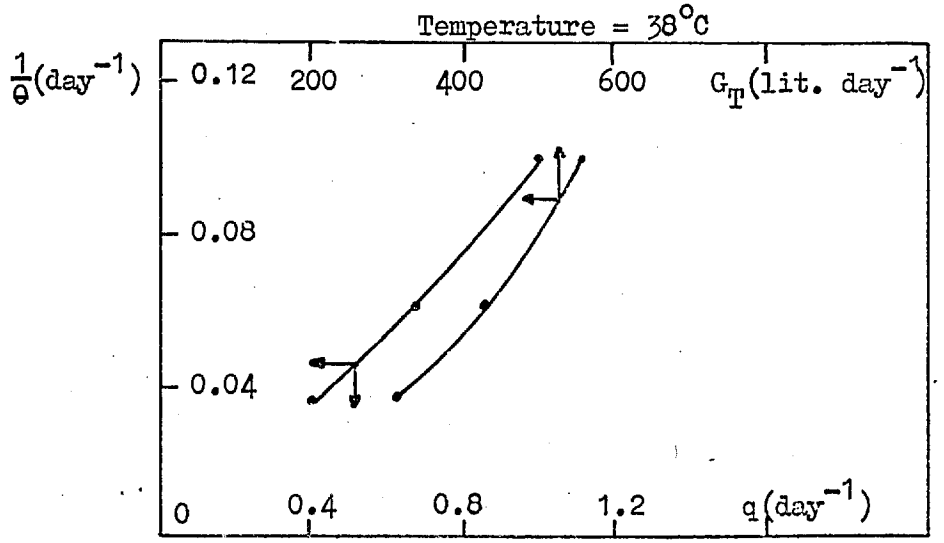
This can be illustrated from a plot of q and G_T versus $\frac{1}{\theta}$, as outlined in Section 3.3. Graph 5.24 shows such a plot for cellulose, grass, cow dung and horse manure digested at a temperature of $38^{o}C$ and an input concentration (dry weight) of 15000 mg. lit^{-1}. The behaviour as shown in Graph 5.24 is typical for the whole range of conditions studied and hence it can be concluded that substrate utilisation and gas production are growth associated processes.

The values of q and G_T used to plot Graph 5.24 are the average values given in Appendix III for the relevant operating conditions.

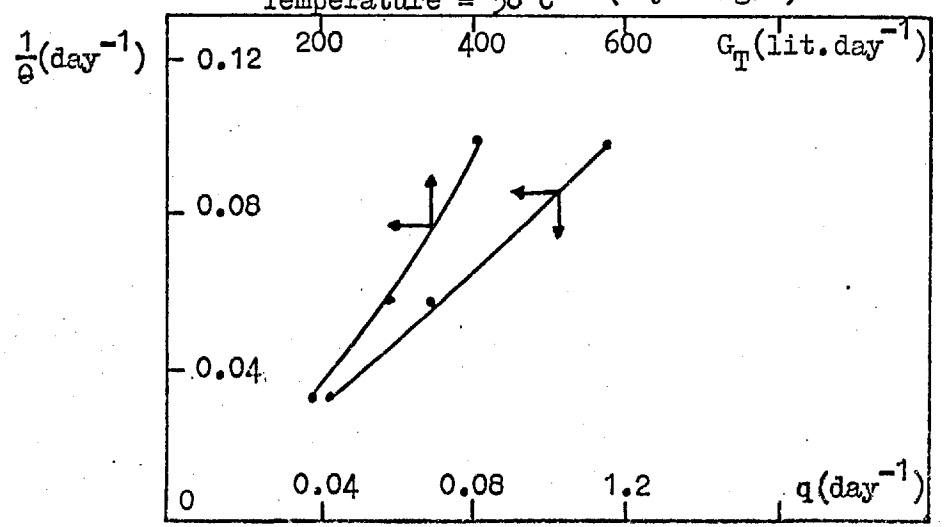
Graph 5.24.1 Cellulose, S_o= 15000 mg.lit^{-1} (dry weight)



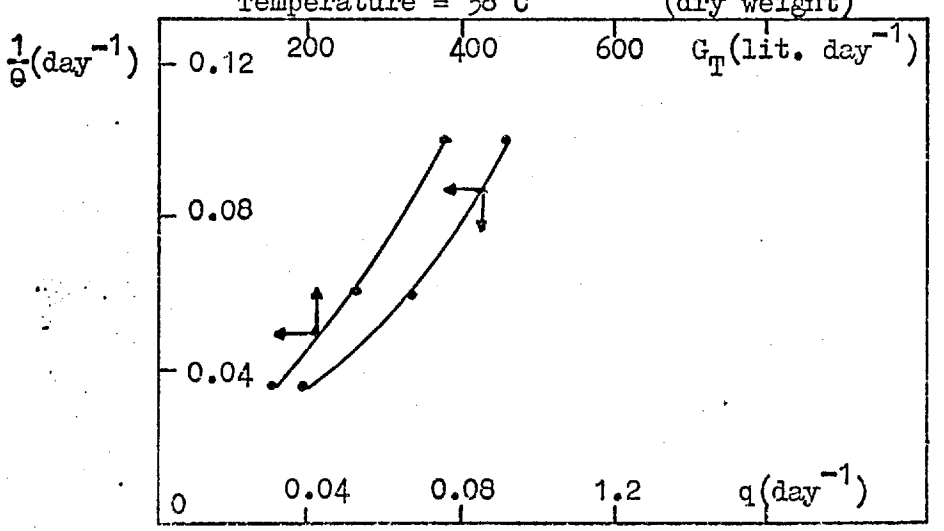
Graph 5.24.2 Grass, S_o= 15000 mg.lit^{-1} (dry weight)



Graph 5.24.3 Cow Dung, S_o= 15000 mg. lit^{-1} (dry weight)



Graph 5.24.4 Horse Manure, S_o= 15000 mg. lit.$^{-1}$ (dry weight)



Graph 5.24 Relationship between substrate removal rate and gas production.

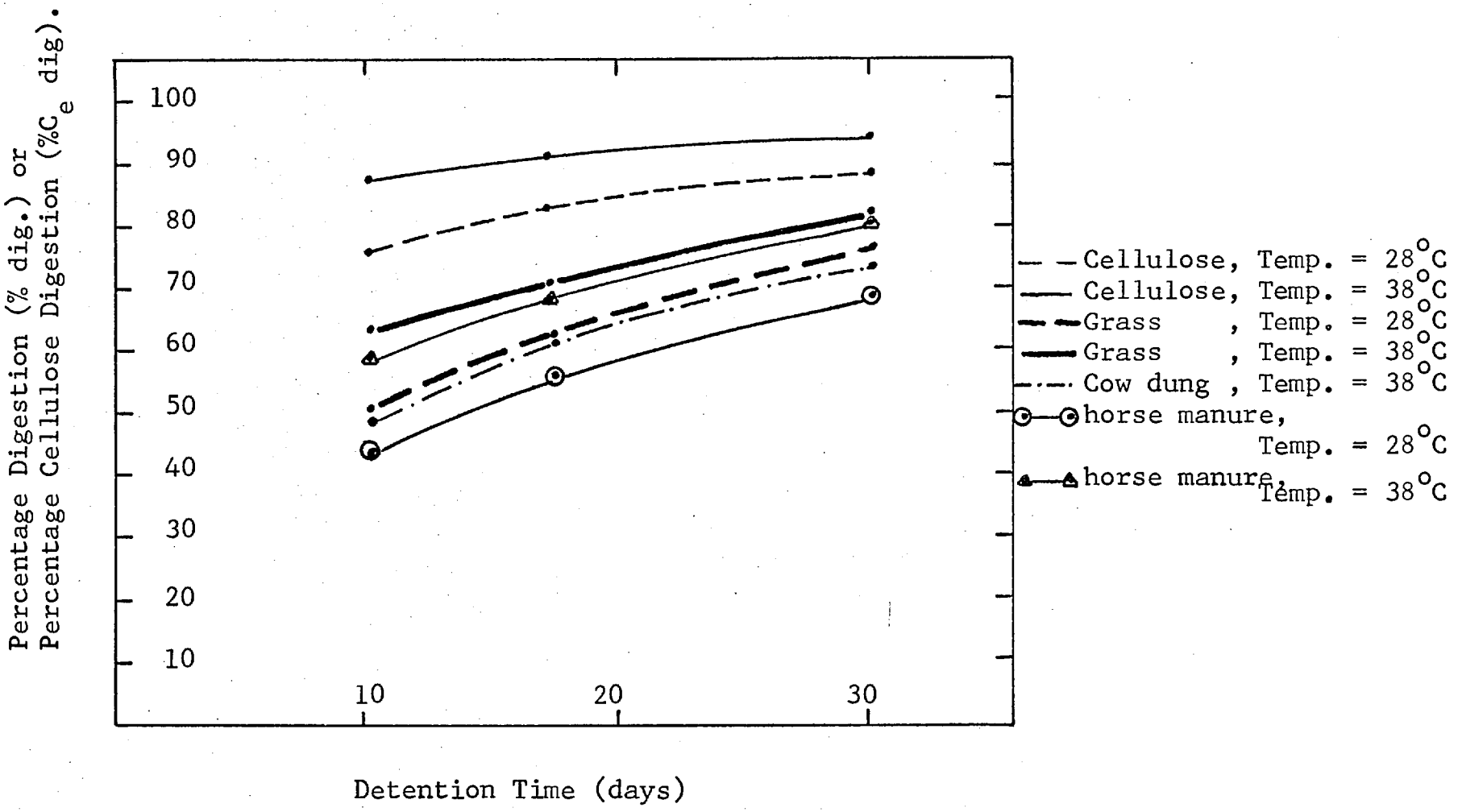
5.2.4 Decomposition Rates

The percentage digestion efficiency (% dig.) is shown in Graphs 5.25, 5.26, 5.27 and 5.28 for the digestion of cellulose, grass, cow dung and horse manure respectively. The complete list of values used for the percentage digestion (% dig.) is given in Appendix III - only the average values have been plotted here. It can be seen from the graphs that for a given digestion efficiency the substrates grass, cow dung,and horse manure require a longer detention time than does cellulose undergoing digestion under the same operating conditions. The percentage digestion plotted in the graphs is the average of the % dig. data obtained for 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$ input concentration (dry weight).

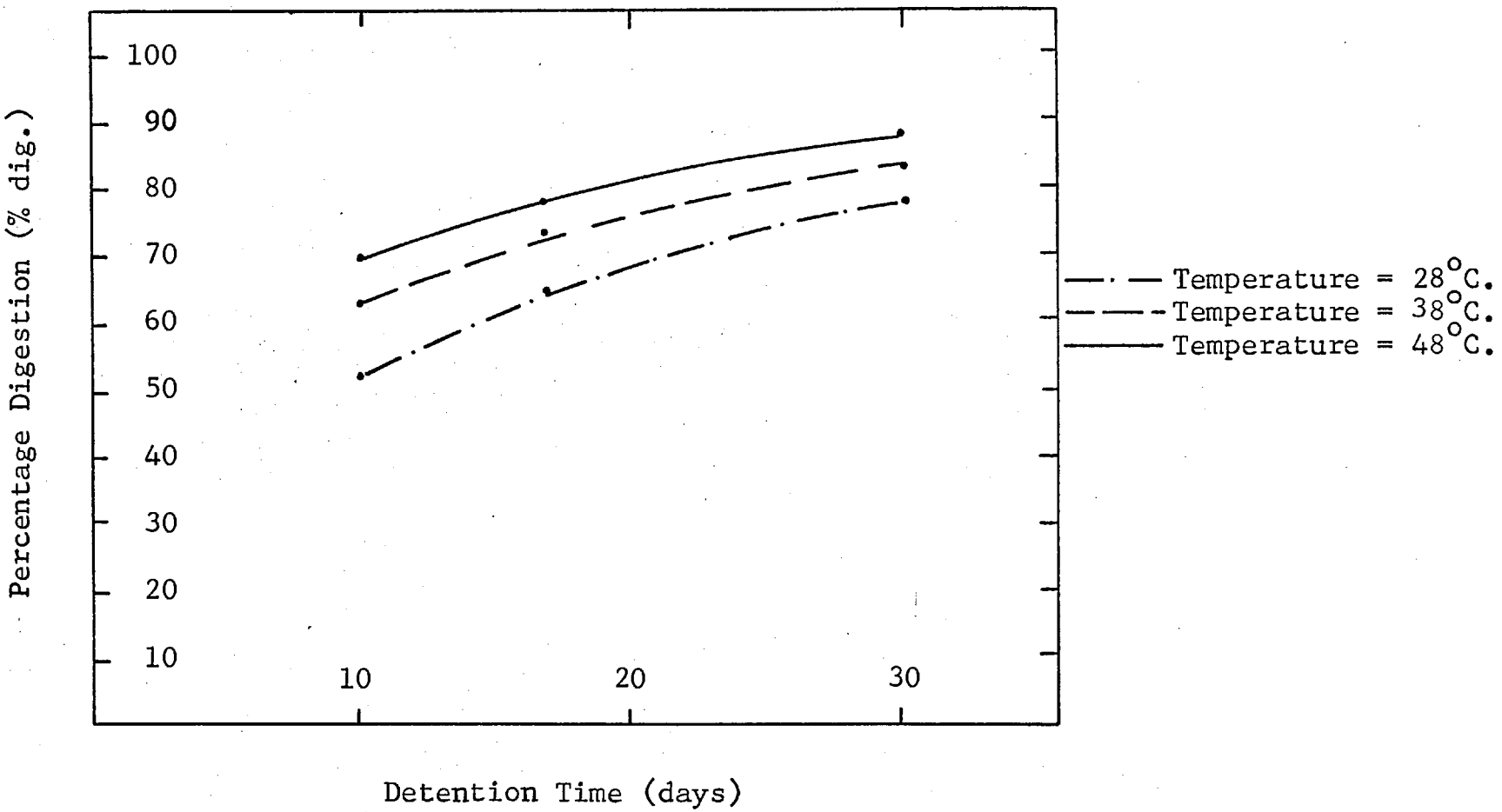
Also plotted in Graph 5.25 is the percentage cellulose digestion (% C_e dig.) occurring in the digestion of grass, cow dung and horse manure. Once more the data is given in Appendix III and the percentage cellulose digestion plotted in Graph 5.25 is the average of the % C_e dig. data obtained for 15000 mg. lit $^{-1}$ and 25000 mg. lit $^{-1}$ input concentration (dry weight) for grass and cow dung.

The digestion of pure cellulose occurs to a far greater extent than does the digestion of cellulose contained in grass, cow dung and horse manure for the same operating conditions (temperature and detention time). In general the % C_e dig. for grass, cow dung and horse manure is 10 to 30% lower than the percentage digestion of pure cellulose.

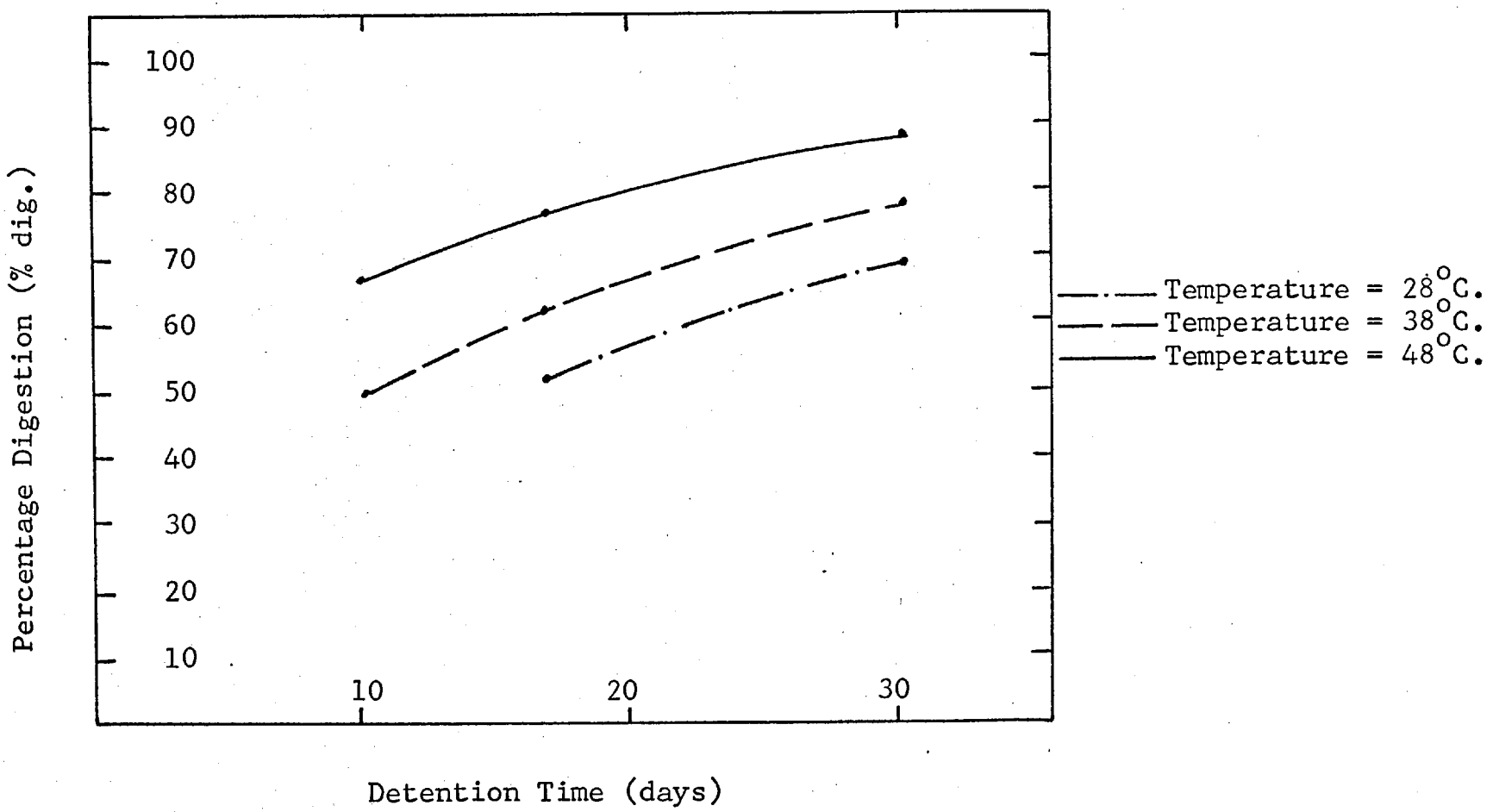
The results of Klein (1972) (Table 2.13) show that the digestion of cellulose in garden debris is lower than the digestion of pure cellulose - 78.5% cellulose destroyed in the digestion of garden debris as compared to 92.4% cellulose destroyed in the digestion of kraft paper (pure cellulose) for the same operating conditions.



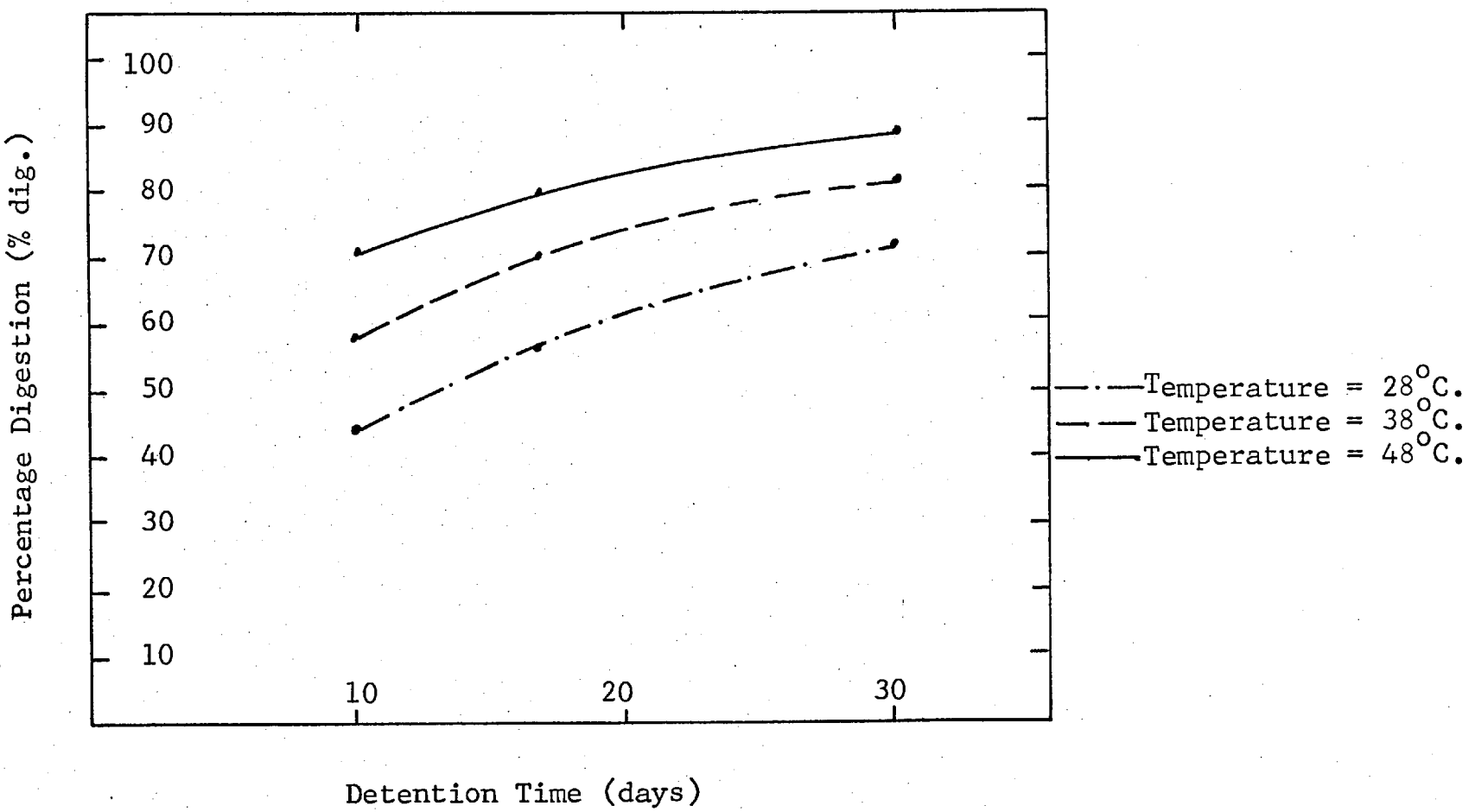
Graph 5.25 Percentage digestion for the digestion of pure cellulose and the percentage cellulose digestion in the digestion of grass, cow dung and horse manure.



Graph 5.26 Percentage digestion for the digestion of Grass.



Graph 5.27 Percentage Digestion for the digestion of Cow Dung.



Graph 5.28 Percentage Digestion for the digestion of Horse Manure.

CHAPTER 6

DISCUSSION

The rate constants obtained in this study represent the effects of temperature and loading rate on the overall degradation of different cellulosic substrates - cellulose, grass, cow dung and horse manure - under controlled nutrient conditions (i.e. carbon, nitrogen and phosphorus ratios) using acclimatised mixed cultures in continuous digestion.

6.1 Removal Rates of Substrates

For the substrates used in this study the removal rate has been correlated to the concentration of substrates present in the digester by two rel ationships:

(i) $q = kS_1$, the rate of substrate removal per unit digester volume per unit cell concentration.
and (ii) $R = KS_1$, the rate of substrate removal per unit digester volume.

As the input concentration was increased from 15,000 mg. lit $^{-1}$ to 25,000 mg. lit $^{-1}$ (dry weight), for all substrates digested, the use of the correlation $q = kS_1$, resulted in a lower value of k being obtained at the higher input concentration; apparently the output substrate concentration is a function of the input substrate concentration.

The use of the correlation $R = KS_1$, satisfactorily described the substrate removal rate over the entire range of operating conditions studied, for all substrates digested; the value of K being a function of the operating temperature.

This correlation ($R = KS_1$) is the same as that used by Gaddy et al (1974) to describe the digestion of liquid cow dung over a limited range of operating conditions. Pfeffer (1974) also used the same correlation (described in terms of gas production) in order to describe the digestion of domestic refuse. This type of correlation has also been used to describe the enzymatic saccharification of paddy hulls using pure and mixed cellulases (Ghose and Das 1971).

The form of digester response, at steady state operation, to increased input substrate concentrations which occurred in this study is not what is normally expected with biological reactions utilising soluble substrates. The findings of this study confirm the findings of Gaddy et al (1974) and Pfeffer (1974) for the digestion of insoluble substrates; however, for the digestion of soluble substrates the correlation, $R = KS_1$, has, generally, not been found to apply - the findings of Grady et al (1975)

for the digestion of mixed soluble substrates in the activated sludge process is a noteable exception to this.

For the digestion of soluble substrates, under steady state conditions, the percentage digestion increases with an increase in the input substrate concentration, providing no nutrient or toxic limitations exist, as shown by Lawrence and McCarty (1967). The response of a conventional digester to a given increase in organic loading depends on how the increase is achieved. Lawrence and McCarty (1967) have shown that if the increase is achieved by increasing the liquid flow rate then the detention time is reduced and a corresponding increase in the effluent substrate concentration occurs. However, they have also shown that a loading increase at constant detention time (i.e. achieved by an increase in the input substrate concentration) does not cause an increase in the effluent substrate concentration. An increase in the biological mass (cells) at steady state conditions is the only kinetic effect produced by such a loading increase. This form of response is found to occur providing the increased organic concentration in the influent stream does not give rise to nutrient limitations or toxic conditions in the digester.

Lawrence and McCarty (1967) have shown that this response is followed in the continuous digestion of volatile acids, although, they did note that population shifts in the mixed culture affected the form of the response to increases in the input substrate concentration - the output substrate concentration did show some variation with increase in the input substrate concentration for digestion at steady state conditions.

In this study it was found that while an increase in the liquid flow rate resulted in an increase in the output substrate concentration, which can be explained in terms of the effect of reducing the detention time, a loading increase at constant detention time (i.e. increase in the input substrate concentration) resulted in an increase in the output substrate concentration to such an extent that the percentage digestion ($100 \times \frac{S_o - S_1}{S_o}$) was independent of the input substrate concentration (i.e. $R = KS_1$), instead of the output substrate concentration being independent of the input substrate concentration, at steady state conditions.

This form of response could be due to several effects such as mass transfer limitations, nutrient limitations, toxic conditions or substrate structure and composition effects; all factors affect substrate availability.

The mass transfer limitations which may exist in the biological degradation of insoluble substrates are:

(i) Diffusional resistance to the transfer of substrate to the active surfaces of the biological floc (cells), as considered by Atkinson (1974).

Gaddy et al (1974) explained the failure of a continuous digester, being fed on liquid cow dung, to follow the response shown by Lawrence and McCarty (1969) by diffusional resistances existing in the biological floc. Gaddy et al proposed that substrate molecules must be transported through a gel (group of cells) before reaction can take place, and cited the work of Atkinson (1974) as evidence for such resistances without offering any data concerning diffusion coefficients or floc sizes apparent in the digesters. The floc sizes considered by Atkinson for the existence of such diffusional limitations are of the order of millimetres and microscopic examination of the digester contents in this study indicated that such gels did not exist and the size ranges for the cells were of the order of microns, suggesting that this form of diffusional resistance is not significant in the digestion process of this study.

(ii) Bulk diffusion - transfer of cells to the substrate by diffusion in the liquid medium. Since in this study well stirred digesters have been used then bulk diffusional resistance of this nature is not a limiting factor.

(iii) Diffusional resistance offered by the substrate pores to the cells. Extra cellular enzymes either excreted by cells or existing on the surface of the cells can only bring about hydrolytic conversion or any other enzymatic activity by being in direct contact with the substrate. The enzymes must diffuse through the pores of the substrate to bring about reaction- substrate voids, tortuous channels, and the size of the pores can affect this diffusional process.

In studies of heterogeneous catalysis diffusional resistances of this kind are considered with respect to reacting species diffusing through porous catalytic solids. The physical situation of such resistances, in this study, is that the catalytic agents are the bacterial cells and the reacting species are the substrates; the substrates are made up of pores or capillary voids (Cowling and Brown 1969) and for grass, cow dung and horse manure

such pores are composed of digestible and indigestible matter. With this physical situation in mind it is possible to consider the diffusional limitation as being given by the Thiele modulus(Φ), previously developed for use in heterogeneous catalysis studies.

The Thiele modulus(Φ) relates the reaction rate constant (K), the effective diffusion coefficient (D_{eff}) of the media through the pores, the effectiveness factor (η) and the radius of the porous solid (R). The effectiveness factor is defined as the ratio of the observed rate of reaction to that which would occur in the absence of diffusion effects within the pores (or the actual reaction rate to that which would occur if all of the surface throughout the inside of the particle were exposed to the cells). For values of $\Phi > 6$ diffusional limitations are significant and for values of $\Phi < .3$ such limitations are considered as being insignificant. Unfortunately experimental determination of D_{eff} or η was not carried out in this study but it is apparent that such a determination would help to resolve the existence of such diffusional resistances.

Nutrient limitations or toxic conditions can give rise to the form of digester response found in this study. Such effects can affect the reaction rate of the substrates by suppression of essential enzyme activity

levels and cell growth. Toxic effects such as excessive volatile acid concentration, cation toxicity or excess ammonia nitrogen concentration were not apparent in the digestion of the substrates in this study since the average conditions - pH, alkalinity and total volatile acid concentration - all lie within the accepted range for normal digestion (refer to 2.2) over the range of operating conditions. The form of digester response could not be explained by population shifts occurring for operation at the higher input substrate concentration since the yield constant showed little variation for steady state operation at either 15,000 mg. lit^{-1} or 25,000 mg. lit^{-1} input substrate concentration.

It has been reported that the enzymatic hydrolysis of cellulosic materials can be affected by micronutrient supply (Chan 1970 and Cowling and Brown 1969). Chan (1970) concludes that micronutrient deficiencies (e.g. iron, vitamin B_{12}, thiamin etc.) do affect the digestion of pure cellulose; on the other hand no data is produced in support of this assertion which suggests that this area requires further study. Poisonous components such as toxic phenolic substrates, present in vegetable wastes, can inhibit the growth and development of cellulolytic microorganisms

according to Cowling (1975); once more, no data is produced by Cowling. Nutrient limitations or toxic conditions both cause cell growth inhibition, possibly reducing enzyme activity levels which ultimately affects substrate digestion.

It has already been shown that diffusional limitations can affect substrate availability as can the presence of fixed solids (lignin) and the complex structure of the substrates. Direct physical contact between the enzymes in the hydrolysis step and the cellulosic substrate molecules is an essential prerequisite to hydrolysis. Since the substrates used in the study are insoluble and structurally complex this contact can be achieved only by diffusion of the enzymes from the cells into the complex structural matrix. Any structural feature, such as the presence of indigestible lignin, that limits the accessibility of the substrate to the enzymes within the fibre diminishes the susceptibility of the digestible components to enzymatic degradation. Evidence for substrate resistance because of structural and composition effects has been noted by Ghose and Kostick (1969), Mandels et al (1974) and Wood (1970). Ghose and Kostick (1969) noted that the most resistant of all substrates undergoing enzymatic hydrolysis are those

in a highly oriented or crystalline state such as cellulose or cellulosic substrates. Mandels et al (1974) found that for substrates containing cellulose with other materials in physical or chemical linkage it is necessary to pretreat the substrate to increase the availability of the cellulose in order to render it more susceptible to enzymatic hydrolysis. Cowling and Brown (1969) suggest that components deposited within the fine capillary structure (such as lignin) of the substrates (in the case of grass, cow dung and horse manure) not only reduce the accessibility of the digestible components but may also affect enzyme activity, once more no data is provided to support the argument.

It is not possible to conclude which of the above effects - diffusional limitations, nutrient limitations, substrate structure and composition effects - affect substrate availability. It is probable that a combination of several effects is apparent in the digestion of the substrates used in this study. The lack of information regarding the digestion of such substrates in mixed culture systems suggests that further studies should be carried out to investigate the significance of the above effects - with a view to investigating the effects separately since interactions between physical/biochemical mechanisms are

inevitable in the digestion process and one effect may mask the presence of other effects.

It is interesting to compare the results of Chan (1970) to the results obtained in this study for the digestion of cellulose. As shown by Graph 5.3.4 the results of Chan were fitted by the correlation $q = kS_1$ for detention times of 10 to 30 days. The value of k obtained using the results of Chan for the digestion of cellulose at $35^{o}C$ and S_o = 13744 mg. lit $^{-1}$ is not too much different from that obtained for the digestion of cellulose at $38^{o}C$ and S_o = 15,000 mg. lit $^{-1}$ in this study, as shown in Table 5.4. Now as noted in section 3.2 for values of the substrate concentration (S_1) less than the saturation constant (K_s) the correlation $q = \frac{q_{max} S_1}{K_s + S_1}$ can be reduced to $q = (\frac{q_{max}}{K_s})S_1$ (i.e. $q = kS_1$). Since Chan obtained a value of K_s equal to 44798 mg. lit $^{-1}$ and the average value of the cellulose concentration (S_1) in the digester at the 10-day detention time for Chan's study was 1539.5 mg. lit, $^{-1}$ which is considerably lower than K_s, then it is not surprising that the correlation $q = kS_1$ adequately fits Chan's results for detention times of 10 to 30 days.

It should be noted that Chan did not test the correlation $q=\left(\frac{q_{max}S_1}{K_s+S_1}\right)$ for its applicability at different input substrate concentrations or for different operating temperatures. The fit of $R = KS_1$ for Chan's results suggests that the rate of cellulose digestion in Chan's study was slightly faster than that obtained in this study (c.f. the values of K in Table 5.4).

From the enzymatic hydrolysis studies of Ghose and Das (1971) a first order reaction rate constant of 0.799 day $^{-1}$ was obtained for the enzymatic saccharification of paddy hulls using a mixed cellulase culture at 30^{o}C. This value is indicative of the slow utilisation of cellulosic substrates in biological reactions in general.

Maximum cell growth rates (μ max) or maximum substrate utilisation rates (q_{max}) have not been obtained in this study because of experimental limitations. However, such values have been obtained by Chan (1970) for the anaerobic digestion of cellulose and by many workers for the digestion (aerobic or anaerobic) of soluble substrates. For example, Jenkins and Menar (1967) obtained a value of μ max equal to 18.7 day $^{-1}$(θ_c^n=0.05 days) for the aerobic

digestion of settled sewage, and Lawrence and McCarty (1969) found a μ max value of 0.345 day $^{-1}$(θ_c^m=2.89 days) for the anaerobic digestion of acetic acid at 35°C. Chan (1970) obtained a value of μ max of 0.334 day $^{-1}$(θ_c^m=2.99 days). Comparisons between the different values must be understood in the light of the different experimental conditions employed by the investigators such as different seed populations and acclimation periods. However, it can be seen that the anaerobic digestion process requires detention times considerably longer than those required for the aerobic process. Generally conventional digesters (aerobic or anaerobic) are operated at detention times of about two to three times above the minimum detention time ($\frac{1}{\mu}_{max}$) so as to prevent bacterial cell washout.

6.2 Comparison of the Decomposition Rates of Cellulose, Grass, Cow Dung and Horse Manure

It has been shown that for a given percentage digestion the substrates grass, cow dung and horse manure require a longer detention time than does cellulose undergoing digestion at the same operating conditions (same temperature and detention time) - this is shown in Graphs 5.25 5.26, 5.27 and 5.28 for the digestion of cellulose, grass, cow dung and horse manure respectively. Graph 5.25 also shows that the percentage cellulose digestion occurring in the digestion of grass, cow dung and horse manure is considerably lower than that for the digestion of pure cellulose at the same operating conditions.

The limitations of this experimental study and the lack of available data in the literature regarding the digestion of such substrates makes it difficult to pinpoint effects for the above behaviour, and hence only possible explanations can be given. It has already been suggested that diffusional limitations, nutrient limitations and the substrate structure and composition can affect substrate availability and similarly such effects could account for the slower rates of digestion of grass, cow dung and horse manure relative to the digestion rate of pure cellulose.

The possibility of the existence of diffusional limitations occurring in the digestion of grass, cow dung and horse manure must be considered with respect to the larger particle size used for such substrates - $d_m \simeq 600\ \mu m$ - as compared to the particle size for cellulose - 15 to 40 μm. The larger size limits substrate accessibility as does the presence of the fixed solids content for such substrates; the chemical linkage between lignin and the carbohydrate fraction gives rise to pores of tight ultrastructure thereby reducing substrate accessibility.

Macro-nutrient limitations do not apparently account for the slower digestion rates of grass, cow dung and horse manure relative to cellulose since adequate Carbon/Nitrogen/Phosphorus ratios were maintained in all digesters. The presence of other constitutents, such as iron, vitamin B_{12}, thiamin etc. in the case of grass, cow dung and horse manure are generally considered as being stimulatory to cell growth although small amounts of phenolic materials present in such substrates are toxic to cell growth. As previously mentioned (refer to 6.1) the presence of such constituents, acting as micronutrients, can influence substrate susceptibility and cell growth in the hydrolysis of cellulosic materials but no quantitative information is available regarding this point.

The slower digestion rates obtained in this study for grass, cow dung and horse manure could also be due to the different degrees of polymerisation of the cellulose molecules in the substrate complex, steric rigidity or to different degrees of crystallinity between the forms of cellulose present in the substrates - Cowling and Brown (1969) suggests that such differences affect substrate accessibility and affect the rate of hydrolysis very considerably, however experimental verification of these factors is not given and requires further investigation.

Comparison of the reaction rate constants (K) for the digestion of grass, cow dung and horse manure (as shown in Tables 5. 5 , 5.6, and 5.7) show that grass is removed at a faster rate than cow dung and horse manure at 28°C and 38°C, but at 48°C the rate constants for grass and horse manure are very similar; it is apparent that the digestion of horse manure is more affected by the operating temperature (E = 8.5 k cal/mole) than is the digestion of grass (E = 6 k cal/mole).

It is worth noting that the fixed solids content of grass is around half that of cow dung and horse manure; moreover, it is digested at a faster rate (at least for

operating temperatures of 28°C and 38°C) and the possibility of the fixed solids content being correlated with the rate of digestion needs further investigation. The above effect suggests the presence of structural or diffusional limitations rather than micronutrient limitations in affecting substrate susceptibility.

The "activity" of a substrate can be defined in terms of the potential gas production and is a useful parameter for comparing the different substrates in relation to the user's requirements. Providing no toxic components or macronutrient deficiencies (C:N ratio) are imposed on the digestion process then substrates capable of producing large quantities of gas per unit weight of substrate added should be used since, as shown by this study, substrates capable of producing only low quantities of gas per unit weight of substrate added to a digester (because of high fixed solids content per unit weight of substrate) may result in slower reaction rates being obtained, not to mention the possibility of the user's gas demands not being met.

In terms of the potential gas production per unit weight of substrate added to a digester system the "activities" of the substrates used in this study are:

(i) cellulose : 540 cm^3/gm. added at S.T.P.
(ii) grass : 411.5 cm^3/gm. added at S.T.P.
(iii) cow dung : 290.4 cm^3/gm. added at S.T.P.
(iv) horse manure : 288.6 cm^3/gm. added at S.T.P.

In terms of the methane-carbon produced per litre of digester capacity per day the activities of the substrates can be compared; for digestion at 38°C and an input concentration of 15000 mg. lit^{-1} (dry weight), for 10 to 30 days detention time the activities are:

cellulose	: 82.6 to 217 mg. CH_4-C/lit. digester capacity-day.	
grass	: 54 to 104	"
cow dung horse manure	:)) 37 to 71 :)	"

The activity expressed as the above is a useful parameter for comparing the digester utilisation efficiency for digesters being fed on the same input substrate concentration (dry weight) - as can be seen a digester fed on cellulose is removing more methane - carbon per unit volume of digester capacity per day relative to digesters fed on the same input substrate concentration (dry weight) of grass, cow dung and horse manure. This reflects the fact that cellulose has a higher potential gas production per unit weight added to the digester.

Rates of digestion for cellulosic substrates may be increased by chemical or physical methods applied to the substrates by increasing substrate availability according to Mandels et al (1974). The same authors concluded that the most successful milling treatment for cellulosic substrates (bagasse, rumen fibres etc.) was ball milling which reduced crystallinity and particle size and increased the digestion rate in enzymatic hydrolysis.

6.3 The Rate Limiting Step in the Digestion of Cellulose, Grass, Cow Dung and Horse Manure

6.3.1 Cellulose

As the detention time of the digesters fed on cellulose was decreased from 30 to 10 days, the total volatile acid concentration showed little change, as shown in Graphs 5.1 and 5.2 for digestion at 28°C and 38°C. However, from the same graphs, it can be seen that the undigested cellulose concentration (S_1) increased considerably as the detention time was decreased. Therefore it appears that the limiting step for the overall digestion of cellulose is the initial hydrolysis step - hydrolysis of cellulose to soluble intermediate products. This agrees with the conclusions of Chan (1970) and Springer (1972) - their findings were based on the digestion of cellulose (Chan used kraft paper pulp powder - 125 μm. diam. particles, and Springer used cellulose obtained from a river bed - 10μm. diam. fibres).

6.3.2 Grass, Cow Dung and Horse Manure

As the detention time was decreased from 30 to 10 days it is seen from the plots of the effluent substrate concentration (S_1) versus detention time that, for digesters fed on grass, cow dung and horse manure, the value of S_1

increased considerably over the detention time range and yet in all cases the total volatile acid concentration was not significantly affected and remained at a low concentration of around 100 mg. lit $^{-1}$ (acetic acid). The above plots are shown in Graphs 5.6, 5.7 and 5.8 for the digestion of grass, Graphs 5.13, 5.14 and 5.15 for the digestion of cow dung, and Graph 5.20 for the digestion of horse manure. It can therefore be concluded that the enzymatic hydrolysis of the insoluble volatile matter represents the rate limiting step in the overall digestion of grass, cow dung and horse manure to methane in this study.

O'Rourke (1968) arrived at a different conclusion for the rate limiting step: On digesting a raw waste containing 28.4 gm. lit^{-1} C.O.D. as lipids, 4.5 gm. lit $^{-1}$ C.O.D. as cellulose and 6.4 gm. lit $^{-1}$ COD as protein, he found that the digestible portion of the cellulose (91% dry weight of the cellulose was found to be digestible) and protein components (59.5% dry weight of the protein was found to be digestible) was destroyed by conversion to volatile fatty acids more rapidly than the volatile acid or lipid fractions of the waste. At solids detention times of 5 days the cellulose and protein fractions were digested by 70% or more whereas the volatile acid and lipid fractions were

hardly removed (the volatile acid concentration was greater at 5 days S.R.T. than that present in the input feed indicating volatile acid accumulation arising from volatile acid production from the more readily digestible components such as cellulose and protein). The data of O'Rourke indicated that the digestion of long-chain and volatile fatty acids was the rate limiting step in the digestion of complex wastes.

It should be noted that the substrate used by O'Rourke was composed of different individual component concentrations than those present in the substrates used in this study; besides the use of pure cellulose the other substrates used in this study were made up of large fractions of cellulosic components (cellulose, he micellulose) and fixed solids with only small amounts of the lipid component present. Similarly, the conclusion of Chan (1970) Springer (1972) and Pfeffer (1974) that the rate limiting step in the digestion of cellulose or cellulosic wastes was the initial hydrolysis step was derived from a study of the digestion of feedstocks containing very little or no lipid component at all. Therefore the conclusion of O'Rourke indicates that the hydrolysis rate of cellulosic wastes could well be promoted by the presence of other

components, such as volatile fatty acids or lipids, to such an extent that the hydrolysis of the insoluble carbohydrate fraction becomes non-limiting. This may suggest a stimulating effect on the hydrolytic enzymes by the presence of such components. The work of O'Rourke also indicates the importance of understanding enzyme interaction and stimulation on the rates of digestion of different components in the digestion of a substrate consisting of such components using mixed cultures.

6.4 Effect of Temperature on the Reaction Rate Constants

6.4.1 Cellulose

The limited data (at 28°C and 38°C) is consistent with the Arrhenius relationship (see Graph 5.5). This is consistent with other data from the literature; from values of the slopes obtained from the Arrhenius plot as given in Table 5.4, it is seen that they are very similar and the use of an average value for the slope gives an activation energy, E, of about 12 k cal/mole for the digestion of cellulose at 28°C and 38°C. For a temperature change of 10°C the values of the rate constants are approximately doubled.

Springer (1972) obtained values of between 16.5 and 17.5 k cal/mole (temperature range of 10°C to 20°C) for the batch digestion of cellulose extracted from river beds. King and Vessal (1969) quote an activation energy of 6.94 k cal/mole for the enzymatic hydrolysis of cellulose by the cellulase Trichoderma viride and showed that the activation energy depends on the crystal lattice form of cellulose. Ghose and Kostick (1969) have shown for amorphous cellulose that the Arrhenius plot is linear over the temperature range of 10-57°C (activation energy = 5.1 k cal/mole) for the enzymatic hydrolysis by T. viride and demonstrated that the effect of temperature on the reaction velocity in the cellulose/T. viride enzyme system (cellulose saccharification) depends on the nature of the cellulosic substrate used.

The difference in the activation energies obtained by various investigators is due to the different physical nature of the cellulosic substrates used; the different operating conditions (seed population, batch or continuous digester operation, acclimation periods etc.) in which the experiments have been carried out; and the different modes of hydrolysis (enzymatic hydrolysis using pure cellulases - aerobic process, or hydrolysis using mixed culture systems as in the anaerobic digestion process).

6.4.2 Grass, Cow Dung and Horse Manure

The reaction rate constants (k or K values) obtained from the digestion of grass at 28°C, 38°C and 48°C obey the Arrhenius relationship, as shown in Graph 5.12. Since the slopes of the plots are similar the use of an average value for the slope gives an activation energy of 6 k cal/mole. Similarly the Arrhenius plots for cow dung (Graph 5.19) and for horse manure (Graph 5.23) show a linear relationship. Once more the use of an average value for the slope gives an activation energy for the digestion of cow dung of 7.4 k cal/mole and 8.5 k cal/mole for horse manure.

Commonly quoted values for the activation energy obtained in cell growth studies usually hold over only small temperature ranges (15°C to 40°C) and the use of the Arrhenius relationship has been found to be a useful correlation for predicting the effect of temperature on cell growth or substrate utilisation over such temperature ranges. Activation energies of 15-25 k cal/mole have been obtained for the digestion of soluble substrates (acetic acid, glucose etc.) in aerobic or anaerobic treatment processes. It therefore appears that the digestion of soluble substrates is more affected by operation at different temperatures as compared to the digestion of the insoluble substrates used in this study.

It is important to realise that differences in the microbial population can be significant at different operating temperatures, for the same initial innoculum, and such differences may result in different activation energies existing for narrow temperature ranges. Thermophiles have enzymes which in general, are much more resistant to conditions which cause denaturation (such as high operating temperatures), just as the enzymes of mesophiles are much more stable than those of psychrophiles: Organisms have enzymes which are stable throughout the temperature range in which they grow, but not at temperatures too much higher. This emphasises the importance of culture acclimatization - the temperature relationship found in this study for digestion of the different substrates would not be expected to follow the Arrhenius relationship for non-acclimated cultures since such cultures may give rise to population shifts resulting in different cell yields and reaction rates as compared to digestion using acclimated cultures. Further, it cannot be assumed that reaction rate constants for operating temperatures higher than $48^{o}C$ or lower than $28^{o}C$ will obey the Arrhenius relationship because of the unknown effects on the cell population e.g. the unknown effects on the enzyme activity levels at different operating temperatures.

It has already been shown that the yield constant was found to be essentially independent of the operating temperature, however this result would not necessarily be obtained for unacclimatised systems or for systems operating at temperatures outside the range used in this study, once more, for the same reasons given above. Lawrence and McCarty (1969) have noted that caution should be exercised in comparing the utilisation kinetics for a given substrate at different temperatures since different microbial populations may be involved.

When considering the effect of digester operation at low temperatures it was found in this study that effective anaerobic treatment was obtained, as shown in Graphs 5.25 5.26, 5.27, and 5.28 for the digestion of cellulose, grass, cow dung and horse manure respectively. For example, digestion at a detention time of 17 days, at 28^{o}C, resulted in the following percentage digestion values being obtained:

Cellulose	:	82 %
Grass	:	65 %
Cow Dung	:	52 %
Horse Manure	:	56 %

6.5 Engineering Significance and Application

The engineering significance of this study lies in the implications of the findings for predicting digester performance. Since it has been argued that the rate limiting step is the hydrolysis step and that the gas produced is proportional to the substrate digested, whilst the percentage digestion is independent of the input substrate concentration, then the digester performance can be predicted either by the use of $R = KS_1$, as already used in this study or by $R = K_g G_1$, as used by Pfeffer (1974) and defined in 3.1.2.4, where R is defined as the rate of substrate removal, given in terms of the gas produced per unit weight of substrate added to the digester, per unit digester volume. The value of K , as used in $R = KS_1$, should equal the value of K_g if gas production is proportional to substrate utilisation, which is the case in this study; hence the kinetics of gas production for the digestion of cellulosic wastes can be described in terms of substrate utilisation or in terms of gas production.

Theeconomic objective for applying the process for gas production should be the maximum stabilisation of the organic feedstocks (by the maintenance of a high treatment efficiency) whilst maintaining, as high as possible, maximum utilisation capacity (defined as the

weight of feedstock utilised per unit volume of digester capacity per day). Now these two parameters conflict with each other; for maximum stabilisation long detention times are required, as shown in Graphs 5.25 to 5.28 inclusive, but for high substrate utilisation capacity. short detention times are required. Therefore a compromise must be used based on a knowledge of such factors as substrate value; immediate gas requirements; loading limitations; quality and quantity of substrate available, etc. Estimates of gas requirements for various applications in less developed countries have been considered by Singh (1972).

The application of the process in the U.K. can be considered from the standpoint of the solid wastes problem. As an example the process may be applied to the waste paper fraction of the organic wastes arising in the U.K., for which the process has not been applied up to now.

The total quantity of waste paper (excluding that portion which is recycled) is about 0.71b/capita-day (most of which is dumped on land or at sea). If this fraction was loaded at a rate of 0.092 lb/ft^3-day (as used in this study for the digestion of pure cellulose) to a 30-day detention time digester at 38^{o}C, a digester of

capacity 2.5 ft^3/capita-day would be required. Conventional heated sludge digesters, used at many sewage works in the U.K., are usually operated at about 0.05 to 0.15 lb V.S./ft^3-day for detention times of 15 to 30 days and are usually designed for 1.5 to 3 ft^3/capita-day. It is therefore possible to digest the paper fraction (75 to 85% cellulose) of the solid wastes arising in the U.K. directly in existing sludge digesters (at present anaerobic sludge digesters are fed on the sludge produced from the activated sludge process) since cellulose digesters and sludge digesters are similar in operating characteristics. This could be an important application in the U.K. since, increasingly, the shortage of suitable sites together with anti-pollution legislation is mitigating the practice of disposal of such wastes on land or at sea.

Further considerations that should be given to the application of the process in developed countries, such as in the U.K., are those regarding competition from a wide range of processes that utilise cellulosic feedstocks to produce valuable products. Examples of such processes are those used to produce single cell protein from cellulosic materials or the production of sugar; Figure 6.1 shows the wide range of products that can be derived from cellulosic feedstocks.

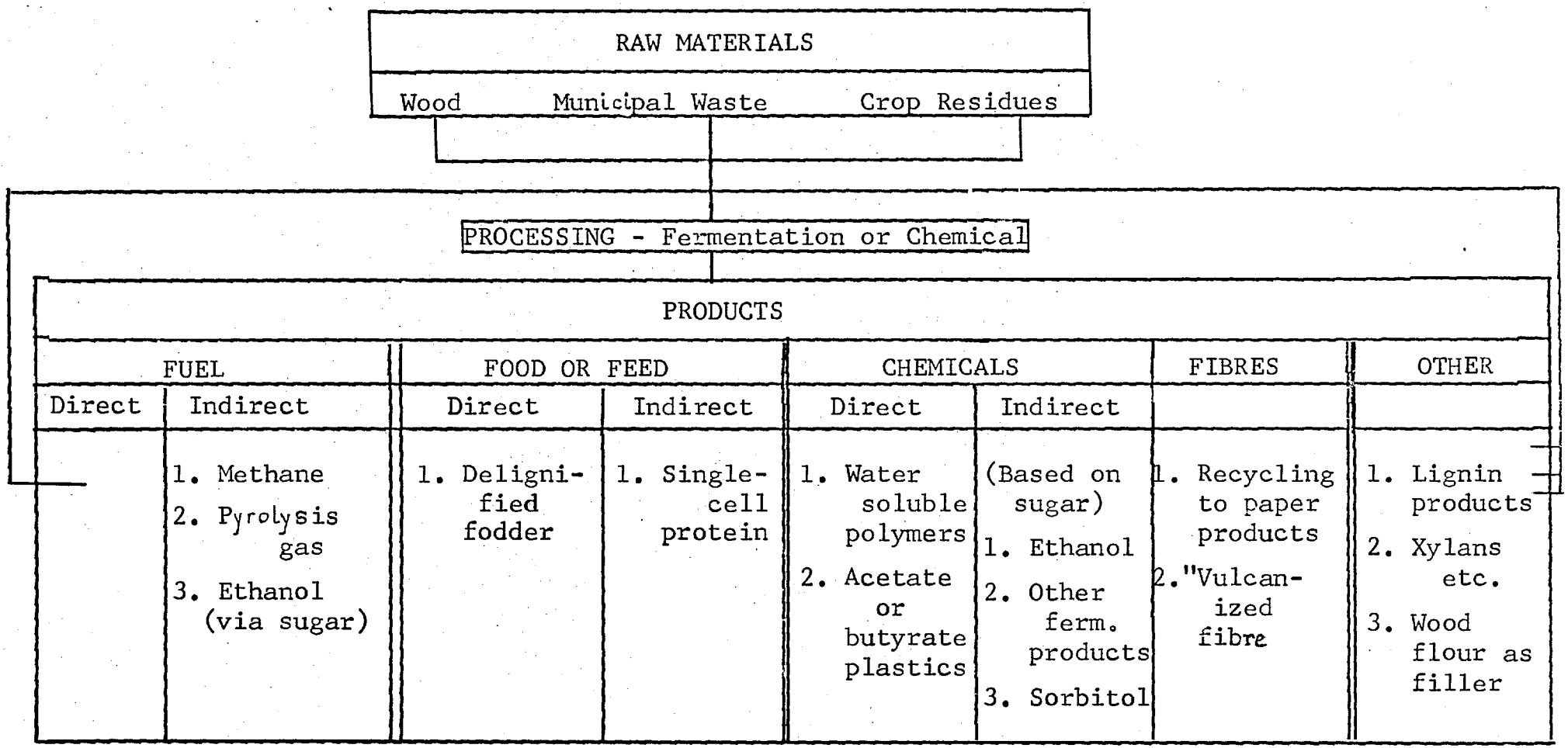


Figure 6.1. Product Chart for Cellulose (Finn 1975)

Dunlap (1975) has compared several such processes by considering the economic value of the products arising from each process based on a pure cellulose feedstock. Table 6.1 shows the results of Dunlap (1975) based on 1974 market prices. The table gives the current values (1974) of several products that can be produced from cellulose in terms of the unit weight of cellulose used (U.S. figures). It must be emphasized that the values given in the table are simply the current market prices (1974) or values of the products multiplied by the efficiency of the particular process to produce the product from cellulose; the values are not related to the cost of production. The compilation shown in Table 6.1 does not account for substrate limitations connected with each process, as an example, low grade cellulosic wastes such as grass or alfafa can be used for fuel (direct) or methane production, however, for single cell protein production or glucose production higher grade substrates, such as paper pulp, are required.

A particular application for the process in the U.K. is for the disposal of very strong (in B.O.D. terms) animal wastes, such as pig wastes. Disposal of such wastes to sewage works or by land spreading is environmentally damaging and can be expensive. The scale and localised

Product or use	Units	Value ($ per unit)	Units produced from 1.0 lb dry cellulose	Value of product from 1.0 lb dry cellulose
Direct burning as fuel	10^6 Btu	0.30-0.80	0.010	0.3-0.8¢
Methane	10^6 Btu	0.80-2.00	0.004	0.3-0.8¢
Ethanol	lb	9.1¢	0.56	5.1¢
Single cell protein	lb	15.25¢	0.45	6.8-11.2¢
Glucose	lb	9.8-26¢	0.80	7.8-20.8¢

Table 6.1 Value of Products from Cellulose
(Dunlap 1975)

production of such wastes do not offer scope for S.C.P. production, which is an expensive and technologically advanced process, and the high water content does not permit the use of the waste as a direct fuel. Since the digestion process produces a useable fuel, an effluent that may be used as a fertilizer and removes the smell and pathogenic qualities of the waste it seems reasonable to expect the digestion process to offer an attractive method for the disposal of such wastes.

In less developed countries the use of cellulosic wastes is controlled more by the need for a cheap fertilizer and a useable supply of energy in very localised areas, such as village communities, as well as by the costs of any particular process which utilises the waste. The amount of interest that has been shown in the process, at present, for application in less developed countries appears to offer scope for the digestion process being more widely adopted in such areas.

CHAPTER 7

CONCLUSIONS

The principal conclusions drawn from this investigation are as follows:

(i) The digestion of each of the following insoluble cellulosic feedstocks - cellulose (crystalline powder), grass, cow dung and horse manure (all substrates previously ground and dried) - in a nutrient rich medium is possible in continuous digester operation at temperatures of 28°C, 38°C and 48°C, without any prior addition of sludge solids (to act as a seed material) in a mixed acclimatised culture.

(ii) As the detention time of the digesters was decreased from 30 to 10 days, the total volatile acid concentration increased only slightly while the digester substrate concentration increased considerably for all substrates digested. Therefore it is concluded that the initial hydrolysis step for the conversion of the input solids to soluble intermediate products is the rate limiting step for the overall conversion of the cellulosic feedstocks to methane.

This conclusion supports the findings of Chan (1970), Springer (1972) - their work being based on the continuous and batch digestion of cellulose respectively - and Pfeffer (1974) for the continuous digestion of domestic refuse (35.8 % cellulose dry weight) with sewage sludge solids. However, this is in contrast with the findings of O'Rourke (1968); he concluded that the rate limiting step was the methanogenic fermentation of the volatile acids and the long chain fatty acids to methane gas for the continuous digestion of a waste containing 44.5 per cent of the total C.O.D. as lipid (12.6 gm./lit.), 15.8 per cent of the total C.O.D. as cellulose (4.5 gm./lit.) and 22.5 per cent of the total C.O.D. as protein (6.4 gm./lit.). Since the wastes used in this study consist largely of cellulosic materials with little or no lipid content, then the findings of O'Rourke could suggest a stimulatory effect on the hydrolysis rates of cellulosic components by the presence of long chain fatty acids. However, no data has been obtained in this study regarding such an effect and therefore further investigation is required.

(iii) The results of this study indicate that the digestion of cellulosic feedstocks can be correlated by a rate equation of the form:

$$R = KS_1$$

where R = rate of substrate removal per unit digester volume, mass/volume-time

S_1 = substrate concentration in the digester, mass/volume.

K = first order rate constant, time $^{-1}$.

This rate equation was found to describe the results obtained for the digestion of cellulose, grass, cow dung and horse manure for input substrate concentrations of 15,000 mg. lit $^{-1}$ and 25,000 mg. lit $^{-1}$, for detention times of 10, 17 and 30 days at temperatures of 28°C, 38°C, and 48°C.

A rate equation of the form:

$$q = kS_1$$

where q = rate of substrate removal per unit digester volume per unit cell concentration, time^{-1}.

k = first order rate constant, volume per mass-time.

did not adequately correlate the results over the full range of operating conditions; the rate constant k was found to decrease with increase in the input substrate concentration fed to the digesters.

(iv) The relationships of $\frac{1}{\theta}$ versus q or the gas production rate G_T, follow the same general pattern over the range of $\frac{1}{\theta}$ studied for all the substrates digested; therefore it appears that the substrate utilisation rate and the gas production rate are growth associated and gas production is proportional to the substrate utilised for all substrates digested.

(v) The rate constants were found to be consistent with the Arrhenius relationship in the temperature range of 28°C to 48°C for all substrates digested.

(vi) The digestion of grass, cow dung and horse manure resulted in a lower percentage digestion, as measured by the change in the substrate concentration (C.O.D. of the volatile matter), at a given detention time and a lower percentage cellulose digestion, as measured by the change in the cellulose concentration in grass, cow dung and horse manure, as compared to the digestion of pure cellulose for the range of operating conditions studied. It was not possible, in the present work, to identify the precise cause of this effect. However, from previous studies, based mainly on the enzymatic hydrolysis of cellulosic wastes using cellulases, substrate ultilisation rates can

be affected by diffusional limitations through the substrate pores, nutrient limitations arising in a mixed substrate/mixed culture system or due to the substrate structure and composition.

Suggestions for further Work

(1) A nutrient rich medium was fed with the substrates to the digesters in this study, however, the effect of micro nutrient concentration levels on substrate utilisation rates is not well understood, especially for mixed substrate (protein, cellulose, he micellulose etc.)/ mixed culture systems and requires further investigation.

(2) The effect of pore size and particle size on substrate utilisation rates requires further study, as previously discussed. Pore sizes can be found from the solute exclusion technique developed by Stone et al (1969). This method employs a series of solute molecules which range in size upwards from glucose with a hydro-dynamic diameter of 8Å, to a dextran of molecular weight 24×10^6 and diameter of 1600Å. These act as a set of molecular probes of the substrate structure and give a complete pore size distribution. The method therefore measures substrate accessibility with the solute molecule and this can be related to substrate utilisation as can the particle size

for pure cellulose substrates or for mixed cellulosic substrates.

(3) An optimisation study based on the following:

(i) process operation optimisation - the optimum operating temperature, nutrient supply, particle size, detention time and loading rate.

(ii) The best mode of operation:- Since the digestion process is a two stage process consisting of at least three steps then it is possible to split the process into one or more steps; the initial hydrolysis of the insoluble feedstocks could be carried out in a separate digester to the subsequent fermentation steps. Such a system could give greater operational control over nutrient supply, loading rate, the choice of the optimum temperature, and toxicity problems for each of the separate steps, although more extensive data concerning the operational characteristics for each step for design purposes is required.

APPENDICES

APPENDIX I

STOICHIOMETRY OF BIOLOGICAL REACTIONS

(McCarty, 1971, 1975)

All bacterially mediated reactions (except photosynthetic ones) consist of two components, a synthesis component and an energy component and they are oxidation-reduction reactions which involve the transfer of electrons; thus they involve an electron donor (which is generally, but not always, considered to be the substrate or waste) and an electron acceptor, and for each a half reaction can be written. The half reactions are written on an electron equivalent basis by dividing each reaction by the number of electrons involved. Three different oxidation half reactions result and from these an overall stoichiometric equation can be obtained. The three oxidation half reactions are:

1. One for the electron donor R_d,
2. One for the electron acceptor R_a,
3. One for the bacterial cells R_c.

The overall reaction (R) can then be obtained as follows:

$$R = R_d - f_e R_a - f_s R_c$$

f_e = portion of the electron donor which is coupled with the electron acceptor,

f_s = portion of the electron donor which is coupled with cell formation,

where $f_e + f_s = 1$,

and $f_e = \frac{A}{1+A}$, $f_s = \frac{1}{1+A}$.

A = ratio of electron equivalents of substrate used for energy to electron equivalent of cells formed,

$$\frac{\Delta G_p/(K^M + 7.5)}{K \times \Delta G_r}$$

ΔG_p = energy required to convert the cell carbon source to an intermediate stage.

For organic electron donors, ΔG_p equals $\Delta G^0(W)_d + 8.54$, where $\Delta G^0(W)_d$ represents the free energy per mole electrons for the electron donor.

$\Delta G_r = (\Delta G^0(W)_d - \Delta G^0(W)_a)$, where $\Delta G^0(W)_a$ represents the free energy per electron equivalent for oxidation half reaction for the electron acceptor.

K represents the efficiency of energy transfer for bacterial growth and is about 0.6.

The free energies may be found from McCarty (1971). The number of electron equivalents in a unit mass of cells can be determined once its composition in terms of the major elements is known. A typical formulation for bacterial cells is given by Hoover and Porges (1952) as $C_5H_7O_2N$.

APPENDIX II

PARTICLE-SIZE DISTRIBUTION OF SUBSTRATES

An I.C.L. laboratory mill was used for particle size reduction. BS410 standard sieves were used for sizing.

1. GRASS

Particle Size Distribution

BS410 Standard Sieve No.	Size mm or μm	Percentage Weight Retained grams	Percentage Weight Accumulated
5	3.35 mm	0	0
14	1.18 mm	5	5
22	710 μm	28.5	33.5
30	500 μm	27.4	60.9
44	355 μm	18.0	78.9
60	250 μm	10.9	89.8
85	180 μm	7.0	96.8
120	125 μm	1.5	98.3
170	90 μm	1.5	99.8

A plot of Percentage Weight Accumulated (in probability scale) versus particle size is shown in Fig. AII.1.

Mean particle size (Accumulated Percentage Weight of 50%)d_{50} = 570 μm.

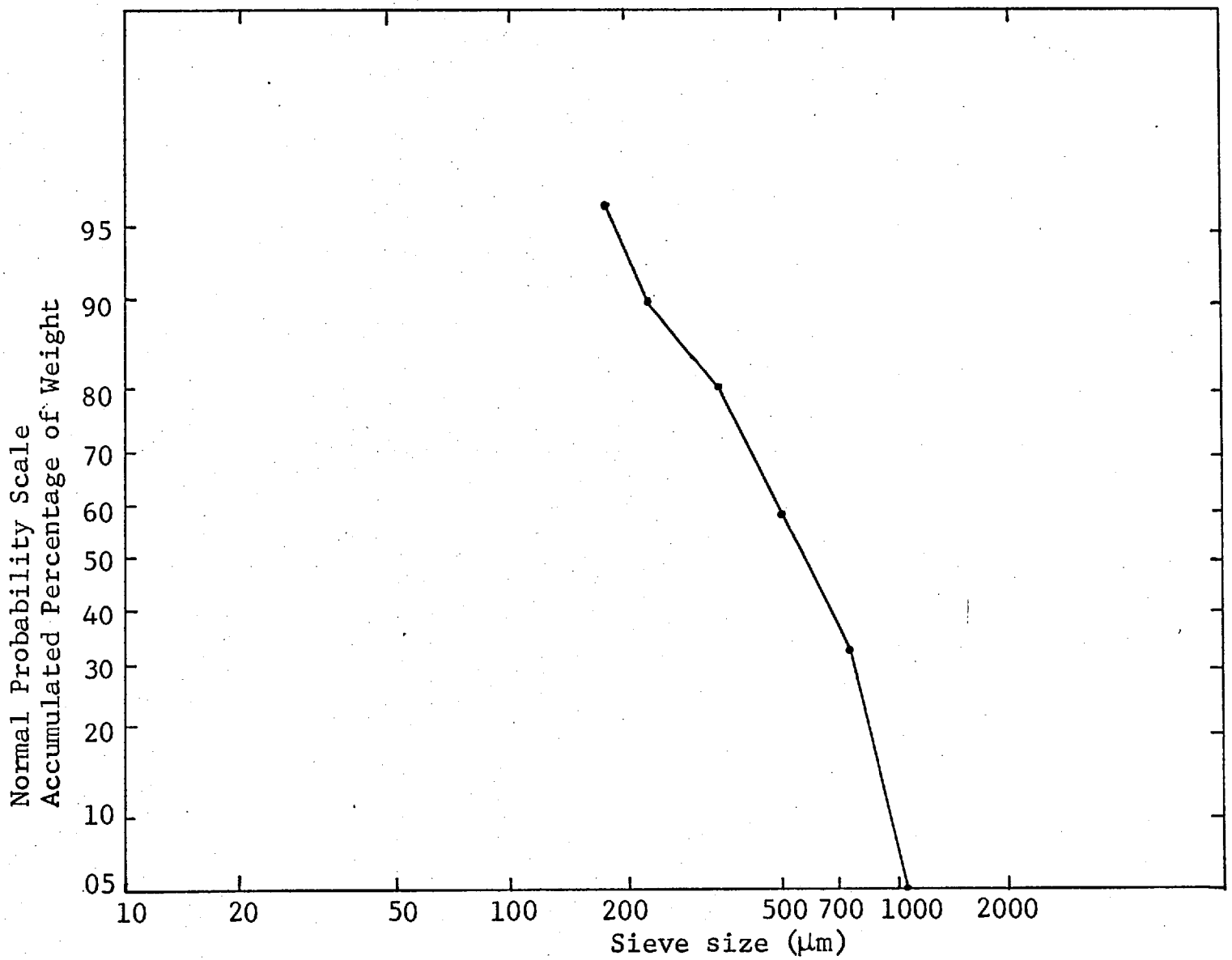


FIGURE AII.1 Particle Size of Grass.

2. COW DUNG

Particle Size Distribution

BS410 Standard Sieve No.	Size mm or μm	Percentage Weight Retained Grams	Percentage Weight Accumulated
5	3.35 mm	-	0
14	1.18 mm	15.0	15.0
22	710 μm	29.0	44.0
30	500 μm	11.4	55.4
44	355 μm	12.2	67.6
60	250 μm	7.0	74.6
85	180 μm	11.8	86.4
120	125 μm	8.9	95.3
170	90 μm	4.2	99.5

A plot of Percentage Weight Accumulated (in probability scale) versus particle size is shown in Figure AII.2.

Mean particle size (Accumulated Percentage Weight of 50%) d_{50} = 690 μm.

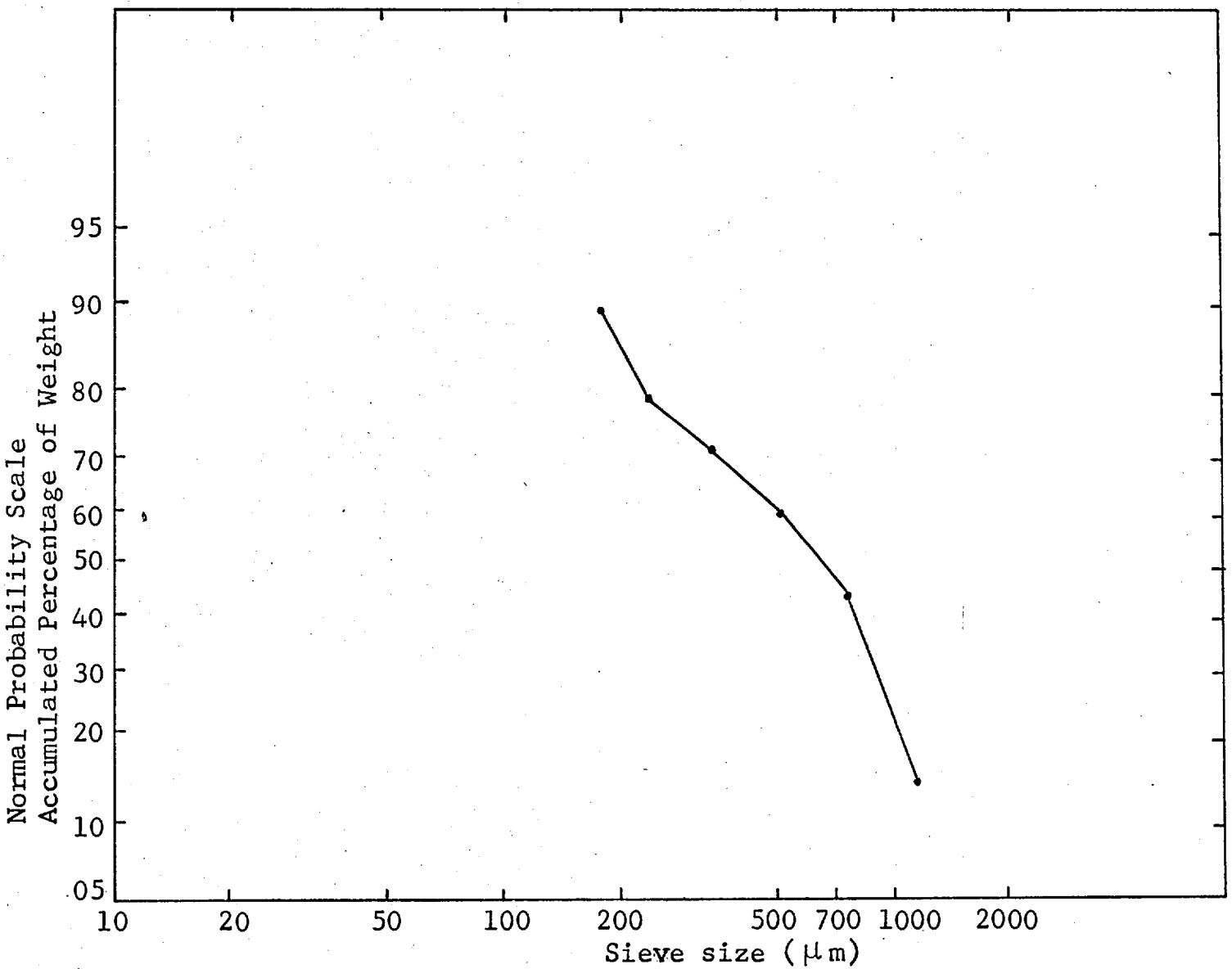


FIGURE AII.2 Particle Size of Cow Dung.

3. HORSE MANURE

Particle Size Distribution

BS410 Standard Sieve No.	Size mm or μm	Percentage Weight Retained grams	Percentage Weight Accumulated
5	3.35 mm	0	0
14	1.18 mm	20.6	20.6
22	710 μm	23.1	43.7
30	500 μm	14.5	58.2
44	355 μm	10.5	68.7
60	250 μm	6.8	75.5
85	180 μm	12.5	88.0
120	125 μm	11.2	99.2
170	90 μm	0.3	99.5

A plot of Percentage Weight Accumulated (in probability scale) versus particle size is shown in Figure AII.3.

Mean particle size (Accumulated Percentage Weight of 50%) d_{50} = 600 μm.

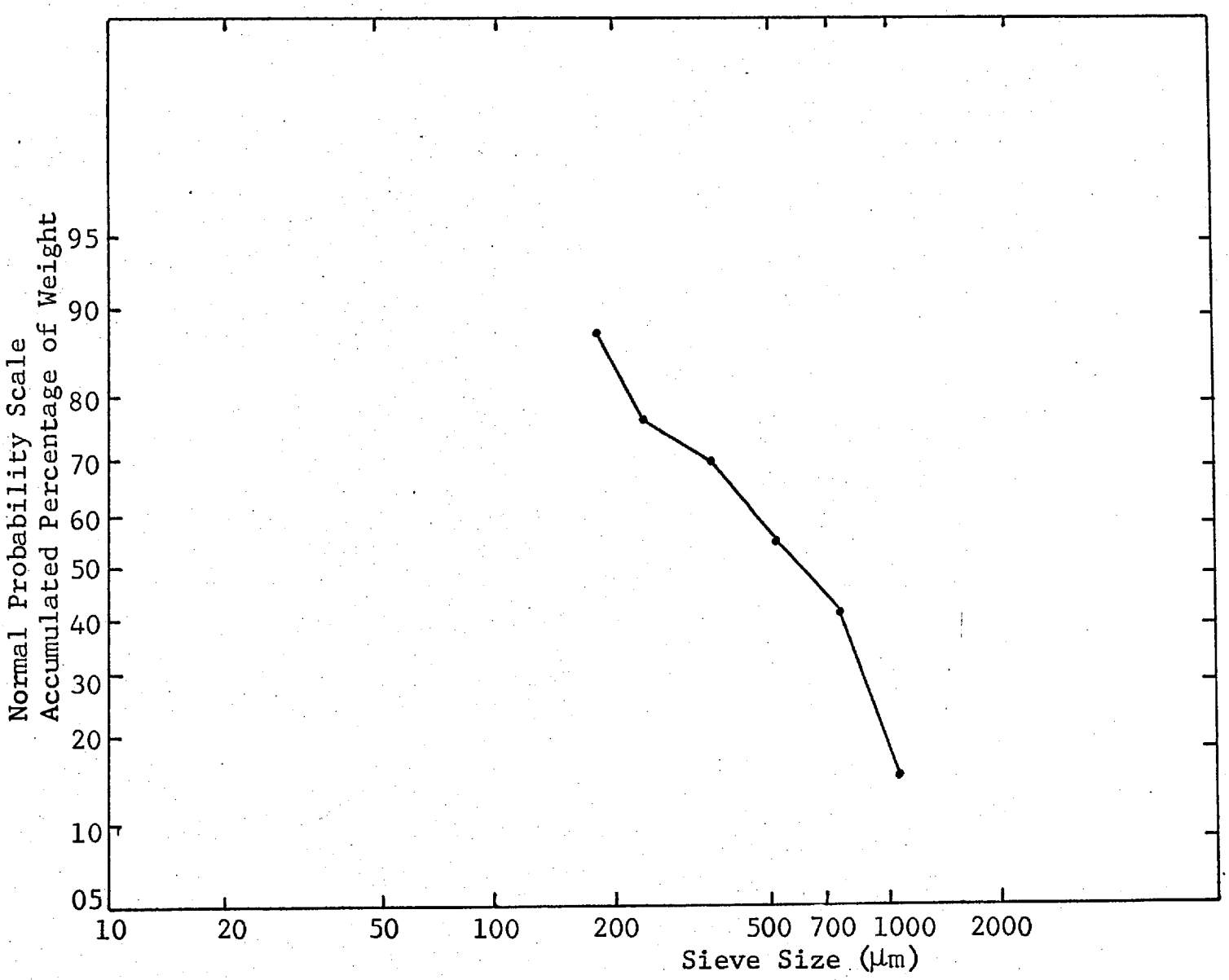


FIGURE AII.3 Particle Size of Horse Manure.

APPENDIX III

GENERAL CHARACTERISTICS DATA

The experimental and calculated data for the digestion of cellulose, grass, cow dung and horse manure is given in the following tables. A list of the symbols given in the tables is shown below:

B.P. - refers to a particular balance period.

S_o - input concentration of substrate (mg/lit. of input liquid).

for cellulose the value of S_o is mg/lit. cellulose x 10^{-3}.

for grass, cow dung and horse manure the value of S_o is mg/lit $COD_{v.m}$ x 10^{-3}.

S_1 - output concentration of substrate (mg/lit. of output liquid sample).

for cellulose the value of S_1 is mg/lit. cellulose x 10^{-3}.

for grass, cow dung and horse manure the value of S_1 is mg/lit. $COD_{v.m}$ x 10^{-3}.

% dig - the percentage change in the input to output substrate concentrations

$$\left(\frac{S_o - S_1}{S_o} \times 100\right)$$

X_1 - VSS concentration of output liquid sample, mg/lit. x 10^{-3}.

q - $\frac{S_o - S_1}{X_1 \times \theta}$ day^{-1}

where θ is the detention time.

R - $\frac{S_o - S_1}{\theta}$ $mg.lit^{-1}$ day^{-1}.

G_T - the total corrected gas production (cm^3 day^{-1}).

% CH_4 - percentage methane content of gas produced (% by volume).

C_i - carbon input to digesters per day - includes carbon in solid and liquid feed, $mg.day^{-1}$.

C_g - carbon content of gas produced, $mg.day^{-1}$.

C_f - carbon content of sample in the filtrate, $mg.day^{-1}$.

C_s - carbon content of residual solids on the filter, $mg.day^{-1}$.

$\%\ C_{rec}$ - percentage carbon recovered, $\frac{(C_g + C_f + C_s)}{C_i} \times 100.$

$\%\ Ce_{dig}$ - percentage cellulose digestion. This figure applies only to the digesters fed on grass, cow dung and horse manure, and is:

$$\frac{\left[\begin{array}{c}\text{Influent Cellulose} \\ \text{Concentration mg/lit} \\ \text{of input feed}\end{array}\right] - \left[\begin{array}{c}\text{Effluent Cellulose} \\ \text{Concentration mg/lit} \\ \text{of output}\end{array}\right]}{\left[\begin{array}{c}\text{Influent Cellulose Concentration} \\ \text{mg/lit of input feed}\end{array}\right]} \times 100$$

The cellulose concentrations in this parameter are determined from the analysis of cellulose for grass, cow dung and horse manure.

The average values for each parameter are shown (Avg.) and the coefficient of variation ($\%\ C_v$) is given for the relevant parameters.

TABLE III.1 EXPERIMENTAL AND CALCULATED DATA

Substrate: CELLULOSE

Temperature: 28°C

Dry Feed Input Concentration: 15000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% C_e dig.
θ = 30 days	1	15.000	2.061	86.3	0.995	0.433	431.3	0.077	445.0	58.0	448.2	238.5	71.3	90.2	89.2	-
	2	"	2.205	85.3	0.877	0.486	426.5	0.068	397.6	57.0	"	213.0	91.2	89.1	87.7	-
	3	"	2.190	85.4	0.934	0.457	427.0	0.073	425.0	58.0	"	235.5	61.1	76.9	90.1	-
	4	"	2.084	86.1	1.194	0.360	430.5	0.092	407.0	59.0	"	218.0	69.1	80.5	83.3	-
	5	"	2.290	84.7	0.872	0.486	423.6	0.068	400.2	58.0	"	239.1	61.5	90.5	87.2	-
	Avg.	"	2.166	85.5	0.974	0.444	427.8	0.0756	415.0	58.0	"	228.8	70.8	85.8	87.5	-
	% Cv.	-	3.8	0.4	12.0	10.5	0.7	-	4.3	-	-	-	14.3	6.6	2.7	-
θ = 17 days	1	"	2.501	83.3	1.29	0.569	735.2	0.103	617.5	59.0	747.0	330.8	186.1	172.3	93.2	-
	2	"	2.491	83.8	1.037	0.709	739.7	0.083	629.1	59.0	"	337.0	154.2	170.1	88.5	-
	3	"	2.287	84.8	1.088	0.687	747.8	0.085	702.7	60.0	"	376.4	142.1	163.4	91.3	-
	4	"	2.152	83.4	0.979	0.771	735.8	0.076	721.3	60.0	"	386.3	147.9	143.2	90.7	-
	5	"	2.693	82.0	1.395	0.519	723.9	0.113	586.4	57.0	"	314.1	139.2	201.3	87.6	-
	Avg.	"	2.425	83.4	1.158	0.651	736.5	0.090	651.4	59.0	"	355.5	153.9	170.0	90.0	-
	% Cv.	-	6.0	1.0	13.6	14.3	1.05	-	7.9	-	-	-	11.0	10.9	1.9	-
θ = 10 days	1	"	3.119	79.2	1.075	1.105	1188.1	0.090	995.3	62.0	1269.9	533.1	312.7	268.1	87.7	-
	2	"	3.401	77.3	1.098	1.055	1159.9	0.095	862.3	62.5	"	461.9	293.1	301.2	83.2	-
	3	"	3.601	75.9	0.998	1.142	1139.8	0.087	892,0	61.1	"	477.7	307.6	297.5	85.2	-
	4	"	3.233	78.4	1.159	1.010	1176.7	0.099	993.2	62.0	"	531.9	263.1	271.3	84.0	-
	5	"	3.630	75.8	0.931	1.220	1136.9	0.081	857.0	62.3	"	459.1	206.2	311.2	74.5	-
	Avg.	"	3.399	77.3	1.052	1.100	1160.3	0.090	919.9	62.0	"	492.7	276.5	289.8	82.9	-
	% Cv.	"	4.6	1.7	7.6	6.6	1.7	-	6.7	-	-	-	14.1	5.9	5.4	-

TABLE III.2 EXPERIMENTAL AND CALCULATED DATA

Substrate: CELLULOSE

Temperature: 38°C

Dry Feed Input Concentration: 15000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% C_e dig.
θ = 30 days	1	15,000	1.155	92.3	1.129	0.409	461.5	0.081	442.7	58	448.2	237.1	75.2	72.1	85.7	-
	2	"	0.992	93.4	0.992	0.47	466.9	0.071	469.8	58	"	251.7	106.2	51.2	91.1	-
	3	"	1.190	92.1	0.913	0.503	460.3	0.066	430.7	56	"	230.7	79.2	67.6	84.3	-
	4	"	1.058	92.9	1.102	0.422	464.7	0.079	489.7	56.6	"	262.2	72.3	50.9	85.9	-
	5	"	0.935	93.8	0.991	0.473	469	0.070	463.1	57	"	248.0	82.1	45.9	83.9	-
	Avg.	"	1.066	92.9	1.025	0.455	464.5	0.073	459.2	57.5	"	245.9	83.0	57.5	86.2	-
	% Cv.	"	9.0	0.7	6.9	7.7	0.7	-	4.5	-	-	-	14.5	17.9	3	-
θ = 17 days	1	"	1.187	92.1	1.278	0.636	812.5	0.092	810.1	60.1	747	434.0	141.9	132.1	94.8	-
	2	"	1.307	91.3	1.032	0.780	805.4	0.075	665.2	58.2	"	309.3	159.7	149.2	82.8	-
	3	"	1.185	92.1	1.309	0.620	812.6	0.094	759	58.9	"	406.6	163.0	136.2	94.4	-
	4	"	1.398	90.7	1.002	0.794	800.0	0.077	709.2	59	"	379.9	130.7	159.7	89.7	-
	5	"	1.151	92.3	1.168	0.697	814.6	0.084	712.4	59	"	381.6	151.0	121.3	87.5	-
	Avg.	"	1.246	91.7	1.158	0.705	809.0	0.084	731.2	59	"	382.2	149.2	139.7	90	-
	% Cv.	-	7.4	-	10.7	10.1	0.7	-	6.8	-	-	-	7.9	9.6	3.5	-
θ = 10 days	1	"	1.678	73.8	1.249	1.070	1332.1	0.093	1093	62	1269.9	585.5	219.0	237.9	82.1	-
	2	"	1.702	70.2	1.290	1.030	1329.7	0.097	1039	62.2	"	556.6	277.0	240.2	84.5	-
	3	"	1.635	81.8	1.456	0.918	1336.5	0.108	1231.2	61	"	659.5	192.1	232.7	85.4	-
	4	"	1.838	74.0	1.068	1.230	1316.1	0.081	114	61	"	596.7	216.0	252.0	84.8	-
	5	"	1.853	76.7	1.168	1.120	1314.7	0.089	1173.5	60.5	"	628.6	209.1	239.1	83.8	-
	Avg.	"	1.741	75.3	1.246	1.060	1325.9	0.094	1130.1	61.3	"	605.4	222.6	240.3	84.1	-
	% Cv.	-	5.0	5.1	10.4	9.7	0.7	-	5.9	-	-	-	12.9	2.7	1.4	-

TABLE III.3 EXPERIMENTAL AND CALCULATED DATA

Substrate: CELLULOSE

Temperature: 38°C

Dry Feed Input Concentration: 25000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% C_e dig.
θ = 30 days	1	25,000	2.107	91.5	2.395	0.319	763.1	0.111	12763	57.5	12444.6	6837.1	1541	2321	85.9	-
	2	"	1.851	92.6	2.301	0.335	771.6	0.099	12501	56.5	"	6696.8	1630	1977	82.8	-
	3	"	1.770	92.9	2.269	0.341	774.2	0.098	13993	56	"	7495.7	1891	19931	91.4	-
	4	"	1.749	93.0	2.398	0.323	775	0.103	12871	59	"	6895	1415	1737.3	80.7	-
	5	"	1.760	92.9	2.229	0.347	774.6	0.096	13962	59	"	7479.3	1371	1775.2	84.5	-
	Avg.	"	1.847	92.6	2.318	0.344	771.7	0.10	13218	57.6	"	7080	1569.6	1959.5	85.2	-
	% Cv.	"	7.3	0.3	2.9	3.2	0.5	-	4.8	-	-	-	11.8	10.7	4.2	-
θ = 17 days	1	"	2.302	90.8	2.401	0.556	1335.1	0.105	20984	60	21952.3	1124	3013	4062.1	83.4	-
	2	"	2.588	89.6	2.436	0.541	1318.3	0.108	21422	61	"	114751	2197	4317.9	81.9	-
	3	"	2.391	90.4	2.561	0.519	1329.9	0.113	22001	62.8	"	11785.8	2421	4013	82.9	-
	4	"	2.502	90	2.213	0.598	1323.3	0.098	21176	61	"	11343.9	2994.3	4220	82.1	-
	5	"	2.340	90.6	2.443	0.545	1332.9	0.107	20337	59	"	10894.4	2810	3861.2	80.0	-
	Avg.	"	2.425	90.3	2.411	0.552	1327.9	0.106	21184	60.8	"	1134.8	2687	4094.8	82	-
	% Cv.	"	4.3	0.5	4.7	4.7	0.4	-	2.5	-	-	-	12.0	3.9	1.4	-
																-
θ = 10 days	1	"	3.261	86.9	2.020	1.076	2173.8	0.092	32562	62	37333.4	17443.4	5215	8313.1	82.7	-
	2	"	3.498	86	2.097	1.025	2150.2	0.098	32796	63.5	"	17568.8	4872	8125.4	81.8	-
	3	"	3.662	85.3	1.831	1.165	2133.8	0.086	30010	63.5	"	16076.2	5610	8731.9	81.5	-
	4	"	3.462	86.2	2.203	0.977	2153.8	0.102	31909	63.5	"	17093.6	5903	8396.0	84.0	-
	5	"	3.591	85.6	2.081	1.03	2140.8	0.097	31550	63	"	16901.2	7704	8621.8	89.9	-
	Avg.	"	3.495	86	2.046	1.055	2150.8	0.095	31765	63.1	"	17016.6	5860	8417.6	84	-
	% Cv.	-	3.9	0.6	6.0	6.0	0.6	-	3.1	-	-	-	16.8	2.7	3.7	-

TABLE III:4 EXPERIMENTAL AND CALCULATED DATA

Substrate: GRASS

Temperature: $28^{\circ}C$

Dry Feed Input Concentration: 15000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% $C_e$dig.
θ = 30 days	1	18.875	4.46	74.9	1.371	0.350	480.5	0.095	307.6	57	391.7	164.7	40.9	99.2	77.8	83.5
	2	"	4.607	75.6	1.272	0.373	475.6	0.089	269.5	55	"	144.3	65.6	129.6	86.7	75.8
	3	"	5.005	73.5	1.019	0.453	462.3	0.073	275.2	56	"	147.3	47.8	143.3	86.4	69.6
	4	"	4.39	76.7	1.163	0.435	482.8	0.08	302.3	57	"	161.9	46.3	118.7	83.4	81.0
	5	"	4.423	76.6	1.234	0.390	481.7	0.085	305.9	57	"	163.8	49.8	124.3	86.2	81.5
	Avg.	-	4.577	75.4	1.212	0.396	476.6	0.084	292.1	56.4	"	156.4	50.1	123.0	84.1	78.3
	% Cv.	"	4.9	-	9.6	8.9	1.6	-	5.6	-	-	-	16.5	11.7	4.0	6.4
θ = 17 days	1	"	6.652	64.7	1.213	0.592	719.0	0.099	419.9	57.8	652.5	224.9	16.5	209.1	41.8	62.1
	2	"	7.918	58.0	0.910	0.707	644.5	0.083	377.1	58	"	201.9	132.9	230.2	86.6	60.9
	3	"	7.516	60.2	1.191	0.561	668.2	0.105	321.0	59.5	"	210.1	153.1	227.3	90.5	61.2
	4	"	5.973	68.3	1.236	0.614	758.9	0.096	413.2	59	"	221.3	147.1	213.4	89.1	64.3
	5	"	7.203	61.8	0.934	0.735	686.5	0.08	392.3	58.2	"	171.9	159.1	243.1	87.9	63.1
	Avg.	"	7.052	62.6	1.097	0.642	695.4	0.091	372.7	58.5	"	206	151.4	231.0	89.2	63.3
	% Cv.	-	9.6	-	13.0	10.5	5.7	-	9.9	-	-	-	7.3	5.9	2	2
θ = 10 days	1	"	10.634	43.7	0.809	1.018	824	0.098	449.8	60.4	1109.2	240.9	240.1	512.9	89.6	51.2
	2	"	9.931	47.4	0.882	1.013	894.3	0.099	515.1	59.6	"	276	201.1	421.9	81.0	58.2
	3	"	8.910	52.8	1.039	0.960	996.5	0.104	451.0	61	"	241.5	309.2	406.5	86.3	56.3
	4	"	9.631	48.9	0.941	1.098	924.3	0.102	473	60	"	253.3	222	417.3	80.4	60.1
	5	"	11.00	41.7	0.815	0.965	787.3	0.103	458.3	59	"	245.6	219.9	532.1	89.9	51.1
	Avg.	"	10.021	47.0	0.937	0.988	885.3	0.101	469.4	60	"	250.7	238.5	458.1	85.4	55.4
	% Cv.	-	7.4	-	10.1	5.5	8.3	-	5.1	-	-	-	15.7	11.6	4.7	6.6

TABLE III.5 EXPERIMENTAL AND CALCULATED DATA

Substrate: GRASS

Temperature: $38^{o}C$

Dry Feed Input Concentration: 15000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% C_e dig.
θ = 30 days	1	18.875	2.907	84.6	1.473	0.361	532.2	0.092	348.0	54	391.7	186.4	76.1	101.2	92.8	86.1
	2	"	3.314	82.4	1.165	0.445	518.7	0.075	295.4	55.5	"	158.1	102.3	97.1	86.8	83.1
	3	"	3.261	82.7	1.280	0.406	520.4	0.081	280	54.5	"	150	82.1	117.1	89.1	76.7
	4	"	3.037	83.9	1.427	0.370	527.9	0.09	356	56	"	150.7	71.2	96.4	81.2	80.9
	5	"	3.176	83.1	1.085	0.482	523.3	0.069	281.3	55	"	190.7	81.3	102.3	95.5	78.6
	Avg.	"	3.139	83.3	1.286	0.413	524.5	0.080	312.3	55	"	167.1	82.6	102.8	89.1	81.1
	% Cv.	-	4.7	-	11.5	11.0	1.0	-	10.6	- -	-	-	12.8	7.2	5.5	4.0
θ = 17 days	1	"	5.073	73.1	1.376	0.811	811.9	0.10	437.9	59.5	652.5	234.6	139.6	225.8	91.9	74.1
	2	"	5.125	72.8	1.254	0.645	808.8	0.0917	413.3	59	"	221.3	153.1	209.1	89.4	67.3
	3	"	5.319	71.8	1.093	0.733	797.4	0.080	388.7	57.5	"	208.2	128.9	217.4	85.0	72.0
	4	"	5.273	72.1	1.224	0.650	800.1	0.09	393.1	57.5	"	210.6	106.0	222.9	82.7	75.6
	5	"	5.055	73.2	1.413	0.575	812.9	0.102	437.8	58.5	"	234.6	131.0	207.2	87.9	63.9
	Avg.	"	5.169	72.6	1.272	0.68	806.2	0.092	413.8	58.4	"	221.8	131.7	216.5	87.4	70.6
	% Cv.	-	2.1	-	8.9	11.9	0.7	-	5.0	-	-	-	11.7	3.4	3.7	6.1
θ = 10 days	1	"	6.897	63.4	1.319	0.907	1197.8	0.109	492.2	60.1	110.9	263.7	201.2	402.6	78.2	60.6
	2	"	7.053	62.6	1.399	0.857	1182.2	0.116	599	60.3	"	320.8	171	441.3	84.1	73.2
	3	"	7.222	61.7	1.039	1.121	1165.2	0.089	565.2	59.5	"	302.7	183	426.1	82.2	65.1
	4	"	7.183	61.9	1.086	1.073	1169.2	0.092	503.3	61.6	"	269.6	148.1	443.9	77.7	60.9
	5	"	7.096	62.4	1.117	1.05	1177.8	0.095	563.5	62	"	301.9	182.0	419	81.4	65.1
	Avg.	"	7.090	62.2	1.188	1.000	1178.4	0.1	544.6	60.5	"	291.6	177	426.6	80.7	63.1
	% Cv.	-	1.6	-	10.3	10.5	1.0	-	7.4	-	-	-	9.8	3.5	3.0	3.3

TABLE III.6 EXPERIMENTAL AND CALCULATED DATA

Substrate: GRASS

Temperature: 48°C

Dry Feed Input Concentration: 15000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% C_e dig.
θ = 30 days	1	18.875	2.593	86.3	1.217	0.445	542.7	0.075	309.6	54	391.7	165.7	83.1	61.7	79.3	87.1
	2	"	2.627	86.1	1.362	0.397	541.6	0.084	310.3	52.5	"	166.2	78.1	68.9	79.9	82.3
	3	"	2.476	86.8	1.430	0.382	546.6	0.086	352.5	53	"	188.2	61.2	89.5	86.6	90.6
	4	"	2.454	87.0	1.396	0.39	547.3	0.084	373.1	53	"	199.8	85.2	77.2	92.4	92.1
	5	"	2.373	87.4	1.214	0.453	550	0.073	340.2	56	"	182.1	70.2	61.2	80.0	89.6
	Avg.	"	2.504	86.7	1.323	0.413	545.6	0.080	337.0	53.7	"	180.5	75.6	71.7	83.6	88.3
	% Cv.	-	3.7	-	6.8	7.0	0.6	-	7.3	-	-	-	11.7	14.8	6.1	3.9
θ = 17 days	1	"	4.269	77.4	1.392	0.617	859.1	0.095	510.3	57	652.5	273.3	120.5	171.1	86.5	81.6
	2	"	4.510	76.1	1.101	0.767	845	0.076	406.9	58.5	"	218.0	128.0	188	81.8	71.2
	3	"	4.330	77.0	1.276	0.670	855.5	0.087	476.6	58	"	255.3	112.6	159.2	80.8	73.1
	4	"	4.335	77.0	1.321	0.647	855.3	0.09	471.2	58	"	252.4	110.2	173	82	78.6
	5	"	4.420	76.8	1.351	0.630	850.3	0.093	503.8	58	"	269.8	132.0	163.3	86.6	83.1
	Avg.	"	4.373	76.9	1.288	0.666	853	0.088	473.7	58	"	253.8	120.7	171	83.5	77.5
	% Cv.	-	2.9	-	6.8	8.0	0.5	-	-	-	-	-	7.0	5.8	2.9	6.0
θ = 10 days	1	"	5.873	68.8	1.446	0.899	1300.1	0.111	761.3	60	1109.2	407.8	191.2	379	88.1	69.1
	2	"	6.039	68.0	1.221	1.05	1283.6	0.095	663.1	58	"	355.2	176.2	365	80.8	66.3
	3	"	6.151	67.4	1.392	0.914	1272.4	0.109	697.8	59	"	373.8	150.7	425	85.6	68.1
	4	"	6.173	67.3	1.119	1.13	1270.1	0.088	628.3	59	"	336.6	181.0	388.3	81.7	63.4
	5	"	6.061	67.8	1.179	1.086	1281.4	0.091	661.0	59	"	354.1	168.0	391.2	82.5	65.2
	Avg.	"	6.059	67.8	1.271	1.016	1281.5	0.099	682.3	59	"	365.5	173.4	389.7	83.7	66.4
	% Cv.	-	1.7	-	9.9	9.1	0.8	-	6.5	-	-	-	7.8	5.1	3.2	3.0

TABLE A III.7 EXPERIMENTAL AND CALCULATED DATA

Substrate: GRASS

Temperature: 28°C

Dry Feed Input Concentration: 25000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% C_e dig
θ = 30 days	1	314.58	7.127	77.3	2.172	0.373	811	0.089	473.4	50.5	652.5	253.6	118.1	193	86.5	62.8
	2	"	6.956	77.8	2.057	0.397	816.7	0.084	433.1	56	"	231.9	107	168.6	77.8	63.5
	3	"	6.813	78.3	2.021	0.406	825	0.081	449	57	"	240.5	122	221.1	89.4	66.3
	4	"	6.620	78.9	1.839	0. 45	827.9	0.074	479.1	58	"	256.6	118.2	183.2	85.5	62.1
	5	"	6.940	77.9	1.975	0.413	817.3	0.080	488.3	57.5	"	258.9	129.2	193.6	89.1	62.9
	Avg.	"	6.891	78	2.013	0.406	818.9	0.081	464.6	57	"	248.3	119.0	193.9	85.6	62.9
	% Cv.	-	2.4	-	7.3	6.2	0.8	-	4.4	-	-	-	5.7	8.8	4.9	5.7
θ = 17 days	1	"	8.476	73	2.344	0.576	1351.9	0.102	627.7	57.3	1087.5	326.3	213.1	442.5	91.2	59.2
	2	"	10.121	67.8	1.920	0.653	1255	0.09	617.7	58.4	"	330.8	221.7	419.3	89.4	56.3
	3	"	10.813	65.6	1.975	0.615	1214	0.096	622.1	57.9	"	311.8	223.0	453.9	90.9	51.2
	4	"	9.485	69.8	2.376	0.547	1292.5	0.107	673.1	57	"	360.5	201.0	436.2	91.7	53.8
	5	"	9.372	70.2	2.077	0.625	1299	0.094	720.9	59.1	"	386.1	177.1	420.5	90.5	60.1
	Avg.	"	9.653	69.3	2.138	0.603	1282	0.098	644.2	57.9	"	345.1	207.2	434.5	90.7	56.1
	% Cv.	"	8.1	-	8.8	6.2	3.6	-	6.2	-	-	-	8.1	3.0	0.8	5.9
θ = 10 days	1	"	14.129	55.0	1.871	0.956	1732.9	0.104	835.9	60	1848.7	447.8	269.3	851.2	81.9	49.6
	2	"	13.582	56.8	2.038	0.877	1787.6	0.113	816.5	59.7	"	437.3	231.7	832.9	81.2	51.2
	3	"	12.175	61.3	1.795	1.074	1928.3	0.093	844	58.1	"	452	249.1	813.2	81.8	59.6
	4	"	12.840	59.2	2.196	0.848	1861.7	0.117	858.3	58	"	459.8	272.1	861.3	86.2	50.3
	5	"	12.428	60.5	1.865	1.02	1903	0.10	981.1	59	"	525.5	240	820.2	85.8	52.6
	Avg.	"	13.031	58.6	1.941	0.955	1842	0.105	865.8	59	"	495.5	252.4	835.7	83.4	52.6
	% Cv.	-	5.6	-	7.5	8.8	3.9	-	6.7	-	-	-	6.3	2.1	2.8	6.9

TABLE III.8 EXPERIMENTAL AND CALCULATED DATA

Substrate: GRASS

Temperature: 38°C

Dry Feed Input Concentration: 25000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% $C_e$dig.
θ = 30 days	1	31,458	4.109	86.9	2.009	0.45	911.6	0.073	515.3	57	652.5	276	102.1	162.1	82.9	86.1
	2	"	5.168	83.5	2.196	0.40	876.3	0.083	490.9	56	"	263	111.4	140.3	78.9	82.8
	3	"	6.450	79.5	2.329	0.358	833.6	0.093	485.3	56	"	259.8	103.2	133.0	86.0	77.3
	4	"	5.921	81.1	2.307	0.37	851.2	0.086	466.3	57	"	249.8	130.1	167	83.8	88.1
	5	"	4.639	83.6	2.093	0.427	893.9	0.078	539.5	56.5	"	242.9	117.1	171.6	81.2	81.3
	Avg.	"	5.257	82.9	2.187	0.40	873.3	0.082	499.5	56.5	"	258.3	112.8	152.8	80.5	83.1
	% Cv.	-	16.1	-	5.6	8.6	3.2	-	5.1	-	"	-	8.3	10.1	3.8	4.5
θ = 17 days	1	"	8.621	72.6	1.912	0.70	1343.3	0.084	675.7	58.5	1087.5	361.9	162.1	371.6	82.3	64.3
	2	"	7.738	75.4	1.923	0.72	1395.3	0.081	689.8	56.5	"	369.5	138.7	359.1	79.7	67.3
	3	"	8.439	73.1	2.146	0.63	1354.1	0.093	639.8	56.5	"	332.4	131.3	382.3	87.8	60.9
	4	"	6.127	80.5	2.207	0.67	1490.0	0.087	737	56	"	394.7	142.3	386.5	84.9	69.2
	5	"	6.590	79.0	2.096	0.697	1462.8	0.084	773.2	57.5	"	414.2	151.1	331.2	82.4	73.8
	Avg.	"	7.503	76.1	2.057	0.685	1409.1	0.085	703	57	"	374.5	145.1	366.1	81.4	67.1
	% Cv.	-	13.2	-	5.8	4.5	4.1	-	6.7	-	-	-	7.3	5.4	4.2	6.5
θ = 10 days	1	"	10.103	67.8	1.938	1.101	2135	0.091	1032.1	58	1848.7	552.8	221.2	783.7	84.3	68.1
	2	"	9.876	68.6	1.873	1.152	2158.2	0.087	976.0	58	"	522.8	256.1	731.2	81.6	58.6
	3	"	11.703	62.7	1.819	1.085	1975.5	0.091	1021.3	57	"	547.1	231.1	777.6	84.2	63.2
	4	"	10.721	65.9	2.189	0.947	2073.7	0.105	1043.1	57	"	558.8	215.1	631.2	86.0	60.9
	5	"	12.107	61.5	1.817	1.065	1935.1	0.093	844.8	57.5	"	452.5	263.2	744.1	78.9	61.9
	Avg.	"	10.90	65.3	1.907	1.077	2055.6	0.093	983.4	57.5	-	526.8	237.2	761.1	81.0	62.5
	% Cv.	-	8.0	-	7.3	6.3	4.2	-	7.4	-	-	-	8.0	8.1	3.9	5.0

TABLE III.9 EXPERIMENTAL AND CALCULATED DATA

Substrate GRASS

Temperature $48^{o}C$

Dry Feed Input Concentration 25000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G'_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% $C_e$dig.
θ = 30 days	1	31.458	4.921	86.3	1.850	0.478	884.5	0.07	523	56.3	625.5	280.1	87.1	162.3	81.1	82.9
	2	"	4.890	86.5	2.205	0.401	885.6	0.083	527.6	55.7	"	282.6	101.2	148.7	81.6	79.3
	3	"	5.138	83.6	2.146	0.409	877.3	0.081	589.0	54	"	315.5	104.5	121.6	83.1	80.1
	4	"	3.615	88.5	2.378	0.390	928.1	0.085	513.1	54.5	"	274.9	90.1	151.7	79.2	82.6
	5	"	3.921	87.5	2.205	0.416	917.9	0.08	608.2	56	"	325.9	107.3	137.9	87.5	80.1
	Avg.	-	4.497	86.4	2.157	0.414	898.7	0.079	552.1	55.2	"	295.7	98.4	144.4	82.5	81.0
	% Cv.	-	13.5	-	7.9	12.9	2.3	-	7.1	-	-	-	8.1	9.6	3.3	1.8
θ = 17 days	1	"	5.857	81.4	2.297	0.653	1505.9	0.09	762.3	56.8	1087.5	408.3	171.2	332.1	83.9	79.8
	2	"	7.021	77.7	1.876	0.766	1437.5	0.077	743.9	57,6	"	398.5	141.2	327.2	79.8	77.3
	3	"	6.437	79.5	2.176	0.676	1471.8	0.087	765.8	5.6	"	410.2	138.2	310.3	79	81.2
	4	"	5.439	82.7	2.396	0.638	1530.5	0.091	787.2	57	"	421.6	147.1	319.0	81.7	75.2
	5	"	7.174	77.2	2.032	0.70	1428.4	0.084	825.0	57.6	"	441.8	155.1	367.3	88.7	79.9
	Avg.	"	6.385	79.7	2.155	0.686	1474.8	0.085	776.8	57	"	416	150.6	331.2	82.6	78.7
	% Cv.	-	10.4	-	8.6	9.2	2.7	-	3.6	-	-	-	7.8	5.9	4.2	2.6
θ = 10 days	1	"	9.873	68.6	1.963	1.099	2158.5	0.091	1021.0	60	1348.7	546.9	227.2	632.1	76.1	62.3
	2	"	9.521	69.7	1.829	1.199	2193.7	0.083	1011	58.6	"	514.5	201.9	667.9	74.9	64.0
	3	"	8.832	71.9	2.396	0.944	2262.6	0.106	1085.6	59.9	"	581.5	241.2	606.7	77.3	66.5
	4	"	8.731	72.2	2.176	1.04	2272.7	0.096	1176.3	58	"	630.2	177.0	691.2	81.0	62.1
	5	"	8.289	72.2	2.176	1.05	2316.8	0.095	1232.1	60.5	"	660.0	215.5	570.2	78.2	68.2
	Avg.	"	9.049	71.2	2.115	1.066	2240.9	0.094	1105.2	59.4	"	586.6	212.5	613.6	77.5	64.6
	% Cv.	""	6.3	-	9.2	7.8	2.5	-	7.8		"	-	10.4	7.7	2.7	3.7

TABLE : III.10 EXPERIMENTAL AND CALCULATED DATA

Substrate — COW DUNG

Temperature — 28°C

Dry Feed Input Concentration — 15000 mg. Lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% C_e dig.
θ = 30 days	1	14.652	5.203	64.5	0.770	0.400	314.7	0.082	185.8	59	359	99.5	91.3	134.5	90.6	62.1
	2	"	5.019	65.7	0.867	0.370	321.0	0.090	188.3	59	"	100.8	98.2	114.4	86.5	60.3
	3	"	5.128	65	0.750	0.423	317.4	0.078	157.9	60	"	84.5	83.1	129.8	82.8	63.9
	4	"	5.003	65.8	0.801	0.401	321.6	0.083	168.0	58	"	90.0	86.8	118.6	82.3	68.2
	5	"	4.816	67.1	0.890	0.368	327.8	0.090	186.2	59	"	99.7	70.3	126.6	81.6	67.3
	Avg.	"	5.034	65.6	0.815	0.392	320.5	0.085	177.2	59	"	94.9	85.9	124.8	84.7	64.4
	% Cv.	"	2.6	-	6.6	5.3	1.3	-	6.8	-	-	-	10.8	5.9	4.0	4.7
θ = 17 days	1	"	7.013	52.1	0.743	0.604	449.3	0.097	239.2	61	598.3	128.1	162.3	242.2	89.0	55.3
	2	"	6.837	53.3	0.771	0.596	459.7	0.099	247.3	60	"	132.5	151.5	227.9	85.6	52.1
	3	"	6.920	52.8	0.768	0.592	454.8	0.100	246.2	60	"	132.0	159.1	236.0	88.1	57.3
	4	"	7.197	50.9	0.762	0.575	438.5	0.102	228.1	60	"	122.0	137.2	250.9	85.3	56.2
	5	"	7.265	50.4	0.738	0.569	434.5	0.100	233.7	61.5	"	125.2	142.0	247.8	86.1	55.7
	Avg.	"	7.046	51.9	0.756	0.591	447.3	0.100	238.9	60.5	"	127.9	150.4	241.0	86.8	55.4
	% Cv.	-	2.4	-	1.8	1.6	2.0	-	3.0	-	-	3.1	6.4	3.4	1.7	3.1

TABLE III.11 EXPERIMENTAL AND CALCULATED DATA

Substrate — COW DUNG

Temperature — 38°C

Dry Feed Input Concentration — 15000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% $C_e$dig.
θ = 30 days	1	14.652	3.300	77.5	1.077	0.350	378.4	0.095	236.9	58.0	359	126.9	68.1	88.1	78.8	73.9
	2	"	3.607	75.4	1.031	0.357	368.1	0.093	231.3	58.5	"	123.9	70.7	97.0	81.2	76.0
	3	"	4.041	72.4	0.855	0.413	353.7	0.080	196.1	58.0	"	105.0	79.1	111.3	82.2	72.5
	4	"	3.939	73.1	0.876	0.407	357.1	0.082	198.3	58.0	"	106.2	53.1	112.1	75.6	74.8
	5	"	4.381	70.1	0.816	0.419	342.3	0.079	178.1	58.0	"	95.4	58.3	127.8	78.4	69.1
	Avg.	"	3.854	73.7	0.931	0.389	359.9	0.085	208.1	58.1	"	111.5	65.9	107.3	79.2	73.2
	% Cv.	-	9.7	-	7.8	7.6	3.4	-	10.8	-	-	-	13.9	10.6	3.0	3.4
θ = 17 days	1	"	6.119	58.2	0.726	0.670	501.9	0.088	249.3	58.5	598.3	133.5	131.2	222.1	81.4	61.2
	2	"	5.730	60.9	0.738	0.711	524.8	0.083	281.6	60.0	"	150.8	117.1	217.2	81.0	65.8
	3	"	5.935	59.5	0.732	0.700	512.7	0.084	279.1	59.0	"	149.5	141.2	220.0	85.4	64.7
	4	"	5.346	63.5	0.751	0.730	547.4	0.081	327.3	60.0	"	175.3	149.2	178.3	83.0	69.2
	5	"	5.320	63.7	0.754	0.728	548.9	0.080	310.6	59.0	"	166.4	129.0	161.6	76.4	68.3
	Avg.	"	5.690	61.1	0.740	0.712	527.1	0.083	289.6	59.3	"	155.1	133.5	199.8	81.4	65.8
	% Cv.	-	5.6	-	1.5	3.1	3.5	-	9.3	-	-	-	8.2	12.5	3.8	4.3
θ = 10 days	1	"	6.976	52.3	0.594	1.290	767.5	0.077	401.0	61.0	1017.2	214.7	248.9	321.3	77.1	53.1
	2	"	7.817	46.6	0.587	1.160	683.5	0.086	389.1	60.0	"	208.4	217.2	407.2	81.9	52.9
	3	"	7.672	47.6	0.817	0.850	698	0.117	407.6	60.5	"	218.3	239.0	376.1	81.9	56.3
	4	"	7.613	48.0	0.731	0.962	703.9	0.104	339.1	60.5	"	181.6	207.2	382.9	75.8	58.1
	5	"	8.087	44.8	0.597	1.100	656.0	0.092	384.2	60.0	"	205.8	221.3	442.8	85.5	53.0
	Avg.	"	7.633	47.8	0.665	1.070	701.8	0.095	392.2	60.4	"	205.7	226.7	386.0	80.4	54.7
	% Cv.	-	4.8	-	13.9	14.3	5.2	-	6.5	-	-	-	6.7	10.3	4.4	3.9

TABLE III.12 EXPERIMENTAL AND CALCULATED DATA

Substrate: COW DUNG

Temperature: 48°C

Dry Feed Input Concentration: 15000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% $C_e$dig.
θ = 30 days	1	14.652	2.128	85.5	0.921	0.453	418.0	0.073	213.5	56.0	359.0	114.3	121.3	105.6	95	79.0
	2	"	1.991	86.4	1.069	0.395	422.0	0.085	224.2	57.0	"	121.0	81.5	86.9	80.3	78.7
	3	"	2.295	84.3	1.053	0.391	411.9	0.085	207.8	58.0	"	111.3	92.1	120.1	90.1	77.2
	4	"	1.898	87.0	1.078	0.394	425.1	0.085	240.6	57.0	"	128.9	73.1	111.1	87.6	83.2
	5	"	1.688	88.4	1.169	0.370	432.1	0.090	257.9	57.0	"	138.2	90.9	97.8	91.0	79.6
	Avg.	"	2.000	86.3	1.058	0.400	421.8	0.084	228.8	57.0	"	122.7	91.8	104.3	88.8	79.6
	% Cv.	-	10.3	-	7.5	6.9	1.6	-	8.0	0	-	-	17.7	10.9	5.5	2.5
θ = 17 days	1	"	3.060	79.1	1.018	0.670	681.9	0.088	356.1	59.0	598.3	190.8	110.1	179.1	80.3	70.2
	2	"	3.696	74.7	0.866	0.740	644.4	0.079	306.0	57.5	"	163.9	120.9	193.0	79.3	68.3
	3	"	3.966	72.9	0.813	0.773	628.6	0.076	317.9	59.0	"	170.3	117.2	210.1	83.2	70.9
	4	"	3.513	76.0	0.929	0.700	655.2	0.084	332.1	58.0	"	177.9	106.7	201.3	81.2	68.7
	5	"	3.725	74.5	0.952	0.670	642.7	0.087	330.2	59.0	"	176.9	123.4	206.0	84.6	67.5
	Avg.	"	3.592	75.4	0.916	0.710	650.6	0.083	328.5	58.5	"	175.9	115.5	197.9	81.8	69.1
	% Cv.	-	8.4	-	7.7	5.7	2.7	-	5.1	-	-	-	5.4	5.6	2.2	1.8
θ = 10 days	1	"	4.810	62.5	1.159	0.969	509.0	0.076	468.2	59.0	1017.2	133.8	144.2	219.0	83.1	58.1
	2	"	5.096	64.7	1.239	0.942	527.0	0.078	472.1	60.0	"	168.5	117.0	187.0	79.0	59.3
	3	"	5.320	63.3	1.112	0.750	515.5	0.072	408.3	59.5	"	164.0	139.2	208.0	85.5	57.6
	4	"	4.701	65.6	1.258	0.993	534.0	0.079	520.3	59.5	"	164.0	156.0	197.0	86.4	61.3
	5	"	5.197	66.8	1.307	0.869	544.0	0.08	447.0	59	"	168.0	109.0	180.0	76.4	62.1
	Avg.	"	5.025	64.6	1.217	0.904	526.0	0.077	463.2	59.2	"	160.0	153.2	198.2	82.1	60.5
	% Cv.	-	4.6	-	5.5	6.0	2.4	-	7.9	-	-	-	12.5	7.1	4.7	3.2

TABLE III.13 EXPERIMENTAL AND CALCULATED DATA

Substrate: COW DUNG

Temperature: 28°C

Dry Feed Input Concentration: 25000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% C_e dig.
θ = 30 days	1	21.420	9.150	62.5	1.159	0.440	509.0	0.076	249.8	60.0	598.0	133.8	144.2	219.0	83.1	58.1
	2	"	8.613	64.7	1.239	0.425	527.0	0.078	314.6	59.0	"	168.5	117.0	187.0	79.0	59.3
	3	"	8.955	63.3	1.122	0.470	515.5	0.072	306.2	60.0	"	164.0	139.2	208.0	85.5	57.6
	4	"	8.397	65.6	1.258	0.420	534.0	0.079	311.0	60.0	"	164.0	156.0	197.0	86.4	61.3
	5	"	8.100	66.8	1.307	0.410	544.0	0.08	313.0	60.0	"	168.0	169.0	180.0	76.4	62.1
	Avg.	"	8.643	64.6	1.217	0.430	526.0	0.077	299.0	59.8	"	160.0	153.2	198.2	82.1	60.5
	% Cv.	"	4.4	-	5.5	4.9	2.4	-	8.3	-	-	-	12.5	7.1	4.7	3.2
θ = 17 days	1	"	13.610	44.3	1.078	0.590	635.8	0.100	319.1	61.0	996.8	171.0	213.5	441.3	82.8	46.1
	2	"	12.925	47.0	1.135	0.596	676.1	0.099	366.1	62.0	"	196.1	207.9	417.9	82.4	49.2
	3	"	12.174	50.1	1.077	0.660	720.0	0.088	406.9	61.0	"	217.9	248.1	388.3	85.7	55.3
	4	"	12.381	49.3	1.276	0.550	708.9	0.106	361.3	61.0	"	193.5	176.3	409.2	78.1	56.7
	5	"	11.870	51.4	1.220	0.605	738.2	0.097	421.6	61.0	"	225.7	221.6	378.3	82.8	54.1
	Avg.	"	12.592	48.4	1.157	0.600	695.6	0.098	375.0	61.4	"	201.0	213.5	407.0	82.4	52.3
	% Cv.	-	4.9	-	6.8	5.9	5.2	-	9.7	0	-	-	10.8	5.5	3.0	7.3

TABLE III.14 EXPERIMENTAL AND CALCULATED DATA

Substrate: COW DUNG

Temperature: 38°C

Dry Feed Input Concentration: 25000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% $C_e$dig.
θ = 30 days	1	24.420	6.726	72.4	1.519	0.390	589.8	0.086	318.3	59.0	598.0	170.5	147.0	188.7	84.6	74.2
	2	"	6.975	71.4	1.522	0.382	581.5	0.088	280.7	59.0	" "	150.4	159.0	222.1	88.8	76.1
	3	"	6.519	73.3	1.607	0.371	596.7	0.09	380.0	58.5	"	203.5	138.0	175.0	86.4	75.7
	4	"	6.830	72.0	1.546	0.379	586.3	0.088	354.4	59.0	"	189.9	132.0	210.0	89.0	78.0
	5	"	6.865	71.8	1.500	0.390	585.1	0.085	319.1	58.0	"	170.9	160.0	206.0	89.8	74.1
	Avg.	"	6.783	72.2	1.539	0.382	587.9	0.087	330.5	58.6	"	177.0	147.2	200.0	87.7	75.9
	% Cv.	-	2.3	-	2.4	1.9	1.0	-	10.3	-	-	-	7.6	8.3	2.2	1.9
θ = 17 days	1	"	10.368	57.5	1.333	0.619	826.6	0.095	461.9	60.0	996.8	247.3	218.1	352.0	82.0	60.2
	2	"	9.131	62.6	1.442	0.623	899.0	0.094	513.2	60.0	"	274.8	238.9	334.0	85.1	65.9
	3	"	9.848	59.6	1.332	0.641	857.1	0.092	497.1	59.0	"	266.2	229.0	380.0	88.0	61.3
	4	"	10.978	55.0	1.095	0.721	790.7	0.081	436.1	59.0	"	233.6	221.0	396.0	85.4	60.9
	5	"	10.125	58.5	1.181	0.710	840.8	0.083	445.5	59.0	"	239.1	219.0	370.0	83.1	63.2
	Avg.	"	10.090	58.6	1.258	0.668	842.9	0.089	471.0	59.4	"	252.2	225.2	366.4	84.7	62.3
	% Cv.	-	6.0	-	9.9	6.6	4.2	-	6.3	-	-	-	3.5	5.9	2.4	3.3
θ = 10 days	1	"	13.890	43.1	1.050	1.000	1053.0	0.100	546.0	61.0	1694.0	292.4	332.0	768.0	82.2	48.7
	2	"	14.376	41.1	1.037	0.970	1004.0	0.103	552.0	62.0	"	295.6	363.5	724.0	81.6	40.9
	3	"	14.609	40.0	1.008	0.970	981.0	0.103	504.4	61.0	"	270.2	317.8	837.0	84.1	51.8
	4	"	13.665	44.0	0.938	1.140	1075.0	0.088	629.2	61.0	"	337.0	390.0	759.0	87.7	50.2
	5	"	13.150	46.1	1.018	1.100	1127.0	0.091	561.5	61.0	"	300.0	321.2	721.0	79.2	49.8
	Avg.	"	13.938	42.8	1.042	1.040	1048.0	0.097	558.6	61.2	"	299.0	344.9	761.8	83.0	48.3
	% Cv.	-	3.7	-	4.8	6.8	4.9	-	7.2	-	-	-	8.0	5.5	3.8	7.9

TABLE III.15 EXPERIMENTAL AND CALCULATED DATA

Substrate: COW DUNG

Temperature: 48°C

Dry Feed Input Concentration: 25000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% C_e dig.
θ = 30 days	1	24.420	3.327	86.4	1.689	0.416	703.1	0.08	396.1	58.0	598.0	212.2	189.2	126.4	88.3	87.2
	2	"	3.393	86.1	1.839	0.381	700.9	0.088	421.3	58.5	"	225.6	147.3	121.5	82.7	88.7
	3	"	4.521	81.5	1.771	0.374	663.3	0.089	376.1	58.0	"	201.4	158.1	158.0	86.5	83.4
	4	"	3.928	83.9	1.794	0.380	683.0	0.088	381.3	59.0	"	204.2	170.3	148.0	86.5	85.1
	5	"	4.738	80.6	1.681	0.390	656.0	0.085	362.1	58.0	"	194.0	168.0	179.0	90.4	86.1
	Avg.	"	3.981	83.7	1.754	0.388	681.2	0.086	387.3	58.0	"	207.5	166.6	146.6	86.9	86.1
	% Cv.	-	14.3	-	3.4	3.8	2.8	-	5.2	-	-	-	8.3	14.3	3	2.1
θ = 17 days	1	"	5.796	76.3	1.843	0.594	1095.5	0.099	575.8	59.0	997.0	308.4	211.0	339.6	86.2	79.8
	2	"	6.376	73.9	1.529	0.694	1061.4	0.085	575.1	59.5	"	308.0	217.0	317.4	84.5	78.7
	3	"	6.289	74.2	1.808	0.588	1066.5	0.100	569.1	60.0	"	304.7	209.0	383.0	90.0	76.3
	4	"	6.697	72.6	1.562	0.660	1042.5	0.088	537.1	59.0	"	287.6	198.0	379.0	86.7	71.8
	5	"	6.517	73.3	1.673	0.630	1053.1	0.093	544.1	59.0	"	291.4	201.0	361.0	85.6	76.8
	Avg.	"	6.335	74.0	1.673	0.633	1063.8	0.093	560.3	59.3	"	300.0	207.2	356.0	86.6	76.7
	% Cv.	-	4.8	-	7.6	6.3	1.7	-	2.9	-	-	-	3.3	6.9	2.1	3.6
θ = 10 days	1	"	9.290	61.9	1.219	1.240	1513.0	0.080	761.3	60.5	1694.0	407.8	291.0	786.0	87.7	63.0
	2	"	8.300	66.0	1.555	1.030	1612.0	0.097	794.5	60.0	"	425.5	257.0	767.0	85.5	68.1
	3	"	9.680	60.3	1.476	1.000	1474.0	0.100	736.1	61.0	"	394.3	268.0	819.0	87.4	63.6
	4	"	8.170	66.5	1.414	1.150	1625.0	0.087	811.1	61.0	"	434.6	238.0	748.0	83.8	68.7
	5	"	7.625	68.7	1.571	0.930	1679.0	0.107	821.4	61.5	"	440.0	237.0	725.0	82.7	66.2
	Avg.	"	8.613	64.7	1.447	1.070	1580.0	0.094	784.9	60.8	"	420.2	258.2	769.0	85.4	66.0
	% Cv.	"	8.8	-	8.8	10.3	4.8	-	4.0	-	-	-	7.8	4.2	2.3	3.5

TABLE III.16 EXPERIMENTAL AND CALCULATED DATA

Substrate: HORSE MANURE

Temperature: 28°C

Dry Feed Input Concentration: 15000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% C_e dig.
θ = 30 days	1	12.508	3.285	73.7	0.838	0.360	307.0	0.091	177.3	58.0	337.5	95.0	95.0	107.0	88.0	70.4
	2	"	3.697	70.4	0.786	0.370	293.0	0.089	170.5	59.0	"	91.4	106.0	108.8	90.7	66.3
	3	"	4.592	63.3	0.682	0.380	263.0	0.086	146.2	59.0	"	78.3	101.0	121.2	89.0	66.3
	4	"	4.321	65.4	0.687	0.390	273.0	0.084	161.2	59.0	"	86.3	77.3	118.0	91.4	67.4
	5	"	4.540	63.7	0.692	0.380	265.0	0.087	170.8	58.0	"	91.9	96.2	127.1	93.4	60.8
	Avg.	"	4.087	67.3	0.738	0.376	280.0	0.087	165.2	58.6	"	88.5	95.2	116.4	90.4	66.2
	% Cv.	-	8.9	-	8.6	2.7	6.1	-	6.5	-	-	-	10.2	6.5	2.1	4.6
θ = 17 days	1	"	5.190	58.5	0.677	0.630	430.0	0.093	223.0	60.0	562.5	119.4	162.8	239.0	92.7	51.5
	2	"	5.542	55.7	0.633	0.640	490.0	0.091	219.0	60.0	"	117.3	169.0	247.6	94.9	59.5
	3	"	5.930	52.6	0.609	0.630	387.0	0.093	221.7	60.0	"	118.7	133.0	272.0	93.1	58.2
	4	"	6.272	49.8	0.542	0.670	367.0	0.087	188.0	61.0	"	100.7	142.5	279.0	92.8	50.4
	5	"	5.996	52.0	0.571	0.670	383.0	0.088	209.0	60.0	"	111.9	118.0	263.7	87.7	54.7
	Avg.	"	5.786	53.7	0.606	0.648	395.0	0.090	212.0	60.0	"	113.6	145.0	260.2	92.2	54.9
	% Cv.	-	6.5	-	7.8	2.9	5.5	-	6.1	-	-	-	13.0	5.7	2.6	6.5
θ = 10 days	1	"	7.409	40.7	0.558	0.910	509.0	0.110	264.2	61.0	956.2	141.5	262.1	496.7	94.1	43.9
	2	"	7.721	38.3	0.552	0.870	478.0	0.115	245.4	61.5	"	131.4	239.0	520.5	93.1	39.6
	3	"	7.926	36.6	0.559	0.820	458.0	0.122	250.0	61.0	"	134.1	240.9	526.0	94.2	32.0
	4	"	6.580	47.4	0.602	0.980	592.0	0.101	303.0	62.0	"	162.4	216.0	462.3	87.9	52.7
	5	"	7.164	42.7	0.622	0.860	534.0	0.116	272.0	61.0	"	145.7	289.0	489.0	96.6	41.9
	Avg.	"	7.360	41.1	0.578	0.888	514.0	0.113	270.0	61.3	"	143.0	249.4	499.0	93.2	42.0
	% Cv.	-	6.3	-	4.8	6.2	9.1	-	7.6	-	-	-	9.8	4.0	3.1	18.3

TABLE III.17 EXPERIMENTAL AND CALCULATED DATA

Substrate: HORSE MANURE

Temperature: 38°C

Dry Feed Input Concentration: 15000 mg. lit^{-1}

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% C_e dig.
θ = 30 days	1	12.508	1.897	84.8	0.982	0.360	353.0	0.093	213.7	59.0	337.5	114.4	91.9	76.1	83.6	82.6
	2	"	2.680	78.6	0.854	0.380	327.0	0.087	196.0	58.5	"	105.1	87.0	89.6	83.4	79.3
	3	"	3.311	73.5	0.772	0.390	306.0	0.084	173.7	58.5	"	93.0	82.8	108	84.0	78.2
	4	"	2.738	78.1	0.807	0.400	325.0	0.083	194.0	58.0	"	103.8	107.0	85.6	87.8	81.1
	5	"	2.219	82.2	0.886	0.380	342.0	0.086	206.3	58.0	"	110.5	76.5	97.8	84.4	81.1
	Avg.	"	2.569	79.4	0.856	0.380	331.0	0.087	196.7	58.4	"	105.3	89.0	91.4	84.6	79.3
	% Cv.	-	18.8	-	8.4	3.7	4.8	-	6.9	-	-	-	11.9	9.8	1.9	3.1
θ = 17 days	1	"	3.876	69.0	0.822	0.610	507.0	0.095	259.6	59.5	562.5	139.0	141.0	210.1	85.5	66.3
	2	"	4.139	66.9	0.747	0.660	492.0	0.089	282.2	59.0	"	151.2	132.8	196.0	85.3	70.4
	3	"	3.928	68.6	0.691	0.730	504.0	0.080	289.0	59.0	"	155.2	147.0	208.7	90.8	71.9
	4	"	3.432	72.6	0.762	0.700	533.0	0.084	289.3	60.2	"	155.0	163.1	179.5	88.5	74.3
	5	"	4.430	64.6	0.673	0.700	475.0	0.083	268.0	59.0	"	143.4	152.0	219.0	91.4	61.9
	Avg.	"	3.961	68.3	0.739	0.680	502.0	0.086	277.6	59.3	"	148.8	147.2	202.7	88,3	68.9
	% Cv.	-	-	7.2	7.2	3.8	3.8	-	4.3	-	-	-	6.9	14.3	2.9	6.3
θ = 10 days	1	"	6.394	48.9	0.617	0.990	611.0	0.101	288.0	61.0	956.2	154.3	238.7	468.0	90.0	47.7
	2	"	5.425	56.6	0.759	0.900	708.0	0.111	364.0	61.5	"	194.9	220.0	439.0	89.3	59.3
	3	"	4.961	60.3	0.789	0.960	754.0	0.104	401.3	61.5	"	214.9	227.0	397.5	87.8	65.6
	4	"	5.530	55.8	0.727	0.960	697.0	0.104	379.0	61.0	"	203.1	202.8	437.0	88.1	58.5
	5	"	5.620	55.0	0.658	1.040	688.0	0.096	355.0	62.0	"	190.0	211.3	479.0	92.0	52.1
	Avg.	"	5.586	55.3	0.710	0.970	692.0	0.103	357.4	61.4	"	191.5	219.9	444.1	89.4	56.6
	% Cv.	-	8.3	-	9.1	4.7	6.6	-	10.7	0	-	-	5.6	6.4	1.7	10.9

TABLE III.18 EXPERIMENTAL AND CALCULATED DATA

Substrate: HORSE MANURE

Temperature: 48°C

Dry Feed Input Concentration: 15000 mg. lit $^{-1}$

DETENTION TIME θ	BP.	S_o	S_1	% dig.	X_1	q	R	$\frac{X_1}{S_o - S_1}$	G_T	% CH_4	C_i	C_g	C_f	C_s	% C_{rec}	% C_e dig.
θ = 30 days	1	12.508	1.329	89.4	0.989	0.370	372.0	0.088	217.7	57.0	337.5	116.6	94.0	64.6	81.5	92.8
	2	"	1.427	86.4	1.000	0.360	360.3	0.093	234.3	56.0	"	125.5	82.3	67.9	81.6	88.2
	3	"	1.535	87.7	1.035	0.350	365.7	0.094	218.7	56.0	"	117.3	98.0	77.0	86.6	81.9
	4	"	1.696	86.4	0.991	0.360	360.4	0.092	222.1	57.0	"	118.9	79.0	82.9	83.2	89.5
	5	"	1.813	85.5	0.981	0.360	356.4	0.092	217.6	57.0	"	116.5	67.5	98.1	83.7	83.4
	Avg.	"	1.560	87.1	1.004	0.360	364.9	0.092	222.1	56.6	"	118.9	84.2	74.1	83.3	87.1
	% Cv.	-	7.6	-	1.9	1.7	1.6	2.1	2.8	-	-	-	12.8	16.9	2.2	4.6
θ = 17 days	1	"	2.554	79.6	0.873	0.670	585.0	0.088	323.0	58.5	562.5	173.0	127.8	169.0	83.5	76.7
	2	"	2.376	81.0	1.003	0.590	596.0	0.099	357.6	58.0	"	191.6	139.0	157.8	86.8	79.3
	3	"	2.589	79.3	0.855	0.680	583.0	0.086	359.5	58.0	"	192.6	117.0	175.0	86.1	71.9
	4	"	2.532	79.7	0.845	0.690	587.0	0.085	336.8	58.2	"	180.4	132.0	176.5	87.0	72.1
	5	"	2.739	78.1	0.880	0.650	574.0	0.09	315.7	59.0	"	170.8	138.6	181.6	87.3	81.4
	Avg.	"	2.558	79.5	0.891	0.650	585.0	0.089	338.5	58.3	"	181.7	131.0	172.0	86.1	76.3
	% Cv.	-	4.5	-	6.4	2.0	1.1	-	5.2	-	-	-	6.2	4.7	1.6	5.0
θ = 10 days	1	"	4.139	66.9	0.951	0.880	836.0	0.113	440.6	59.5	956.2	236.0	172.0	377.6	83.5	70.2
	2	"	3.876	69.0	0.800	1,080	863.0	0.092	449.0	59.0	"	240.5	198.8	358.0	86.8	71.7
	3	"	3.365	73.0	0.952	0.960	914.0	0.104	452.5	59.4	"	242.4	187.1	329.0	86.1	72.9
	4	"	2.980	76.1	1.013	0.940	952.0	0.106	541.5	60.0	"	290.0	209.8	310.0	87.0	78.4
	5	"	4.100	67.2	0.875	0.960	840.0	0.104	439.0	59.0	"	235.2	173.5	379.8	87.3	70.1
	Avg.	"	3.692	70.4	0.918	0.960	881.0	0.104	464.5	59.4	"	248.7	188.2	351.0	86.1	70.1
	% Cv.	"	12.2	-	8.0	7.6	5.1	-	8.3	-	-	-	7.7	7.8	1.6	4.2

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LIST OF MAIN SYMBOLS

List of symbols as defined in Chapter 3 and subsequent chapters.

b = cell decay coefficient, time $^{-1}$.

D = dilution rate, time $^{-1}$.

E = activation energy of a reaction.

G_o = maximum gas produced per unit weight of volatile solids added, volume/mass.

G_1 = potential gas production of digester output stream for a continuous digester per unit weight of volatile solids added, volume/mass.

K = first order rate constant, time $^{-1}$.

K_g = first order rate constant based on gas production per unit weight of volatile solids added, time $^{-1}$.

K_s = saturation constant, concentration of substrate at which the specific growth rate is half the maximum specific growth rate, mass/volume.

K_T = a reaction rate constant.

k = first order rate constant, volume/mass-time.

k' = maximum rate of substrate utilisation per unit weight of microorganisms, at high substrate concentration, time $^{-1}$.

k'' = first order rate constant with respect to substrate concentration - as a limiting case of the Monod equation, for low substrate concentration, volume/mass-time.

Q = hydraulic flow rate through digester, volume/time.

q = rate of microbial substrate utilisation per unit digester volume per unit cell concentration, time $^{-1}$.

q' = flow rate at which solids separator underflow is recycled to the digester, volume/time.

R = rate of substrate utilisation per unit digester volume, mass/volume-time.

r = rate of microbial substrate utilisation per unit digester volume, mass/volume-time.

r'_v = volumetric recycle ratio.

S_o = input substrate concentration, mass/volume.

S_1 = output substrate concentration, mass/volume.

T = absolute temperature, deg K.

V = digester volume, volume.

w = flow rate of the fraction of the biological solids seperator underflow which is wasted from the system, volume/time.

Y = yield constant.

X_1 = cell concentration in digester, mass/volume.

X_o = input cell concentration to digester, mass/volume.

X_e = cell concentration in the clarified overflow from the solids separator, mass/volume.

X_r = cell concentration in the underflow from the solids separator, mass/volume.

θ = detention time, $(\frac{V}{Q})$, time.

θ_c^m = minimum biological solids detention time at which $S_1 = S_o$, time.

$(\theta_c^m)_{lim}$ = limiting value of θ_c^m for $S_o \gg K_s$.

μ = specific growth rate, rate of change of cell concentration $\frac{dx}{dt}$, per unit of cell concentration X, $= \frac{1}{X} \frac{dx}{dt}$.

μ_{max} = the maximum specific growth rate.

ΔC_s = change between input and output substrate concentration.

$COD_{v.m.}$ = chemical oxygen demand of the volatile matter (chemical oxygen demand per unit weight of volatile matter oxidised).

V.S.S. = volatile suspended solids.

$\frac{dG_1}{dt}$ = rate of change of gas production per unit weight of volatile solids added to digester, volume/mass-time.

$\frac{dS_1}{dt}$ = rate of change of substrate concentration, mass/volume-time.

$\frac{dX_1}{dt}$ = rate of change of cell concentration, mass/volume-time.

d_m = mean particle size diameter (d_{50}).