FACTORS INFLUENCING THE PRODUCTION OF METHANE DURING THE ANAEROBIC DIGESTION OF POULTRY WASTES

by

ANDREW RICHARD WEBB

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ABSTRACT

Factors Influencing Methane Production During the Anaerobic Digestion of Poultry Wastes.

A.R. WEBB

The semi-continuous digestion of poultry litter and manure was carried out in 51 digesters at 35°C to determine the potential of each waste for methane production, and to investigate the relationships between gas yield (GY) and loading rate (LR). GY's obtained were 0.327 m^3 kg VS added^{-1} for litter and 0.397 m^3 kg VS added^{-1} for manure. During the digestion of litter, GY increased both with increasing retention time (RT) and influent concentration. For manure GY increased with influent concentration up to 2.6 - 4.1% VS after which further increases caused reductions in GY due to ammonia inhibition which reached 4274 mgl^{-1} of NH_4^+ - N.

The effects of raised concentrations of NH_4^+ - N on manure batch digesters seeded with sludge adapted to different levels of NH_4^+ - N was investigated by shock loading NH_4Cl or NH_4HCO_3. High adapted seed was more tolerant of NH_4Cl than low adapted seed whereas the opposite was true for NH_4HCO_3 which at low levels had a stimulatory effect on low adapted seed.

Long term effects of raised NH_4^+ - N concentrations were examined by adding NH_4Cl to semi-continuously fed manure digesters. Increasing NH_4^+ - N concentrations to 3062 mgl^{-1} and 4824 mgl^{-1} reduced GY's to 88% and 73% of control levels respectively. After periods of up to 22 weeks exposure to these concentrations GY's failed to regain untreated values indicating that complete adaptation had not occurred.

The potential for digestion of poultry wastes at high solids concentrations (up to 27% TS) was tested in a packed bed type digester. Successful hydrolysis and acidogenesis occurred but methanogenesis was inhibited by NH_4^+ - N concentrations of up to 13,314 mgl^{-1}. Replacing the liquor allowed intiation of methane production during the digestion of manure but not litter.

A study of Monod growth kinetics revealed that the increase in GY obtained with increasing influent concentration was due to the dependence of digester effluent concentration on RT but not influent concentration. Models were developed to describe the uninhibited digestion of litter and the inhibited digestion of manure.

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CHAPTER 1

INTRODUCTION

1.1 Methanogenesis

Methanogenesis is the process by which complex organic compounds are converted anaerobically by bacteria to methane and other end products. The biological formation of methane is common in nature and methane bacteria are readily found in anaerobic environments where organic matter is being decayed such as fresh water and marine sediments at low temperatures, and animal digestive systems at higher temperatures. Since methane is poorly soluble and inert under anaerobic conditions it has the ideal non-toxic properties of an end product and readily escapes from the anaerobic environment.

Methane may be produced in the caecum of non-ruminants, but the best known methanogenic animal digestive system is the rumen of herbivores where anaerobic digestion forms an integral part of the animals' metabolism.

Short chain fatty acids produced by fermentation are removed by absorption through the rumen wall and metabolised by the host, while methane is produced almost exclusively from carbon dioxide and hydrogen and is removed by belching. In aquatic sediments and other gastrointestinal environments acetate is converted to gas and only about one third of the methane produced is generated from carbon dioxide and hydrogen.

1.2 Biochemistry of Anaerobic Digestion

Organic compounds are utilised by the microbial population in anaerobic degradation as source of carbon and nitrogen for cell synthesis and a source of energy for growth. Energy is provided by the oxidation of substrates which is accomplished by a series of dehydrogenation reactions which produce the step-wise transfer of hydrogen from substrate to a carrier molecule, normally NAD.

$$NAD^+ + 2\ (H) \rightarrow NADH + H^+$$

Under anaerobic conditions the products of anaerobic fermentations are determined by the need to find an acceptor molecule to absorb surplus hydrogen generated by the energy-yielding reactions of the bacteria.

The effective anaerobic degradation of complex organic matter to methane is the result of the combined and co-ordinated metabolic activity of a consortium of bacterial species. The complex organic compounds of the substrate are first liquefied by fermentative bacteria to simple soluble organics such as acids and alcohols. These simpler compounds are metabolised by acidogenesis to short chain fatty acids (formate, acetate, propionate and butyrate) hydrogen and carbon dioxide. If the hydrogen concentration is low, obligate proton reducing acetogenic bacteria convert propionate and butyrate to acetate and hydrogen (Bryant 1979).

The energy yielding substrate for the majority of methanogenic bacteria is hydrogen which by reduction of CO_2 yields CH_4. Some methane bacteria also have the capability of converting acetate to CH_4 and CO_2. Homoacetogenic or hydrogen consuming bacteria convert formate or H_2 and CO_2 into acetate. When the pH drops below 7.0 these bacteria may replace the methane bacteria so acetate builds up and CH_4 production is inhibited.

If the hydrogen concentration is high or the pH low, acidogenic bacteria will produce butyric or propionic acid instead of acetate which reduces further H_2 production. However if long chain fatty acids accumulate in the medium the final methanogenesis stage may become inhibited.

1.3 Microbiology of Anaerobic Digestion

In sewage sludge 10^8 - 10^9 hydrolytic bacteria have been detected per ml (Mah and Sussman, 1968, Toerien and Siebert 1967) of which 10^7 show proteolytic activity (Siebert and Toerien 1969), such as Eubacterium, and 10^5 show cellulolytic activity (Maki, 1954) such as Clostridium. All genera have not been identified but Gram negative non sporing obligate anaerobic rods predominate (Burbank et al, 1966) although other types can be routinely isolated.

According to McInery et al (1978) up to 4.2 x 10^6 H_2 producing acetogenic bacteria may be present per ml of sewage sludge. These have not been generically identified but a Gram negative rod has been isolated which metabolises 3-8 carbon fatty acids to acetate and hydrogen, but can only be cultured in the presence of H_2 utilising species.

Populations of 10^5 - 10^6 per ml of homoacetogenic bacteria have been reported by Ohwaki et al (1977) and Braun et al (1979) of which Clostridium and Acetobacterium are recognised genera. These bacteria catabolise both H_2 and CO_2 or multi-carbon compounds such as sugars.

The methanogenic bacteria as a group are more diverse than the other three groups involved in the process. Genera isolated to date are limited to the catabolism of one and two carbon compounds. Populations of 10^8 per ml have been detected in sewage sludge and Methanobacterium, Methanospirillum, Methanosarcina and Methanococcus are present at concentrations of 10^6 - 10^8 per ml (Smith, 1966). Another genus Methanothrix which metabolises acetate but not H_2 and CO_2 is present in concentrations of 10^5 - 10^6 per ml (Zeikus,1980).

1.4 Application of Anaerobic Digestion to Waste Treatment.

Essentially the same naturally occuring degradation process can be exploited in the treatment of organic wastes which cannot be disposed of by physical methods such as incineration. Materials presenting possibilities for treatment by anaerobic digestion (AD) include domestic and agricultural wastes, food processing wastes, and industrial effluents from, for example breweries, distilleries and pulp or paper factories.

As a method of waste treatment AD offers a number of potential benefits. The process provides a high degree of waste stabilisation, thus reducing the pollutional load, and produces a low volume of excess sludge. The end products are less offensive in odour and are homogenised which facilitates subsequent handling. The numbers of pathogenic organisms are reduced and pests such as rodents and flies are less attracted to the

digested effluent. The fertilising constituents of the raw material are conserved and methane is produced as a by-product which can be used as an energy source to drive the process.

1.5 Agricultural Wastes

In countries of the Far East such as India and China AD has been widely used for many years for the treatment of human and animal wastes. Although in the West AD has been used in conjunction with aerobic methods for the treatment of domestic sewage for at least 60 years, before 1976 the use of AD for the treatment of animal wastes was considered inadequate and too costly.

However the attitude to AD has changed dramatically due to a number of recent developments. These include a sharp increase in energy prices and stricter legislation pertaining to pollution from agricultural sources. Coupled with the trend towards more intensive agriculture these factors make AD as a solution to waste disposal problems a more attractive proposition.

The 13 million cattle, 8 million pigs and 117 million poultry in England produce annually approximately 114, 11.5 and 4.6 million tonnes of excreta per year respectively. (ADAS 1983). This presents a major disposal problem and much research has been devoted to the investigation of agricultural wastes as a substrate for AD, most digestion tests of agricultural wastes have been concerned with piggery and cattle excreta while less attention has been paid to poultry wastes and the data on the digestion of poultry litter is particularly scarce. This investigation therefore should provide valuable information on the potential of AD for the treatment of poultry litter and manure.

1.6 Types of Digester

There are three basic designs for anaerobic digesters. The simplest and most common is the single stage mixed reactor in which the waste is transferred regularly to the digestion tank where it remains for the

retention period of the system and escapes over a weir system to an overflow tank. Gas is taken off from the top of the digester and stored in a gas holder. The single stage digester in which digesting biomass is mixed with influent by mechanical stirrers, gas recirculation or pumping, is termed the high rate digester. High rate digestion reduces the theoretical retention time (RT) and allows increased loading rates.

A major limitation in high rate digestion is the growth rate of the microorganisms involved in the process. As the RT is decreased the rate of removal of bacteria from the system is increased. When the RT is short enough the rate at which the essential bacterial species are removed becomes faster than their growth rate and the balance of the process is upset. A solution of this problem is provided by the anaerobic contact process in which washout of essential bacteria is prevented by concentrating the biomass in a settling chamber and recirculating back to the digestion tank. The mean cell RT is therefore significantly greater than the hydraulic RT. This process provides a high treatment efficiency without requiring excessive digester volumes.

An alternative method of obtaining a long bacterial RT together with a high flow rate and short hydraulic RT is by use of an inert solid in the anaerobic filter (See for example McCarty, 1969). This configuration consists of a tower filled with packing material which has a high surface area and a high void ratio. The waste liquor is pumped upwards through the filter matrix and flows off at the top. The packing provides a surface onto which bacteria attach thus eliminating the need for solids separation and sludge recycle. This type of reactor is most suited to the treatment of fast flowing soluble wastes.

The original basic digester designs are now being superceded by a new generation of upflow anaerobic reactors. In the upflow anaerobic sludge blanket (UASB) process, waste water is allowed to flow gently upwards through a bed of flocculent bacteria. (See for example Lettinga, 1980). At the top of the reactor is a gas-solids separator which collects gas and prevents excess carry over of suspended solids in the effluent.

This process affords effective treatment of wastewater at very high loading rates.

In the anaerobic fluidised bed the settling rate of the biomass can be determined by the operator rather than the bacteria themselves, which are grown as a film on the surface of fluidised sand particles (Mosey, 1982a). Small compact reactors can be used containing a high concentration of biomass.

Besides employing methods which effectively increase the biomass RT or biomass concentration in the digester, it has been attempted to improve upon the efficiency of the conventional high rate digester by operating the process in two phases in which the non-methanogenic and methanogenic stages of digestion take place in isolated optimised environments (See for example Ghosh etal, 1975, Ghoshand Klass, 1978). Phase separation can be achieved by selective inhibition of methane formers, by dialysis separation of the acid and methane producing cultures, or by kinetic control of the two groups of organisms by adjustment of dilution rates and cell mass recycling. Culturing the non-methanogenic and methanogenic bacteria in two separate reactors is claimed to result in improved overall process efficiency, reaction rate and operational control.

1.7 Digester Operation

There are a number of parameters which influence digester performance, the alteration of which will result in significant changes in operating characteristics. These include the degree of anaerobiosis, pH, nutritional content of the substrate, temperature, retention time, and presence or absence of inhibitors.

1.7.1 Anaerobiosis

The methane formers appear to be the group of bacteria most sensitive to changes in environmental conditions and, being obligate anaerobes a small amount of oxygen is inhibitory to these bacteria. It is essential therefore that a highly reduced environment be maintained to promote their growth.

1.7.2 pH

The methanogens are also sensitive to the pH of the culture medium. The optimum range for methane production is between 6.8 and 7.5 (Kirsch and Sykes, 1971). When the pH drops below 6.6 significant inhibition of methanogenic bacteria occurs, and at about 6.2 the acid conditions exhibit acute toxicity. This pH however does not stop acid production and the fermentative bacteria continue to produce acids until the pH drops to 4.5 or 5.0, when the digester is said to be 'stuck' or 'sour'. Inhibition of the methanogens alone therefore results in overproduction or under-consumption of volatile fatty acids (VFA's) which can thus provide an indication of digester performance and may be monitored routinely.

1.7.3 Temperature

Since rates of reaction are temperature dependent, if the operating temperature is increased (within limits) shorter RT's can be used for the same efficiency of operation. The optimum temperature for bio-gas production in the mesophilic range is between 35^{o} and 40^{o} C, and in the thermophilic range 55^{o} - 60^{o} C. However methane production at thermophilic temperatures is uneconomical due to the high energy requirement, so mesophilic digestion is normally practised.

1.7.4 Waste Composition

The bacteria involved in AD require sufficient concentrations of nitrogen, phosphorus, trace minerals and degradable carbon for optimum growth. Agricultural wastes normally contain adequate levels of these materials, but one or more may be lacking in certain industrial effluents which would result in retarded digestion.

The three main digestible components of wastes fed to digesters are carbohydrates, proteins and fats. The largest proportion is constituted by carbohydrates of which the main component is cellulose. Cellulose is a highly ordered polymer of glucose linked by β glucosidic bonds and is

normally associated with lignin or polysaccharides such as pectin and other hemicelluloses. Hemicelluloses are polymers of galactose, mannose, xylose and other sugars, and are readily degradable. Lignin on the other hand is virtually non-degradable anaerobically, and by its association with cellulose affords a degree of protection against bacterial attack, so the digestion of cellulose is slow and complete degradation may take a number of weeks.

Proteins are hydrolysed during digestion to peptides and amino acids which may then be deaminated with the formation of NH_3, CO_2 and VFA's. Peptides, amino acids and NH_3 can all be reconverted to microbial protein. Excretory products such as urea in mammals and uric acid in birds, which will be present in agricultural wastes will also be degraded in the digester to NH_3 and CO_2. Purines and pyrimidines, constituents of all biomass will be present in sludge fed to digesters and are converted to VFA's, CO_2 and H_2. Nitrates are reduced to nitrites, then NH_3, thus providing an additional source of nitrogen.

Triglycerides and membrane lipids are degraded by lipases to free fatty acids which are converted possibly by β oxidation, to acetic acid and water.

1.7.5 Biodegradability

In addition to digestible material, all wastes will contain certain amounts of non-biodegradable or refractory matter such as lignin and ash. Rather than measuring the proportion of each biodegradable component in a waste to determine its digestibility, an overall assessment can be obtained by the BOD (biological oxygen demand) or COD (chemical oxygen demand) tests. These are measurements of the quantity of oxygen required to oxidise unit volume of the waste by bacterial or chemical action respectively, and are therefore indicative of its polluting potential.

Although these tests are conducted aerobically, they give an indication of digestibility under anaerobic conditions and can be used for this purpose.

However since both tests are time consuming, an approximation of biodegradability can be obtained by the volatile solids (VS) determination (Section 2.6.2) which is faster. The VS measurement however, has the disadvantage that it includes material such as lignin which although volatilised at 500°C is not biodegradable anaerobically.

1.7.6 Inhibitors

A variety of materials can be toxic to methanogenic bacteria if present in high enough concentrations. These include, in ascending order of potency, the light metal cations Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}. When more than one of these metals is present the combined effect on the methanogenic bacteria may be synergistic or antagonistic.

Ammonia may demonstrate inhibitory effects on methane production which are increased at high pH values when the free NH_3 form predominates over NH_4+. Other materials which are toxic to digestion above certain levels are heavy metals and sulphides. Concentrations of soluble sulphide ranging from 50 to 100 mgl^{-1} can be tolerated with little or no acclimation, but concentrations of 150 mgl^{-1} may cause digester failure (Mosey and Hughes, 1975). Additionally there are many organic materials that can be inhibitory to the process. These range from organic solvents to alcohols and long chain fatty acids.

There are a variety of possible mechanisms by which an inhibitor can cause a reduction in the metabolic activities of a cell (Edwards, 1970). These are summarised below:

1) A change in the chemical activity of one or more chemical species essential to the cell's nutrition by complexing or reaction with the inhibitor.
2) The alteration of the cell's permeability.
3) A change in the activity of enzymes.
4) The dissociation of enzymes or metabolic aggregates.
5) The synthesis of enzymes affected by interaction with the genome or transcription process.

6) Interference with motility, biosynthesis of excreted metabolites, or cell wall synthesis.

1.7.7 Retention Time

The length of time the waste material remains inside the reactor influences the extent to which it is degraded. The RT is calculated by $\frac{\text{digester volume}}{\text{volume of waste fed per day}}$. Increasing the RT therefore increases the efficiency of the process, but increases the size and cost of the reactor if a fixed volume of waste is to be treated each day. A compromise must be reached therefore which gives an adequate degree of stabilisation at an acceptable cost.

The quantity of material fed to a digester per day is expressed as Loading Rate (LR), the weight of solids added to unit volume of digester per day, and is a function of RT and the concentration of the waste.

1.7.8 Efficiency

The efficiency of the anaerobic digestion process is defined as the degree of waste stabilisation achieved and the volume of biogas produced during stabilisation. The latter is determined by the former since biogas is produced by the degradation of organic matter. Stabilisation is measured by reduction in BOD, COD or solids concentration, while gas production is expressed as a yield, either gas volume produced per unit weight of material (usually VS) destroyed or added. The gas yield (GY) in terms of VS destroyed should be constant for a given substrate, but GY in terms of VS added is dependent on the parameters discussed above.

In addition to these parameters, a survey of the literature on sewage digesters has indicated that feed concentration may also influence GY (Hawkes and Horton, 1981). It was found that during the digestion of sewerage, GY increased not only with increasing RT, but also with increasing influent concentration. These relationships were presented in

the form of the LYRS diagram which is a plot of biogas yield v LR for a range of influent VS concentrations and RT's, where L=LR, Y=GY, R=RT, and S=Influent solids (Figure 1.1). The fact that GY increased with increasing influent concentration has implications in digester design and operation, since besides producing more biogas and giving a greater degree of waste stabilisation, the digestion of a concentrated substrate requires a smaller digester and will therefore be more economical.

Additionally the energy required to raise the temperature of the smaller volume of concentrated waste will be less than that required for a larger volume of dilute waste. However there may be a limit to the maximum concentration at which a waste can be digested due to inhibitors, either in the influent or produced during digestion, which will increase in concentration as the influent concentration increases.

One of the main objectives of this investigation was to ascertain the validity of the observations on sewage digestion on a laboratory scale, and to quantify the effects of RT and feed concentration on GY using poultry wastes as substrates. It was also intended to investigate whether the mechanism which causes increased GY's is a consequence of bacterial growth kinetics. If so this would allow the development of a kinetic model which could predict the performance of digesters operating on poultry wastes under varying operating conditions.

The systematic investigation of digestion at different RT's and influent concentrations has been reported by other workers using poultry and other agricultural wastes. These include reports by Gramms et al (1971), Van Velsen (1977), Hobson et al (1980), Morrison et al (1980), Shih and Huang (1980), Huang and Shih (1981), and Aubart and Fauchille (1983). However these investigations were conducted chiefly to determine the optimum conditions for digestion and were not concerned with the kinetics of gas production.

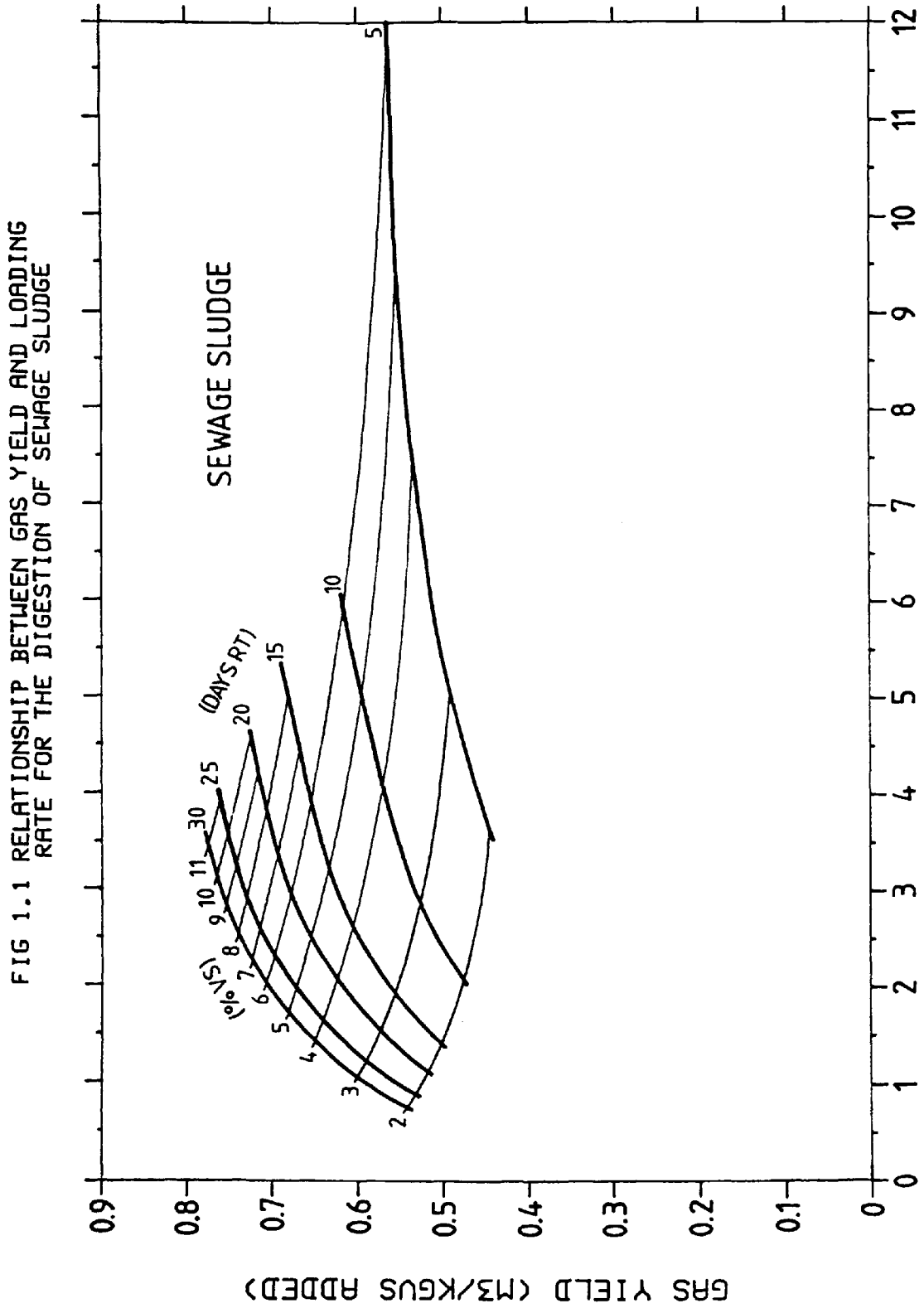


FIG 1.1 RELATIONSHIP BETWEEN GAS YIELD AND LOADING RATE FOR THE DIGESTION OF SEWAGE SLUDGE

CHAPTER 2

MATERIALS AND METHODS

2.1 Start Up

The digesters used in this investigation were seeded with effluent from three laboratory scale digesters already operating on poultry litter, which were originally seeded from sewage sludge or a pilot scale digester operating on the Polytechnic site on litter, according to Hawkes and Young (1980). All subsequent digesters were then seeded with mixed effluent from those currently operating.

2.2 Waste Material

Deep litter based on sawdust was collected from the floor of huts housing laying hens on a local smallholding. A layer of approximately 15cm normally accumulated on the floor of the hen houses. Poultry manure was obtained from Brantridwr Poultry Farm, Caerphilly, where the battery houses were cleared out by conveyor each week. Both types of waste material were stored frozen until required for making feed.

2.3 Data Analysis

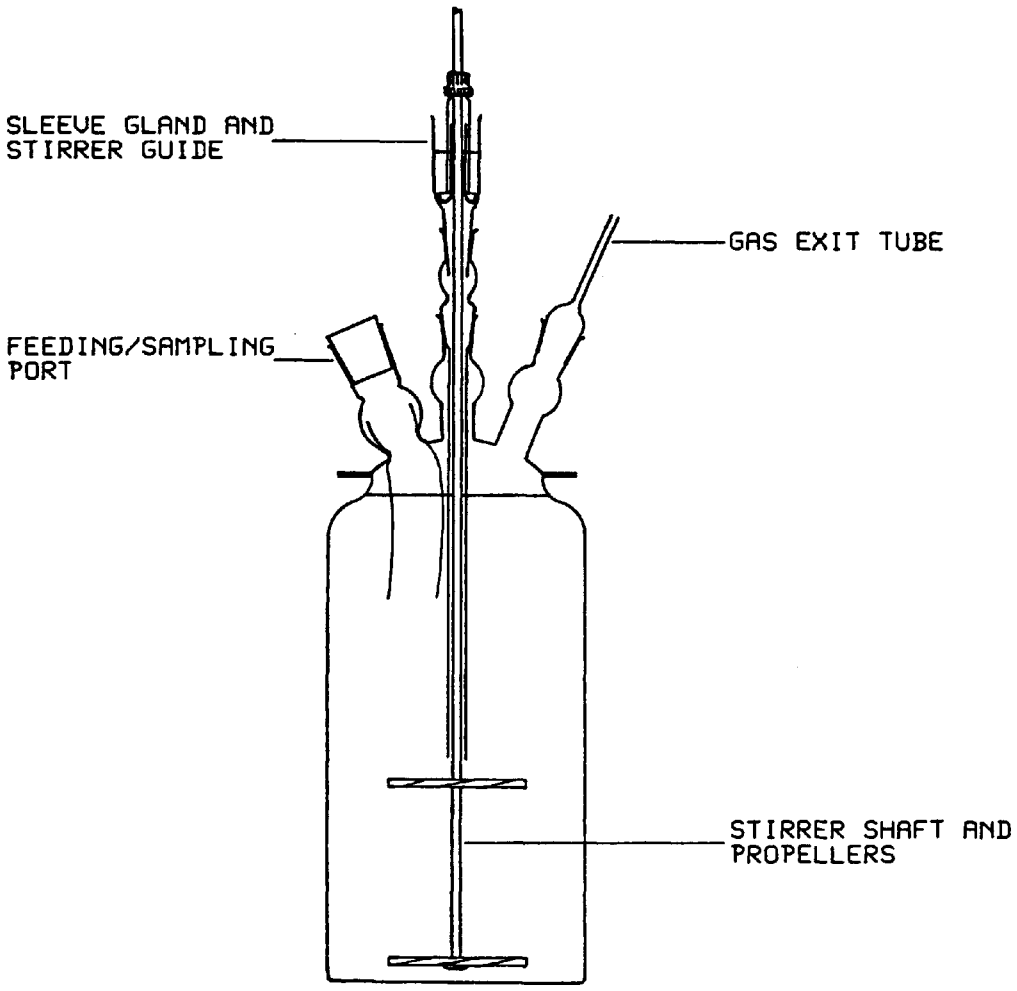
Data collected were entered routinely into a database management system with a processing language, developed at the Polytechnic of Wales. Figures were plotted using a Servogor 281 plotter (BBC Goerz Electro GMBH, Vienna, Austria). Student's T Test was employed for the statistical comparison of sets of data.

2.4 Apparatus

2.4.1 Semi Continuous Digesters

The semi-continuously fed digesters used for the digestion tests of poultry litter and manure, and for the investigation of the long term effects of ammonia on digestion, were constructed from Quickfit glassware (Corning Ltd, Stone, UK) as previously described (Figure 2.1, Hawkes and

FIG 2.1 FIVE LITRE LABORATORY ANAEROBIC DIGESTER



Young, 1980). Five-litre flat bottomed, wide necked culture vessels were fitted with multisocket flanged lids having four 19/26 ports and one 34/35 port. All joints were greased with silicone vacuum grease and the lid secured with a flange clip. A stirrer shaft of 6mm diameter stainless steel was fitted with two propellers made from 60 mm diameter stainless steel discs, one positioned at the end of the shaft and the second 60mm above. The shaft entered the digester through a stirrer guide and sleeve gland containing diethylene glycol as lubricant and gas seal. The contents were stirred by a stirrer motor (Citenco Ltd, Borehamwood, UK) at 600rpm for approximately five minutes before removing a sample from the digester and again after adding fresh material (feeding).

The largest port in the lid was fitted with a polythene tube approximately 180mm long which was sealed to the lid, and dipped below the level of the digester contents. Sampling of contents and feeding were performed through this tube. Removal of effluent was achieved using a glass tube attached to a 100 ml plastic syringe. A siphon incorporating water filled flasks was connected during removal of effluent and feeding, while the gas meter was disconnected to prevent air entering the digester. The digesters were maintained at 35^{o}C by immersion in a water bath heated by a Grant FI5 flowheater (Grant Instruments Ltd, Cambridge, UK).

Gas samples were removed for analysis by a 25 ml syringe via a 17 gauge needle inserted through a rubber stopper in a port of the lid into the digester head space and closed externally by rubber tubing and a Hoffman clip. The two remaining ports in the lid accommodated a greased Quickfit stopper to act as a pressure release point, and the gas exit tube.

2.4.1.1 Operation

Batches of digester feed were prepared weekly by macerating waste material with water in an Atomix Blender (MSE, Crawley, UK) at maximum speed for one minute to give a stock feed of required concentration, and stored at 4^{o}C until the stock feed was diluted again where necessary before feeding to the digesters. Digesters were operated in pairs and treated identically to check the reproducibility of results. They were fed once

daily from Monday to Thursday, and a double amount on Friday to compensate partly for the lack of feeding over the weekend. Samples of digester contents were removed five days per week and stored in sealed containers at 4°C and analysed the same day. Samples of feed were analysed at the same time.

Data which were collected during the initial stages of each experiment were discarded and results used only after the digesters had stabilised.

The construction and operation of the batch and high solids digesters are described in Sections 5.2 and 7.2 respectively.

2.5 Analytical Techniques

2.5.1 Total Solids

The total solids (TS) content of fresh and digested poultry waste was determined by drying to constant weight in a microwave oven (Hitachi MR6050) set on 'defrost'. The sample to be dried (normally 25 ml) was placed in a preweighed 250 ml pyrex beaker to prevent loss of sampling during boiling, reweighed then heated to constant weight in the microwave oven, which normally took two hours. The beaker and sample were weighed again when cool, and the weight of dry matter as a percentage of the original wet sample weight calculated.

2.5.2 Volatile Solids

The volatile solids content was determined according to standard methods (HMSO, 1972). Dried material from the TS determination was transferred to a preweighed and ignited crucible, then reweighed before and after ignition in an electric furnace (Carbolite Eurotherm, Sheffield, UK) at 500°C for 30 minutes. The weight of volatile material lost on ignition was expressed as a percentage of the dried sample.

For a comparison and discussion of solids determinations using the microwave and conventional oven see sections 3.2.1 and 3.4.1.

2.5.3 pH

The pH of influent and effluent was measured by standard methods (HMSO 1972) using a radiometer pH meter 26 (Radiometer, Copenhagen, Denmark) fitted with glass and calomel electrodes. The pH of digester effluent was determined as soon as possible after sampling to minimise the effects on pH of dissolved CO_2 leaving solution when removed from the CO_2 and CH_4 environment of the digester.

2.5.4 Total Alkalinity

The total alkalinity of feed and effluent was determined by titrating a stirred 50 ml sample with 1M HCl to pH 4.5 measured by electrode (HMSO, 1972). Total alkalinity as mgl^{-1} of Ca CO_3 is the volume of HCl used (ml) x 1000.

2.5.5 Ammonium Nitrogen

Ammonia was initially extracted by diluting samples of influent or effluent 50 : 50 with 0.1M HCl (Byrne and Power, 1974) before centrifuging to remove solid material. 1-5 ml of supernatant was transferred to a Markham still and after the pH was raised to 13 by addition of 2 ml 10M NaOH, steam was passed through the sample until approximately 20 ml of distillate had been collected in a beaker containing 5 ml of boric acid indicator. This was titrated against standard 0.01M HCl. The concentration of NH_4^+-N (mgl^{-1} as N) in the original sample was calculated by: Titre (ml HCl) - blank x 13999.5 x Molarity of HCl x dilution ÷ sample vol (ml), where the blank is the volume of HCl required to bring 5 ml of boric acid indicator + 15 ml H_2O to the end point.

Boric acid indicator was made up as follows according to standard methods (HMSO, 1972). A solution of boric acid was made by dissolving 20g

in warm water and diluting to approximately 1 litre. After the addition of 20 ml 0.5 gl^{-1} methyl red and 0.4 ml 15 gl^{-1} methylene blue the solution was mixed well. One drop of 0.1M NaOH changes the colour of 20 ml of the solution from purple to green.

2.5.6 Total Nitrogen

The total nitrogen of fresh and digested poultry waste was determined by Kjeldahl's method in which the nitrogen of a material is converted quantitatively to NH_3 which is then measured by the method above. Two ml of Kjeldahl reagent was added to 0.5g - 2.0g of wet sample in a 25 ml digestion flask and heated over a low flame to give a clear colourless solution. After cooling, the solution was transferred quantitatively into a Markham still, and NH_3 content determined as above with the exception that 10 ml NaOH was added to raise the pH of the sample.

Kjeldahl reagent was made up by dissolving 1gSeO_2 in 100 ml 50% H_2SO_4.

2.5.7 Holocellulose

The method used for holocellulose determination was a modification of the procedure described by Wise et al (1946) which is a delignification process yielding cellulose and hemicellulose.

A sample of fresh or digested poultry waste containing approximately 0.5g solid matter, diluted if necessary to give a volume of 30 ml, was measured into a 100 ml conical flask. After adding 1 ml 10% (V/V) acetic acid and 0.3g $NaClO_2$ and mixing, the flask was placed in a water bath at 75°C. The same amounts of acetic acid and $NaClO_2$ were added after one, two and three hours. After four hours the flask was removed and cooled in ice cold water. The sample was centrifuged, the supernatant discarded and pellet resuspended in water. This washing procedure was repeated twice with water, three times with acetone, and once with ether. After the final centrifugation, the pellet was washed into a weighed beaker using

the minimum amount of ether. The ether was allowed to evaporate at ambient temperature, and drying was completed in an oven at 105°C for 30 minutes. The beaker and sample were reweighed and the ash content of the sample determined using the VS method (Section 2.5.2), and subtracted from the weight of holocellulose. The holocellulose content was expressed as a percentage of the wet sample weight.

2.5.8 Alpha Cellulose

Breakdown of holocellulose was carried out with 24% KOH which dissolves hemicellulose to leave a relatively pure form of cellulose (α-cellulose), which is recovered as a white product. The procedure was adapted from Allen's (1974) modification of the method described by Bath (1960), and allows the determination of both holocellulose and α-cellulose.

The sample was treated according to the holocellulose method, and the available holocellulose was weighed into a 50 ml conical flask. After the addition of 20 ml 24% KOH, the flask was stoppered and placed in a water bath at 20°C mixing gently at intervals. After two hours the sample was centrifuged and the pellet washed with water twice, then 5% acetic acid, water again, acetone and finally ether. The ether was allowed to evaporate and the sample was dried in an oven at 105°C for 30 minutes, then left to cool in a dessicator before weighing. The ash content was determined and substracted from the sample weight to give the weight of α-cellulose. The percentage of α-cellulose in the original sample

$$= \frac{\text{weight of holocellulose (g) x weight of } \alpha\text{-cellulose (g) x 100}}{\text{holocellulose sub sample (g) x sample weight (g)}}$$

2.5.9 Sulphate

The method used for the determination of sulphate was a gravimetric method in which Ba SO_4 is precipitated in the presence of HCl, which was adapted from standard methods (HMSO, 1972).

The sample was filtered and a known volume measured into a beaker. HCl (50%) was added until the pH dropped to 3.75 then an additional 2 ml were added and the solution boiled for 20 seconds. After removing from the heat 15 ml hot $BaCl_2$ were added slowly by pipette into the centre of the stirred solution. The solution was filtered by vacuum through ashless filter paper and the precipitate washed with hot water. The filter paper and precipitate were transferred to a pre-ignited and weighed crucible and dried over a low flame, then placed in a furnace. The temperature was increased to 800°C for 1-2 hours and after cooling in a dessicator the crucible and sample were weighed. Sulphate concentration as SO_4^{2-} (mgl^{-1})

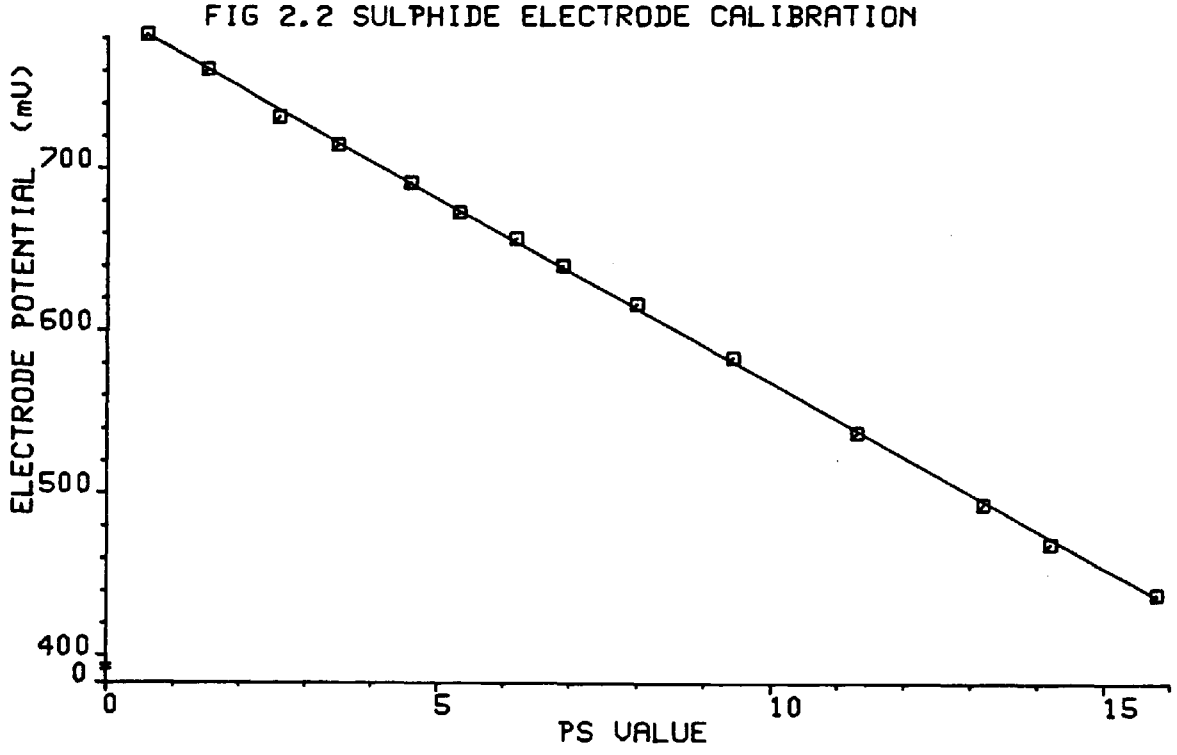
$$= \frac{\text{weight of } BaSO_4 \text{ (mg)} \times 412}{\text{sample volume (ml)}}$$

2.5.10 Sulphide

Sulphide ions (S^{2-}) were measured by direct readings from a calibrated sulphide electrode constructed from a silver electrode according to Mosey and Hughes (1975). Electrode potentials formed in sulphide solutions are proportional not to the total concentration of dissolved sulphide (H_2S) + (HS^-) + (S^{2-}), but to the negative logarithm of the activity of the divalent sulphide ion (S^{2-}). Therefore $pS = -\log_{10} A_s 2-$ where A is activity which for S^{2-} is 0.6. Electrode readings were measured in millivolts and converted to pS units using a calibration curve prepared as follows.

A 50 ml beaker containing a solution of Na_2S of known molarity was stirred by magnetic stirrer without vortex formation. A pH electrode, a saturated calomel electrode and a Ag/AgS electrode were placed in the solution. The reference electrode was connected to the calomel terminal of the pH meter and, depending on the measurement required, either the glass or the sulphide electrode was connected to the glass terminal. The pH value of the solution and the potential between the sulphide and reference electrode were recorded, sufficient 0.1M HCl was added to reduce the pH of the solution by approximately one unit, and the new pH value, the new potential between the sulphide and reference electrode, and the volume of acid were recorded. This procedure was repeated until

FIG 2.2 SULPHIDE ELECTRODE CALIBRATION



the pH of the solution dropped to below 4.0.

A calibration curve of the electrode is shown in Figure 2.2. The initial concentration of the Na_2S solution was 4.13M S^{2-}, measured by flame photometry for Na^+. The nomograph given by Mosey and Hughes (1975) was used to calculate the pS values associated with each relevant pH and sulphide concentration. The plot of electrode potential against pS produces a straight line. From Figure 2.2 mv readings were converted to pS values using the following formula : $pS = \frac{mv - 795.2}{- 22.74}$

2.5.11 Volatile Fatty Acids

A colorimetric method was used for the determination of VFA conc. which is based on the reaction of carboxylic acids with ethanediol in the presence of H_2SO_4, to produce esters which in turn are converted to hydroxamic acids by reaction with hydroxylamine. Hydroxamic acids react with ferric chloride to produce complexes of characteristic colour, the density of which is dependent on the original VFA concentration (Montgomery et al, 1962).

Acidic ethanediol was prepared by mixing 30 ml ethanediol with 4 ml 50% H_2SO_4. 1.7 ml of the fresh solution was added to 0.5 ml filtered sample of influent or effluent in a test tube and mixed thoroughly. The tubes were heated in a boiling water bath for exactly three minutes then cooled immediately in ice cold water. After adding 0.5 ml hydroxyammonium chloride solution and 2.0 ml 4.5 M NaOH to each tube, the contents were mixed then transferred to 25 ml volumetric flasks containing 10 ml acidic $FeCl_3$ solution. Acidic ferric chloride was prepared by dissolving 20g $FeCl_3 . 6H_2O$ in 500 ml water, adding 20 ml concentrated sulphuric acid, and diluting to 1 litre. The volume was made up to 25 ml with water and the contents mixed thoroughly. The flasks were left to stand for five minutes unstopped to allow dissolved gases to escape then the contents were transferred to cuvettes and the optical density measured at 500 nm within one hour using a Varian Techtron Model 635 spectrophotometer

(Varian Associates, Ca, USA). The machine was zeroed using a blank of 0.5 ml water treated in the same way as the samples, and a calibration curve was prepared using a series of dilutions of acetic acid. The concentration of VFA's in the samples were read from the optical density on the calibration curve.

2.5.12 Biological Oxygen Demand

The BOD_5 of the influent and effluent was determined using a manometric apparatus (Hach model 2173). The sample was diluted by approximately 40 fold for influent, and between 3 and 10 for effluent, to give a BOD_5 in the range 0 - 350 mgl^{-1}, and 157 ml added to one of the bottles on the apparatus. Two drops of 45% KOH were added to the seal cup resting in the mouth of the bottle, which was then allowed to equilibrate on the apparatus base which stirs the sample by means of a magnetic stirring bar. After 30 minutes the bottle was connected to a closed end Hg manometer and Hg column zeroed against a BOD scale. The apparatus was incubated at 20°C for five days. The volume of O_2 utilised in bacterial oxidation of organic matter is measured by manometer, and CO_2 produced during the process is absorbed by KOH in the seal cup. The BOD_5 of the sample is the reading on the manometer scale after five days incubation multiplied by the dilution employed.

2.5.13 Gas Volume

The volume of biogas produced by the digesters was measured using a 0.25 l wet type gas meter (Wright and Co, Ltd, London, UK). Meter readings were normally taken five times per week at the same time each day.

2.5.14 Gas Composition

Gas samples were analysed for CH_4 and CO_2 by two different gas chromatographic methods. The first method used for the poultry litter digestion experiments, and the second two groups of the manure digestion experiments, employed a Perkin Elmer 452 gas chromatograph fitted with a

2m column packed with Porapak T 100 - 120 mesh, with an oven temperature of 60°C and N_2 carrier gas. The detector was a katharometer.

The second method employed the same column and oven temperature in a Varian 6000 GC (Varian Associates, Ca, USA) with a thermal conductivity detector, connected to a Data Station 4100. The carrier gas was He.

The H_2S content of the biogas was analysed using a Dräger Multi Gas Detector 21/31 (Drägerwerk AG, Lübeck, FRG) with the appropriate detector tubes for H_2S.

CHAPTER 3

THE SEMI CONTINUOUS ANAEROBIC DIGESTION OF POULTRY LITTER

3.1 Introduction

Few reports on the digestibility of poultry litter have appeared in the literature. The earliest documented investigation appears to be that undertaken by Farag et al (1970) who reported successful batch digestion of poultry droppings with clay loam soil and sawdust beddings at 30°C. In batch digestion tests at 25°C and 35°C Hassan et al (1975a, 1975b) added sawdust to poultry manure to simulate broiler litter to test whether the inbalance in the C:N ratio of manure could be corrected by the addition of an exogenous carbon source. A slight stimulatory effect was observed, but it was concluded that sawdust would have little effect on gas production unless RT's longer than 35 days were employed.

In a comparison of the digestibility of chicken and turkey wastes, Hills (1982) reported lower gas production by turkey litter than manure in batch digestion tests at 35°C. Research on the digestion of poultry litter has been undertaken at the Polytechnic of Wales since 1974 at laboratory scale (Hawkes and Young, 1980), and pilot scale (Hawkes et al , 1976), and has indicated the potential of this material for digestion in continuously fed digesters at mesophilic temperatures. Conversely at thermophilic temperatures (60°C) poor efficiency of digestion of poultry litter was reported by Shih and Huang (1980).

In addition to providing information on the digestion of litter at 35°C at different influent concentrations and RT's, the purpose of this study was to investigate the GYLR relationships observed in sewerage digestion and discussed in Section 1.7.8.

3.2 Materials and Methods

3.2.1 Comparison of Microwave and Conventional Ovens for Solids Determinations

Approximately 1.3l of poultry litter feed was prepared, and after

mixing thoroughly, 20 ml samples were transferred to 60 preweighed 100 ml beakers. TS and VS determinations were made on 30 of these according to the microwave method described in Sections 2.5.1 and 2.5.2. The remaining samples were dried in a conventional oven at 105°C overnight before volatilising as above.

3.2.2 Digester Operation

Eight semi-continuously fed digesters operated in pairs were used in this investigation. The experiments fell into two groups. In the first group digesters were operated at RT's between 15 and 29 days, and influent concentrations of 2% and 4% VS, which gave LR's of between 0.68 and 2.72 KgVS $m^{-3}d^{-1}$ (Table 3.1). In the second group the operating conditions were 12 and 29 days RT, 1% and 5% influent VS, and 0.34 - 4.20 KgVS $m^{-3}d^{-1}$ LR (Table 3.2). In the text, RT's and influent concentrations will be rounded to the nearest whole number. Tables 3.1 and 3.2 also show the duration of the experiments and the period of stable operation of each digester. The frequency of analyses made on the performance of each digester and the composition of the influent and effluent, for both groups of experiments, is shown in Table 3.3.

Table 3.3 Frequency of Analyses

	Frequency				
Analysis	5 days per week	Weekly	Once per 1-2 weeks	Once per 2-3 weeks	Once per 3 weeks
Gas production	1,2				
Gas composition	1,2				
TS and VS		1,2			
pH		1	2		
pS			1,2		
NH_4^+ - N			1	2	
Alkalinity			1,2		
Cellulose				1	2
Total nitrogen				2	1
BOD					1,2

1 = Measurement made on 1st group digesters.

2 = Measurement made on 2nd group digesters.

Table 3.1

Operating conditions for group 1 experiments

Digester no.	1,2	3,4	5,6	7,8	9,10	11,12	13,14	15,16
Retention time (days)	19.44	29.17	16.67	14.58	14.58	16.67	29.17	19.44
Influent TS (%)	6.44 0 5	6.44 0 5	3.22 0 6	3.22 0 6	6.54 0.26 7	6.54 0.26 7	3.27 0.13 7	3.27 0.13 7
Influent VS (%)	4.00 0.09 5	4.00 0.09 5	2.01 0.05 6	2.01 0.05 6	3.96 0.08 7	3.96 0.08 7	1.98 0.04 7	1.98 0.04 7
Loading rate (kg VS m^{-3} d^{-1})	2.06 0.05 5	1.37 0.03 5	1.21 0.03 6	1.38 0.03 6	2.72 0.06 7	2.38 0.05 7	0.68 0.01 7	1.02 0.02 7
Duration of experiment (days)	105	105	70	56	84	84	84	84
Period of stable operation (days)	35	35	42	42	49	49	49	49
Litter batch while stable	2	2	2	2	3	3	3	3

Values are mean, standard deviation, number of measurements

Table 3.2

Operating conditions for group 2 experiments

Digester no.	17,18	19,20	21,22	23,24
Retention time (days)	29.17	11.67	29.17	11.67
Influent TS (%)	1.55 0.11 11	1.55 0.11 11	7.76 0.05 11	7.76 0.05 11
Influent VS (%)	0.98 0.03 11	0.98 0.03 11	4.90 0.13 11	4.90 0.13 11
Loading rate (kg VS m^{-3} d^{-1})	0.34 0.01 11	0.84 0.02 11	1.68 0.05 11	4.20 0.11 11
Duration of experiment (days)	112	112	112	112
Period of stable operation (days)	77	77	77	77
Litter batch while stable	3,4	3,4	3,4	3,4

See legend to table 3.1

3.3 Results

3.3.1 Comparison of Microwave and Conventional Ovens

No further changes in weight were observed after drying the samples in the microwave oven for two hours and the conventional oven overnight.

Both TS and VS determinations were higher for samples dried in the microwave than conventional oven. The mean microwave TS was 8.13% $\pm$ 0.57 SD which is significantly greater than the mean conventional oven TS of 7.78% $\pm$ 0.44 SD at the 2% level. The mean volatile fraction of TS after microwave drying was 67.00% $\pm$ 2.46 SD which is not significantly different to the mean VS measured after conventional oven drying of 66.32% $\pm$ 1.75 SD. The mean VS as a percentage of the wet weight therefore, was 5.45% $\pm$ 0.42 SD for samples dried in the microwave, which is significantly greater at the 1% level than the mean of 5.17% $\pm$ 0.33 SD for samples dried in the conventional oven.

3.3.2 Digester Operation

3.3.2.1 Comparison of paired Digesters

The performance of paired digesters is compared in Figure 3.1 in which weekly gas volume is plotted against week number for two pairs of digesters operated at 1% influent VS; digesters 17 and 18 at a 29 day RT, and digesters 19 and 20 at a 12 day RT. As the figure indicates there was sometimes a consistent difference in gas production between the two digesters of a pair, although both were fed identically. In Figure 3.1 digester 17 produced more gas than digester 18, and digester 19 more than digester 20. During the 11 week stable period the mean weekly gas volumes were for digesters 17 and 18, 3.77 and 3.22 litres respectively, (a significant difference at the 1% level), and for digesters 19 and 20, 7.49 and 6.90 litres respectively, (an insignificant difference).

FIG 3.1 COMPARISON OF PAIRED DIGESTERS

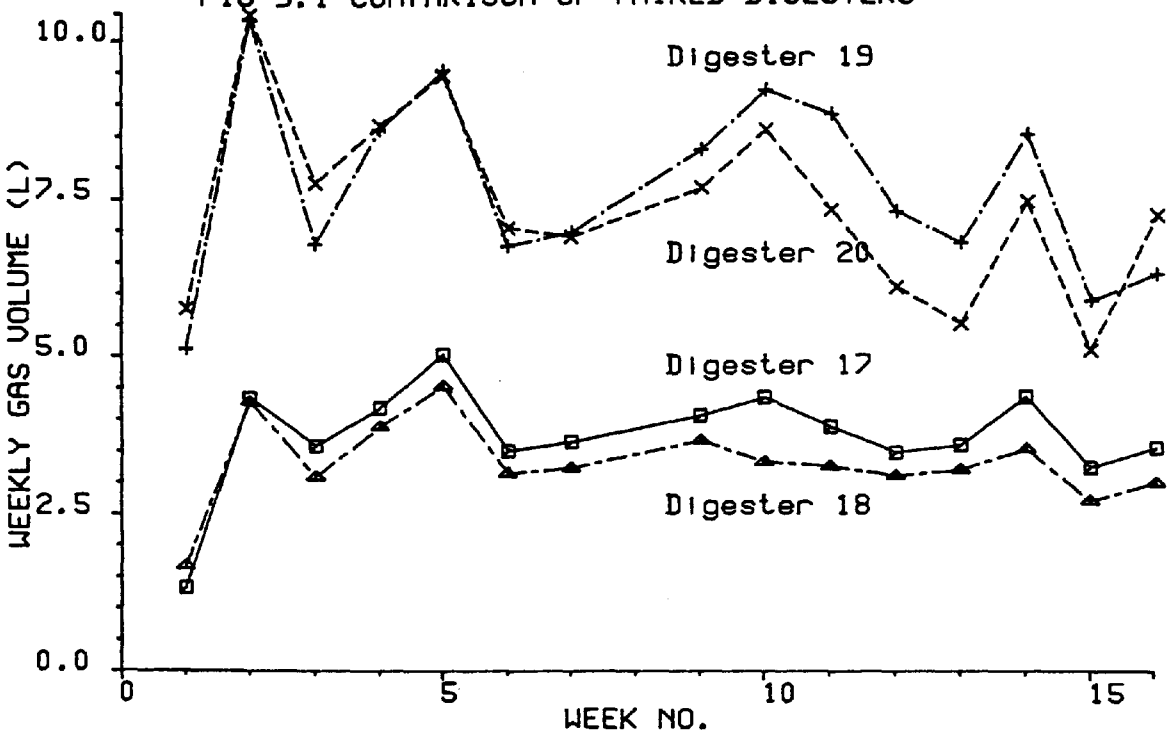
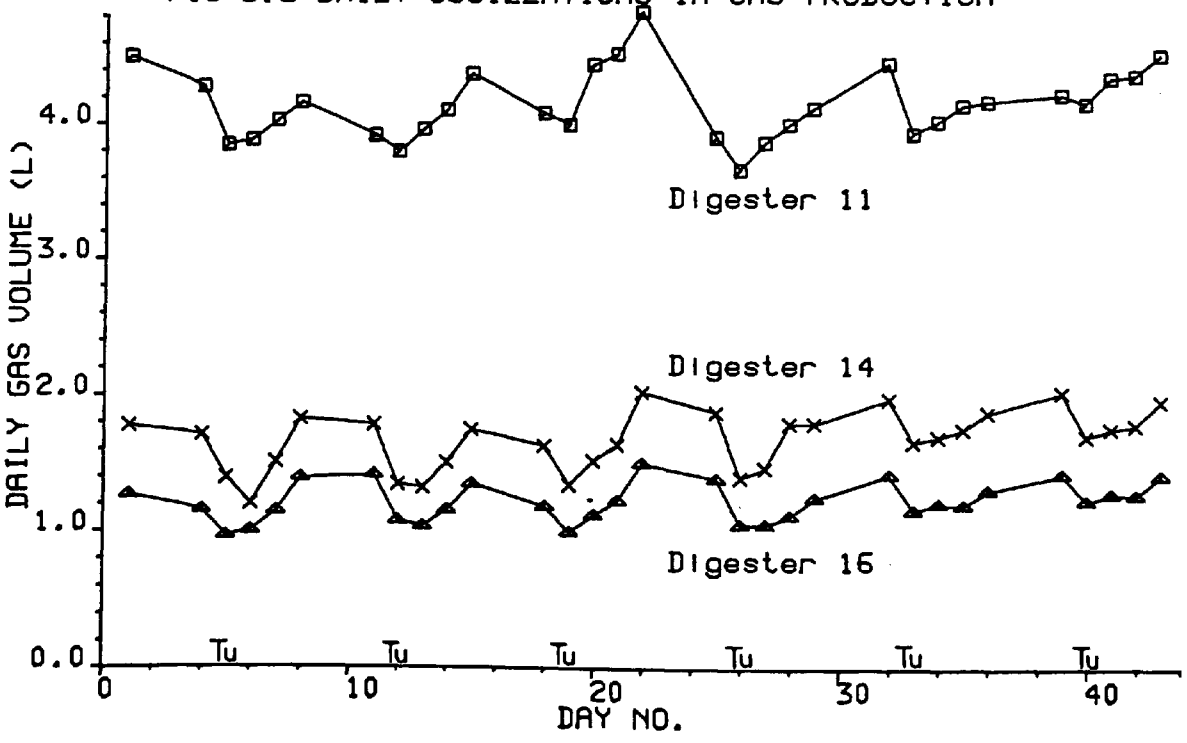


FIG 3.2 DAILY OSCILLATIONS IN GAS PRODUCTION



3.3.2.2 Daily Oscillations in Gas Production.

Figure 3.2 is a plot of daily gas production for digesters 11, 14 and 16 for weeks 8-13 of operation. These digesters were operated at a 17d RT and 4% influent VS, a 29d RT and 2% influent VS, and a 19d RT and 2% influent VS respectively. Oscillations in daily gas production during each week which followed a distinctive pattern are noticeable. A low point in gas production during the week was reached on Tuesday which increased gradually to a maximum on Friday. The mean daily gas production over the weekend, recorded on Monday, was normally lower than the volume recorded on Friday. On occasions when meter readings were taken on Saturday, the gas production was higher than that on Friday. These oscillations were observed in all experiments, particularly when stabilised, and appeared to be independent of influent concentration and RT.

3.3.2.3 Digester Stabilisation.

The stabilisation of a digester after start-up is illustrated in Figure 3.3 for weeks 1-13 of digester 17, operated at a 29d RT and 1% influent VS. For effluent TS, alkalinity, and pH there was a marked decrease from the start of the experiment, followed by a levelling off as the digester became adapted to the operating conditions. Effluent pS increased from start-up since the sulphide concentration decreased with the other parameters. The stabilisation of gas production was normally characterised not by a general increase or decrease, but by a more consistent rate of production as the experiment progressed.

3.3.3 Analyses

The characteristics of the influent for each pair of digesters during the stable periods are shown in Table 3.4, and of the effluent of each pair in Tables 3.5 and 3.6.

3.3.3.1 Solids Determinations

The fresh solids composition of the four batches of litter used in this

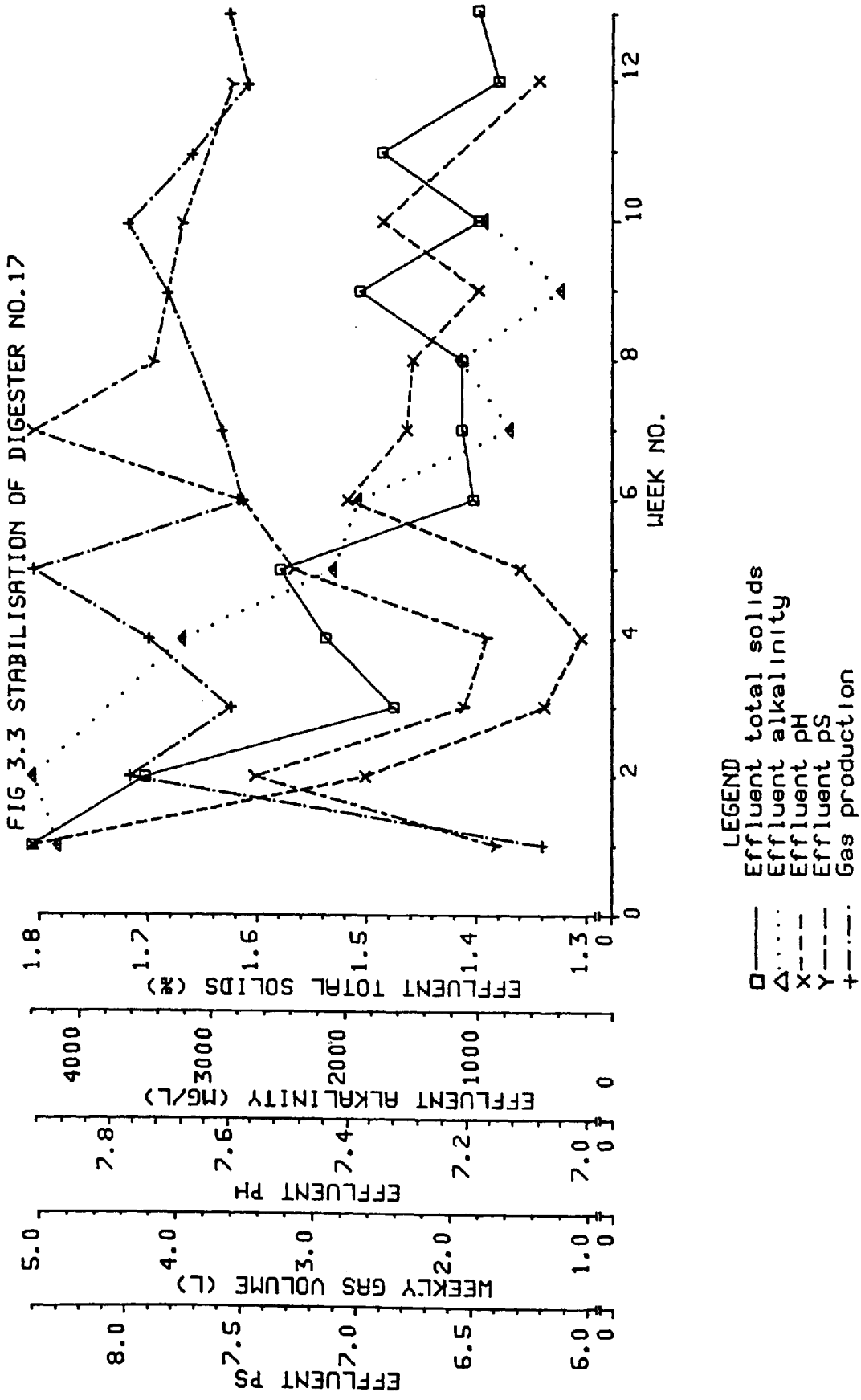


FIG 3.3 STABILISATION OF DIGESTER NO.17
EFFLUENT TOTAL SOLIDS (%)
EFFLUENT ALKALINITY (MG/L)
EFFLUENT PH
WEEKLY GAS VOLUME (L)
EFFLUENT PS
WEEK NO.
LEGEND
Effluent total solids
Effluent alkalinity
Effluent pH
Effluent pS
Gas production

Table 3.4

Influent characteristics for all experiments

Digester no.	1-4	5-8	9-12	13-16	17-20	21-24
Total solids (%)	6.44 0 5	3.22 0 6	6.54 0.26 7	3.27 0.13 7	1.55 0.11 11	7.76 0.53 11
Volatile solids (%)	4.00 0.09 5	2.01 0.05 6	3.96 0.08 7	1.98 0.04 7	0.98 0.03 11	4.90 0.13 11
Total nitrogen (mgl^{-1})	3748 25 2	1874 13 2	3572 145 3	1786 290 3	654 140 4	3270 700 4
Ammonium nitrogen (mgl^{-1} as N)	1006 26 3	489 29 4	1038 222 4	519 111 4	176 84 4	880 420 4
Total alkalinity (mgl^{-1})	8380 245 4	4184 107 5	8934 912 3	4467 456 3	1649 553 7	8245 2765 4
pS	13.94 0.50 3	13.64 0.50 3	19.90 4.16 6	19.60 4.16 6	16.71 2.72 8	17.36 2.93 7
Holocellulose (%)	- - -	- - -	2.91 0.57 4	1.45 0.28 4	0.77 0.20 3	3.85 0.98 3
BOD_5 (mgO_2l^{-1})	6464 471 3	3393 373 4	4498 678 3	2294 339 3	1580 238 5	7900 1190 5

See legend to table 3.1

Table 3.5

Effluent characteristics for group 1 experiments

Digester no.	1,2	3,4	5,6	7,8	9,10	11,12	13,14	15,16
Total solids (%)	5.62 0.32 10	5.67 0.47 10	2.65 0.09 12	2.76 0.15 12	6.11 0.32 14	5.56 0.27 14	2.68 0.12 14	2.68 0.12 14
Volatile solids (%)	2.92 0.22 10	2.88 0.27 10	1.35 0.05 12	1.42 0.08 12	3.16 0.15 14	2.87 0.15 14	1.35 0.19 14	1.33 0.07 14
Total nitrogen (mgl^{-1})	3350 8 2	2872 32 2	1709 270 3	1769 106 2	3336 186 6	3259 226 6	1772 261 6	1656 136 5
Ammonium nitrogen (mgl^{-1} as N)	1222 164 6	1106 127 6	647 86 8	670 56 8	1416 63 8	1352 86 8	768 33 8	759 48 8
Free ammonia (mgl^{-1} as N)	26.9 6.2 6	29.9 4.4 6	8.9 1.7 8	10.2 1.3 8	39.9 5.0 8	36.6 4.5 8	17.4 2.5 6	14.1 1.5 6
Total alkalinity (mgl^{-1})	10988 468 8	10319 586 8	5295 410 10	5445 259 10	11625 277 6	11042 213 6	5758 102 6	5558 180 6
pH	7.29 0.04 10	7.37 0.07 10	7.08 0.04 12	7.13 0.04 12	7.41 0.03 14	7.39 0.03 14	7.32 0.06 12	7.20 0.04 12
pS	7.60 0.15 6	7.70 0.08 6	8.22 0.10 6	8.22 0.08 6	7.44 0.21 14	7.51 0.21 14	8.06 0.22 12	8.09 0.23 12
Holocellulose (%)	- - -	- - -	- - -	- - -	1.49 0.16 4	1.18 0.19 6	0.45 0.03 6	0.51 0.08 6
BOD_5 (mgO_2l^{-1})	1248 17 3	1308 0 1	830 25 2	876 148 2	1187 135 4	1249 56 4	876 50 4	853 208 4

See legend to table 3.1

Table 3.6

Effluent characteristics for group 2 experiments

Digester no.	17,18			19,20			21,22			23,24		
Total solids (%)	1.36	0.09	22	1.38	0.08	22	6.44	0.50	22	6.76	0.59	22
Volatile solids (%)	0.66	0.05	22	0.71	0.05	22	3.19	0.28	22	3.63	0.31	22
Total nitrogen (mgl^{-1})	793	108	8	682	135	8	3267	208	8	3300	376	8
Ammonium nitrogen (mgl^{-1} as N)	409	68	8	342	30	8	1502	99	8	1389	139	8
Free ammonia (mgl^{-1} as N)	8.0	3.3	8	4.3	1.2	8	64.5	11.1	8	44.0	6.5	8
Total alkalinity (mgl^{-1})	3238	285	14	2779	195	14	12286	898	14	12268	619	14
pH	7.21	0.11	16	7.04	0.10	16	7.55	0.07	16	7.44	0.06	14
pS	8.03	0.39	14	8.02	0.39	16	6.44	0.13	16	6.37	0.18	16
Hollocellulose (%)	0.25	0.02	8	0.27	0.03	8	1.55	0.28	8	1.65	0.32	8
BOD_5 (mgO_2l^{-1})	464	101	5	563	105	5	2210	205	6	3026	436	4

See legend to table 3.1

investigation is shown in Table 3.7.

Table 3.7 Solids Composition of Litter Batches

Batch	Date of Collection	TS (%)	VS (% of TS)	VS (%)
1	20.3.80	51.79	60.63	31.40
2	30.10.80	54.84	61.80	33.89
3	12.2.80	50.25	57.88	29.08
4	3.8.81	81.42	64.71	52.69

Batches 1-3 had a similar water content ranging from 50.25 - 58.84% TS (mean 52.29%), while batch 4 was much dryer at 81.42% TS. Variations in the VS content of the litter batches were relatively small ranging betwen 57.88 and 64.71% of TS, with mean of 61.26%.

The TS content of the effluent varied from 1.36% at a 29d RT and 1% influent VS, to 6.76% at 12d RT and 5% influent VS. (Tables 3.5 and 3.6). Figure 3.4 shows the relationship between effluent TS and LR for all digesters. Each point represents one determination made on one digester during its stable period. Regression lines connect determinations made on digesters operated at equal RT's. At each RT effluent TS increased with LR, and was therefore dependent on the influent TS content. Changing the RT at constant influent concentration appeared to have little effect on effluent TS, although at 4% influent VS the effluent TS at 15d RT was higher than that at the other RT's, (6.11% TS compared with a mean of 5.62% TS for 29, 19 and 17d RT's).

The proportion of the TS which was volatile was relatively constant for all conditions and varied only between 48.2% and 53.6%, so the VS content of the wet weight followed the same pattern as that of the TS, and was dependent on influent concentration. Effluent VS varied between 0.66% at 12d RT and 1% influent VS, and 3.63% at the same RT and 5% influent VS. The relationship between effluent VS and LR is shown in Figure 3.5. As with TS, the VS content of the effluent at 15d RT and 4% influent VS was slightly higher than at the other RT's and the same influent concentration.

FIG 3.4 RELATIONSHIP BETWEEN EFFLUENT TS AND LR

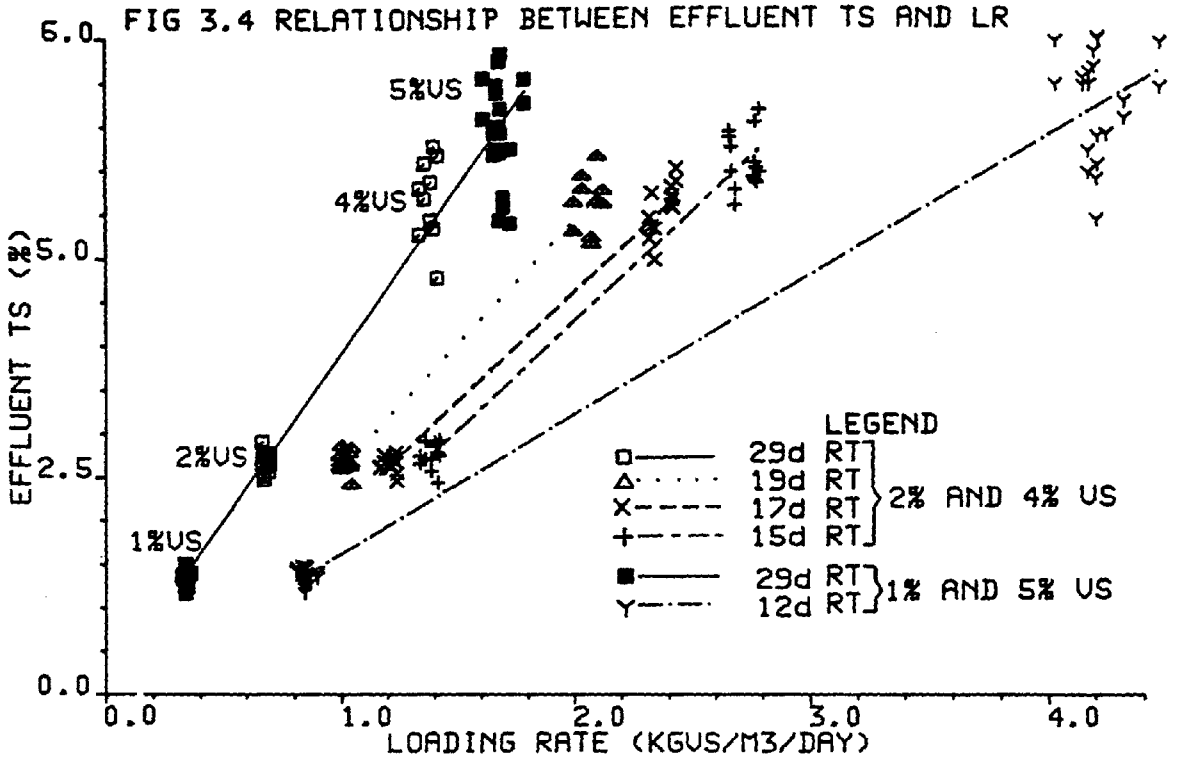
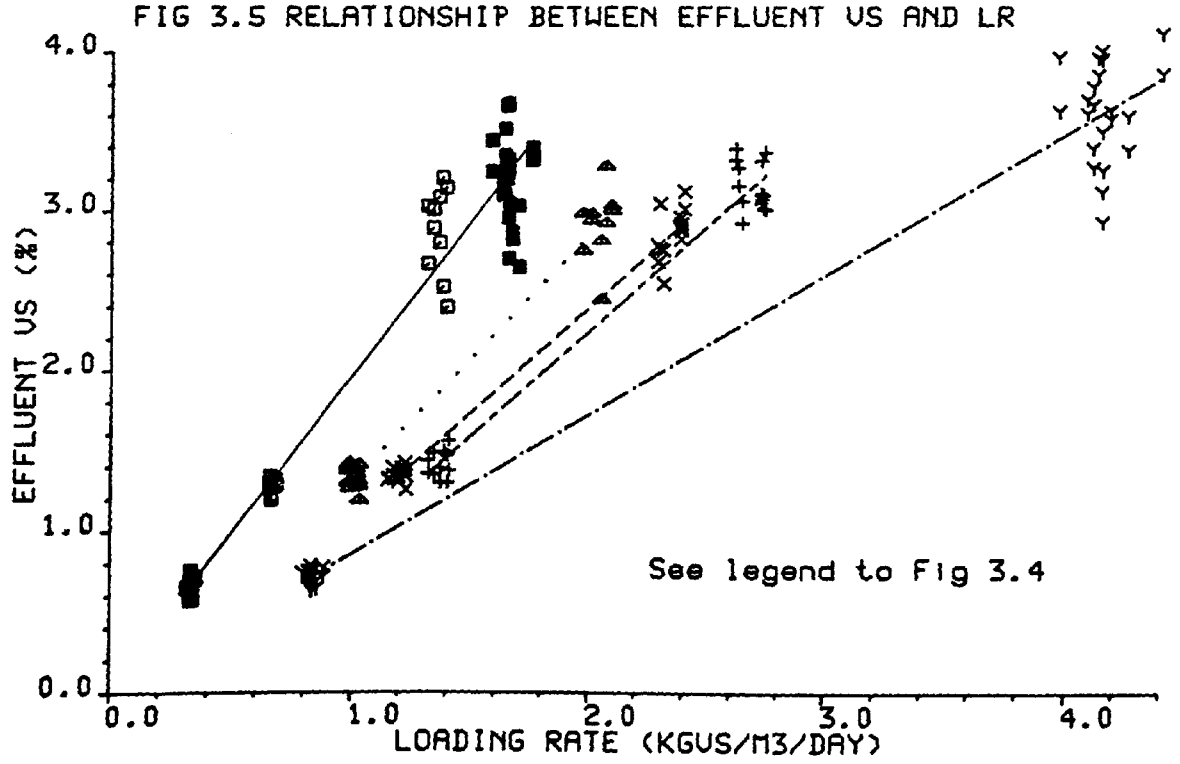


FIG 3.5 RELATIONSHIP BETWEEN EFFLUENT US AND LR



3.3.3.2 Total Nitrogen

The mean total nitrogen content of the four batches of litter used was, as a percentage of the dry weight, 4.62%, 5.72%, 5.08% and 3.18% respectively, with an overall mean of 4.65%.

The total nitrogen content of the effluent varied from 680mgl^{-1} at 12d RT and 1% influent VS to 3350 mgl^{-1} at 19d RT and 4% influent VS (Tables 3.5 and 3.6). The relationship between effluent nitrogen and LR is shown in Figure 3.6. At each RT the effluent nitrogen concentration increased linearly with influent concentration and LR. However the nitrogen concentration of the effluent at 19, 17 and 15d RT and 4% influent VS (mean of 3315 mgl^{-1} nitrogen) was higher than that at 5% influent VS (mean of 3284 mgl^{-1}) and not lower as expected.

3.3.3.3 Ammonium Nitrogen

The mean NH_4^+ - N content of the different batches of litter varied between 0.85% and 1.50% of the dry weight, with an overall mean of 1.18%, and an average constituted 25.22% of the total nitrogen.

The concentration of NH_4^+ - N in the effluent varied between 342 mgl^{-1} at 12d RT and 1% influent VS, and 1502 mgl^{-1} at 29d RT and 5% influent VS, (Tables 3.5 and 3.6). The relationship between effluent NH_4^+ - N and LR is shown in Figure 3.7. At each RT effluent NH_4^+ - N increased with increasing LR, and was therefore dependent on influent solids concentration, but was higher at 15 d RT and 4% influent VS than at 12d RT and 5% influent VS. The effect of RT on effluent NH_4^+ - N was inconsistent. At 1%, 2% and 5% influent VS the NH_4^+ - N concentration increased with increasing RT, but this was reversed at 4% influent VS. The proportion of the total nitrogen in the effluent constituted by NH_4^+ - N varied between 36.5% and 51.6%, with a mean of 42.8%.

FIG 3.6 RELATIONSHIP BETWEEN EFFLUENT TN AND LR

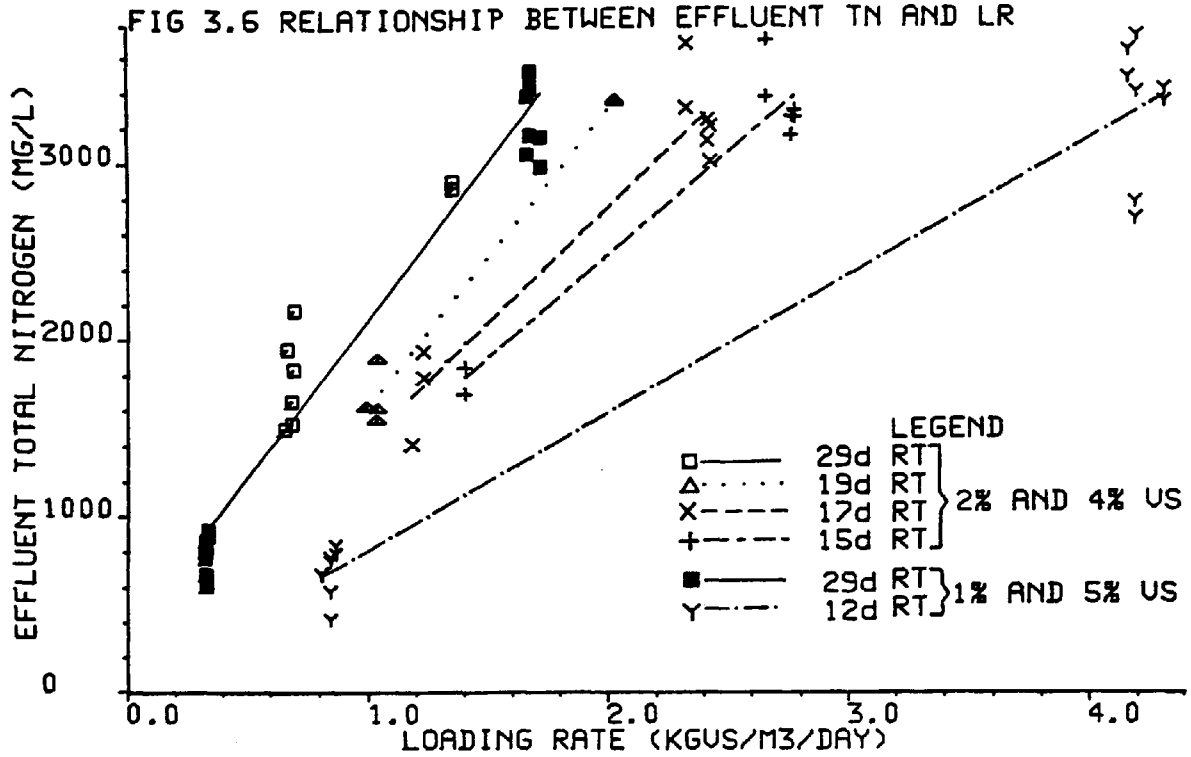
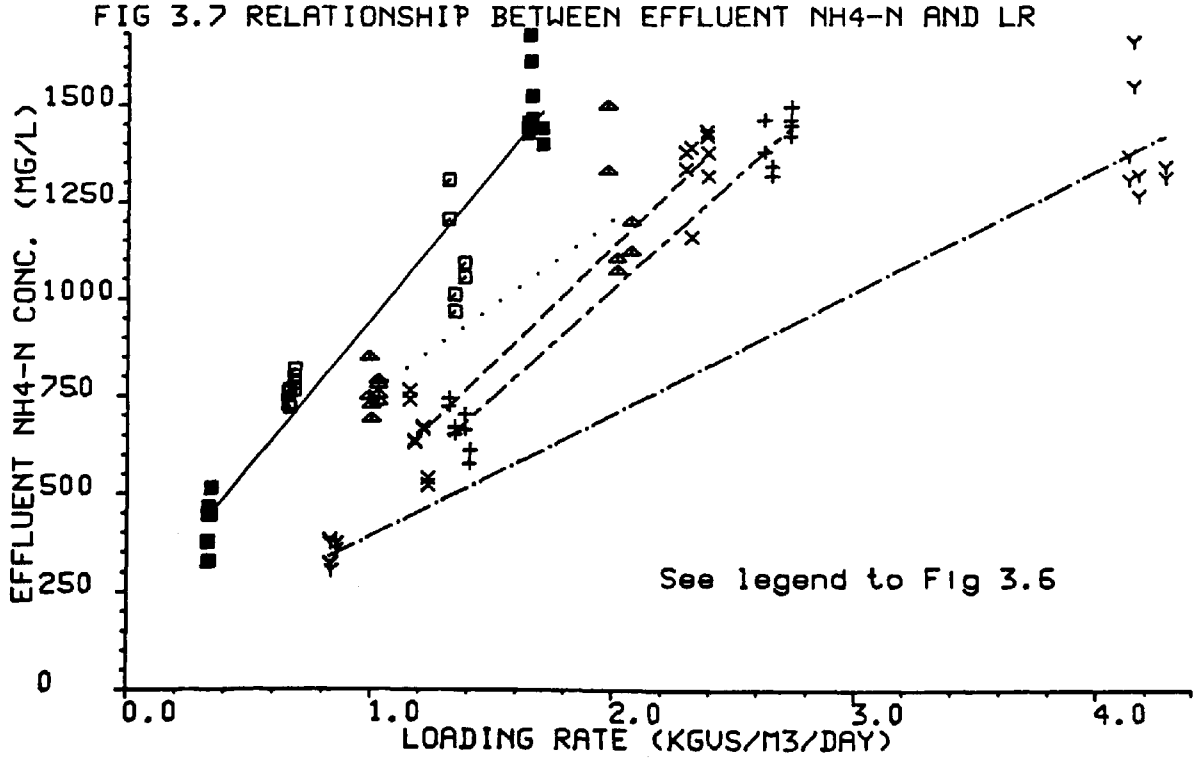


FIG 3.7 RELATIONSHIP BETWEEN EFFLUENT NH4-N AND LR



3.3.3.4 Total Alkalinity

The mean alkalinity of 8% TS stock feed varied between batches from 5657 - 10,567 mgl^{-1} as $Ca\ CO_3$ with an overall mean of 8827 mgl^{-1}.

The alkalinity of the effluent varied between 2779 mgl^{-1} at 12d RT and 1% influent VS, and 12,286 mgl^{-1} at 29d RT and 5% influent VS (Tables 3.5 and 3.6). The relationship between effluent alkalinity and LR is shown in Figure 3.8. At constant RT alkalinity increased with LR i.e. influent solids concentration. There was a marginal increase in effluent alkalinity with increasing RT at 1%, 2% and 5% influent VS, but as with NH_4^+ - N, the reverse was true for 4% influent VS.

3.3.3.5 pH

The stock feed pH varied between 6.85 and 8.62 with a mean value of 7.67 ± 0.45 SD. The pH of the influent was not routinely measured and cannot be calculated from the dilution of the stock feed, which was employed to determine the influent concentrations of the other parameters measured.

The pH of the effluent varied from 7.04 at 12d RT and 1% influent VS, to 7.55 at 29d RT and 5% influent VS. (Tables 3.5 and 3.6). The relationship betwen effluent pH and LR is presented in Figure 3.9. Effluent pH increased with increasing influent concentration and RT, although digesters at 15d and 17d RT gave apparently anomalous results as regards RT.

3.3.3.6 Sulphide

The mean pS value of 8% TS stock feed varied between batches from 17.71 to 28.92 with a mean of 21.26. The concentration of sulphide in the stock feed was on average 607 mgl^{-1}, which is equivalent to 0.76% of the dry weight. The pS of the effluent ranged from 6.37 units at 12d RT and 5% influent VS, to 8.03 units at 17d RT and 2% influent VS, (Tables 3.5 and 3.6). Effluent pS is plotted against LR in Figure 3.10 for digesters operated at 2% and 4% influent VS, and Figure 3.11 for digesters at 1% and 5% influent VS. The two groups are plotted separately since there is a difference of approximately 0.8 pS units between the two

FIG 3.8 RELATIONSHIP BETWEEN EFFLUENT ALKALINITY AND LR

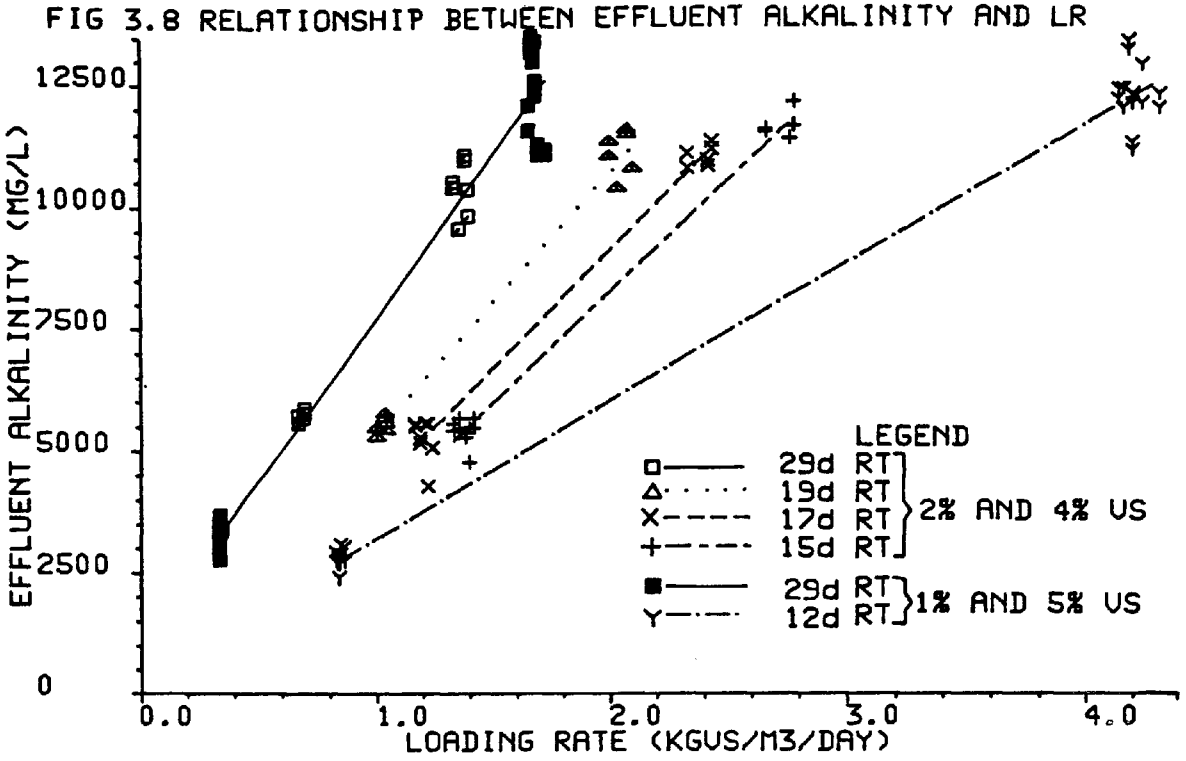
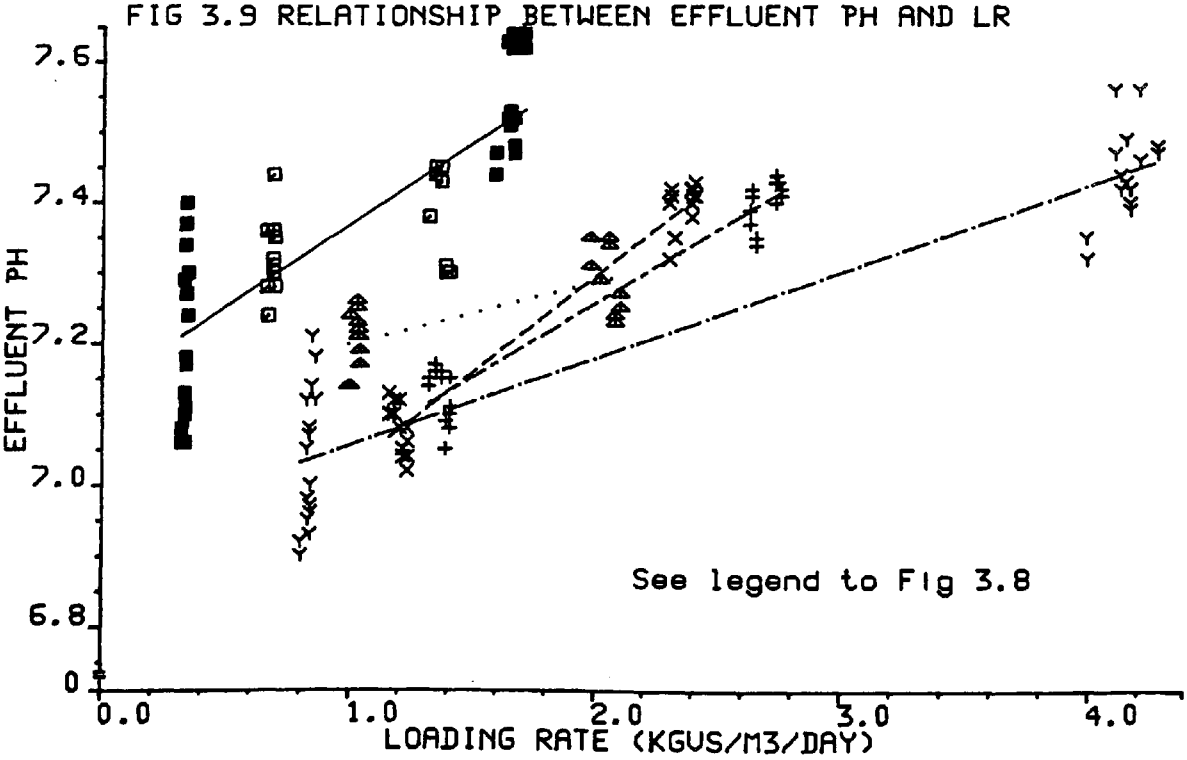


FIG 3.9 RELATIONSHIP BETWEEN EFFLUENT PH AND LR



3.10 RELATIONSHIP BETWEEN EFFLUENT pS AND LR FOR DIGESTERS OPERATED AT 2% AND 4% INFLUENT VS

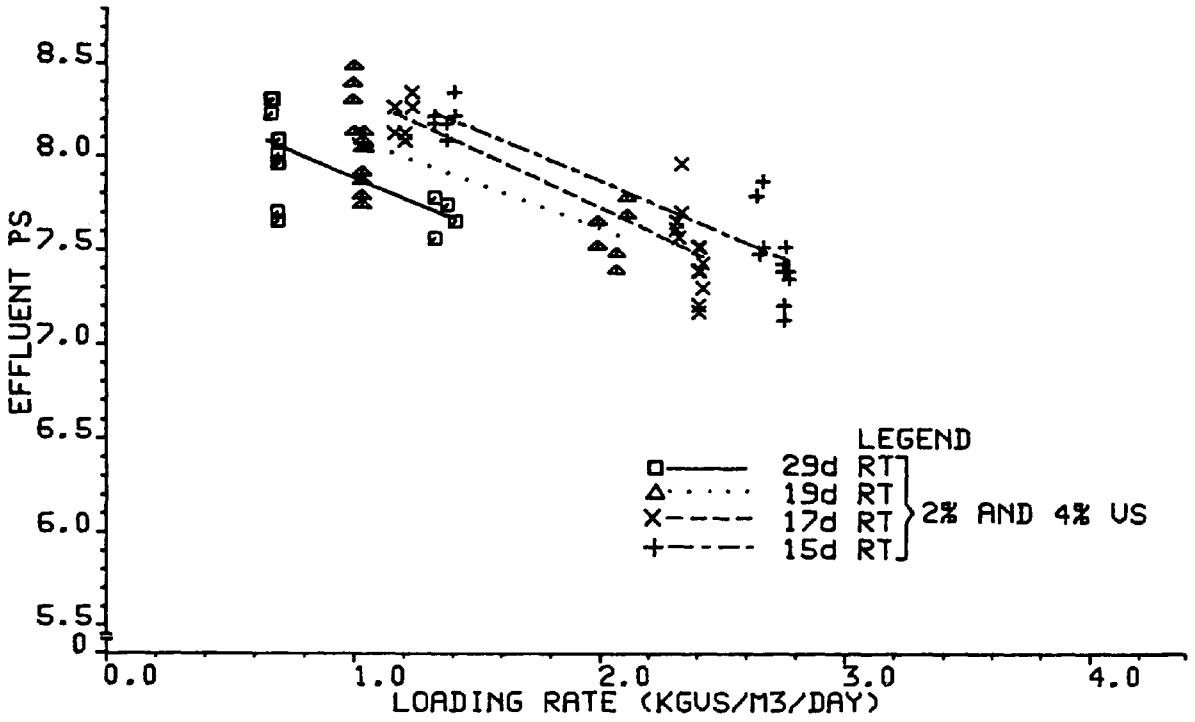
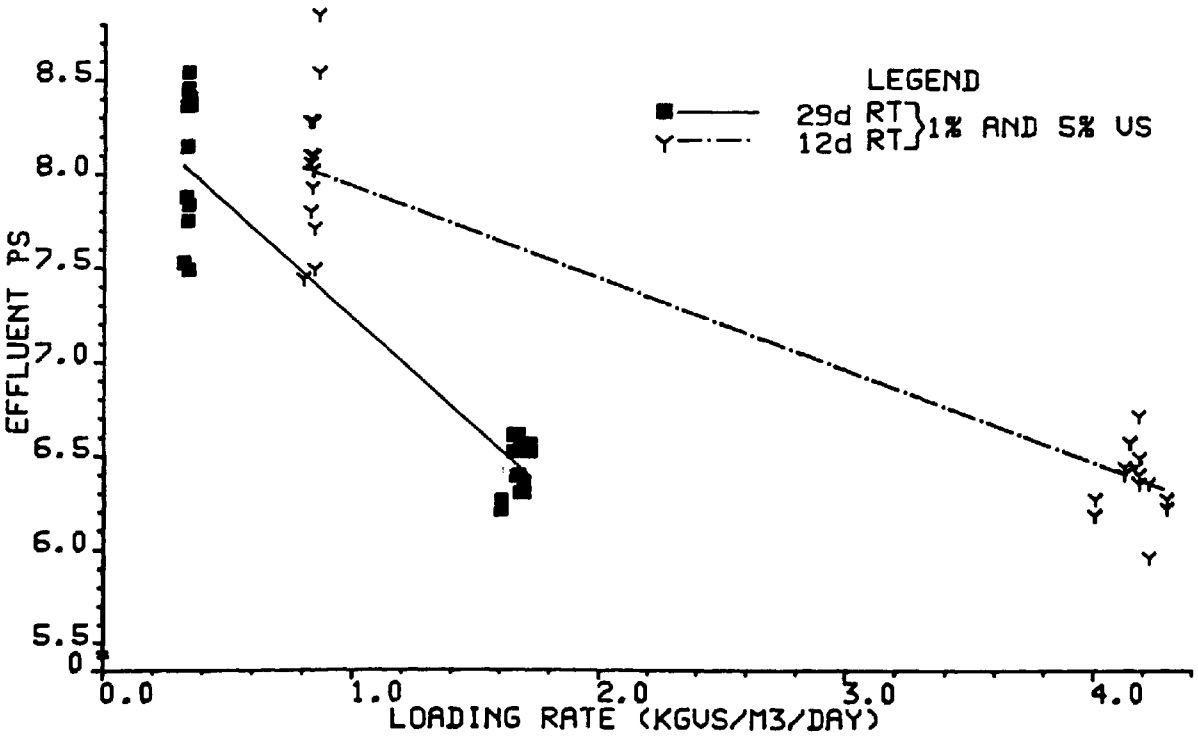


FIG 3.11 RELATIONSHIP BETWEEN EFFLUENT pS AND LR FOR DIGESTERS OPERATED AT 1% AND 5% INFLUENT VS



groups of experiments. However when considered separately, the results indicate that at constant RT the effluent pS decreased with increasing LR. i.e. the effluent sulphide concentration was dependent on the influent solids concentration. The effect of changing RT on digester pS was inconsistent. At 1%, 4% and 5% influent VS, increasing the RT increased the pS value, while the opposite was true at 2% influent VS.

3.3.4 Digestion Efficiency

The efficiency of digestion for experiments in Group 1 is shown in Table 3.8, and for experiments in Group 2 in Table 3.9.

3.3.4.1 Gas Composition

The mean proportion of CH_4 in the digester gas varied only slightly between 57.7% at 29d RT and 4% influent VS, and 61.0% at 29d RT and 1% influent VS, with an overall mean of 59.2%. The remainder was composed mainly of CO_2 with low levels of H_2S (less than 1%). The effect of changing RT and influent concentration on gas composition is not clear when the results from all experiments are considered together. However when the digesters which were operated concurrently are considered separately, i.e. digesters 1-8, 9-16, 17-24, apart from experiments at 1% influent VS, when the gas composition was practically constant, increasing both RT and influent concentration tended to reduce the CH_4 content of the gas. (Tables 3.8 and 3.9).

3.3.4.2 Biogas Yields

The mean weekly biogas yields for experiments at 2% and 4% influent VS varied from 0.320 m^3 kg VS added^{-1} at 15d RT and 2% influent VS, to 0.372 m^3 kg VS added^{-1} at 29d RT and 4% influent VS, with an overall mean of 0.343 m^3 kg VS added^{-1}, (Table 3.8). The mean biogas yields are plotted against LR in Figure 3.12. Each point represents the mean GY of each pair of digesters during the stable periods. Regression lines connect points of equal RT, and the vertical bars are $\pm$ one standard deviation. The figure

Table 3.8

Digestion efficiency for group 1 experiments

Digester no.	1,2	3,4	5,6	7,8	9,10	11,12	13,14	15,16
Biogas yield (m^3 kg VS added^{-1})	0.347 0.009 9	0.372 0.007 10	0.339 0.014 11	0.320 0.012 8	0.331 0.013 14	0.341 0.014 14	0.353 0.025 14	0.341 0.021 14
Methane yield (m^3 kg VS added^{-1})	0.200 0.007 9	0.215 0.008 10	0.199 0.011 11	0.188 0.008 8	0.198 0.009 14	0.202 0.008 14	0.210 0.016 14	0.207 0.015 14
Total solids reduction (%)	12.8 4.9 10	11.9 7.3 10	17.7 2.9 12	14.4 4.6 12	6.4 5.0 14	14.9 3.5 14	17.8 4.5 14	17.8 5.0 14
Volatile solids reduction (%)	27.0 5.2 10	28.0 7.1 10	32.9 1.5 12	29.3 4.2 12	20.1 4.5 14	25.1 7.6 14	34.7 2.3 14	32.9 4.0 14
Holocellulose reduction (%)	- - -	- - -	- - -	- - -	50.6 8.6 3	58.9 6.5 5	69.3 6.1 5	63.9 13.6 5
BOD_5 reduction (%)	81.6 0.1 2	80.7 0 1	75.5 0.8 2	74.2 4.4 2	74.1 1.5 2	72.8 0.7 2	61.8 0.6 2	62.8 6.1 2
Gas composition (% CH_4)	57.9 1.6 10	57.7 1.39 10	58.5 1.2 12	58.7 4.6 12	59.9 1.7 14	59.3 0.7 14	59.5 0.6 14	60.5 1.3 14

See legend to table 3.1

Table 3.9

Digestion efficiency for group 2 experiments

Digester no.	17,18	19,20	21,22	23,24
Biogas yield (m^3 kg VS added^{-1})	0.297 0.037 20	0.245 0.04 20	0.337 0.022 16	0.300 0.029 16
Methane yield (m^3 kg VS added^{-1})	0.182 0.025 18	0.150 0.027 18	0.195 0.014 16	0.177 0.021 16
Total solids reduction (%)	12.0 5.2 22	11.0 4.7 22	16.8 7.9 22	12.9 4.9 22
Volatile solids reduction (%)	32.3 4.8 22	27.3 5.2 22	34.7 6.1 22	25.9 6.3 22
Holocellulose reduction (%)	69.4 8.1 8	67.3 10.2 8	61.0 16.5 8	57.6 19.8 8
BOD_5 reduction (%)	72.1 4.8 8	68.8 5.1 4	71.7 1.9 4	61.7 3.0 4
Gas composition (% CH_4)	61.0 1.5 20	60.8 2.0 20	57.7 1.1 20	58.4 1.7 20

See legend to table 3.1

FIG 3.12 RELATIONSHIP BETWEEN BIOGAS YIELD AND LOADING RATE FOR 2% AND 4% INFLUENT VS

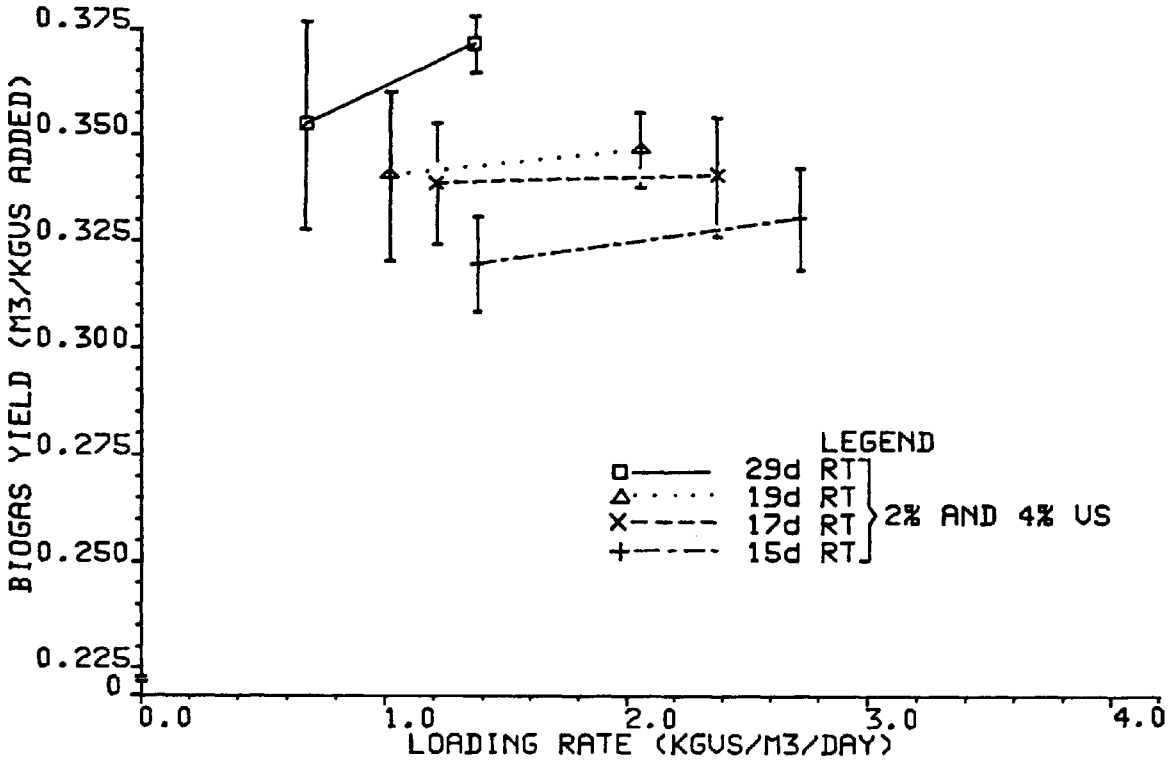
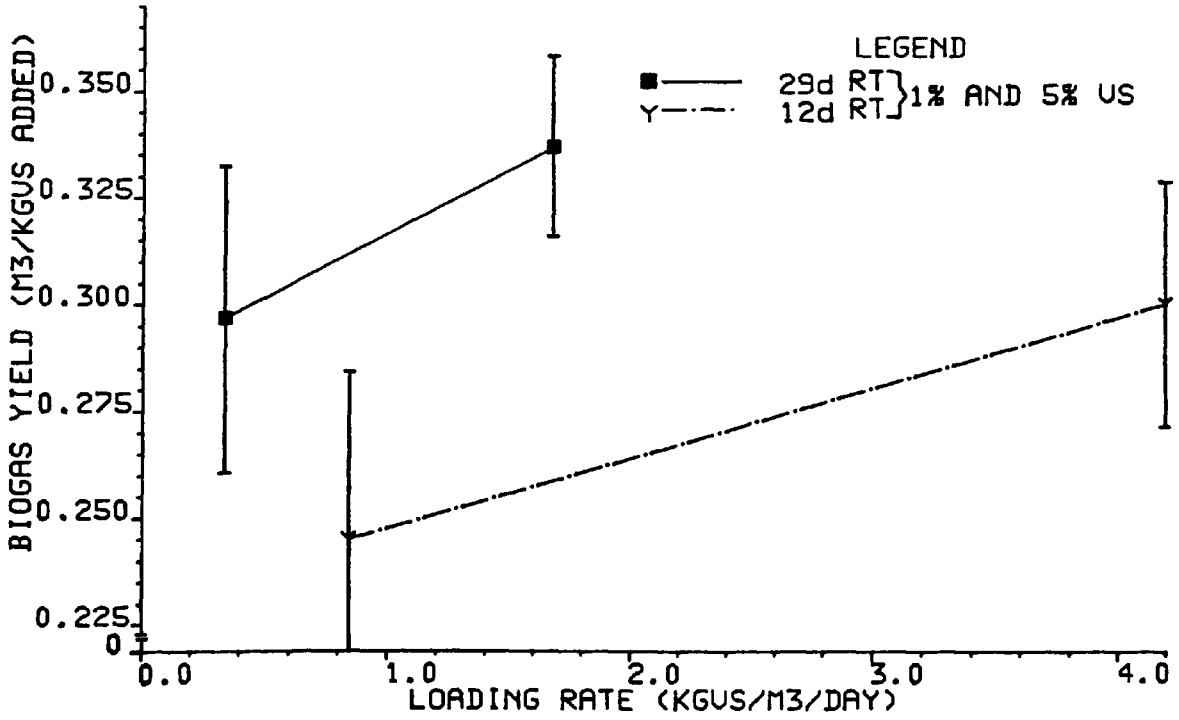


FIG 3.13 RELATIONSHIP BETWEEN BIOGAS YIELD AND LOADING RATE FOR 1% AND 5% INFLUENT VS



illustrates that at constant influent concentration the GY increased with increasing RT, and at constant RT, the GY increased with increasing influent concentration. This increase in GY with increasing RT and influent concentration was significant at 29d and 15d RT, but insignificant at 19d and 17d RT.

The mean weekly biogas yields obtained at 1% and 5% VS influent concentration varied between 0.245 m^3 kg VS added^{-1} at 12d RT and 1% influent VS, and 0.337 m^3 kg VS added^{-1} at 29d RT and 5% influent VS, with an overall mean of 0.295 m^3 kg VS added^{-1} (Table 3.9). The GY's are plotted against LR in Figure 3.13. As with the experiments in the first group the biogas yields obtained increased both with increasing influent concentration and RT. The GY's at 29d RT were significantly higher, at the 1% level, than those at 12d RT, and GY's at 5% influent VS significantly higher, at the 1% level, than those at 1% influent VS.

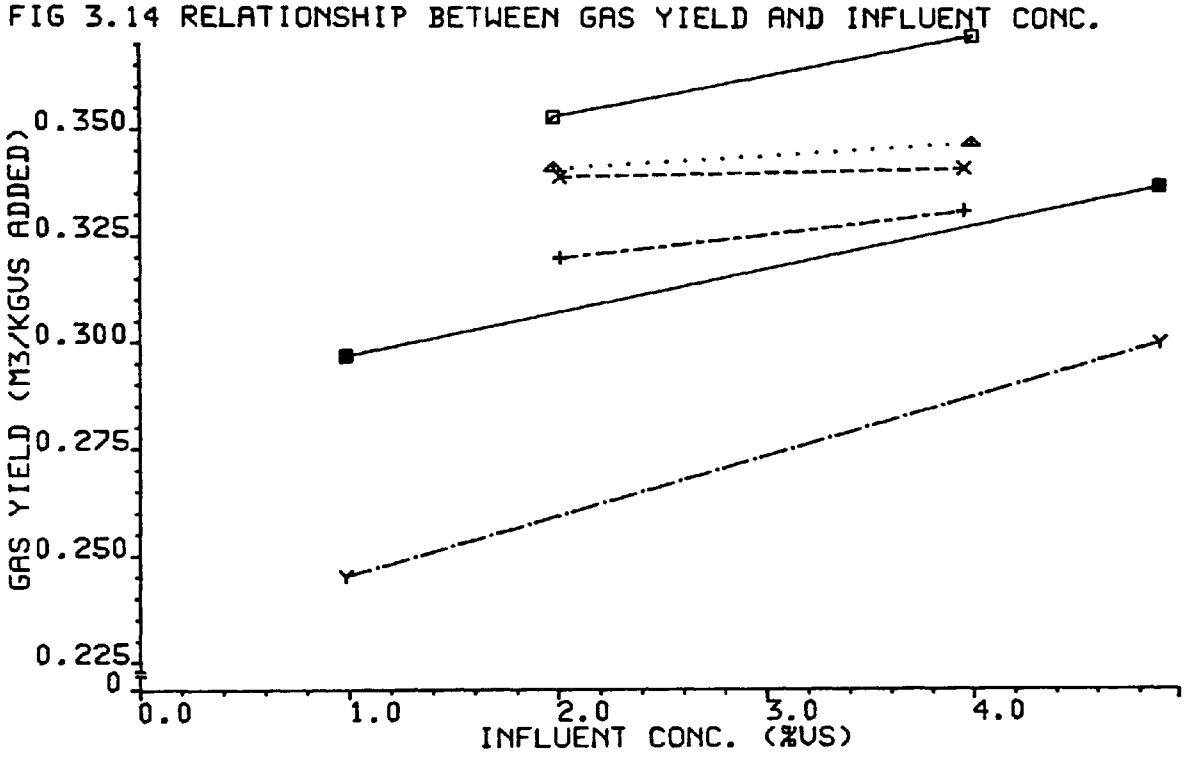
From Figure 3.12 and 3.13 it would appear that the GY's obtained from the second group of experiments are too low to be compatible with those of the first group. The biogas yields from all experiments are plotted against influent concentration for different RT's in Figure 3.14, which shows more clearly the effects of influent concentration and RT on GY, and the apparent low values at 1% and 5% VS.

3.3.4.3 Methane Yields

The mean weekly methane yields calculated from the gas composition and biogas yields during the stable periods of each experiment are plotted against LR in Figure 3.15 for experiments in the first group, and Figure 3.16 for experiments in the second group. Methane yields varied from 0.150 m^3 kg VS added^{-1} at 12d RT and 1% influent VS, to 0.215 m^3 kg VS added^{-1} at 29d RT and 4% influent VS, with a mean of 0.194 m^3 kg VS added^{-1} (Tables 3.8 and 3.9).

Although the variation in gas composition under different operating conditions was small, there was a tendency for the CH_4 content of the gas

FIG 3.14 RELATIONSHIP BETWEEN GAS YIELD AND INFLUENT CONC.



LEGEND

- 29d RT, 19d RT, 17d RT, 15d RT } 2% AND 4% VS
- 29d RT, 12d RT } 1% AND 5% VS

FIG 3.15 RELATIONSHIP BETWEEN METHANE YIELD AND LOADING RATE FOR 2% AND 4% INFLUENT VS

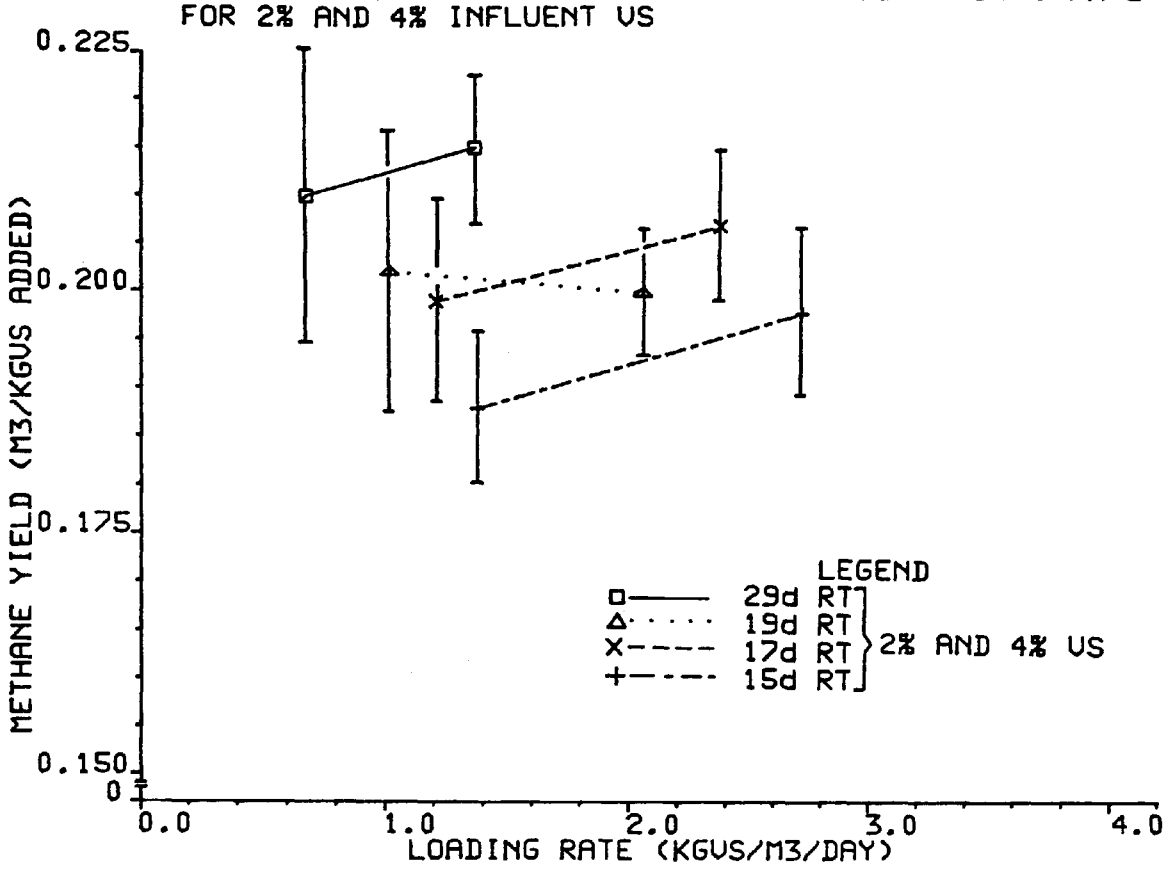
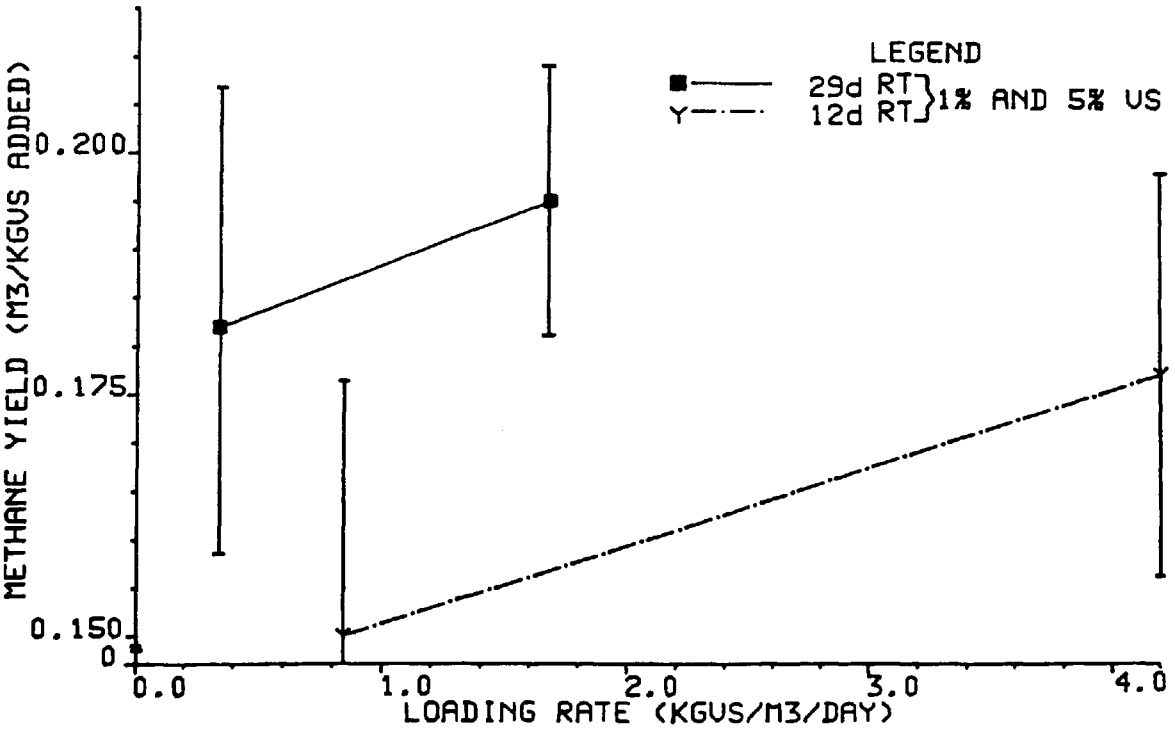


FIG 3.16 RELATIONSHIP BETWEEN METHANE YIELD AND LOADING RATE FOR 1% AND 5% INFLUENT VS



to decrease with increasing GY which reduced the effect of increasing RT and influent concentration. However as with biogas yield the methane yield increased with increasing influent concentration and RT, except at 19d RT where increasing the influent concentration from 2% to 4% VS reduced the methane yield from 0.207 to 0.200 m^3 kg VS added^{-1}.

Because there was little difference in gas composition between experiments in the two groups the incompatability of the GY's is still evident when expressed in terms of CH_4 production.

3.3.4.4. Destruction of Solids

The reductions in TS which were achieved were between 6.41% at 15d RT and 4% influent VS, and 17.84% at 29d RT and 2% influent VS, with a mean of 13.86% for all conditions tested (Tables 3.8 and 3.9). The effects of changing the RT and influent concentration on TS destruction were inconsistent. In experiments at 1%, 2% and 5% influent VS, TS reduction increased with increasing RT, but at 4% influent VS this was not observed. In the first group of experiments TS reduction increased with decreasing influent concentration, whereas in the second the opposite was true.

Because of the small variation in the volatile fraction of the TS (48.2 - 53.6%), the destruction of VS followed a similar pattern to that of TS, although at 4% influent VS increasing the RT increased VS reduction. The range of VS destruction was between 20.11% at 15d RT and 4% influent VS, and 34.74% at 29d RT and 2% influent VS, with a mean of 29.18%.

3.3.4.5 Cellulose

The holocellulose determination was carried out only on the second and third batches of litter. As a percentage of the dry weight the holocellulose content of the two batches was 46.8% and 46.5% respectively, with a mean of 46.7%. A limited number of α-cellulose determination were made during the second group of experiments. On average α-cellulose constituted 37.9% of the litter holocellulose.

The holocellulose content of the effluent varied from 0.25% of the wet weight, at 29d RT and 1% influent VS, to 1.65% at 12d RT and 5% influent VS. (Tables 3.5 and 3.6). Effluent holocellulose was dependent on influent concentration and decreased slightly with increasing RT. The proportion of α-cellulose in the effluent holocellulose was 52.6%.

The destruction of holocellulose increased from 57.6% at 12d RT and 5% influent VS to 69.38% at 29d RT and 1% influent VS, with an overall mean of 62.2% (Tables 3.8 and 3.9). Holocellulose destruction increased both with increasing RT and decreasing influent concentration.

3.3.4.6 Biological Oxygen Demand

The BOD of 8% TS stock feed made from the four batches of litter was 3364, 6438, 7123 and 8642 mgl^{-1} O_2 respectively, with a mean figure of 6392 mgl^{-1}.

The BOD of the effluent varied from 464 mgl^{-1} at 29d RT and 1% influent VS, to 3026 mgl^{-1} at 12d RT and 5% influent VS (Tables 3.5 and 3.6). Increasing the influent concentration raised the effluent BOD, but the effect of RT was inconsistent. At 1% and 5% influent VS increasing the RT reduced the BOD, while at 2% and 4% influent VS the opposite was observed.

A high degree of BOD destruction was achieved during digestion, which ranged from 61.7% at 12d RT and 5% influent VS to 81.6% at 19d RT and 4% influent VS, with a mean of 71.5% (Tables 3.8 and 3.9). However the effects of RT on BOD reduction were inconsistent. In the first group of experiments RT had little effect on BOD destruction, while in the second group increasing the RT from 12 to 29 days increased destruction from 68.8% to 72.1% at 1% influent VS, and from 61.7% to 71.7% at 5% influent VS. When the influent concentration was increased from 2% to 4% VS there was an increase in BOD reduction at 29d and 19d RT. Changing the influent concentration at other RT's had little effect except at 12d when the BOD destruction was reduced.

3.4 Discussion

3.4.1 Comparison of Microwave and Conventional Ovens

There are two possible explanations for the microwave oven giving higher TS results than the conventional oven. These are:

i) Drying in the microwave is inefficient and water is retained in the samples,

or

ii) Evaporation of volatile matter occurs during drying in the conventional oven.

Both possibilities would lead to higher TS and VS measured by microwave.

To determine if the microwave oven was less efficient than the conventional oven, samples were first dried in the microwave then after weighing were transferred to the conventional oven for a further period of drying. No further weight loss was observed which suggests that explanation i) is incorrect. However this result does not confirm the second explanation since volatile matter may have been expected to be lost when the sample was transferred to the conventional oven.

To test explanation ii), TS determinations were made by both methods on samples of effluent to which had been added 5ml glacial acetic acid. The TS content of control samples (without added acetic acid) was again higher in the microwave dried samples (mean 1.931%) than those dried by conventional oven (mean 1.917%). The acetic acid dosed samples dried in the microwave had a higher TS content (2.055%) than that of the control, while the dosed samples dried in the conventional oven had a lower TS content (1.804%) than the control. It appears therefore that acetic acid was lost during drying in both ovens, but to a higher degree in the conventional oven. Thus the evaporation of volatile matter from samples dried in the conventional oven may account for the lower solids figures obtained by this method. Since drying by microwave oven is faster and appears to retain more volatile material than the conventional oven, this method was employed for all TS determinations.

3.4.2 Digester Operation

3.4.2.1 Comparison of Paired Digesters

Since all external factors such as temperature and feed composition were identical for both digesters operated as a pair, the difference in performance observed in paired digesters must have been due to internal factors such as the biological properties of the contents, or the physical characteristics of the digesters. At the start of each experiment both digesters in a pair received seed of identical composition and hence identical bacterial populations. However it is possible that during the course of an experiment the two populations may diverge to produce strains with slightly differing physiological characteristics including the generation of CH_4. There were no consistent differences however, in the chemical properties of the contents of paired digesters which may be expected from differing physiologies.

All digesters were built to the same specifications but slight variations in the speed of stirring and impeller construction occurred. These variations could have led to differences in mixing efficiency and allowed accumulation of solids in poorly mixed digesters, thus increasing the solids RT and gas production. During the period of accumulation the solids content of the effluent would be lower than expected, but if it was a gradual process it would be difficult to detect. Consistent differences in effluent solids occurred between paired digesters, but these were not related to differences in GY. Nevertheless differences in the levels of sediment between pairs were observed occasionally although not recorded. Since it was evident that a range of GY's could be obtained from a given set of operating conditions, digesters were always operated in pairs to obtain more representative results.

3.4.2.2 Daily Oscillations in Gas Production

The daily oscillations in gas production (Figure 3.2) were probably a consequence of the feeding schedule employed, i.e. a single daily feed from Monday to Thursday, a double feed on Friday, and none on Saturday

and Sunday. From these oscillations inferences can be made about the rate of breakdown of material to produce gas. Since the daily gas volume increased progressively from Tuesday to Friday, the gas volume measured on Friday must have been produced from the material fed over at least the previous four days. Similarly the gas volume recorded on the other days in the week was also the result of gas produced from litter fed at least four days previously. Since the gas volume measured on Tuesday (produced from a single feed the day before and a double feed four days before) was less than that recorded on Wednesday (from single feeds one and two days previously), the contribution to gas production from two days before was greater than from a double feed four days previously. The maximum gas production during the week was recorded on Saturday since this gas was produced from feeds on Friday (double), Thursday, Wednesday and Tuesday, a total of five feeds.

It can be concluded therefore, that gas production and digestion of litter continues for at least four days, but the fastest rate of breakdown occurs within the first three days then subsequently decreases. Apart from the generation of oscillations in gas production the schedule of feeding employed appeared to have no detrimental effects on digester performance.

3.4.2.3 Digester Stabilisation

The main criterion used for determing when a digester had stabilised was gas production, since this was the parameter most relevant to this investigation. However other factors such as effluent composition and gas composition were also taken into consideration. In digester 17 the effluent TS concentration was high initially but decreased rapidly to constant levels (Figure 3.3). This was because the initial concentration of the digester seed (approximately 2% TS) was higher than that of the influent (approximately 1.6% TS), so during the experiment the concentration of the digester contents decreased from 2% TS to approx. 1.36% TS at steady state as the contents were replaced.

Since the solids content of a digester affects the levels of alkalinity, sulphide and pH these parameters also decreased from start up to constant levels. Since gas production is dependent mainly on the input of feed rather than the digester contents, this did not follow the same trend as the other parameters but fluctuated initially before becoming more stable.

Digesters are normally assumed to have reached a steady state condition after one or two RT's have elapsed, since by this time the digester contents should have been completely replaced. If the digester is not closely monitored this is a reasonable stipulation, but at long RT's it may be an excessive period of time to wait before results are obtained. A more precise method of determining the stability is by closely monitoring the performance of the digester.

3.4.3 Analyses

3.4.3.1 Solids Determinations

The chemical and physical properties of litters are subject to a variety of factors such as age, climatic conditions during production and storage, type and density of birds, and type of litter base material used (El-Sabban, 1969). Since the litter used in this investigation was always collected from the same source some of the problems were avoided, but because batches were collected at different times of the year, the composition was affected by age and climatic conditions. Thus batch 4 which was collected during the dryer weather of the Summer had a lower water content than the other batches collected at other times of the year (Table 3.7).

Variations in litter TS were compensated for by diluting down to a constant concentration. No compensation was made for fluctuations in litter VS, but these were small and led only to slight differences in LR's between experiments conducted using different batches.

Both the TS and VS of the effluent from the digesters operating at 15d RT and 4% influent VS were higher than expected from the results at other RT's. (Figures 3.4 and 3.5). Both digesters of the pair operating at these conditions had a high effluent TS content (5.98% and 6.25%) so it would be unlikely that the cause was a characteristic of the apparatus such as inefficient mixing. An abnormally high effluent solids concentration may be indicative of a low GY since destruction of solid matter results in gas production. The GY did decrease with decreasing RT and at 4% influent VS the lowest GY was obtained at 15d RT, but a gradual increase in effluent solids with decreasing RT would have been expected rather than a practically constant value at 29d, 19d and 17d RT, and a sharp increase to 15d RT.

3.4.3.2 Total Nitrogen

The nitrogen content of the litter used in this study (4.65% of dry weight) was slightly lower than the 5% reported by Caswell et al (1978), but higher than other values in the literature of 2.48% (Farag et al, 1979), 3.2% (Shih and Huang, 1980), and 2.50% - 3.38% (Riley, 1968). This demonstrates the variation which can be obtained in litter nitrogen content and probably reflects the different proportions of carbonaceous base material in different litters which dilutes the nitrogen rich manure.

The nitrogen content of digesters operated at 1% and 5% influent VS was lower than expected from the concentrations obtained at 2% and 4% influent VS (Figure 3.6). This was because effluent nitrogen is determined by influent nitrogen, and during the stable periods the nitrogen content of the litter used for experiments at 1% and 5% VS was lower than for experiments at 2% and 4% VS (4.17% compared with 5.58% of dry weight).

3.4.3.3 Ammonium Nitrogen

Since uric acid in poultry litter is readily degraded to NH_3 during storage (Schefferle, 1965a, b), a wide variation in NH_4^+ - N content was obtained between batches. Apart from a small amount of nitrogen lost as

NH_3 gas, the total nitrogen content of litter remains constant during storage, so the proportion constituted by NH_4^+ - N fluctuates. The mean value was 25.2% which is comparable with figures reported in the literature of 15% (Farag et al, 1970), 16% (Caswell, 1978) and 31.3% (Shih and Huang, 1980).

The proportion of NH_4^+ - N in the total nitrogen increased during digestion to 42.8%. This indicates that the amount of NH_4^+ - N used in microbial cell synthesis is less than that produced by protein and uric acid degradation. Effluent NH_4^+ - N was related to the influent solids concentration rather than the influent NH_4^+ - N since this was variable, but since ammonia is produced by the metabolism of nitrogenous compounds, effluent NH_4^+ - N must be dependent on the influent nitrogen concentration and can be predicted from this, or the nitrogen content of the dry litter. The relationship between effluent NH_4^+ - N and influent nitrogen is shown in Figure 3.17. Thus:

$$\text{Effluent } NH_4^+ - N \ (mgl^{-1}) = \text{influent nitrogen } (mgl^{-1}) \times 0.317 + 200$$

From Figure 3.7, increasing the RT at 1%, 2% and 5% influent VS increased the effluent NH_4^+ - N concentration probably due to a greater degree of breakdown of nitrogenous compounds to NH_3 over a longer period of time. At 4% influent VS however, the effluent NH_4^+ - N content decreased with increasing RT. This appears to be an anomalous result since the degradation of nitrogen containing compounds would not be expected to be lower at longer RT's.

3.4.3.4 Alkalinity

There was a wide variation in the alkalinity of the stock feed although this was always made up to 8% TS. This variation coincided with the variation in stock feed NH_4^+ - N concentration which suggests that the two parameters are related. The same relationship was also observed in the effluent. Alkalinity is plotted against NH_4^+ - N for both stock feed and effluent in Figure 3.18. The alkalinity of the influent and effluent

FIG 3.17 RELATIONSHIP BETWEEN EFFLUENT NH4-N AND INFLUENT TN

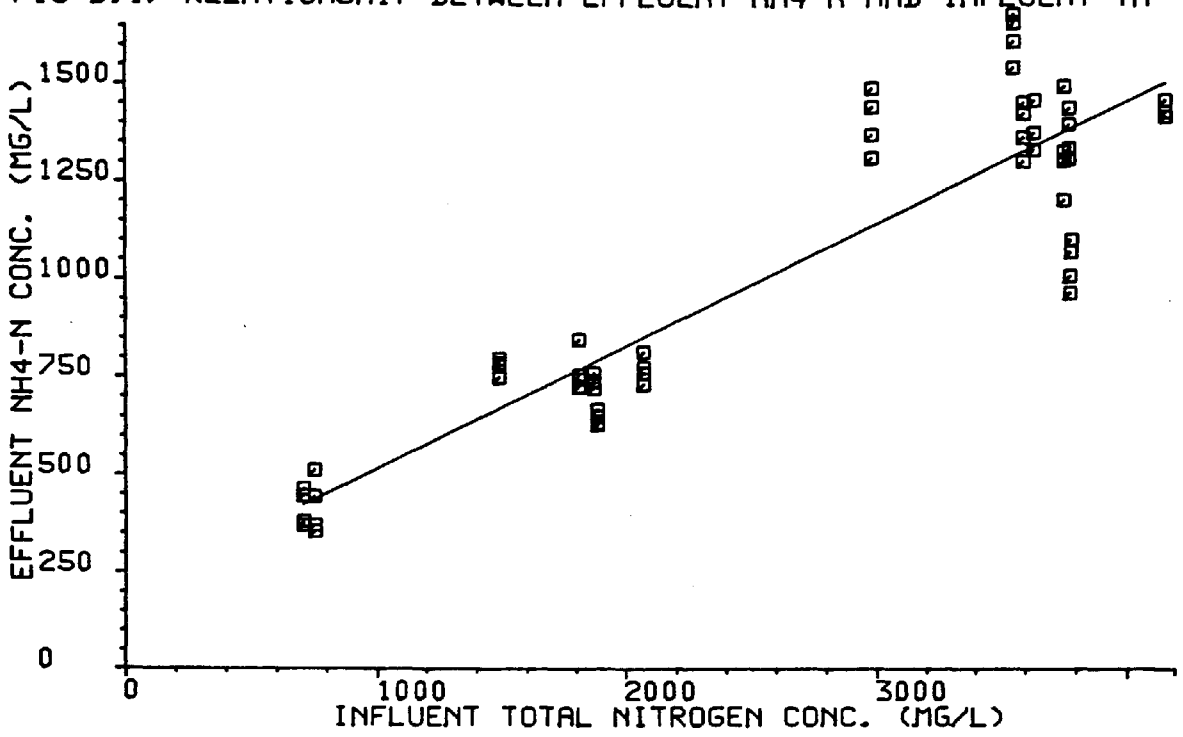
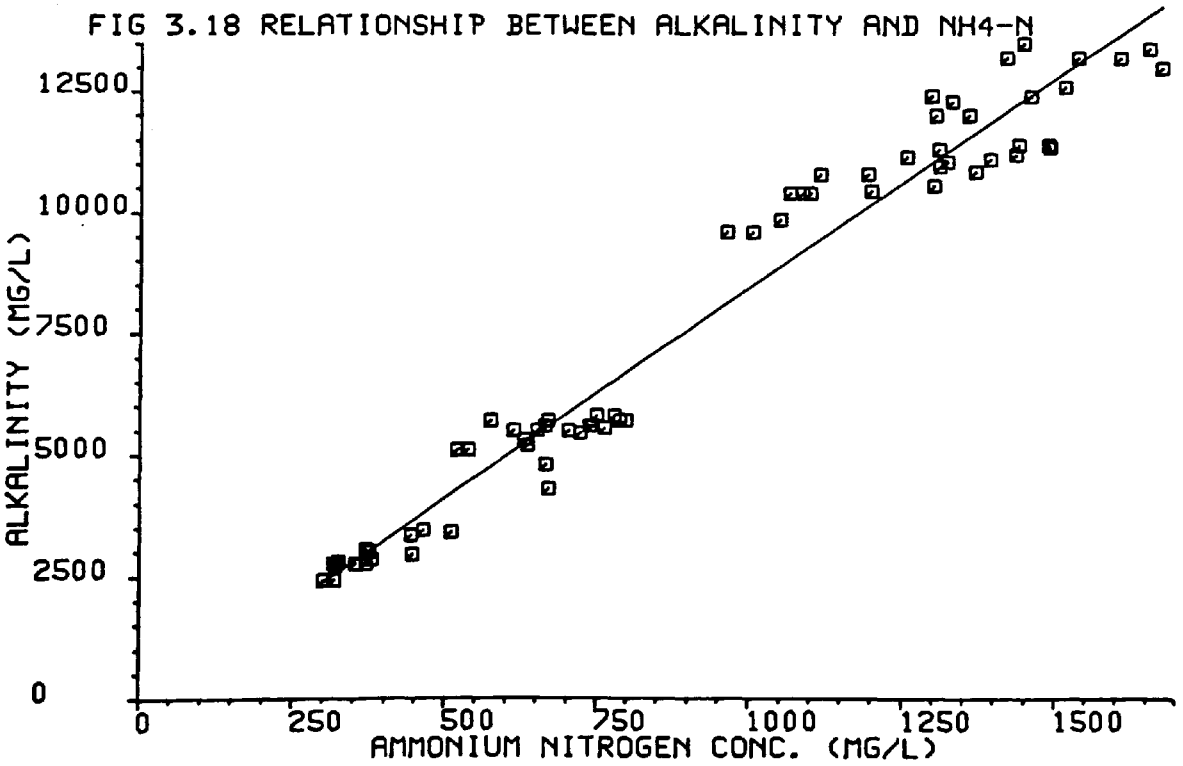


FIG 3.18 RELATIONSHIP BETWEEN ALKALINITY AND NH4-N



therefore can be predicted from the NH_4^+ - N concentration by the equation:

$$\text{Alkalinity } (mgl^{-1}) = NH_4^+ - N\ (mgl^{-1}) \times 8.61 - 213$$

The relationship between alkalinity and NH_4^+ - N has also been noted by Melbinger and Donnellon (1971) in sewage digestion, who observed that 23% of the alkalinity was in the form of NH_4^+ - N. i.e. an NH_4^+ - N concentration of 1000 mgl^{-1} was accompanied by an alkalinity of 4349 mgl^{-1}.

3.4.3.5 pH

According to Kirsch and Sykes (1971) the optimum pH range for digestion is 6.8 - 7.5. In the poultry litter digesters this range was exceeded at only one set of operating conditions (29d RT, 5% influent VS) when the mean pH was 7.55. The efficiency of digestion at this pH did not appear to be affected since GY, and solids and BOD destruction were higher than at the other operating conditions in the same group.

In common with effluent alkalinity, the relationship between pH and LR was similar to that between NH_4^+ - N and LR, and pH increased with influent concentration but was affected by changes in RT (Figure 3.9). Digester pH therefore increased with, and may be dependent on NH_4^+ - N concentration, due to its alkaline nature in solution (Figure 3.19):

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

The effect of NH_4^+ - N on pH has also been observed by Albertson (1961), Melbinger and Donnellon (1971), and Braun et al (1981). As the pH increases the equilibrium between NH_3 and NH_4^+ shifts to the left and increases the concentration of NH_3. Thus as the concentration of NH_4^+ - N in the digester increases with influent concentration, so does the proportion of NH_4^+ - N which is in the form of free NH_3. This increases the possibility of ammonia toxicity at high influent concentrations since free NH_3 is more toxic than NH_4^+ (McCarty and McKinney, 1961a, McCarty, 1964).

FIG 3.19 RELATIONSHIP BETWEEN EFFLUENT PH AND NH4-N

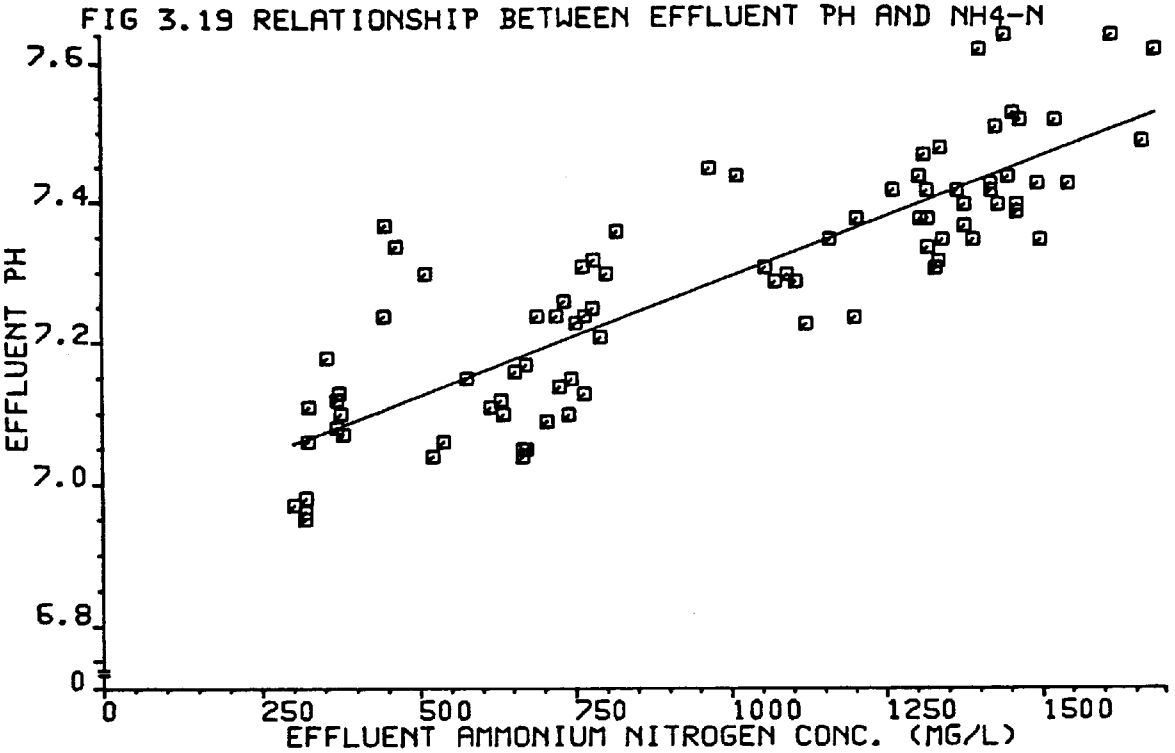
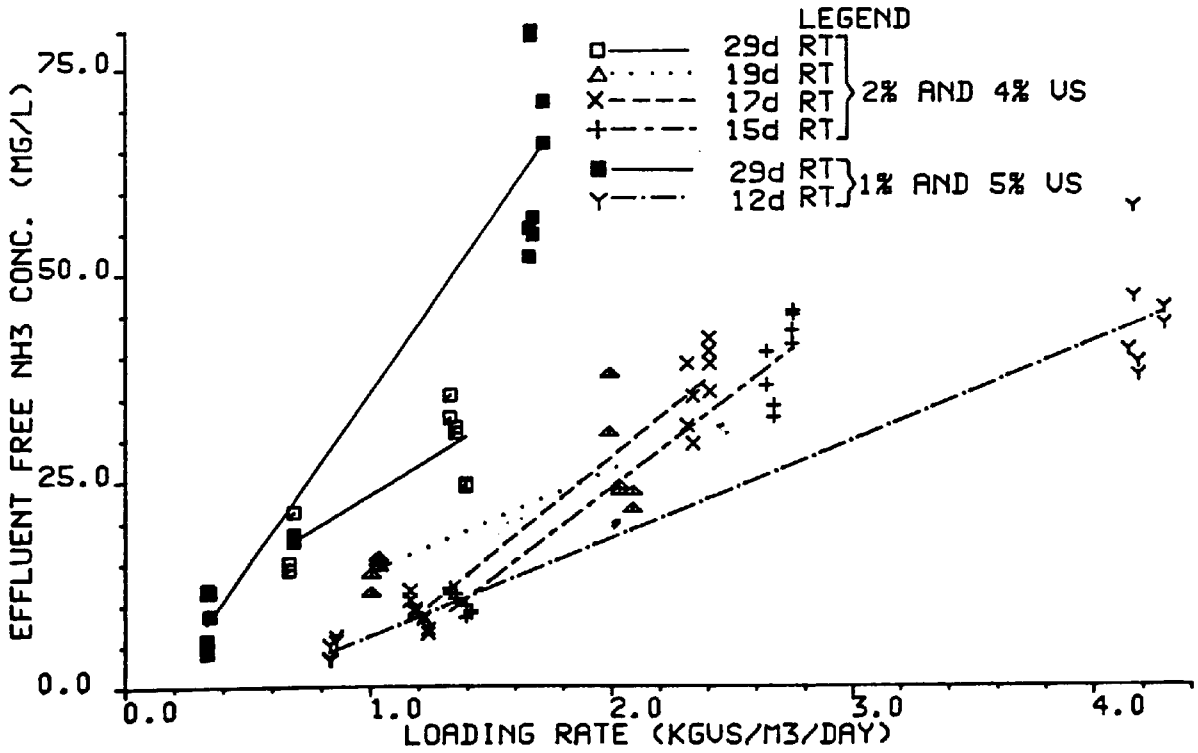


FIG 3.20 RELATIONSHIP BETWEEN FREE AMMONIA AND LR



The concentration of dissolved gaseous NH_3 can be calculated from the NH_4^+ - N concentration and the pH using the following formula derived from equilibrium constants (McCarty and McKinney, 1961a).

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

The free NH_3 concentrations in the litter digesters varied between 4.3 mgl^{-1} as N at 12d RT and 1% influent VS, and 64.5 mgl^{-1} at 29d RT and 5% influent VS (Tables 3.5 and 3.6).

The relationship between free NH_3 and LR is shown in Figure 3.20.

3.4.3.6 Sulphide

The mean pS value of 8% TS stock feed of 21.26 units is equivalent to less than 10^{-16} mgl^{-1} S^{2-}. During digestion pS values decreased sharply as sulphates and other sulphur containing compounds in the feed were reduced, to values between 6.37 and 8.03 units which represent 2.28×10^{-2} mgl^{-1} S^{2-}, and 4.98×10^{-4} mgl^{-1} S^{2-} respectively. These concentrations are lower than those reported by Mosey and Hughes (1975) for sewage digesters which had pS values of 9 - 10.

The concentration of S^{2-} in digesters is dependent primarily on the concentration of sulphur containing compounds in the influent and therefore influent solids concentration (Figures 3.10 and 3.11). However in the litter digesters at each RT the S^{2-} concentration increased by a greater proportion than the increase in influent VS. i.e. a doubling of influent VS caused more than a two fold increase in S^{2-}. This was possibly because digester S^{2-} levels are also influenced by digester pH which controls the equilibrium between the soluble and insoluble forms of sulphide. Thus:

$$H_2S \rightleftharpoons H^+ + HS^- \qquad HS^- \rightleftharpoons H^+ + S^{2-}$$

An increase in pH causes a decrease in gaseous H_2S and/or an increase in soluble sulphides including S^{2-}, and a decrease in pS. Since digester pH increased with increasing influent concentration (Figure 3.9) this may have produced an additional increase in S^{2-} to that caused by increasing influent VS.

It would be expected that at a constant influent concentration, an increase in RT would increase digester S^{2-} (decrease pS), since a longer period of digestion would allow a more complete reduction of sulphur containing compounds in the influent. However this was only observed at 2% influent VS, while at the other influent concentrations a slight decrease in S^{2-} was obtained. This was possibly due to the production of sulphides other than S^{2-} during digestion.

The S^{2-} concentrations of the digesters in the second group of experiments were too high to be compatible with those of the first group of experiments. This was presumably because the feed for the second group had a higher content of sulphur containing compounds than the first group. The S^{2-} concentration of the second group feed was not significantly higher than the first, but before digestion most of the sulphur is in the form of sulphates or sulphur containing proteins. Sulphate determinations however were not made routinely so a comparison cannot be made.

3.4.4 Digestion Efficiency

3.4.4.1 Gas Composition

The mean CH_4 content (59.2%) of the biogas produced during this investigation is comparable with the figures reported for batch digestion tests of turkey litter at 35°C (53-58% CH_4) by Hills (1982) and chicken litter at 30°C (64-65% CH_4) by Farag (1970). However this value (59.2%) is higher than the figures obtained in continuously fed litter digesters of 53% CH_4 at 35°C (Hawkes and Young, 1980) and 35-49% CH_4 at 60°C (Shih and Huang, 1980). However the decreased solubility of CO_2 at high temperatures could influence the values obtained in the latter study.

Additionally the GY's reported by Shih and Huang were also comparatively low indicating that efficient digestion was not occurring at 60°C, possibly due to overloading at the high LR's employed (7.9 - 23.3 kg VS m^{-3} day^{-1}). In this thermophilic study CH_4 composition increased both with increasing RT an influent concentration. In the present mesophilic investigation however, influent concentration had the same effect, but RT tended to reduce the methane content. This result is in agreement with Pfeffer (1980) who states that digestion at shorter RT's should produce a gas of higher CH_4 content since the liquid throughput rate controls the amount of CO_2 removed from the digester in the form of bicarbonate.

A relationship should exist between the bicarbonate alkalinity of the effluent, the pH and the CO_2 in the digester gas which depends on the dissociation of carbonic acid:

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

Thus an increase in bicarbonate alkalinity and or H^+ concentration shifts the equilibrium to the left and causes an increase in the CO_2 of the gas. At a constant CO_2 content an increase or decrease in bicarbonate alkalinity will be compensated for by the opposite change in H^+ concentration. Thus pH should increase with increasing alkalinity.

In the litter digesters due to the influence of NH_4^+ - N, both alkalinity and pH increased with increasing RT and influent concentration, so a constant gas composition would be expected. Only slight differences in composition were obtained but there was a tendency for the CO_2 content to increase with influent VS and RT.

3.4.4.2 Gas Yields

The mean biogas yields obtained varied between 0.245 and 0.373 m^3 kg VS added^{-1}, with a mean of 0.327 m^3 kg VS added^{-1}. The values are lower than the GY of 0.409 m^3 kg VS^{-1} reported previously for the digestion of poultry litter at 35°C (Hawkes and Young, 1980), but are considerably higher than those obtained in the thermophilic study of litter digestion

by Shih and Huang (1980) of 0.0398 - 0.0643 m^3 kg VS $added^{-1}$. These latter authors attributed the low GY's to the high lignin content (20.5%) of the litter which being non-digestible did not provide the carbon source for methanogenesis. However the low efficiency is probably also due to the RT's used in the investigation, (3-10 days) being too short for the degradation of cellulose which constitutes a large proportion of the volatile matter of litters. These short RT's combined with the high influent concentrations (3-9% VS) used may have resulted in overloading as stated previously.

Although the differences in GY's obtained at different operating conditions in the present study were small and not significant in all cases, definite trends did emerge concerning the relationship between GY and LR, namely that for constant influent concentration GY increased with increasing RT, and for constant RT GY increased with increasing influent concentration, (Figures 3.12 and 3.13). This confirms the relationships postulated by Hawkes and Horton (1981) for sewage digestion.

The first group of experiments were conducted at RT's of 15-29 days and influent concentrations of 2% and 4% VS, a doubling of both RT and influent VS. Such an increase in operating conditions for the digestion of sewage sludge would be expected to produce a 30% increase in GY, but for the digestion of poultry litter the increase was only 16%.

The second group of experiments was carried out at a wider range of operating conditions to attempt to reproduce the results of the first group, and obtain more significant differences between GY's. A wider range of GY's was obtained and the differences between GY's at different operating conditions were all significant. However the GY's produced in the second group were lower than expected considering the results from the first group. This apparent incompatability may have been due to differences in the composition of the influent between the two groups, caused by differences between batches of litter. During the stable periods the stock feed of the second group had lower concentrations of total nitrogen, NH_4^+ - N and alkalinity, but higher concentrations of

holocellulose (Table 3.4). Higher levels of poorly digestible material and lower levels of nitrogen may have resulted in the lower GY's of the second group of experiments, although the biodegradable content of the stock feed was not particularly low.

An increase in GY is obtained with increasing RT since a longer period of exposure to bacterial activity allows a greater degree of degradation and hence a higher gas production and yield. The increase in GY with increasing influent solids concentration is more difficult to explain, but has been noted previously for the digestion of cattle manure (Bousfield et al, 1979). These authors attributed the effect to low concentrations of ammonia (412 mgl^{-1} as N) causing less than optimum digestion. Thus the rate of digestion at low influent concentrations could be limited by ammonia levels. As the influent concentration increases, the rate of digestion and hence GY, increases to a point when the optimum concentration of ammonia is reached. In studies on the effect of ammonia levels on rumen fermentation however, the optimum concentration has been reported as less than 250 mgl^{-1} as N (Mehrez et al, 1977), lower than the lowest levels in both cattle and litter studies.

3.4.4.3 Destruction of Solids

The mean destruction figures of 13.9% of TS and 29.2% of VS obtained during the digestion of litter are lower than those reported by Farag (1979) at 30°C, of 23.3% of TS and 34.4% of VS. The low VS reduction figure of 18.4% at 60°C given by Shih and Huang (1980) reflects the low GY's obtained in that study. Solids destruction should be related to GY since gas is produced during the degradation of solid matter. In the present investigation this was the case for increasing RT and TS destruction at 1%, 2% and 5% influent VS, and for VS destruction at all influent concentrations. However an increase in solids destruction with increasing influent concentration was obtained only in the second group (at both RT's for TS, but only 29d RT for VS). It must be noted however that the correlation between GY and solids destruction may be difficult to detect since the variation in solids destruction produced by the slight

differences in GY's obtained may be obscured by the experimental errors incurred.

3.4.4.4 Cellulose

The holocellulose content of litter batches 2 and 3 (46.8% and 46.5% of TS) was similar to the figure of 50.26% of TS reported by Shih and Huang (1980), while the proportion of holocellulose constituted by α-cellulose was slightly lower in the present study (37.9% compared with 47.1%).

The destruction of holocellulose increased with increasing RT as expected, but not with increasing influent concentration. The elevated GY's at high influent concentrations therefore, were not the result of a greater degree of holocellulose degradation. The α-cellulose content of the holocellulose increased from 37.9% to 52.6% during digestion which indicates that hemicellulose was digested more readily than α-cellulose. This was probably due to the fact that α-cellulose is always associated with lignin or polysaccharides which protect it against bacterial attack.

3.4.4.5 Biological Oxygen Demand

The mean BOD of 8% TS litter of 6392 mgl^{-1} O_2 is equivalent to 7990 mgl^{-1} at 10% TS which is lower than the value of 10,350 mgl^{-1} given by Laak (1970) for broilers and laying hens.

As with solids destruction the removal of BOD should be directly related to GY. In this investigation the expected increase in BOD destruction with RT was observed only in the second group of experiments, and with increasing influent concentration for 29d and 19d RT from 2% to 4% influent VS. Accurate measurements of BOD are difficult to obtain. The BOD apparatus employed was sensitive to temperature fluctuations and often subject to leaks, both leading to errors. Since the BOD test is primarily used for waste waters of relatively low BOD, samples of fresh and digested litter had to be greatly diluted to adjust the BOD to that of wastewaters.

These high dilutions inevitably led to a magnification of errors incurred in sample preparation. Additionally since the BOD is a lengthy test and was not always successful, only a small number of determinations could be made during each experiment. It was not always possible therefore, to obtain accurate measurements of BOD reduction, or to demonstrate the relatively small differences in reduction between different sets of operating conditions.

3.5 Conclusions

1. The stable digestion of poultry litter of mean VS content of 61.3% of TS was achieved at 35°C using 51 digesters operated at RT's between 12 and 29 days and influent concentrations of 1.55% - 7.76% TS (0.98 - 4.90% VS) producing LR's of between 0.34 and 4.20 kg VS m^{-3} d^{-1}.

2. Feeding the digesters once daily on the first four days of the week, a double amount on the 5th day, and none on the 6th or 7th days, generated reproducible oscillations in gas production during the course of the week.

3. Biogas yields of 0.245 - 0.372 m^3 kg VS $added^{-1}$ were obtained, with a mean of 0.327 m^3 kg VS $added^{-1}$. GY's increased with increasing RT and influent concentration, although the differences between GY's produced under different conditions were not always significant.

4. Reductions of TS, VS and BOD were on average 13.9%, 29.2%, and 71.5% respectively, but did not correlate well with GY.

5. The mean CH_4 content of the gas produced was 59.2%. The remainder was composed mainly of CO_2 with less than 1% H_2S. The CH_4 content tended to decrease with increasing RT and influent concentration.

6. Digester pH varied between 7.04 and 7.55, and alkalinity between 2779 mgl^{-1} and 12,730 mgl^{-1} as $CaCO_3$. Both were influenced by digester NH_4^+ - N concentration and could be predicted from this.

7. Digester NH_4^+ - N concentration ranged from 342 mgl^{-1} to 1502 mgl^{-1} as N. Since digester pH increased with increasing NH_4^+ - N concentration, the proportion of the latter in the free NH_3 form increased also to a maximum of 60 mgl^{-1} as N.

CHAPTER 4

THE SEMI-CONTINUOUS ANAEROBIC DIGESTION OF POULTRY MANURE

4.1 Introduction

The potential of AD for the treatment of poultry manure has been investigated by a number of workers in batch and continuously fed digesters at both laboratory and large scale, and at mesophilic and thermophilic temperatures. Batch digestion tests include those reported by Farag (1970) at 30°C, Hassan et al (1975a) at 25.5°C, Hassan et al (1975b) at 30°C, and by Badger et al (1979) at 37°C in a comparison of the digestibility of animal wastes with crops. Adderley et al (1976) investigated the possibility of separating the fermentative and methanogenic stages of poultry manure batch digestion using different temperatures for each stage, while Yang and Chan (1977) reported the replacement of the initial hydrolysis step by acid and heat treatment before digestion at 30° - 33°C.

The digestion of poultry manure in daily fed laboratory digesters has been compared with cattle and piggery wastes by Hart (1963) at temperatures of 23° and 35°C, and by Gramms et al (1971) at 32.5°C, while Aubart and Fauchille (1983) have examined the effects of influent concentration and RT on gas production and COD reduction at 37°C. In the thermophilic range, Huang and Shih (1981) have reported an optimum temperature of 50°C for poultry manure digestion in laboratory scale daily fed digesters, while Savery and Cruzan (1972) obtained efficient digestion at 52°C in a 35 l farm digester operated in batch mode, but retarded efficiency when operated continuously.

On a larger scale a 150 l pilot digester has been operated successfully on poultry manure at 35°C at the Rowett Research Institute, Aberdeen (Hobson et al 1980), and a 3.5 m^3 plant on a poultry farm has been used by Morrison et al (1980) to investigate the effects of LR and RT on the efficiency of the process. Other reports of digesters which have been operated successfully on poultry farms include those by Patel and Patel (1971) who report on a low technology digester in India operated at ambient temperature, and by Dugan et al (1972) who describe a pilot digester which

formed part of an integrated waste management system in the USA. The operation of full scale poultry manure digesters has been documented by Rockey et al (1978), and Converse et al (1980) who carried out a detailed investigation of a 97 m^3 digester at 35°C.

The aims of the poultry manure digestion experiments in this chapter were similar to those of the litter experiments in the preceding chapter in that information was sought on manure digestion as well as GY - LR relationships. In some of the investigations detailed above (for example Gramms et al 1971, Hassan et al 1975a, b, Hobson et al, 1980) the efficiency of manure digestion was reduced at high LR's which was assumed to be due to inhibition by high concentrations of NH_4^+ - N caused by the high nitrogen content of poultry manure. Thus it seemed probable that ammonia inhibition would occur in the present study which would modify the GY - LR relationships.

4.2 Materials and Methods

4.2.1 Digester Operation

The semi-continuously fed digesters used in the litter experiments were used for the digestion of manure and operated in the same manner. Mixed effluent from the litter digesters provided the seed. The experiments were in three groups of 3 or 4 pairs of digesters. In the first two groups digesters were operated at a RT of 29 days and influent concentrations between 0.72% and 7.20% VS, giving LR's of 0.25 - 2.47 kg VS m^{-3} d^{-1} (Table 4.1). In the third group the RT was 15 days and influent concentrations of 0.68% - 6.84% VS, with LR's of 0.47 - 4.69 kg VS m^{-3} d^{-1}. As with the experiments in Chapter 3, RT's and influent concentrations are rounded to the nearest whole number in the text. The frequency of analyses made on the performance of each digester and the composition of the influent and effluent is shown in Table 4.2.

Table 4.1 Operating Conditions For Group 1

Group	1											
Digester no.	1,2			3,4			5,6			7,8		
Retention time (days)	29.17			29.17			29.17			29.17		
Influent TS (%)	1.00	0.00	9	3.99	0.02	9	6.99	0.03	9	9.98	.047	9
Influent VS (%)	0.72	0.02	9	2.88	0.07	9	5.04	0.13	9	7.20	0.18	9
Loading rate (kg VS m^{-3} d^{-1}).	0.26	0.01	9	0.99	0.03	9	1.73	0.04	9	2.47	0.06	9
Duration of experiment (days)	161			161			161			161		
Period of stable operation (days)	70			70			70			70		
Manure batch while stable	1			1			1			1		

Values are mean, standard deviation, no. of measurements.

Table 4.1 continued

Group	2								
Digester no.	9,10			11,12			13,14		
Retention time (days)	29.17			29.17			29.17		
Influent TS (%)	2.00	0.00	8	4.00	0.00	8	6.00	0.00	8
Influent VS (%)	1.31	0.41	8	2.62	0.08	8	3.93	0.12	8
Loading rate (kg VS m^{-3} d^{-1})	0.45	0.01	8	0.90	0.02	8	1.35	0.04	8
Duration of experiment (days)	140			140			140		
Period of stable operation (days)	63			63			63		
Manure batch while stable	2			2			2		

Values are mean, standard deviation, no. of measurements.

Table 4.1 continued

Group	3								
Digester no.	15,16			17,18			19,20		
Retention time (days)	14.58			14.58			14.58		
Influent TS (%)	1.00	0.01	7	5.98	0.04	7	9.96	0.07	7
Influent VS (%)	0.68	0.02	7	4.10	0.12	7	6.84	0.20	7
Loading rate (kg VS m^{-3} d^{-1})	0.47	0.01	7	2.81	0.08	7	4.69	0.14	7
Duration of experiment (days)	98			98			98		
Period of stable operation (days)	56			56			56		
Manure batch while stable	3			3			3		

Values are mean, standard deviation, no. of measurements.

Table 4.2 Frequency of Analyses

Frequency

Analysis	5 times per week	3 times per week	Weekly	Once per 1-2 weeks	Once per 2 weeks	Once per 2-3 weeks	Once per 3 weeks	Infrequently
Gas production	1,2,3							
Gas composition		1,2,3						
TS and VS			1,2,3					
pH			2	1	3			
NH_4^+ - N			2	3		1		
Alkalinity						1,2		
Cellulose							1	
Nitrogen							1	
BOD								1

1 = measurement made on 1st group digesters

2 = measurement made on 2nd group digesters

3 = measurement made on 3rd group digesters

4.3 Results

4.3.1 Digester Operation

Digesters operated on poultry manure showed similar characteristics to those operated on litter in that consistent differences in gas production occurred between paired digesters, and daily oscillations in gas production were produced which were repeated each week.

4.3.1.1 Comparison of paired digesters

In Figure 4.1 a plot of weekly gas production, digester 8 consistently produced higher gas volumes than digester 7, and digester 4 greater volumes than digester 3. Digesters 7 and 8 were both operated at 29d RT and 7.2% influent VS and during the period of stable operation produced mean weekly gas volumes of 31.201 and 32.991 respectively, a difference which is significant at the 5% level. Digesters 3 and 4 were operated at 29d RT and 2.9% influent VS and during the stable period produced mean weekly gas volumes of 13.591 and 15.051 respectively, which is significant at the 1% level.

4.3.1.2 Daily oscillations in gas production

The oscillations in gas production during the week are shown in Figure 4.2 for digesters 10, 12 and 14 over weeks 12 to 17 of operation. These digesters were all operated at 29d RT and influent concentrations of 2%, 4% and 6% TS. The minimum gas production was measured on Tuesday but increased progressively during the week to a maximum on Friday. The mean daily gas production over the weekend and recorded on Monday was frequently the second lowest of the week.

4.3.1.3 Digester stabilisation

The stabilisation of digester 7 after start up is illustrated in Figure 4.3. This digester was filled with seed of approx 3% TS from litter

FIG 4.1 COMPARISON OF PAIRED DIGESTERS

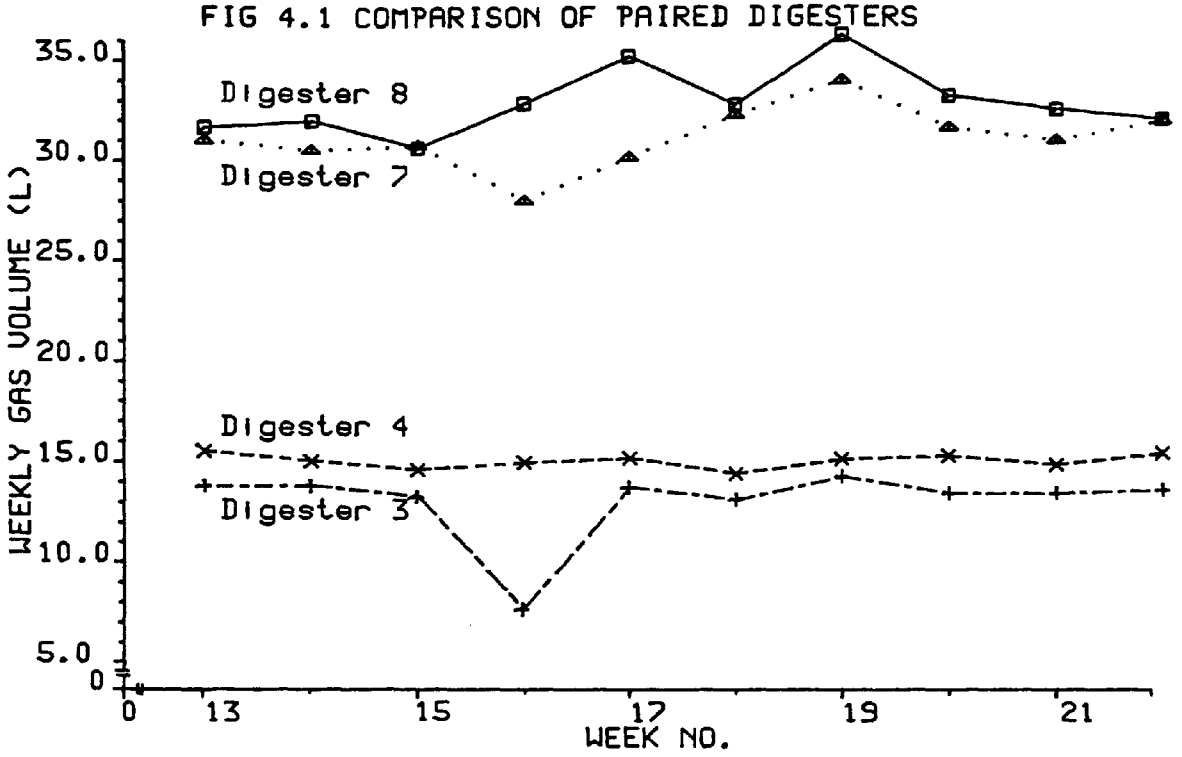
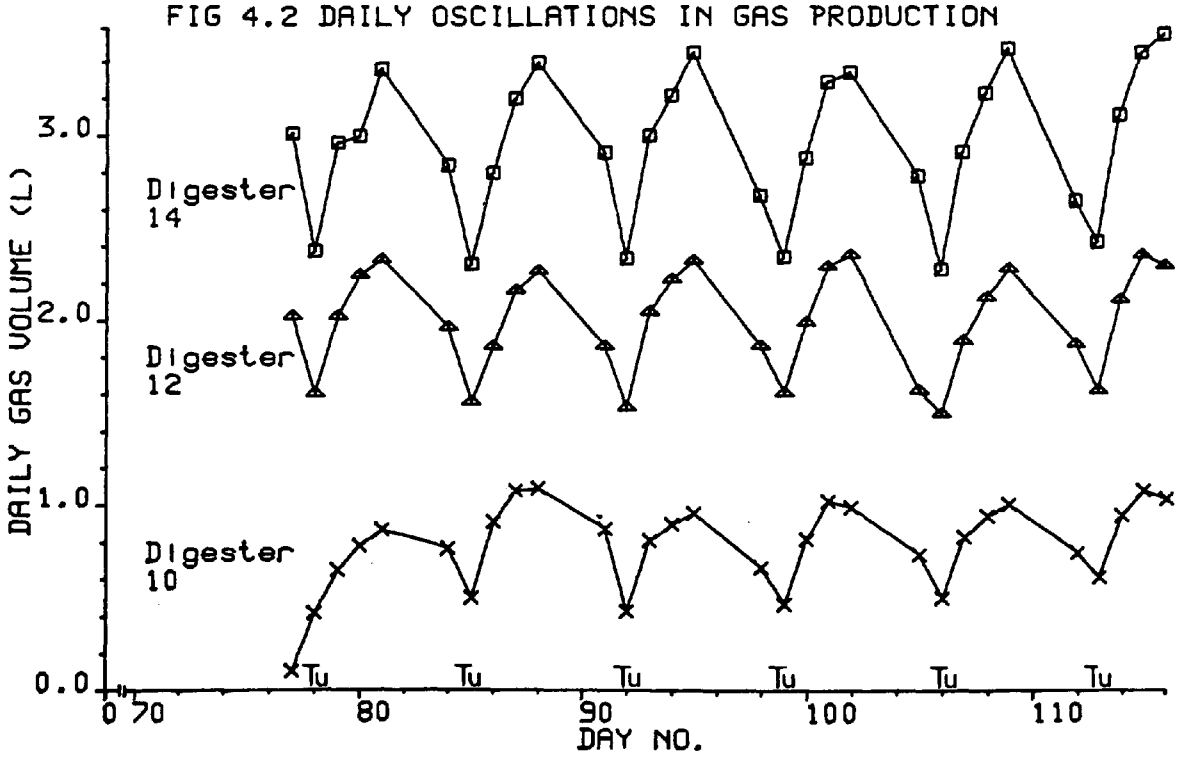


FIG 4.2 DAILY OSCILLATIONS IN GAS PRODUCTION



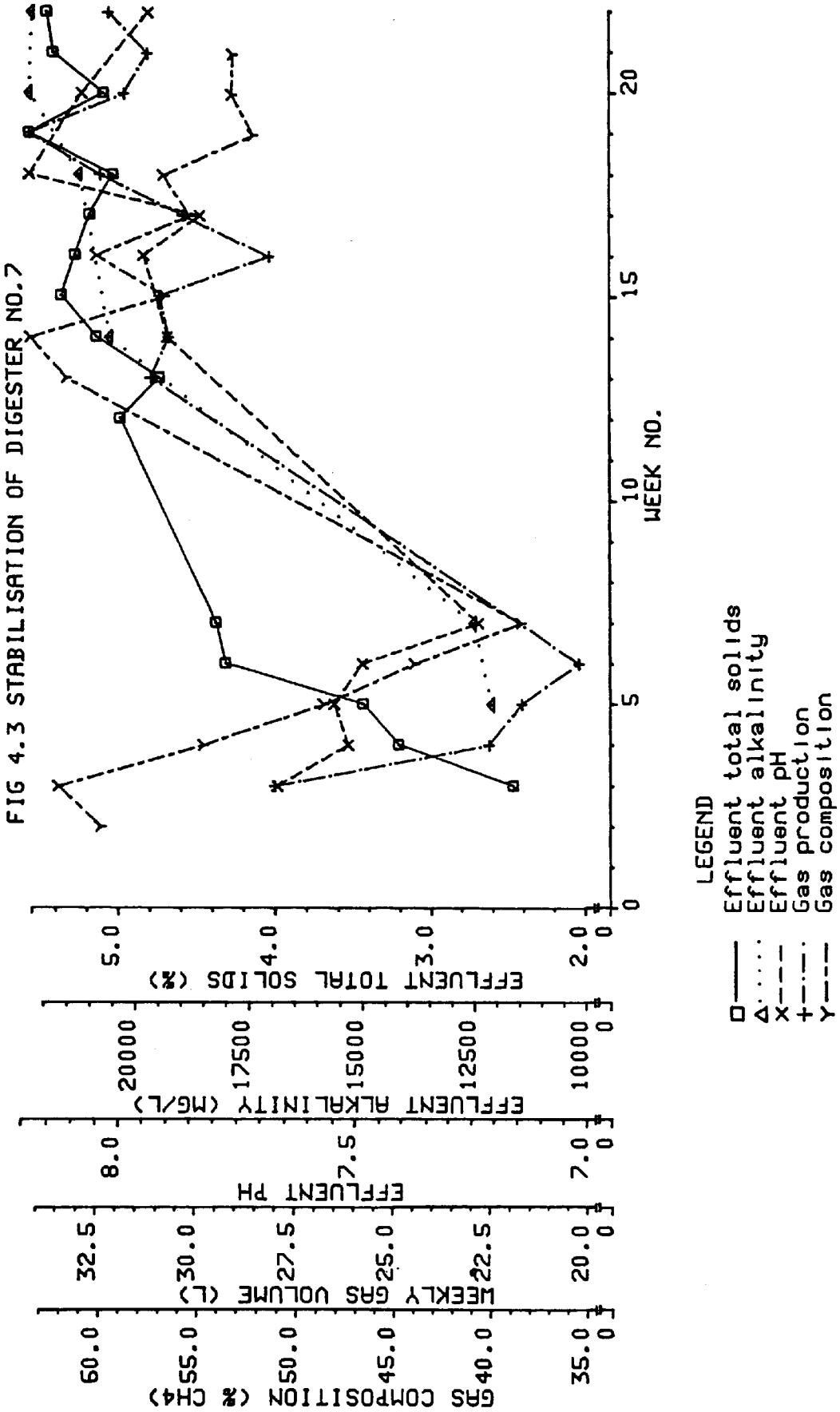


FIG 4.3 STABILISATION OF DIGESTER NO.7
WEEK NO.
0
5
10
15
20
EFFLUENT TOTAL SOLIDS (%)
2.0
3.0
4.0
5.0
EFFLUENT ALKALINITY (MG/L)
10000
12500
15000
17500
20000
EFFLUENT PH
7.0
7.5
8.0
WEEKLY GAS VOLUME (L)
20.0
22.5
25.0
27.5
30.0
32.5
GAS COMPOSITION (% CH4)
35.0
40.0
45.0
50.0
55.0
60.0
LEGEND
Effluent total solids
Effluent alkalinity
Effluent pH
Gas production
Gas composition

digesters and fed with 10% TS (7.2% VS) manure at 29d RT. There was a break in feeding from weeks 8 - 11 inclusive during which no measurements were taken, but the temperature was maintained at 35°C.

Effluent TS and alkalinity increased rapidly during the first 14 weeks of operation, while pH, weekly gas production and CH_4 content of the gas decreased in the first 7 weeks before regaining high levels after the break. Gas production and pH remained stable but the gas composition decreased towards the end of the experiment.

4.3.2 Analyses

The composition of the influent for each pair of digesters during the stable periods is shown in Table 4.3, and for the effluent from each pair in Table 4.4.

4.3.2.1 Solids determinations

Three batches of manure collected from the same source were used in this investigation. Table 4.5 shows the TS and VS content of the fresh manure and the date of collection.

Table 4.5 Solids composition of manure batches

Batch	Date of collection	TS (%)	VS (% of TS)	VS%
1	14.10.81	38.59	70.59	27.24
2	14.5.82	47.29	60.00	28.37
3	27.10.82	29.40	63.31	18.61

Since the stock feed was always made up to a constant TS content (10%), variations in TS of different batches were eliminated, but no compensation was made for variations in VS. This led to relatively wide variations in influent VS between batches of manure at constant TS. For clarity therefore,

Table 4.3 Influent Characteristics For Group 1

Group	1											
Digester no.	1,2			3,4			5,6			7,8		
Total solids (%)	1.00	0	9	3.99	0.02	9	6.99	0.03	9	9.98	0.05	9
Volatile solids (% of TS)	72.07	1.92	9	72.07	1.92	9	72.07	1.92	9	72.07	1.92	9
Volatile solids (%)	0.72	0.02	9	2.88	0.07	9	5.04	0.13	9	7.20	0.18	9
Total nitrogen (mgl^{-1})	542	56	3	2166	225	3	3791	393	3	5415	562	3
Ammonium nitrogen (mgl^{-1} as N)	214	139	4	865	555	4	2141	971	4	2141	1388	4
Total alkalinity (mgl^{-1})	981	478	4	3926	1910	4	6870	3343	4	9814	4776	4
pH		-			-			-		7.37	0.53	6
Holocellulose (%)	0.35	0.01	3	1.41	0.04	3	2.47	0.02	3	3.53	0.09	3
BOD (mg O_2 l^{-1})	1267	99	3	5067	394	3	8867	690	3	12667	986	3

See legend to table 4.1

Table 4.3 continued

Group	2								
Digester no.	9,10			11,12			13,14		
Total solids (%)	4.00	0	8	6.00	0.01	8	6.00	0.01	7
Volatile solids (% of TS)	65.57	2.05	8	65.57	2.05	8	65.57	2.05	7
Volatile solids (%)	1.31	0.41	8	2.62	0.08	8	3.93	0.12	7
Total nitrogen (mgl^{-1})	1344	0	1	2691	0	1	4035	0	1
Ammonium nitrogen (mgl^{-1} as N)	986	95	8	1974	191	8	2960	287	8
Total alkalinity (mgl^{-1})	5342	568	3	10700	1138	3	16042	1706	3
pH	7.59	0.08	4	7.55	0.06	4	7.84	0.05	8
Holocellulose (%)		-			-			-	
BOD (mg O_2 l^{-1})		-			-			-	

See legend to table 4.1

Table 4.3 continued

Group	3								
Digester no.	15,16			17,18			19,20		
Total solids (%)	1.00	0.01	7	5.98	0.04	7	9.96	0.07	7
Volatile solids (% of TS)	68.76	2.36	7	68.76	2.36	7	68.76	2.36	7
Volatile solids (%)	0.68	0.02	7	4.10	0.12	7	6.84	0.20	7
Total nitrogen (mgl^{-1})	552	0	1	3309	0	1	5515	0	1
Ammonium nitrogen (mgl^{-1} as N)	167	20	4	999	118	4	1666	197	4
Total alkalinity (mgl^{-1})	-			-			-		
pH	-			-			7.45	0	1
Holocellulose (%)	-			-			-		
BOD (mg O_2 l^{-1})	-			-			-		

See legend to table 4.1

Table 4.4 Effluent Characteristics For Group 1

Group	1											
Digester no.	1,2			3,4			5,6			7,8		
Total solids (%)	0.44	0.07	18	1.73	0.08	18	3.79	0.25	18	5.33	0.27	18
Volatile solids (% of TS)	55.30	3.32	18	55.88	1.23	18	57.75	2.09	18	61.44	2.20	18
Volatile solids (%)	0.25	0.04	18	0.96	0.05	18	2.19	0.18	18	3.28	0.23	18
Total nitrogen (mgl^{-1})	692	91	6	2342	47	6	3995	274	6	5678	127	6
Ammonium nitrogen (mgl^{-1} as N)	465	34	8	1749	101	8	2880	150	8	4122	219	8
Free ammonia (mgl^{-1} as N)	8.0	1.7	8	86	23	8	225	66	8	435	132	8
Total alkalinity (mgl^{-1})	2362	79	8	9056	132	8	15317	946	8	21763	641	8
pH	7.18	0.09	12	7.64	0.09	12	7.80	0.13	12	7.97	0.12	12
Holocellulose (%)	0.10	0.20	6	0.55	0.06	6	1.17	0.12	6	1.74	0.09	5
BOD (mgl^{-1})	289	70	3	1085	153	2	2418	111	2	2630	0	1

See legend to table 4.1

Table 4.4 continued

Group	2								
Digester no.	9,10			11,12			13,14		
Total solids (%)	0.96	0.08	16	2.15	0.14	16	3.26	0.14	16
Volatile solids (% of TS)	46.95	1.39	16	47.88	0.90	16	51.47	0.72	16
Volatile solids (%)	0.45	0.04	16	1.03	0.07	16	1.68	0.08	16
Total nitrogen (mgl^{-1})	-			-			-		
Ammonium nitrogen (mgl^{-1} as N)	1135	76	16	2298	88	16	3325	115	16
Free ammonia (mgl^{-1} as N)	34	4.0	15	124	13	16	260	28	16
Total alkalinity (mgl^{-1})	7128	745	6	14313	967	6	21167	416	6
pH	7.42	0.03	15	7.68	0.04	16	7.84	0.04	16
Holocellulose (%)	-			-			-		
BOD (mgl^{-1})	-			-			-		

See legend to table 4.1

Table 4.4 continued

Group	3								
Digester no.	15,16			17,18			19,20		
Total solids (%)	0.49	0.08	12	3.30	0.24	12	5.54	0.40	12
Volatile solids (% of TS)	60.47	2.32	12	62.05	1.73	12	62.57	2.54	12
Volatile solids (%)	0.29	0.05	12	2.05	0.18	12	3.47	0.28	12
Total nitrogen (mgl^{-1})		-			-			-	
Ammonium nitrogen (mgl^{-1} as N)	502	30	7	2727	206	9	4274	133	8
Free ammonia (mgl^{-1} as N)	7.0	0.1	4	138	17	6	291	53	6
Total alkalinity (mgl^{-1})		-			-			-	
pH	7.09	0.04	6	7.65	0.08	6	7.78	0.08	6
Holocellulose (%)		-			-			-	
BOD (mgl^{-1})		-			-			-	

See legend to table 4.1

influent concentration will be expressed in terms of TS rather than VS.

The TS content of the effluent varied between 0.44% at 29d RT and 1% influent TS, and 5.54% at 15d RT and 10% influent TS (Table 4.4). The second group of experiments was carried out at the same RT as the first, repeating 4% influent TS to test reproducibility of results, as well as two new influent concentrations (2% and 6% TS). The TS of the effluent from the 4% influent TS digesters was higher for the second group of experiments than the first (2.15% compared with 1.73%) so the results do not appear to be compatible. This is demonstrated in Figure 4.4 which shows the relationship between effluent TS and LR. Regression lines connect results from each group of experiments. The regression lines for the first and second group are quite distinct and not coincident as would be expected from varying the influent concentration at the same RT. At the same LR therefore the effluent TS was higher for the second group than the first.

Since the solids concentration of a digester influences the concentration of other components such as NH_4^+ - N, alkalinity and H^+, these were also higher in the second than first group of experiments at the same LR, and therefore a distinct regression line was produced by each group in Figures 4.7, 4.8 and 4.9.

In the third group the effluent TS was slightly higher than that of the other two groups at the same influent concentrations. For all groups effluent TS increased with increasing LR, and was therefore dependent on influent concentration.

The proportion of TS in the effluent which was volatile was higher for the third group (mean 61.70%) than the first (mean 57.59%) which in turn was higher than that of the second group of experiments (mean 48.77%) (Table 4.4). The relationship between effluent VS and LR is shown in Figure 4.5,At 29d RT effluent VS varied from 0.24% at 1% influent TS to 3.28% at 10% influent TS. At these influent concentrations the effluent VS was higher at 15d RT, with 0.29% at 1% influent TS, and 3.47% at 10% influent TS.

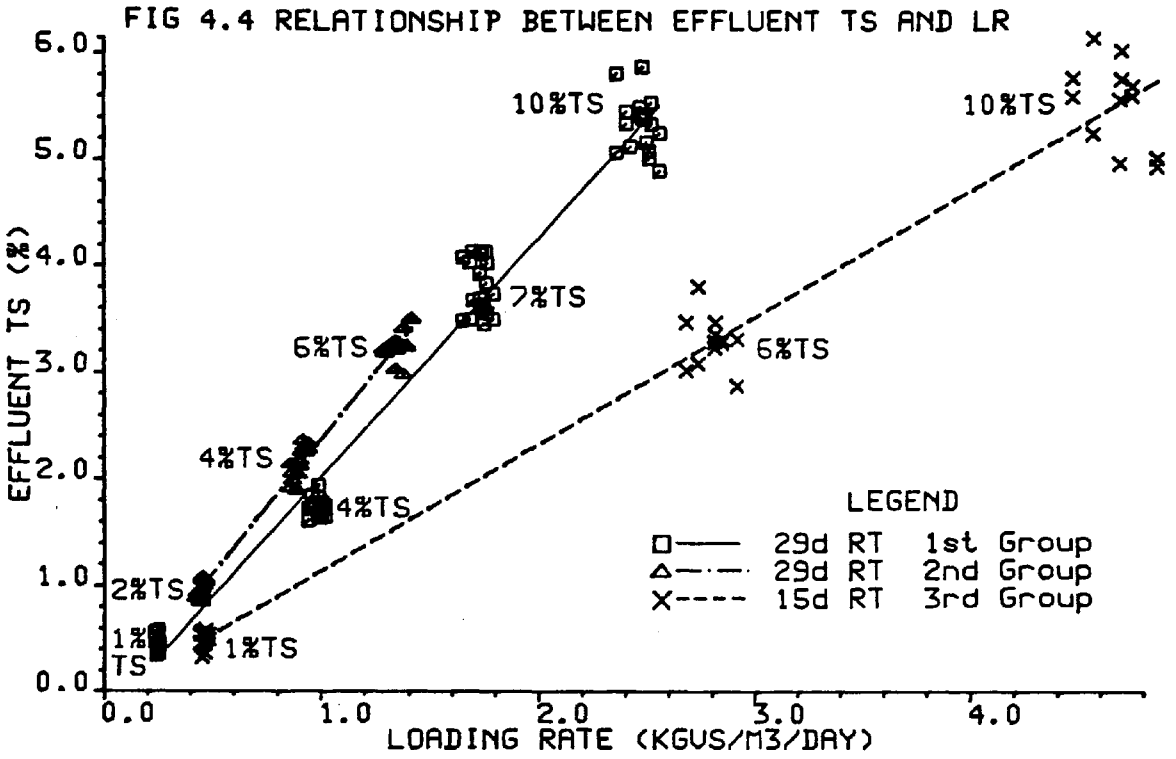


FIG 4.4 RELATIONSHIP BETWEEN EFFLUENT TS AND LR
EFFLUENT TS (%)
LOADING RATE (KGVS/M3/DAY)
10%TS
10%TS
7%TS
6%TS
6%TS
4%TS
4%TS
2%TS
1%TS
1%TS
LEGEND
29d RT 1st Group
29d RT 2nd Group
15d RT 3rd Group

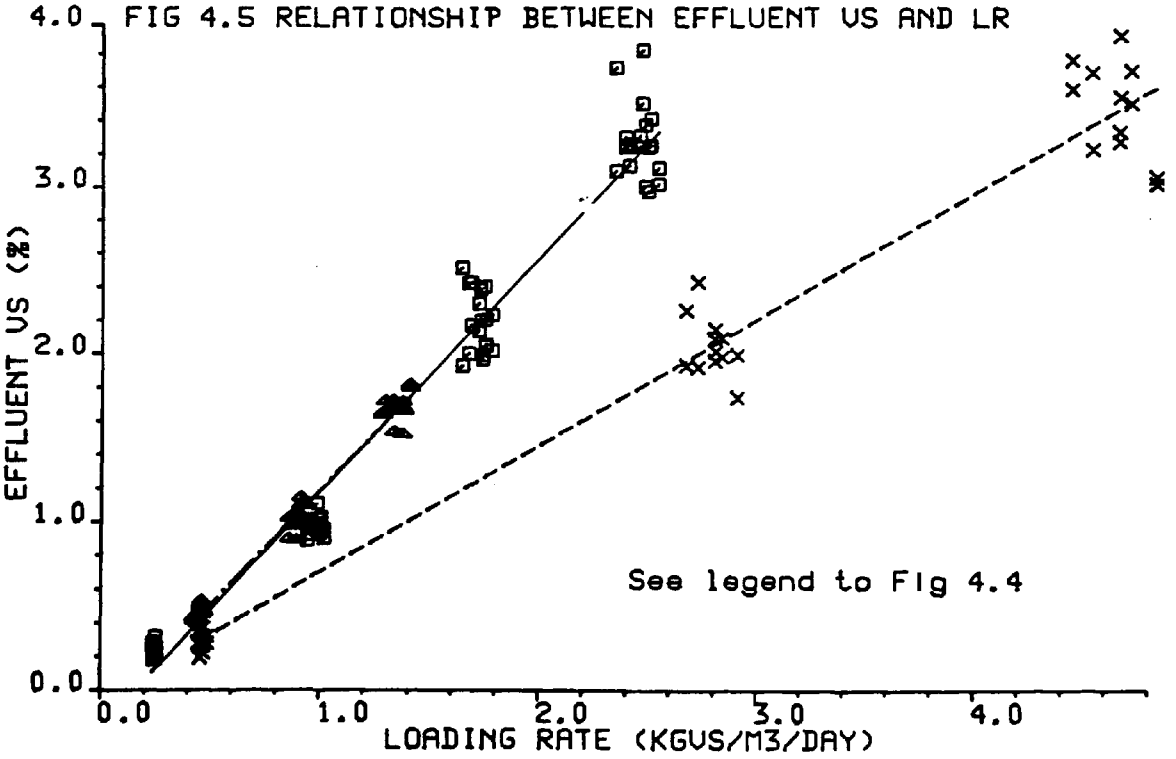


FIG 4.5 RELATIONSHIP BETWEEN EFFLUENT VS AND LR
EFFLUENT VS (%)
LOADING RATE (KGVS/M3/DAY)
See legend to Fig 4.4

4.3.2.2 Total nitrogen

The mean total nitrogen content of the 3 batches of manure was 5.52% 6.73% and 5.58% of the dry weight respectively, with overall mean of 5.94%. The high nitrogen content of the second batch led to a high influent nitrogen concentration for the second group of experiments. (Table 4.3).

Nitrogen was determined in the effluent of the first group of experiments only. It was dependent on LR and increased from 692 mgl^{-1} at 1% influent TS to 5678 mgl^{-1} at 10% influent TS (Figure 4.6).

4.3.2.3 Ammonium nitrogen

The NH_4^+ - N content of the manure varied widely between batches. In 10% TS stock feed the mean NH_4^+ - N concentration for the 3 batches was 2152 mgl^{-1}, 4511 mgl^{-1} and 1673 mgl^{-1} respectively, with an overall mean of 2779 mgl^{-1}, which is equivalent to 2.78% of the dry weight. This figure represents 45.4% of the total nitrogen.

The concentration of NH_4^+ - N in the effluent varied from 465 mgl^{-1} at 29d RT and 1% influent TS to 4274 mgl^{-1} at 15d RT and 10% influent TS (Table 4.4). The relationship between effluent NH_4^+ - N and LR is shown in Figure 4.7. For each group of experiments NH_4^+ - N increased with influent concentration. At the same influent concentration effluent NH_4^+ - N was higher for the second than the first group, which in turn was slightly lower than that of the third group. NH_4^+ - N constituted 71.7% of the total nitrogen in the effluent.

4.3.2.4 Total alkalinity

The alkalinity of the effluent varied between 2362 mgl^{-1} at 1% influent TS, and 21,763 mgl^{-1} at 10% influent TS (Table 4.4) the relationship between alkalinity and LR is presented in Figure 4.8. In both groups of experiments alkalinity was dependent on LR, but at the same influent

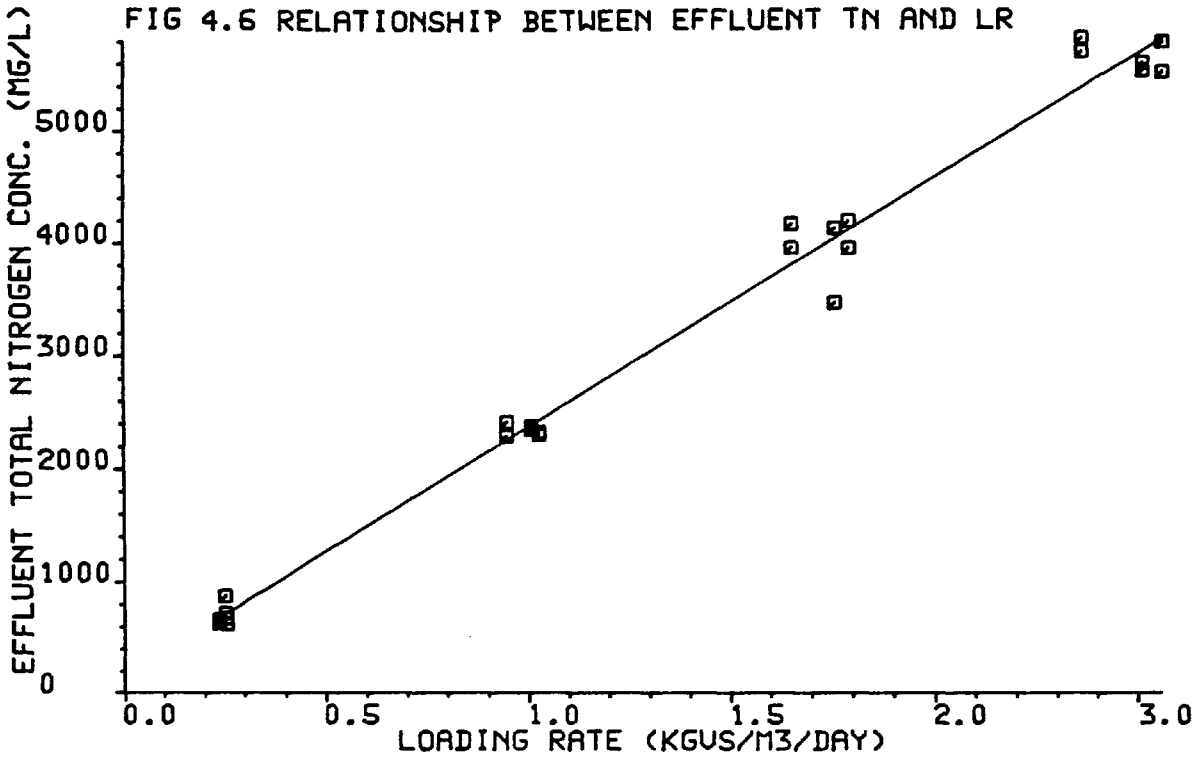


FIG 4.6 RELATIONSHIP BETWEEN EFFLUENT TN AND LR
EFFLUENT TOTAL NITROGEN CONC. (MG/L)
0
1000
2000
3000
4000
5000
0.0
0.5
1.0
1.5
2.0
3.0
LOADING RATE (KGVS/M3/DAY)

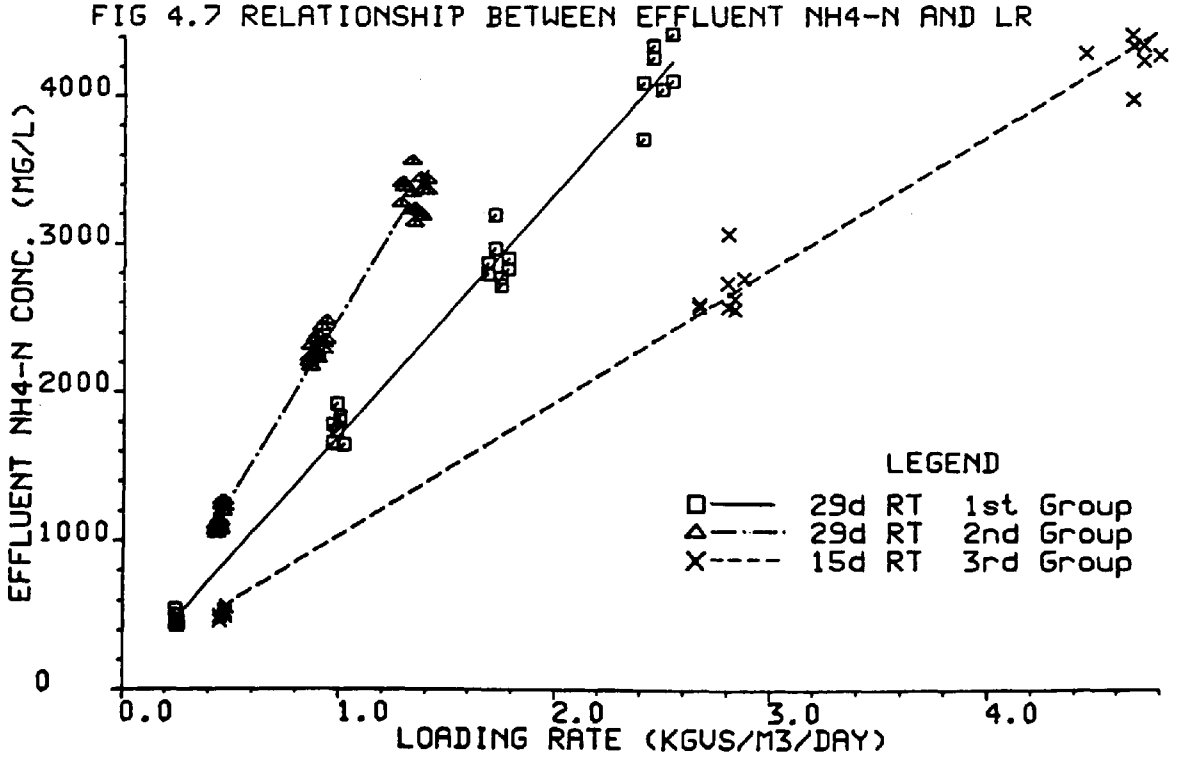


FIG 4.7 RELATIONSHIP BETWEEN EFFLUENT NH4-N AND LR
EFFLUENT NH4-N CONC. (MG/L)
0
1000
2000
3000
4000
0.0
1.0
2.0
3.0
4.0
LOADING RATE (KGVS/M3/DAY)
LEGEND
29d RT 1st Group
29d RT 2nd Group
15d RT 3rd Group

FIG 4.8 RELATIONSHIP BETWEEN EFFLUENT ALKALINITY AND LR

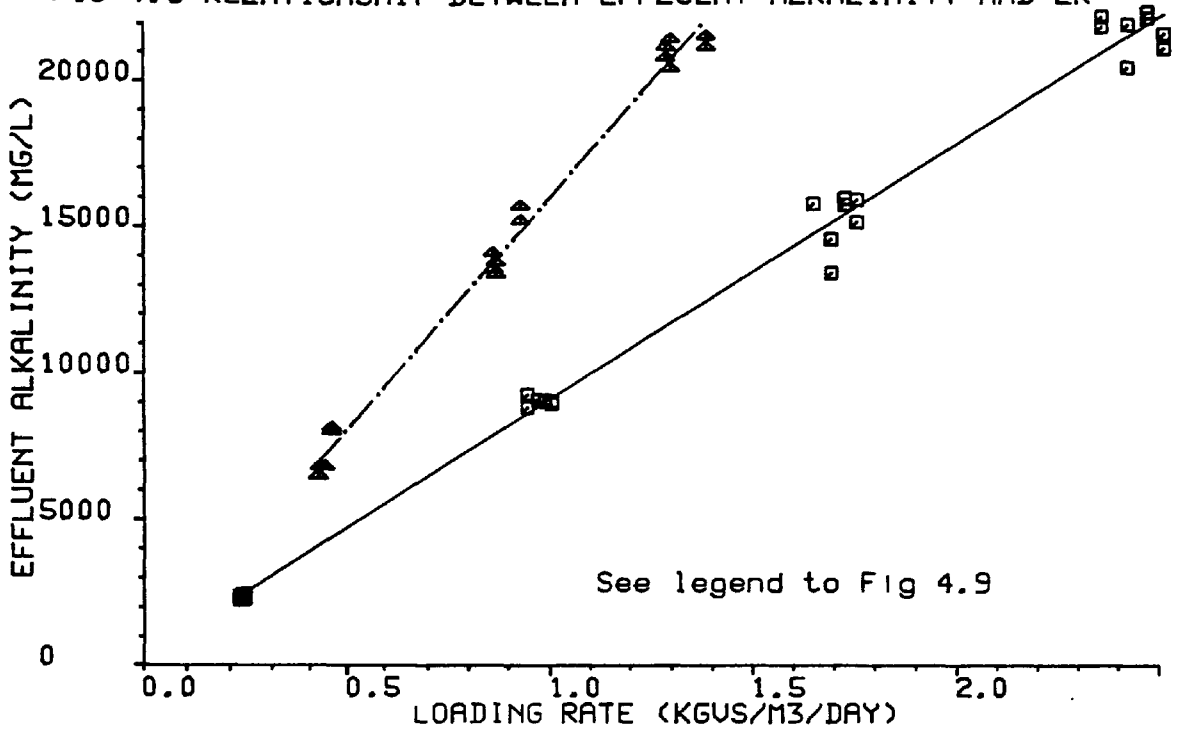
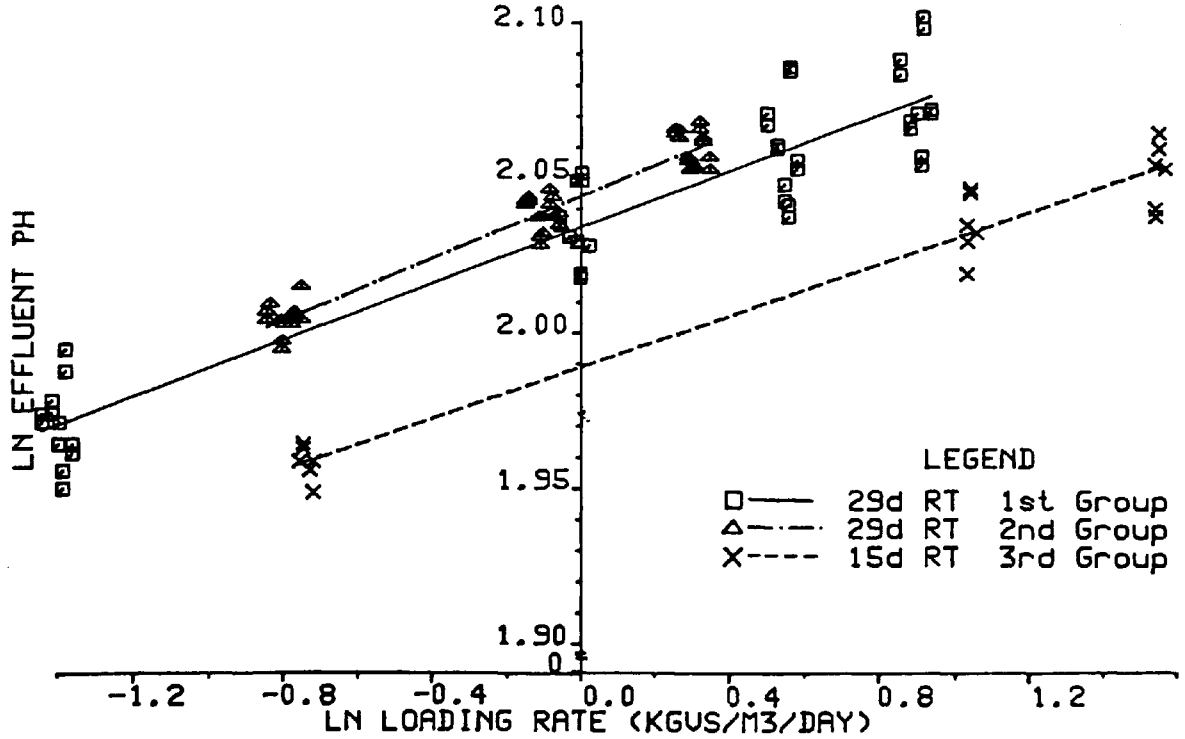


FIG 4.9 RELATIONSHIP BETWEEN EFFLUENT pH AND LR



concentration alkalinity was higher for the second than the first group.

4.3.2.5 pH

The mean pH of the stock feed made from the 3 batches of manure was 7.19, 7.51 and 7.31 respectively, with overall mean of 7.34.

Effluent pH varied from 7.09 at 15d RT and 1% influent TS to 7.97 at 29d RT and 10% influent TS (Table 4.4). A linear relationship between pH and LR is obtained in a log-log plot (Figure 4.9). Effluent pH was dependent on LR, but as with the other parameters measured the second group of experiments gave higher values than the first at the same influent concentration.

Shortening the RT from 29 to 15 days reduced the pH, thus at 10% influent TS the pH decreased from 7.97 to 7.78, and at 1% influent TS from 7.18 to 7.09.

4.3.3 Digestion Efficiency

The efficiency of digestion for all experiments is shown in Table 4.6.

4.3.3.1 Gas composition

The mean proportion of CH_4 in the biogas varied by only 5.5% from 56.0% at 29d RT and 10% influent TS, to 61.5% at 15d RT and 1% influent TS, with an overall mean of 58.6% CH_4. The difference was made up by CO_2 with less than 1% H_2S present.

The relationship between gas composition and LR (Figure 4.10) shows that increasing the influent concentration reduced the proportion of CH_4 in the biogas. The second group of experiments produced similar results to the first, but reducing the RT to 15 days increased the CH_4 composition.

Table 4.6 Digestion Efficiency For Group 1

Group	1											
Digester no.	1,2			3,4			5,6			7,8		
Biogas yield (m^3 kg VS added^{-1})	0.370	0.017	18	0.416	0.025	17	0.380	0.017	13	0.376	0.017	13
Gas composition (% CH_4)	60.21	2.66	41	56.47	3.41	43	56.64	6.17	44	56.04	7.49	42
Methane yield (m^3 kg VS added^{-1})	0.221	0.014	16	0.228	0.034	16	0.211	0.024	11	0.207	0.015	16
Total solids reduction (%)	55.86	7.26	18	56.81	2.06	18	45.79	3.54	18	46.60	2.65	18
Volatile solids reduction (%)	66.74	5.05	18	66.50	1.89	18	56.48	4.08	18	54.41	3.65	18
Holocellulose reduction (%)	71.96	5.52	5	61.25	3.65	6	52.78	5.23	6	48.88	4.79	6
BOD reduction (%)	77.2	5.5	3	78.6	3.0	2	72.7	1.3	2	79.2	0.0	1

See legend to table 4.1

Table 4.6 Digestion Efficiency For Group 2

Group	2								
Digester no.	9,10			11,12			13,14		
Biogas yield (m^3 kg VS added^{-1})	0.404	0.036	14	0.437	0.017	16	0.428	0.014	16
Gas composition (% CH_4)	59.22	1.57	38	58.52	2.19	36	57.70	2.22	37
Methane yield (m^3 kg VS added^{-1})	0.239	0.024	14	0.257	0.015	16	0.246	0.011	16
Total solids reduction (%)	52.15	3.82	16	46.35	3.60	16	45.74	2.31	16
Volatile solids reduction (%)	65.76	2.32	16	60.85	2.14	16	57.39	1.92	16
Holocellulose reduction (%)	-			-			-		
BOD reduction (%)	-			-			-		

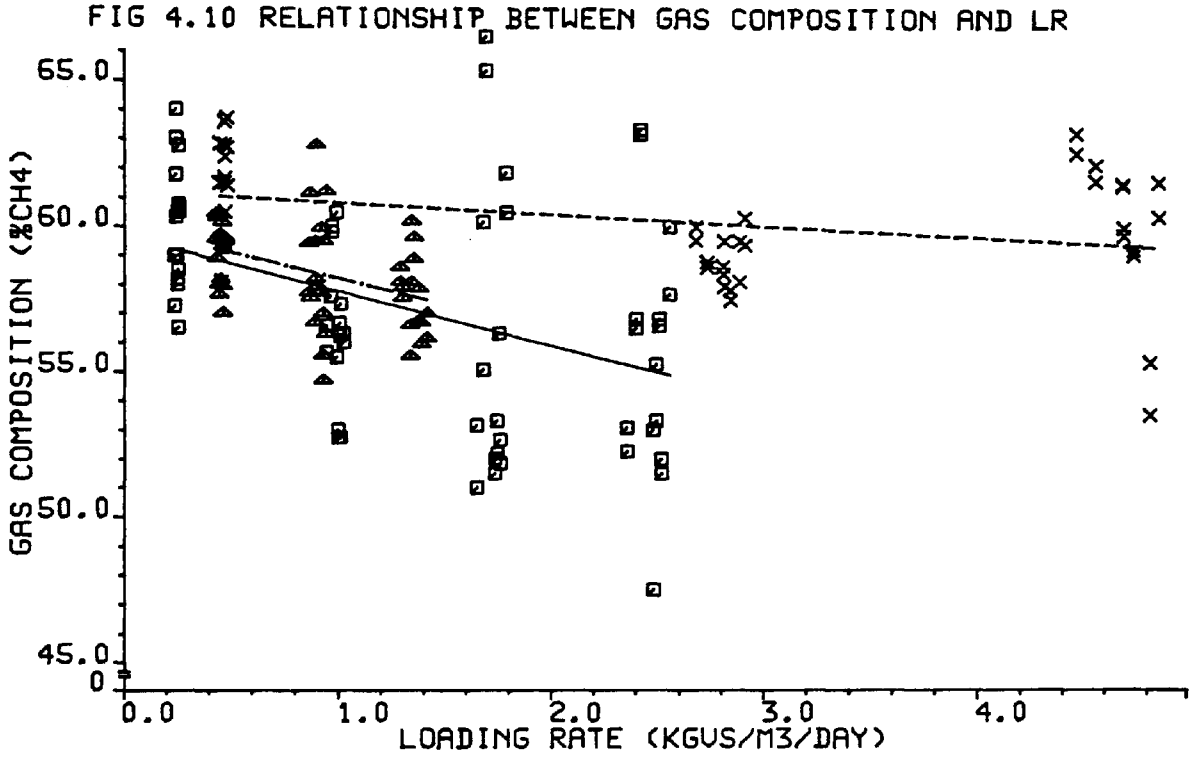
See legend to table 4.1

Table 4.6 Digestion Efficiency For Group 3

Group	3								
Digester no.	15,16			17,18			19,20		
Biogas yield (m^3 kg VS added^{-1})	0.346	0.032	10	0.410	0.017	12	0.399	0.010	9
Gas composition (% CH_4)	61.50	2.70	34	58.97	2.59	33	60.67	4.00	34
Methane yield (m^3 kg VS added^{-1})	0.211	0.020	10	0.241	0.012	12	0.233	0.016	9
Total solids reduction (%)	51.26	8.06	12	44.88	3.88	12	44.49	3.83	12
Volatile solids reduction (%)	56.74	7.64	12	49.73	5.09	12	48.97	4.98	12
Holocellulose reduction (%)		-			-			-	
BOD reduction (%)		-			-			-	

See legend to table 4.1

FIG 4.10 RELATIONSHIP BETWEEN GAS COMPOSITION AND LR



LEGEND

□—— 29d RT 1st Group
△—·—· 29d RT 2nd Group
X---- 15d RT 3rd Group

4.3.3.2 Biogas yields

Biogas yields varied from 0.346 m^3 kg VS added^{-1} at 15d RT and 1% influent TS, to 0.437 m^3 kg VS added^{-1} at 29d RT and 4% influent TS, with overall mean of 0.397 m^3 kg VS added^{-1} (Table 4.6). Figure 4.11 shows the relationship between mean GY and LR for each pair of digesters. For each group of experiments as the influent concentration was raised the GY increased from a minimum value at the lowest concentration to a maximum, before decreasing to an intermediate value at the highest concentration. Comparing the GY's obtained at the same influent concentrations but different RT's, at 1% and 6% TS GY's were higher at 29d RT, but at 10% TS GY's were higher at 15d RT.

4.3.3.3 Methane yields

The effect of the CH_4 content of the biogas on the yield is shown in Figure 4.12, a plot of methane yield against LR. Since the CH_4 composition decreased with increasing influent concentration, the increase in GY's at low LR's was reduced, and the decrease in GY's at higher LR's magnified. Similarly due to the decrease in CH_4 content with increasing RT, where an increase in RT caused an increase in GY this was reduced, but where a decrease in GY was produced this effect was enlarged. The overall mean methane yield was 0.229 m^3 kg VS added^{-1}.

4.3.3.4 Destruction of solids

The destruction of TS varied from 44.5% at 15d RT and 10% influent TS, to 56.8% at 29d Rt and 4% influent TS, with a mean of 49.0%. (Table 4.6). The relationship between TS destruction and LR (Figure 4.13) shows that at the same influent concentration destruction was higher during the experiments of group 1 than group 2, which in turn had higher destruction figures than group 3. Within each group TS removal decreased with increasing LR.

The relationship between VS reduction and LR (Figure 4.14) followed a

FIG 4.11 RELATIONSHIP BETWEEN BIOGAS YIELD AND LOADING RATE

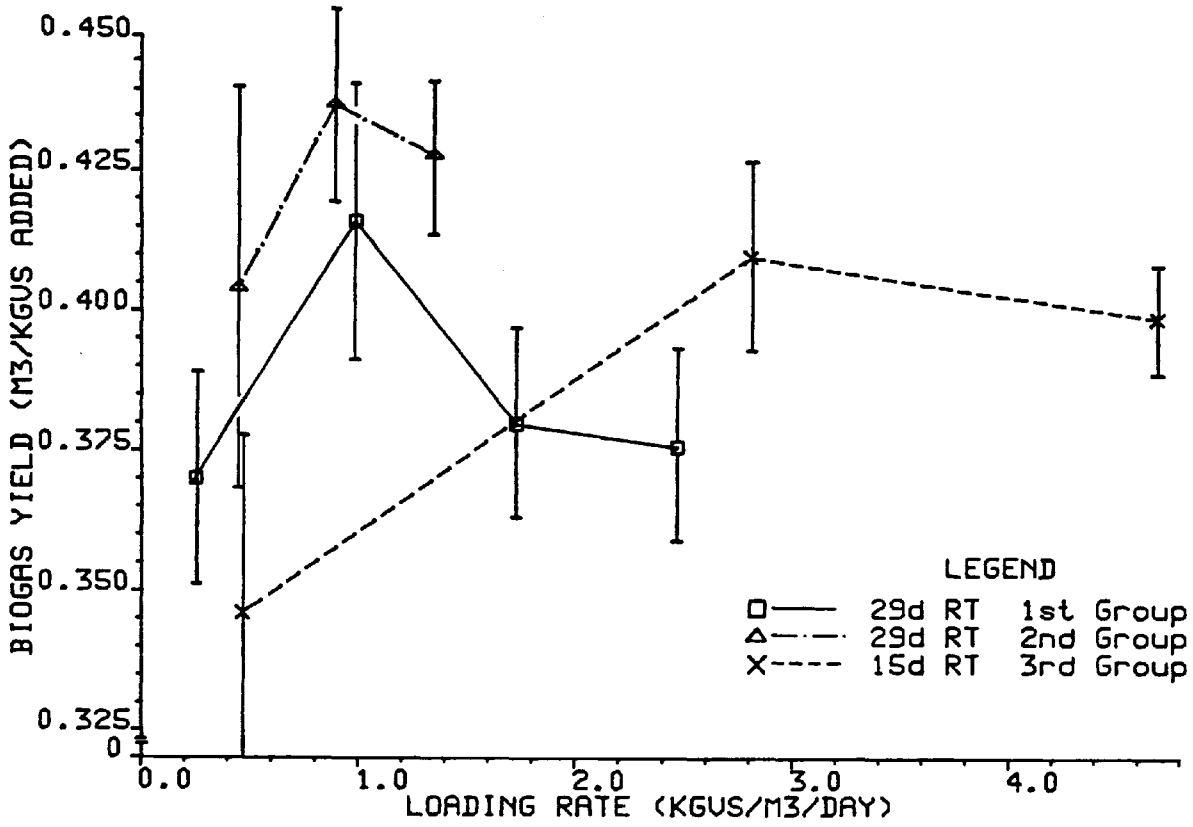


FIG 4.12 RELATIONSHIP BETWEEN METHANE YIELD AND LOADING RATE

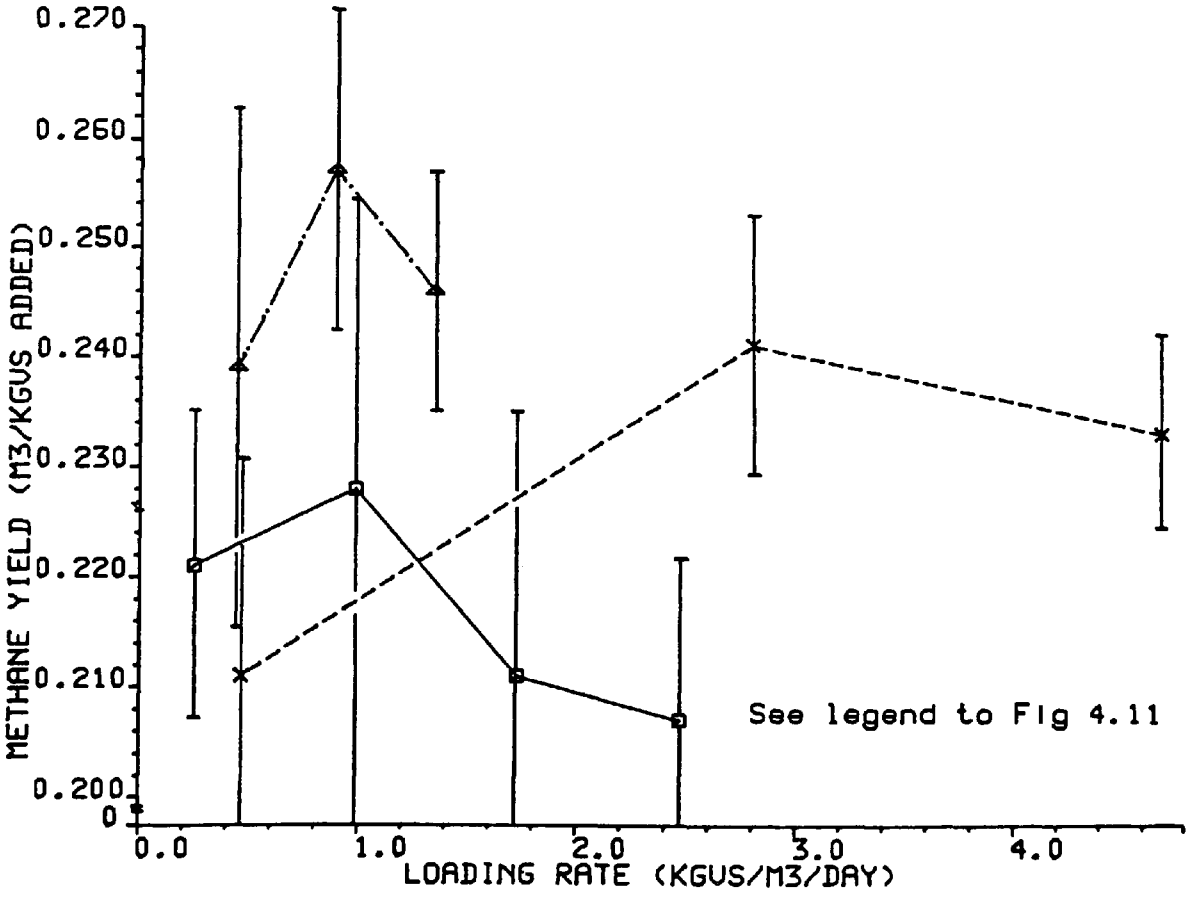


FIG 4.13 RELATIONSHIP BETWEEN DESTRUCTION OF TS AND LR

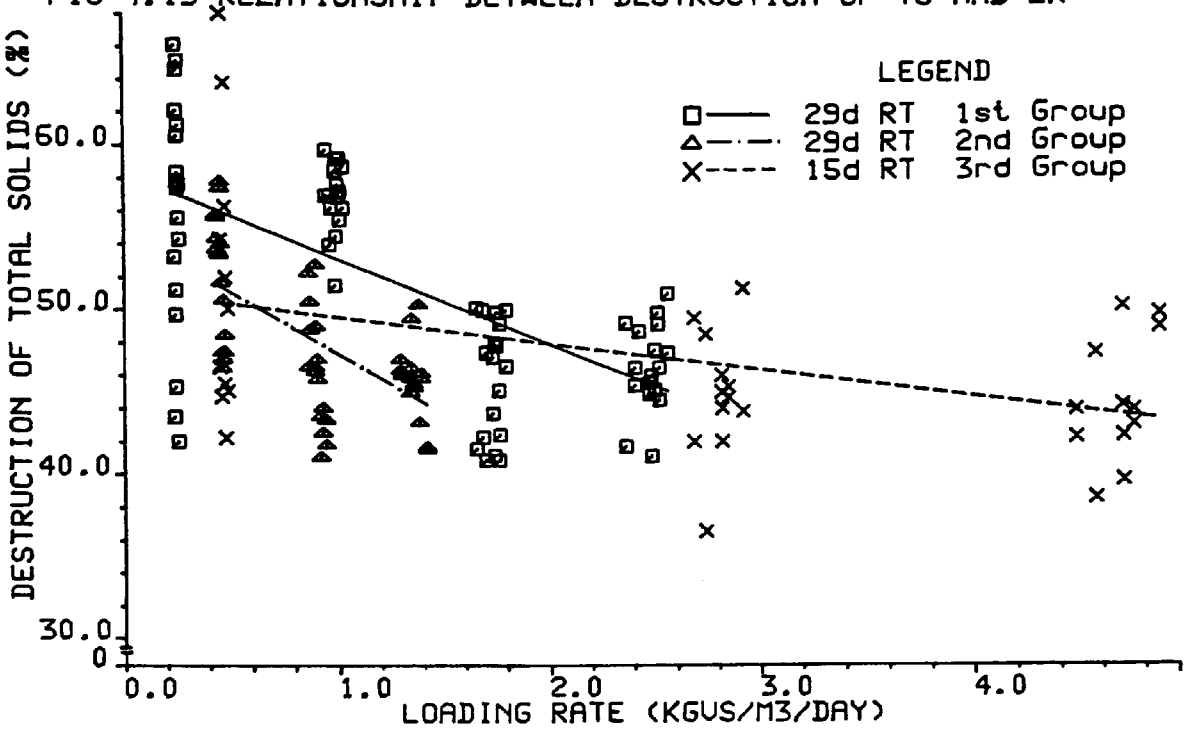
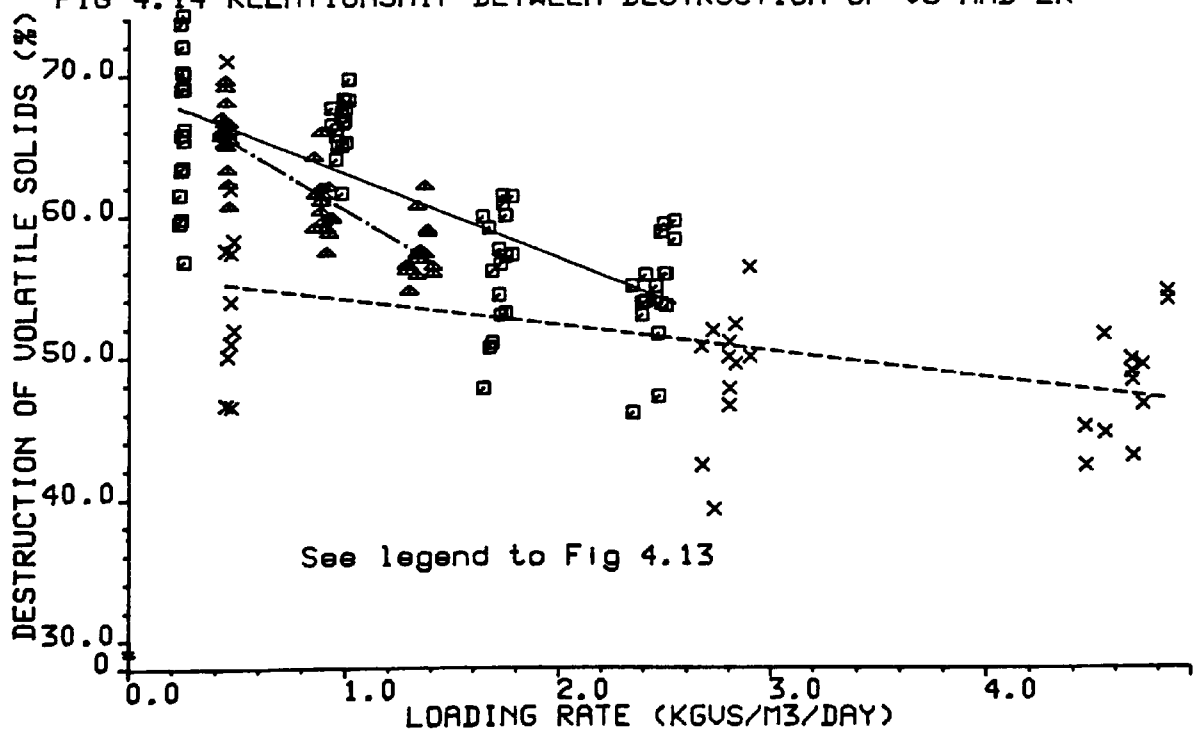


FIG 4.14 RELATIONSHIP BETWEEN DESTRUCTION OF VS AND LR



See legend to Fig 4.13

similar pattern to that of TS reduction and LR. The minimum value of 49.0% destruction was achieved at 15d RT and 10% influent TS, and the maximum of 66.5% at 29d Rt and 4% influent TS. (Table 4.6). The mean VS destruction was 58.3%.

4.3.3.5 Cellulose

Holocellulose determinations made only during the first group of experiments gave a mean value of 3.59% for 10% TS stock feed which is equivalent to 35.9% of the dry weight. Effluent holocellulose increased with increasing influent concentration from 0.10% at 1% influent TS, to 1.74% at 10% TS (Table 4.4). Holocellulose destruction increased with decreasing influent concentration from 48.9% to 72.0%, with a mean value of 58.7% (Table 4.6).

4.3.3.6 Biological oxygen demand

Measurements of BOD were also only made during the first group of experiments. The mean BOD in 10% TS stock feed was 12,358 mgl^{-1}. Effluent BOD increased with increasing influent concentration from 289 mgl^{-1} at 1% TS to 2630 mgl^{-1} at 10% TS. Influent concentration had little effect on the destruction of BOD which varied between 72.7% and 79.2%, with a mean of 76.9% (Table 4.6).

4.4 Discussion

4.4.1 Digester Operation

4.4.1.1 Comparison of paired digesters

The possible causes of variation in the performance of paired digesters operated on poultry manure are presumably the same as those proposed for litter digestion in Section 3.4.2.1, namely the divergence of bacterial populations or differences in the physical characteristics of the digesters.

As with the litter digesters there was no substantial evidence for the divergence of populations. Slight differences were observed in effluent NH_4^+ - N, pH and alkalinity, between digesters 7 and 8, but these were inconsistent and insignificant.

Similarly there was no evidence to suggest accumulation of solids which might lead to increased gas production. Because the variations in the performance of digesters operated on litter under identical conditions were repeated for manure, the policy of operating digesters in pairs was continued.

4.4.1.2 Daily oscillations in gas production

The oscillations in gas production by the poultry manure digesters produced by the schedule of feeding (Figure 4.2) were similar to those generated in litter digestion (Figure 3.2) with the exception that in manure digestion the volume measured on Monday was frequently the second lowest of the week, whereas in litter digestion this was often the second highest.

The gas volume produced over the weekend was generated by material fed during (at least) the previous 4 days i.e. a single feed on Thursday and double on Friday. Since this volume was highest for litter, the contribution to gas production from 3 and 4 days previously must be higher for litter than manure. This suggests that litter has a lower content of rapidly digestible material which is presumably due to its high content of lignocellulosics. It would be expected therefore that the increase in daily gas volume on Thursday and Friday would be less pronounced during manure digestion than litter digestion. A small increase in gas production between Thursday and Friday during manure digestion is observable in some instances e.g. days 102 and 116.

4.4.1.3 Digester stabilisation

The TS and alkalinity of the effluent of digester 7 increased during the course of the experiment as the contents of low initial concentration

were replaced by influent of higher concentration. The decrease in pH, gas production and composition during the first 7 weeks is indicative of digester failure by overloading which was brought about by feeding 10% TS to the digester seeded with only 3% TS effluent. The break in feeding allowed the digester to recover and on recommencement of feeding pH and gas production stabilised although gas composition showed a slight decrease again.

Digester 8 operated identically to digester 7 showed the same signs of failure and recovery as did digesters 5 and 6 operated at the same RT but lower influent concentration (7% TS), although in these digesters the initial decrease was not so pronounced.

4.4.2 Analyses

4.4.2.1 Solids determinations

The water content of freshly collected manure was greater than that of the litter (61.6% compared with 40.4%) possibly because litter contains dry wood shavings and sawdust, while the proportions of TS which were volatile were similar (64.63% and 61.26% respectively). As with litter, batches of manure collected during warmer weather had the highest solids content (Table 4.5). The volatile fraction of the influent TS for each group of experiments was higher than the corresponding measurements on the fresh batches of manure (Tables 4.3 and 4.5). This was due to the fact that when the manure was diluted with water to produce influent, dense material such as grit settled out at the bottom of the container and was not included in the solids determination. Since grit is non-volatile, the volatile fraction of the influent TS was increased. For purposes of preparing stock feed of desired concentration, solids determinations were always made on diluted rather than solid manure to take into account the loss of grit.

It would be expected that at constant RT and a similar range of influent TS concentrations, effluent TS concentrations would be identical.

However in the first two groups of experiments, effluent TS was higher for the first than second groups (Figure 4.4). This was due to the fact that during the periods of stable operation a different batch of manure was used for each group. Batch 1 used for the first group of experiments had a higher proportion of volatile matter than batch 2 (72.07% compared with 65.57% of TS), which at the same influent TS led to a higher LR, so the two regression lines in Figure 4.4 are naturally displaced. Additionally this meant that a greater proportion of solids were available for digestion in the first group, so over the same period of time more solids were removed which led to lower effluent TS figures and higher TS destruction figures. (Tables 4.4 and 4.6).

Because of the higher proportion of volatile matter in the influent for the first than second groups, the effluent from first group digesters was higher also. Combined with the lower effluent TS figures, the VS content of the effluent was similar to that of the second group (Figure 4.5).

The third group of experiments was carried out at the shorter RT of 15 days so less solids degradation took place, producing effluent of higher TS and VS than the other two groups but lower destruction figures (Tables 4.4 and 4.6).

4.4.2.2 Total nitrogen

The mean total nitrogen content of the manure used in this study (5.94% of dry weight) was higher than the values reported in the literature for poultry manure of 4.6% by Huang and Shih (1981), 5.14% by Hart (1963) and 5.77% by Hills (1982), but lower than the range of 7.1 - 8.6% given by Aubart and Fauchille (1983). The nitrogen content was higher than that of the litter (although only by 0.77%) due to the diluting effects of the carbonaceous sawdust present in litter.

4.4.2.3 Ammonium nitrogen

There was a wider variation in the NH_4^+ - N content of batches of manure than total nitrogen. Batch 2 which had the highest stock feed nitrogen

content also had the highest proportion of NH_4^+ - N, which was approximately double that of other batches. This was presumably due to a greater degree of uric acid degradation during storage. However the mean proportion of total nitrogen constituted by NH_4^+ - N (45.4%) was comparable to the figure of 43.5% reported by Gramms et al (1971). The value of 45.4% increased during digestion to a mean of 71.7% which indicates as with litter digestion that more NH_4^+ - N was produced during uric acid and protein degradation than was utilised in microbial cell synthesis.

In both the influent and effluent the proportion of total nitrogen attributable to NH_4^+ - N was much higher for manure than litter, which therefore contains a larger proportion of organic nitrogen.

At the same influent solids concentration, the effluent from group 2 experiments had a higher NH_4^+ - N concentration than the other 2 groups (Figure 4.7). This was because the influent of the second group had the highest total nitrogen content and this, rather than influent NH_4^+ - N which changes during storage, determines effluent NH_4^+ - N concentration. Effluent NH_4^+ - N can be predicted from influent total nitrogen by the following relationship obtained from Figure 4.15: Effluent NH_4^+ - N (mgl^{-1}) = Influent total nitrogen (mgl^{-1}) x 0.730 + 129.

4.4.2.4 Total alkalinity

There was a 2.5 fold difference in the alkalinity of stock feed between manure batches 1 and 2 (9871 mgl^{-1} and 24,688 mgl^{-1} respectively). Batch 2 had higher concentrations of all parameters measured, but only in alkalinity was there such a large difference from other batches. This may have contributed to a higher effluent alkalinity for group 2 than group 1 experiments (Figure 4.8), and a slightly different relationship between alkalinity and NH_4^+ - N (Figure 4.16). For a given NH_4^+ - N concentration alkalinity was higher in digesters of the second group, so besides NH_4^+ - N other factors must be involved in the production of alkalinity. Combining the results from both groups:

$$\text{Alkalinity } (mgl^{-1}) = NH_4^+ - N\ (mgl^{-1}) \times 5.55 + 389$$

FIG 4.15 DEPENDENCE OF EFFLUENT NH4-N ON INFLUENT TN

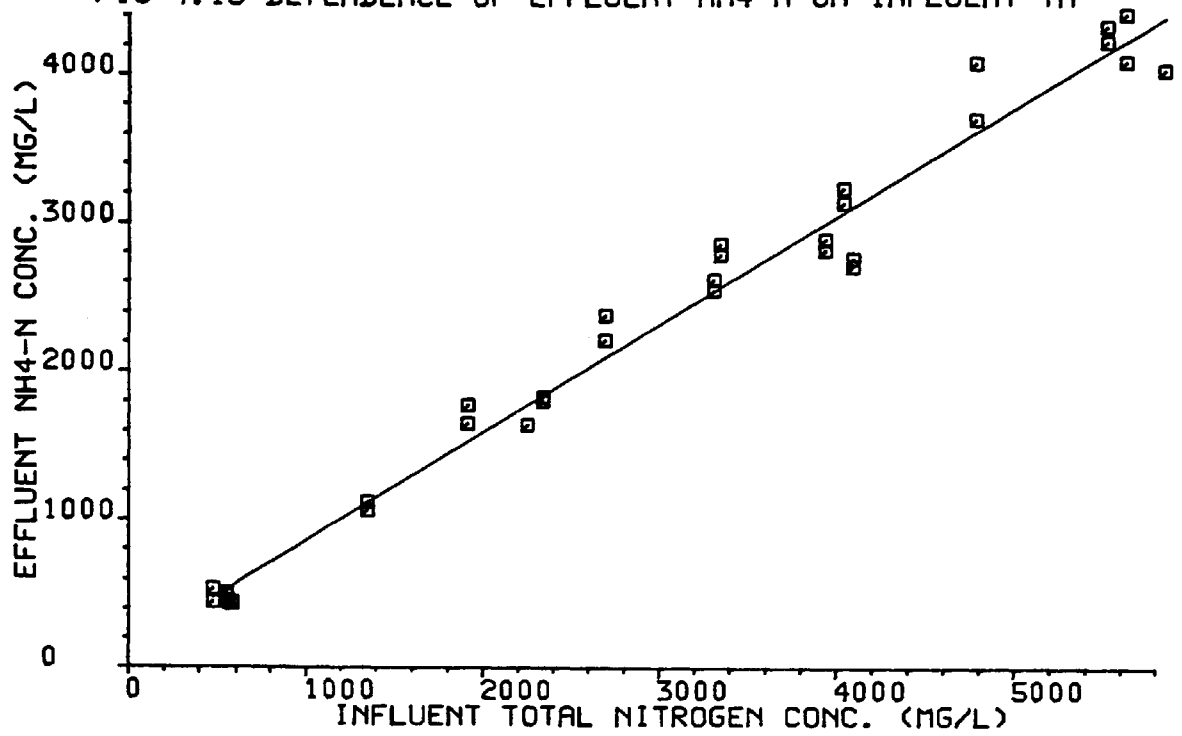
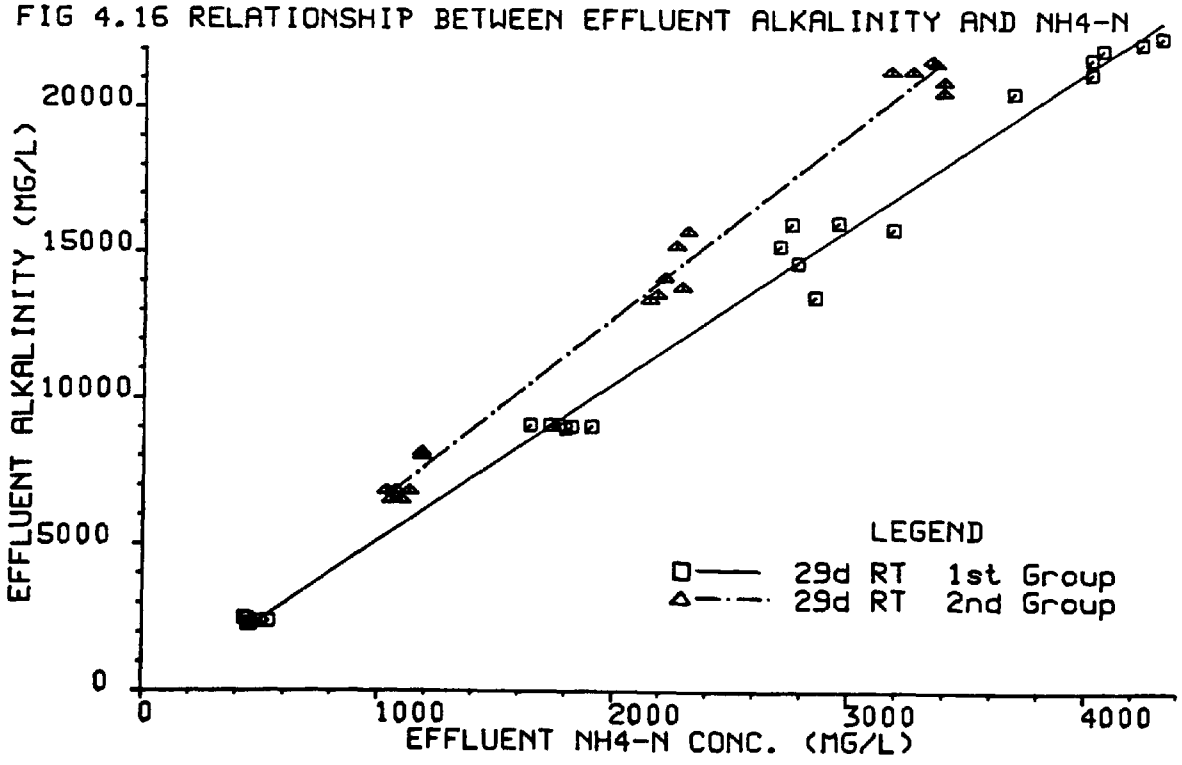


FIG 4.16 RELATIONSHIP BETWEEN EFFLUENT ALKALINITY AND NH4-N



4.4.2.5 pH

Effluent pH increased with LR and was higher in the second group of experiments than the first possibly due to the influence of NH_4^+ - N which followed the same trend. Effluent pH also increased with increasing RT, but an increase in NH_4^+ - N with RT was only observed between the second and third group of experiments. The relationship between effluent pH and NH_4^+ - N is presented in log-log form in Figure 4.17. At a constant NH_4^+ - N concentration, pH was different for each group, so although NH_4^+ - N may be the main influence on digester pH, as with alkalinity other factors appear to be involved. These may include VFA's which were not measured routinely, but in both litter and manure digesters were normally present in concentrations less than 1000 mgl^{-1} as acetic acid.

Both digester pH and alkalinity increased with increasing LR and were higher for the second than first group of experiments due largely to the influence of NH_4^+ - N. It follows then that pH and alkalinity are related but are not necessarily interdependent. (Figure 4.18). The concentrations of free ammonia in the effluent for all experiments are shown in Table 4.4, and the logarithmic relationship between free NH_3 and LR in Figure 4.19.

4.4.3 Digestion efficiency

4.4.3.1 Gas composition

The mean biogas CH_4 content of 58.6% was within the ranges reported by other workers for poultry manure digestion, but lower than the 69% reported by both Hobson et al (1979) and Aubart and Fauchille (1983). Compared with other animal wastes the value obtained in this study for manure was slightly lower than the 59.2% CH_4 for litter digestion (this study), but similar to the figures reported in the litterature for cattle and swine manures, although the figure of 69% reported by Hobson et al (1979) was again appreciably higher. Digestion efficiencies are given for poultry manure and other agricultural wastes in Table 4.7.

FIG 4.17 RELATIONSHIP BETWEEN EFFLUENT PH AND NH4-N

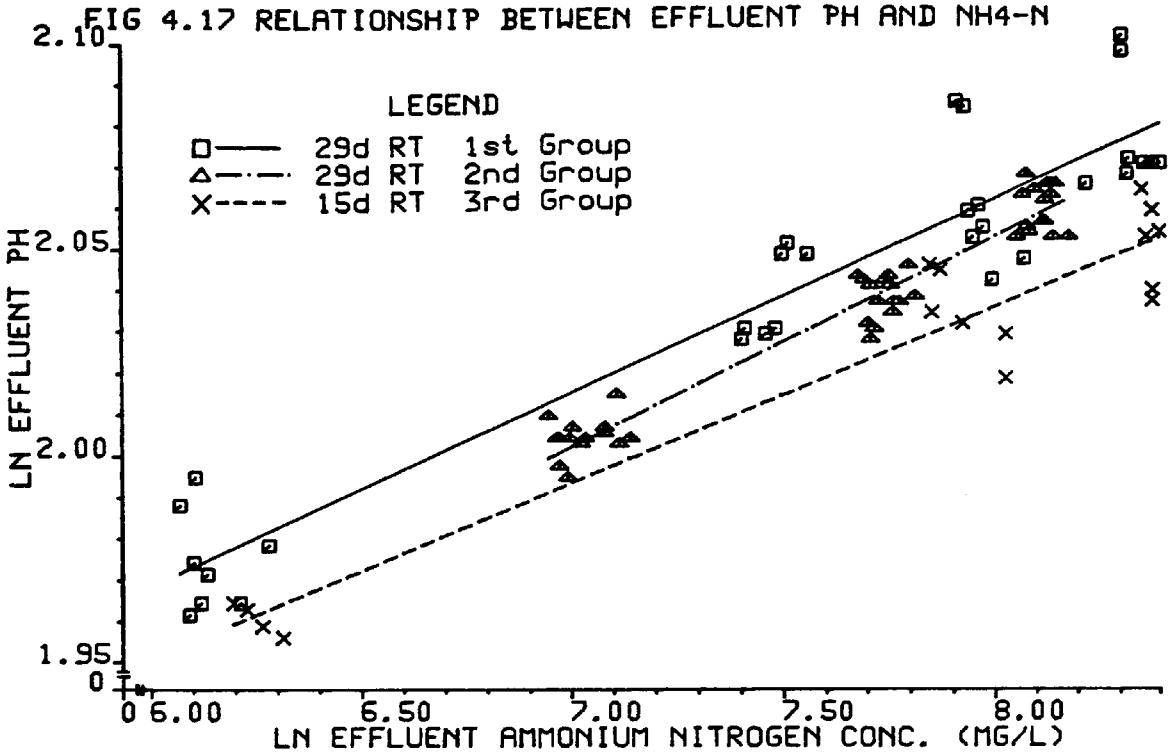


FIG 4.18 RELATIONSHIP BETWEEN EFFLUENT pH AND ALKALINITY

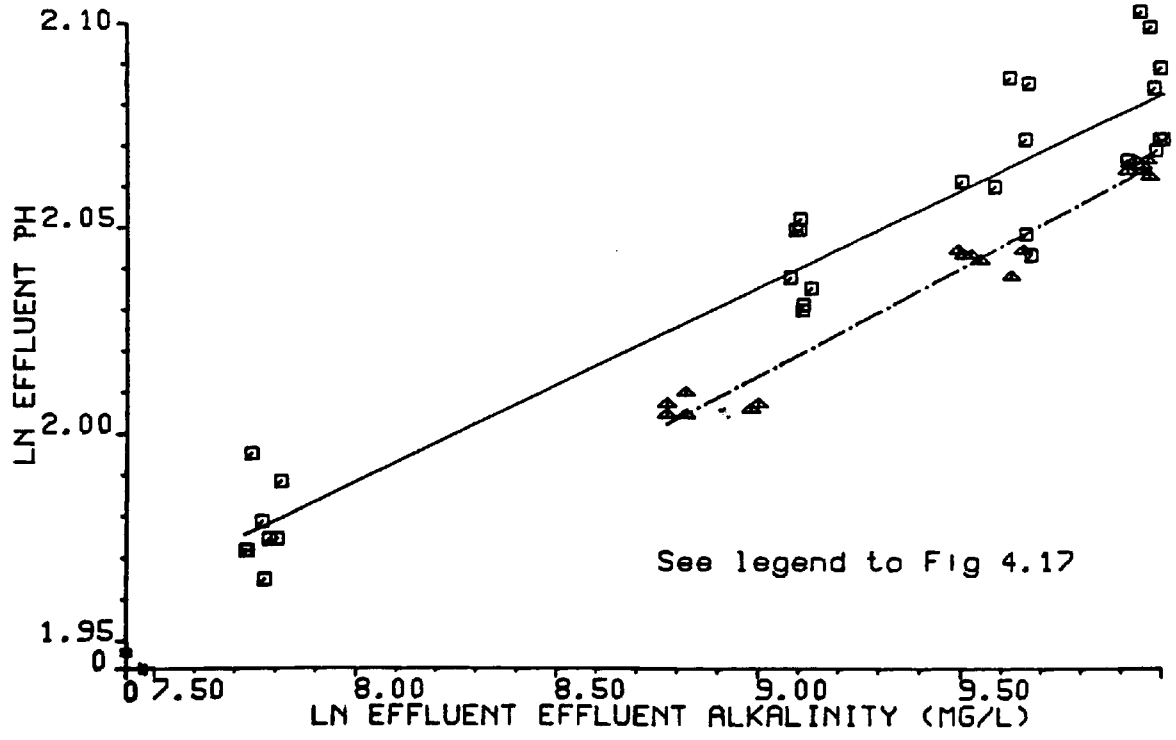
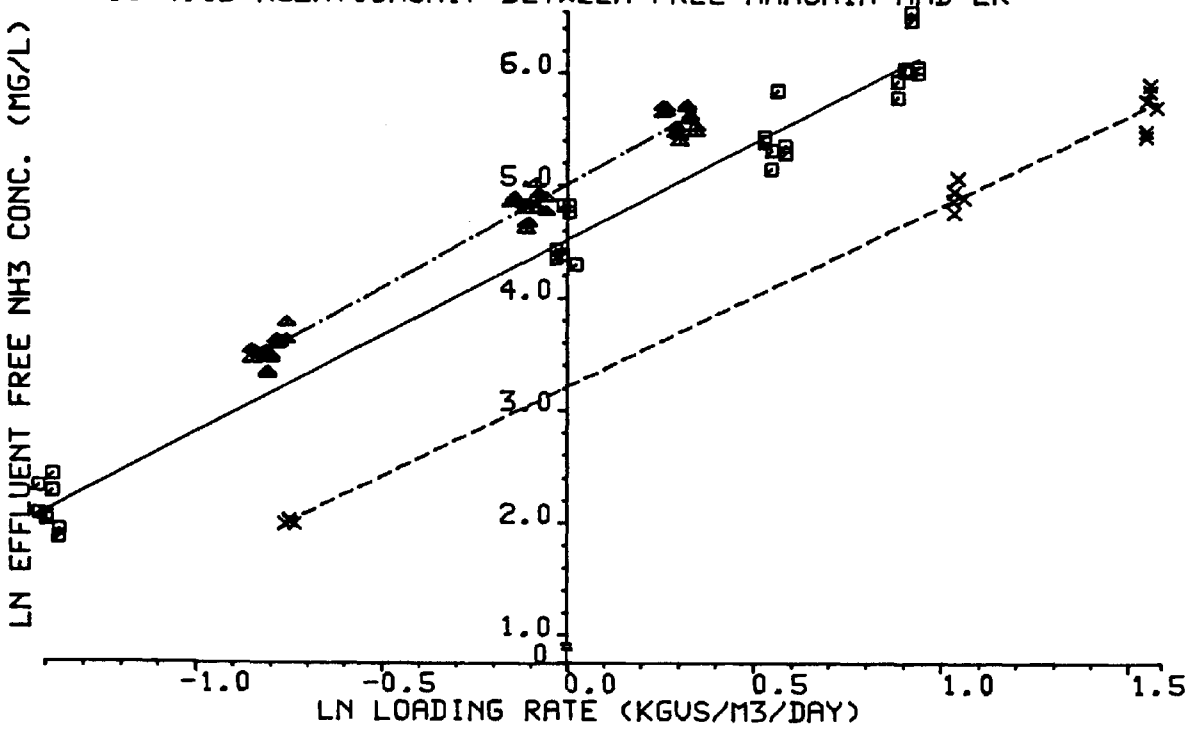


FIG 4.19 RELATIONSHIP BETWEEN FREE AMMONIA AND LR



LEGEND

□—— 29d RT 1st Group
△—·—· 29d RT 2nd Group
X---- 15d RT 3rd Group

Table 4.7 Comparison of Digestion Efficiencies for Different Agricultural Wastes

Waste Material	Operating Conditions			
	Temp. (^{o}C)	RT (days)	Influent VS (%)	LR (kg VS m^{-3} d^{-1})
Poultry Litter	35	12-29	0.98 - 4.90	0.34 - 4.20
	35	35	4.06	1.16
	60	3-10	7.0	7.0 - 23.3
Cattle Manure	32.5	10-15	1.92 - 5.76	1.92 - 3.84
	35	25.3-26.3	8.23	1.95 - 3.41
	35	20	3.5 - 7.7	-
Pig Manure	32.5	10-15	1.92 - 5.76	1.92 - 3.84
	35	10	1.4 - 4.9	-
Poultry Manure	35	15-29	0.70 - 7.20	0.25 - 4.7
	37	4-30	2.56 - 5.45	0.85 - 7.27
	35	36-46	6.38 - 8.18	1.65 - 2.29
	32.5	10-15	1.92 - 5.76	1.92 - 3.84
	35	22.5-26.1	10.71	2.77 - 4.88
	35	15	3-6	2-4
	35	18-20	2.8 - 8.75	-
	55	4-10	7.0	7-17.5

Table 4.7 Continued

Waste Material	Digestion Efficiency				
	Gas composition (% CH_4)	Gas Yield (m^3 kg VS added^{-1})	Destruction of TS (%)	Destruction of VS (%)	Destruction of BOD (%)
Poultry Litter	57.7 - 61.0	0.245 - 0.372	6.4 - 17.8	20.1 - 34.7	61.7 - 81.6
	53	0.415	-	-	-
	35.2 - 54.8	0.040 - 0.055	-	16.4 - 20.0	-
Cattle Manure	61.4 - 65.8	-	-	17.7 - 26.7	-
	57 - 66	-	33.5 - 58.9	15.4	76.8 - 88.9
	55 - 60	0.271 - 0.371	15 - 30	-	66.77
Pig Manure	58.0 - 60.8	-	-	49.2 - 60.9	-
	69	0.429	40	-	83
Poultry Manure	56.0 - 61.5	0.346 - 0.437	44.5 - 56.8	49.0 - 66.5	7.27 - 79.2
	68.9	0.32 - 0.627	-	29.1 - 61.5	-
	58.5 - 64.4	0.37 - 0.51	43.5 - 58.1	51.4 - 65.3	-
	52.5 - 58.0	-	-	57.0 - 67.8	-
	48-49	-	57.4 - 71.5	32.0 - 44.8	35.6-74.4
	55.5 - 62.6	0.43 - 0.44	-	56.5 - 62.8	-
	69	0.414 - 0.686	23-57	-	79-84
	60.1 - 64.7	0.125 - 0.187	-	1.21 - 19.1	-

Table 4.7 Continued

Waste Material	Source
Poultry Litter	This study
	Hawkes and Young, 1980
	Shih and Huang, 1980
Cattle Manure	Gramms et al, 1971
	Hart, 1963
	Hobson et al, 1979
Pig Manure	Gramms et al, 1971
	Hobson et al, 1979
Poultry Manure	This study
	Aubart and Fauchille, 1983
	Converse et al, 1980
	Gramms et al, 1971
	Hart, 1963
	Hills, 1982
	Hobson et al, 1979
	Huang and Shih, 1981

Since both effluent alkalinity and pH increased with increasing LR, little variation would be expected in gas composition, due to the bicarbonate buffer system. However in common with litter digestion (section 3.4.4.1) the proportion of CO_2 in the biogas did tend to increase with increasing influent concentration and RT (Figure 4.10). This confirms the litter results and is in agreement with the gas composition and RT relationship proposed by Pfeffer (1980).

4.4.3.2 Biogas yields

The biogas yields of 0.346 - 0.437 m^3 kg VS added^{-1} obtained in this study were slightly below the values reported in the literature for the mesophilic digestion of poultry manure (Table 4.7). However the very long RT's used by Converse et al (1980), and the slightly higher operating temperature employed by Aubart and Fauchille (1983) may contribute to the differences in these cases. Very low GY's were obtained from the digestion of poultry manure at 55°C by Huang and Shih (1981) which indicates its unsuitability for digestion at that temperature, although the relatively short RT's and high influent concentration employed may have contributed to the low yields. By reducing the temperature to 50°C, these authors found that the GY increased to 0.381 m^3 kg VS added^{-1} at 6% influent VS and 4d RT.

In comparison with other animal wastes, poultry manure gives similar GY's to swine manure, but higher GY's than both cattle manure and poultry litter. (Table 4.7). The high lignocellulose content could account for the low litter GY, and less than optimum concentrations could possibly cause low GY's during cattle manure digestion (Bousfield et al, 1979).

To increase the possibility of obtaining significant differences between GY's, the influent concentrations used in the first group of experiments covered as wide a range as possible (1 - 10% TS) at a single RT. However in contrast to sewage sludge digestion (Hawkes and Horton, 1981) and litter digestion (this study) a progressive increase in GY with influent concentration was not obtained. Instead, after an initial increase

to a maximum, GY's decreased (Figure 4.11). To confirm these results and to determine more precisely the optimum concentration for digestion, the second group of experiments was carried out at the same RT, but influent concentrations covering a narrower range; one the same as the optimum of the first group (4% TS), and one either side of this (2% and 6% TS). The results obtained followed the same pattern as those of the first group in that the optimum influent concentration was in the middle of the range used (4% TS). However the GY's of the second group are higher than those of the first, so the results from the two groups appear to be incompatible.

As with the incompatibility of results between groups of experiments in the investigation of litter digesters, the most likely cause of the incompatibility of manure results is variations between batches. Different batches of manure with varying compositions were used during the stable periods of each group which may have led to different results under similar operating conditions.

The third group of experiments was carried out using the same influent concentrations as the first two groups, but at a shorter RT to test whether RT affected the relationship between GY and LR. The same pattern emerged as in the first two groups, and the maximum GY was obtained at the median influent concentration of 6% TS (Figure 4.11). As expected from a shorter RT, the GY's obtained at 1% and 6% TS were lower for the third than first two groups, but at 10% influent TS, GY's were higher at the shorter RT, an apparently anomalous result.

The fact that instead of a progressive increase in GY, a decrease was obtained after a certain influent concentration had been reached, was possibly due to inhibition of digestion either by a components of the feed or by a substance produced during digestion. Inhibition by high influent concentrations resulting in reduced GY's has been recorded for the digestion of poultry litter (Shih and Huang, 1980) and manure (Gramms et al 1971, Bousfield et al 1979, Hobson et al 1980, Huang and Shih 1981, and Aubart and Fauchille, 1983) which was in some cases attributed to toxic

concentrations of NH_4^+ - N (Gramms et al 1971, Bousfield et al 1979, and Hobson et al 1980). A threshold level of 1500 mgl^{-1} NH_4^+ - N is thought to exist below which no inhibitory effects on gas production are observed, but above which inhibition begins (McCarty and McKinney, 1961a, McCarty 1964). This threshold level was exceeded in all digesters in the present study except at the lowest influent concentration in each group. There is evidence to suggest however, that ammonia inhibition may be more directly related to the concentration of free NH_3 than the ammonium on, NH_4^+ (McCarty and McKinney, 1961a). The concentration of free NH_3 in the manure digesters increased logarithmically with increasing LR from 8 mgl^{-1} to 435 mgl^{-1} as N (Figure 4.19). In the digesters which exhibited depressed GY's levels of free NH_3 were 225 mgl^{-1} and 435 mgl^{-1} (first group), 260 mgl^{-1} (second group) and 291 mgl^{-1} (third group), so assuming free NH_3 is the toxic agent concentrations above 225 mgl^{-1} may be inhibitory, but the threshold level must be lower. Assuming that little or no inhibition occurred when the GY was increasing with influent concentration, free NH_3 concentrations of 86 mgl^{-1}, 124 mgl^{-1} and 138 mgl^{-1} are not inhibitory, so the threshold level for inhibition must lie between 138 mgl^{-1} and 225 mgl^{-1} as N. This range is higher than the figure of 80 mgl^{-1} reported by Anderson et al (1982), but similar to the 150 mgl^{-1} determined by McCarty and McKinney (1961a) as causing total inhibition of digestion. The latter study involved shock loading non-adapted bacteria with NH_4^+ - N in batch digesters, while in the present work the bacteria were able to adapt to high NH_3 concentrations over a period of time. Thus the threshold level for inhibition of adapted bacteria was similar to the concentration causing death to non-adapted organisms.

The effect of changing the RT and influent concentration on the digestion of poultry manure has been investigated by other workers in laboratory and full scale digesters at various temperatures. Under all conditions tested at constant influent concentrations longer RT's have produced higher GY's but the effect of influent concentration on GY at constant RT has been inconsistent. In some reports increasing the influent concentrations caused a decrease in GY (Huang and Shih) 1981, Bousfield et al 1979), while in others an increase followed by a decrease was observed (Aubart and Fauchille 1983, Hills 1982), and in two a progressive increase in GY was reported but only at certain RT's (Converse et al 1980, Morrison

et al 1980). From the examination of cases where an increase in influent concentration caused an increase in GY, there does not appear to be a common set of operating conditions responsible. However the effect of both RT and influent concentration on GY may be a consequence of the kinetics of microbial growth, the implications of which are discussed in Chapter 8.

4.4.3.3 Destruction of solids

The mean destruction figures of 49.0% of TS and 58.3% of VS obtained in this study are both comparable with the figures reported in the literature for the mesophilic digestion of poultry manure, and higher than the 12.1 - 19.1% for VS destruction achieved for digestion at 55°C by Huang and Shih (1981) (Table 4.7). In the latter study low GY's were obtained so a high degree of solids destruction would not be expected.

In comparison with the digestion of other animal wastes at mesophilic temperatures, the destruction of poultry manure solids is similar to that of pig manure, and higher than both cattle manure and poultry litter. This would be expected since solids reduction is related to GY. In the present study however, solids destruction did not correlate well with GY. In the first group of experiments the destruction of both TS and VS followed a similar pattern to GY, and increased initially with increasing influent concentration to a maximum at 4% TS, before decreasing again. In the other 2 groups however, the initial increase in GY was not reflected by solids reduction which decreased progressively with increasing influent concentration.

4.4.4.4 Cellulose

Holocellulose constituted 35.9% of the dry weight of the manure which is appreciably lower than the figure of 52.9% TS given by Huang and Shih (1981), and as expected lower than the 47.0% determined for litter in Chapter 3. As with the litter digestion experiments there was little correlation between holocellulose destruction and GY for manure.

4.4.4.5 Biological oxygen demand

The BOD of the manure used in this investigation (12,358 mgl^{-1} at 10% TS) was lower than the figure of 19,733 mgl^{-1} reported by Hart (1963), but higher than the 7,990 mgl^{-1} obtained for 10% TS litter (section 3.3.4.6). Litter has a lower BOD due to diluting effects of inert lignocelluloses, present at higher concentrations than in manure, which are not degraded in the 5 day BOD test.

The BOD of poultry manure is comparable to that of cattle manure (13,158 mgl^{-1} at 10% TS) but substantially lower than that of pig manure (22,581 at 10% TS) (Mosey, 1982b). Since BOD destruction is related to GY, poultry manure BOD removal is greater than that of poultry litter and cattle manure, but lower than for pig manure (Table 4.7). For poultry manure digestion alone, some correlation was observed between GY and BOD destruction, although only a limited number of BOD determinations were made. BOD destruction followed the initial increase and then decrease of GY with increasing influent concentration, but whereas GY then decreased further BOD destruction increased to a maximum value (Table 4.6).

4.5 Conclusions

1. The digestion of poultry manure was achieved in 5 l digesters operated at 35°C, RT's of 15 and 29 days, and influent concentrations of between 1% and 10% TS (0.70% and 7.20% VS), producing LR's of 0.34 - 3.42 kg VS m^{-3} d^{-1}.

2. The feeding regime of 6 feeds from Monday to Friday produced a pattern of daily gas volume fluctuation which was slightly different to that established in litter digesters, and indicated the presence of a component in manure which is rapidly digested.

3. Biogas yields obtained were between 0.346 and 0.437 m^3 kg VS added^{-1} with a mean of 0.397 m^3 kg VS added^{-1}. GY's increased initially with increasing influent concentration, but then decreased after a maximum had been reached, possibly due to inhibition caused by toxic concentrations of free NH_3. The threshold concentration for inhibition appeared tobe between 138 mgl^{-1} and 225 mgl^{-1} of NH_3 as N.

4. The destruction of TS, VS and BOD was on average 49.0%, 58.3%, and 76.9% respectively, but did not always correlate with GY.

5. The CH_4 content ofthe gas produced varied between 56.0% and 61.5% of the total. The remainder was composed mainly of CO_2 with less than 1% H_2S. As with litter digestion the proportion of CH_4 in the gas decreased with increasing RT and influent concentration.

6. The pH of the digester contents varied between 7.09 and 7.97, and alkalinity between 2,362 mgl^{-1} and 21,763 mgl^{-1}. Both were related to the digester NH_4^+ - N concentration which was between 465 mgl^{-1} and 4,274 mgl^{-1} as N.

CHAPTER 5

THE EFFECTS OF SHOCK LOADING AMMONIA ON THE BATCH DIGESTION OF POULTRY MANURE

5.1 Introduction

Information in the literature concerning the inhibition of digestion by ammonia produced during the digestion of highly concentrated industrial and agricultural wastes has been obtained by a number of methods. These include reports on naturally occurring concentrations of ammonia in sewage sludge digesters (e.g. Albertson 1961, Melbinger and Donnellon 1971), and piggery manure digesters (e.g. van Velsen 1979b, Fischer et al 1975), artificially raised levels in continuously fed digesters (e.g. Albertson 1961, Kroeker et al 1979), and in batch digesters (e.g. Braun et al 1981, Hobson and Shaw 1976, McCarty and McKinney 1961a, van Velsen 1979a).

The results are complicated by the fact that inhibition caused by a given concentration of NH_4^+ - N may be dependent on the substrate provided for digestion, the form in which it is added, the period (if any) of acclimation (van Velsen, 1979a), pH (McCarty and McKinney, 1961a), and the presence of other cations which may exhibit antagonistic or synergistic effects with NH_4^+ - N. (Kugelman and Chin, 1971). Thus the results obtained may depend on the method of investigation adopted, and consequently much of the information on NH_4^+ - N is conflicting and ambiguities still exist concerning threshold levels, the degree of inhibition caused by a given concentration, and the maximum levels which can be tolerated after accumation.

The aims of this investigation were, for the batch digestion of poultry manure at 35°C, to quantify the inhibitory effects produced by specific concentrations of NH_4^+ - N, ascertain the threshold and upper limit for inhibition, and investigate the responses of seeds adapted to different NH_4^+ - N levels to increased concentrations. Digesters were shock loaded with NH_4^+ - N in the form of NH_4Cl or NH_4HCO_3 with sodium acetate or glucose as substrate.

In the first 10 experiments the NH_4^+ - N concentration of the digesters was raised by the addition of NH_4Cl which was replaced by NH_4HCO_3 in experiments 11 - 17. Tables 5.2 and 5.3 show the percentage increases in NH_4^+ - N and the final concentrations employed in the two sets of experiments.

In experiments 1 - 10, 400ml seed was added to each digester with 500 ml water and allowed to equilibrate for 30 minutes in a water bath at 35°C. Each experiment consisted of one control digester and one treated digester. As substrate for digestion 6.919g CH_3COONa (representing 3000 mgl^{-1} acetate in the digester) was dissolved in 100ml water and added to each digester. The amount of NH_4Cl required to give the desired concentration of NH_4^+ - N was dissolved with CH_3COONa before adding to the treated digesters. The digester contents were mixed by shaking, a 50ml sample removed for immediate pH measurement, and a 10ml sample frozen for VFA determination. Digesters were then flushed with nitrogen for one minute then sealed and connected to the gas meter. Gas samples were taken at intervals during the experiment for analyses of composition. Experiments were stopped after 140 - 148 hours, the pH measured and a sample of the contents frozen for VFA determination.

Experiments 11 - 17 were conducted in the same manner but with the following modifications. Each experiment consisted of a control digester and up to three treated digesters which were all provided with one litre of seed. Sodium acetate was replaced as a substrate by 2g glucose, and the gas produced was passed through a solution of KOH before the volume was measured. No gas composition determinations were made, and the duration of the experiments was reduced to 90 hours.

The degree of inhibition produced by NH_4^+ - N in the treated digesters was determined by the reduction in gas production as a percentage of the control gas column i.e.

$$\text{Degree of inhibition (\%)} = \frac{\text{control volume - treated volume}}{\text{control volume}} \times 100$$

Table 5.2 Digesters Treated with NH_4Cl.

Experiment	1	2	3	4	5	6	7	8	9	10
Adapted level of NH_4^+ - N (mgl^{-1})	1244	1075	1113	960	3390	3372	3395	3343	3394	3192
% increase in NH_4^+ - N concentration	50	100	200	290	104	20	50	100	200	290
Final concentration of NH_4^+ - N (mgl^{-1})	1866	2150	3339	3744	3744	4215	5093	6686	10182	12449

Table 5.3 Digesters Treated with NH_4HCO_3.

Experiment	11		12	13	14		15		16			17		
Adapted level of NH_4^+ - N (mgl^{-1})	3066		3066	3066	3066		4287		4450			4340		
% increase in NH_4^+ - N concentrations	10	25	50	104	100	200	5	15	50	75	100	20	150	200
Final concentration of NH_4^+ - N (mgl^{-1})	3373	3833	4599	6251	6132	9198	4501	4930	6675	7788	8900	5208	10850	13020

5.2 Materials and Methods

Batch digesters were constructed from Quickfit glassware in the same manner as the semi-continuous digesters described in Section 2.4.1 except that the capacity was reduced to 1 litre and there was no facility for automatic mixing by stirrer motor. Digester temperature was maintained at 35°C by immersion in a water bath heated by a control unit. The gas meters were as described in Section 2.4.1 but were provided with a remote read out facility which allowed continuous monitoring of gas production when connected via a relay amplifier (Pepperl and Fuchs, Mannheim, W. Germany) to a chart recorder.

Seed was provided by stable semi-continuous fed digesters operated on poultry manure used for the investigation of GY - LR relationships (Chapter 4), which were adapted to different levels of NH_4^+ - N. Table 5.1 shows the source of seed, the NH_4^+ - N concentration to which it was adapted, and the period of adaptation.

Table 5.1 Characteristics of seed.

Experiments using seed	Treatment	Digesters providing seed	RT (days)	Influent conc. (% VS)
1- 4	NH_4Cl	9,10	29	1.3
5-10	NH_4Cl	13,14	29	3.9
11-14	NH_4HCO_3	17,18	15	4.1
15-17	NH_4HCO_3	19,20	15	6.8

Adapted level of NH_4^+ - N (mgl^{-1})	Period of adaptation (days)
1098	49
3348	79
3066	44
4359	43

5.3 Experiments Involving Addition of NH_4Cl

5.3.1 Results

The NH_4^+ - N content of the seed used in experiments 1 - 4 varied between 960 mgl^{-1} and 1244 mgl^{-1} as N with a mean of 1098 mgl^{-1}, and in experiments 5 - 10 between 3192 mgl^{-1} and 3395 mgl^{-1} with a mean of 3348 mgl^{-1}. The performance of control and treated digesters is presented in Table 5.4 for the low adapted seed, and Table 5.5 for the high adapted seed.

5.3.1.1 Control Digesters

The total volume of biogas produced by control digesters seeded with low adapted sludge over 140-148 hours of experiments varied between 595 ml and 895 ml. Figure 5.1 shows a typical pattern of gas production by control digesters containing low adapted seed. The CH_4 content of the gas produced was between 82.1% and 83.5% of the total (mean 82.9%), so the volume of CH_4 produced was between 488ml and 741ml.

The control digesters seeded with sludge adapted to high concentrations of NH_4^+ - N produced gas volumes of 705 ml-1060ml during 142 - 145 hours of experiment. Gas production by control high adapted seed is presented in Figure 5.2. The CH_4 content of the biogas was between 78.4% and 89.1% of the total (mean 83.9%) which gives CH_4 volumes of between 589ml and 939ml.

The gas production by digesters seeded with high adapted sludge was characterised by a high initial rate of production which gradually decreased during the experiment as substrate was removed (Figure 5.2). Conversely, gas production by low adapted seed was low at the start of the experiment but increased exponentially for up to 100 hours before levelling off after which the rate was maintained for the rest of the experiment (Figure 5.1).

Table 5.4 NH_4Cl Treatment and Performance of Seed Adapted to 1098 mgl^{-1} NH_4^+ - N.

Experiment	1 Control	1 Treated	2 Control	2 Treated	3 Control	3 Treated	4 Control	4 Treated
% increase in NH_4^+ - N concentration	-	50	-	100	-	200	-	290
Final concentration of NH_4^+ - N (mgl^{-1})	-	1866	-	2150	-	3339	-	3744
Total gas volume produced (ml)	895	635	765	490	595	170	810	155
Inhibition of gas production (%)	-	29.1	-	35.9	-	71.4	-	80.9
Gas composition (% CH_4)	82.8	71.9	83.0	75.3	82.1	48.7	83.5	65.1
Volume of CH_4 production (%)	741	457	635	369	488	83	677	101
Inhibition of CH_4 production (%)	-	38.4	-	41.9	-	83.0	-	85.1
pH Start	8.11	7.86	7.81	7.39	7.66	7.18	7.61	7.29
pH End	7.76	8.00	8.02	7.71	8.02	7.73	-	-
Free NH_3 (mgl^{-1}) Start	72	153	31	60	23	57		82
Free NH_3 (mgl^{-1}) End	32	211	51	150	53	203	-	-

Table 5.5 NH_4Cl Treatment and Performance of Seed Adapted to 3348 mgl^{-1} NH_4^+ - N.

Experiment	5		6		7		8		9		10	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
% increase in NH_4^+ - N concentration	-	10	-	20	-	50	-	100	-	200	-	290
Final concentration of NH_4^+ - N (mgl^{-1})	-	3744	-	4215	-	5093	-	6686	-	10182	-	12449
Total gas volume produced (ml)	1060	1065	705	665	815	735	720	605	820	390	785	25
Inhibition of gas production (%)	-	-0.5	-	5.7	-	9.8	-	16.0	-	52.4	-	96.8
Gas composition (% CH_4)	88.6	84.3	89.1	73.9	78.4	67.3	81.8	69.9	82.6	36.9	83.0	33.5
Volume of CH_4 produced (ml)	939	898	628	491	639	495	589	423	677	144	652	8
Inhibition of CH_4 production (%)	-	4.4	-	21.8	-	22.6	-	28.2	-	78.8	-	98.7
pH start	7.93	7.47	8.15	7.78	7.77	7.57	7.96	7.48	7.88	7.06	8.08	7.00
pH end	8.13	7.89	8.16	7.89	8.01	8.05	7.95	7.64	8.18	7.73	7.93	7.50
Free NH_3 start (mgl^{-1})	130	125	215	287	90	214	138	228	116	132	174	141
Free NH_3 end (mgl^{-1})	207	328	220	370	157	646	135	330	232	618	129	445
VFA Concentration at end (mgl^{-1} acetate)	1,100	1,150	1,750	1,740	1,540	1,650	1.450	1.540	1.360	2,530	1,410	2,780

FIG 5.1 GAS PRODUCTION BY CONTROL AND NH4CL TREATED DIGESTERS SEED ADAPTED TO 1098mg/L NH4-N

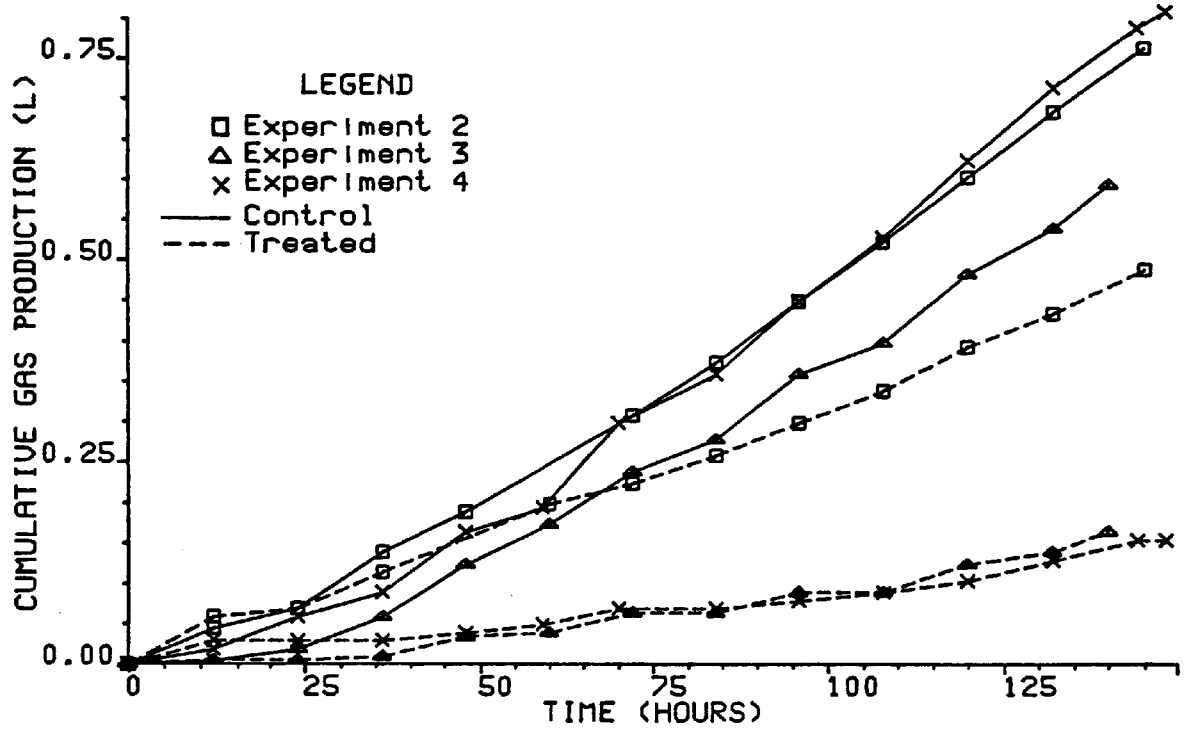
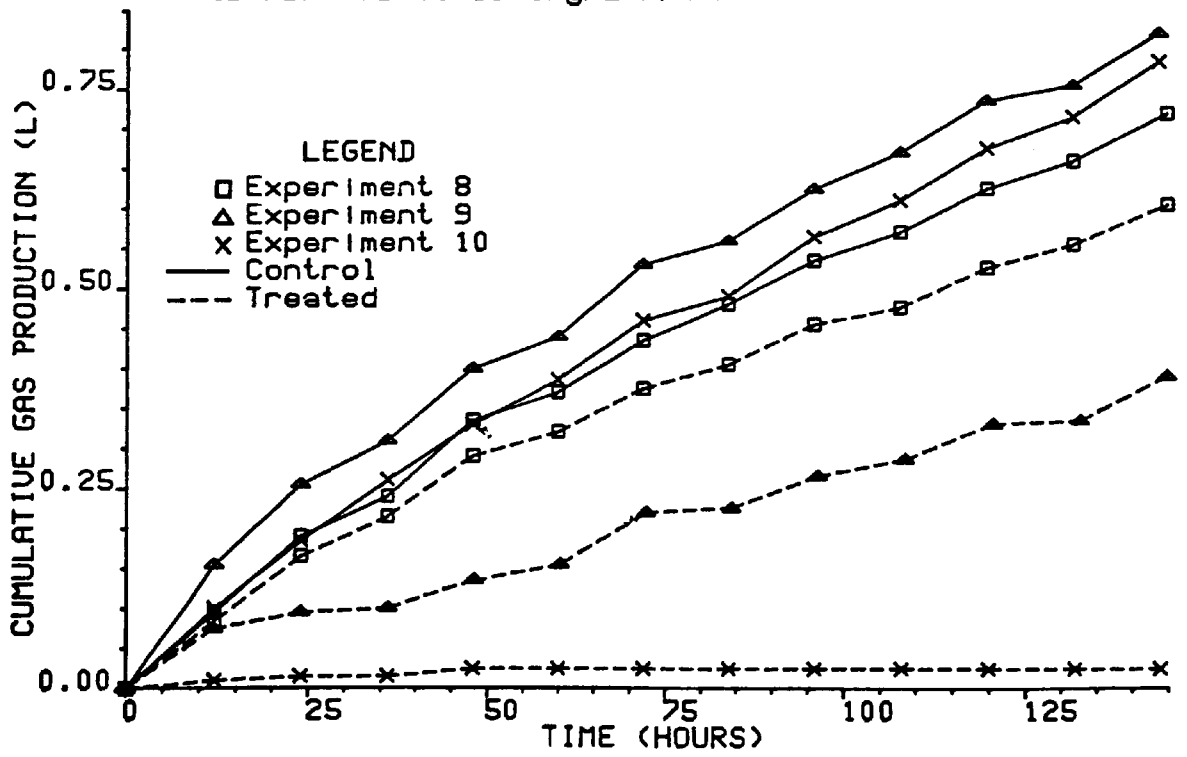


FIG 5.2 GAS PRODUCTION BY CONTROL AND NH4CL TREATED DIGESTERS SEED ADAPTED TO 3348mg/L NH4-N



The VFA concentration was measured only in digesters using high adapted seed. The concentrations in control digesters at the end of the experiments were between 1100 mgl^{-1} and 1750 mgl^{-1} as acetic acid (Table 5.5)

The mean pH of the low adapted control digesters at the start of the experiments was 7.80. This increased during experiments 2 and 3, but decreased during experiment 1. The pH was not measured at the end of experiment 4.

At the beginning of the experiments using high adapted seed the control pH was between 7.77 and 8.15, which increased during experiments 5,6, 7 and 9 to between 8.18, but decreased during experiments 8 and 10 to 7.93 and 7.95 respectively.

5.3.1.2 Treated Digesters

The volume of biogas produced by low adapted treated digesters decreased with increasing amounts of NH_4Cl added from 635ml at a 50% increase in NH_4^+ - N concentration, to 155ml at a 290% increase. Examples of the cumulative gas production by these digesters are shown in Figure 5.1. The degree of inhibition of digestion, measured by the percentage decrease in gas production compared with the control digester, increased from 29.1% (experiment 1) to 80.9% (experiment 4). The inhibition of gas production is plotted against percentage increase in NH_4^+ - N concentration and final concentration for low adapted seed in Figure 5.3. From this figure it can be deduced that 50% inhibition would be produced by a 140% increase in NH_4^+ - N concentration to approximately 2600 mgl^{-1}.

The biogas volume produced by high adapted treated seed decreased progressively with increasing NH_4^+ - N concentration, from 1065 ml at a 10% increase in NH_4^+ - N, to 25ml at a 290% increase in NH_4^+ - N concentration. Fig 5.2 shows the cumulative gas production by these digesters. Inhibition of digestion increased from -0.47% in experiment 5

FIG 5.3 INHIBITION OF GAS PRODUCTION BY NH4CL

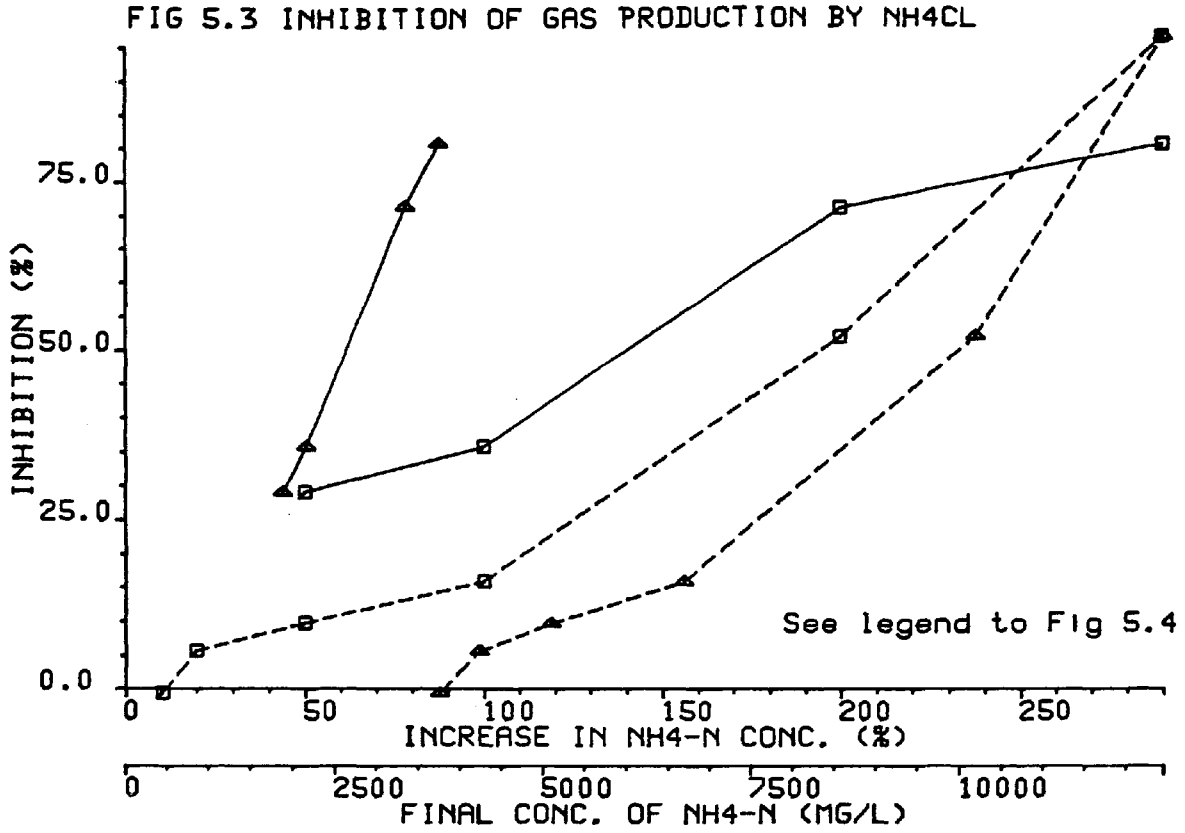
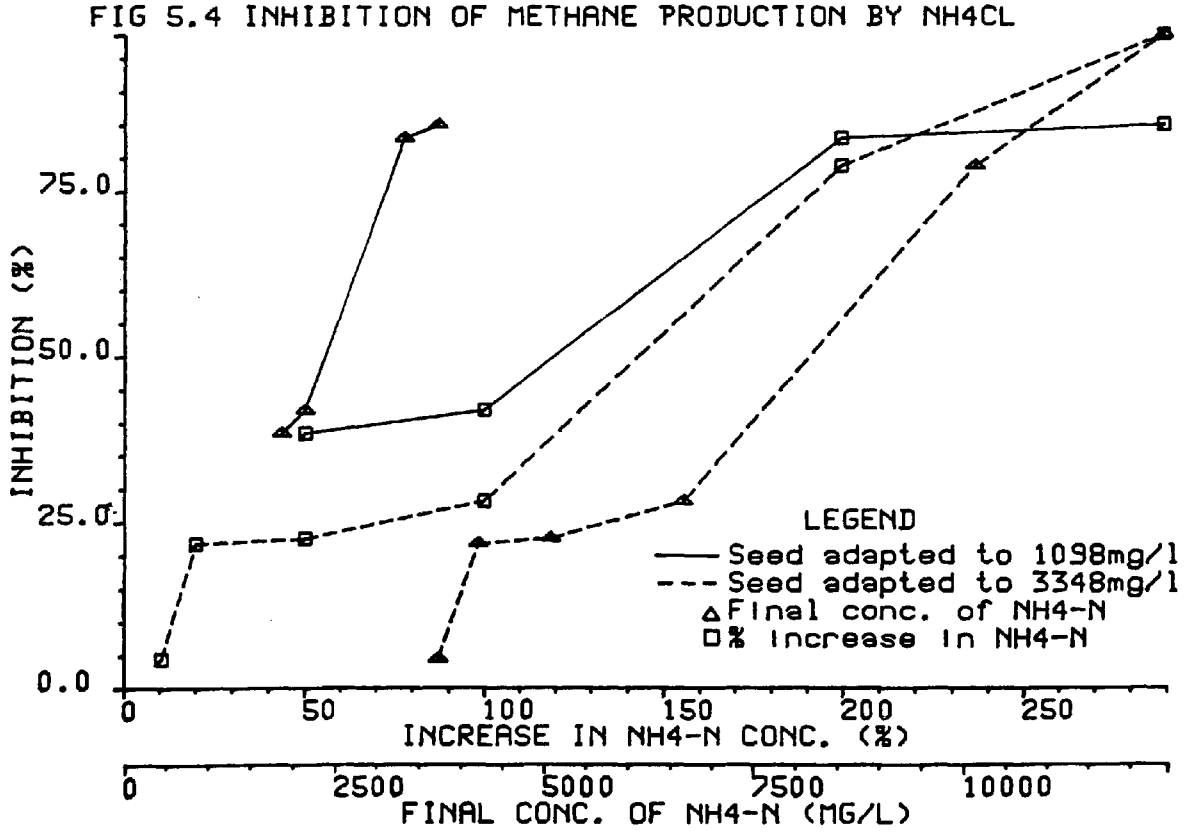


FIG 5.4 INHIBITION OF METHANE PRODUCTION BY NH4CL



(where the negative sign indicates that addition of NH_4Cl caused a greater gas production than the control) to 96.8% in experiment 10 where digestion was almost completely inhibited. Inhibition is plotted against percentage increase in NH_4^+ - N and final concentration in Figure 5.3. Thus 50% inhibition would be produced by increasing the NH_4^+ - N concentration by 196% to approx. 10,000 mgl^{-1}.

The CH_4 content of the biogas produced by seed adapted to low concentrations of NH_4^+ - N was reduced by the addition of NH_4Cl and varied between 48.7% and 75.3%, but was not proportional to the increase in NH_4^+ - N concentration (Table 5.4).

The volume of CH_4 produced tended to decrease with increasing amounts of NH_4Cl added, but was higher for a 290% increase than a 200% increase in NH_4^+ - N. However, the percentage reduction in CH_4 volume, when compared with the control, increased progressively with NH_4^+ - N concentration from 38.4% at a 50% increase to 85.1% at a 290% increase. Inhibition of CH_4 production is plotted against percentage increase in NH_4^+ - N and final concentration in Figure 5.4.

The addition of NH_4Cl also reduced the CH_4 content of the gas produced by high adapted treated digesters when compared with the control, and the degree of reduction increased with increasing amounts added. Thus the gas composition dropped from 84.3% CH_4 at a 10.4% increase, to 33.5% CH_4 at a 290% increase. The volume of CH_4 therefore also decreased with increasing NH_4^+ - N concentration, although the volume produced at a 50% increase was slightly higher than at a 20% increase, nevertheless the percentage reduction in CH_4 production when compared with the control increased progressively with increasing addition of NH_4Cl from 4.4% to 98.7% (Figure 5.4).

At the end of the experiments the VFA content of high adapted treated digesters increased with increasing amounts of NH_4Cl added from 1150 mgl^{-1} to 2780 mgl^{-1}. The percentage increase over control VFA's also increased with NH_4Cl additions from 4.54% to 97.2%, although in experiment 6 the VFA level was less than that of the control digester. (Table 5.5).

The pH of all treated digesters was lower than that of their respective controls at the start of the experiments, but increased as the experiments progressed. However since the pH of the control digesters also increased during the experiments, after 140-148 hours in most cases the control pH was higher than that of the treated digesters. (Tables 5.4 and 5.5).

5.3.2 Discussion

5.3.2.1 Control Digesters

Although the source of the seed was the same for the experiments of each group, it was collected only when required and therefore was not identical for each experiment. Thus NH_4^+ - N content, rate of gas production, and pH of control digesters varied between experiments. However a control digester was operated during each experiment on the same seed as the treated digester, so variations between the seed collected at different times were eliminated. The composition of the gas produced by control digesters appeared to be unaffected either by the source of the seed or when it was collected.

The differences in rates of gas production between the two seeds was possibly due to the fact that the high adapted seed had approx 3 times the concentration, and therefore 3 times the concentration of bacteria than the low adapted seed. The exponential increase in gas production by low adapted seed could represent an exponential increase in bacterial numbers before growth became limited by substrate concentration as in the high adapted seed.

On average the digester pH at both the start and the end of the experiments was higher for high adapted than low adapted control digesters, which reflects the pH of the continuously fed digesters which provided the seed. The effect on pH of incubating the seed with CH_3COONa for up to 148 hours was inconsistent, but in most cases the pH increased by up to 0.2 units possibly due to the utilisation of acetate and formation of NaOH and $NaHCO_3$.

5.3.2.2 Treated Digesters

In the digesters adapted to low levels of NH_4^+ - N some degree of inhibition of gas production was observed in all experiments. Therefore the threshold level for inhibition of this seed must be below the lowest concentration tested which was 1866 mgl^{-1}, a 50% increase over the adapted level. However, since gas production occurred in all experiments the concentration to cause total inhibition must be greater than the highest concentration tested, 3744 mgl^{-1}, a 290% increase.

This latter concentration was the lowest tested on high adapted seed and represented a 10% increase. No inhibition was produced by this concentration, instead a slight stimulating effect was observed in comparison with the control digester. The threshold level for inhibition was between 3744 mgl^{-1} and 4215 mgl^{-1} NH_4^+ - N, a 10% - 20% increase. The greatest percentage increase in NH_4^+ - N used in the experiments on low adapted seed (290%) was also the highest tested on high adapted seed, and gave a final concentration of 12, 449 mgl^{-1} NH_4^+ - N. This concentration caused almost complete inhibition of gas production.

Comparing the responses of the two different seeds, when the NH_4^+ - N concentration was increased to the same final concentration, the low adapted seed exhibited a greater degree of inhibition than the high adapted seed. Thus from Figure 5.3 when the NH_4^+ - N level is increased to 4000mgl^{-1}, greater than 80% reduction in gas production by low adapted seed can be expected, but less than 5% by high adapted seed.

Alternatively, the same degree of inhibition was produced in both seeds by a greater increase in NH_4^+ - N concentration, and a higher final concentration for high than low adapted seed. Thus 50% inhibition of high adapted seed would be produced by an increase to a final concentration of approx 10,000 mgl^{-1}, and of low adapted seed by an increase to 2600 mgl^{-1} (Figure 5.3). Seed adapted to high concentrations of NH_4^+ - N, therefore is more tolerant of increases than that adapted to low concentrations. Additionally there does not appear tobe an absolute threshold concentration

for ammonia inhibition, instead the concentration of NH_4^+ - N above which inhibition occurs depends on the levels to which the bacteria are adapted. Consequently the degree of inhibition of gas production caused by specific concentrations of NH_4^+ - N cannot be predicted unless the levels are known to which the seed is already adapted.

When the response of the seeds to proportionate increases in NH_4^+ - N is examined, apart from the maximum increase when inhibition was greater for the high adapted seed, a greater degree of inhibition was produced in low adapted digesters (Figure 5.3). Thus a 100% increase in NH_4^+ - N concentration caused 36% inhibition to the low adapted, but only 16% to the high adapted seed. The seed adapted to 3348 mgl^{-1} NH_4^+ - N therefore was not only more tolerant of absolute increases, but also of proportionate increases in NH_4^+ - N.

As well as reducing the total volume of gas produced, increasing the NH_4^+ - N concentration caused a decrease in the proportion of CH_4 in the gas and a consequent increase in CO_2 content, so when the gas production is expressed as volume of CH_4, the inhibitory effect of NH_4^+ - N is magnified. When compared with the control digesters the inhibition of CH_4 production increased with NH_4^+ - N concentrations. Since the net effect was a reduction in both total volume of gas produced and the CH_4 content, this suggests that the methanogenic bacteria which produce CH_4 and CO_2, rather than other groups of bacteria which produce only CO_2, was the group which sustained the inhibition.

The inhibition of methanogenesis results in the accumulation (or non utilisation) of VFA's which are precursors of CH_4 production. Since the concentration of VFA's measured in NH_4Cl treated digesters was higher than in control digesters and tended to increase with increasing NH_4^+ - N concentration, this increases the liklihood that ammonia was inhibiting the metabolism of the methanogenic bacteria.

Higher levels of VFA's in the treated than control digesters may have contributed to the lower pH values at the end of the experiments, although

the pH of treated digesters was also lower at the start. This is in contrast to results obtained from the semi-continuous digesters (Chapters 3 and 4) where high NH_4^+ - N concentrations produced high pH levels. Since as stated in Section 3.4.3.5 the toxicity of ammonia increases with increasing pH, the inhibitory effects of NH_4^+ - N concentrations produced by the addition of NH_4Cl may not be as severe as the same naturally occurring levels. The free NH_3 levels calculated at the start and end of each experiment are shown in Tables 5.4 and 5.5. Inhibition was proportional to the amount of NH_4Cl added, pH and free NH_3 however were not, so inhibition was not proportional to free NH_3 levels. This suggests that if free NH_3 is the toxic agent the pH results were not reliable, which is possible since only one measurement was made at the start and the end of each experiment, and no information was available on pH during the course of the experiments.

5.4 Experiments Involving Additional of $NH_4\ HCO_3$

In the experiments described in Section 5.3 ammonia was administered in the form of NH_4Cl. The highest concentration of NH_4^+ - N tested was 12,449 mgl^{-1} and involved the addition of 42.685g NH_4Cl which gave a Cl^- concentration of 28,321 mgl^{-1} in the digester. Although Cl^- is one of the least toxic inorganic ions (Mosey, 1974), it is possible that a proportion of the inhibition observed was attributable to Cl^-.

To eliminate the possibility of Cl^- toxicity, in this section ammonia was introduced as $NH_4\ HCO_3$, which may be reduced in a digester with the resultant production of CO_2 (Albertson, 1961):

$$NH_4HCO_3 + CH_3COOH \longrightarrow CH_3COONH_4 + H_2CO_3$$

$$H_2CO_3 \rightleftharpoons H_2O + CO_2$$

Since the evolution of CO_2 from NH_4HCO_3 would complicate the interpretation of results, all CO_2 produced was removed by passing the gas through a solution of KOH so the volume of CH_4 alone was measured.

Each digester was provided with 6.919g CH_3COONa as substrate for digestion which produces concentrations of 3000 mgl^{-1} CH_3COO^- and 1169 mgl^{-1} Na^+ in the digesters. Na^+ has been reported to be stimulatory to digestion at concentrations of 100 mgl^{-1} - 200 mgl^{-1} and inhibitory at 3500 mgl^{-1} - 5500 mgl^{-1} (McCarty, 1964), so the levels in the batch digesters probably had little effect on gas production. Additionally since CH_3COONa was added to both control and treated digesters any effect of Na^+ was eliminated. However there still remained the possibility of antagonism or synergism between Na^+ and NH_4^+ which has been shown to affect digestion (Kugelman and McCarty 1965, Kugelman and Chin 1971). Since the concentration of NH_4^+ was different in each batch digester the degree of antagonism or synergism would vary also. To exclude any possibility of these effects occurring, sodium acetate was replaced by glucose as a substrate in these experiments.

5.4.1 Results

The NH_4^+ - N concentration of the seed used in experiments 11 - 14 was 3066 mgl^{-1}, while in experiments 15 - 17 the seed NH_4^+ - N concentration varied between 4287 mgl^{-1} and 4450 mgl^{-1} (mean 4359 mgl^{-1}). The performance of control and treated digesters is presented in Table 5.6 for the 3066 mgl^{-1} adapted seed and Table 5.7 for the 4359 mgl^{-1} adapted seed.

5.4.1.1 Control Digesters

The total volume of CH_4 produced by the lower adapted control digesters in 90 hours varied between 580ml and 830ml. Figure 5.5 shows a typical pattern of gas production by these digesters. The control digesters adapted to the higher NH_4^+ - N level produced between 835ml and 1935ml CH_4 in 90 hours (Figure 5.6).

The pH of the lower adapted control digesters decreased during the experiments from between 7.61 and 7.65 to between 7.55 and 7.61 (Table 5.6), while the pH of the higher adapted control digesters increased from 7.62 - 7.74 to 7.75 - 7.89 (Table 5.7).

Table 5.6 NH_4HCO_3 Treatment and Performance of Seed Adapted to 3066 mgl^{-1} NH_4^+ - N.

Experiment	11			12		13		14		
	Control	Treated	Treated	Control	Treated	Control	Treated	Control	Treated	Treated
% increase in NH_4 - N concentration	-	10	25	-	50	-	104	-	100	200
Final concentration of NH_4^+ - N (mgl^{-1})	-	3373	3833	-	4599	-	6251	-	6132	9198
Total CH_4 volume produced (ml)	580	825	725	695	780	830	510	805	675	515
Inhibition of CH_4 production (%)	-	-42.2	-25.0	-	-12.2	-	38.6	-	16.1	36.0
pH Start	-	-	-	7.61	7.65	7.65	7.62	7.64	7.60	7.62
pH End	7.57	7.59	7.63	7.59	7.66	7.55	7.66	7.61	7.69	7.79
Free NH_3 (mgl^{-1}) Start	-	-	-	141	232	155	294	151	276	433
Free NH_3 (mgl^{-1}) End	128	148	185	135	238	123	323	141	427	641

Table 5.7 NH_4HCO_3 Treatment and Performance of Seed Adapted to 4359 mgl^{-1} NH_4^+ - N.

Experiment	15			16				17			
	Control	Treated	Treated	Control	Treated	Treated	Treated	Control	Treated	Treated	Treated
% increase in NH_4^+ - N concentration	-	5	15	-	50	75	100	-	20	150	200
Final concentration of NH_4^+ - N (mgl^{-1})	-	4501	4930	-	6675	7788	8900	-	5208	10850	13020
Total CH_4 volume produced (ml)	835	605	840	1325	825	590	175	1935	1720	625	185
Inhibition of CH_4 production (%)	-	27.5	-0.6	-	37.7	55.5	86.8	-	11.1	67.7	90.4
pH Start	7.74	7.76	7.73	7.63	7.62	7.61	7.62	7.62	7.66	7.61	7.65
pH End	7.75	7.76	7.75	7.84	7.89	7.71	7.74	7.89	7.90	7.84	7.88
Free NH_3 (mgl^{-1}) Start	266	293	299	215	314	359	419	204	269	499	657
Free NH_3 (mgl^{-1}) End	272	293	313	348	586	451	553	381	467	848	1116

5.4.1.2 Treated Digesters

The total volume of CH_4 produced by the lower adapted treated digesters varied between 510ml and 830ml, but was not proportional to the amount of NH_4HCO_3 added. The cumulative CH_4 production by these digesters in experiments 13 and 14 is presented in Figure 5.5.

The inhibition of CH_4 production by lower adapted digesters is plotted against percentage increase in NH_4^+ - N and final concentration of NH_4^+ - N in Figure 5.7. Up to a 50% increase in NH_4^+ - N concentration (4599 mgl^{-1}) CH_4 production by treated digesters was greater than by control digesters, so the effect of NH_4HCO_3 on CH_4 production was stimulatory, and the decrease in production when compared to the control was negative. The maximum degree of stimulation (-42%) was obtained at the lowest levels of NH_4^+ - N tested (3373 mgl^{-1}, a 10% increase), and decreased with increasing NH_4^+ - N concentration. The point at which NH_4^+ - N became inhibitory was between 4599 mgl^{-1} and 6132 mgl^{-1} (50% and 100% increase). The maximum degree of inhibition obtained was 38% at 6251 mgl^{-1}, a 104% increase. Increasing the NH_4^+ - N concentration above this level to 9198 mgl^{-1} (a 200% increase) failed to increase inhibition further. The total inhibition of CH_4 production therefore was not achieved.

The digesters adapted to 4359 mgl^{-1} NH_4^+ - N treated with NH_4HCO_3 produced 175ml - 1720ml CH_4 in 90 hours, and in each separate experiment CH_4 production decreased with increasing NH_4^+ - N concentration, although when taken as a whole CH_4 production was not proportional (inversely) to the amount of NH_4HCO_3 added (Table 4.7). The inhibition of CH_4 production is plotted against percentage increase in NH_4^+ - N concentration and final concentration in Figure 5.7. At the lowest level of NH_4^+ - N tested (a 5% increase to 4501 mgl^{-1}) CH_4 production was inhibited by 27.5%. When NH_4^+ - N concentration was raised by 15% to 4930 mgl^{-1} however, no inhibition was observed. Increases in NH_4^+ - N above this level caused a progressive increase in inhibition to 86.8% at 8,900 mgl^{-1}, a 100% increase in NH_4^+ - N concentration. Further increases failed to increase the degree of inhibition significantly, and the maximum inhibition of 90.4% was

FIG 5.5 CH4 PRODUCTION BY CONTROL AND NH4HCO3 TREATED DIGESTERS. SEED ADAPTED TO 3066mg/L NH4-N

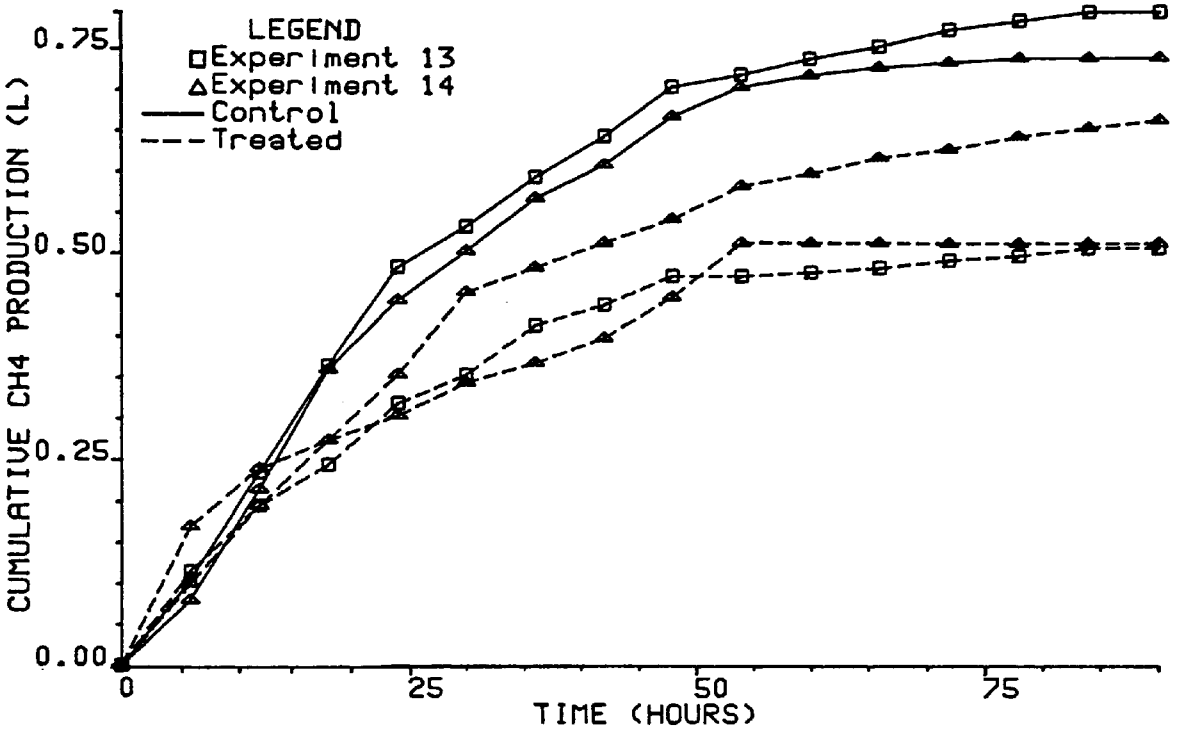


FIG 5.6 CH4 PRODUCTION BY CONTROL AND NH4HCO3 TREATED DIGESTERS. SEED ADAPTED TO 4359mg/L NH4-N

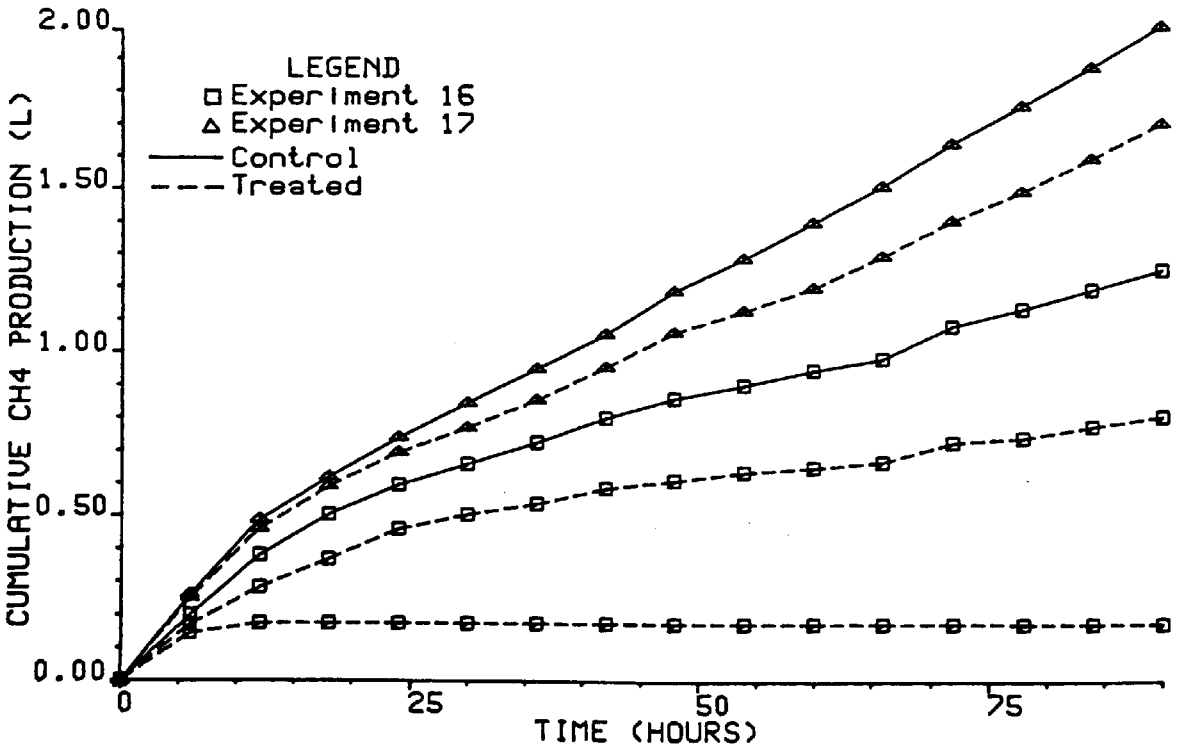
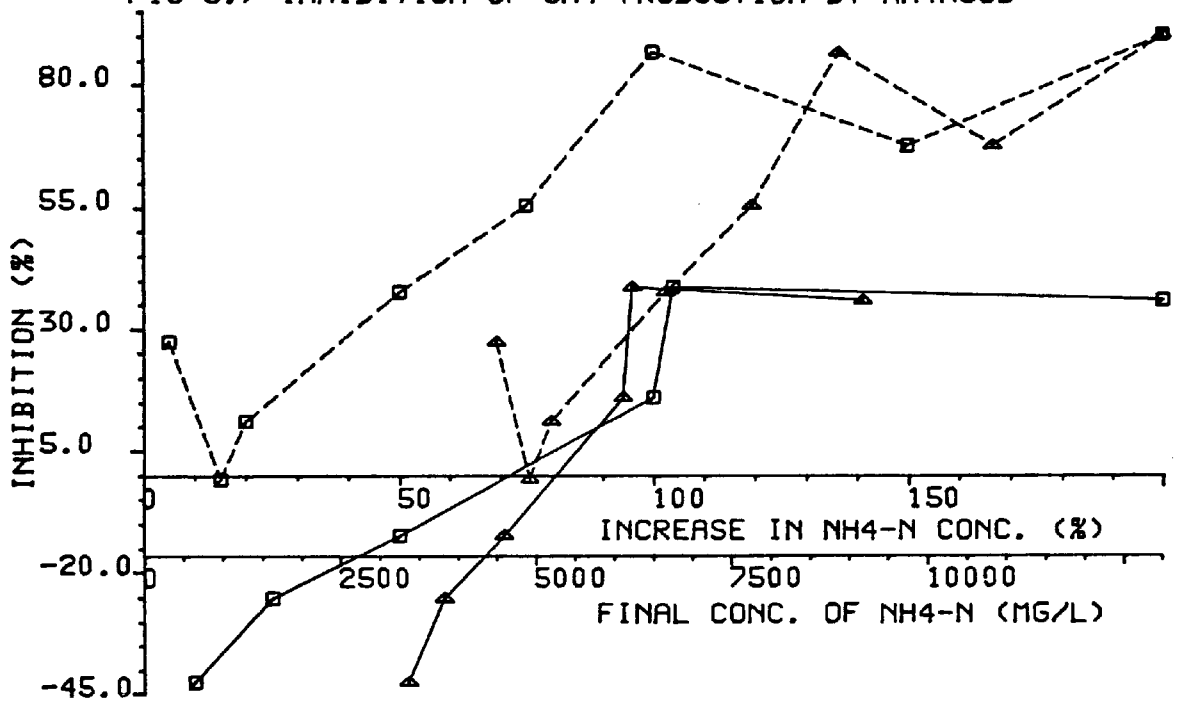


FIG 5.7 INHIBITION OF CH4 PRODUCTION BY NH4HCO3



LEGEND

—— Seed adapted to 3066mg/l
--- Seed adapted to 4359mg/l
△ Final conc. of NH4-N
□ % Increase in NH4-N

obtained at a concentration of 13,020 mgl^{-1}, a 200% increase. From Figure 5.7 50% inhibition of CH_4 production would be caused by a 67% increase to 7,500 mgl^{-1} NH_4^+ - N.

The pH of the lower adapted treated digesters increased during the experiments from 7.60 - 7.65 to 7.66 - 7.79. Digester pH was not related to NH_4^+ - N concentration at the start of the experiments, but at the end NH_4^+ - N levels caused a high pH. The pH of the higher adapted treated digesters followed the same pattern as the lower adapted digesters and increased from 7.61 - 7.66 to 7.71 - 7.90 during the experiments. Digester pH did not appear to be related to NH_4^+ - N concentration (Tables 5.6 and 5.7).

5.4.2 Discussion

5.4.2.1 Control Digesters

As with the experiments in which digesters were treated with NH_4Cl, variations in pH, NH_4^+ - N concentration and gas production occurred between control digesters due to the fact that the seed was collected from the daily fed digesters at different times.

CH_4 production by both the 3066 mgl^{-1} and 4359 mgl^{-1} NH_4^+ - N adapted control digesters was characterised by a high initial rate which decreased after 12 - 24 hours (Figures 5.5 and 5.6). In the higher adapted digesters this reduced rate was maintained for the rest of the experiment, while in the lower adapted digesters the rate decreased progressively and gas production levelled off towards the end of the experiments. CH_4 production by the higher adapted control digesters therefore, appeared not to be limited during the experiments, but CH_4 production by the lower adapted digesters was limited towards the end. On a TS basis the concentration of the lower adapted seed was six tenths that of the higher adapted seed (3.37% compared with 5.68% TS), so presumably the concentration of bacteria was also six tenths of the higher adapted seed. If bacterial numbers had been limiting CH_4 production in the lower adapted digesters an increase in

production rate from the start of the experiment would have been expected (as noted in Section 5.3.2.1), and not the gradual decrease which was obtained. Therefore it is more likely that substrate concentration was limiting CH_4 production rather than bacterial concentration.

All digesters were provided with 2g glucose as a substrate which would be expected to produce a volume of 800ml CH_4 when completely converted to gas. All higher adapted control digesters produced a greater volume than this, so besides glucose the substrate already present in the seed must have contributed to gas production. The lower adapted control digesters had only six tenths of this endogenous substrate, which was all metabolised within 90 hours limiting gas production, and resulting in a lower total CH_4 volume.

5.4.2.2 Treated Digesters

In experiment 11 the volume of CH_4 produced by the control digester from glucose and endogenous substrates was appreciably less than the volume of at least 800ml expected before gas production stopped. Because the control volume was low, the treated digesters in this experiment produced greater volumes, and hence the effect of NH_4HCO_3 appeared to be stimulatory. It is recognised that addition of salts to digesters at low concentrations can stimulate digestion, but inhibit at high concentrations (McCarty, 1964). The stimulatory effect must increase from zero when no salt is added to a maximum at the optimum concentration, then decrease to zero again when the concentration is increased further. However in experiment 11 the stimulatory effect of NH_4HCO_3 did not increase initially to a maximum, but decreased progressively as the concentration was raised (Figure 5.7). The performance of the control digester in experiment 11 therefore is questionable, and the degree of inhibition recorded for 10% and 25% increases in NH_4^+ - N erroneous results. Disregarding these two results leaves the 50% increase in NH_4^+ - N the lowest concentration tested producing a 12% stimulation of CH_4 production.

Increasing the NH_4^+ - N concentration of the higher adapted digester by 5% produced 27.5% inhibition. This result appears to be anomalous since a lower degree of inhibition was obtained from 15% and 20% increases (Figure 5.7). Excluding this result, the threshold level for inhibition was between 4930 mgl^{-1} and 5208 mgl^{-1} NH_4^+ - N, which is slightly lower than the threshold of 4599 mgl^{-1} - 6132 mgl^{-1} obtained for the lower adapted seed.

Raising the NH_4^+ - N concentration in both absolute and proportionate terms produced a greater degree of inhibition in the seed adapted to 4359 mgl^{-1} than 3066 mgl^{-1}. Thus an increase to 6000 mgl^{-1} caused approx. 25% inhibition to higher adapted but only approx. 13% to lower adapted seed. A 100% increase produced inhibition of 87% and 61% respectively.

The fact that the degree of inhibition caused by specific concentrations of NH_4^+ - N was not absolute, but was dependent on the adapted level of the seed, is in agreement with the findings in Section 5.3. However in contrast to those results, in the experiments in this section the lower adapted seed was more tolerant of NH_4^+ - N than the higher adapted seed. This result is unexpected and appears to be anomalous. However a possible explanation could be that the tolerance of a seed to increases in NH_4^+ - N concentration increases with adapted level, but only up to a point. When this adapted concentration is exceeded the ability of a seed to tolerate NH_4^+ - N increases may be reduced, so inhibition produced by the same NH_4^+ - N concentration may be greater in a high adapted than in a low adapted seed. This may reflect the cumulative effects of high levels of NH_4^+ - N on an adapted seed over long periods of time which could increase sensitivity to NH_4^+ - N. Further work however, would be required to confirm this hypothesis.

As with experiments involving the addition of NH_4Cl, pH of digesters treated with NH_4HCO_3 was not proportional to the NH_4^+ - N concentration, which would have been the case with naturally produced levels of NH_4^+ - N. Thus the inhibitory effects of NH_4^+ - N concentrations produced by the addition of NH_4HCO_3 may not be as great as those produced naturally occurring concentrations.

5.6 Comparison of Methods

Two different methods were employed for the investigation of ammonia toxicity described in Sections 5.3 and 5.4. In each method ammonia was introduced in a different form, different substrates were provided, and different methods used for the measurement of CH_4 production.

Acetate, provided as a substrate for the NH_4Cl addition experiments, is metabolised directly by methanogenic bacteria to CH_4 and CO_2. Inhibition of acetate metabolism by methanogens would result in a decrease in gas production, the proportions of CH_4 and CO_2 remaining constant. However other groups of bacteria are present in the seed which produce CO_2, so to determine whether the methanogenic, non-methanogenic or both stages are affected the gas composition must be measured. If the methanogenic bacteria alone are inhibited the proportion of CH_4 in the gas will decrease as well as the total gas volume produced, but if the fermentative or acidogenic bacteria are affected then the proportion of CO_2 will decrease. If all groups are affected the total gas volume will decrease but the gas composition will remain constant. The gas composition was determined at intervals to take into account any variations during the experiments, but removal of samples from the digester head space may cause inaccuracies in the measurement of the relatively small volumes of gas produced.

Glucose was provided as a substrate in the experiments employing NH_4HCO_3 as a source of ammonia. On digestion glucose is initially converted by fermentative and acidogenic bacteria to acetate which then serves as a substrate for CH_4 and CO_2 production by methanogens. The reduction in gas production caused by NH_4HCO_3 could be due to inhibition of any stage in this process, so analysis of gas composition is again required to determine the point at which ammonia acts. Because all CO_2 produced was absorbed the gas composition was not known and the reduction of CH_4 production alone was measured, which could be due to inhibition of the methanogenic, or any of the preceding stages. So although absorption of CO_2 to allow measurement of CH_4 production takes into account any changes in gas composition during the experiment, and avoids problems that

may be caused by sampling, it does not permit identification of the stages susceptible to ammonia inhibition.

Ammonia toxicity has been investigated by other workers using batch digesters and a variety of substrates, seeds and sources of ammonia. In most reports the inhibitory effects caused by different concentrations of NH_4^+ - N was not quantified, and either no attempt was made to determine which stage of the process was affected (Braun, 1981), or it was assumed that the final methanogenic stage was the most susceptible and this was the stage investigated (Hobson and Shaw 1976, McCarty and McKinney 1961a, van Velsen 1979a).

Braun (1981) reported that the source as well as the concentration of NH_4^+ - N influenced gas production from piggery manure during 90 days incubation at 30°C. Increasing the NH_4^+ - N concentration from the control level of 1638 mgl^{-1} as N up to 2788 mgl^{-1} by addition of NH_3 or NH_4HCO_3 caused a delay in gas production of up to 80 days (suggesting adaptation), and a lower total gas production. Adding NH_4Cl to give final concentrations of 2436 mgl^{-1}, 3157 mgl^{-1}, and 3990 mgl^{-1} produced a faster initial rate of gas production, but apart from the lowest concentration a lower total volume than the control. Neither gas composition nor digester pH was reported so the affected stage or free NH_3 concentations cannot be ascertained.

McCarty and McKinney (1961a) also tested the toxic effects of ammonium salts on sludge containing active acetate utilising bacteria in batch digesters. Acetate, Cl^- and HCO_3^- ammonium salts were added with CH_3COONH_4 as substrate, and acetate utilisation recorded for up to 11 days. Gas composition was not reported, but pH was measured to calculate the concentrations of free NH_3. NH_4HCO_3 raised digester pH which caused a lower rate of utilisation than the control and death of the bacteria after 10 days. NH_4Cl and CH_3COONH_4 produced higher initial rates than NH_4HCO_3, but lower than the control. It was concluded that concentrations of free NH_3 above 150 mgl^{-1} resulted in death of the bacteria.

The toxic effects of ammonia on CH_4 production by pure cultures of Methanobacterium formicicum were investigated by Hobson and Shaw (1976) who increased NH_4^+ - N levels by adding NH_4Cl and monitored CH_4 formation for 10 days at 38°C. It was found that 2471 mgl^{-1} NH_4^+ - N produced some degree of inhibition when compared with the control, and 3294 mgl^{-1} caused total inhibition. No attempt was made to distinguish between NH_4^+ and free NH_3.

A study conducted by van Velsen (1979a) appears to be the only one in which seeds adapted to different levels of NH_4^+ - N were tested for susceptibility to ammonia inhibition.

Digested sewage sludge and piggery manure adapted to 815 mgl^{-1} and 2420 mgl^{-1} NH_4^+ - N respectively were subjected to loadings of NH_4Cl, and CH_4 production from fatty acid substrates recorded for up to 85 days incubation at 30°C. Increasing the NH_4^+ - N level of the lower adapted seed caused a lag in gas production which increased as the NH_4^+ - N concentration was raised from 730 mgl^{-1} to 4990 mgl^{-1}. It was suggested that this lag phase was a period of acclimation by the bacteria to higher NH_4^+ - N levels. When the seed adapted to higher NH_4^+ - N concentrations was subjected to levels of between 605 mgl^{-1} and 3075 mgl^{-1}, no lag phase was observed. Digester pH was not measured during the experiments, but at the start the pH was adjusted to 7.0, so the influence of ammonia on pH and the consequent effect on toxicity was eliminated.

In the experiments conducted over the relatively long periods of 85 and 90 days (Braun 1981, van Velsen 1979a) the toxic effects of ammonia were complicated by acclimation by the bacteria which was identified by a lag phase in gas production. The present work was concerned solely with the effects of shock loading ammonia on bacterial metabolism so the experiments were of short duration and no lag phases were observed. Acclimation by bacteria in semi-continuously fed digesters to increasing levels of NH_4^+ - N is reported in Chapter 6.

5.7 Conclusions

1. In poultry manure batch digesters provided with CH_3 COONa as substrate and shock loaded with NH_4Cl, the total gas and CH_4 production was inhibited by a degree proportional to the amount of NH_4Cl added.

2. No absolute threshold concentration for ammonia inhibition was observed. Instead the level at which inhibition began depended on the concentration of NH_4^+ - N to which the seed was adapted.

3. Seed adapted to 3348 mgl^{-1} NH_4^+ - N was more tolerant of ammonia than that adapted to 1098 mgl^{-1}, when the concentration was increased by the same proportion, or to the same level.

4. The addition of NH_4Cl to raise ammonia levels did not produce an increase in digester pH which was observed in Chapter 3 and 4 with naturally occurring high levels of NH_4^+ - N. Due to the more toxic effects of NH_3 than NH_4^+ therefore, the inhibition caused by NH_4^+ - N concentrations produced by NH_4Cl may not be as great as the same levels produced naturally.

5. The residual VFA concentration increased with the amount of NH_4Cl added and therefore degree of inhibition. In conjunction with the inhibition of CH_4 production this suggests that the methanogens are the group affected by ammonia.

6. In batch digesters provided with glucose as substrate and seed adapted to 3066 mgl^{-1} NH_4^+ - N, low doses of NH_4HCO_3 (up to 4600 mgl^{-1} concentration) had a stimulatory effect, but higher doses an inhibitory effect on CH_4 production.

7. When seed adapted to 4359 mgl^{-1} NH_4^+ - N was subjected to NH_4 HCO_3 shock loadings, no stimulatory effect was observed. Instead inhibition of CH_4 production occurred which increased with the amount of NH_4 HCO_3 added.

8. In contrast to experiments in which NH_4Cl was added to raise the NH_4^+ - N concentration, the lower adapted seed appeared to be more tolerant of increases in ammonia levels than the higher adapted seed.

9. In common with NH_4Cl, the addition of $NH_4\ HCO_3$ to raise ammonia levels did not have the same effect on pH as naturally produced concentrations.

THE LONG TERM EFFECTS OF AMMONIA ON THE SEMI-CONTINUOUS DIGESTION OF POULTRY MANURE

6.1 Introduction

Shock loadings of NH_4^+ - N are applicable to situations where ammonia is suddenly introduced into a waste treatment system. However this is not normally the case for a toxicity situation in waste treatment. Usually the toxic material is present in the waste on a continuous basis and its concentration will slowly rise and remain at some equilibrium value in the digester. Such conditions allow adaptation of the microbial population of the digester to the toxic agent. This was observed by Kugelman and Chin (1971) reporting on an investigation of the toxic effects of light metal cations on daily and slug fed digesters. Daily fed digesters showed greater tolerance to the same concentrations of cations than slug fed digesters. Van Velsen (1979a) demonstrated that a period of acclimation was required before gas production started by seed adapted to 815 mgl^{-1} NH_4^+ - N when the NH_4^+ - N concentration was raised to 4,990 mgl^{-1}, but that seed adapted to 2,420 mgl^{-1} required no acclimation. In studies on the digestion of concentrated sewage sludge. Melbinger and Donnellon (1971) concluded that acclimation could prevent ammonia inhibition at concentrations above 1,799 mgl^{-1} as N if the rate of acclimation exceeded that of the formation of NH_4^+ - N.

The present study was conducted to investigate the long term effects of raised ammonia concentrations on the performance of continuously fed digesters, and to test whether a period of acclimation would allow the bacterial population of the digesters to adapt to these concentrations.

6.2 Materials and Methods

These experiments were conducted using 3 or 4 semi-continuously fed poultry manure digesters operated at 29d RT and an influent concentration of 4% TS (2.62% VS) giving a LR of 0.9 kg VS $m^{-3}d^{-1}$. Two digesters, C1 and C2, were operated normally as controls as described in Section 2.4.1.1,

and were actually digesters 11 and 12 in the investigation of GY - LR relationships for poultry manure (Chapter 4), but were continued after these experiments had ended. The treated digesters, T1 and T2, were dosed with NH_4Cl to raise the NH_4^+ - N concentrations above control levels. The amount of NH_4Cl added was adjusted to give the desired NH_4^+ - N concentrations, and dissolved in the influent before feeding. The levels were maintained for up to 20 weeks to allow comparison with control digesters.

The experiment was conducted in two phases. In the first phase (weeks 13 - 28) NH_4^+ - N concentrations of the treated digesters (T1 and T2) were increased to mean levels of 2,752 mgl^{-1} and 3,062 mgl^{-1}, which were 20.9% and 30.6% greater than control levels respectively (Table 6.1). In the second phase (weeks 33 - 54) the mean NH_4^+ - N concentrations in digesters T2 and T3 were 4,837 mgl^{-1} and 4,811 mgl^{-1} respectively (135% and 134% greater than control levels).

6.3 Results

The variation in effluent concentrations and digestion efficiencies for each digester during the experiment are shown in Figures 6.1 - 6.10 and mean values during each phase in Table 6.1.

6.3.1 Ammonium Nitrogen

The NH_4^+ - N levels in the effluent of control digesters, C1 and C2, increased from start-up to 2,194 mgl^{-1} and 2,226 mgl^{-1} respectively by week 13. Digester C1 was stopped in week 22 since the equipment was required for another experiment. During the first phase (weeks 13 - 28) the mean effluent concentrations of C1 and C2 were 2,277 mgl^{-1} and 2,475 mgl^{-1} respectively. In the transition period between the two phases the NH_4^+ - N concentration of C2 decreased to 1,942 mgl^{-1}, and during the second phase (weeks 33 - 54) this digester had a mean concentration of 2,060 mgl^{-1} NH_4^+ - N.

Table 6.1 Variation in Effluent Composition and Digestion Efficiencies for Control and Treated Digesters

	Phase 1											
Digester	C1			C2			T1			T2		
Ammonium nitrogen concentration (mgl^{-1})	2277	61	9	2344	99	14	2752	147	9	3062	174	16
Gas Yield (m^3 kg VS add^{-1})	0.431	0.012	9	0.444	0.018	16	0.251	0.038	9	0.393	0.028	16
Gas composition (% CH_4)	56.8	1.2	10	59.1	1.5	16	57.1	1.66	9	57.4	1.9	15
Methane Yield (m^3 kg VS add^{-1})	0.246	0.011	9	0.263	0.013	16	0.143	0.022	9	0.227	0.015	15
Effluent TS (%)	2.21	0.13	8	2.19	0.12	14	2.20	0.11	8	2.30	0.13	14
Destruction of TS (%)	44.8	3.2	8	44.6	4.0	14	44.5	3.2	8	42.4	3.5	14
Effluent VS (%)	1.08	0.10	8	1.08	0.09	14	1.08	0.07	6	1.19	0.09	14
Destruction of VS (%)	59.1	3.0	8	58.5	5.4	14	58.4	2.8	6	55.4	3.0	14
Effluent alkalinity (mgl^{-1})	14209	910	3	14417	1214	3	14306	746	3	13917	1063	3
Effluent pH	7.70	0.06	8	7.69	0.09	14	7.67	0.04	8	7.62	0.10	14
Effluent free NH_3 concentration (mgl^{-1})	129			130			145			144		

Values are mean, standard deviation, number of measurements.

Table 6.1 continued

	Phase 2								
Digester	C2			T2			T3		
Ammonium nitrogen concentration (mgl^{-1})	2060	240	17	4837	137	17	4811	140	16
Gas Yield (m^3 kg VS add^{-1})	0.496	0.027	18	0.353	0.034	18	0.372	0.054	18
Gas composition (% CH_4)	60.0	0.6	17	60.0	0.9	17	60.1	0.6	17
Methane Yield (m^3 kg VS add^{-1})	0.298	0.014	17	0.213	0.019	17	0.225	0.031	17
Effluent TS (%)	2.12	0.34	18	3.19	0.33	17	3.27	0.39	16
Destruction of TS (%)	46.7	8.2	18	20.6	8.1	18	18.9	9.3	16
Effluent VS (%)	1.21	0.23	18	2.23	0.27	18	2.22	0.30	16
Destruction of VS (%)	59.5	7.2	18	25.3	7.9	18	25.4	8.6	16
Effluent alkalinity (mgl^{-1})	10488	635	16	10922	1193	16	11163	1063	16
Effluent pH	7.53	0.07	17	7.43	0.10	17	7.43	0.07	17
Effluent free NH_3 concentration (mgl^{-1})	79			147			146		

Values are mean, standard deviation, number of measurements.

FIG 6.1 VARIATION IN AMMONIUM NITROGEN CONC. OF CONTROL DIGESTERS DURING EXPERIMENT

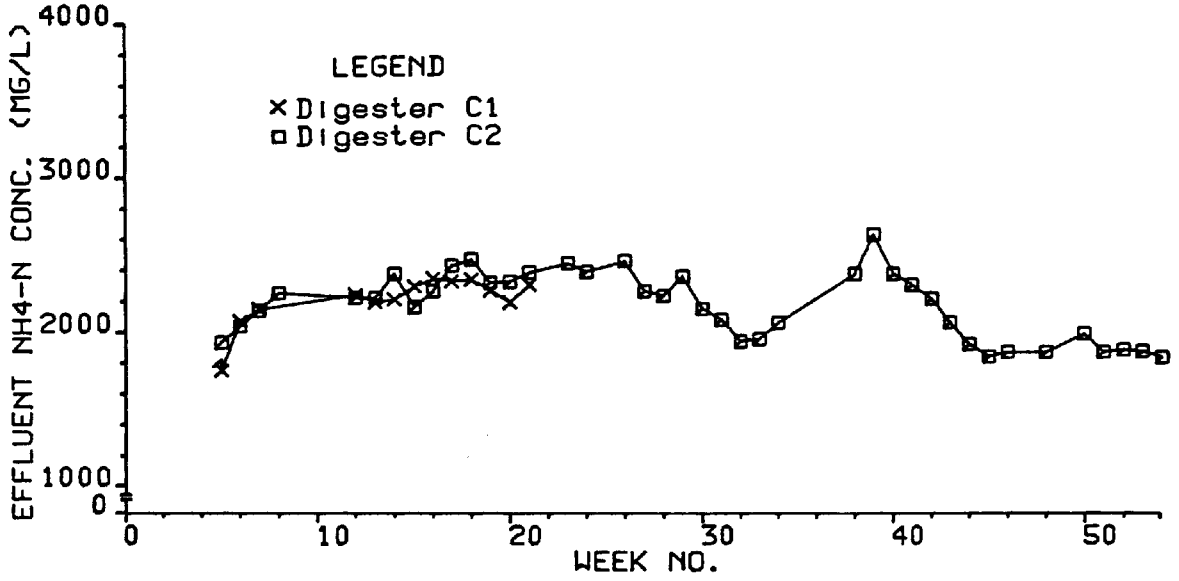
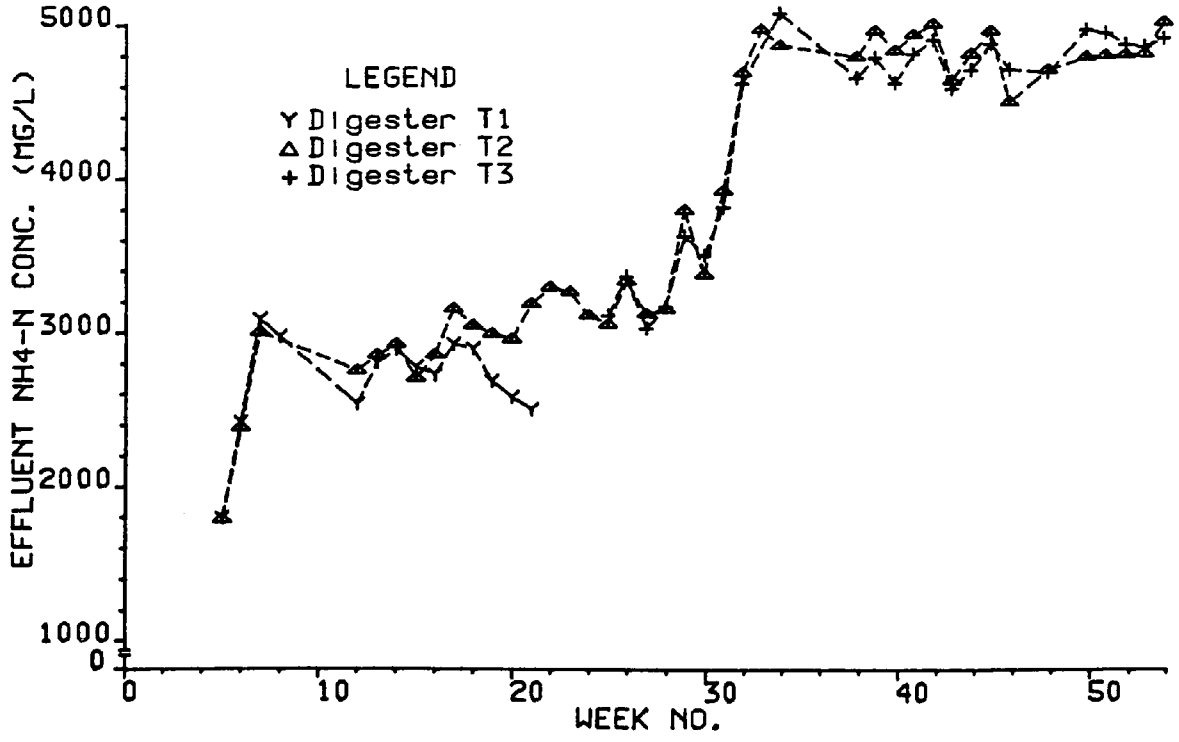


FIG 6.2 VARIATION IN AMMONIUM NITROGEN CONC. OF TREATED DIGESTERS DURING EXPERIMENT



In the sixth week of the experiment daily additions of NH_4Cl to T1 and T2 were initiated and by week 13 NH_4^+ - N levels had reached 2,805 mgl^{-1} and 2,856 mgl^{-1} respectively (Figure 6.2). Because equipment was required for another experiment and the performance of digester T1 was poorer than that of T2, no more additions of NH_4Cl were made to T1 after week 18, and the digester was stopped with C1 in week 22. While NH_4Cl was being added to T1 during the first phase the mean NH_4^+ - N concentration was 2,752 mgl^{-1} which was 20.9% greater than that of C1 during the same period. The mean concentration of T2 during Phase 1 was 3,062 mgl^{-1}, 30.6% greater than that of C2. After week 28 additions of NH_4Cl to T2 were increased and during Phase 2 the mean NH_4^+ - N concentration was 4,837 mgl^{-1}, an increase of 135% over C2 levels.

An additional treated digester, T3, was started up on week 25 seeded with effluent from T2 with the same daily NH_4Cl additions, to provide a duplicate for T2 and replace T1. The mean NH_4^+ - N concentration of T3 during Phase 2 was 4,811 mgl^{-1} which was 134% greater than the C2 level.

6.3.2 Gas Yields

The GY's of C1 were consistently lower than those of C2 in the first 8 weeks of the experiment (Figure 6.3). During weeks 9 and 10 feeding was stopped but the digesters continued to produce gas. Both control digesters recovered quickly when feeding was resumed in week 11 and produced similar GY's with means for Phase 1 of 0.431 m^3 kg VS $added^{-1}$ (C1), and 0.444 m^3 kg VS $added^{-1}$ (C2). Between weeks 29 and 34 the GY's produced by C2 remained relatively stable, then dropped to 0.456 m^3 kg VS $added^{-1}$ in week 38 after a second break in feeding of 3 weeks. The mean control GY for the second phase was 0.496 m^3 kg VS $added^{-1}$.

Up to the eighth week GY's of the treated digesters were between those of the controls, but produced less gas during the break (Figure 6.4). After the first break the GY of T1 increased initially but then dropped to 0.186 m^3 kg VS $added^{-1}$ by week 15, followed by a further increase to 0.283 m^3 kg VS $added^{-1}$ in week 21. The mean GY of T1 during Phase 1 was

FIG 6.3 VARIATION IN GAS YIELD OF CONTROL DIGESTERS DURING EXPERIMENT

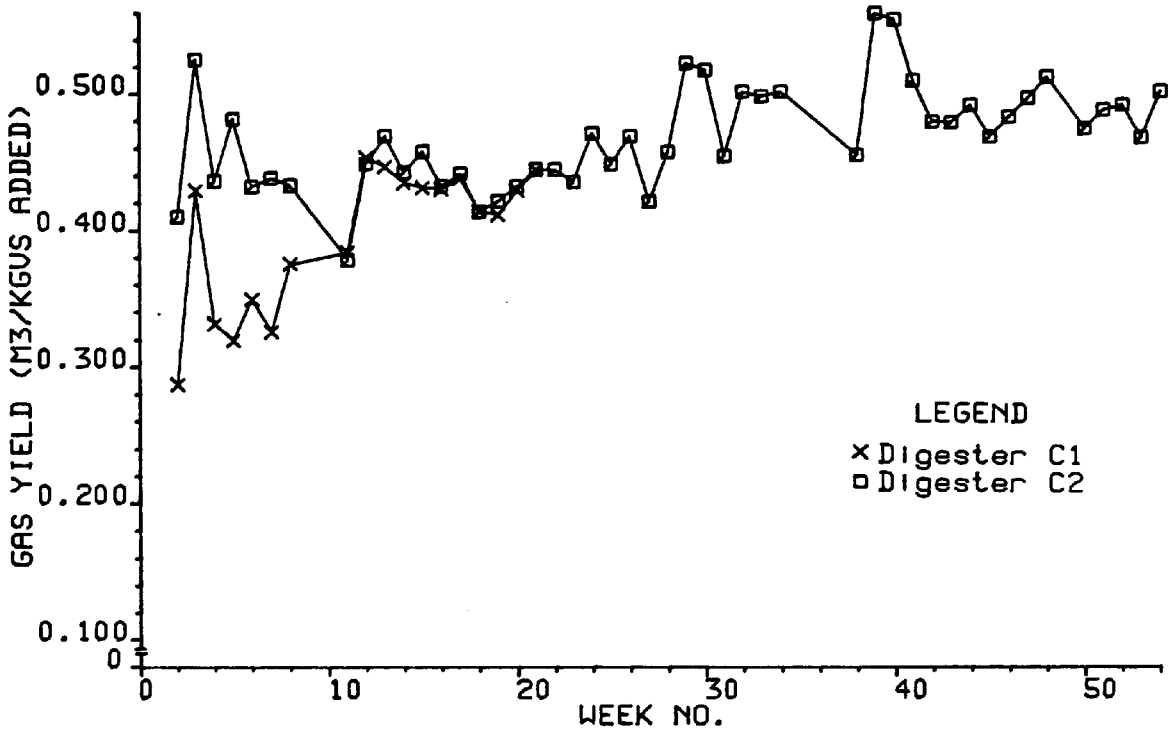
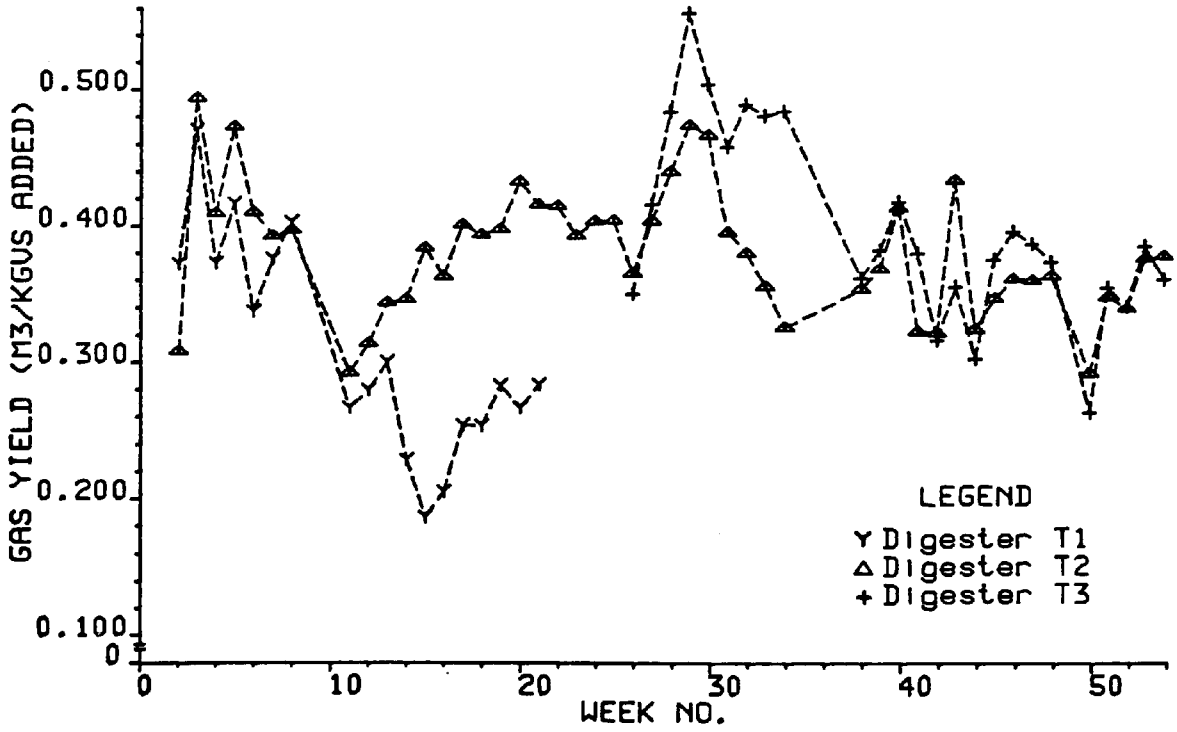


FIG 6.4 VARIATION IN GAS YIELD OF TREATED DIGESTERS DURING EXPERIMENT



0.251 m^3 kg VS added^{-1}, a 48% reduction in GY compared with C1.

GY's of T2 increased slowly from week 10 and by week 20 had reached control levels, only to drop again in the following 6 weeks. Control GY's were again approached in weeks 27 and 28, and during Phase 1 T2 produced a mean GY of 0.393 m^3 kg VS added^{-1}, a decrease in the C2 GY of 11.5% GY's of T2 dropped sharply from weeks 29 to 34 and during Phase 2 were on average 0.353 m^3 kg VS added^{-1}, a 28.8% reduction of the control value.

Digester T3 produced similar GY's to C2 upto week 34 then decreased to T2 levels which were then followed closely to week 54. The mean GY of T3 during Phase 2 was 0.372 m^3 kg VS added^{-1} which is 25.0% less than the control GY.

6.3.3 Gas Composition

Due to the low reproducibility of the method used for the analyses of gas composition during the first phase there were relatively large fluctuations in the CH_4 content of the gas (Figures 6.5 and 6.6). Digester C1 consistently produced gas of lower CH_4 content than C2 with means for Phase 1 of 56.8% and 59.1% respectively, the difference made up by CO_2. Treated digesters produced intermediate values of 57.1% (T1) and 57.4% (T2).

In Phase 2 the second of the gas chromatographic methods described in Section 2.5.14 was used for analyses and measurements were more reproducible. After an initial decrease the CH_4 content of the gas from all digesters increased to between 60.5% and 60.7% by week 54. The gas composition was similar for control and treated digesters with means of 60.0% (C2), 60.0% (T2) and 60.1% (T3).

6.3.4 Total Solids

The TS content of the effluent from control and treated digesters followed the same pattern during Phase 1 and increased from start-up for

FIG 6.5 VARIATION IN COMPOSITION OF GAS PRODUCED BY CONTROL DIGESTERS DURING EXPERIMENT

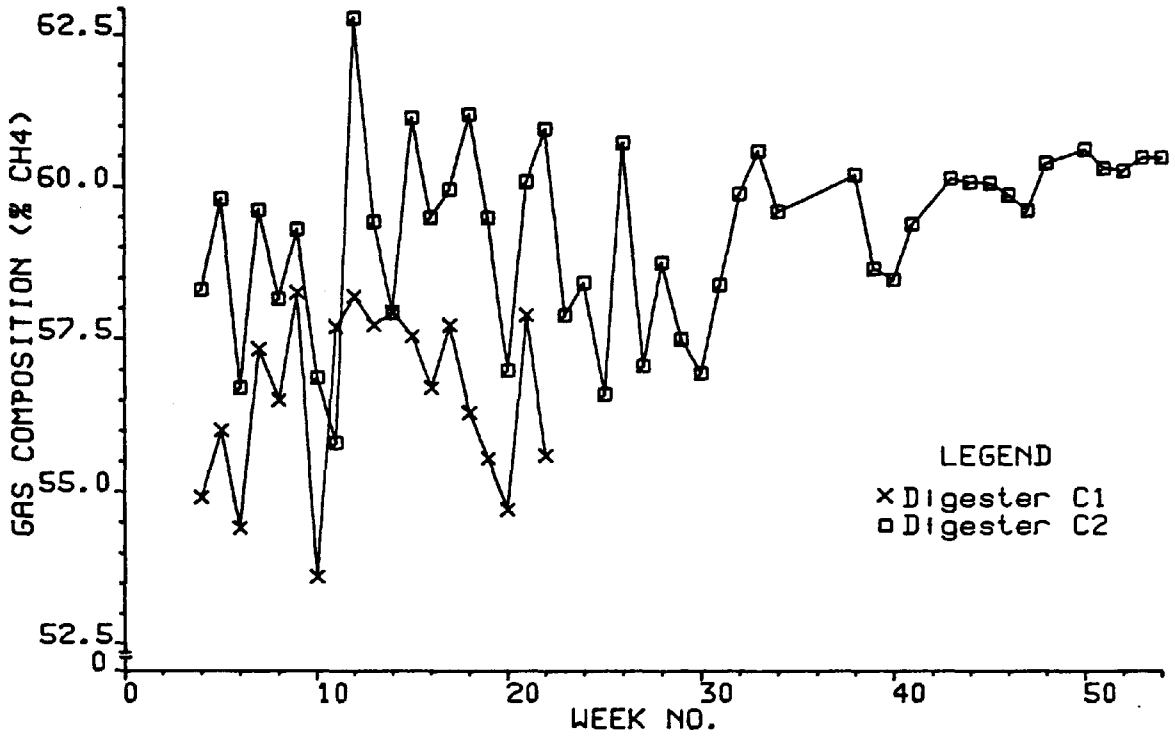
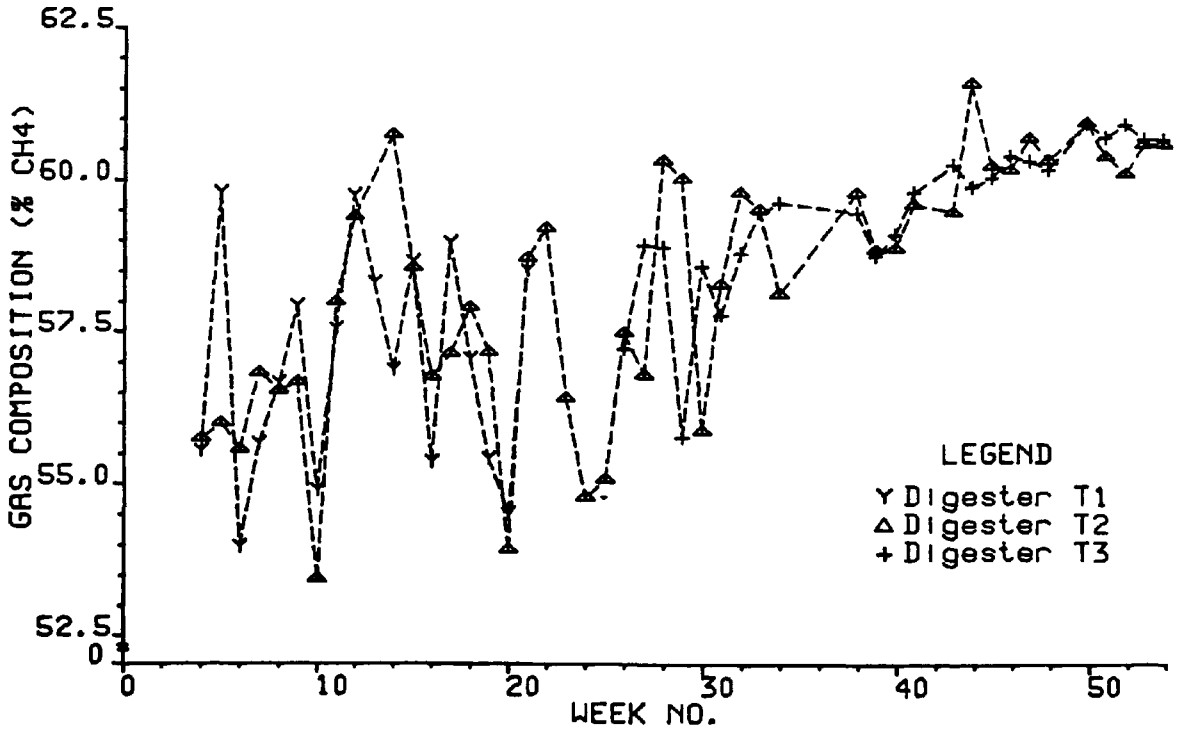


FIG 6.6 VARIATION IN COMPOSITION OF GAS PRODUCED BY TREATED DIGESTERS DURING EXPERIMENT



7 weeks then became more stable until week 28 (Figure 6.7). The mean effluent TS of the control digesters during this period was 2.20%, and of the treated digesters 2.25%.

After week 28 the control digester effluent TS decreased to 1.49% on week 41, then increased in the next two weeks and remained stable between 1.79% and 2.62% for the remainder of Phase 2 with a mean value of 2.12%. The TS in the effluent of the treated digesters increased between weeks 30 and 32 then dropped to approx. 2.60% in week 38 before increasing steadily to week 54. The effluent TS of T2 and T3 was consistently higher than that of C2 during Phase 2 with a mean of 3.23%.

The destruction of TS was slightly higher for control than treated digesters during Phase 1 (44.7% and 43.4% respectively) but more than double that of treated digesters in Phase 2 (46.7% and 19.8% respectively).

6.3.5 Volatile Solids

The variation in digester effluent VS content was similar to that of effluent TS (Figure 6.8). In Phase 1 the VS of the effluent from T1 was equal to that of control digesters, while T2 effluent VS was higher by 0.11%. During Phase 2 the effluent VS of both treated digesters was higher by 1.02% on average than that of the control.

As with TS destruction, the destruction of VS was slightly higher for control digesters during Phase 1, and more than double that of treated digesters in Phase 2 (Table 6.1).

6.3.6 Total alkalinity

The alkalinity of all digesters increased rapidly during the first 19 weeks from between 7,200 mgl^{-1} and 9,100 mgl^{-1} to between 15,000 mgl^{-1} and 15,750 mgl^{-1} (Figure 6.9). No alkalinity measurements were made in weeks 20 - 37. The alkalinity of all digesters increased from week 38 to week 39, but then decreased slowly for the next 11 weeks and levelled off

FIG 6.7 VARIATION IN TOTAL SOLIDS CONC. OF CONTROL AND TREATED DIGESTERS DURING EXPERIMENT

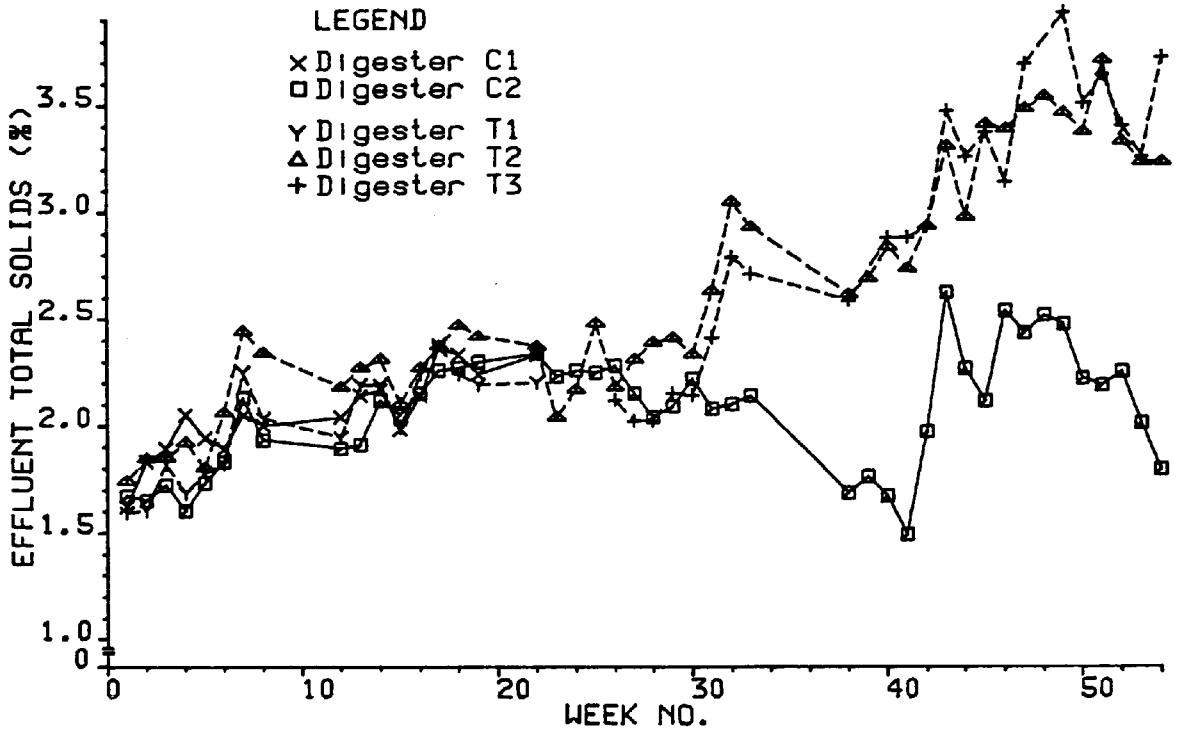


FIG 6.8 VARIATION IN VOLATILE SOLIDS CONC. OF CONTROL AND TREATED DIGESTERS DURING EXPERIMENT

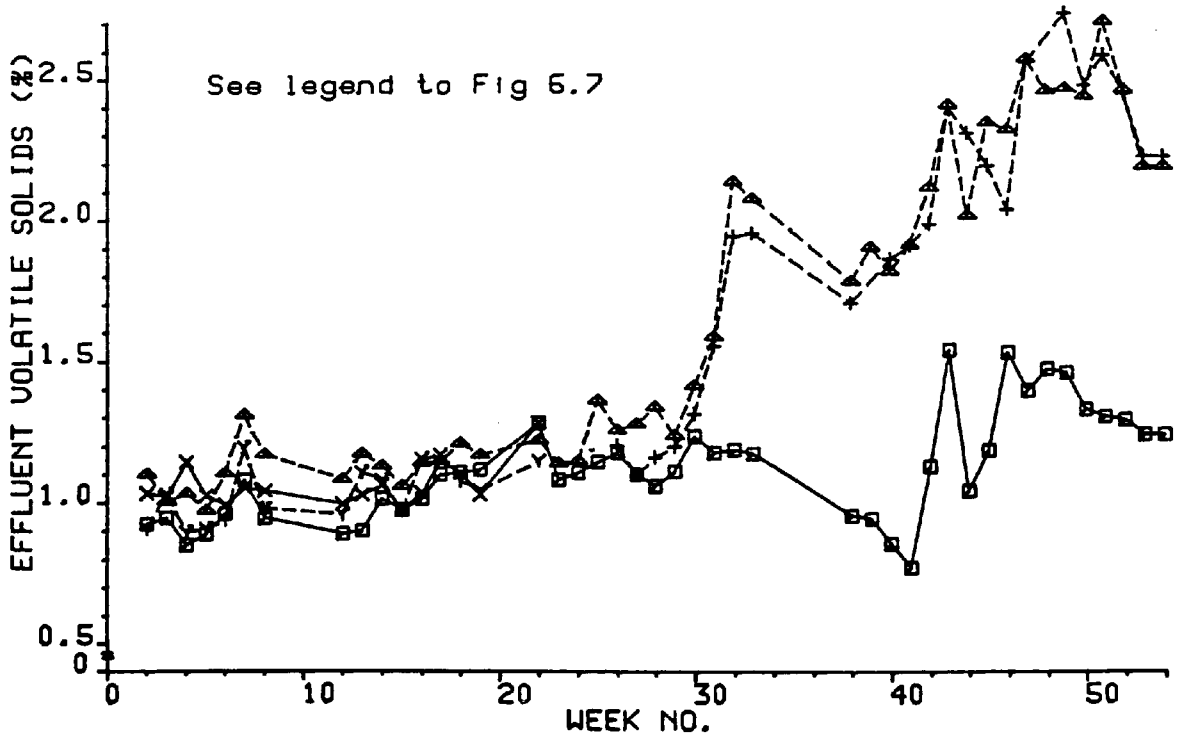


FIG 6.9 VARIATION IN ALKALINITY OF CONTROL AND TREATED DIGESTERS DURING EXPERIMENT

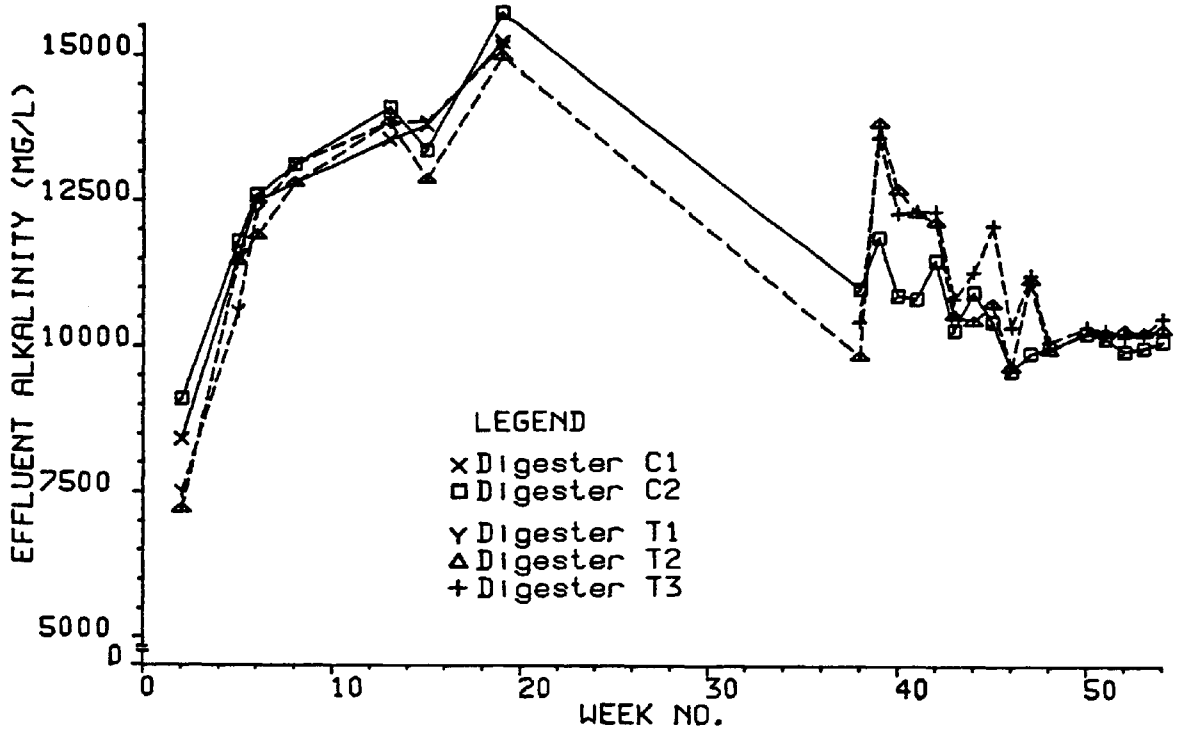
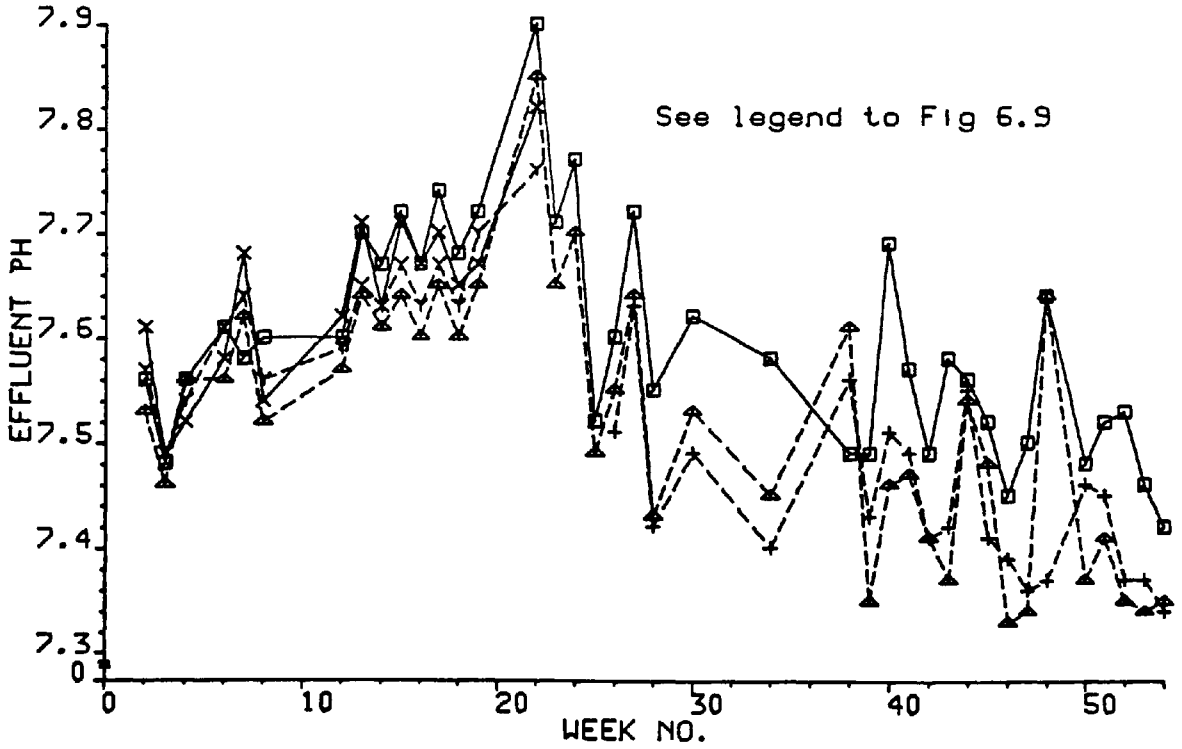


FIG 6.10 VARIATION IN pH OF CONTROL AND TREATED DIGESTERS DURING EXPERIMENT



at week 50. The alkalinity was similar for control and treated digesters during Phase 1 at approx. 14,200 mgl^{-1} on average, but during Phase 2 the alkalinity of T2 and T3 was higher than that of C2 (11,043 mgl^{-1} (mean) compared with 10,488 mgl^{-1}).

6.3.7 pH

From start-up the effluent pH of all digesters increased to a maximum of between 7.76 and 7.90 on week 22 after which it decreased sharply to week 28, and then more gradually to week 54 (Figure 6.10). During both phases the pH of the control digesters was higher than that of the treated digesters by up to 0.1 unit (Table 6.1).

6.3.8 Volatile Fatty Acids

The concentration of VFA's in the digesters was not measured routinely and only a limited number of determinations were made during each phase. In Phase 1 all VFA concentrations were below 600 mgl^{-1} with treated values slightly higher than control values.

In Phase 2 VFA concentrations reached 1,550 mgl^{-1} and again the concentration was greater in treated than control digesters, although in both phases the difference between control and treated values was not significant.

6.4 Discussion

6.4.1 Gas Yields

The interpretation of results obtained during Phase 1 is complicated by the break in feeding during weeks 9 and 10 soon after the NH_4^+ - N concentrations of the treated digesters had been increased. The recovery of gas production by the control digesters after the break was rapid and two weeks after feeding had been resumed GY's had regained previous levels (Figure 6.3). However, due to the combination of raised NH_4^+ - N levels

and the break in feeding, the recovery of the treated digesters was slower than that of the controls (Figure 6.4).

In an attempt to demonstrate that the poor performance of digester T1 was due to raised NH_4^+ - N concentrations, additions of NH_4Cl were stopped after week 18, and NH_4^+ - N levels in this digester began to decrease. However the progressive increase in GY expected from reduced NH_4^+ - N concentrations was not obtained, so either the toxic effects of ammonia are not reversible, or the performance of this digester was affected by other factors besides ammonia. The pH of T1 was consistently higher than that of T2 which may have influenced ammonia inhibition, but the mean free NH_3 concentrations between weeks 11 and 21 were not significantly different (145 mgl^{-1} TI, 144 mgl^{-1} T2). It is possible that T1 may have recovered fully given time, but since equipment was required for another experiment, this digester was stopped in week 22 in favour of T2 which was allowed to continue.

During Phase 1 neither treated digester showed obvious signs of adaptation to raised NH_4^+ - N levels which would be characterised by achieving control GY's after a period of reduced yields. In weeks 27 and 28 however, the GY's of T2 improved and did approach control levels, but in week 28 the GY's of C2 and T3 also increased suggesting that the improvement was due to variations in feed composition during these weeks and not adaptation.

When NH_4Cl additions were increased in week 29, the GY of T2 decreased progressively in the following 5 weeks and levelled off when the NH_4^+ - N concentration had reached 4800 mgl^{-1}. Digester T3 however, maintained control GY's from start up for these 5 weeks before dropping to T2 levels after the second break in feeding. The reason for this slow response is not clear. It cannot be due to the requirement by the bacteria in a new digester for a period of exposure to raised NH_4^+ - N levels before the effects are observed, since the seed was provided by digester T2 which had been subject to high concentrations of NH_4^+ - N for the previous 23 weeks. However since it took 4 weeks to collect enough effluent from

T2 to fill the new digester, by the time daily feeding began the biological and chemical composition of the contents of T3 would not have been identical to that of T2, so a different response would not be unexpected.

As in Phase 1 neither treated digester showed signs of adaptation to raised NH_4^+ - N levels by improvement of GY's even after 22 weeks exposure. When comparing GY's of the treated digesters with those of C2 it must be noted that during stabilisation digester C2 produced higher GY's than all other digesters and may have continued to produce more gas than the treated digesters without the addition of NH_4Cl, thus increasing the apparent toxic effect of ammonia. However during stabilisation the GY's of the other control digester (C1) were lower than all other digesters, but after the break in feeding the GY's were very close to those of C2. Thus it is possible that in the absence of NH_4Cl additions the GY's of the treated digesters after the break would also have approached those of C2.

Of the four digesters started up, those giving intermediate GY's were the ones selected for NH_4Cl treatment since they showed no tendency for excessively high or low gas production.

In the study conducted by Braun (1981) the NH_4^+ - N concentration of continuously fed piggery manure digesters was raised to 3,000 mgl^{-1} by feeding manure of high NH_4^+ - N content. This depressed gas production and was accompanied by an increase in pH and VFA concentration which could be reversed by adjustment of the pH to 7.4. Neither the increase in NH_4^+ - N concentration nor the decrease in GY were quantified, but after pH adjustment NH_4^+ - N levels remained around 3,000 mgl^{-1} and GY's remained depressed and did not regain previous levels.

By adding urea to laboratory digesters fed on a synthetic substrate Kroeker et al (1979) raised NH_4^+ - N levels to between 2,000 mgl^{-1} and 5,000 mgl^{-1} (108 mgl^{-1} - 256 mgl^{-1} NH_3). After a period of two months acclimation CH_4 production was inhibited by a degree proportional to the NH_4^+ - N concentration.

Thus it appears that in digesters operated with elevated NH_4^+ - N levels gas production is depressed, and even after two months acclimation does not improve or regain previous or control levels. Complete adaptation therefore does not occur. Progressive increases in NH_4^+ - N concentration depress CH_4 production until a level is reached when production ceases. This concentration was found by Kroeker et al (1979) to be 6,700 mgl^{-1} although the bacteria were shown to be still viable.

6.4.2 Gas Composition

It is not clear why digester C2 consistently produced gas of higher CH_4 content than C1 during the first phase. The pH of the digesters was similar during this phase, but the alkalinity of C2 was higher than that of C1. However this should reflect a lower CH_4 content for C2 and not higher as observed. Although consistent, differences in gas composition were small and since there was a wide variation for each digester differences were not significant.

During Phase 2 there was much less variation in the composition of the gas from all digesters since a more sensitive and accurate machine was used for analyses. Additionally the time between taking the sample from the digester and analyses was reduced, thus decreasing the possibility of gas diffusing out of the syringe and improving accuracy. Even with the increased accuracy of gas analyses and higher NH_4^+ - N levels, no significant differences were observed between control and treated digesters. The CH_4 content of all digesters increased gradually from week 38 until the end of the experiment. This coincided with a decrease in both alkalinity and pH (Figures 6.9 and 6.10) which should reflect a constant gas composition so an increase would not be expected. Thus the depression of GY's caused by raised NH_4^+ - N levels was the result of both CH_4 and CO_2 production being reduced by equal proportions. This suggests that in addition to the methanogens, the fermentative and acidogenic bacteria were also inhibited by NH_4Cl additions. This is in contrast to the results obtained by Kroeker et al (1979) where the CH_4 content of the gas decreased gradually as the NH_4^+ - N concentration was raised to 7,000 mgl^{-1}.

However differences between the two studies are not unexpected due to the different materials used as a source of ammonia.

Since there were no significant trends in gas composition or significant differences between control and treated digesters during the present study, methane yields followed the same pattern as biogas yields.

6.4.3 Total Solids

The increase in effluent TS of all digesters at the beginning of the experiments was due to the fact that they were started up with seed of lower TS (approx. 1.7%) than the influent (4%). Digester TS therefore increased until stable conditions were attained by week 7 at 2.1% - 2.4% TS (Figure 6.7). Most digesters showed a decrease in effluent TS after the first break in feeding since gas was still being generated and solids destroyed during the break.

In Phase 1 raised NH_4^+ - N concentrations had no observable effects on effluent TS although more solid material must have been degraded in control GY's to produce higher GY's.

From weeks 30 to 32 the effluent TS of the treated digesters increased sharply above control levels which was probably caused by the increase in dose rate of NH_4Cl, and was accompanied by a decrease in GY. After the break during weeks 34 to 37 there was again a reduction in the effluent TS of all digesters as observed in Phase 1.

Although reduced GY's must be due to the lower destruction of solids in the treated digesters, the lower figures may only have been apparent and due to the residue of NH_4Cl in the effluent which increased TS. In the second phase the NH_4^+ - N concentrations of T2 and T3 were raised by 2,777 mgl^{-1} and 2,751 mgl^{-1} respectively above that of the control digester. These increases represent weights of 53.05g and 52.55g of NH_4Cl respectively in each digester, equivalent to TS increases of 1.06% and 1.05%. When substracted from the TS values obtained for T2 and T3, the

actual TS drops to 2.13% and 2.22% respectively, only slightly higher than the control TS of 2.12%. When used to recalculate TS destruction these figures produce 46.5% and 44.2%, slightly lower than the control value of 46.7%. Thus it appears that the differences observed in TS destruction figures between control and treated digesters was due to the presence of NH_4Cl in the treated digesters, rather than a smaller amount of solid material degraded.

6.4.4 Volatile Solids

Since the residue from NH_4Cl is volatilised at 500°C, the effluent VS of the treated digesters during Phase 2 was artificially high and VS destruction artificially low. Subtracting the amount of TS attributable to NH_4Cl from the VS results gives a figure of 1.17% VS for both treated digesters which is slightly lower than the 1.21% of the control, and a VS destruction of 60.8%, slightly higher than that of the control value of 59.5%.

The effect is noticeable of breaks in feeding on effluent VS which decreased during the break since volatile matter was destroyed during gas production (Figure 6.8).

6.4.5 Total alkalinity

The rapid increase in the alkalinity of all digesters during the first 7 weeks of the experiment was probably due to the increase in digester TS, which influences alkalinity, over this period (Figures 6.9 and 6.7). However after digester TS had levelled off by week 8, alkalinity continued to increase until week 19. Similarly the decrease in alkalinity from week 39 to 49 has no obvious explanation since no such trend occurred in digester solids nor NH_4^+ - N content during this period.

From the relationships observed in Chapters 3 and 4 between NH_4^+ - N and alkalinity, the alkalinity of the treated digesters would be expected to be higher than that of the controls, In Phase 1 there were no

significant differences in alkalinity between control and treated digesters, and in Phase 2 the alkalinity of T2 and T3 was higher than that of C2 but by only 550 mgl^{-1}, where a difference of 16,600 mgl^{-1} would be expected. Thus concentrations of NH_4^+ - N artificially raised by addition of NH_4Cl do not have the same effect on alkalinity as naturally produced concentrations. This fact was confirmed by alkalinity determinations made on a 100ml sample of C2 effluent to which NH_4Cl was added in 0.5g increments. Alkalinity actually decreased slightly with increasing addition of NH_4Cl.

6.4.6 pH

The increase in pH of all digesters to week 22 then decrease to week 54 were accompanied by and possibly caused by the same changes in digester alkalinity (Figures 6.9 and 6.10). An increase in both pH and alkalinity should reflect a constant gas composition according to the equilibrium which exists between the CO_2 in the digester gas and the alkalinity of the digester contents. The gas composition did fluctuate but the variation was low when compared with that of pH and alkalinity of the digester contents.

In Chapters 3 and 4 it was observed that high NH_4^+ - N levels resulted in a high digester pH, and in the study conducted by Braun (1981) when the NH_4^+ - N concentration of continuously fed piggery manure digesters was increased by feeding manure of high NH_4^+ - N content, the digester pH increased to 8.0. However in the present study raising NH_4^+ - N levels artificially had the opposite effect which was also noted in the batch digestion tests in Chapter 5, and confirmed by adding NH_4Cl to a 100ml sample of effluent. The addition of NH_4Cl therefore raises the digester NH_4^+ - N concentration, but does not affect the pH in the same way as ammonia produced naturally by uric acid and protein degradation. As stated in Section 5.3.2.2 since pH affects ammonia toxicity, the inhibitory effects of artificially raised NH_4^+ - N levels may not be as great as the same naturally produced concentrations.

The free NH_3 concentrations of the treated digesters remained constant throughout the experiment even though the NH_4^+ - N level was increased from approx. 3,000 mgl^{-1} to 4,800 mgl^{-1} (Table 6.1). This was due to the reduced pH during Phase 2 which also affected the control digester, and was probably due to changes in feed composition. The NH_4^+ - N concentration of C2 also decreased in Phase 2 and combined with the reduction in pH produced a decrease in free NH_3 from 130 mgl^{-1} in Phase 1 to 79 mgl^{-1} in Phase 2. Therefore the free NH_3 levels of the treated digesters, although constant throughout the experiment, were 11.5% greater than that of the control digesters during Phase 1 and 86% greater during Phase 2. Thus when compared with the controls the treated digesters would be expected to give a poorer performance during the second phase. When comparing the performance of treated digesters in the two phases, the GY's decreased despite a constant free NH_3 concentration. This suggests that the NH_4^+ ion is also involved in ammonia toxicity which has been reported by McCarty (1964) above NH_4^+ - N concentrations of 3,000 mgl^{-1}. Thus it appears that in the present investigation inhibition produced by NH_4Cl was related to total NH_4^+ - N rather than free NH_3 levels alone.

6.4.7 Volatile Fatty Acids

The fact that the concentration of VFA's in the treated digesters was not significantly higher than in the control digesters suggests that the methanogens were not the only bacterial group to be inhibited by NH_4Cl additions. This is in agreement with the observation that the proportion of CO_2 in the gas produced did not increase when the GY of the treated digesters was depressed.

6.5 Conclusions

1. Raising the NH_4^+ - N concentration of two semi-continuously fed poultry manure digesters by 21% and 31% above control levels to 2,752 mgl^{-1} and 3,062 mgl^{-1} respectively caused a slow and incomplete recovery of gas production after a two week break in feeding.

2. After 6 and 16 weeks exposure to these NH_4^+ - N concentrations the GY's of the treated digesters failed to reach control levels and remained depressed by 42% and 12% respectively. However the greater depression did not appear to be entirely due to the effects of NH_4^+ - N since the GY did not increase substantially when the NH_4^+ - N level was reduced.

3. When NH_4^+ - N concentrations were increased by 135% over control levels to 4824 mgl^{-1} mean, gas production was reduced by 25% - 27%. After 22 weeks exposure to this concentration gas production again failed to recover.

4. The composition of the gas produced was unaffected by the elevated NH_4^+ - N concentrations, and the levels of VFA's in the treated digesters was not significantly higher than in the control digesters which indicates that the depression of GY's was not due to the inhibition of methanogenic bacteria alone.

5. Raising NH_4^+ - N levels by addition of NH_4Cl did not have the effect of increasing digester pH and alkalinity which was observed for naturally produced high concentrations of NH_4^+ - N in Chapters 3 and 4.

6. The destruction of TS and VS was lower in treated than control digesters mainly due to the presence of NH_4Cl residue in the effluent of treated digesters which raised the solids content.

CHAPTER 7

THE DIGESTION OF POULTRY WASTES AT HIGH SOLIDS CONCENTRATIONS

7.1 Introduction

Many potential substrates for AD are produced in a relatively dry state. These include crop residues, animal wastes using crop residues for bedding, and poultry wastes. Conventional high rate digestion in stirred tank reactors involves diluting the waste with large quantities of water. This renders the process less economical since energy is required to heat up the volume of water as well as the waste material itself. The process economics can be improved by digesting the waste in an undiluted state or with the addition of the minimum volume of water.

Jewell (1982) reporting on investigations of the dry (15% TS) fermentation of corn stover and wheat straw at laboratory and pilot scale, at mesophilic and thermophilic temperatures, concluded that crop residues can be digested in a dry state without generating major water needs or problems. The successful digestion of dairy manure at high solids concentrations in digesters fed on alternate days has been demonstrated by Hills (1980) , and in batch digesters by Wong-Chong (1975), but the latter author reported the failure of poultry manure digestion at solids concentrations of 20% TS or higher, due to the formation of high levels of NH_4^+ - N.

Rijkens (1981) has achieved complete digestion of solid organic matter at 35°C in batch reactors by percolating water through the decaying solid mass. The effluent was treated in a conventional continuous digester before recirculation to the batch digester. The investigation of the single stage batch digestion of solid poultry wastes by percolating liquor through a bed of waste is reported here, while the possibility of digesting poultry litter in a 2 stage process was examined by testing the digestibility of the liquor in daily fed and batch digesters.

7.2 Materials and Methods

7.2.1 Apparatus

A 10 litre cylindrical digester was constructed from a flat perspex sheet as shown in Figure 7.1. The waste material to be digested was placed in a nylon mesh bag and supported on a perforated perspex plate at the bottom of the digester. A second perforated plate above the waste dispersed the liquor which was circulated through the digester by a peristaltic pump (Watson Marlow Ltd, Falmouth, UK) via rubber tubing. Silicone rubber tube was used through the pump.

Samples of liquor were removed and replaced by syringe via a'T' piece inserted in the rubber tubing. A siphon system prevented entry of air during sampling while the gas meter was clamped off. Ports in the lid were used for the gas exit tube to the meter and a manometer. The gas produced was sampled from a port in the side of the digester above the bed of waste material.

The digester was enclosed in a polystyrene cabinet to minimise heat loss. Two methods of heating were tested. The first was electrothermal tape wrapped round the digester body which was controlled manually via a transformer, and the second was a domestic fan heater controlled automatically via a temperature sensor within the digester and a thermostat. The temperature was measured by thermocouples inserted through ports in the side of the digester body.

The material to be digested was weighed into a nylon mesh bag and placed in the digester with diluted effluent from poultry manure digesters as seed and liquor. The lid was sealed with clear silicone sealant (Loctite, Welwyn, UK), bolted down and the system flushed with nitrogen for two minutes before the pump was switched on. The silicone tubing passing through the pump was replaced at intervals to avoid leaks caused by wear. Each time the liquor was sampled for analysis the same volume of fresh diluted effluent was introduced into the digester to maintain the liquor volume.

FIG 7.1 HIGH SOLIDS DIGESTER

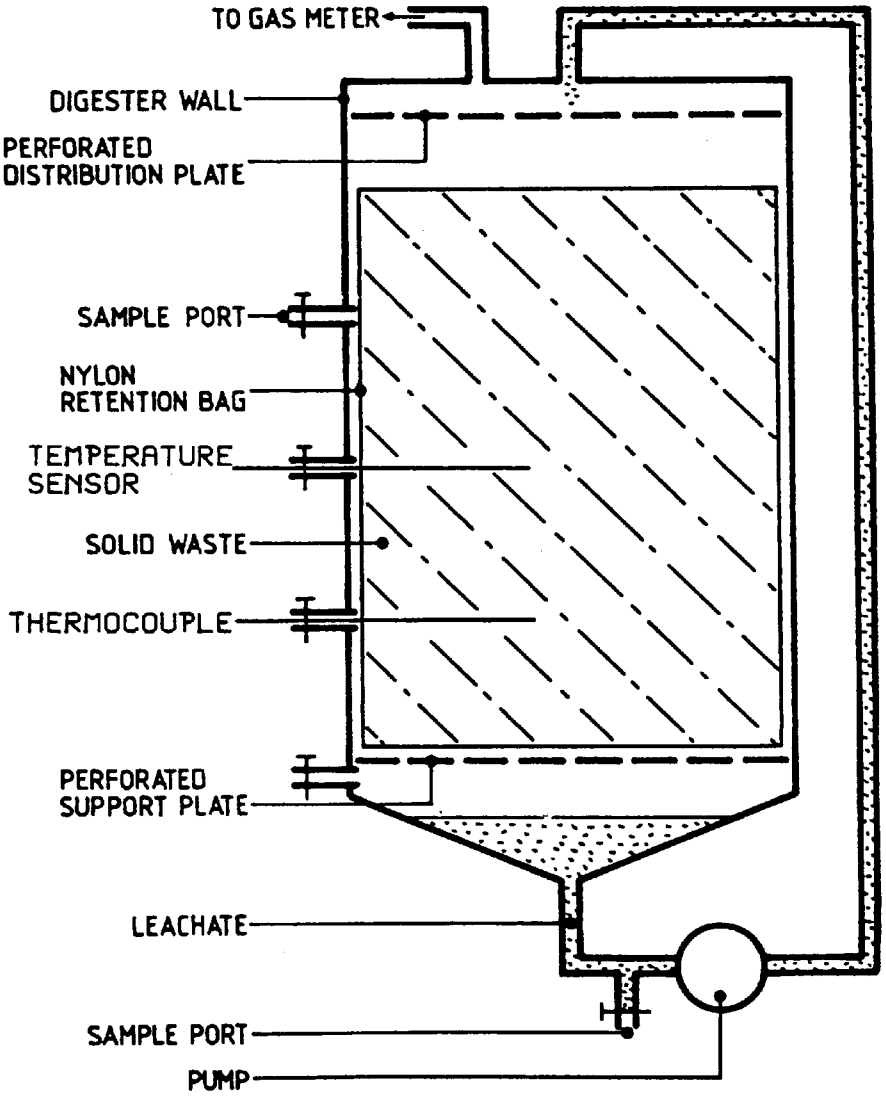


Table 7.1 Digester Loading at Start-up.

Experiment	Waste type	Wet Weight of waste (kg)	Solids content of waste		Volume of seed (1)	Volume of water (1)	Overall solids content of digester		Duration of experiment (days)
			% TS	% VS			% TS	% VS	
1	Manure	2.7	47.3	60.0	1.5	1.5	22.8	13.7	28
2	Manure	2.0	47.3	60.0	1.5	1.5	19.4	11.6	51
3	Litter	2.9	75.3	89.1	2.65	2.65	27.1	24.0	66

7.2.2 Operation

Three high solids digestion experiments were conducted; the first two on poultry manure, and the third on poultry litter. The solids content and weight of raw material used in each experiment are shown in Table 7.1 together with the overall digester solids concentration taking into account both raw waste and liquor.

All digesters were seeded with mixed effluent from poultry manure digesters operated at 29d RT and influent concentrations between 2% and 6% TS, diluted with the same volume of water. Liquor was sampled several times each week for solids, pH, alkalinity, NH_4^+ - N and VFA determinations. Gas production was monitored daily and composition measured at intervals.

Three experiments were conducted to determine the potential of liquor from Experiment 3 for digestion in daily fed digesters. To a 2 litre conical flask was added 500ml diluted mixed effluent as seed, together with 800ml water and 200ml liquor diluted 50 : 50 with water. After flushing with nitrogen for 30 seconds the flask was incubated in a water bath at 35°C. Each day the flask was fed with 200ml diluted liquor after removal of the same volume of contents. Gas production was monitored daily.

7.3 Results

7.3.1 Experiment 1

7.3.1.1 Start-up

The digester was filled with 2.7 kg frozen poultry manure (batch 2 of the semi-continuous digestion experiments in Chapter 4), and seeded with 1.5l mixed effluent diluted with 1.5l water. The initial percolation rate of approx. 100ml per min. was found to be too high so this was reduced then increased gradually until an optimum of 20ml per min. was obtained.

The electrothermal tape used to heat the digester for most of this experiment produced a temperature gradient of approx. 8°C within the digester. The temperature stabilised at approx 41°C at the bottom and approx. 33°C at the top of the digester. After 17 days the tape developed a fault and the internal temperature dropped to 21° - 22°C. While the digester was not being heated a leak was observed in the digester caused by localised heating by the tape (up to 70°C) which melted the perspex cement in the seam of the digester body. The digester was repaired and from day 22 onwards a fan heater, which did not produce localised heating, replaced the tape and maintained a more uniform internal temperature of between 28° and 31°C, with only 1°C difference between top and bottom. The experiment was stopped after 28 days. Figure 7.2 shows the cumulative gas production and variation in liquor composition during the course of the experiment.

7.3.1.2 Gas production

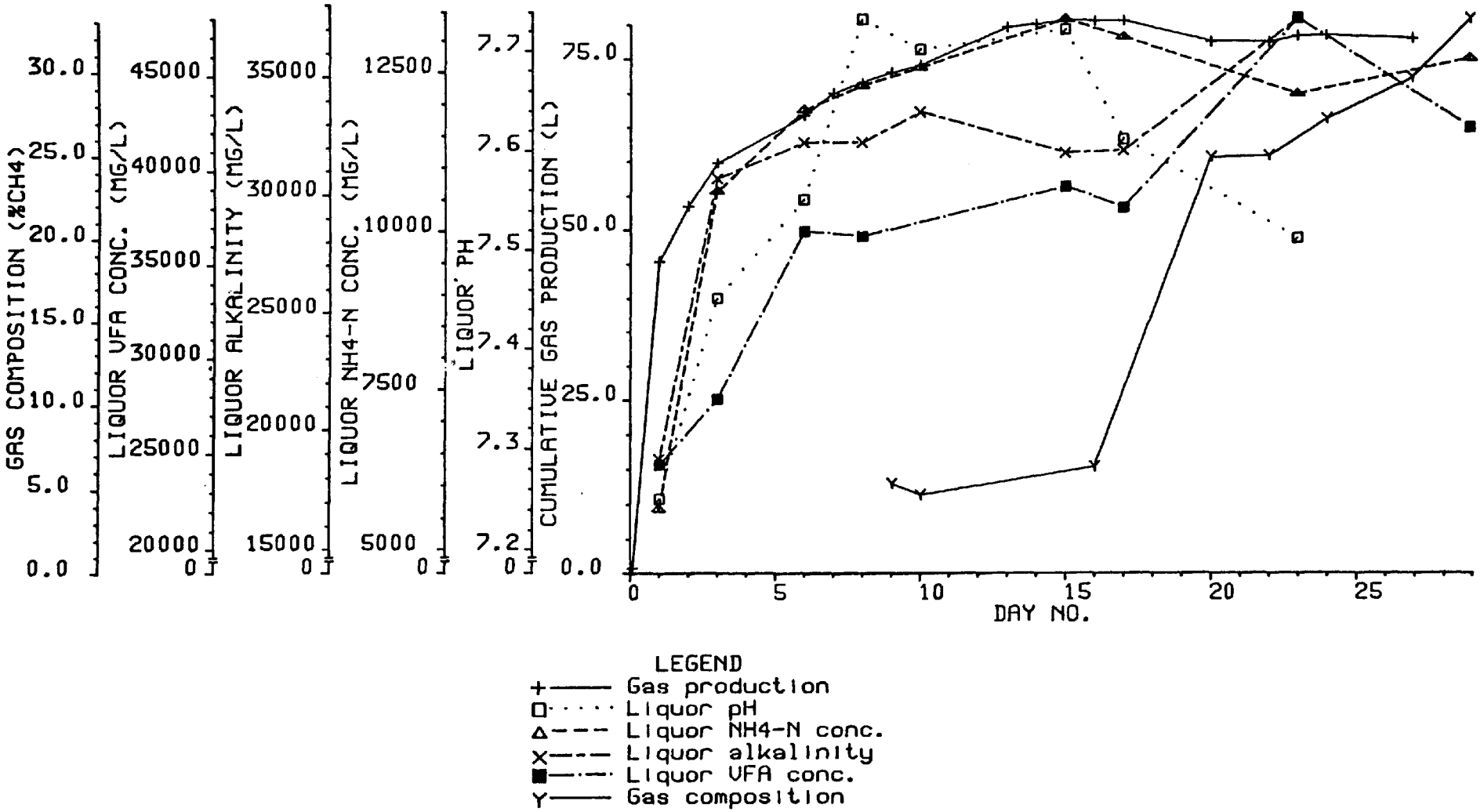
The rate of gas production was very high initially and in the first 20 hours 45 litres had been recorded, although expansion of gas during heating up may have contributed to the volume (Figure 7.2). The rate decreased as the experiment progressed and after 15 days when 80.5l had been measured gas production stopped. There was an apparent decrease of approx. 3l in the total gas volume produced between the 17th and 22nd days, followed by an increase of approx. 1l in the next 24 hours.

In the first 16 days methane constituted only approx. 5% of the gas produced with the difference made up by carbon dioxide. For the rest of the experiment the methane content of the gas rose to approx. 29%.

7.3.1.3 Liquor Composition

The TS content of the liquor increased from 1.5% at start up to 2.5% after 6 days. This level was maintained until the end of the experiment. The volatile fraction of the TS was relatively constant at between 41.1% and 55.6%.

FIG 7.2 VARIATION IN GAS PRODUCTION AND LIQUOR COMPOSITION DURING EXP.1



The concentrations of NH_4^+ - N, VFA's and alkalinity all increased from start-up and reached levels of 11,907 mgl^{-1}, 36,765 mgl^{-1} and 32,300 mgl^{-1} respectively by day 6 (Figure 7.2). These levels were maintained for the rest of the experiment for NH_4^+ - N, but only up to the 17th day for VFA's and alkalinity which then increased to 47,970 mgl^{-1} and 37,400 mgl^{-1} respectively by the 28th day.

The liquor pH increased from 7.25 to 7.73 after 8 days before dropping rapidly to 7.51 after 23 days.

7.3.2 Experiment 2

7.3.2.1 Start-up

Two kilograms of poultry manure and 1.5l seed from the same sources as in Experiment 1 were used in this experiment, with 1.5l water. The digester and its contents were allowed to reach ambient temperature before it was sealed and percolation started. The percolation rate was maintained at 20ml min^{-1} and the temperature by fan heater between 30^o and 31^oC throughout the experiment.

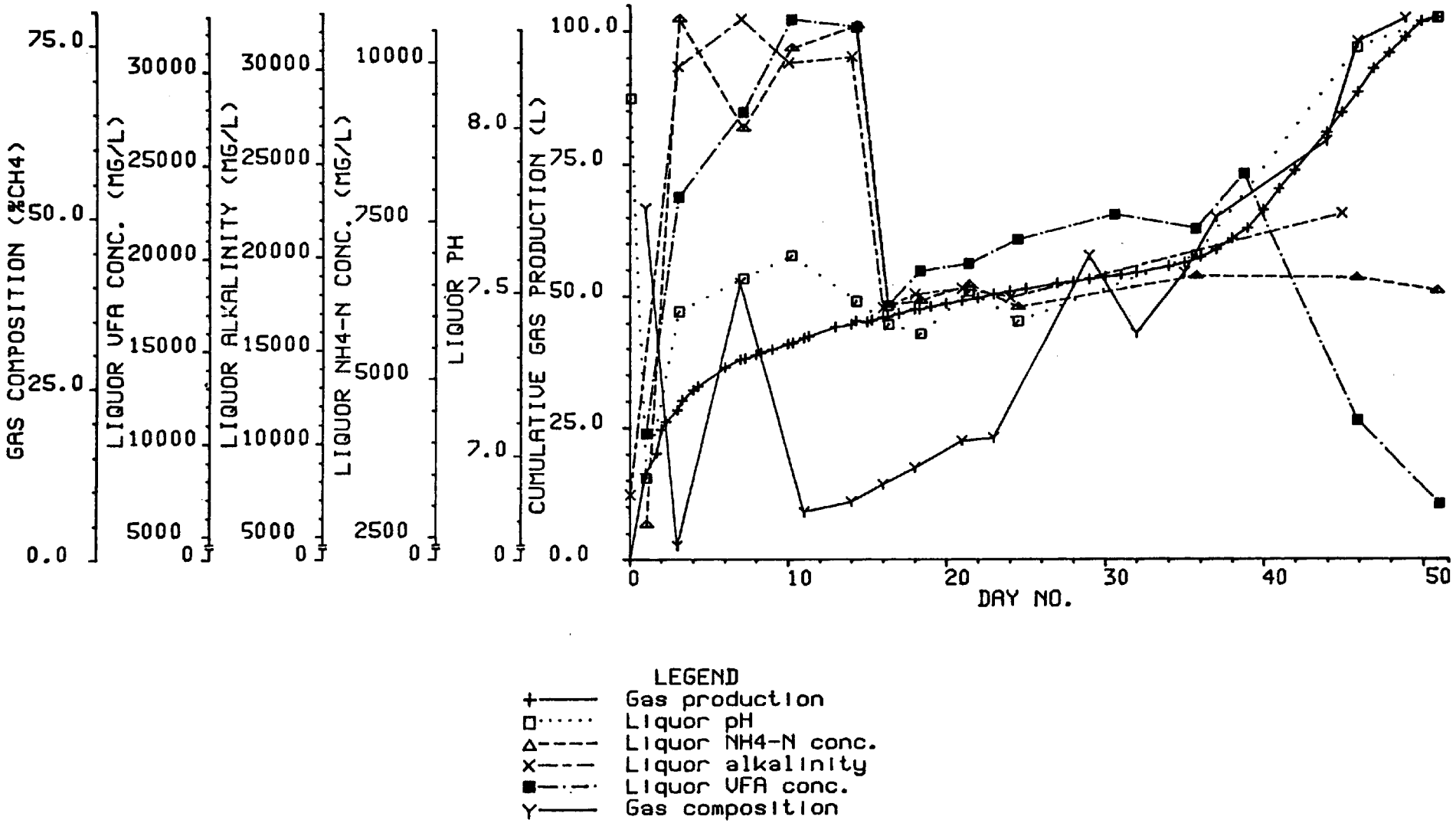
After 14 days 1.8l liquor was replaced by the same volume of fresh diluted effluent (pH 8.15, alkalinity 7,800 mgl^{-1}), and the digester was allowed to run for a further 37 days. Figure 7.3 shows the cumulative gas production and the variation in liquor composition during the course of the experiment.

7.3.2.2 Gas Production

The rate of gas production was high initially, but decreased progressively up to day 34 by which time 55.5l had been produced. After the 34th day the rate of production increased again and remained constant for the rest of the experiment. The total gas volume produced in 51 days was 102.25l.

During the first 7 days the methane content of the gas fluctuated

FIG 7.3 VARIATION IN GAS PRODUCTION AND LIQUOR COMPOSITION DURING EXP.2



widely between 2.2% and 51.7% of the total. Between days 11 and 23 the methane composition increased gradually from 7.0% to 17.9% then more rapidly to a maximum of 79.1% on the 49th day.

7.3.2.3 Liquor Composition

The TS content of the liquor increased from 0.6% at start-up to 3% after 3 days then varied between 2.0% and 3.1% up to day 14, and 1.1% - 1.5% after the liquor had been replaced.

The concentrations of NH_4^+ - N, VFA's and alkalinity in the liquor followed the same pattern during most of Experiment 3 and increased sharply at the start to maxima of 10,668 mgl^{-1}, 32,900 mgl^{-1} and 32,700 mgl^{-1} respectively, then decreased when the liquor was changed to approx. 60% of previous levels. Towards the end while the NH_4^+ - N concentration remained constant the VFA content decreased to 6,767 mgl^{-1} and alkalinity increased to 24,400 mgl^{-1}.

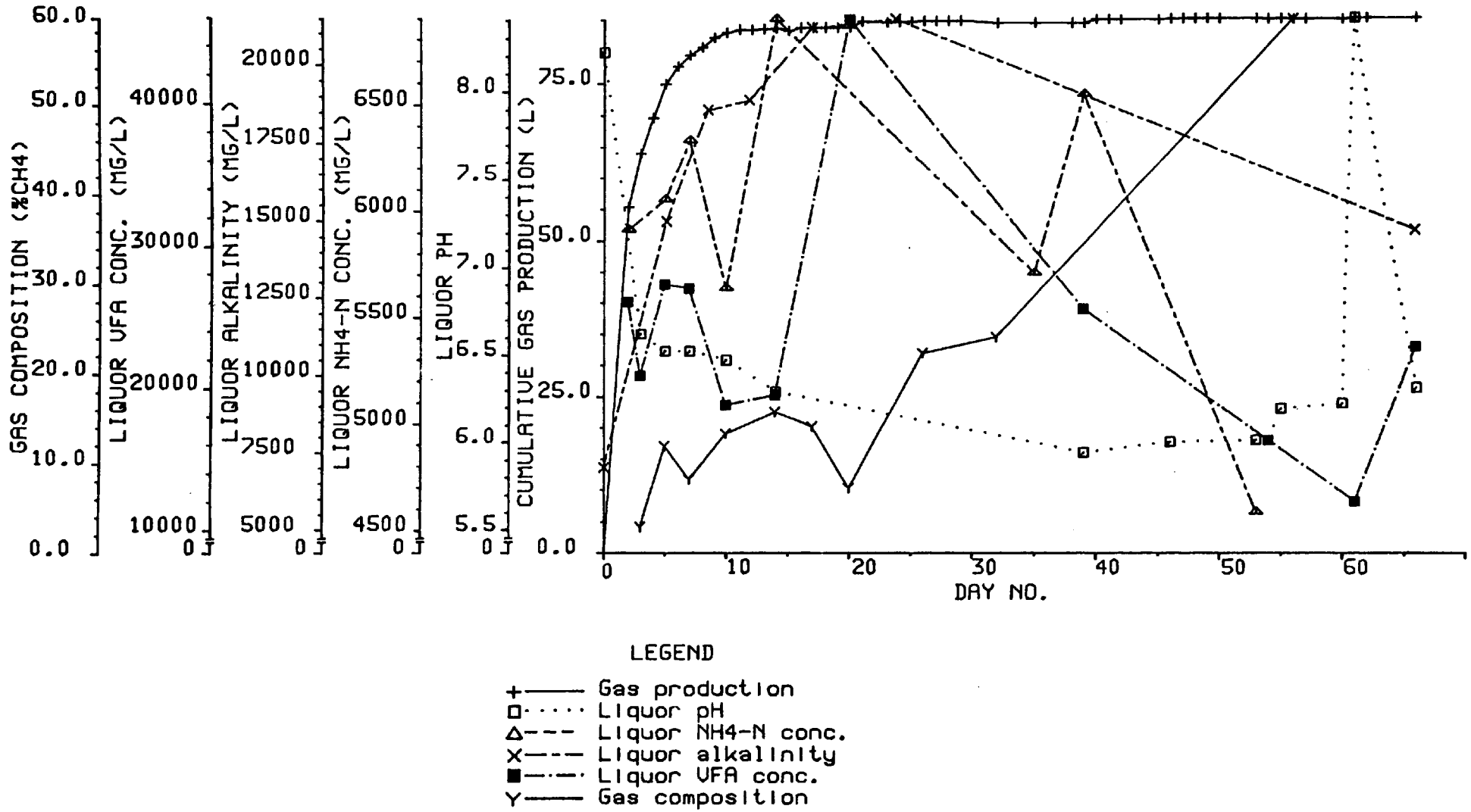
The liquor pH dropped from 8.09 at start up to 6.93 on day 1. This was followed by a gradual rise to 7.61 then a further increase to 8.33 when the digester was stopped.

7.3.3 Experiment 3

7.3.3.1 Start-up

The digester was loaded with 2.9 kg poultry litter and seeded with 2.651 mixed effluent from the same source as Experiments 1 and 2, with 2.651 water. The digester and its contents were allowed to warm up to 30°C overnight before the experiment was started. The temperature was maintained by fan heater between 29° and 32°C, and the percolation rate at 20ml min^{-1}. during the 66 days of operation. The liquor was replaced by diluted mixed effluent after 20 days and 39 days. The cumulative gas production and variation in liquor composition is shown in Figure 7.4.

FIG 7.4 VARIATION IN GAS PRODUCTION AND LIQUOR COMPOSITION DURING EXP.3



7.3.3.2 Gas Production

The initial rate of gas production was very high. In the first 45 hours 55l had been measured, and after 9 days the total volume was 82l. The rate of production decreased dramatically from the 10th day onwards and gas production stopped on the 48th when 85.2l had been recorded.

For the first 21 days methane constituted between 3% and 16% of the gas produced while CO_2 made up the difference. From day 26 to day 32 the methane content increased to approx. 23% after which no measurements were made until day 56 when the figure had reached 59.6%.

7.3.3.3 Liquor Composition

Solids determinations were made on the liquor in the first 14 days of the experiment only. As with the previous experiments the TS increased from start up (0.9%) to a constant higher level (4.2%). The volatile fraction of the TS remained constant at approx. 56%.

During the first 20 days the levels of NH_4^+-N, VFA's and alkalinity in the liquor increased to maxima of 6,895 mgl^{-1}, 45,868 mgl^{-1} and 21,450 mgl^{-1} respectively. These concentrations decreased progressively when the liquor replaced to 4,585 mgl^{-1}, 12,056 mgl^{-1} and 14,700 mgl^{-1} respectively.

The liquor pH decreased from 8.22 at start-up to 6.62 on day 3 then more gradually to 5.94 on day 39. Between days 54 and 57 200ml 1M NaOH was introduced into the liquor in an attempt to lower H^+ concentrations below inhibitory levels.

The pH increased slightly to 6.22 and a further 240ml NaOH was added on day 60. This raised the liquor pH briefly to 8.41, but 5 days later it dropped back to 6.31.

7.3.3.4 Digestibility of Liquor

At the dilutions tested all attempts to initiate gas production from poultry litter liquor, in both daily fed and batch mode, failed.

7.4 Discussion

7.4.1 Experiment 1

Since the digester was not allowed to reach operating temperature before the experiment was started some of the initial gas production would have been attributable to the expansion of gas within the digester as its temperature increased. However assuming a temperature increase of $40^{o}C$ and an internal gas volume of 5l, from the expansion coefficient for air of 3.662×10^{-3} the volume of gas attributable to expansion would have been only 0.732l which is insignificant when compared with the 45l recorded in the first 20 hours. In future experiments the digester was allowed to reach operating temperature before percolation started. The apparent decrease then increase in total gas volume after the 17th day may also have been partly due to contraction and expansion caused by heater failure and recommencement of heating 5 days later (Figure 7.2).

The TS content of the liquor increased from start-up due to leaching out of solids from the bed of manure. However the TS did not exceed 2.6% since most suspended solids were filtered out of the liquor during percolation through the bed. Possibly because of this the liquor TS and VS did not reflect the destruction of manure solids which must have occurred during gas production.

The rapid production of gas in the first 3 days resulted in very high concentrations of NH_4^+ - N in the liquor produced by breakdown of nitrogenous material in the manure. The concentrations were within the range expected from an effective solids concentration of 22.8% TS in the digester and inhibitory to the methanogenic bacteria, so the CH_4 content of the gas produced was low. The acidogenic and fermentative bacteria appeared to be less affected by NH_4^+ - N and continued to produce CO_2 for 15 days. High concentrations of VFA's therefore accumulated in the liquor which may

themselves have been toxic to the methanogens. Because of the high buffering capacity of the liquor generated by NH_4^+-N, the high VFA levels did not reduce the pH which in fact increased due to the influence of NH_4^+-N. Conversely the pH did not reach levels expected from the high NH_4^+-N concentrations due to the opposing influence of the VFA's. The combination of pH and NH_4^+-N produced high concentrations of free NH_3 which reached a maximum of 790 mgl^{-1}. Towards the end of the experiment there was a drop in pH which may have been due to the coincident increase in VFA concentration at constant NH_4^+-N level, although alkalinity also increased here.

7.4.2 Experiment 2

As with the first experiment liquor TS increased from start-up due to the leaching out of solids from the bed of manure. When the liquor was changed there was an immediate drop in TS which was maintained for the rest of the experiment.

The rate of gas production at the start was lower than that of the first experiment possibly due to the lower operating temperature of 31°C, but continued throughout the 51 days of operation (Figure 7.3). In the first 24 hours CH_4 and CO_2 were produced in equal proportions but high concentrations of VFA's (10,600 mgl^{-1}) were present in the liquor produced by fermentation and acidogenesis, and leached out of the bed. This depressed the pH from 8.09 at start-up to 6.93 on day 1. The microbial degradation of manure also led to high liquor NH_4^+-N levels which by the 3rd day reached 10,668 mgl^{-1}, the level expected from an effective solids concentration of 19.4% TS. These concentrations were inhibitory to the methanogenic bacteria so the rate of CH_4 production decreased. The high NH_4^+-N levels also raised the buffering capacity of the liquor to 30,200 mgl^{-1} and the pH to 7.44. Higher pH values would have been expected from these high NH_4^+-N concentrations, but as in Experiment 1 were not attained due to the opposing influence of VFA's.

The fermentative and acidogenic bacteria appeared to be less susceptible to ammonia inhibition as CO_2 production was still occurring and the VFA

concentration of the liquor increased to 23,400 mgl^{-1} - 32,700 mgl^{-1} due to non utilisation by the methanogens. When the liquor was replaced on day 15 to reduce inhibitory concentrations of NH_4^+-N there was an immediate reduction in levels of NH_4^+-N, VFA's and alkalinity, but there was no improvement in gas production. The pH was unaffected since the relative proportion of the liquor components remained unchanged. Fifteen days later total gas production rose sharply. The liquor VFA levels consequently dropped which allowed the pH to rise.

Replacing the liquor therefore, appeared to alleviate the toxicity problems and gas production was still continuing when the experiment was stopped. However this solution increases the volume of effluent which must be disposed of, and the volume of liquid which must be heated, thus eliminating some of the advantages of this type of digester. Additionally if the total volume of water that is required to dilute liquor ammonia concentrations to below toxic levels reduces the effective solids content of the digester to 10% TS or lower, a conventional stirred tank reactor may be more suitable for the treatment of this waste.

Very high concentrations of NH_4^+-N (10,000 mgl^{-1}) also prevented the successful batch digestion of poultry manure attempted by Wong-Chong (1975) at high solids concentrations (20% TS) and temperatures between 21° and 29°C. This author however reported the successful digestion of cattle manure at the same concentration due to the lower nitrogen content which did not lead to high digester NH_4^+-N concentrations.

7.4.3 Experiment 3

Poultry litter was used in this experiment since its low nitrogen content should lead to liquor NH_4^+-N concentrations lower than those produced by poultry manure. However the NH_4^+-N levels obtained (maximum 6,895 mgl^{-1}) still appeared to be high enough to inhibit methanogenesis since CH_4 production was low and liquor VFA concentration increased to 45,868 mgl^{-1} (Figure 7.4).

Possibly because of the relatively low NH_4^+-N levels in comparison with the VFA concentration, the liquor pH dropped to 5.94 where H^+ may have become toxic to all groups of bacteria. Since the concentration of free NH_3 is reduced at low pH levels, the inhibitory effect of NH_4^+-N may have been diminished, although at NH_4^+-N concentrations greater than 3000 mgl^{-1}, NH_4^+ can also become toxic (McCarty, 1964). Replacing the liquor reduced the concentrations of all components measured in the liquor (apart from pH) to comparatively low levels but failed to reinitiate gas production possibly due to the still toxic levels of H^+.

Introducing volumes of NaOH calculated to raise the pH of the liquor to 7.5 had only a small effect and larger volumes increased the pH only briefly before the NaOH was neutralised during percolation through the bed of litter. Gas production did not recommence therefore, and the total volume produced remained at 85.21.

7.5 The Potential of Poultry Wastes for High Solids Digestion

It is possible that poultry wastes would prove to be suitable substrates for two-phase digestion since it appeared that hydrolysis and acidogenesis proceded well in each experiment reported here. The leachate from a high solids digester containing high concentrations of soluble organics could be transferred to a secondary digester for the methanogenic stage after dilution to reduce NH_4^+-N and VFA concentrations to below toxic levels. The second stage could theoretically be operated at short RT's since the substrate is already solubilised so a fixed film or UASB reactor may be suitable. The removal of leachate from the bed of waste also eliminates the possibility of ammonia inhibition of the first stage bacteria.

The elimination of the toxicity problem by removal and separate treatment of leachate was employed by Rijkens (1981) in the percolated high solids batch digestion of a mixture of sugarbeet pulp, wheat-straw and cow manure (PSM) at 35°C. Low pH caused by accumulation of VFA's was avoided by treating the leachate in a conventional continuously fed

digester before recirculating to the batch digester. When the capacity of the methanogens to break down VFA's met their production rate the process became self sustaining and percolation could be stopped.

However in the present investigation when liquor was tested for digestibility it was not possible to initiate gas production presumably because the concentrations of NH_4^+-N and possibly VFA's were still too high despite dilution. Higher dilutions therefore would be necessary for the treatment of poultry waste leachate in a secondary digester. As stated previously this would tend to render the process less economical since more energy would be required to heat up the greater volume of liquid.

In Rijken's (1981) study the NH_4^+-N concentration of the liquor was not reported, but since the waste materials used had a low nitrogen content it can be assumed that ammonia levels were also low. In the absence of inhibitory concentrations of NH_4^+-N the digestion of PSM may be possible without the need for a secondary reactor to eliminate VFA's, since a mixture of dairy cattle manure and straw has been digested successfully in a percolated packed bed digester without the separate treatment of leachate at the Polytechnic of Wales (Hall, 1983), and the digestion of dairy manure at 20% TS has been demonstrated in a conventional batch digester by Wong-Chong (1975) and in semi-continuously fed digesters by Hills (1980).

7.6 Conclusions

1. The operation of a percolated packed bed type of digester on poultry wastes produced efficient hydrolysis and fermentation, but methanogenesis was inhibited by high NH_4^+-N and possibly VFA concentrations which reached 13,314 mgl^{-1} and 47,970 mgl^{-1} respectively during poultry manure digestion, and 6,895 mgl^{-1} and 45,868 mgl^{-1} respectively during litter digestion.

2. The pH of the liquor of the manure digesters was normally between 7.25 and 7.72 despite the high VFA concentrations, due to the high buffering capacity of the liquor (up to 37,400 mgl^{-1} HCO_3^-) and high

NH_4^+-N levels. The pH of the litter digester liquor dropped to inhibitory levels (5.94) possibly due to the relatively low NH_4^+-N and alkalinity concentrations in comparison with VFA levels.

3. Replacing the liquor with fresh digester effluent and water reduced liquor NH_4^+-N, VFA and alkalinity concentrations and allowed the recovery of manure, but not litter digestion where the low pH was unaffected by the change.

4. It was not possible to raise the pH of the liquor of the litter digester permanently by addition of 1M NaOH due to neutralisation during percolation through the litter bed.

5. Attempts to digest diluted liquor from the litter digester in a secondary unit failed presumably due to still toxic concentrations of NH_4^+-N, thus preventing the digestion of litter as a two phase process. Further liquor dilution would be necessary for successful two phase operation.

CHAPTER 8

KINETICS AND MODELLING OF POULTRY WASTE DIGESTION

8.1 Introduction

For the AD process to be economically viable, digester design and operation must be optimised for maximum efficiency of gas production and waste stabilisation, and minimum capital cost. Optimisation can be facilitated by computer simulation of digestion using models which describe the progression of substrate utilisation, bacterial growth, and product formation. Kinetic models are able to predict digester performance under varying operating conditions, and situations such as inhibition which may lead to process failure.

Biological process models are often based on the culture theory of Monod (1949) which describes the growth of homogeneous cultures on simple substrates. Modified Monod kinetics have been used in the development of models describing AD presented by Lawrence and McCarty (1969), Andrews and Graef (1971), and Hill and Barth (1977). These are dynamic models which possess the ability to predict process stability at start-up or under transient conditions resulting from changes in process inputs. However these models may require up to 15 kinetic parameters which need to be evaluated for each set of operating conditions or waste type, although a simplified dynamic model has been presented by Hill (1983) for the methane fermentation of animal wastes. These dynamic models are able to simulate the steady-state as well as the non steady-state, but have not been employed to investigate steady-state relationships between process efficiency and operating conditions.

A steady state substrate utilisation model has been proposed by Chen and Hashimoto (1978) which is based on the Contois (1959) model derived using an enzyme kinetics analogy. This model has the advantage of simplified inputs and can accurately predict volumetric methane yields for a wide range of operating conditions for dairy, beef and swine wastes. This model possesses the limited ability to predict inhibition, but will not predict process failure due to inhibition which is important when

dealing with materials high in organic and nitrogen content such as agricultural wastes (Hill, 1983).

The examination of microbial growth kinetics presented here was undertaken to assertain whether the GY - LR relationships observed in sewerage digestion (Hawkes and Horton, 1981) and obtained in digestion tests of poultry wastes (Chapters 3 and 4 this study) are a consequence of kinetics, and to develop a model which describes the relationships for poultry wastes in non-inhibited and inhibited states.

8.2 Microbial Growth Kinetics

8.2.1 Specific Growth Rate

In a simple homogeneous batch culture consisting of a well mixed batch of inoculum and dispersed biomass free from concentration gradients, the increase in biomass resulting from the growth and death of microorganisms (dx) in an infinitely small time interval (dt) is proportional to the amount of x present and to the time interval:

$$dx = \mu x.dt - Kd\ x.dt \qquad (1)$$

$$\text{hence } \frac{dx}{dt} = x\ (\mu - kd) \qquad (2)$$

where μ is the specific growth rate, or rate of growth per unit amount of biomass, with dimensions 1/time, Kd is the specific death rate (1/time), and x is the concentration of microorganisms (gl^{-1}).

The increase in weight of biomass per unit weight of substrate consumed is termed the growth yield Y, with dimensions gg^{-1}, thus

$$Y = -\frac{dx}{dS} \qquad (3)$$

where the negative sign indicates that x increases as S decreases. The assumption that under constant conditions in batch cultures Y is constant was shown to hold by Monod (1949). It follows from equation (3) that

$$dx = dSY \tag{4}$$

and substituting into equation (2)

$$\frac{dS}{dt} = \frac{\mu x - Kdx}{Y} \tag{5}$$

8.2.2 Effect of Substrate Concentration on Growth Rate

The growth rate of bacteria in a medium varies with the concentration of limiting substrate in the manner shown in Figure 8.1. The specific growth rate μ, is approximately proportional to the substrate concentration S, when this is low, but tends to a maximum value μm, as S increases. Monod (1949) showed that the relation of bacterial growth rate to substrate concentration fitted the equation

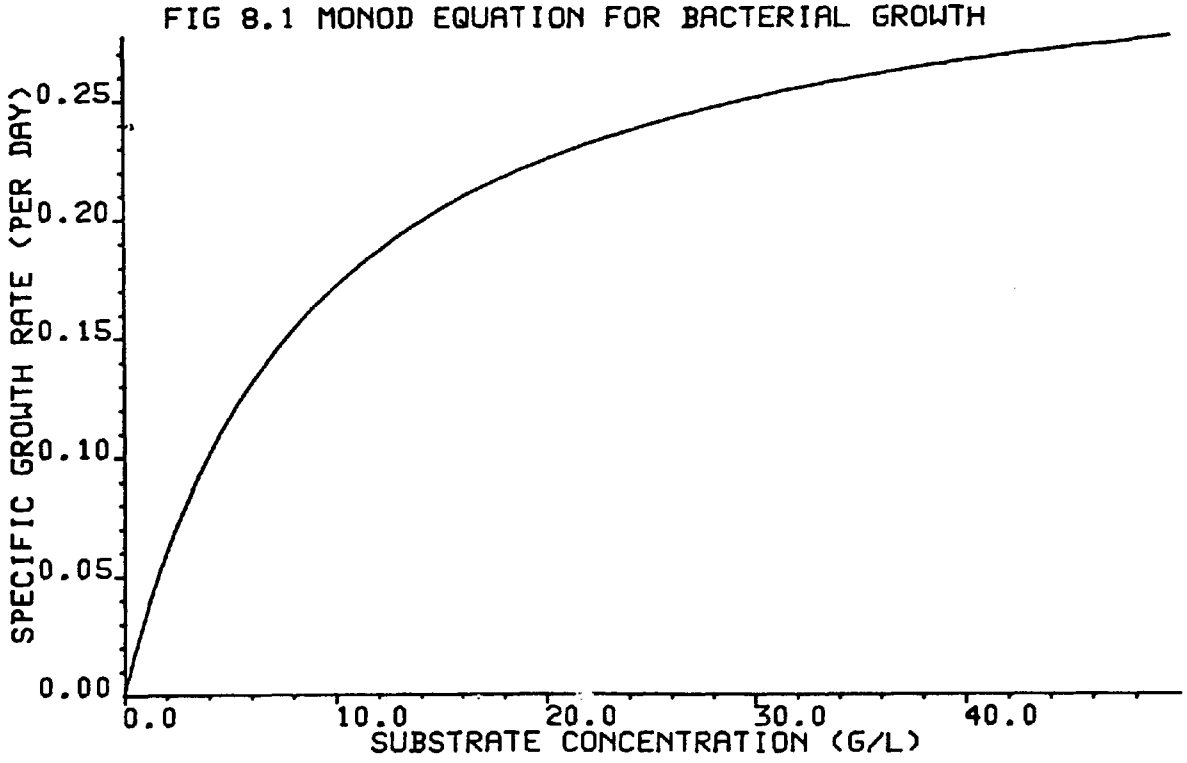
$$\mu = \frac{\mu m S}{S + Ks} \tag{6}$$

which is termed the Monod equation where Ks is a saturation constant which is numerically equal to the substrate concentration at which μ is half of the maximum value. Figure 8.1 is a plot of this equation where $\mu m = 0.326\ d^{-1}$ and $Ks = 8.9\ gl^{-1}$.

8.3 Chemostat Culture

The culture of microorganisms can be prolonged by continuous addition of fresh medium and continuous harvesting of the product. One such type of continuous flow culture is the chemostat, which ideally consists of a perfectly mixed suspension of biomass into which medium is introduced at a constant rate, and culture volume maintained constant by removal of culture at the same rate. Chemostat culture allows the growth rate to be

FIG 8.1 MONOD EQUATION FOR BACTERIAL GROWTH



adjusted to any value between 0 and the maximum, an advantage over simple batch culture where the growth rate is limited to the doubling time of the biomass.

Let the chemostat contain constant volume of culture V, with cell concentration x and substrate concentration S. Fresh culture medium of substrate concentration Sr is fed at flow rate F. The flow rate per unit volume (F/V) is the dilution rate D, which is the reciprocal of RT. The balance of the biomass is

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

For an infinitely small time interval dt, this balance for the whole culture is

$$V.dx = V.\mu x.dt - V.Kdx.dt - Fx.dt \qquad (7)$$

Dividing throughout by V.dt we obtain

$$\frac{dx}{dt} = x\,(\mu - D - Kd) \qquad (8)$$

At steady state when $\frac{dx}{dt} = 0$

$$\mu = D + Kd \qquad (9)$$

Substituting equation (9) into equation (6)

$$S = \frac{Ks\,(D + Kd)}{\mu m - D - Kd} \qquad (10)$$

Equation (10) shows that the steady state substrate concentration in the chemostat is independent of the concentration of inflowing substrate (Sr), and cell concentration (x), and for a given organism and medium the value of S is determined solely by the dilution rate, D.

8.3.1 Critical Dilution Rate

When the dilution rate is increased, μ tends to μm until a point is reached termed the critical dilution rate (Dc) when $Dc + Kd = \mu m$. The growth of biomass cannot keep pace with the replacement of culture volume and washout occurs, so at steady state the biomass concentration = 0, and concentration of S = Sr.

The Monod equation now becomes

$$\mu = Dc = \frac{\mu m Sr}{Sr + Ks} - Kd \qquad (11)$$

and at Dc, $Sr \gg Ks$

8.4 Application of Continuous Culture Kinetics to Anaerobic Digesters

Daily fed laboratory scale anaerobic digesters such as those used for the digestion of poultry wastes in Chapter 3 and 4 differ from the chemostat in the following respects.

a) The substrate is a complex non-homogeneous mixture of dissolved and suspended solids which have different degrees of bio-degradability.
b) The biomass consists of a combination of many species of interdependent bacteria.
c) Introduction of fresh substrate and removal of culture is not continuous, and only performed at intervals.
d) Mixing is not perfect.
e) Biomass may be introduced with substrate additions.

Despite these important differences between the chemostat and the anaerobic digester, the growth kinetics of chemostat continuous flow culture have been successfully applied to the growth of biomass in continuously fed digesters, and models based on Monod kinetics have been described by Kugelman and Chin (1971), Andrews and Graef (1971) and Hill (1983).

8.5 Poultry Litter Model

The Monod based model proposed here consists of an expression for biogas yield in terms of RT, influent concentration and LR, and the kinetic constants Ks, μm and Kd.

8.5.1 Derivation.

The substrate destroyed during microbial growth is the sum of that which is utilised in cell synthesis and that which is destroyed during energy production. During digestion the energy yielding reactions produce CH_4 and CO_2. Thus only a fraction of substrate destroyed is converted to gas while the remainder is used in cell synthesis. Let this fraction be K_e which is constant for a particular waste, then the substrate used for energy production is given by K_e (Sr - S), and the volume of gas produced from this amount of substrate destroyed is $K_g K_e$ (Sr - S) where K_g is a constant which is the volume of gas produced per unit weight of substrate destroyed during energy production, with dimensions lg^{-1}. Let Kg. Ke = Kc which is equivalent to the volume of gas produced per unit weight of total substrate destroyed, i.e. GY in terms of substrate destroyed, which for a given substrate is constant. Thus

$$\text{Volume of gas produced} = Kc\ (Sr - S) \tag{12}$$

and GY in terms of substrate added is

$$GY = Kc\ \frac{(Sr - S)}{Sr} \tag{13}$$

which becomes

$$GY = Kc\ \left(1 - \frac{S}{Sr}\right) \tag{14}$$

Substituting the expression for S (equation (10)) into equation (14) we have

$$GY = Kc\left[1 - \left(\frac{Ks\ (D + Kd)}{Sr\ (\mu m - D - Kd)}\right)\right] \qquad (15)$$

which is the basic equation of the model.

Using the relationship between LR, Sr and RT ($LR = \frac{Sr}{RT}$) equation (15) can be used to generate families of curves of constant Sr and RT in a plot of GY against LR (Figure 8.2), where the substrate concentration is in $gVSL^{-1}$ and Kc is 1gVS $destroyed^{-1}$. This figure generated by computer, shows the same qualitative relationship between GY and LR as those obtained during the digestion of poultry litter (Figures 3.12 and 3.13) in that GY increases both with increasing Sr and RT. The relationships generated by the model can be rendered quantitative by giving numerical values to the 4 constants involved.

8.5.2 Determination of Constants

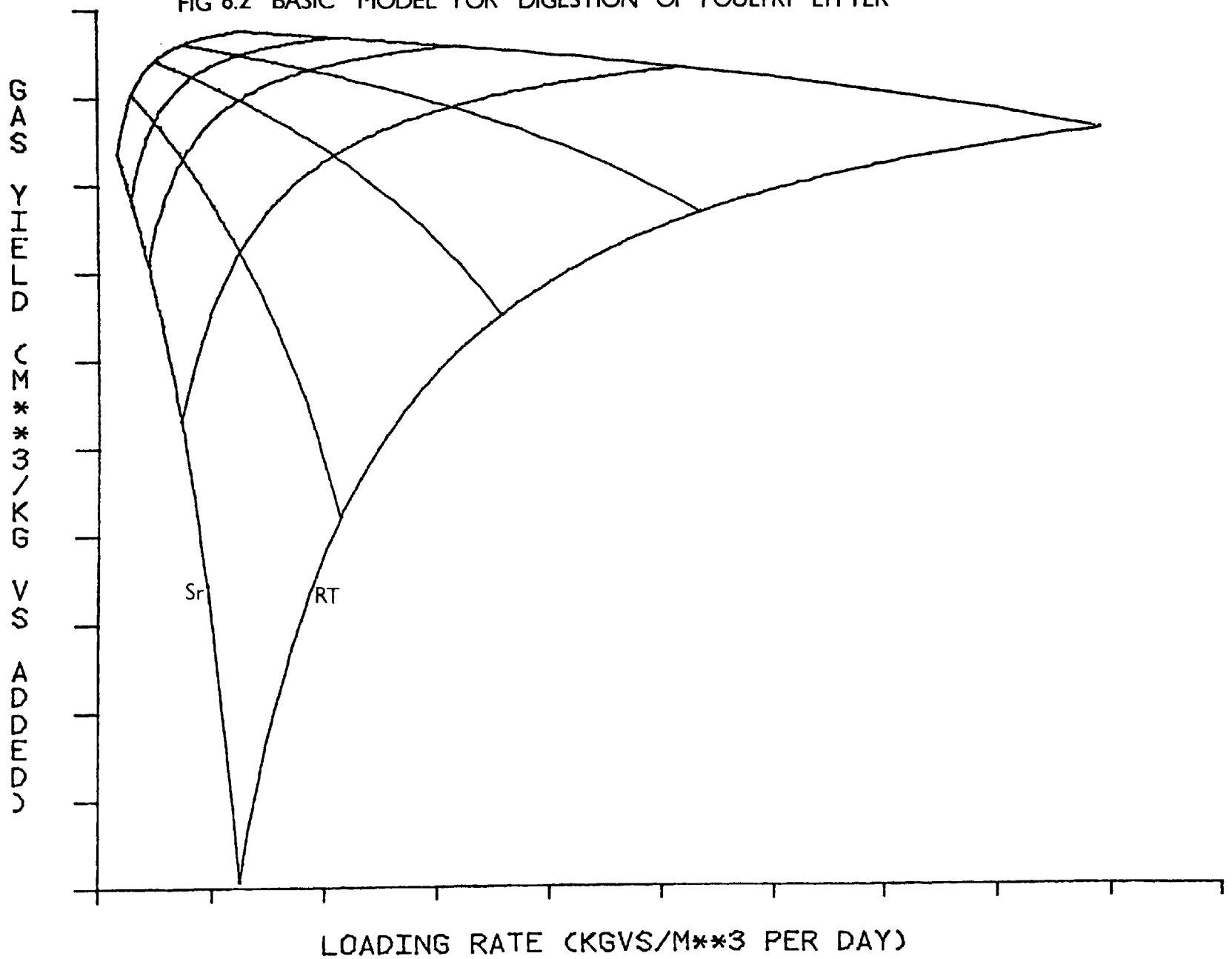
Kc Conversion Constant

Kc is equivalent to the maximum attainable GY in terms of substrate added and can be determined theoretically or experimentally. An estimation can be made from the proportion of digestible components in a waste material and the calculated volumes of gas produced during complete digestion of these components. The main digestible components of agricultural wastes are polysaccharides, proteins and lipids. Theoretical GY's obtained from these materials have been calculated by a number of workers examples of which are given in Table 8.1.

Table 8.1 Theoretical GY's from the Major Components of Poultry Litter and Manure.

Waste Component	Theoretical GY's (1g $destroyed^{-1}$)			Proportion of component in waste (% of VS)	
	Hawkes (1983)	Mosey (1982b)	Badger *et al* (1979)	Litter[1]	Manure[2]
Poly-Saccharides	0.75	0.79	0.886	38.2	40.2
Proteins	0.99	0.96	0.587	20.3	28.8
Lipids	1.42	1.44	1.535	1.5	1.5

FIG 8.2 BASIC MODEL FOR DIGESTION OF POULTRY LITTER



1 Data of Shih and Huang (1980)
2 Data of Huang and Shih (1981)

Using the analysis of poultry litter given by Shih and Huang (1980), and assuming Nitrogen x 6.25 = protein, ether extract represents lipid, and cellulose is the main gas yielding polysccharide, GY's of 0.509, 0.518, and 0.481 m^3 kg VS $destroyed^{-1}$ are obtained for litter from the figures of Hawkes (1983), Mosey (1982b) and Badger et al (1979). These estimated GY's are high since not all the material digested will be utilised in energy and hence gas production, a proportion will be required for cell synthesis. A more accurate estimation of Kc can be obtained experimentally.

Measurement of the total volume of gas produced by a known weight of substrate in a batch digester gives the value of Kc, or over a shorter time period log of cumulative gas volume can be plotted against $\frac{1}{\text{time}}$ to produce a straight line, where the intercept is the gas volume at infinite RT. In continuously or semi-continuously fed digesters Kc can be calculated directly from the volume of gas produced and the weight of VS destroyed. This involves the measurement of gas volume and both influent and effluent VS so may be less accurate than the above methods. Alternatively, using the data obtained from continuously fed digesters Kc can be determined by plotting for constant influent concentration, log GY against LR where the intercept is the GY at infinite RT, or GY against LR where again the intercept is Kc. Using the litter digestion data from Chapter 3, Kc determined by these two methods was in both cases 0.400 $lgVS^{-1}$.

μm Maximum Specific Growth Rate

Most values reported in the literature for the experimental determination of μm for the acid phase of digestion have been within the range $3.8d^{-1}$ - $30d^{-1}$ depending on the substrate used (Ghosh et al 1975, Ghosh 1981), but much lower for methane production from acetate at $0.327d^{-1}$ - $0.49d^{-1}$ (Hobson and McDonald 1980, Lawrence and McCarty 1969, Ghosh et al 1975). This suggests that the rate of the process as a whole is

determined by the growth rate of the methanogenic bacteria. Thus Yang and Chan (1977) have reported a value of $0.37d^{-1}$ for μm for the production of CH_4 from poultry manure. Hashimoto et al (1980) have shown that for both phases combined, temperature is the primary factor affecting μm and that the type of substrate and concentration have no detectable effect. The effect of temperature on μm can be described by the following empirical relationship

$$\mu m\ (d^{-1}) = 0.013T - 0.129 \qquad (16)$$

which holds at temperatures between 20° and 60°C. Thus at 35°C μm is $0.326d^{-1}$ which is the value used in the model described here.

Kd Specific Death Rate

The specific death rate has been excluded from some kinetic models describing the AD process in order to maintain the simplicity of the model (see for example Andrews and Graef 1971, Chen and Hashimoto 1978). However Kd is included in the model presented here to give a more complete description of the process, and since the model is based on one equation its inclusion does not render the model un-manageable.

For modelling purposes Kd has been assumed to be a constant fraction of μm (e.g. Hill 1983, Hill and Nordstedt 1980), and in studies where kd has been determined experimentally during VFA degradation the fraction has been between 0.025 and 0.118 of μm (Kugelman and Chin 1971, Lawrence and McCarty 1969, Mosey 1974). In accordance with other models and for ease of manipulation, Kd here is assumed to be one-tenth of μm, i.e. $0.0326\ d^{-1}$.

Ks Saturation Constant

There is a wide variation in the values reported in the literature for Ks which suggests that this constant may vary with waste type. Higher values, indicating lower digestibility, have been reported for complex substrates such as sewage sludge and poultry manure, than simple substrates

such as VFA's, presumably because the latter can be degraded more readily.

The value of Ks can be determined which, with a value of 0.41 g^{-1} for Kc, 0.326 d^{-1} for μm, 0.0326 d^{-1} for Kd, and the operating conditions used in the litter digestion experiments (Chapter 3) will give the GY obtained in each experiment. Rearranging equation (15) an expression for Ks is given by

$$Ks = Sr \left[\frac{(\mu m - D - Kd)}{D + Kd} \cdot \frac{1}{Kc} - \frac{GY}{Kc} \right] \qquad (17)$$

The values obtained for Ks for each experiment are shown in Table 8.2.

Table 8.2 Values of Ks Calculated for Litter Digestion Experiments.

Sr (g VSl^{-1})	RT (days)	GY (l gVS $added^{-1}$)	Calculated Ks (gl^{-1})
40.0	19.4	0.347	15.3
40.0	29.2	0.372	10.8
20.1	16.7	0.339	7.7
20.1	14.6	0.320	8.9
39.6	14.6	0.331	15.2
39.6	16.7	0.341	14.7
19.8	29.2	0.353	9.0
19.8	19.4	0.341	8.4
9.8	29.2	0.297	9.8
9.8	11.7	0.245	6.7
49.0	29.2	0.337	29.9
49.0	11.7	0.300	21.5

It can be seen from Table 8.2 that Ks is directly proportional to Sr, and therefore as Sr increases the deviation between theoretical and experimental results increases. For the experiments of both groups, the best fit to all data is given by the Ks value determined for the lowest GY obtained, which for the experiments in the first group is 8.9 gl^{-1}, and in the second group 6.7 gl^{-1}.

8.5.3 Predicted GY - LR Relationships

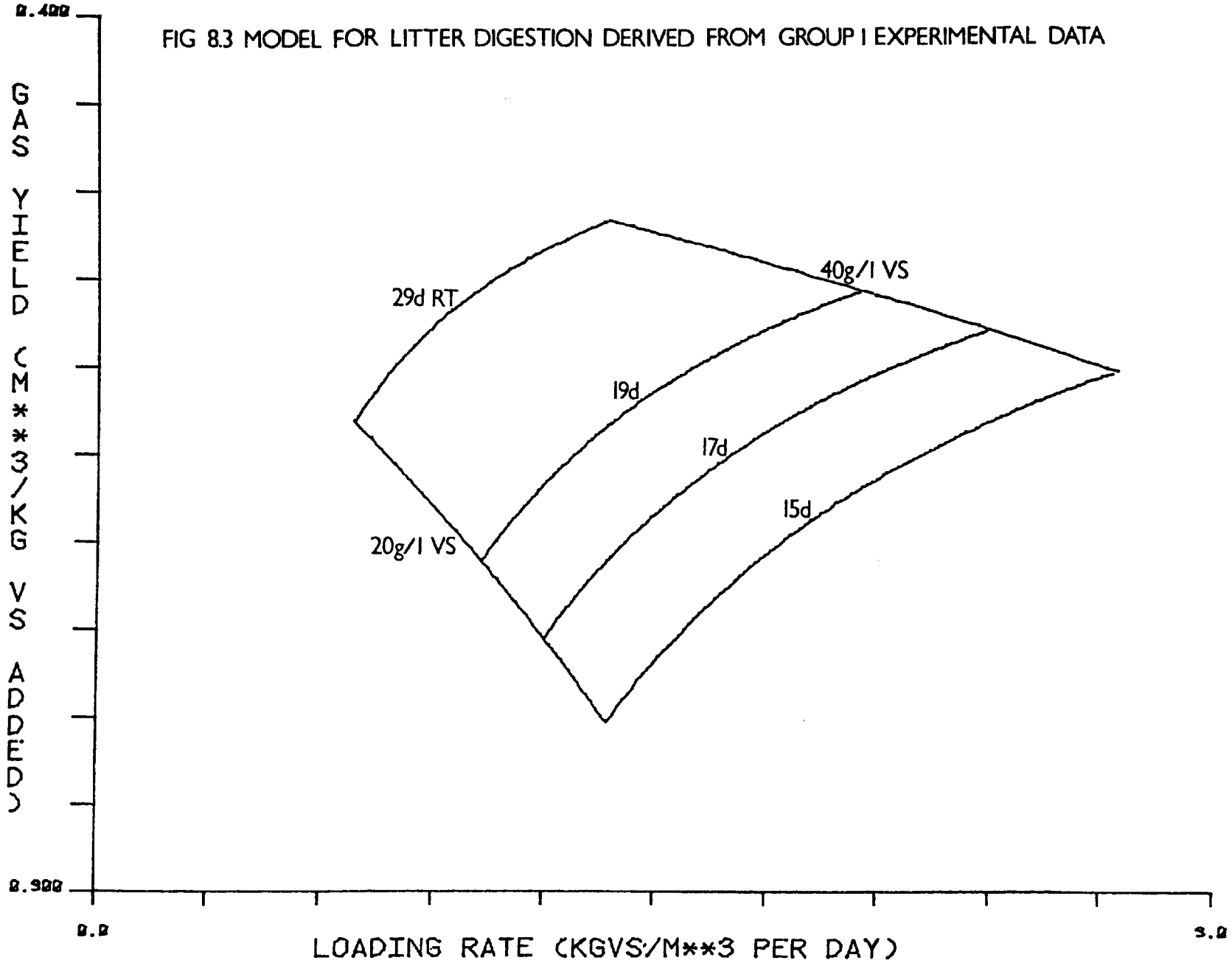
Figures 8.3 and 8.4 are GY - LR plots generated by computer using the model, for the two groups of experiments respectively, and values of Kc, μm and Kd as stated above and the appropriate value for Ks for each group (compare with Figs 3.12 and 3.13). Table 8.3 compares GY's obtained experimentally with those predicted by the model.

Table 8.3 Comparison of Experimental and Model GY's.

Sr (g VSl^{-1})	RT (days)	Experimental GY (1 gVS add^{-1})	Model GY (1g VS add^{-1})	Deviation (%)
40.0	19.4	0.347	0.369	6.3
40.0	29.2	0.372	0.377	1.3
20.1	16.7	0.339	0.329	-3.0
20.1	14.6	0.320	0.320	0
39.6	14.6	0.331	0.360	8.8
39.6	16.7	0.341	0.365	7.0
19.8	29.2	0.353	0.354	0.3
19.8	19.4	0.341	0.338	-0.9
9.8	29.2	0.297	0.336	13.1
9.8	11.7	0.245	0.245	0.0
49.0	29.2	0.337	0.386	14.5
49.0	11.7	0.300	0.369	23.0

For first group experiments the best fit to experimental data was obtained at 2% influent VS where the derivation from experimental GY's was 3% or less in each case. The GY produced by the model at 14.6d RT was exactly the same as the experimental GY since this was the set of operating conditions chosen for the selection of Ks. Apart from the 1.3% deviation at 29.2d RT, the predicted GY's were less accurate at 4% influent VS, and increased to between 6% and 9%.

FIG 8.3 MODEL FOR LITTER DIGESTION DERIVED FROM GROUP I EXPERIMENTAL DATA



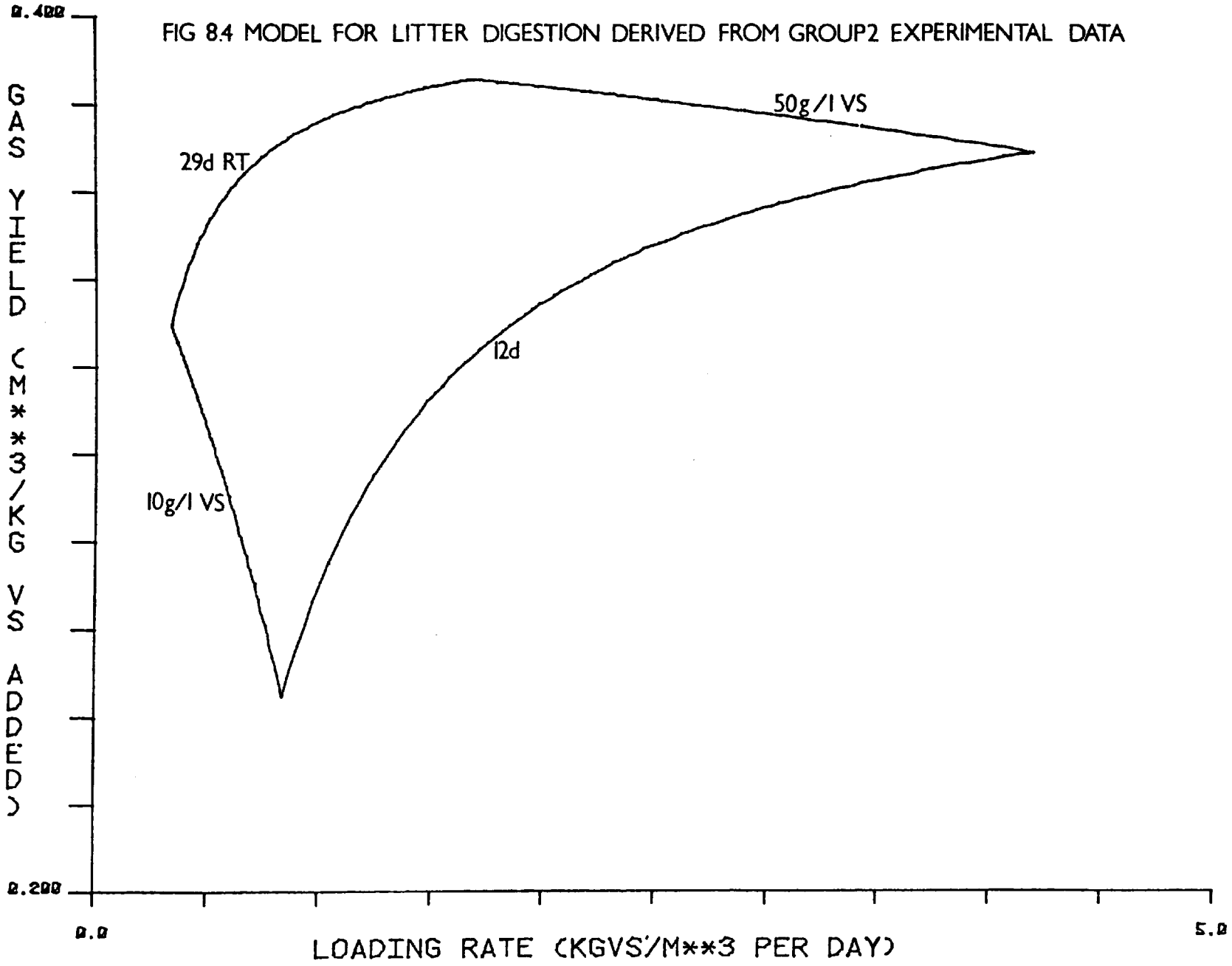


FIG 8.4 MODEL FOR LITTER DIGESTION DERIVED FROM GROUP2 EXPERIMENTAL DATA
0.400
0.200
GAS YIELD (M**3/KG VS ADDED)
50g/l VS
29d RT
12d
10g/l VS
0.0
5.0
LOADING RATE (KGVS/M**3 PER DAY)

As with the predicted GY at the lowest influent concentration and shortest RT for the first group of experiments, the GY predicted by the model for 11.7d RT and 1% influent VS was exactly that of the experimental data. However other predicted GY's in the second group deviated from experimental GY's by 13% - 23%.

The limits of RT and influent concentration can be extended outside the ranges used in the litter digestion experiments to give a more complete view of the GY - LR relationships as in Figure 8.5 generated using a Ks of 8.9 gl^{-1} and other constants as stated above, and operating conditions of 7 - 50d RT and 7 - 50 gl^{-1} influent VS.

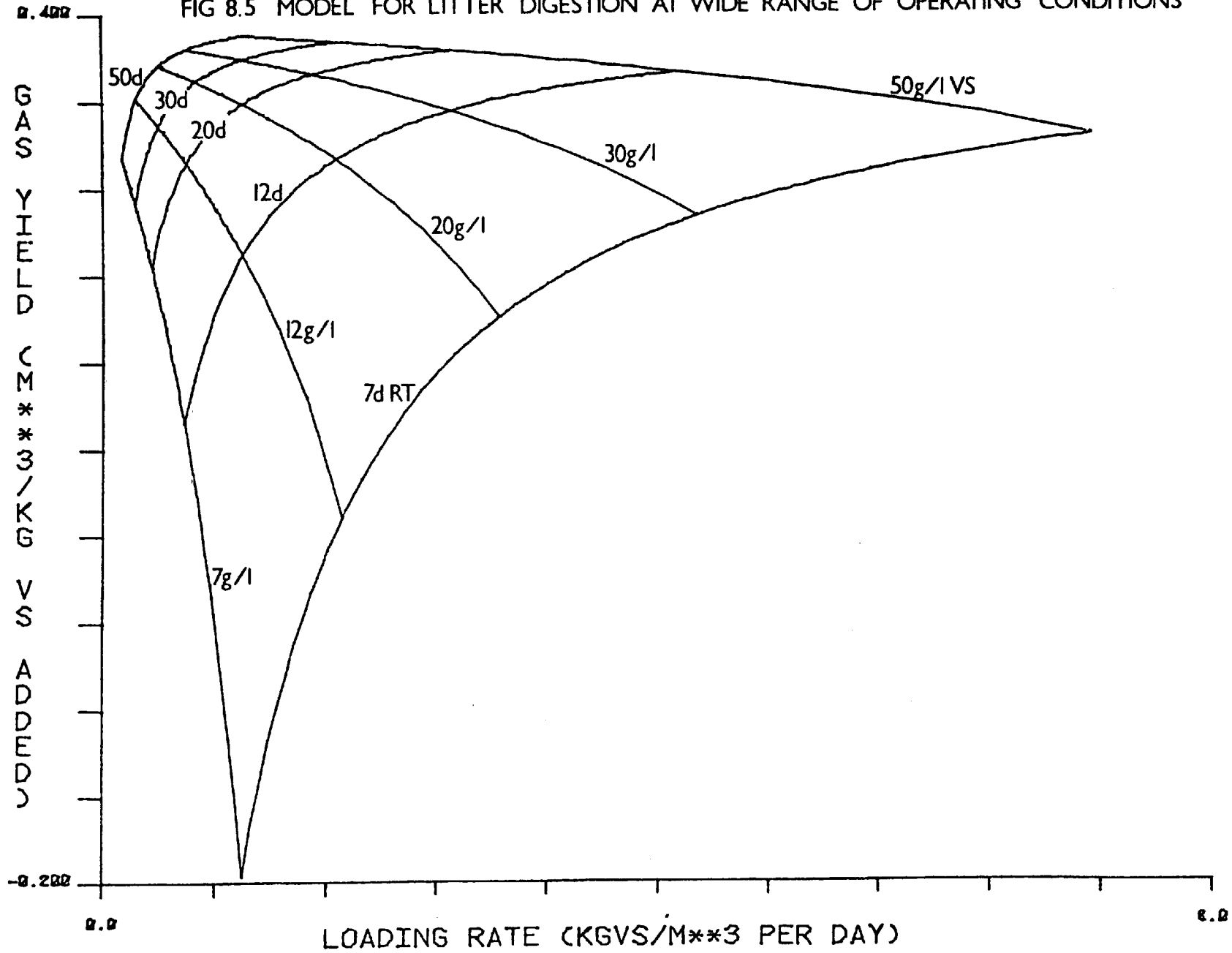
At constant RT GY increases with increasing influent concentration and asymptotes to a maximum GY of 0.4 lg VS $added^{-1}$ at infinite LR. At constant influent concentration GY increases with increasing RT to the maximum GY attainable at that concentration at infinite RT or zero LR. This maximum GY increases with increasing influent concentration, but only reaches the theoretical maximum of 0.4 lg VS $added^{-1}$ at infinite Sr. This is due to the specific death rate which is constant and has a greater effect on GY at low influent concentrations. This figure can be used to determine the optimum conditions for process efficiency. Increasing both influent concentration and RT raises the efficiency, but a point is reached in both cases where further improvement in efficiency is too costly in terms of extra time, digester volume, or concentration required.

8.6 Poultry Manure Model

The same basic equation (15) used in the model of litter digestion is employed to describe the digestion of manure, but with different values for some of the constants and the incorporation of an inhibition term.

8.6.1 Determination of Constants

FIG 8.5 MODEL FOR LITTER DIGESTION AT WIDE RANGE OF OPERATING CONDITIONS



Kc Conversion Constant

The same methods are available for the determination of Kc for manure as for litter. Kc would be expected to be higher for manure due to its lower content of refractory material. Using the analysis of manure given by Huang and Shih (1981), and the three reported GY's from separate components, the theoretical maximum GY's for manure digestion are 0.607, 0.615 and 0.548 lg VS destroyed^{-1} (Table 8.1).

The experimental method employed for the determination of Kc for litter cannot be applied to manure since insufficient data was obtained at the same RT and influent concentration during the manure digestion experiments. Additionally since at high influent concentrations GY's may be depressed due to inhibition, the results obtained may be misleading. Similarly the determination of Kc by batch digestion may produce a low value due to the build up of inhibitory concentrations of NH_3.

Values of Kc obtained from continuously fed poultry manure digesters at mesophilic temperatures have varied between 0.456 - 0.537 lg VS dest^{-1}. (Gramms et al, 1971) and 0.589 - 0.663 lg VS dest^{-1}. (Hart, 1963). Taking into consideration theoretical and reported values, and GY's obtained during the present study, Kc for manure was estimated as 0.500 lg VS^{-1} for the purpose of development of the model.

μm, Kd Specific Growth and Death Rates

Since the poultry manure digesters were operated at the same temperatures as the litter digesters, the same values can be given to these constants. i.e. $\mu m = 0.326\ d^{-1}$, $Kd = 0.0326\ d^{-1}$.

Ks Saturation Constant

In the litter digestion experiments Ks determined for the shortest RT and lowest influent concentration gave the best fit to all data, so to determine Ks for manure digestion, data at 14.6d RT and 6.8 gl^{-1} VS may be used. If it is assumed that at this concentration no inhibition by

ammonia occurs, equation (17) can still be used to determine Ks. Using the above values for μm and Kd, and the GY of 0.346 lg VS add^{-1} obtained at 14.6d RT and 6.8 gl^{-1} influent VS, a value of 4.65 gl^{-1} is produced for Ks.

8.6.2 Inhibition Term

The major difference between the results from the digestion of litter and manure was that at high influent concentrations manure GY's became depressed due to inhibition by ammonia. In order to apply the litter model to manure therefore, a term must be incorporated which takes into account the inhibition of digestion at high concentrations.

The microbial response to growth inhibition can be based on the kinetics of enzyme inhibition in which it is assumed that biomass behaves as an enzyme reacting with growth limiting substrate, thus both competetive and non-competetive types of inhibition are possible. Ammonia will be considered as an inhibitor added to the substrate rather than the substrate itself causing inhibition, or the inhibitor being produced during digestion.

In competetive inhibition the inhibitor competes with growth limiting substrate at the same enzyme site for up-take by the biomass. While μm is unaffected, Ks is increased by a factor Ki which is proportional to Sr and greater than 1. The Monod equation now becomes

$$\mu = \frac{\mu m\ S}{S + KsKi} \qquad (18)$$

and the model equation is

$$GY = Kc \left[1 - \left(\frac{KsKi\ (D + Kd)}{Sr\ (\mu m - D - Kd)}\right)\right] \qquad (19)$$

In non-competetive inhibition the inhibitor combines with the biomass at a different site to the substrate without affecting the affinity for the substrate. Ks is unaffected but μm is decreased by factor Ki which is

proportional to Sr and less than 1.

The modified Monod equation is

$$\mu = \frac{\mu m \; Ki \; S}{S + Ks} \qquad (20)$$

and the model equation

$$GY = Kc \left[1 - \left(\frac{Ks \; (D + Kd)}{Sr \; (\mu m \; Ki - D - Kd)} \right) \right] \qquad (21)$$

Alternatively the effect of the inhibitor may be to increase Kd by factor Ki which is proportional to Sr and greater than 1. Ks and μm are unaffected. In this case the model equation becomes

$$GY = Kc \left[1 - \left(\frac{Ks \; (D + Kd \; Ki)}{Sr \; (\mu m - D - Kd \; Ki)} \right) \right] \qquad (22)$$

Rearranging equations (19), (21) and (22) an expression can be obtained for each type of inhibition for Ki in terms of RT, Sr, GY and kinetic constants. The equation can be solved for each manure experiment and a value for Ki obtained. From the relationship between Ki and Sr, Ki can be predicted for any influent concentration. The relationship was determined for the second and third group of experiments only since the results from the first group were incompatible with these. For the three inhibition models, expressions for Ki are as follows:

Inhibition Affecting Ks

$$Ki = \frac{Sr \; (\mu m - D - Kd)}{Ks \; (D + Kd)} \cdot \left(1 - \frac{GY}{Kc} \right) \qquad (23)$$

When Ki is plotted against Sr a straight line is obtained with slope $\frac{\mu m - D - Kd}{Ks \; (D + Kd)} \cdot \left(1 - \frac{GY}{Kc} \right)$ and intercept through the origin. The relationship between Ki and Sr is

$$Ki = 0.087 \times Sr + 0.624 \qquad (24)$$

Inhibition Affecting μm

$$Ki = \left[\frac{Ks\ (D + Kd)}{Sr\ (1 - \frac{GY}{Kc})} + D + Kd \right] \div \mu m \qquad (25)$$

The relationship between Ki and Sr is not linear, but a linear relationship may be approached by plotting Ki against ln Sr (model a)) or ln Ki against ln Sr (model b)). Thus

$$Ki = (\ln Sr \times -0.237) + 1.329 \qquad (26)$$

or

$$Ki = \text{antilog}\ (\ln Sr \times -0.369 + 0.561) \qquad (27)$$

Inhibition Affecting Kd

$$\frac{D + KiKd}{\mu m - D - KiKd} = 1 - \frac{GY}{Kc} \cdot \frac{Sr}{Ks} \qquad (28)$$

The relationship between Ki and Sr is linear with

$$Ki = 0.0665 \times Sr + 1.239 \qquad (29)$$

These expressions for Ki were substituted into the appropriate model equations (19), (21) or (22) and GY's calculated for the operating conditions used in the manure digestion experiments.

8.6.3 Predicted GY - LR Relationships

The model GY's are compared with the experimentally determined GY's in Table 8.4, and plotted against LR for a wider range of operating conditions in Figures 8.6 - 8.9. For each model the smallest deviation

Table 8.4 Comparison of Experimental and Model GY's

Sr ($gVSl^{-1}$)	RT (days)	Experimental GY ($lgVS\ add^{-1}$)	Ks Inhibition		μm Inhibition a)	
			GY ($lgVS\ add^{-1}$)	Deviation (%)	GY ($lgVS\ add^{-1}$)	Deviation (%)
7.2	29.2	0.370	0.396	7.0	0.399	7.8
28.8		0.416	0.435	4.6	0.449	7.9
50.4		0.380	0.440	15.8	0.451	18.7
72.0		0.376	0.422	12.2	0.439	16.8
13.1	29.2	0.404	0.419	3.7	0.429	6.2
26.2		0.437	0.433	-0.5	0.447	2.3
39.3		0.428	0.438	2.3	0.452	5.6
6.8	14.6	0.346	0.313	-9.5	0.311	-10.1
41.0		0.410	0.393	-4.1	0.371	-9.5
68.4		0.399	0.399	0.0	-0.198	-149.6

Table 8.4 Comparison of Experimental and Model GY's

Sr ($gVS l^{-1}$)	RT (days)	μm Inhibition b) GY ($lgVS\ add^{-1}$)	μm Inhibition b) Deviation (%)	Kd Inhibition GY ($lgVS\ add^{-1}$)	Kd Inhibition Deviation (%)
7.2	29.2	0.396	7.0	0.376	1.7
28.8		0.445	7.0	0.441	6.0
50.4		0.454	19.5	0.440	15.8
72.0		0.458	21.8	0.422	12.2
13.1	29.2	0.423	4.7	0.418	3.5
26.2		0.443	1.4	0.440	0.7
39.3		0.451	5.4	0.443	3.5
6.8	14.6	0.308	-13.1	0.291	-15.9
41.0		0.369	-10.0	0.412	0.5
68.4		0.317	-20.6	0.372	-6.8

from experimental results was obtained for the data of the second group of experiments. The best fit to all experimental data was provided by the Ks and Kd inhibition models, while both μm inhibition models produce the greatest deviations (Table 8.4).

Although the Ks inhibition model (equation (19)) gave a good fit to experimental data, increasing the influent concentration at constant RT produced a progressive increase in GY which levelled off at high LR's, but did not then decline with further increases in influent concentration (compare Figure 8.6 with Figure 4.11). In this model therefore, the inhibition term was not strong enough to overcome the stimulatory effect of increasing influent concentration of GY, so although it gave a good fit to experimental data, outside these operating conditions errors would become enlarged.

The depression of GY's at high influent concentrations however was obtained to varying degrees with the other three models. The largest inhibitory effects of Sr on GY were provided by μm inhibition model a) (equation (21) and Figure 8.7). At each RT after a certain influent concentration had been reached GY dropped rapidly, and at 14.6d RT and 68.4 gl^{-1} Sr a negative yield was obtained. Thus the effect of the inhibition term in this model was too large and it produced the greatest deviation from experimental results.

The second μm inhibition model (equation (21)) gave a more gradual reduction in GY with increasing influent concentration, but at RT's longer than 12 days and LR's up to 5.0 kg VS m^{-3} d^{-1}, the decline in GY was so slight as to be almost undetectable (Figure 8.8). At long RT's therefore, the inhibition term was not strong enough and since errors were relatively large this model appeared to be unsuitable.

The Kd inhibition model (equation (22)) gave a close fit to experimental data (Table 8.4) and at all RT's gave a moderate reduction in GY after a certain optimum Sr had been reached (Figure 8.9). This appeared to be the model which most accurately described the digestion of poultry manure under the experimental operating conditions, and conditions outside

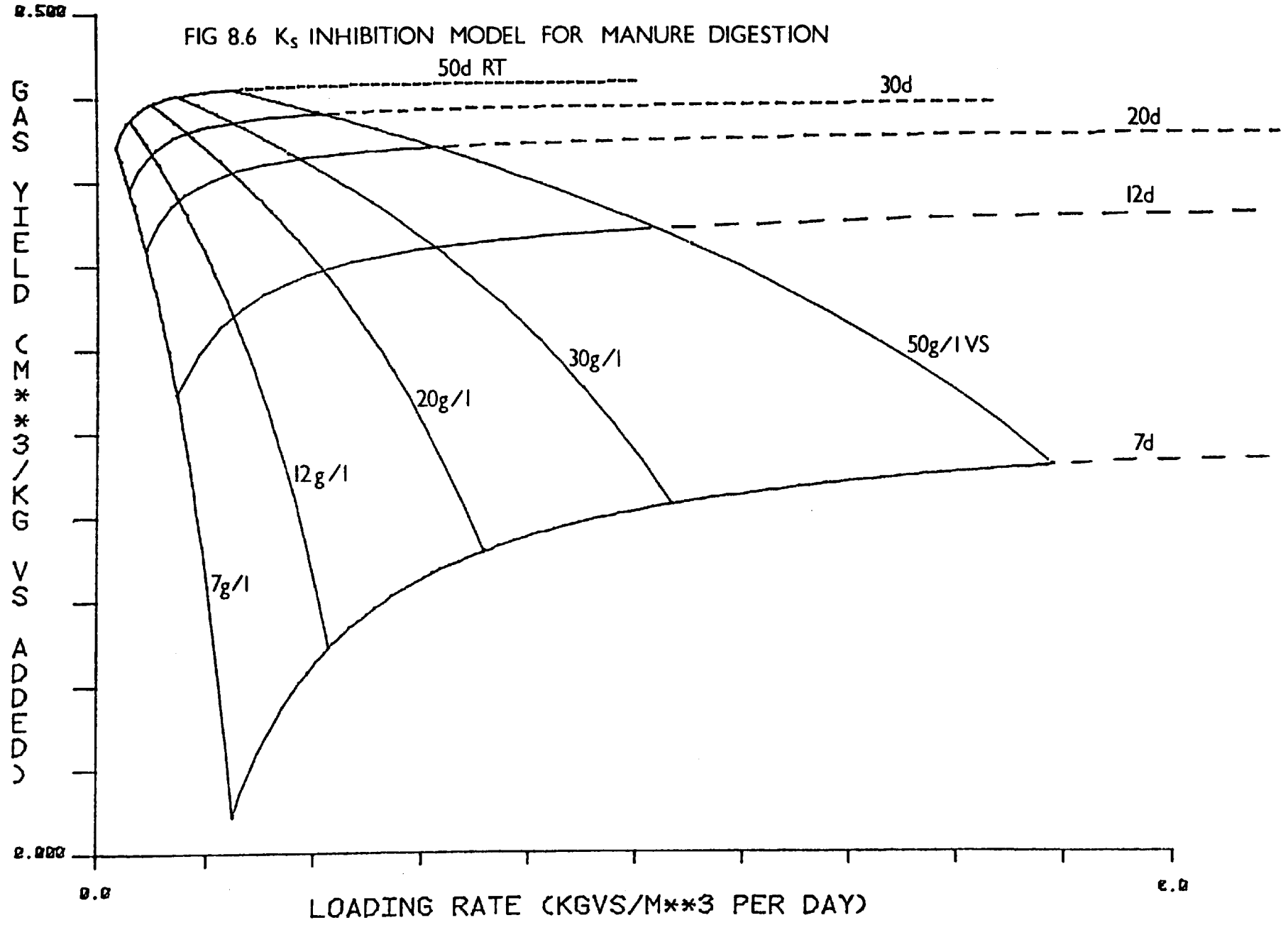


FIG 8.6 K_s INHIBITION MODEL FOR MANURE DIGESTION
50d RT
30d
20d
12d
7d
50g/l VS
30g/l
20g/l
12g/l
7g/l
GAS YIELD (M**3/KG VS ADDED)
0.500
0.000
0.0
LOADING RATE (KGVS/M**3 PER DAY)

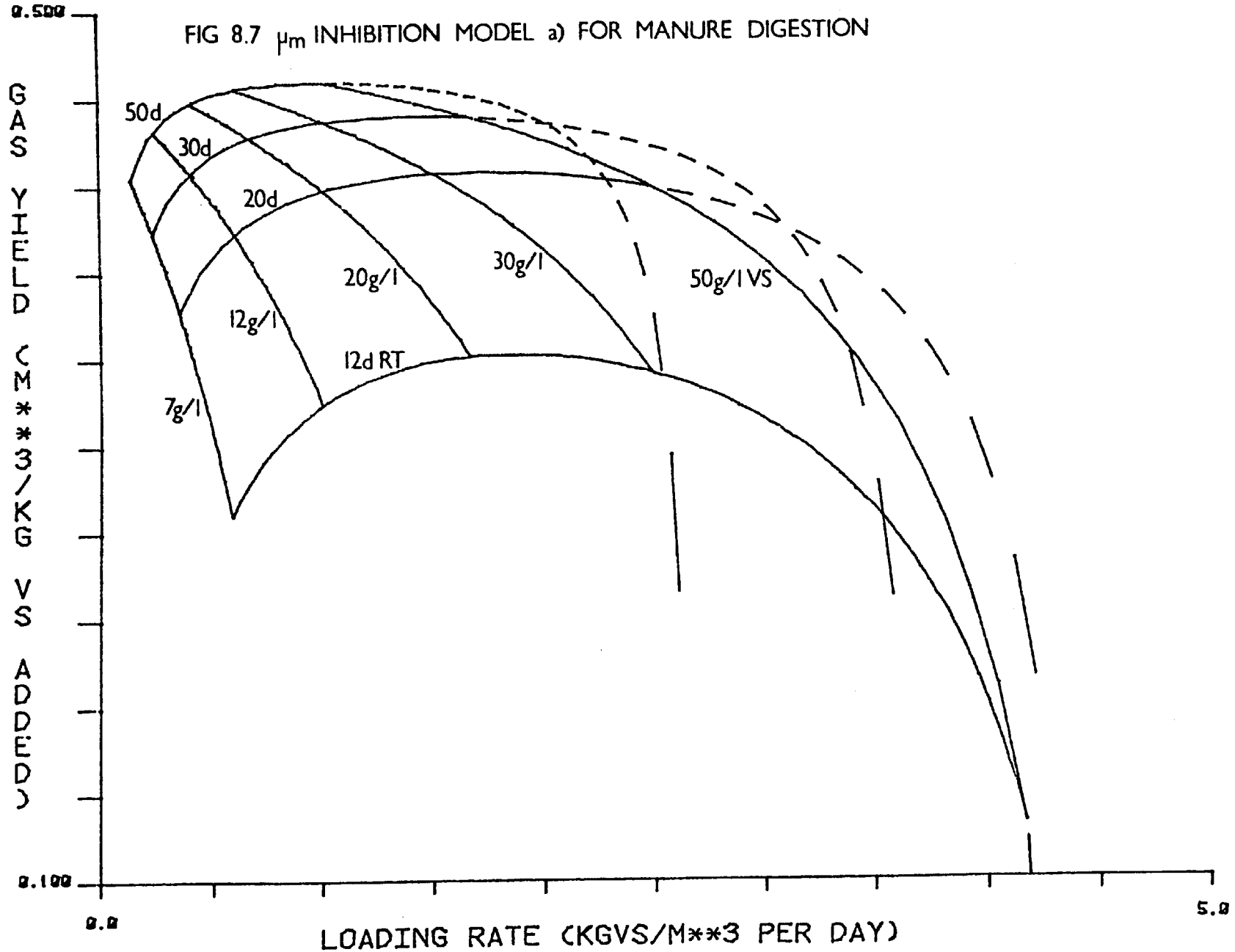


FIG 8.7 μm INHIBITION MODEL a) FOR MANURE DIGESTION
0.500
0.100
0.0
5.0
GAS YIELD (M**3/KG VS ADDED)
LOADING RATE (KGVS/M**3 PER DAY)
50d
30d
20d
12d RT
20g/l
30g/l
50g/l VS
12g/l
7g/l

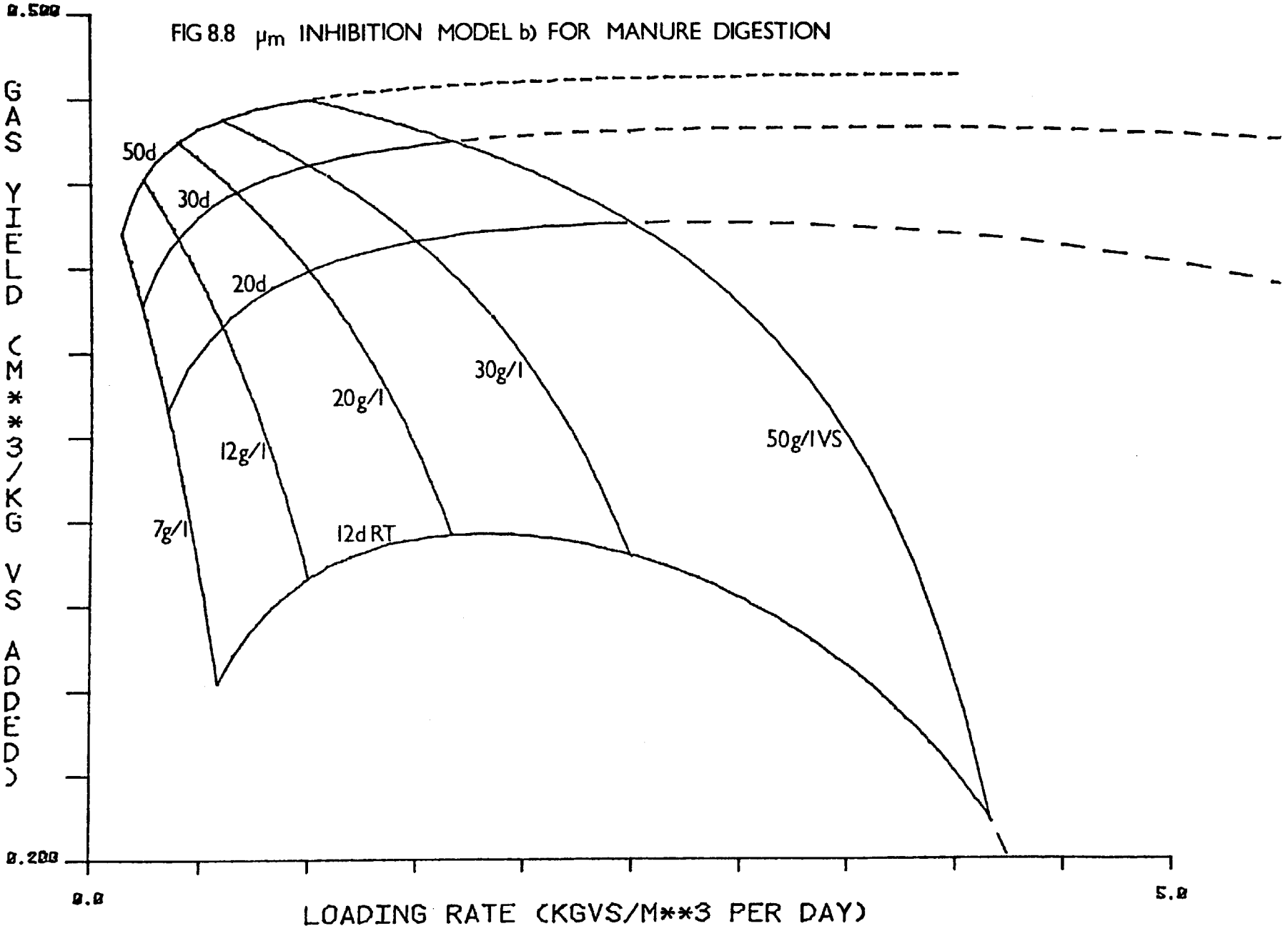
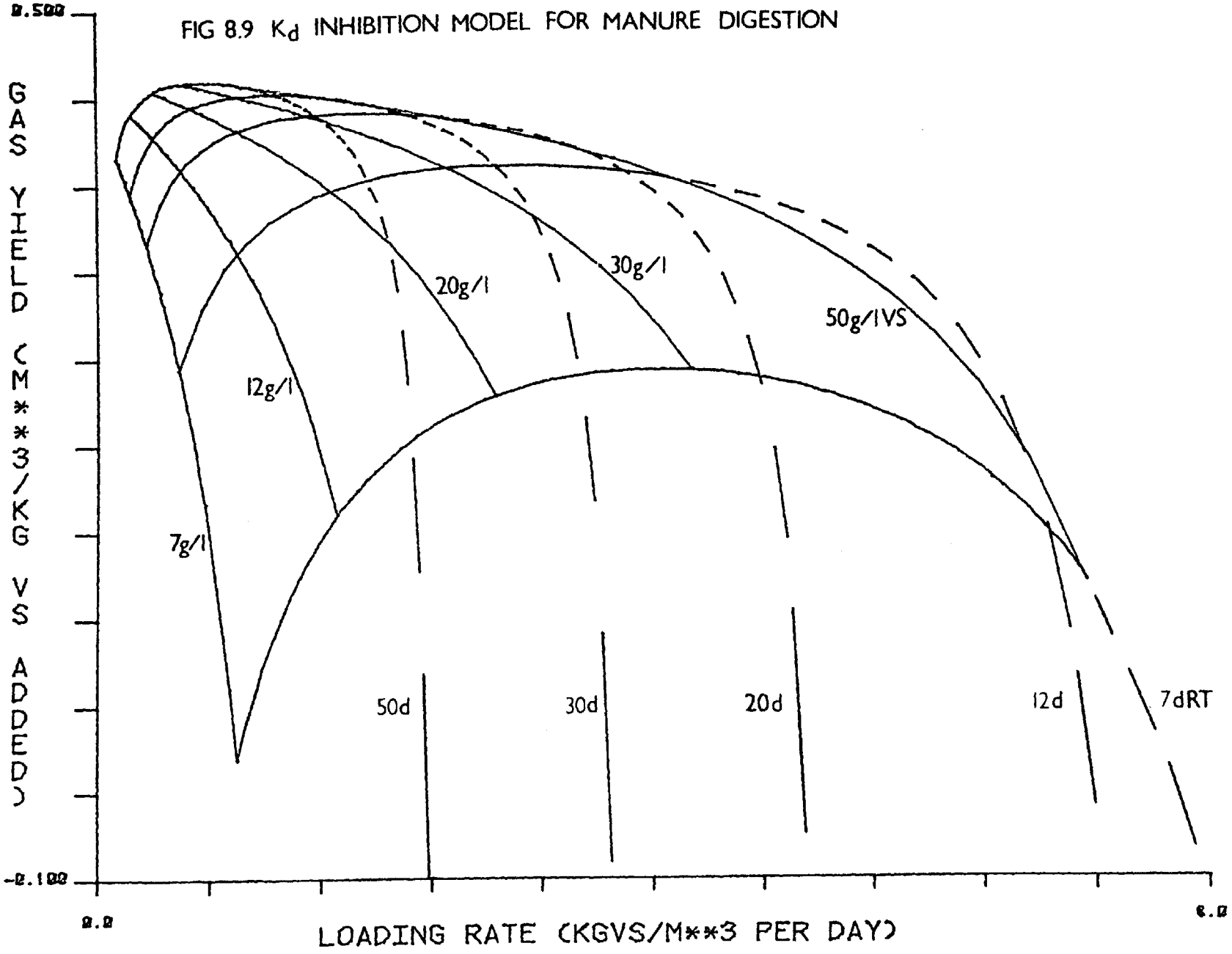


FIG 8.8 μm INHIBITION MODEL b) FOR MANURE DIGESTION
0.500
0.200
GAS YIELD (M**3/KG VS ADDED)
50d
30d
20d
30g/l
20g/l
12g/l
7g/l
12d RT
50g/lVS
0.0
5.0
LOADING RATE (KGVS/M**3 PER DAY)

FIG 8.9 K_d INHIBITION MODEL FOR MANURE DIGESTION



this range. Figure 8.9 can be used to predict the optimum conditions for maximum efficiency of digestion. The influence of RT on GY is not affected by the inhibition term, so at constant Sr a longer RT gives a greater GY and hence degree of waste stabilisation, but will require a larger digester. At constant RT the optimum Sr is the highest Sr before the inhibitory effects of ammonia begin to depress the GY. This optimum point increases with increasing RT, thus at 7d RT the optimum SR is 30 g VSL^{-1}, and at 20d RT the optimum Sr is 50g $VS1^{-1}$.

8.7 Discussion

According to the litter model (and manure model before inhibition reaches a certain level) GY increases not only with increasing RT, but also with increasing Sr. Longer RT's increase the GY since the substrate can be more completely degraded over a longer period of time. Additionally from equation (10) S is dependent on D ($\frac{1}{RT}$) and when RT increases S decreases. Thus according to equation (13) a decrease in S at constant Sr will increase the amount of substrate destroyed and therefore the volume of gas produced and also gas yield.

Since S is independent of Sr and constant at constant D (equation (10)), according to equation (13) when Sr is increased, the amount of substrate destroyed will increase as will gas production and gas yield. Both characteristics of the model examined here therefore are consequences of the dependence of S solely on D expressed by equation (10) which is derived from the Monod equation and given that at steady state $\mu = D + Kd$. This should apply to all models based on Monod kinetics.

Although there is evidence to suggest that the S-D relationship may hold only for pure cultures (Grady et al, 1972), it has been shown to be true for the digestion of glucose (Ghosh and Pohland, 1974) and fatty acids (Hobson 1983, Lawrence and McCarty 1969), and Monod based models have been used successfully to describe AD of simple soluble substrates in completely mixed fermenters (Andrews and Graef 1971, Kugelman and Chin 1971).

However in the digestion of complex substrates such as animal wastes the S-D relationship appears to break down and S, measured by VS for example, becomes dependent primarily on influent concentration, and D has only secondary significance (see for example Converse et al 1980, Hart 1963, Hashimoto 1982, Hill and Barth 1977, and Sections 3.3.3.1 and 4.3.2.1 in the present study). This is because animal wastes are heterogeneous in nature and are composed of both soluble and insoluble material. The S-D relationship does not hold for insoluble VS such as lignocelluloses which tend to settle out in the digester and accumulate due to incomplete mixing. The amount of insoluble VS in the digester will be determined by Sr. Since by weight insoluble VS is much greater than soluble VS, the VS determination made on the digester will reflect the insoluble VS content, and will therefore be proportional to Sr. For the insoluble VS D has only secondary significance so from equation (13) at constant RT, GY from insoluble VS will be constant at all influent concentrations. However the soluble VS in the digester is determined primarily by D so the GY from this material will increase with Sr, and when combined with the GY from insoluble VS the overall effect will be an increase in GY with Sr. Thus for any waste material, which is not subject to inhibition, GY will increase with increasing Sr but by a degree related to the fraction of digester VS which is soluble. Most of the soluble VS such as VFA's, is biodegradable and readily converted to gas, but only a proportion of insoluble VS such as cellulose, is biodegradable and this requires a long period for digestion. Soluble VS therefore, has a greater effect on gas production than insoluble VS, so the effect of GY increasing with Sr becomes more apparent. However due to the insoluble biodegradable content of poultry litter the increase in GY with Sr obtained by experiment was not as great as that predicted by the model (Section 8.5.3).

Thus all models based on Monod kinetics describe the digestion of the soluble biodegradable fraction of the substrate alone, and cannot predict gas production by insoluble substrate, so their application to the digestion of complex animal wastes is limited.

The fact that solid matter is retained within a digester has been taken into account by Hill and Barth (1977) in the development of a model which predicted the dependence of total digester substrate on Sr and the secondary effects of RT, during the digestion of poultry manure. However the steady-state relationships between GY and LR were not examined, but assuming that $\mu = D + Kd$ at steady-state, from this model soluble digester substrate concentration is dependent on D alone and GY will therefore increase with increasing Sr.

In a generalised substrate utilisation model proposed by Chen and Hashimoto (1978) which was derived not from Monod kinetics but by using an enzyme kinetics analogy, effluent substrate concentration is given by

$$S = \frac{Sr\ K}{RT/RT_m - 1 + K} \qquad (30)$$

where K is a kinetic constant and RT_m the minimum possible RT. Thus S is dependent on Sr as well as RT. However GY (B) in terms of gas volume produced per gCOD added is

$$B = Bo \left[1 - \left(\frac{K}{RT/RT_m - 1 + K}\right)\right] \qquad (31)$$

where Bo is the GY at infinite RT, i.e. Bo = Kc. Thus GY is a function of RT and kinetic constants and is independent of Sr. In the derivation of this model the Monod equation was replaced by a kinetic equation which relates specific growth rate to substrate concentration

$$\mu = \frac{\mu m\ S/Sr}{K + (1-K)\ S/Sr} \qquad (32)$$

Thus μ is a function of both Sr and S, whereas in the Monod equation only S is involved. The consequence of this is the dependence of S on both RT and Sr and the dependence of GY on RT alone.

The model developed by Lavagno et al (1983) which is based on the steady state equilibria of the hydrolysis, acidogenic and methanogenic

stages of the process has been used to examine steady state relationships between gas production and operating conditions. GY increased with increasing RT, levelling off at approx. 30 days, but increasing Sr caused only an initial increase in GY, and after an influent concentration of approx 5 gl^{-1} was reached, GY declined rapidly. This model therefore predicts a decrease in GY with increasing Sr rather than the increase obtained from Monod kinetics. This suggests the possibility of substrate inhibition, but in the Lavagno model no function describing inhibition is included.

In the poultry manure model proposed in this study, inhibition was described in terms of Sr i.e. as substrate inhibition, rather than NH_4^+-N concentration, although the latter can be predicted from the former, and the effects of inhibition were assumed to increased progressively with increasing Sr rather than exhibiting a threshold level above which inhibition begins. In the results obtained during the digestion of poultry manure there appeared to be a threshold Sr above which GY began to decrease (Figure 4.11), but this was the result of the inhibitory effects of NH_4^+-N overcoming the stimulatory effects of increasing Sr.

The Chen and Hashimoto (1978) model includes a kinetic parameter K which increases when the CH_4 production rate decreases and thus indicates some form of inhibition. In an analysis of the kinetics of CH_4 production using this model, Hill (1982) determined K for poultry manure and found evidence for a threshold concentration for substrate inhibition of 40 g VSl^{-1}. This value is similar to the threshold of 30 g VSl^{-1} - 50 g VSl^{-1} at 7d-20d RT obtained from the manure model (Section 8.6.2), but as stated above the threshold may be only apparent and not a real effect.

The dynamic model developed by Andrews and Graef (1971) includes a term to take into account inhibition by high concentrations of unionised VFA's. In the present study free NH_3 rather than VFA's was thought to be the inhibiting agent since even at the highest Sr tested, the concentrations of VFA's was less than 1,000 mgl^{-1}. The inhibition by free NH_3 as well as unionised VFA's was included in Hill and Barth's (1977) modification of

the Andrews and Graef model which accurately predicted the performance and failure during start up of poultry manure digesters operated at 25°C, RT's of 15d - 45d and Sr's of 48 g VSl^{-1} - 144 g VSl^{-1}. High levels of VFA's (11,980 mgl^{-1} - 17,212 mgl^{-1}) were produced in the failed digesters which were accompanied by NH_4^+-N concentrations between 1,800 mgl^{-1} and 2,785 mgl^{-1}. However in the present study poultry manure digesters operated under similar conditions did not fail at start-up even though NH_4^+-N levels were as much as two fold higher. Consequently VFA concentrations were much lower in the present study than in Hill and Barth's failed digesters, but comparable to the levels in their failed digesters. The Hill and Barth (1977) model therefore does not accurately describe the performance of the digesters in the present study, although the discrepancy may be due to the temperature difference between the two investigations since several of the constants including μm are temperature dependent.

In an investigation of the effects of inhibitors on the kinetics of acetate degradation, Kugelman and Chin (1971) found that increasing the K^+ concentration to 0.2M reduced μm by half from $0.36d^{-1}$ to $0.18d^{-1}$, while raising Na^+ levels to 0.35M had the same effect on μm, but also doubled Kd from $0.065d^{-1}$ to $0.13d^{-1}$, as well as reducing Y, the growth yield constant. Thus the aspect of microbial growth kinetics which is affected by an inhibitor may depend on the nature of the inhibitor, and one or more kinetic constant may be involved.

Several possibilities, although no combinations, were considered in the inhibition model proposed in the present study which fitted the experimental results to varying degrees. No experimental evidence is available to suggest that AD inhibitors affect Ks but when Ks was increased with increasing Sr a good fit was obtained to experimental data, but outside this range of operating conditions predicted GY's deviated from the expected pattern. Varying μm inversely with Sr produced large deviations between experimental and predicted data, while the model which described the digestion ofmanure most accurately was that in which inhibition affected Kd (equation (22)). A similar approach was adapted by Hill et al (1983) who were able to increase the accuracy of the description of the performance of inhibited digesters by assuming that the inhibitory

effect of VFA's was to raise Kd. This constant was varied initially between 0.1 μm and μm, then given a Monod type function in which

$$Kd = \frac{Kd\ max}{1 + \frac{Kid}{VFA}} \qquad (32)$$

From these two investigations therefore it appears that the best description of the inhibition of AD by high substrate concentrations may be provided by considering that the Kd of the micro-organisms increases in proportion to the influent concentration. Kd therefore may be the aspect of microbial kinetics which is affected by high influent concentrations.

8.8 Conclusions

1. The increase in GY obtained with increasing Sr and RT observed during the digestion of sewage sludge and poultry litter can be explained by Monod based microbial growth kinetics.

2. Monod kinetics applies only to soluble substrates, so the degree to which heterogeneous materials such as animal wastes follow the predicted pattern depends on the proportion of biodegradable material present in the waste which is soluble.

3. A steady-state model based on Monod kinetics was developed to predict GY's which may be obtained at varying RT's and Sr's for the digestion of poultry litter.

4. A similar model was developed to describe the digestion of poultry manure which incorporated a term taking into account the depression of GY's at high Sr's due to inhibition by NH_4^+-N.

5. Of the 3 types of inhibitor action considered which affected the constants Ks, μm or Kd, the type which increased Kd with Sr gave the best overall description of poultry manure digestion.

Nomenclature

Symbol	Parameter	Units
B	Gas yield	1 gCOD added^{-1}
Bo	Gas yield at infinite RT	1 gCOD added^{-1}
D	Dilution rate	d^{-1}
Dc	Critical dilution rate	d^{-1}
F	Flow rate	ld^{-1}
GY	Gas yield	1 gVS added^{-1}
K	Kinetic parameter	Dimensionless
Kc	Volume of gas produced per unit weight of total substrate destroyed	lg^{-1}
Kd	Specific death rate	d^{-1}
Ke	Fraction of substrate utilised in energy production	
Kg	Volume of gas produced per unit weight of substrate utilised in energy production	lg^{-1}
Ki	Inhibition coefficient	Dimensionless
Ks	Saturation coefficient	gl^{-1}
LR	Loading rate	g VS l^{-1} d^{-1}
RT	Retention time	d
RT_m	Minimum retention time	d
S	Digester substrate concentration	gl^{-1}
Sr	Influent substrate concentration	gl^{-1}
V	Digester liquid volume	l
X	Biomass concentration	gl^{-1}
Y	Growth yield	gg^{-1}
μ	Specific growth rate	d^{-1}
μm	Maximum specific growth rate	d^{-1}

CHAPTER 9

THE IMPLICATIONS OF AMMONIA AND INFLUENT CONCENTRATIONS FOR THE ANAEROBIC DIGESTION OF POULTRY WASTES.

9.1 Ammonium Nitrogen

During the course of this investigation the importance of NH_4^+-N in poultry waste digestion has become evident. The digester NH_4^+-N concentration is determined by the concentration of total nitrogen in the influent which therefore provides a means for prediction if the latter is known. Besides providing a nitrogen source for bacterial growth and limiting the growth of methanogens at low levels (Bryant et al 1971), ammonia is a potential inhibitor and can reach toxic concentrations at high LR's. Additionally digester ammonia contributes to alkalinity and influences pH, both important parameters in process stability. Alkalinity determines the ability of the digester to withstand changes in pH which may otherwise reduce performance and cause process failure. Digester pH increases with increasing NH_4^+-N concentration which thus increases the possibility of ammonia toxicity since the proportion of the more toxic free NH_3 form is raised. Free NH_3 is more toxic than NH_4^+ presumably because unionised forms are able to pass through the bacterial cell membrane more easily than ionised forms.

9.2 Ammonia Inhibition

In the digestion tests of poultry manure there appeared to be a threshold concentration for ammonia inhibition which was between 2,727 mgl^{-1} and 2,880 mgl^{-1} NH_4^+-N or 138 mgl^{-1} and 225 mgl^{-1} NH_3 (Section 4.4.3.2). However this threshold was only apparent and was caused by the inhibitory effects of ammonia overcoming the stimulatory effects of increasing Sr on GY. Actual thresholds for ammonia inhibition were observed in the shock loading experiments (Sections 5.3.2.2 and 5.4.2.2), and evidence was produced which suggested that the threshold level depended on the NH_4^+-N level to which the bacteria were adapted.

It is possible however that the effects of ammonia when shock loaded in the form of ammonium salts to batch digesters may be different to the same concentrations occurring naturally in daily fed digesters, due to the possibility of adaptation and the tendency for NH_4^+ salts to reduce rather than raise the pH. The long term experiments (Chapter 6) give a more realistic representation of the inhibitory effects that may have been produced by ammonia in the daily fed manure digesters. Raising the NH_4^+-N concentration of the treated digesters from approx. 2,300 mgl^{-1} to 3,062 mgl^{-1} produced a 12% inhibition of gas production, while a further increase to 4,837 mgl^{-1} produced 29% inhibition. The control digesters in these experiments were actually digesters 11 and 12 in the second group of daily fed manure experiments which were operated at 29d RT and influent concentration of 2.62% VS, and had a mean NH_4^+-N content of 2,298 mgl^{-1} (Table 4.4). Digesters 13 and 14 in this group were operated at the same RT but greater Sr of 3.93% VS, and had an NH_4^+-N concentration of 3,325 mgl^{-1}. According to the long term experiments and assuming a linear relationship between NH_4^+-N concentration and degree of inhibition, an NH_4^+-N level of 3,325 mgl^{-1} in digesters 13 and 14 would be expected to produce 14% inhibition of GY. However compared with digesters 11 and 12 a reduction of only 2.1% in GY was obtained (Table 4.6). This was due to the fact that an increase in Sr which besides increasing digester NH_4^+-N concentrations, which depresses the GY, also has a stimulatory effect on GY which is not obtained when NH_4^+-N concentration alone is raised as in the long term experiments. The stimulatory effect of raising Sr therefore reduced the inhibitory effect of NH_4^+-N in digesters 13 and 14 from an expected 14% to only 2.1%. The effect of increasing Sr from 2.62% VS to 3.93% VS therefore was an increase of approx. 12% in GY.

The GY obtained from the digestion of poultry manure at varying influent concentrations and RT's therefore, is the result of the combination of the beneficial effect of Sr and the inhibitory effect of ammonia produced at that LR. The model proposed for manure digestion (equation (22), Section 8.6.2) can predict the GY obtained at any combination of RT's and Sr's and takes into account the effect of Sr and NH_4^+-N concentration on GY. For simplicity the model proposed for

litter digestion (equation (15) Section 8.5.1) does not contain an inhibition term although ammonia may produce inhibitory effects at all but the lowest levels. The maximum levels of NH_4^+-N and free NH_3 obtained in the litter digesters were 1502 mgl^{-1} and 64.5 mgl^{-1} respectively, which are both lower than the apparent thresholds for inhibition in the manure digesters, so no inhibitory effects of ammonia were observed during litter digestion.

9.3 Effect of Ammonia on pH

It was observed in Sections 5.3.2.2, 5.4.2.2 and 6.4.6 that the inhibitory effects of NH_4^+-N concentrations produced by the addition of NH_4^+ salts may not be as great as the same naturally occurring concentrations. This is because in untreated digesters pH increases with NH_4^+-N concentration (Sections 3.3.3.5 and 4.3.2.5) thus increasing the level of the more toxic free NH_3, whereas in digesters treated with NH_4^+ salts this effect is not observed. Ammonia produced naturally from proteins or uric acid degradation reacts with water to form NH_4^+ and OH^- ions thus raising the pH.

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \qquad (1)$$

Adding NH_4Cl or NH_4HCO_3 to raise the NH_4^+-N concentration of poultry manure digesters failed to raise the pH significantly, but the pH values which would have resulted from the same naturally produced NH_4^+-N levels can be estimated from Figure 4.17. For instance the NH_4^+-N levels of digester T2 in the long term experiments (Chapter 6) were raised to 3062 mgl^{-1} and 4,837 mgl^{-1} by the addition of NH_4Cl. According to the relationship from Figure 4.17,

$$pH = \text{antilog } (\ln NH_4^+\text{-N conc. } (mgl^{-1}) \times 0.0452 + 1.691)$$

so the expected pH values for T2 would be 7.80 and 7.96 respectively whereas values of 7.62 and 7.43 were actually obtained. Similarly expected pH values can be calculated for the shock loading experiments.

Apart from the lowest NH_4^+-N levels, the pH values obtained were much lower than predicted for naturally occurring concentrations. It must be noted however, that CH_3 COONa or glucose which were also added may have had some effect on the pH in these digesters, and that the relationship presented in Figure 4.17 was determined for semi-continuous and not batch digesters.

9.4 Effect of Ammonia on Alkalinity

In addition to affecting digester pH, naturally produced NH_4^+-N also influenced the alkalinity of the digesters in Chapters 3 and 4. The elevated pH caused by NH_4^+-N allows CO_2 in the digester head space to dissolve in the digester liquid producing bicarbonate which therefore raises the alkalinity.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \qquad (2)$$

Carbonic acid reacts with OH^- produced in equation (1) to produce more bicarbonate

$$OH^- + H_2CO_3 \rightleftharpoons HCO_3^- + H_2O \qquad (3)$$

However in the long term experiments, raising the NH_4^+-N concentration by adding NH_4Cl did not have the same effect on alkalinity as naturally produced levels, and was approximately the same in control and treated digesters (Section 6.3.6). This is because as stated above NH_4Cl does not raise digester pH so the equilibria in equations (2) and (3) remain to the left and less HCO_3^- is produced. As with pH, the alkalinity produced by the same naturally occurring NH_4^+-N levels can be estimated from the relationship determined during the semi-continuous digestion of poultry manure shown in Figure 4.16.

$$\text{Alkalinity } (mgl^{-1}) = 5.55 \times NH_4^+\text{-N } (mgl^{-1}) + 389$$

Thus at NH_4^+-N concentrations of 3,062 mgl^{-1} and 4,837 mgl^{-1}, alkalinities of 17,383 mgl^{-1} and 27,234 mgl^{-1} would be expected whereas values of 13,917 mgl^{-1} and 10,922 mgl^{-1} were obtained experimentally for digester T2 (Table 6.1).

A more suitable method of determining the effects of ammonia on digestion would be to increase NH_4^+-N levels naturally by adding to the influent nitrogen containing compounds such as Proteins, amino acids, uric acid or urea which would be metabolised in the digester with the formation of ammonia and consequent increase in pH. This method however has the disadvantage that these materials can contribute to gas production and may reduce the apparent effects of NH_4^+-N.

9.5 High Solids Digestion

The NH_4^+-N concentrations produced in the liquor of the high solids digester reached 13,314 mgl^{-1} and 10,570 mgl^{-1} in the manure experiments (Figures 7.2 and 7.3), and 6,895 mgl^{-1} in the litter experiment (Figure 7.4). These concentrations were higher than those tested in the long term experiments, but similar to the levels used in the shock loading experiments. The highest NH_4^+-N levels tested by shock loading were 12,449 mgl^{-1} and 13,020 mgl^{-1} which caused almost complete inhibition of CH_4 production (98.7% and 90.4% inhibition respectively), while 10,182 mgl^{-1} and 10,850 mgl^{-1} produced 78.8% and 67.7% inhibition, and 6,686 mgl^{-1} and 6,675 mgl^{-1} produced 28.2% and 37.7% inhibition (Tables 5.5 and 5.7). Although some degree of adaptation may have occurred during start-up of the high solids digesters as NH_4^+-N concentrations built up in the liquor, the shock loading experiments indicate that the methanogenic bacteria would have been highly inhibited by the NH_4^+-N levels occurring there. Indeed even the high solids litter digester which had the lowest NH_4^+-N concentrations produced insignificant volumes of CH_4 after the maximum level had been reached. However in the second high solids manure digester when the liquor had been replaced and the NH_4^+-N concentration had dropped to approx. 6,200 mgl^{-1}, CH_4 production resumed after 15 - 20 days and continued to the end of the

experiment. These 15 - 20 days may have represented a lag period as observed by Van Velsen (1979a) during which the bacteria were able to adapt to the reduced NH_4^+-N level. In this situation therefore where a period of acclimation is necessary, shock loading experiments do not give an accurate indication of the effect of an inhibitor, but are nevertheless useful in predicting inhibition when the inhibitor is introduced in one dose.

It is not known if the rate of gas production by this high solids manure digester after acclimation reached levels which would have been achieved in the absence of ammonia. i.e. if complete adaptation had occurred. The results from the long term experiments (Figure 6.4) suggest that a period of acclimation, although affording the bacteria a degree of tolerance to the inhibitor, does not allow a rate of gas production approaching that of the uninhibited state to be attained. When a digester is said to be adapted to a potential inhibitor therefore, it may merely imply that the seed has undergone a period of exposure or acclimation to that inhibitor and thus become acclimated without becoming fully adapted.

9.6 Inhibition of Methanogenic Bacteria

The methanogens are considered to be the most sensitive group of bacteria involved in the AD process and would be expected to be the group most susceptible to ammonia inhibition. In the semi-continuous digestion tests of both litter and manure, as Sr and therefore digester NH_4^+-N concentrations increased, there was a tendency for the proportion of CH_4 in the gas produced to decrease thus indicating methanogen inhibition (Tables 3.8, 3.9 and 4.6). This effect was more pronounced in the manure digesters, but has since been reconfirmed for litter digestion at the Polytechnic of Wales.

Indication that the methanogenic rather than hydrolytic and acetogenic bacteria were inhibited by ammonia was provided again by shock loading NH_4Cl to manure batch digesters. Raising the NH_4^+-N

concentration reduced total gas production and CH_4 content of the gas in comparison with control digesters. Additionally there was an accumulation of VFA's in the inhibited digesters (Table 5.5).

The methane forming bacteria were again inhibited by high NH_4^+-N levels in the initial stages of the high solids digestion tests where only a small proportion of the total gas produced was CH_4. CO_2 was still produced by other groups of bacteria which were unaffected or inhibited to a lesser degree than the methanogens. Due to the inability of these bacteria to remove VFA's, very high concentrations of these compounds accumulated in the liquor.

In the long term experiments involving NH_4Cl additions however, gas production by treated digesters was reduced but the CH_4 content of the gas was not significantly different to that of control digesters. Additionally the VFA concentration was similar in treated and control digesters. Both these factors suggest that methanogenic and non-methanogenic groups of bacteria were equally susceptible to ammonia inhibition and in this respect the results contradict the evidence from the other experiments.

9.7 VFA Inhibition

There is conflicting evidence concerning the inhibition of VFA's to methanogenic bacteria. The subject has been reviewed by Kugelman and Chin (1971), and Kroeker *et al* (1979). Buswell (1947) and Schlenz (1947) concluded that VFA's are toxic to CH_4 bacteria but not to acid formers at concentrations greater than 2000 mgl^{-1}, while McCarty and McKinney (1961a, b) showed that high VFA concentrations are the result of unbalanced fermentation, and not the cause. Buswell and Morgan (1962) reported that propionic rather than acetic acid was the toxic agent. This was investigated further by McCarty *et al* (1964) who provided evidence which indicated that propionate retarded acid formers, and that VFA's were not toxic to CH_4 bacteria at concentrations occurring in malfunctioning digesters. Andrews and Graef (1971) have suggested that VFA toxicity

may be due to the unionised portion of the molecule which like free NH_3 can pass through the cell membrane more readily than the ionised form, and is thus related to digester pH which controls the equilibrium between ionised and unionised VFA's (UVFA's)

$$CH_3\ COOH \rightleftharpoons CH_3\ COO^- + H^+ \qquad (5)$$

At 35°C the ionisation constant has a value of 1.73×10^{-5}, Thus

$$\frac{[CH_3\ COOH]}{[CH_3\ COO^-]} = \frac{[H^+]}{1.73 \times 10^{-5}} \qquad (6)$$

A decrease in pH (increase in H^+ concentration) shifts the equilibrium to the left and increases the proportion of VFA's in the more toxic unionised form. This hypothesis was confirmed by Kroeker et al (1979) who concluded that process inhibition by ammonia appeared to be the result of excessive concentrations of free NH_3 rather than NH_4^+, but that process toxicity (as defined by total cessation of microbial activity) was caused by UVFA's at concentrations between 30 mgl^{-1} and 60 mgl^{-1} as acetic acid. This occurred when the pH of digesters inhibited by high NH_4^+-N concentrations was reduced to lower the level of free NH_3. High concentrations of VFA's, present due to the inhibition of methanogens, were converted by the low pH to the unionised form which exceeded toxic levels.

This situation however, should not occur naturally in continuously fed digesters inhibited by ammonia since high NH_4^+-N concentrations would lead to high digester pH and alkalinity. VFA's may accumulate but UVFA's would not reach toxic levels due to the high pH and buffering capacity which would resist any pH changes.

In the semi-continuous and batch digesters of the present study, VFA concentrations were not high enough to produce the reported toxic concentrations of UVFA's at the pH values obtained even in the shock

loading experiments when the NH_4^+-N concentration was increased to 12,449 mgl^{-1} and CH_4 production was inhibited by 98.7%. However in all the high solids digestion experiments VFA levels were high enough to raise UVFA concentrations above the threshold limit of 30 mgl^{-1} specified by Kroeker et al, and gas production was severely retarded, although in the second experiment the bacterial population was still viable since when the liquor was replaced gas production resumed (Figure 7.3). This suggests that the concentrations of UVFA's present were not toxic, but is not definite since when the liquor was replaced fresh seed was introduced which may have taken over gas production from the original bacteria.

9.8 Ammonia Desorption

The problem of ammonia toxicity can be avoided by diluting the digester influent so that the concentrations of ammonia produced in the digester are below toxic levels. However as stated previously this has a detrimental effect on the economics of the process.

An alternative method of eliminating the problem is to remove the ammonia as gas from the digester liquid by desorption. However ammonia can only be expelled as gas in its undissociated form and at mesophilic temperatures at the pH range where most digester operate (6.8-7.5) only a small proportion of the total NH_4^+-N is in the form of free NH_3. Thus at 35°C and pH 7.0

$$\left[NH_3\right] = \left[NH_4^+\text{-}N\right] \times 0.014 \qquad (7)$$

The volume of gas available for stripping can be increased by recirculating many times through the digester when the gas is used for mixing, and if the pH of the digester liquid is raised the efficiency of absorption can be increased. This can be accomplished by adding a base continuously to the digester, or by removing CO_2 from the gas stream and using CO_2 free gas for mixing and further stripping of CO_2. Ammonia can be captured by stripping from the gas by a solution of phosphoric acid which results in the formation of $NH_4 HPO_3$, a valuable fertiliser.

The feasibility of stripping ammonia from digesting piggery manure has been investigated by Schmid et al (1975) who found that the removal of CO_2 from biogas and recycling to the digester could raise the digester pH to 8.0, and that at 35°C significant amounts of ammonia could be stripped from the waste. The authors however do not comment on the effects of the raised pH or reduced NH_4^+-N concentration on digester performance. The removal of ammonia by desorption therefore, may have a role to play in the digestion of poultry wastes, particularly manure at high solids concentrations, although the effect on GY and digestion efficiency can only be ascertained by experiment.

9.9 Influent Concentration

In Chapters 3 and 4 it was demonstrated that for the digestion of poultry wastes increasing both RT and Sr would increase the GY. However for wastes of high nitrogen content such as poultry manure raising Sr will also increase the concentration of toxic ammonia in the digester, and a point will be reached where the inhibitory effect of NH_3 will overcome the beneficial effect of increasing Sr. This point represents the optimum Sr for maximum GY at each RT and can be predicted for poultry manure using the model proposed in Section 8.6.2. Since GY is directly related to solids destruction this point will also represent the optimum conditions for waste stabilisation, but not necessarily for net energy gain and process economics which are influenced by additional factors such as digester size, and operating and environmental temperature. The effect of temperature on digestion may be incorporated into the model by determining the relationship between the kinetic constants and temperature as reported by Hashimoto et al (1980) for μ_m. Kc is obviously temperature dependent, but by what degree and whether other constants are affected must be determined experimentally.

The overall process efficiency may be improved by raising the optimum Sr by reducing the digester ammonia concentration, thus increasing GY and reducing the size of the digester and heating requirements. This may possibly be achieved by desorption as outlined in the previous section.

Alternatively operation of the process in 2 stages as suggested in Section 7.5 may result in greater efficiency or reduced treatment time comprising a high solids, long RT first stage followed by a low solids short RT second stage. However the feasibility fo the two methods of improvement and the practical details may only be determined by experiment.

In conclusion mesophilic anaerobic digestion has potential as a method of treatment of poultry wastes and is able to reduce the BOD and solids contents by significant amounts with the production of gas of up to 61% CH_4 which may be used as an energy source to offset the cost of the rpocess. Due to the high cellulose content of poultry wastes long RT's are desirable to give a high degree of stabilisation, although at RT's as short as 12 days efficiency is still high.

The efficiency and economics of the process are also increased by raising the influent concentration, but the optimum concentration will be determined by the nitrogen content of the raw waste due to the formation of toxic ammonia, although concentrations of up to 4,270 mgl^{-1} NH_4^+-N can be tolerated with little reduction in efficiency.

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