ANAEROBIC DIGESTION OF PIGGERY WASTE.

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A.F.M. van Velsen

ANAEROBIC DIGESTION OF PIGGERY WASTE

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op woensdag 20 mei 1981
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen.

STELLINGEN

1. De standaard methode ter bepaling van het droge stof en organische stof gehalte geeft voor de praktijk van de anaërobe gisting doorgaans niet de juiste informatie. Het is doelmatiger het substraat te karakteriseren aan de hand van CZV-bepalingen.

Dit proefschrift.

2. De resultaten van de experimenten van McCarty en McKinney naar de giftigheid van kationen op het anaërobe gistingsproces hebben slechts een zeer beperkte geldigheid, omdat in de betreffende experimenten onvoldoende tijd is genomen voor aanpassing van de bakteriepopulatie.

McCarty P.L. & McKinney R.E., 1961. Salt toxicity in anaerobic digestion.
J. Water Pollut. Control Fed. 33, 399.

3. Bij de vergisting van complexe substraten is de hydrolyse doorgaans de snelheidsbepalende stap. Ten onrechte wordt er bij het opstellen van kinetische modellen voor het anaërobe gistingsproces dikwijls van uitgegaan, dat de methaanvorming snelheidsbepalend is.

Andrews J.F., 1969. Dynamic model of the anaerobic digestion process.
J. Sanit. Eng. Div., SA 1, 95.
Novak J.T. & Ramesh M.S., 1975. Stimulation in anaerobic degradation.
Water Res., 9, 963.

Dit proefschrift.

4. Met het oog op de toenemende schaarste aan energie en minerale grondstoffen dient, in aansluiting op de toepassing van anaërobe zuivering van afvalwater, de terugwinning van waardevolle mineralen als ammoniumstikstof te worden nagestreefd. Het proces van NH_3 strippen, gevolgd door absorptie, biedt in dit opzicht uitstekende perspektieven.

5. Uit het feit, dat defosfatering op rioolwaterzuiveringsinstallaties in principe de P-belasting van het oppervlaktewater sterker kan reduceren dan het weglaten van fosfaat uit wasmiddelen, concludeert het VNO zeer ten onrechte, dat prioriteit moet worden gegeven aan defosfatering op zuiveringsinstallaties.

Selectief defosfateringsbeleid gewenst. Milieuhygiëne 12 (1981) 1, 1.

6. De scepsis van gevestigde instituten t.a.v. nieuwe ontwikkelingen is dikwijls niet alleen gebaseerd op wetenschappelijke argumenten.

7. Het bestaan van monopolieposities op de afzetmarkt vormt geen stimulans voor de terugwinning en het hergebruik van waardevolle componenten uit afval.

8. Het is een fundamenteel recht voor iedereen desgewenst te kunnen beschikken over alle informatie met betrekking tot zijn/haar leefmilieu.

9. De invoer van grote hoeveelheden tapioca uit Thailand zou kunnen worden uitgelegd als een vorm van ontwikkelingssamenwerking, omdat het een gunstige invloed heeft op het inkomen van de Thaise boeren. Strikt genomen is het echter een verhullende vorm van uitbuiting, omdat de door ons betaalde prijzen te laag zijn om de aan de grond onttrokken mineralen weer aan te vullen met kunstmest.

Asseldonk J.S.O van, Faber D.C. en Stolwijk H.J.J., 1980. De betekenis van tapioca voor de landbouwsector in Thailand en Nederland. Landbouwk. Tijdschr. 92, 202.

10. Het instituut 'kroondocent' heeft zich niet of nauwelijks aangepast aan de veranderde omstandigheden in het wetenschappelijk onderwijs. Het verdient aanbeveling dit instituut te vervangen door een systeem van gedeelde verantwoordelijkheid op basis van specifieke capaciteit en ervaring.

11. De liefde van stadsmensen voor het platteland gaat kennelijk niet door de neus.

A.F.M. van Velsen, Anaerobic digestion of piggery waste.
Wageningen, 20 mei 1981.

VOORWOORD

Bij het verschijnen van dit proefschrift wil ik graag allen bedanken die op enigerlei wijze hebben bijgedragen aan de totstandkoming van dit werk.

Het heeft mij verheugd dat Prof. Fohr als promotor wilde optreden.

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Het onderzoek werd voor 50% gefinancierd door het Fonds Hinderpreventie Veeteeltbedrijven.

Ontwerp omslag: Willem Baljet

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1 INTRODUCTION

1.1 GENERAL INTRODUCTION

The present-day economical system of the developed countries is based on an almost unlimited supply of fossil energy and minerals at low costs. This system has forced up the consumption of raw materials to such high levels that the finite natural resources threaten to give out. The consequent threat of a world fossil energy and mineral shortage has stimulated the search for systems producing minerals and energy from renewable resources. The modern economical system also has resulted in the production of large amounts of industrial, domestic and agricultural wastes. Although wastes may contain useful and valuable components, including potential energy, recovery often could not compete with the low prices of these products on the world market, thus attributing to wastes a negative economical value. The consequent disposal of large amounts of waste materials has resulted in the pollution of the natural environment on a mondial scale.

In combining both these problems the Western society is faced with, it more and more becomes clear that wastes should be considered as raw materials rather than as unwanted materials of no or even a negative value. Because of this there is a need for appropiate processes capable to convert wastes into useful products, e.g. energy, food and minerals.

Anaerobic digestion of organic matter is one of the technologies available now to reduce the environmental pollution while at the same time valuable products are produced. In anaerobic digestion organic matter is microbiologically converted to a mixture of methane and carbon dioxide ('biogas') which represents a very useful fuel. According to a global scheme of Beek (1976) for mondial energy relations, the human activity in forestry, animal keeping and human food results in a waste stream of 0.7 TW, which chiefly is of organic nature. Assuming that these wastes can be converted to methane at a 50 % efficiency, the potential energy recovery can be estimated at 0.35 TW, which is about 5 % of the present world fossil energy consumption. Moreover, other elements of the digestible wastes such as nitrogen, phosphorus, potassium etc. remain in the digested residues, which enables the re-use of these compounds as fertilizer.

As compared to other processes directed towards the valorization of organic wastes, e.g. methanol-, ethanol- and SCP-production, anaerobic digestion is particularly suited for treating wet complex organic wastes, because the gaseous endproducts spontaneously escape from the liquid phase, which saves the need for additional separation processes.

In nature, anaerobic digestion is part of the carbon cycle as it plays a role in the mineralization of organic material. Anaerobic decomposition of organic materials occurs at all places where organic materials accumulate and the supply of oxygen is insufficient for aerobic degradation, e.g. in marshes and lake sediments.

The fact that rottening organic material will produce an inflammable gas has been known for centuries, particularly in the phenomenon of marsh gas. The occasional dancing flames of this gas - ignited perhaps by sparks from a nearby fire - seen at night, have given rise to many legends. As early as 1930, Van Helmont (Partington, 1960) mentioned, among 15 different kinds of gas, an inflammable gas that evolves during putrefaction and also is contained in intestinal gas. Volta in 1776 (Priestley, 1790) was the first who recognized the close relationship between the decaying vegetation in the sediments of lakes and streams and the appearance of combustible gas.

The research on anaerobic digestion may be considered to have begun in 1808, when Davy (1814) collected methane from cattle manure in a retort under vacuum. Davy was not interested in the production of energy; his experiments were directed towards an evaluation of the fertilizing value of digested and undigested manure. Gayon (Dubaquie, 1943) in investigating the digestion of manure in 1883 - 1884, collected such large amounts of gas, that Pasteur suggested to utilize the gas produced from horse manure for improving street lightning in Paris (Le Figaro, 1884). However, as a matter of fact the first utilization of gas produced in anaerobic digestion occurred in Exeter (England) in 1895, where the gas from a 'carefully designed' septic tank was used for street lightning (Liebmann, 1956). Since then, anaerobic digestion of domestic sewage sludge has been increasingly applied. The main objective of sewage sludge digestion has always been - and still is - the stabilization of excess primary and secundary sludge in domestic sewage treatment. The methane produced in this process sometimes was considered as an useful byproduct to cover part of the energy requirements of the treatment plant, but frequently it was simply flared. The digestion process was also utilized for producing energy from agricultural residues (manure, straw) whenever fossil energy was in short supply, e.g. during and shortly after World War II in France, Algeria and Germany (Tietjen, 1975). However, in the post-war period, energy from digestion of agricultural residues could not compete with cheap fossil energy and the direct interest in the process diminished. On the other hand, the development of manure digestion processes has been continued in some Asian countries (India, Korea, China), which has resulted in an extensive small-scale application of the process in these countries (Pyle, 1980).

The energy crisis of 1973 forced the developed countries to realize their dependence on finite natural resources. This and the consequent prospect of ever-increasing prices of raw materials in the near future, has strongly revived the interest and the research into anaerobic digestion as an energy-producing method and an energy-saving waste treatment technology.

The main objective of the present investigation was to assess the energy recovery and malodour abatement potentials of piggery waste digestion.

1.2 OBJECTIVES OF MANURE DIGESTION

The main objectives of applying manure digestion in the Netherlands are

1. the abatement of malodour nuisance during storage and spreading of animal slurries and
2. energy recovery.

Until recently, animal husbandry was characterized by a close relation between the number of animals kept and the area of cultivated land to produce the animal feed. The import of large amounts of animal feed seriously disrupted this relation and resulted in a change-over from traditional animal husbandry to the system of intensive animal keeping. In regions where intensive animal housing units are concentrated, much more manure is produced than can be utilized for fertilizing the nearby cultivated land, thus creating a serious manure-surplus problem.

For the purpose of reducing the labour requirements, intensive animal production units commonly are equipped with slatted floors, beneath which the combined droppings, viz. faeces, urine and spillage water, are collected and stored. During storage of the slurry malodorous compounds are formed, which has to be attributed to the occurrence of an incomplete anaerobic fermentation (Spoelstra, 1978). The spreading of these slurries frequently causes malodour nuisance, especially in densely populated regions (Voorburg, 1978). When subjecting manure to anaerobic digestion under controlled conditions, most of the digestible organic matter is eliminated, resulting in the production of a stabilized material without offensive odours.

Because of the production of an useful fuel, anaerobic manure stabilization provides an attractive alternative for conventional, energy-consuming aerobic treatment systems. According to V.d. Hoek (1977) aeration requires a minimum energy input of 36 kWh per pig place per year to prevent malodorous nuisance during storage and spreading of pig manure.

The other, and in many cases prime object of animal manure digestion is the recovery of biogas, which consists mainly of methane (60-75%) and carbon dioxide (25-40%). The gas produced is an useful fuel, either for heating or for driving combustion engines, e.g. to generate electricity. The production of energy is particularly important for farms having a high energy consumption, such as pig and poultry breeding units and dairies. Depending on the farm size and the manure handling system applied, anaerobic digestion may provide sufficient energy to cover the farm needs at production costs that can compete with the present-day retail price of fossil fuels (Hashimoto and Chen, 1980; Poelma, 1981 personal communication).

However, in spite of its favourable prospects, the process can only play a minor role in the national energy supply. In Table 1.1. the animal manure production in the Netherlands has been estimated together with the maximum obtainable gross methane yield. Table 1.1 indicates that, in case all manure produced in the Netherlands is properly digested, the annual methane production amounts to 720 × 10^6 m^3. This corresponds to an energy production of 0.85 GW, which is about 1% of the present-day national energy consumption (Nota Energiebeleid, 1979).

Table 1.1 Potential methane production from animal wastes, produced in the Netherlands.

	manure production[a] (10^6kg TS* $year^{-1}$)	methane production (m^3 kg^{-1} TS*added)	total methane production (10^6 m^3 $year^{-1}$)
cattle	3 135	0.10 - 0.13[b,c,d]	300 - 400
pigs	1 080	0.15 - 0.20[e,f]	150 - 200
poultry	552	0.20 - 0.24[c,e]	100 - 120
		total	550 - 720

a. Landbouwcijfers 1980
b. Baader et al., 1978
c. Badger et al., 1979
d. Hayes et al., 1980
e. Hobson et al., 1980
f. van Velsen, this publication

* Total solids

An additional benefit of anaerobic digestion, in which only little interest has been shown, concerns the use of digested residues for fertilizer purposes. Upon digestion, manure retains its fertilizer value and becomes easier to handle. The extensive small-scale practice of manure digestion in countries like India (Singh, 1972) and Kenya (Hutchinson, 1972) has shown that the fertilizing properties of manure even may improve through anaerobic digestion, at least under the local conditions. A closer examination of the available data learns that these observations have to be attributed to the addition of straw and other carbon-rich material to the manure feed in such amounts that the C to N ratio in the feed increases to approx. 30. In consequence, the ammonia nitrogen present in the residue is almost completely incorporated in bacterial biomass, thus producing a fertilizer which does not rapidly release N, and therefore reduces the nitrogen losses during spreading. Given the needs for fertilizer and minerals, it becomes clear that this benefit of anaerobic digestion is mirrored at in the developing countries (Pyle, 1980).

At present such an improvement of the fertilizer value is of minor importance in developed countries, such as in the Netherlands, because these countries largely rely on chemical fertilizers for their high agricultural production. However, with the raising prices of chemical fertilizers, the production of slow release organic fertilizers may become increasingly important in these countries as well.

1.3 MICROBIOLOGY AND BIOCHEMISTRY OF ANAEROBIC DIGESTION

The effective conversion of complex organic matter to methane depends on the combined activity of a miscelanneous microbial population, consisting of diverse genera of obligate and facultative anaerobic bacteria. As a result of the coordinated activity of the different bacterial groups, anaerobic digestion as a whole is a stable process.

According to the present knowledge, complete anaerobic digestion of organic matter proceeds according to the scheme depicted in Fig. 1.1 (McInerney et al., 1980). The responsible microbial population is comprised of at least four trophic groups of bacteria, each with a distinct carbon catabolizing function: hydrolytic bacteria (1), H_2-producing acetogenic bacteria (2), methanogenic bacteria (3) and homo-acetogenic bacteria (4).

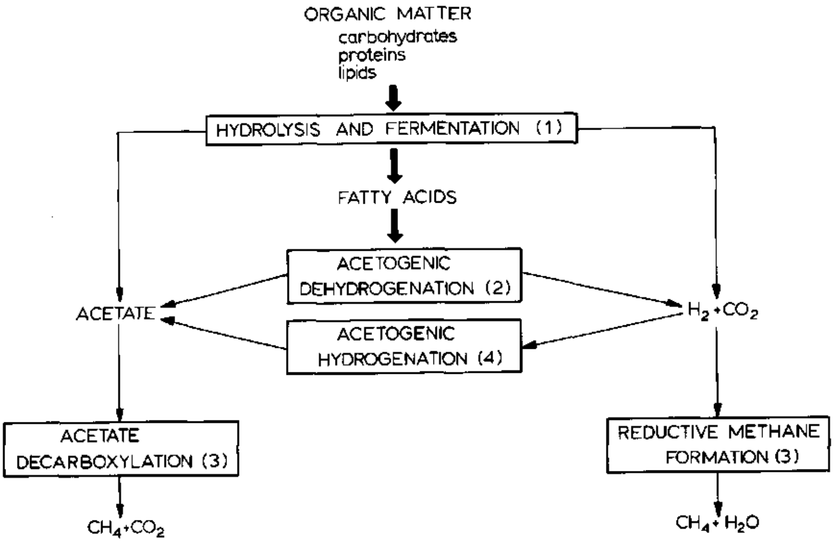

Fig. 1.1 Scheme for the complete anaerobic degradation of organic matter according to McInerney et al. (1980).

Hydrolytic bacteria (group 1) ferment organic polymers, e.g. proteins, polysaccharides and lipid material, into compounds with a low-moleculair weight, such as volatile fatty acids, organic acids, alcohols, hydrogen, carbon dioxide, ammonia nitrogen and sulphide. They possess the ability to produce and excrete hydrolysing enzymes (exo-enzymes), enabling them to utilize high-molecular, even undissolved compounds. According to the type of exo-enzymes produced, hydrolytic bacteria isolated from anaerobic digester populations are classified in lipolytic bacteria, e.g. *Anaerovibrio lipolytica* (Hobson et al., 1974), proteolytic bacteria, e.g. *Clostridia* spp., *Micrococcus* spp. (Hobson et al., 1974) and *Eubacterium* (Zeikus, 1980) and cellulolytic bacteria, e.g. *Ruminococcus* spp., *Butyrivibrio fibrisolvens* (Hobson et al., 1974) and *Clostridium thermocellum* (Ng et al., 1977). According to Hobson and Shaw (1974) streptococci are the predominant fermentative bacteria in piggery waste digesters. The total population of hydrolytic bacteria in mesophilic sewage sludge amounts to 10^8 - 10^9 ml^{-1} (Kirsch, 1969; Mah and Sussman, 1968; Toerien and Siebert, 1967).

The obligate H_2-producing acetogenic bacteria (group 2) degrade propionate and longer chain fatty acids, alcohols, aromatics and other fermentation products. They produce acetate, hydrogen and carbon dioxide (from odd numbered carbon sources). Because of their metabolic activity, the acetogenic bacteria form an intermediate group linking the fermentative and methanogenic stage. Since the catabolic reactions of the H_2-producing acetogens are thermodynamically unfavourable unless the hydrogen partial pressure is kept extremely low (below 2×10^{-3} atm. and 9×10^{-5} atm. for the degradation of butyrate and propionate, respectively), these organisms can grow only in the presence of hydrogen-utilizing bacteria, e.g. methanogens and sulphate reducing organisms (Zeikus, 1980; McInerney et al., 1980). Thus, in cocultured growth, the hydrogen metabolism of methanogens provides thermodynamically favourable conditions for H_2-producing acetogens. The classic example of a H_2-producing acetogen is the S-organism isolated from the "*Methanobacillus omelanskii*" mixture. Bryant et al. (1967) demonstrated that *M. omelanskii*, which had been regarded as an ethanol-utilizing methanogen, in fact is a syntrophic association of two bacterial species, viz. the S-organism catabolizing ethanol to acetate and hydrogen and *Methanobacterium* strain MOH. Recently McInerney et al. (1980) reported that interspecies hydrogen transfer reactions also occur in the methane formation from propionate and longer chain fatty acids. Populations of 4.2×10^6 H_2-producing acetogens per ml mesophilic sewage sludge have been reported (Zeikus, 1980).

The methanogenic bacteria (group 3) produce methane from acetate and one-carbon compounds, e.g. hydrogen/carbon dioxide (carbon monoxide) mixtures, methanol, formate and methylamine (Balch et al., 1979). Owing to their ability to produce a reduced gaseous endproduct, methane, that spontaneously escapes from the liquid phase, the methanogens are the "key"-

organisms in the anaerobic digestion process providing thermodynamically favourable conditions for the preceeding non-methanogenic stages. Methanogens are featured by the relatively slow growth rate, the requirement of strictly anaerobic environments (redox potential below about -300 mV) and the utilization of only a narrow range of substrates as their energy source. Yet, the methanogens appear to show a wide microbial diversity. Balch et al. (1979) proposed a taxonomic scheme, based on comparative cataloguing of the 16S ribosomal RNA and substrates used (Table 1.2). They classified the methanogens in three orders, viz. *Methanobacteriales*, *Methanococcales* and *Methanomicrobiales*. In mesophilic sewage sludge digesters populations have been reported of approx. 10^8 methanogens per ml (Zeikus, 1980).

Table 1.2 Proposed taxonomic scheme for methanogenic bacteria of Balch et al. (1979) based on comparative cataloguing of the 16S ribosomal RNA and substrates used.

	Type strain	Substrates for growth and CH_4 production
Order I. *Methanobacteriales* (type order)		
Family I. *Methanobacteriaceae*		
Genus I. *Methanobacterium* (type genus)		
1. *Methanobacterium formicicum* (neotype species)	MF	H_2, formate
2. *Methanobacterium bryantii*	M.o.H.	H_2
Methanobacterium bryantii strain M.o.H.G.		H_2
3. *Methanobacterium thermoautotrophicum*	ΔH	H_2
Genus II. *Methanobrevibacter*		
1. *Methanobrevibacter ruminantium* (type species)	MI	H_2, formate
2. *Methanobrevibacter arboriphilus*	DH1	H_2
Methanobrevibacter arboriphilus strain AZ		H_2
Methanobrevibacter arboriphilus strain DC		H_2
3. *Methanobrevibacter smithii*	PS	H_2, formate
Order II. *Methanococcales*		
Family I. *Methanococcaceae*		
Genus I. *Methanococcus*		
1. *Methanococcus vannielii* (neotype species)	SB	H_2, formate
2. *Methanococcus voltae*	PS	H_2, formate
Order III. *Methanomicrobiales*		
Family I. *Methanomicrobiaceae* (type family)		
Genus I. *Methanomicrobium* (type genus)		
1. *Methanomicrobium mobile* (type species)	BP	H_2, formate
Genus II. *Methanogenium*		
1. *Methanogenium cariaci* (type species)	JR1	H_2, formate
2. *Methanogenium marisnigri*	JR1	H_2, formate
Genus III. *Methanospirillum*		
1. *Methanospirillum hungatii*	JF1	H_2, formate
Family II. *Methanosarcinaceae*		
Genus II. *Methanosarcina* (type genus)		
1. *Methanosarcina barkeri* (type species)	MS	H_2, CH_3OH, CH_3NH_2, acetate
Methanosarcina barkeri strain 227		H_2, CH_3OH, CH_3NH_2, acetate
Methanosarcina barkeri strain W		H_2, CH_3OH, CH_3NH_2, acetate

The H_2-consuming acetogenic or homo-acetogenic bacteria (group 4) show a mixotrophic metabolism and catabolize both hydrogen/carbon dioxide and multicarbon compounds. They can produce acetate and longer chain volatile fatty acids from hydrogen/carbon dioxide mixtures, but are successfully outcompeted for hydrogen by the methanogenic bacteria in the gastro-intestinal environment (Prins and Lankhorst, 1977). The exact role of homo-acetogenic bacteria in anaerobic digestion is not yet clear. According to Zeikus (1980), the net result of homo-acetogenic metabolism in anaerobic digestion is the maintenance of low hydrogen partial pressures, thus contributing to process stability. The present known homo-acetogenic bacteria all belong to the genera *Clostridium* and *Acetobacterium* (Zeikus, 1980). Ohwaki et al. (1977) and Braun et al. (1979) reported populations of 10^5 - 10^6 homo-acetogenic bacteria per ml in sewage sludge.

The above mentioned scheme for anaerobic digestion describes the process on the basis of distinguished trophic groups of bacteria found in anaerobic digester populations. As the metabolism of these groups is closely interlinked, the scheme is very useful for understanding the process, but because of its complexicity it is not suited for describing the course of the process from routine analyses.

A simplified scheme, shown in Fig. 1.2 is proposed for describing the digestion of complex, undissolved substrates. In the scheme, anaerobic digestion is split up into three steps, viz. hydrolysis of undissolved compounds, acid formation and methane formation. The conversion degree of the

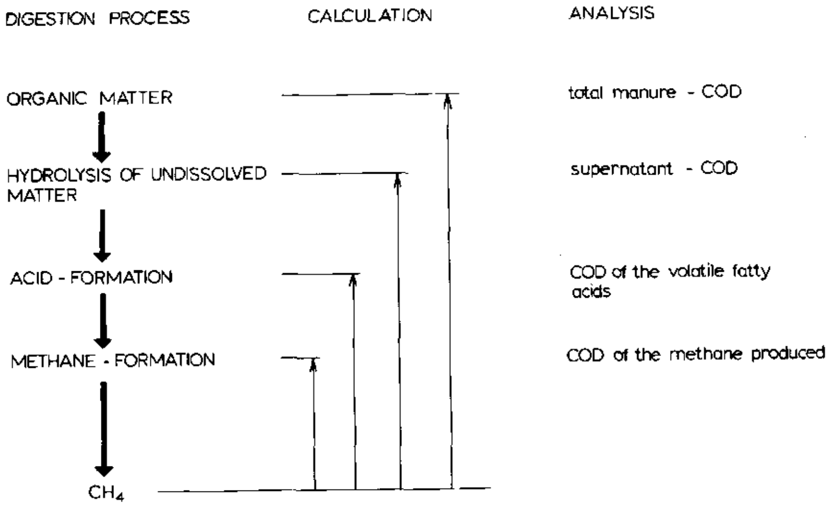

Fig. 1.2 Simplified scheme for describing the anaerobic degradation of organic material on the basis of chemical routine analyses.

distinguished steps in continuous experiments can be roughly estimated from routine analyses, by expressing the analytical data in terms of chemical oxygen demand (COD).

According to the proposed scheme, hydrolysis is confined to the liquefaction of undissolved organic compounds and exclusively indicates the net activity of hydrolysing exo-enzymes produced by fermentative bacteria. Acid formation indicates the extent to which the feed-COD is converted into volatile fatty acids. For this purpose, it has been assumed (a) that methane is exclusively produced from endproducts of fermentative and acetogenic bacteria and (b) that volatile fatty acids are the predominant process intermediates as was stated before by Hobson et al. (1974). Methane formation indicates which part of the feed-COD is ultimately converted to methane. Methane formation thus expressed is a measure of the overall process efficiency under the circumstances investigated.

The proposed scheme provides a means to get insight in the conversion degree of the distinguished process steps under the conditions investigated. However, it should be emphasized that the three distinct steps do not coincide with the trophic microbial groups discussed before. In consequence, conclusions on the basis of the simplified scheme do not necessarily demonstrate the activity of the distinguished trophic groups.

2.1 MALODOURS IN ANAEROBICALLY STORED PIGGERY WASTES

Odours are hardly accessible to measurement, because both their strength and their nature can be estimated only by sensory perception. As a consequence, methods have been developed for defining odours on the basis of the subjective perception of panel members. These sensorial methods, e.g. olfactometry, indicate the odour strength (threshold dilution level) rather than the sensorial appreciation of the odour. To describe odour quality, the subjectivity of the human language is introduced as well (Harper et al., 1968). Furthermore, reliable sensorial measurements need rather large panels to preclude the response of one individual, which makes them costly and unsuited for routine analyses.

Odour can be defined more objectively by giving the complete spectrum of contributing components, including their concentrations. For anaerobically stored piggery wastes, this is impossible because of the great number of compounds involved. Spoelstra (1978), in compiling literature data, mentioned over sixty compounds identified in both the air of swine confinement units and anaerobically stored piggery wastes. Furthermore, such a spectrum of malodorous compounds, supposing it is complete, does not give any information about the nature of the malodorous sensation.

Attempts to overcome the practical problems involved in estimating malodours of anaerobically stored piggery wastes, include the search for correlations between the malodour strength and the concentration of one or more components, which contribute to the piggery waste malodour. As the concentration of such compounds can be determined instrumentally, such an approach might provide a cheap and fast method for estimating malodours. However, such a direct relationship is difficult to understand, because the volatilization of acid and alkaline compounds depends on the pH level of the wastes (Spoelstra, 1978).

According to Schaefer et al. (1974) the main compounds responsible for the bad smell of piggeries are volatile fatty acids (especially butyric acid), diacethyl, phenol, *p*-cresol, indole and skatole. A mixture of these odorous compounds was selected by a panel as having similarity with the odour from piggery wastes. However, when correlating odour intensity with the concentration of these components in the air from piggeries it appeared that a significant correlation was obtained only with *p*-cresol (Schaefer, 1977). Spoelstra (1978) investigated the formation of volatile fatty acids, phenol, *p*-cresol, 4-ethylphenol, indole and skatole at anaerobic storage of piggery wastes and demonstrated that these components result from the activity of fermentative bacteria. He further presented evidence that simple phenols (phenol and *p*-cresol) and indoles (indole and skatole) originate from the degradation of tyrosine and tryptophan, respectively.

In the underlying study the concentration of the malodorous compounds presented by Schaefer et al. (1974) and Spoelstra (1978), viz. volatile fatty acids, phenol, *p*-cresol, 4-ethylphenol, indole and skatole, have been used to indicate the reduction of piggery waste malodours at anaerobic digestion.

1.5 OUTLINE OF THE INVESTIGATION

In view of the motives underlying the use of piggery waste digestion, the investigation was directed to assess the potentials of anaerobic digestion to reduce malodours and to produce energy.

Chapter 3 describes the start-up of piggery waste digesters seeded with anaerobic sewage sludge. As the adaption of anaerobic sewage sludge to piggery waste appeared to be related to an increase of the ammonia nitrogen concentration beyond the presumed inhibitory level of approx. 1500 mg l^{-1} (McCartey et al., 1961), the effect of high ammonia nitrogen concentrations on the digestion process is emphasized.

In Chapter 4 attention is given to the effects of the manure concentration and the detention time on the digestion of piggery waste.

Experiments on the influence of the process temperature are presented and discussed in Chapter 5.

Chapter 6 contains a general discussion on the anaerobic digestion of piggery wastes. From the results obtained proposals are made for improving the process efficiency.

The results of some preliminary experiments on the elimination of phenol and *p*-cresol under anaerobic, methanogenic conditions are presented and discussed in an Appendix.

2 MATERIALS AND METHODS

2.1 ANALYSES

2.1.1 Determination of Total Solids (TS) and Volatile Solids (VS)

The TS concentration was determined by drying a 10-ml sample in a 105°C stove overnight. The VS content of the sludge was calculated by determining the weight losses at ignition of the dried sample at 600°C for 4 hours. Since part of the volatile matter may be lost in this procedure for measuring the TS content (Miner and Smith, 1975) the method was checked by performing the following experiments.

a. The condensate vapors that are released during the drying procedure were collected and analysed for their ammonia nitrogen, volatile fatty acid (VFA) and chemical oxygen demand (COD) concentrations. It turned out that all the ammonia nitrogen and part of the VFA were found in the condensate. The COD-content of the distillate corresponds to the total VFA content of the sample.

b. To a digested sample containing no VFA a weighted amount of VFA, corresponding to the VFA concentration in raw manure, was added. The pH of the solution was close to the manure-pH. Both solutions, with and without VFA, were analysed in triplicate on TS by drying at 105°C overnight. The results indicated that approx. 90% of the VFA evaporate during the drying procedure.

Although it is evident that the standard method for TS determination does not suffice, we still applied the method mainly for reason of comparison with other reports. The TS and VS analyses mentioned in this publication, therefore, are not corrected for the weight losses. However, as the VFA-losses affect TS mass balances, the TS and VS analyses for drawing up mass balances have been corrected as will be indicated in the text.

2.1.2 Determination of the volatile fatty acids (VFA)

VFA have been assayed directly from the aqueous phase of centrifugated (14000 *g*, 10 min.) samples using a Becker gas chromatograph Model 417 equipped with a glass column and flame ionisation detector. The glass column (1 m × 4 mm i.d.) was packed with Chromosorb W-AW (80-100 mesh) coated with 20% Tween 80. Flow rates of the carrier gas, nitrogen saturated with formic acid, of hydrogen and of air were 60, 45 and 350 ml min^{-1} respectively. Column temperature was 115°C. The injection port and detector temperature were 170°C.

2.1.3 Determination of the chemical oxygen demand (COD)

COD was estimated according to Standard Methods (Am. Society, 1965). The liquid phase-COD (COD_s) was determined on supernatant samples prepared by centrifugation at 14000 *g* for 10 minutes.

2.1.4 Determination of ammonia nitrogen

Two methods were used to determine the ammonia nitrogen concentration.

a. Steam distillation at pH 7.4 into a boric acid solution and titration with standard acid.

b. Distillation at pH 7.3, with lead hydrocarbonate as a catalyser, into a boric acid solution and titration with a standard acid.

2.1.5 Determination of total nitrogen

The total nitrogen concentration was determined by a Kjelldahl-type destruction, followed by neutralization with NaOH and distillation at pH 7.3 into a boric acid solution and titration with a standard acid.

The destruction procedure was as follows: a well mixed 10-ml sample was boiled in concentrated sulphuric acid with Selenium Mixture GR as a catalyser, up to clearness of the sample.

2.1.6 Determination of the pH

A Knick pH-meter was used for pH measurements.

2.1.7 Determination of phenol, p-cresol, 4-ethylphenol, indole and skatole

The concentration of these aromatic compounds was determined using two methods.

a. According to the method described by Spoelstra (1978).
Two ml of a freshly prepared 6% $NaHCO_3$ solution and 1.00 ml of a standard solution of 5-decanol in ether were added to a sample of slurry of about 5 g. 5-Decanol was used as an internal standard in the gaschromatographical analysis. Subsequently, the sample was extracted four times with 20 ml of ether. The emulsion, which was usually formed during the ether extraction, was broken by gentle centrifugation. The combined ethereal fractions were evaporated under reduced pressure at a maximum temperature of 25°C to a volume of a few millilitres. The residual ether was dried by adding anhydrous $CaSO_4$, and transferred to a 10-ml tube. The volume was further reduced to 0.5 - 1 ml by holding the test tube in a waterbath of 30°C. Approximately 1 microlitre of the final ethereal solution was injected for gas-chromatographical analysis.

The gas chromatograph used was a Varian 2240 model equipped with a flame ionisation detector. A column of stainless steel (3m × 3mm o.d.) was used, packed with 10% SE 30 on Chromosorb W-AW (80 - 100 mesh). The operational data were: temperature, oven 140°C; injection port 150°C; detectorblock 190°C; flow of carrier gas, nitrogen, of air and of H_2 40, 300 and 30 ml min $^{-1}$, respectively.

The peak heights obtained were measured relative to the peak height of the internal standard and compared with a calibration curve, which had been obtained by the same procedure as described above with mixtures of known concentrations of phenol, *p*-cresol, 4-ethylphenol, indole and skatole in water.

b. A gas chromatographical method applied to supernatant samples (14000 *g*, 10 minutes) without further treatment. The clear samples were introduced in a glass column (2m × 4 mm i.d.) packed with 5% FFAP on Chromosorb G-AW-DMCS. The column was installed in a Becker 756 gas chromatograph equipped with a flame ionisation detector. Column temperature was 200°C and that of the injection port and detectorblock 240°C. Flow rates of the carrier gas, nitrogen, of H_2 and of air were 75, 50 and 235 ml min^{-1}, respectively.

The peak heights obtained were measured relative to the peak heights of mixtures of known concentrations of these aromatic compounds in water.

2.1.8 Determination of hemicellulose, cellulose and lignin

The concentration of hemicellulose, cellulose and lignin in dried samples was determined according to the method described by de Wit (1980).

One gram of washed solids was supplied with 50 ml of 0.1 N H_2SO_4 and transferred to a glass tube which was sealed and heated for 6 hours at 100°C. During hydrolysis the tube was shaken frequently. After cooling, the slurry was centrifugated. The supernatant was used for carbohydrate analysis. The amount of hemicellulose was estimated by measuring the reducing sugars present in the hydrolysate. Reducing sugars were determined according to the method of Somogyi and Nelson (Somogyi, 1952) as described by Hodge and Hofreiter (1962).

After washing with acetone and air drying, the residue of the hemicellulose determination was transferred to a tube which was supplied with 2 ml of 72% sulphuric acid and left at 30°C for 1 hour in order to solubilize the cellulose. Subsequently, 50 ml of water was added, the glass tube sealed and the contents hydrolysed completely at 100°C within 6 hours. After cooling, the slurry was spun down and the supernatant analysed for glucose which resulted from the hydrolysis of cellulose.

The residue of the cellulose hydrolysis was washed twice with deionized water and dried at 103°C until constant weight. The dried samples were subsequently heated at 550°C for 6 hours whereupon the ash content was measured. Oven-dry sample minus ash content was defined as lignin.

2.1.9 Gas analysis

The methane content of the gas was determined using one of the following methods.

a. Gas chromatographically on a Fraktovap Model M partitioner equipped with a dual column arrangement of Molecular Sieve 30/60 mesh 5A and silica gel 30/60 mesh.

b. Entrapping of the carbon dioxide of the gas in a KOH solution. This analysis is performed with an Orsatt apparatus. As all remaining gas is assumed to consist of methane, this analytical method provides only a rough estimate of the methane content of the gas.

c. Combustion of the gas in a portable combustible gas meter calibrated for methane.

2.2 MATERIALS

2.2.1 Manure and seed materials

The piggery wastes used in the experiments consisted of combined faeces, urine and spillage water, obtained from different piggeries. Because of the inevitable variations in the composition of the feedstock, the most relevant chemical characteristics of the manure used are mentioned in each Chapter separately.

This also holds for the origin and the chemical characteristics of the seed materials.

2.2.2 Volatile fatty acids stock solution

The volatile fatty acids stock solution (Chapter 3) contained 12 g l^{-1} acetic acid, 7 g l^{-1} propionic acid and 4 g l^{-1} n-butyric acid. The solution was neutralized with NaOH.

2.3 EXPERIMENTAL EQUIPMENT

2.3.1 Batch experiments

The batch experiments described in Chapter 3 were performed in 1-litre glass bottles, placed in a temperature-controlled room of 30 ± 2°C. The digestion units were shaken by hand once a day. The gas production was determined by means of a brine displacement system.

The equipment used for the batch experiments, described in the Appendix, consisted of 5-litre glass bottles, placed in a temperature-controlled room of 30 ± 2°C. Mechanical mixing was provided for 15 s every 20 min. The gas produced was led through a 1% NaOH solution in order to entrap volatile aromatic compounds. In the gas outlet system only poly-ethylene tubes were used. The gas production was estimated with a brine displacement system.

2.3.2 Daily fed laboratory experiments

The laboratory experiments were performed in digesters with a volume of 1.5, 2, 5, 6 and 45 litres. The experimental arrangement of the digesters was further almost identical. The digester system is shown in Fig. 2.1.

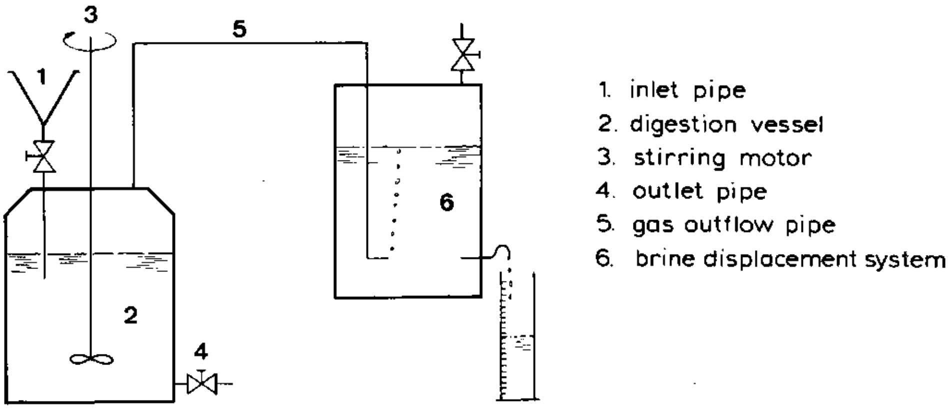

Fig. 2.1 Laboratory digester system

The fresh manure was added at the top of the digester whereas the effluent was discharged at the bottom. The gas production was estimated with a brine displacement system using brine with a pH of 1 in the experiments with 1.5-litre and 2-litre digesters and by means of a wet gas meter in the experiments with 5-litre, 6-litre and 45-litre digesters.

The digestion units except those with a volume of 45 litres were placed in a temperature-controlled room of 30 ± 2°C. The experiments at deviating temperatures were conducted in 5-litre digesters equipped with a water jacket. Digestion temperature in these digesters was regulated by circulating thermostated water through the jacket. The 45-litre digesters were heated electrically. Temperature in these digesters was regulated with a thermocouple in the digester contents.

The digestion units were mixed mechanically at 100 rev min^{-1} for 15 s every 4 min. At this stirring intensity the small digesters (1.5 - 6 litres) were mixed up completely, but the stirring capacity in the 45-litre digesters was not sufficient to achieve a complete mixing of the digester contents.

2.3.3 Small pilot plant experiments

The small pilot plant consisted of two identical insulated digestion units with a volume of 240 litres each. The digester system is shown in Fig. 2.2.

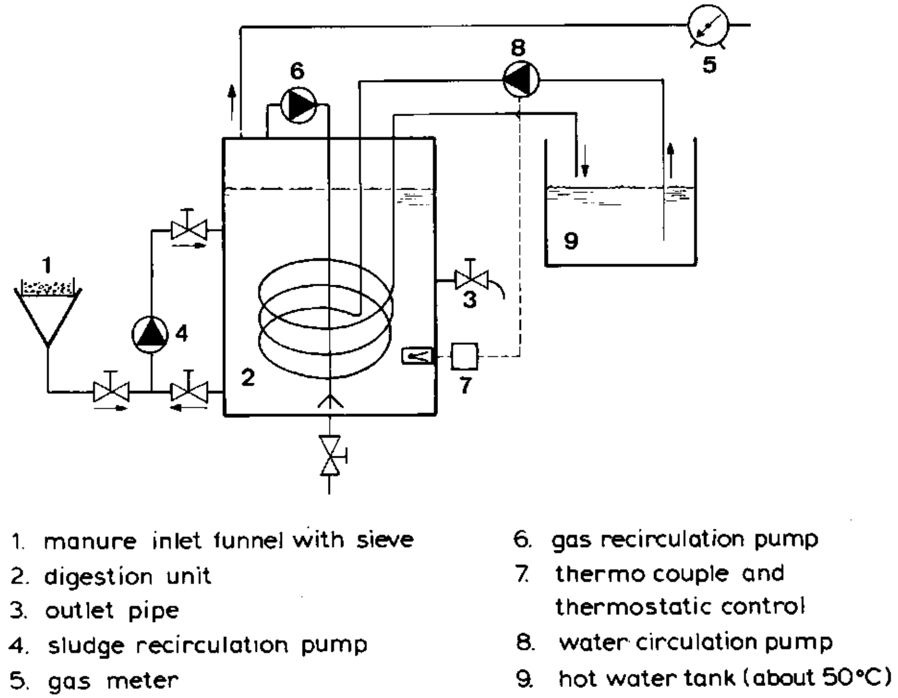

Fig. 2.2 Small pilot plant digester (240 litres).

Mixing was achieved by continuous gas recirculation using a gas flow of about 3 litres min^{-1}. Furthermore the digester contents were mixed by recirculating the digester contents with a sludge pump (capacity approx. 5 m^3 h^{-1}) once a day for 15 min. The daily feed was added during sludge recirculation. To prevent damage to the sludge pump the manure feed was sieved through a 0.5-cm sieve to remove hairs, straw etc.

The digester heating system consisted of a hot water tank (temperature of the water 50°C), a water circulation pump and stainless steel heat exchange piping inside the digester. The digestion temperature (30 - 32°C) was controlled by means of a thermocouple and a thermostatic control, connected with the water circulation pump. Gas production was determined with a wet gas meter.

2.3.4 Pilot plant experiments

Pilot plant experiments were conducted in a 6-m³ insulated steel digester. A scheme of the experimental pilot plant is shown in Fig. 2.3.

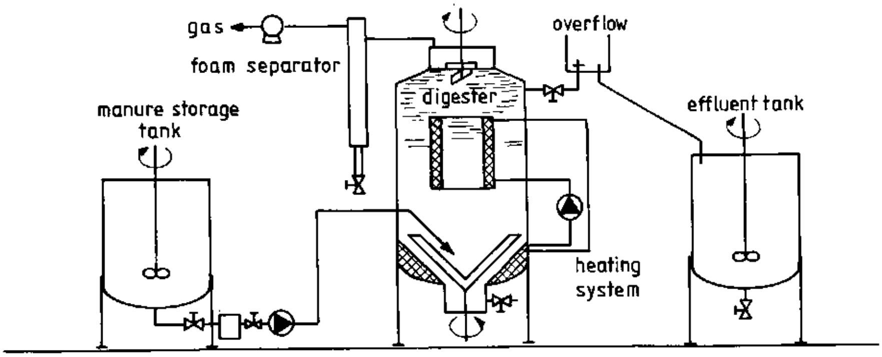

Fig. 2.3. Experimental pilot plant digester (6 m^3)

The contents of the digester were kept at a constant temperature by recirculating electrically heated water (35°C) through the digester bottom and an internal heat exchanger. Mixing was accomplished by intermittent mechanical stirring at the bottom of the digester (0.7 rev min^{-1}) and at the top of the digester (30 rev min^{-1}). Fresh manure was added from a manure holding tank at the bottom of the digester by means of an adjustable mono-pump. The fresh manure displaced an equal amount of the digester contents via an overflow system at the upper part of the digester into an effluent storage tank. The gas was discharged at the top of the digester. Gas production was measured with a dry gas meter.

2.4 EXPERIMENTAL PROCEDURES

The laboratory and 240-litre pilot plant digesters were fed daily except during the weekends when only one feed was supplied, although of a double amount. The feeding procedure was as follows. After determining the gas production a fixed volume (determined by the detention time) of the mixed digester contents was discharged. Then the manure was added according to the organic and hydraulic load. Before and during the feeding procedure the digester contents were mixed.

In the 6-m^3 digester fresh manure was added every 30 min for 1 min. In view of the feeding system the pilot plant digester can be considered as a continuously fed system.

The duration time of all daily fed and continuous experiments was at least three times the hydraulic detention time in order to approach a steady state and to avoid the influence of variations in the manure composition.

2.5 CALCULATIONS

2.5.1 Calculation of the degree of hydrolysis, acid formation and methane formation in continuous experiments.

The digestion of undissolved material can be supposed to proceed in three subsequent steps, viz. hydrolysis, acid formation and methane formation (Chapter 1.3). For the calculation of the conversion degree of the separate steps at the end of the daily fed and continuously fed experiments, when a steady state condition had been approached, all experimental results are converted to COD-values (Fig. 1.2).

The degree of hydrolysis, acid formation and methane formation can be calculated then by the following equations:

Hydrolysis (%) = 100 (G+S)/M
Acid formation (%) = 100 (G+V)/M
Methane formation (%) = 100 G/M

in which: G = COD removed via methane gas (g l^{-1} manure)
The COD-value of the digester gas is calculated by multiplying the volumetric methane production (at 30°C and a pressure of 720 mm Hg) by 2.46.
S = COD supernatant (g l^{-1})
M = total manure COD (g l^{-1})
V = COD, corresponding with the VFA concentration (g l^{-1}).
The VFA-COD is calculated by multiplying the concentration (in g l^{-1}) of acetic acid, propionic acid, (n- and iso-) butyric acid and (n- and iso-) valeric acid with a factor of 1.06, 1.51, 1.82 and 2.04 respectively and summing up the COD-values of the separate VFA.

In the calculation it is assumed (1) that in hydrolysis only undissolved organic matter* is converted into dissolved fragments, (2) that the VFA are exclusively formed by acid-forming bacteria and (3) that all methane produced originates from the endproducts of the acid formation.

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* Arbitrarily defined as the material that is precipitated during centrifugation at 14000 g for 10 minutes.

2.5.2 Calculation of the concentration of undissociated ammonia nitrogen

The fraction of undissociated ammonia under a given set of conditions can be calculated by the following equation:

$$NH_3\text{-}N = NH_4^+\text{-}N \; \frac{10^{pH}}{kb/kw + 10^{pH}}$$

in which: NH_3-N = concentration of undissociated ammonia nitrogen (mg l^{-1})

NH_4^+-N = concentration of total ammoniacal nitrogen (mg l^{-1})

k_b = dissociation constant of aqueous ammonia

k_w = ionization constant for water

The values of k_b and k_w at different temperatures were derived from Weast (1972).

3 ADAPTATION OF DIGESTED SEWAGE SLUDGE TO HIGH AMMONIA NITROGEN CONCENTRATIONS

3.1 INTRODUCTION

In anaerobic digestion the greater part of the oxygen demanding organic matter is eliminated from the liquid phase through the formation of methane. Therefore the process efficiency largely depends on the metabolism rate of the methanogenic organisms. In view of specific features of the methane-producing organisms, such as the long generation time and sensitivity to environmental factors, the start-up of the digestion process is of great importance, especially that of the methanogenic stage.

With respect to the feasibility of the on-farm application of digestion a proper start-up procedure, i.e. not requiring intensive supervision, should be on hand. This condition seems to be met for digesters treating ruminant wastes, because these wastes as a rule contain sufficient anaerobic organisms, acid-forming as well as methane-forming, to start the digestion process without seeding. However, in the manure of non-ruminants such as pigs and poultry, the concentration of methanogenic bacteria is relatively low. Together with the low growth rate of these organisms, this will result in an extended period of digester start-up, unless seed material is added (Hobson and Shaw, 1973). Therefore, seeding is recommended of digesters treating pig and poultry wastes.

Undoubtedly the mixed liquor from a well-working digester treating the same type of manure is the most suitable seed material. However, up to now anaerobic digestion of farm wastes still is not extensively applied and therefore, other seed materials should be considered. An attractive material for this purpose is digested sewage sludge from municipal sewage plants (1) because this material is the only enriched methanogenic inoculum generally available in large quantities and (2) because it may be expected to be a suitable seed material for the digestion of substrates being as complex as raw sewage sludge, viz. piggery wastes.

Being a microbial process, methanogenesis is influenced by a variety of environmental factors. An important factor in this respect is ammonia nitrogen, because exceptionally high ammonia nitrogen concentrations frequently occur in piggery wastes, e.g. exceeding 3000 mg l^{-1}.

Evidently a minimum amount of ammonia is always required in order to achieve favourable conditions for growth, because ammonia nitrogen is an essential nutrient for most anaerobic organisms (Mah et al., 1977). Ammonia nitrogen concentrations up to 1500 mg l^{-1}, which frequently occur in sewage sludge digester, are supposed to have no adverse effects on the methane formation. However, the effect of ammonia nitrogen at concentrations in excess of 1500 mg l^{-1} is not clear at present. Literature data concerning the effect of these ammonia nitrogen concentrations on methane formation are rather conflicting.

McCarty and McKinney (1961) stated that ammonia nitrogen concentrations between 1500 and 3000 mg l^{-1} are inhibitory at pH levels above 7.4, whereas ammonia nitrogen concentrations in excess of 3000 mg l^{-1} are toxic at all pH values. These threshold levels were confirmed by Hobson and Shaw (1976) in investigations on the influence of ammonia nitrogen on a pure culture of *Methanobacterium formicicum*. Albertson (1961) studied the digestion of sewage solids and reported an ammonia nitrogen inhibitory level of approx. 1250 mg l^{-1}. According to McCarty and McKinney (1961) the ammonium ion toxicity is related to the concentration of undissociated ammonia in the solution. The critical concentration for inhibition of methanogenesis appeared to be approx. 140 mg l^{-1}. On the other hand many investigators recently reported satisfactory anaerobic digestion at ammonia nitrogen concentrations considerably exceeding 1500 mg l^{-1}, even in combination with pH values in the range 7.5 - 8. These results were obtained in the digestion of concentrated sewage sludge (Melbinger and Donnellon, 1971) and animal wastes (Gramms et al., 1971; Lapp et al., 1975; Hobson and Shaw, 1976; Kroeker et al.,1976; Converse et al., 1977; Fisher et al., 1977). The successful digestion at ammonia nitrogen concentrations in excess of 1500 mg l^{-1} was attributed to an adaptation of the methanogenic organisms (Melbinger and Donnellon, 1971; Lapp et al., 1975), to cation antagonism (Lapp et al., 1975) or to a combination of both.

This Chapter deals with the results of laboratory and pilot-plant studies concerning the adaptation of digested sewage sludge to piggery waste. The main objective was to establish practical directions for starting up anaerobic digesters for piggery waste and similar substrates.

The sensitivity of the microbial population, both adapted and unadapted to piggery waste, to ammonia nitrogen concentrations up to 3500 mg l^{-1} was tested in batch-type experiments.

The final part of the investigation on the adaptation, described in Section 3.3, concerns the influence of ammonia nitrogen concentrations in the range 2070 - 5290 mg l^{-1} on the continuous digestion of piggery wastes by a microbial population, which was well adapted to this material.

3.2 START-UP PROCEDURE WITH DIGESTED SEWAGE SLUDGE AS SEED MATERIAL

3.2.1 Procedures

The influence of the organic loading rate on the adaptation of digested sewage sludge to piggery waste was investigated both in four 4-litre digesters (Section 2.3.2) and in the 6-m^3 pilot plant (Section 2.3.4). The digestion units were filled completely with digested sewage sludge. The seed materials for the laboratory and pilot plant experiments were obtained from the municipal sewage plant at Zeist and Ede, respectively. Some chemical characteristics of both seed sludges are summarized in Table 3.1. Before starting the feeding with piggery waste the digesters were warmed up to the digestion temperature of 30°C.

Table 3.1 Chemical characteristics of the seed materials used in the start-up experiments.

	Laboratory	Pilot plant
TS (g l^{-1})	36.0	31.6
VS (g l^{-1})	22.2	21.5
Ammonia nitrogen (mg N l^{-1})	900	336
Total ammonia (mg N l^{-1})	n.d.	1420
COD total (mg l^{-1})	n.d.	16900
COD supernatant (mg l^{-1})	700	980
COD of the VFA (mg l^{-1})	0	18
Acetic acid conc. (meq l^{-1})	0	0.3
Propionic acid conc. (meq l^{-1})	0	0

In order to investigate the effect of the loading rate upon the adaptation, different initial loading rates have been applied in the laboratory experiments. Since in these experiments manure was used with a TS concentration of about 60 g l^{-1}, the desired loading rate could be obtained by adjusting the detention time. In the course of the experiments the loading rate was increased gradually. The experiments were continued until a stable digestion process had been achieved at a 15-days detention (loading rate 4.0 kg TS m^{-3} day $^{-1}$). Based on the results of the laboratory experiments the pilot plant was operated at an initial loading rate of approx. 2.5 kg TS m^{-3} day^{-1} (detention time 20 days). At day 67 the loading rate was increased to approx. 3.5 kg TS m^{-3} day^{-1} (detention time 15 days).

The manure used in the investigation consisted of both urine and faeces. Relevant chemical characteristics of the manure are contained in Table 3.2.

Table 3.2 Chemical characteristics of the manure used in the start-up experiments.

	Laboratory		Pilot plant		
	day 0-147	day 147-200	average	maximum	minimum
TS (g l^{-1})	57.5	61.7	58.3	93.9	29.5
VS (g l^{-1})	38.2	46.3	43.9	64.6	21.6
pH	n.d.	n.d.	7.03	7.92	6.4
Ammonia nitrogen (mg N l^{-1})	1675	2480	1200	1800	720
Total nitrogen (mg N l^{-1})	3400	4230	2680	3800	1570
COD total (mg l^{-1})	58500	60000	51750	116500	28000
COD supernatant (mg l^{-1})	15900	23500	8440	16250	4100
COD of the VFA (mg l^{-1})	8700	15200	4775	8700	1580
Acetic acid (meq l^{-1})	65	100	40.3	72.7	13.2
Propionic acid (meq l^{-1})	28	4	9.1	17.7	3.4

The manure used in the laboratory experiments was obtained from piggeries with a slatted-floor system and contained no litter material. If necessary the manure was diluted with tap water to a TS concentration of about 60 g l^{-1}. The experiments were conducted with two different batches of manure, which were stored at 4°C. From analyses made twice a week during the storage period it appeared that storage at 4°C hardly affects the chemical composition of the manure.

The piggery waste used in the pilot plant experiment was obtained from an experimental farm where the manure solids and the manure liquid were discharged separately. Before digestion both fractions were combined in appropriate proportions. The manure in this case partly consisted of saw dust and other litter materials. As the manure concentration varied considerably, only the maximum, the minimum and the average values are given in Table 3.2.

Before as well as after the adaptation of digested sewage sludge to piggery waste the microbial populations were tested for their sensitivity to ammonia nitrogen in concentrations up to 3500 mg l^{-1}. The tests were performed in five 1-litre batch digesters (Section 2.3.1) at a temperature of 30°C. One series of digesters was seeded with digested sewage sludge from the municipal sewage plant at Zeist. The ammonia nitrogen concentration of the seed material was 815 mg l^{-1}. The other series was conducted with digested piggery waste, adapted to 2420 mg l^{-1} ammonia nitrogen. The sludge was obtained from a well-balanced laboratory digester, which was fed with piggery waste for a period of 6 months. Originally the piggery waste digester was seeded with digested sewage sludge of the same origin as the sludge used in the first series of the tests.

The digestion units were seeded with 250 ml of the seed material. After addition of 500 ml tap water, ammonium chloride was supplied from a concentrated stock solution to increase the ammonia nitrogen concentration to the desired level. The ammonia nitrogen concentrations employed are summarized in Table 3.3. Feed was added by supplying 100 ml of a neutralized VFA stock-solution (Section 2.2.3). Then the digesters were filled up to 1 litre with tap water and subsequently the digester-pH was adjusted to 7.0 with HCl or NaOH. At the start of the experiments nitrogen gas was passed through the digester contents to assure anaerobic conditions. The course of the methane formation was followed by measuring the gas production (daily) and the VFA concentration in the digester contents (2 or 3 times a week).

Table 3.3 Ammonia nitrogen concentrations employed in the batch experiments (in mg N l^{-1}).

Digested sewage sludge	Digested piggery manure
730	605
1210	1230
2360	1790
3520	2410
4990	3075

3.2.2 Results

The course of the gas production, the VFA concentration, the ammonia nitrogen concentration and the pH of the mixed liquor in the start-up experiments are shown graphically in Fig. 3.1, together with the loading rates applied.

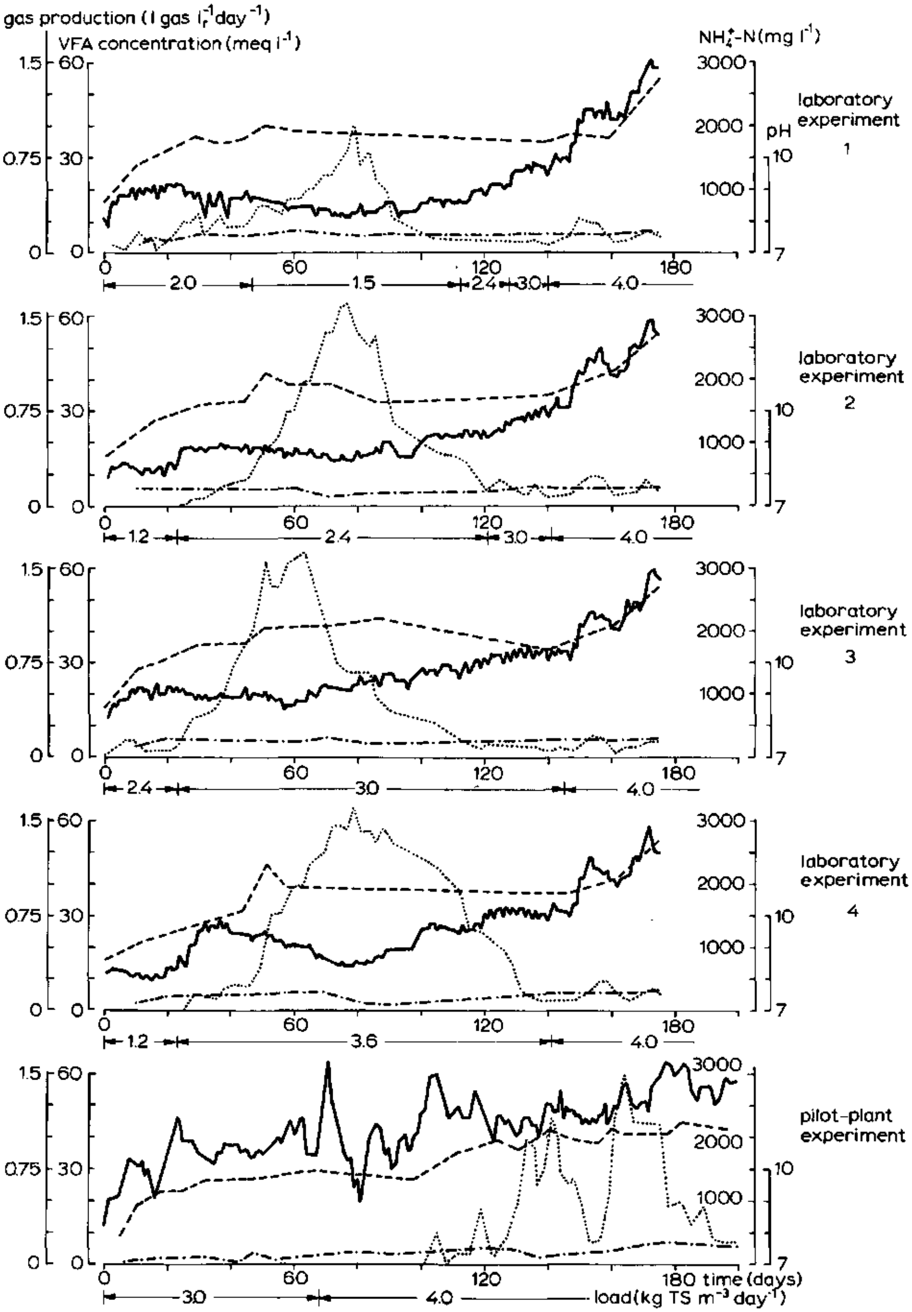

Fig. 3.1 Course of the digestion process in the start-up of the laboratory and pilot plant experiments. ———— gas production; .... VFA concentration; - - - - - - - ammonia nitrogen concentration; -.-.-.-. pH.

From these results it appears that the course of the process was very similar in all experiments. The results reveal an immediate start of the gas production after supply of the feed and no appreciable accumulation of VFA in the first weeks after the start of the experiments. However, in due time the VFA concentration increased in all experimental units, indicating that the digestion process passed a certain imbalance. Nevertheless, the feeding of the digesters was continued at the same level. In spite of this the digestion process in all digesters recovered within 3 months. Some of the more characteristic features of the start-up period as observed in the laboratory experiments, such as the maximum total VFA, the maximum acetic acid and propionic acid concentrations and the duration of the period of process imbalance, are summarized in Table 3.4. For this purpose we defined the digestion process as being in imbalance at VFA concentrations in excess of 10 meq l^{-1}.

Table 3.4 Summary results of the laboratory start-up experiments.

	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Load at acclimation (kg TS $m^{-3}day^{-1}$)	2.0 - 1.5	2.4	3.0	3.6
Sludge load (kg COD kg^{-1}VS day^{-1})	0.070	0.084	0.105	0.126
Duration of adaptation period (days after initial feeding)*	93	117	106	131
Duration period of imbalance (days)*	48	71	80	84
Maximum VFA conc. (meq l^{-1})	40	64	65	64
Maximum acetic acid conc. (meq l^{-1})	38	50	42	55
Maximum propionic acid conc. (meq l^{-1})	8	14	22	27

* The digestion process is arbitrarily considered to be imbalanced at VFA concentrations in excess of 10 meq l^{-1}.

In comparison with the laboratory experiments, where the increase in the VFA concentration started approx. 25 days after the initial feeding, the increase in the VFA concentration in the pilot plant experiment was significantly delayed because it took place after 110 days.

The smell of the digester effluent in the period of unbalanced digestion as determined by personal observation was rather obnoxious and more or less similar to that of fresh manure. However, after recovery of the process the odour was significantly less objectionable than that of the fresh manure. The methane production before and after the period of imbalance is contained in Table 3.5. From these data it appears that the digestion process has not been seriously affected by the temporary upset. The higher gas yield as found in the pilot plant experiment relative to that in the laboratory experiments should be attributed to the presence of litter materials in the manure used in the pilot plant experiment.

Table 3.5 Methane production before and after the period of imbalanced digestion in the start-up experiments.

	Laboratory				Pilot plant
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	
Before period of process imbalance					
Load (kg TS m^{-3} day^{-1})	2.0	1.2	2.4	1.2	2.5
CH_4 production (m^3 CH_4 kg^{-1}TS added)	0.173	0.207	0.181	0.195	0.227
After period of process imbalance					
Load (kg TS m^{-3} day^{-1})	2.4	3.0	4.1	3.6	3.5
CH_4 production (m^3 CH_4 kg^{-1}TS added)	0.186	0.184	0.165	0.172	0.265

After recovery of the process the organic loading rate was increased in all experiments to approx. 4 kg TS m^{-3} day^{-1} by increasing the detention time stepwise to 15 days (Fig. 3.1). At day 147 it was necessary to use manure of a slightly different chemical composition in the laboratory experiments. Both these changes did not affect the process stability. At the end of the experimental period the digestion process proceeded very similarly in all laboratory digesters and the results were also in accordance with those of other experiments conducted at comparable process circumstances (Chapter 4) as can be seen in Table 3.6.

Table 3.6 Comparison of the average results at the end of the start-up experiments with those of other experiments at similar process circumstances (Chapter 4). Manure concentration 60 g l^{-1}; detention time 15 days; temperature 30°C.

	'typical' results	results of the start-up experiments
CH_4 production (m^3 CH_4 kg^{-1}TS added)	0.197	0.202
pH	7.8	7.55
Ammonia nitrogen (mg N l^{-1})	2675	2750
TS reduction (%)	30.8	31.6
VS reduction (%)	39.2	42.6
VFA concentration (meq l^{-1})	3.5	5.0
Acetic acid (meq l^{-1})	3.5	3.1
Propionic acid (meq l^{-1})	0	1.3

The results of the batch experiments with digested sewage sludge and digested piggery waste are shown in Figs. 3.2 and 3.3, respectively. The results presented in Fig. 3.2 indicate that methanogenesis in digested sewage sludge adapted to 815 mg l^{-1} ammonia-nitrogen takes place at all ammonia nitrogen concentrations investigated, even at a concentration of 4990 mg l^{-1}. However, an increasing lag phase occurs when the sludge is inoculated at increasing concentrations, viz. extending to 50 days at an ammonia nitrogen concentration of 4990 mg l^{-1}. Once the methane formation has begun, the VFA were eliminated simultaneously except at 4990 mg l^{-1} ammonia nitrogen when propionic acid was not eliminated at all, even not after 90 days of incubation.

In digested piggery waste acclimated to 2420 mg l^{-1} ammonia nitrogen methane production started immediately after the incubation at all ammonia nitrogen concentrations investigated in the range 605 - 3075 mg l^{-1}. In these experiments the course of the gas production as well as that of the VFA elimination are very similar. Acetic acid and butyric acid were eliminated simultaneously and immediately after incubation, whereas the breakdown of propionic acid started only 18 days after incubation. The methane content of the gas collected during the whole experimental period, ranged from 90 to 95%. The pH measured at the end of the experiments was in the range 7.2 - 7.4 for the experiments with digested sewage sludge and in the range 7.5 - 7.6 in the experiments with digested piggery waste.

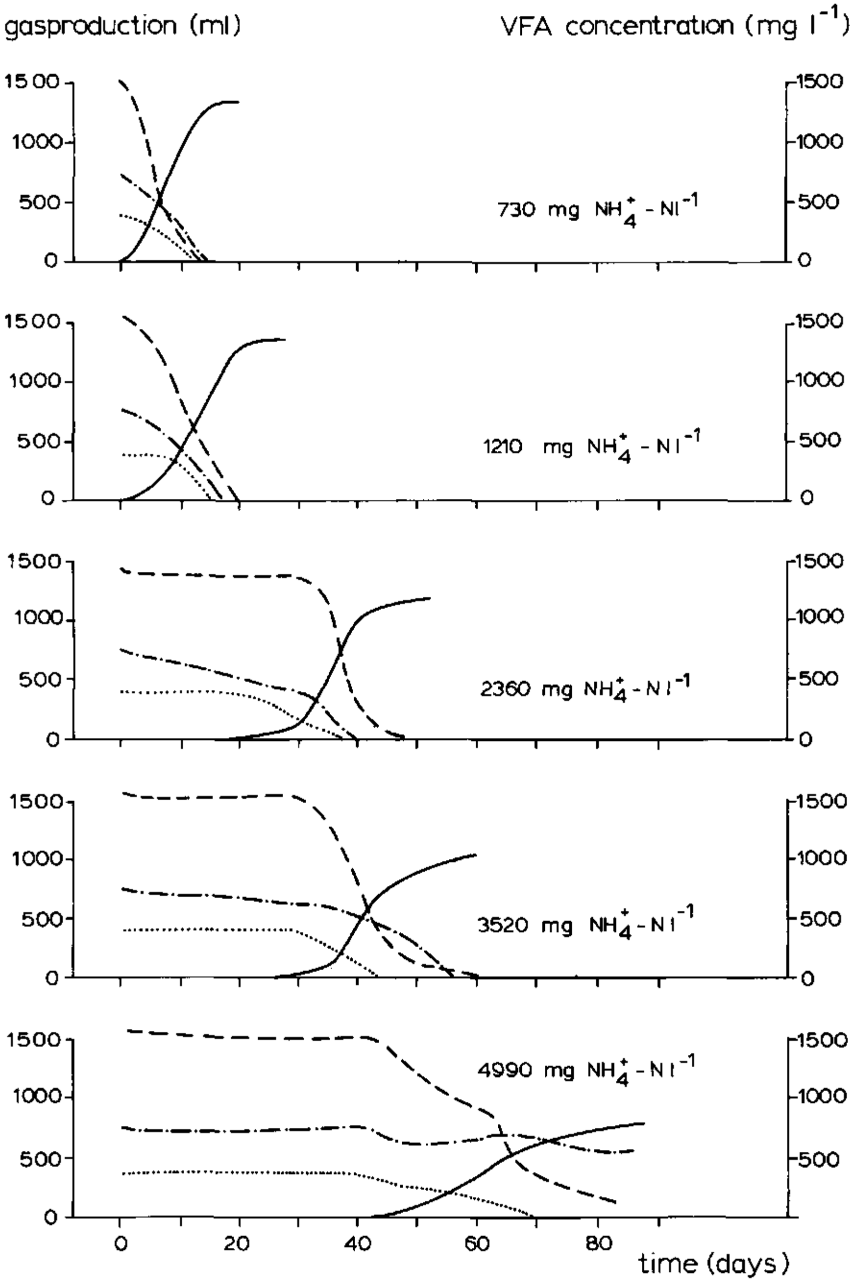

Fig. 3.2. Cumulative gas production and the course of the VFA elimination by digested sewage sludge at increasing ammonia nitrogen concentrations. VS concentration 6.8 g l^{-1}. ———— gas production; - - - - - acetic acid concentration; -.-.-.-.-. propionic acid concentration; .... n-butyric acid concentration.

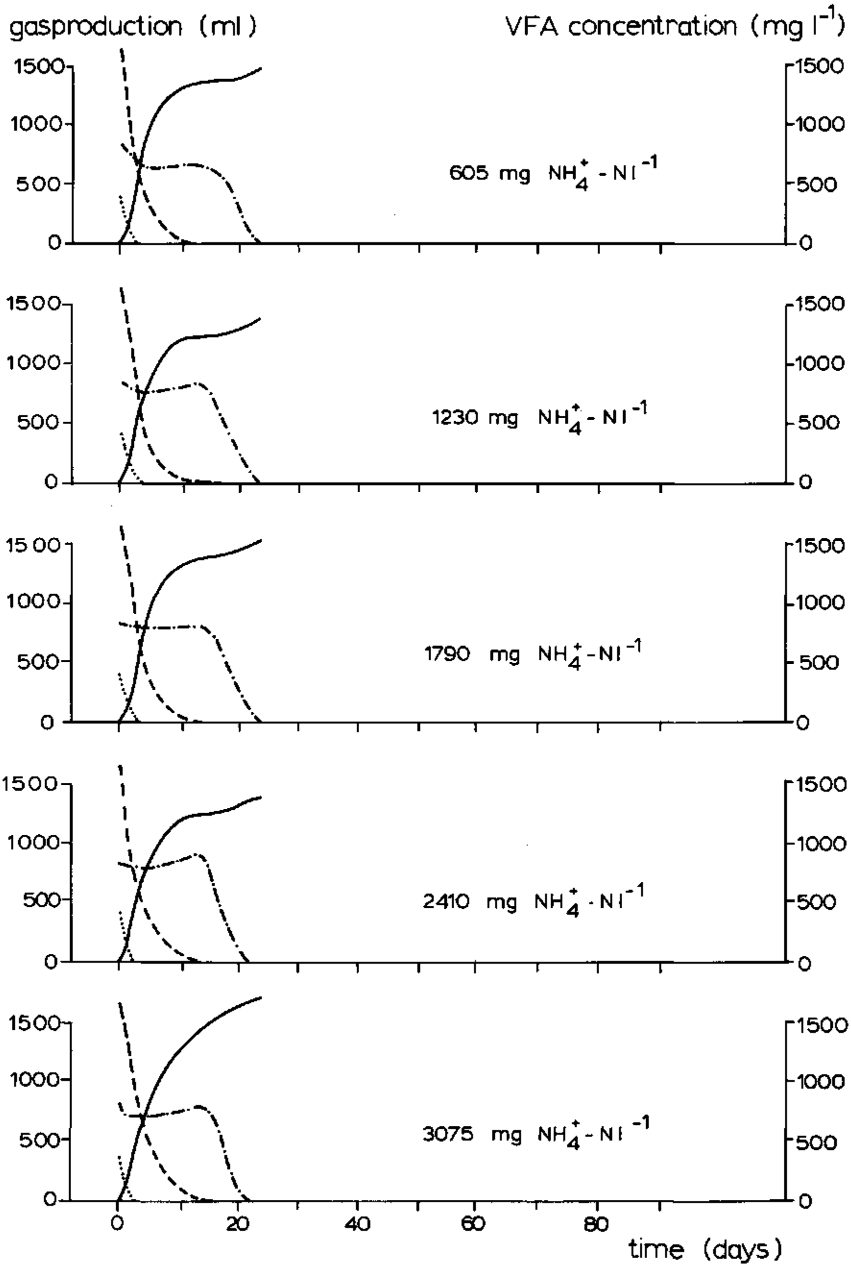

Fig. 3.3. Cumulative gas production and the course of the VFA elimination by digested piggery waste at increasing ammonia nitrogen concentrations. VS concentration 9.8 g l^{-1}. ———— gas production; - - - - - acetic acid concentration; -.-.-.-.-.-.- propionic acid concentration; .... n-butyric acid concentration.

3.2.3 Discussion

Digested sewage sludge apparently is a fairly suitable material for seeding a piggery waste digester because methane formation starts immediately upon feeding with piggery waste. The methane production reached its maximum level already in the first week after the start of the feeding (Table 3.5). These observations demonstrate that the anaerobic organisms present in digested sewage sludge are capable to metabolize the digestible constituents of piggery waste without any adaptation.

On the other hand all start-up experiments are characterized by the occurrence of a period of process imbalance as indicated by the increase in the VFA concentration. An increase in the VFA concentration in anaerobic processes reveals that the methane formation rate is insufficient to metabolize the VFA offered. As the sum of the COD removed via the methane gas and the VFA-COD present in the mixed liquor remained almost unchanged during the whole experimental period, it is evident that the accumulation of VFA in these experiments should be attributed to an inhibition of the methane-forming organisms. Since the seed material was well adapted to piggery waste, the inhibition of the methane formation can be attributed to changes in the environmental conditions, resulting from the gradual displacement of the original mixed liquor by piggery waste. A significant change in the environmental conditions concerns the increase in the ammonia nitrogen concentration of the digester contents. In all start-up experiments the VFA concentration only started to increase when the ammonia nitrogen concentration of the digester contents reached a level of approx. 1700 mg l^{-1}. This coincidence is the more striking because the presumed critical ammonia nitrogen concentration of approx. 1700 mg l^{-1} is reached after 3 weeks in the laboratory experiments, but only after 15 weeks in the pilot plant experiment. The experimental results, therefore, evidently point to an ammonia nitrogen inhibition at concentrations exceeding 1700 mg l^{-1}.

A further proof for the role of ammonia nitrogen in the methanogenesis of digested sewage sludge at concentrations above the threshold value of 1700 mg l^{-1} is found in the results of the batch experiments with digested sewage sludge (Fig. 3.2). Here the VFA were metabolized almost immediately at ammonia nitrogen concentrations of 730 and 1210 mg l^{-1}, but at a concentration of 2360 mg l^{-1} the microbial population required a lag phase of 25 days. This sharp increase in the lag phase at ammonia nitrogen concentrations between 1210 and 2360 mg l^{-1} once again points to the above mentioned threshold ammonia nitrogen concentration somewhere in this range, beyond which a prolonged lag phase is required for the adaptation of the sludge. The existence of such a critical threshold level is in accordance with the results of Melbinger and Donnellon (1971). Ammonia nitrogen here appeared to be toxic or inhibitory at concentrations above 1700 - 1800 mg l^{-1} only if its formation rate increases more rapidly than the adaptation of the methane-forming organisms.

Furthermore, literature data on the digestion of farm wastes indicate that the digestion of wastes with an ammonia nitrogen concentration below the threshold level of 1700 mg l^{-1} does not result in an accumulation of VFA (Fisher et al., 1977; Gramms et al., 1971; Miner and Smith, 1975) whereas the digestion of wastes with an ammonia nitrogen concentration above this level results in an increase of the VFA concentration in course of the experiment (Miner and Smith, 1975; Lapp et al., 1975; Kroeker et al., 1976; Schmid and Lipper, 1969; Hart, 1963). Unfortunately in none of the investigations cited above the course of the ammonia nitrogen concentration during the start-up process has been monitored. Therefore, these reports do not allow to conclude at what ammonia nitrogen concentration the VFA concentration started to increase. The increase in the VFA concentration made some workers to conclude that ammonia nitrogen concentrations in excess of 1700 mg l^{-1} effected process failure (Schmid and Lipper, 1969; Miner and Smith, 1975) or process inhibition (Gramms et al., 1971).

On the other hand various other investigators reported satisfactory digestion of animal wastes at ammonia nitrogen concentrations considerably exceeding 1700 mg l^{-1} in long term experiments (Lapp et al., 1975; Lapp et al., 1975; Kroeker et al., 1976; Fisher et al., 1977; Converse et al., 1977; Summers and Bousfield, 1976; Hobson et al., 1976). The successful digestion at high ammonia nitrogen concentrations, even in combination with pH levels above 7.5, should be attributed to the ability of the microbial population to adapt to these circumstances. As appears from the results of both the daily fed start-up experiments and the batch experiments, the ammonia nitrogen inhibition of the methane formation at concentrations in excess of 1500 - 1700 mg l^{-1} only is temporary provided the methanogenic organisms are allowed to acclimate. The results of the batch experiments, showing a significant difference in the sensitivity to high ammonia nitrogen concentrations between digested sewage sludge and digested piggery waste, clearly indicate that the adaptation of digested sewage sludge to piggery waste in fact concerns an adaptation to the high ammonia nitrogen concentration present in these wastes. Contrary to digested sewage sludge the microbial population acclimated to piggery waste is well adapted to all ammonia nitrogen concentrations investigated, although in all cases a lag phase is required for the elimination of propionic acid. However, as the lag phase is almost similar in all experimental units of this series, the retarded breakdown of propionic acid in these experiments may be caused by an inhibition of either the H_2-producing acetogenic or the methanogenic organisms. As the experiments were carried out with mixed cultures the results do not reveal which group of organisms is predominantly inhibited.

The effect of ammonia nitrogen on adapted sludge can be deduced from the specific gas production rate, k (in ml gas g^{-1}VS day^{-1}) obtained in the batch experiments. The *k*-values at different ammonia nitrogen concentrations are shown in Fig. 3.4. The *k*-values suggest the existence of a linear relationship between *k* and the ammonia nitrogen concentration. Assuming such a linear relationship a regression line has been computed and depicted in Fig. 3.4. The correlation coefficient, r^2, of this regression line is 0.73.

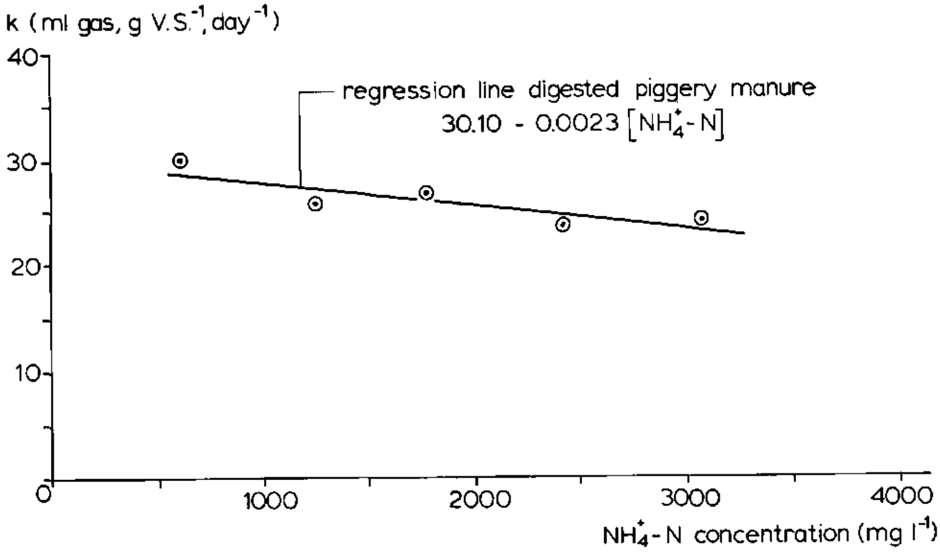

Fig. 3.4. Maximum specific gas production rate (k) of digested piggery waste at increasing ammonia nitrogen concentrations.

The successful digestion at high ammonia nitrogen concentrations seems to conflict with the experimental results of McCarty and McKinney (1961) and Hobson and Shaw (1976), who found that ammonia nitrogen is inhibitory at about 1500 mg l^{-1} and toxic at about 3000 mg l^{-1}. However, a more close comparison of our results with those of McCarty et al. and Hobson et al. suggest that the inhibitory ammonia nitrogen concentration, as found by McCarty et al. and Hobson et al., correlates with the critical concentration of 1700 mg l^{-1} observed in our experiments. The threshold levels reported by McCarty et al. and Hobson et al. were deduced from experiments lasting only 30 days and 10 days, respectively. In the light of the extended lag phase observed in the present investigations, we are the opinion that the experiments of McCarty et al. and Hobson et al. were run over a too short period of time to allow an adaptation of the microbial population.

The results obtained in the present study show that anaerobic sludge, once it is adapted to the ammonia nitrogen threshold level of about 1700 mg l^{-1}, is also adapted to much higher concentrations. The increase of the ammonia nitrogen concentration in the start-up experiments after day 147 did not cause any accumulation of VFA in the digester contents. Accordingly no lag phase occurred in the batch experiments with digested piggery waste when sludge adapted to 2420 mg l^{-1} ammonia nitrogen was inoculated at a concentration of 3075 mg l^{-1}.

The results of the laboratory start-up experiments reveal that an increase in the initial loading rate is accompanied with both an increase in the VFA concentration and an extension of the period of imbalanced digestion (Table 3.4). Since in these experients 6% TS manure has been used an increase of the organic loading rate is accomplished by the application of a shorter detention time, resulting in an accelerated increase in the ammonia nitrogen concentration. Therefore the results do not allow to conclude whether the increased loading rate or the accelerated rise in the ammonia nitrogen concentration predominates with respect to the height of the VFA concentration and the duration of the period of process imbalance.

Although the initial loading rate markedly affects the degree of process imbalance it apparently has not any clear effect on the final digestion characteristics, because the performance of the 4 laboratory digesters at the end of the experiments is very similar. Moreover, as the results were very similar to those of other experiments at similar process circumstances it seems reasonable to conclude that the start-up procedure is especially important to provide optimum conditions for the adaptation of the organisms to the increasing ammonia nitrogen concentrations, but that the initial loading rate does not influence the operation of the digester once the microbial population has been adapted.

With respect to the procedure to be followed in the first start-up of full scale digesters emphasis should be given to the initial sludge load (in kg COD kg^{-1}VS day^{-1}) as directive and not to the space load (in kg COD m^{-3} day^{-1} or kg TS m^{-3} day^{-1}), because the quantity of seed sludge available for start-up frequently is insufficient to fill up the digester completely. The results of the start-up experiments (Table 3.4) indicate that a digester can be started up succesfully at sludge loads up to 0.126 kg COD kg^{-1}VS day^{-1}. The digester should be fed at this loading rate for a period of 2.5 months in order to allow the sludge to adapt. During the start-up of a piggery waste digester a fall of the pH below pH 7 is very unlikely because as a rule sufficient ammonia nitrogen and other buffering agents are present in this waste to neutralize the VFA, even in case of severe accumulation of these intermediates. After 2.5 months the loading rate can be increased stepwise, e.g. according to the procedures outlined in Fig. 3.1, to the desired level.

3.3 THE INFLUENCE OF AMMONIA NITROGEN ON CONTINUOUS DIGESTION OF PIGGERY WASTE BY ADAPTED SLUDGE

3.3.1 Procedures

The influence of ammonia nitrogen at concentrations in the range 2070 - 5290 mg l^{-1} on the continuous digestion of piggery waste was investigated in three 45-litre laboratory digesters (Section 2.3.2). At the start of these experiments the digestion units were filled completely with digested piggery waste and fed with 6% TS manure at a 15-days detention time (organic load 4 kg TS m^{-3} day^{-1}). The seed material originated from the 6-m^3 pilot plant digester when it was operated at a temperature of 30°C and at a 15-days detention (loading rate 4 kg TS m^{-3} day^{-1}). The digester population already was adapted to 1900 mg l^{-1} ammonia nitrogen.

The piggery waste used in the experiment was obtained from an experimental farm where the manure solids and the manure liquid were discharged separately. For the experiments both fractions were combined in appropiate proportions and stored at a temperature of 4°C. In the three parallel experiments the same manure was used. Some chemical characteristics of the manure, including the concentration of the aromatic malodorous compounds, are represented in Tabel 3.7. To raise the ammonia nitrogen concentration urea was added to the manure feed used in experiments 2 and 3 at an amount of 4 g and 8 g per litre manure, respectively.

Table 3.7 Chemical characteristics of the manure used in the experiments on the influence of ammonia nitrogen on the digestion of piggery waste.

TS (g l^{-1})	61.8
VS (g l^{-1})	51.9
Ammonia nitrogen (mg N l^{-1})	1325
Total nitrogen (mg N l^{-1})	3230
COD total (mg l^{-1})	64130
COD supernatant (mg l^{-1})	7750
VFA (meq l^{-1})	70.4
Phenol (mg l^{-1})	18.4
p-Cresol (mg l^{-1})	32.9
4-Ethylphenol (mg l^{-1})	5.1
Indole (mg l^{-1})	4.9
Skatole (mg l^{-1})	1.5

3.3.2 Results

The gas production, the gas composition and some chemical characteristics of the digester effluent at the end of the experiments are summarized in Table 3.8, together with the process conditions applied. In Table 3.8 the values of TS, VS and COD total are not represented since they are inexpressive as a result of the incomplete mixing of the digester contents in the 45-litre digesters (Section 2.3.2).

Table 3.8 Summary results at steady state operation of laboratory experiments concerning the influence of ammonia nitrogen on the digestion of piggery waste. Temperature 30 ± 2°C; detention time 15 days; organic load 4.1 kg TS m^{-3} day^{-1}; duration of the experiments 100 days.

Urea added to the manure (g l^{-1})	0	4	8
Gas			
Gas production (l gas l_r^{-1} day^{-1})	1.708	1.416	1.126
(m^3 gas kg^{-1} TS added)	0.415	0.344	0.273
Gas composition (% CH_4)	64.3	69.1	73.6
Methane production (m^3 kg^{-1} TS added)	0.267	0.237	0.201
COD removed via gas (g COD l^{-1} manure)	40.52	36.10	30.59
Digester effluent			
pH	7.46	7.69	7.88
Ammonia nitrogen (mg N l^{-1})	2070	3760	5290
Total nitrogen (mg N l^{-1})	3520	5460	6890
COD supernatant (mg l^{-1})	3870	4680	6130
COD of the VFA (mg l^{-1})	98	213	1673
VFA (meq l^{-1})	1.5	3.5	25.1
Acetic acid (meq l^{-1})	1.4	3.4	23.6
Propionic acid (meq l^{-1})	0.1	0.1	1.2
COD total reduction (%)	63.2	56.3	47.7
COD_s reduction (%)	50	38.7	20.9
Reduction of the VFA-COD (%)	98.2	96.1	69.3

The results of Table 3.8 indicate that the gas production at 2070 mg l^{-1} ammonia nitrogen is considerably higher, viz. 33%, than that at 5290 mg l^{-1}. However, as the digester gas contains more methane at increasing ammonia nitrogen concentrations, the decrease of the methane production is only

24.5% over this concentration range. The increase of the methane content of the gas at raising ammonia nitrogen concentrations can be explained from the higher digester-pH, effectuated by the higher bicarbonate alkalinity More of the carbon dioxide produced in the digestion process dissolves in the digestion mixed liquor in that case and consequently the biogas is richer in methane. The experimental results further show an evident increase in the concentration of dissolved process intermediates upon raising ammonia nitrogen concentrations, i.e. the supernatant COD increases from 3870 mg l^{-1} at 2070 mg $^{-1}$ ammonia nitrogen to 6900 mg l^{-1} at 5290 mg l^{-1} ammonia nitrogen and the concentration of the VFA from 1.5 meq l^{-1} to 25.1 meq l^{-1}. The increase in the VFA concentration mainly concerns acetic acid which points to a slight inhibition of the methane-forming organisms under these circumstances.

The less complete conversion of piggery waste ingredients at high ammonia nitrogen concentrations also appears from the relative concentrations of the malodorous compounds at the various ammonia nitrogen concentrations (Fig. 3.5). There is a sharp increase in the concentration of the malodorous compounds, especially that of the aromatic compounds, at increased ammonia nitrogen concentrations.

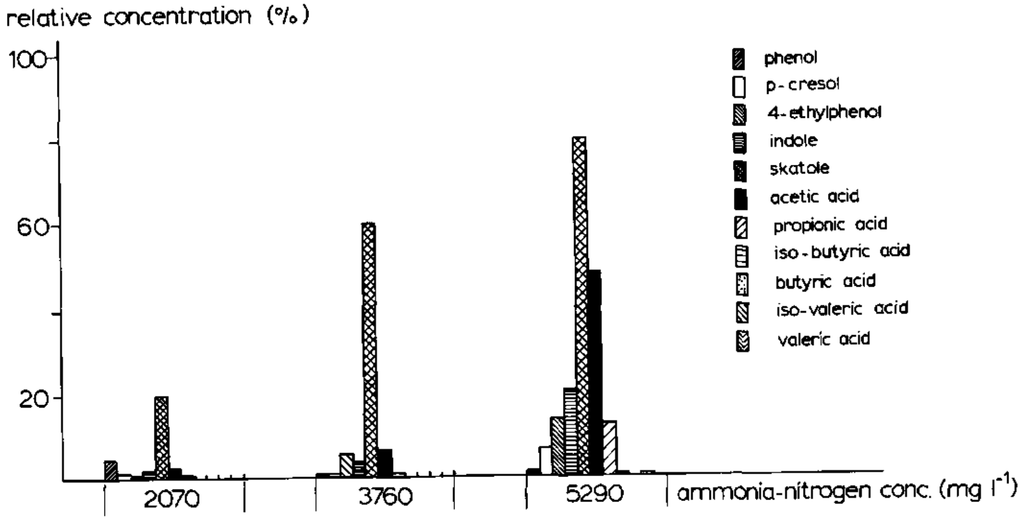

Fig. 3.5 Relative concentration of malodorous compounds at various ammonia nitrogen concentrations. The concentration has been expressed in relation to the concentration *in raw manure (Table 3.7). Temperature 30°C; detention time 15 days;* organic load 4.1 kg TS m^{-3} day^{-1}.

3.3.3 Discussion

The experimental results indicate that methanogenesis proceeds satisfactorily even at an ammonia nitrogen concentration of 5290 mg l^{-1} and a pH of 7.88, despite the fact that under these circumstances the concentration of undissociated ammonia nitrogen is 310 mg l^{-1}, which is appreciably higher than the toxic level of 140 mg l^{-1} mentioned by McCarty and McKinney (1961). On the other hand a rise in the ammonia nitrogen concentration clearly affects the digestion process; the methane production is lower and the concentration of the process intermediates becomes higher. A closer examination of the results learns that the increased concentration of process intermediates is insufficient to account for the decreased methane production. Obviously the addition of urea not only affects the metabolism rate of the methane-forming organisms but also that of the hydrolytic bacteria. The addition of urea results in an increased ammonia nitrogen concentration and consequently in a higher pH of the medium. As the culture-pH affects both the enzyme concentration and the enzyme activity (Hobson et al., 1974; Dean, 1972; Bull, 1972) no conclusions can be drawn on the question which factor predominates in the inhibition of the separate steps of the digestion process, either it can be the increased ammonia nitrogen concentration or the higher culture-pH or a combination of both.

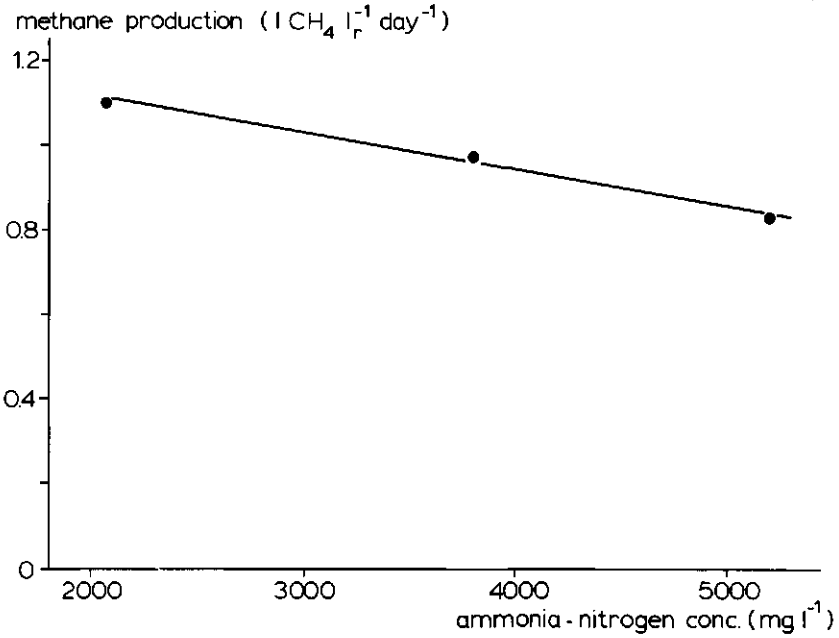

Fig. 3.6 Methane production rate at various ammonia nitrogen concentrations. Temperature 30 ± 2°C; detention time 15 days; organic load 4.1 kg TS m^{-3} day^{-1}.

The effect of ammonia nitrogen on the methane production rate is shown in Fig. 3.6. Conform the results of the batch experiments with digested piggery wast and VFA as feed (Fig. 3.4) the experimental results of Fig. 3.6 show a linear relationship between the ammonia nitrogen concentration and the methane production rate, although it should be taken in mind that the pH was different in the various experiments.

Referring to the practical application of the digestion process these results implicate that the loading rate should be decreased as the ammonia nitrogen concentration increases in order to achieve the same degree of stabilization and methane formation. This especially holds for ammonia nitrogen concentrations exceeding 5000 mg l^{-1} (concentration undissociated ammonia nitrogen 300 mg l^{-1}). As the obtainable degree of manure stabilization may also be influenced by ammonia nitrogen it is doubtful whether or not a satisfactory reduction of the manure malodours can be obtained at these high ammonia nitrogen concentrations.

From long-term experiments with piggery waste in the 6-m^3 digester it turned out that at an ammonia nitrogen concentration slightly above 5000 mg l^{-1} and a loading rate of 3.5 kg TS m^{-3} day^{-1} (detention time 20 days) the methane production decreases slowly in the course of several months. Similar observations were made in a 300-m^3 full scale piggery waste digester. The gradual decrease of the methane production under these circumstances may be explained from the wash-out of methane-forming bacteria since their activity and hence their growth rate decreases at these high ammonia nitrogen concentrations.

4 INFLUENCE OF DETENTION TIME AND MANURE CONCENTRATION

4.1 INTRODUCTION

A basic knowledge of the effects of the loading rate on the digestion process is essential for assessing the optimum digester design and operation. However, the loading rate of a digester, expressed in kg TS m^{-3} day^{-1}, can not be considered as a single process parameter but is fixed by the detention time and the manure feed concentration. Both these feeding parameters may influence the digestion process independently.

The manure feed concentration affects a number of important environmental factors of the digestion process, such as the pH, the ammonia nitrogen concentration, the hydrogen sulphide concentration and the VFA concentration. Together the complex of these factors influences the metabolic activity of the microbial population, including the production, secretion and activity of exocellular enzymes (Dean, 1972; Bull, 1972; Demain, 1972).

The other loading parameter, i.e. the manure detention time, influences the digester population as well. Since the digestion of concentrated substrates like piggery wastes is performed in mixed reactors, the sludge detention time in these systems is equal to the manure detention time applied. Hence the detention time together with the growth rate of each individual type of organism under the prevalent environmental conditions determines which organisms can sustain in the digester and which not.

In order to determine the influence of the loading rate on the digestion process the effects of both loading parameters should be investigated independently. This Chapter deals with the results of laboratory and small pilot plant experiments on the influence of the manure detention time on the digestion of piggery waste at varying TS concentrations.

4.2 PROCEDURES

The laboratory experiments with piggery waste at TS concentrations of 4%, 6% and 9% were performed in digesters with a volume of 1.5, 2, 5, and 6 litres (Section 2.3.2). In addition the digestion of 6% TS piggery waste was studied in two 240-litre pilot plant digesters (Section 2.3.3).

The manure concentrations and the detention times investigated are summarized in Table 4.1. The laboratory experiments were carried out in two series, which have been indicated also in Table 4.1. The first series laboratory and pilot plant experiments were seeded with digested sewage sludge, which was well adapted to piggery waste as it was fed with 6% TS piggery waste at a 40-days detention time for more than 6 months. In the second series laboratory experiments seed sludge from the pilot plant experiment D_2 was used after a stable digestion had been established at a 20-days detention time.

Table 4.1 Manure concentrations and detention times applied in the experiments.

Detention time (days)	Manure concentration (g TS l^{-1})			
	40	60		90
		laboratory	pilot plant	
40			D_1	
20		B_1^{*}, B_2^{**}	D_2	C_1^{**}
16		B_3^{*}		
15	A_1^{*}		D_3	C_2^{**}
12.5		B_4^{*}		
12			D_4	
10	A_2^{*}	B_5^{*}		C_3^{**}

* First series of experiments

** Second series of experiments

Table 4.2 Chemical characteristics of the piggery waste used in the experiments.

	6% TS waste			4% TS waste	9% TS waste
	minimum	maximum	average		
TS (g l^{-1})	58.7	63.5	61.4	40.9	92.1
VS (g l^{-1})	40.6	46.7	43.6	29.1	65.4
Ammonia nitrogen (mg N l^{-1})	1780	2635	2090	1390	3140
COD total (mg l^{-1})	64500	104000	80300	53500	120500
COD supernatant (mg l^{-1})	17500	23300	19400	12900	29000
VFA (meq l^{-1})	107	170	134	89	201
Aromatic compounds					
Phenol (mg l^{-1})	12.6	55.4	25.2	16.8	37.8
p-Cresol (mg l^{-1})	118.2	195.6	149.0	99.3	223.5
4-Ethylphenol (mg l^{-1})	5.7	14.4	11.2	7.5	16.8
Indole (mg l^{-1})	0.6	3.7	2.2	1.5	3.3
Skatole (mg l^{-1})	33.4	48.7	39.6	26.4	59.4

The piggery waste used in the experiments consisted of urine and faeces and contained no or only minor amounts of bedding or litter. The manure, obtained from an experimental farm was supplied at a rather constant TS concentration of approx. 110 g l^{-1} and was diluted to the desired level with tap water.

Table 4.2 summarizes some important chemical characteristics of raw manure, including the concentration of the aromatic odorous compounds.

During the experimental period the analysis of both the manure and the digester effluent were carried out according to the scheme shown in Table 4.3.

Table 4.3 Scheme of the analytical measurements.

	Daily	3 times a week	Weekly	Fortnightly	Incidentally
Gas production	X				
Gas composition			X		
pH		X			
COD total					X
COD supernatant				X	
VFA		X			
Ammonia nitrogen			X		
Total solids			X		
Volatile solids			X		
Aromatic compounds					X

4.3 RESULTS

The results of the laboratory experiments with 4%, 6% and 9% TS piggery waste together with the experimental conditions applied are summarized in Tables 4.4, 4.5 and 4.6, respectively. The data in these Tables concern the average values of the analytical results during the last 4 weeks of the experiments, when the digestion processes had reached a steady state. The values for the COD reduction mentioned in Tables 4.4 to 4.7 have been calculated from the amount of COD removed via the methane gas and the initial manure-COD.

Table 4.4 Results obtained at steady state operation of laboratory digesters fed with 40 g TS l^{-1} piggery waste.

	Exp. A_1	Exp. A_2
Working digester volume (litres)	1.5	1.5
Duration experiment (days)	92	60
Detention time (days)	15	10
Organic load (kg TS $m^{-3}day^{-1}$)	2.8	4.2
(kg COD $m^{-3}day^{-1}$)	3.5	5.3
Gas		
Gas production (l gas l_r^{-1} day^{-1})	0.650	0.713
(m^3 gas kg^{-1}TS added)	0.232	0.170
Gas composition (% CH_4)	84.9	82.8
Methane production (m^3 kg^{-1}TS added)	0.197	0.141
COD removed via gas (g COD l^{-1}manure)	20.37	14.47
Digester effluent		
pH	7.6	7.45
Ammonia nitrogen (mg N l^{-1})	1700	1830
TS (g l^{-1})	30.3	32.5
VS (g l^{-1})	19.8	21.7
COD supernatant (mg l^{-1})	6200	10000
VFA (meq l^{-1})	12.9	38.6
Acetic acid (meq l^{-1})	4.5	4.1
Propionic acid (meq l^{-1})	4.1	33.1
COD total reduction (%)	44.4	31.5
TS reduction (%)	28.4	23.2
VS reduction (%)	31.9	25.3
COD_s reduction (%)	63.1	40.0
VFA reduction (%)	88.6	61.6

The methane content of the digester gas, produced in the experiments A_1, A_2, B_3 and B_4, is high in comparison with the gas produced in the other experiments. This difference has to be attributed to the use of a brine displacement system to collect the digester gas in the former experiments. Part of the carbon dioxide dissolves in the brine solution!

In all laboratory digesters mixing was necessary to prevent the formation of a floating layer. Nevertheless, in some digesters a tough floating layer was formed, which could only be disrupted by continuous stirring for 10 minutes. Scum formation did not occur except under conditions of digestion imbalance. In none of the laboratory experimental units retarded digestion was observed except in experiment C_3, where 9% TS piggery waste was added at a 10-days detention time.

The conditions and the results of the 240-litre pilot plant experiments are summarized in Table 4.7. One digester was run at various detention times, viz. 20, 15 and 12 days, whereas the reference digester was run at a 40-days detention time. Continuous gas recirculation was applied in both digesters. In this way a uniform digester temperature could be maintained whereas the formation of a floating layer was prevented. No scum formation was observed except during start-up periods and after digestion disturbance.

Table 4.5 *Results obtained at steady state operation of laboratory digesters fed with* 60 g TS l^{-1} piggery waste.

	Exp. B_1	Exp. B_2	Exp. B_3	Exp. B_4	Exp. B_3
Working digester volume (litres)	6	5	2	2	6
Duration experiment (days)	102	109	92	94	93
Detention time (days)	20	20	16	12.5	10
Organic load (kg TS m^{-3}day^{-1})	3.2	3.2	4.0	5.1	6.4
(kg COD m^{-3}day^{-1})	4.0	4.0	5.0	6.4	8.0
Gas					
Gas production (l gas l_r^{-1} day^{-1})	0.897	0.810	0.930	1.032	1.300
(m^3 gas kg^{-1}TS added)	0.280	0.253	0.232	0.202	0.203
Gas composition (% CH_4)	73.8	75.6	83.4	83.0	77.1
Methane production (m^3 kg^{-1}TS added)	0.207	0.191	0.193	0.168	0.157
COD removed via gas (g COD l^{-1}manure)	32.6	30.1	30.5	26.3	24.7
Digester effluent					
pH	7.7	7.8	7.9	7.8	7.7
Ammonia nitrogen (mg N l^{-1})	2510	2740	2620	2580	2635
TS (g l^{-1})	49.1	47.3	49.4	51.2	53.2
VS (g l^{-1})	33.9	29.7	34.2	33.9	35.4
COD supernatant (mg l^{-1})	6750	5700	8500	9150	8600
VFA (meq l^{-1})	3.1	5.5	10.7	17.6	23.4
Acetic acid (meq l^{-1})	2.9	5.5	5.3	10.0	11.0
Propionic acid (meq l^{-1})	0	0	5.2	7.3	11.0
COD total reduction (%)	44.6	41.7	41.7	36.4	34.2
TS reduction (%)	22.6	25.5	22.2	19.5	16.2
VS reduction (%)	22.2	31.9	21.6	22.2	18.8
COD_s reduction (%)	75.0	75.5	66.0	63.4	63.0
VFA reduction (%)	97.7	95.9	92.0	86.9	82.6

Table 4.6 Results obtained at steady state operation of laboratory digesters fed with 90 g TS l^{-1} piggery waste.

	Exp. C_1	Exp. C_2	Exp. C_3
Working digester volume (litres)	5	5	5
Duration experiment (days)	109	109	109
Detention time (days)	20	15	10
Organic load (kg TS m^{-3} day^{-1})	4.8	6.4	9.5
(kg COD m^{-3} day^{-1})	6.0	8.0	12.0
Gas			
Gas production (l gas l_r^{-1} day^{-1})	1.120	1.448	0.534
(m^3 gas kg^{-1}TS added)	0.233	0.226	0.056
Gas composition (% CH_4)	74.7	74.4	67.6
Methane production (m^3 kg^{-1}TS added)	0.174	0.169	0.038
COD removed via gas (g COD l^{-1}manure)	41.2	39.8	8.9
Digester effluent			
pH	7.9	7.9	7.55
Ammonia nitrogen (mg N l^{-1})	3950	3910	3980
TS (g l^{-1})	77.2	79.2	85.9
VS (g l^{-1})	49.6	51.5	57.3
COD supernatant (mg l^{-1})	12000	13000	30600
VFA (meq l^{-1})	18.9	20.1	201.3
Acetic acid (meq l^{-1})	18.1	18.0	111.0
Propionic acid (meq l^{-1})	0.8	1.7	63.5
COD total reduction (%)	31.7	30.5	6.8
TS reduction (%)	19.0	16.9	9.8
VS reduction (%)	24.2	21.3	12.4
COD_s reduction (%)	66.4	63.6	14.3
VFA reduction (%)	90.6	90.0	0.2

Due to a failure in the temperature control system the digestion temperature in experiment D_2 temporarily (24 hrs) increased to approx. 50°C shortly after reducing the detention time from 40 to 20 days. This resulted in a severe imbalance of the digestion process as was indicated by a sudden decrease of the gas production, a low methane content of the digester gas and a strong increase in the VFA concentration. To accomplish process recovery no feed was supplied for 3 days, followed by a period in which the supply of the feed was increased stepwise: for a period of 2 days 6 litres of manure per day, then for a period of 3 days 9 litres per day and subsequently with a daily quantity of 12 litres according to the orginal detention time of 20 days. The course of the process recovery is shown in Fig. 4.1. Although gas production and gas composition soon restored, the VFA concentration reached high values, especially that of propionic acid. However, after 5 weeks the propionic acid concentration decreased rapidly, which indicated that the recovery of the digestion process had been completed.

Table 4.7 Results obtained at steady state operation of 240-litre pilot plant experiments with 60 g TS l^{-1} piggery waste.

	Digester II	Digester I		
	Exp. D_1	Exp. D_2	Exp. D_3	Exp. D_4
Working digester volume (litres)	240	240	240	240
Duration experiment (days)	200	100	92	40
Detention time (days)	40	20	15	12
Organic load (kg TS m^{-3} day^{-1})	1.6	3.2	4.2	5.3
(kg COD m^{-3} day^{-1})	2.0	4.0	5.3	6.7
Gas				
Gas production (l gas l_r^{-1} day^{-1})	0.420	0.850	1.030	1.180
(m^3 gas kg^{-1}TS added)	0.262	0.265	0.245	0.223
Gas composition (% CH_4)	73.9	75.2	76.4	76.3
Methane production (m^3 kg^{-1}TS added)	0.194	0.199	0.187	0.170
COD removed via gas (g COD l^{-1}manure)	30.5	31.5	29.0	26.6
Digester effluent				
pH	n.d.	n.d.	n.d.	n.d.
Ammonia nitrogen (mg N l^{-1})	2970	2820	2680	2320
TS (g l^{-1})	45.3	41.3	48.5	48.6
VS (g l^{-1})	28.5	27.2	31.1	31.5
COD supernatant (mg l^{-1})	6600	6350	6550	6100
VFA (meq l^{-1})	4.2	4.2	3.5	4.7
Acetic acid (meq l^{-1})	4.2	4.0	3.5	3.9
Propionic acid (meq l^{-1})	0	0.2	0	0.8
COD total reduction (%)	49.0	44.3	43.9	40.3
TS reduction (%)	28.7	35.0	23.6	23.5
VS reduction (%)	34.6	37.6	28.7	27.8
COD_s reduction (%)	61.2	74.1	62.9	65.5
VFA reduction (%)	96.9	96.9	97.4	96.5

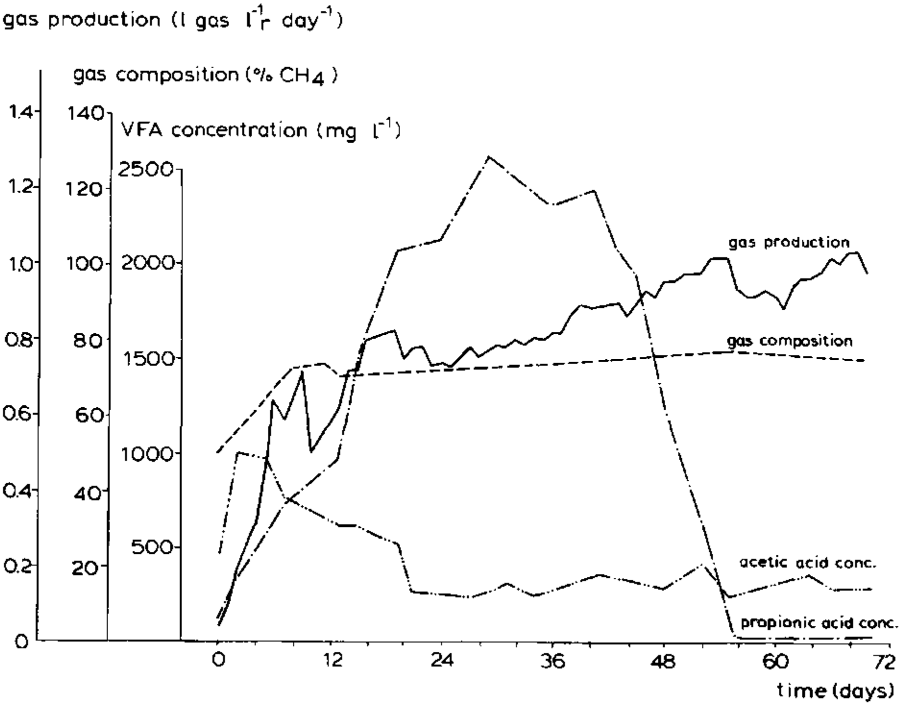

Fig. 4.1 Course of some process parameters after process disturbance due to a temperature shock from 30°C to 50°C for 24 hrs in experiment D_2. Feeding rate after temperature shock: day 0-3 no feed; day 4-5 6 l day^{-1}; day 6-8 9 l day^{-1}; from day 9 12 l day^{-1} according to the original detention time of 20 days.

The concentration of the malodorous compounds, viz. VFA, phenol, *p*-cresol, 4-ethylphenol, indole and skatole, has been determined in raw manure as well as in the digested manure at the end of some experiments. The results of these analyses are shown in Fig. 4.2. The smell of the digester effluent of the pilot plant experiments in no case resembled the objectionable odours of raw piggery waste. In this respect digested piggery waste from the laboratory units was less satisfactory.

The concentration of the malodorous compounds has been analysed also in digested piggery waste, which had been stored at temperatures of 4°C and 20°C for a period of 1, 2 and 3 months. The digested material was obtained

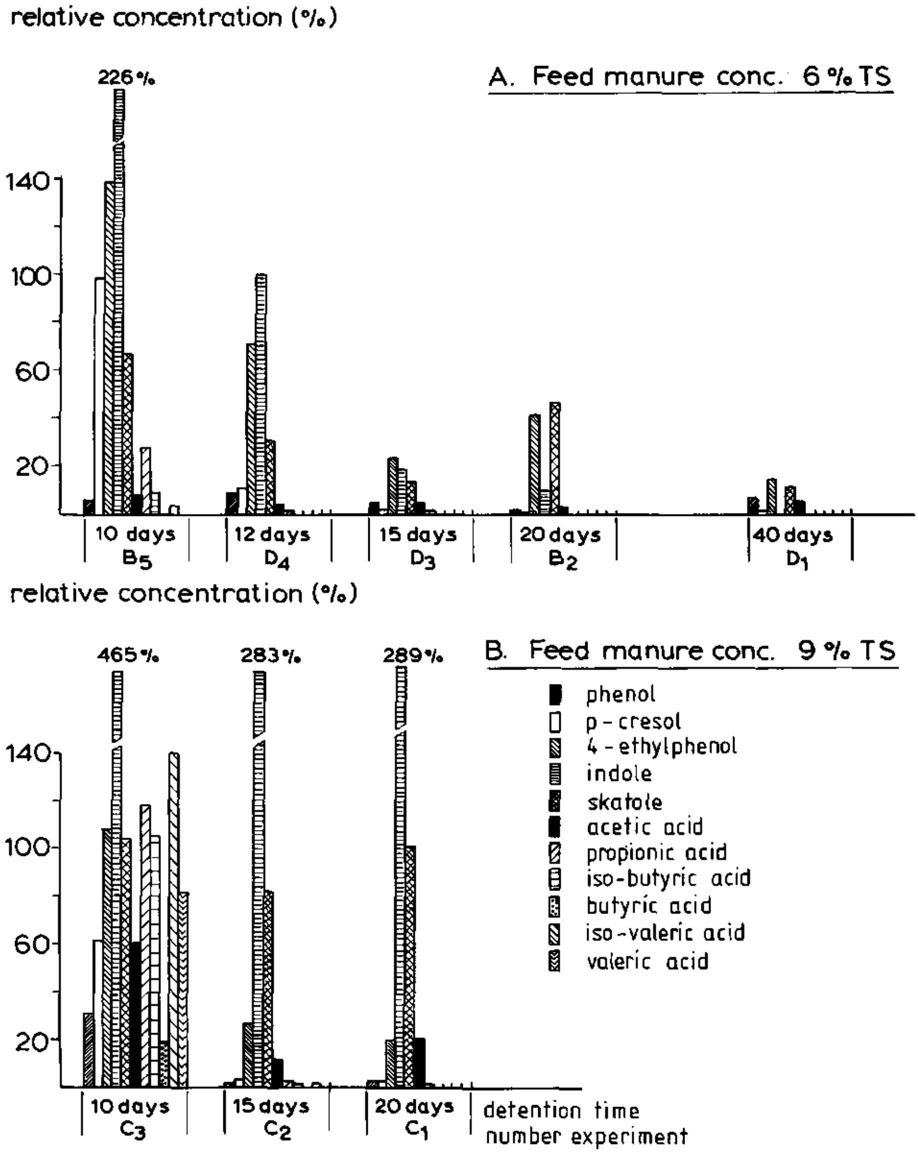

Fig. 4.2 Concentration of malodorous compounds present in digested piggery waste. The concentration has been expressed in relation to the concentration in raw manure (Table 4.2).

from an experimental digestion unit treating 6% TS piggery waste at a 15-days detention time at 30°C. The analytical results, shown in Table 4.8, reveal that upon storage the concentration of the malodorous compounds decreases further.

Table 4.8 Effect of the temperature on the concentration of malodorous compounds (in mg l^{-1}) during storage of digested piggery waste. Digestion conditions: temperature 30°C; detention time 15 days; manure concentration 6% TS.

	digested manure	storage at 4°C			storage at 20°C		
storage time (months)		1	2	3	1	2	3
acetic acid	1300	180	220	140	22	49	9
propionic acid	105	14	23	19	<1	<1	<1
iso-butyric acid	7	<1	3	2	<1	<1	<1
butyric acid	<1	<1	<1	<1	<1	<1	<1
iso-valeric acid	5	6	8	2	<1	<1	<1
valeric acid	<1	<1	<1	<1	<1	<1	<1
phenol	0.3	0.4	0.5	0.4	<0.1	<0.1	<0.1
p-cresol	1.9	0.2	0.2	0.2	<0.1	<0.1	<0.1
4-ethylphenol	5.2	0.9	0.9	0.9	1.7	0.3	<0.1
indole	0.7	0.2	<0.1	<0.1	<0.1	<0.1	<0.1
skatole	12.6	3.9	2.2	1.2	6.1	3.5	0.9

4.4 DISCUSSION

4.4.1 Process efficiency

The above results indicate that piggery waste can be digested successfully at loading rates from 1.6 - 6.4 kg TS m^{-3} day^{-1}, i.e. 1.1 - 4.4 kg VS m^{-3} day^{-1}. However, application of a space load of 9.5 kg TS m^{-3} day^{-1}, i.e. 6.5 kg VS m^{-3} day^{-1}, as in experiment C_3 results in process failure as reflected in the strong decrease of the CH_4-yield. These values of the applicable loading rate are in accordance with those reported in literature. Loehr (1969), in summarizing available information, reported that animal wastes could be successfully digested at loading rates in the range of 1.6 - 6.4 kg VS m^{-3} day^{-1}. More recently Hobson et al. (1979) and Fisher et al.

(1977) also obtained evidence that loading rates up to 6 kg VS m^{-3} day^{-1} can be applied at a temperature of 35°C*.

The COD reduction obtained in the pilot plant experiments varied from 40.3% at a 12-days detention time to 49% at a 40-days detention time, which is slightly lower than the COD reduction of 49-53% reported by Hobson et al. (1979) and of 54.6% reported by Gramms et al. (1971). The same applies for the reduction of the volatile solids. In the pilot plant experiment a VS reduction of only 27.8 - 37.6% was found, whereas Gramms et al. (1971), Hobson et al. (1979) and Kroeker et al. (1975) reported a VS reduction of 60.9%, 45% and 44%, respectively. These differences should be attributed to the different process conditions, such as pH, temperature and ammonia nitrogen concentration, as well as to differences in the manure composition, in the degree of mixing in the digester and in the analyses used. In the laboratory experiments the VS reduction varied from 21.3 - 31.9% under conditions of balanced digestion. Furthermore the results, shown in Tables 4.4 -4.7, indicate that the TS and VS reductions tend to decrease at decreasing detention times. However, due to difficulties in representative sampling and the errors implicitly coupled with the standard TS and VS analyses* only little if any value can be attached to the TS and VS reduction as parameters for describing animal waste efficiency.

At stable digestion the gas production varied from 0.200 - 0.280 m^3 of gas per kg TS added, corresponding to a CH_4-production of 0.146 - 0.207 m^3 kg^{-1} TS added. At similar process conditions Hobson et al. (1979) reported a methane production of 0.207 m^3 kg^{-1} TS added.

The methane production as related to the total solids fed to the digesters has been plotted versus the detention time in Fig. 4.3. From Fig. 4.3 it appears that at detention times exceeding 15 days there is only a slight increase in the methane production - and consequently also in the degree of stabilization of the waste - at piggery waste concentrations of 60 and 90 g TS l^{-1}. On the other hand the methane production decreases sharply at detention times shorter than 15 days, especially in the experiments with 90 g TS l^{-1} manure. The far less sharp decrease in the methane production in the experiments with 60 g TS l^{-1} manure indicates that this manure concentration provides more favourable environmental conditions than the 90 g TS l^{-1} manure.

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* When expressing the digester load in terms of TS or VS one should keep in mind that upon storage of wet manure part of the solid organic material is converted into volatile compounds such as VFA and ammonia nitrogen. As the greater part of these volatile compounds evaporate during the standard TS and VS analyses, the duration of the storage period seemingly affects the TS and VS loading rate of an anaerobic digester. Hence the use of COD-values should be preferred over TS and VS values for expressing the digester loading rate. The present study reveals that loading rates up to 8 kg COD m^{-3} day^{-1} can be applied.

4.4.2 Reduction of malodorous compounds

According to Schaefer et al. (1974) the main compounds, responsible for the objectionable piggery waste odour are compounds such as VFA, phenol, *p*-cresol, 4-ethylphenol, indole and skatole. These compounds are formed through microbiological activity in stored wet piggery wastes (Spoelstra, 1978). However, under the anaerobic conditions prevailing in methane digesters these compounds are effectively eliminated (Fig. 4.2). The experimental results, indicating a considerable reduction of the odorous compounds and hence of the manure malodours, are in accordance with the observations of Hobson et al. (1979) and Welsh (1977) who reported that anaerobic digestion substantially reduces the piggery waste odours.

The experimental results summarized in Fig. 4.2 indicate

1. that the elimination of malodorous compounds increases at increasing detention times,
2. that the VFA are eliminated faster than the aromatic compounds and
3. that a better elimination, especially of indole and skatole, is obtained if the TS levels in the digester feed are lower.

In the experiments with 40 g TS l^{-1} manure a decrease of the detention time from 15 to 10 days results in an increase in the VFA concentration, especially of propionic acid. Unfortunately the concentration of the aromatic malodorous compounds has not been determined in these experiments.

With the 6% TS piggery waste the VFA concentration in the pilot plant experiments was 3.5 - 4.2 meq l^{-1} at all detention times investigated, viz. 40, 20, 15 and 12 days. In the laboratory experiments the VFA concentration increased from 3.1 meq l^{-1} at a 20 days detention time to 23.4 meq l^{-1} at a 10-days detention time. In the latter experiment the increase in the VFA concentration again mainly concerns propionic acid. Concerning the aromatic compounds phenol is almost completely eliminated at a 10-days detention time, but the reduction of the *p*-cresol concentration was only 2% at a 10-days detention time, whereas it increased to 98% at a 12-days detention time. The explanation for this remarkably better elimination at a 12-days detention time may be (1) that the organisms responsible for the *p*-cresol degradation have - under these conditions - a generation time between 10 and 12 days and/or (2) that at a 12-days detention time all easily degradable constituents of the manure feed have been reduced to a low concentration, so that the metabolization of less easily degradable substrates, such as aromatic compounds, is stimulated. At a 10-days detention time the indole concentration increased with a factor of 2.66 relative to its concentration in the raw manure, indicating that the indole formation surpasses its elimination rate. However, increasing the detention time to 15 days results in an effective indole elimination of 76%. In summarizing the results of the experiments with 60 g TS l^{-1} piggery waste it appears that the elimination of odorous compounds markedly improves by increasing the detention time in the range 10 - 15 days. As a further reduction of these compounds is not manifest at detention times beyond 15 days (Fig. 4.2), the optimum detention time with respect to the reduction of the manure odour is approx. 15 days. These results are in accordance with those of Welsh et al. (1977) who performed olfactory studies on undigested and digested pig manure and concluded that the most effective odour reduction through anaerobic digestion occurs at or beyond a 12-days detention time.

In the experiments with 90 g TS l^{-1} manure the VFA concentration was approx. 18 meq. l^{-1} at detention times of both 20 and 15 days. However, at a 10-days detention time there was no reduction in the VFA concentration; on the contrary, the propionic acid concentration in the digestion liquid was even higher than in the manure feed. Contrary to the effective elimination of the simple phenols (phenol, *p*-cresol, 4-ethylphenol) at detention times of 15 and 20 days, no elimination of indole and skatole occurred in the experiments with 90 g TS l^{-1} manure, even not at a 20-days detention time. These poor results should be attributed to the fact that too high space loads were applied and consequently only an acceptable elimination can be achieved of the easily degradable organic compounds. These results indicate that the digestion of piggery waste at high TS concentrations should be dissuaded when a reduction of the manure odours is the prime object of the process application.

4.4.3 Process balance

As volatile fatty acids are important intermediates in the conversion of complex organic matter to methane and carbon dioxide, the VFA concentration is an appropiate indicator for process imbalance (Kroeker et al., 1975; McCarty, 1964).

The VFA concentration in the pilot plant experiments was 3.5 - 4.2 meq l^{-1}, whereas the VFA consisted exclusively of acetic acid. In the laboratory experiments the VFA concentration at stable digestion conditions was in the range 3.1 - 38.6 meq l^{-1}. Here, at a 20-days detention time acetic acid was the mere volatile fatty acid present, whereas at shorter detention times the increase of the VFA concentration mainly resulted from an increase in the propionic acid concentration.

With respect to the process recovery after the temporary upset, shown in Fig. 4.1, we found that considerably more time was involved in the recovery of the propionic acid degradation than of the other VFA. Hobson et al. (1974) also observed that at failing digestion conditions propionic acid was the predominant VFA. From all these observations it appears that propionic acid is a critical factor in anaerobic digestion of piggery wastes and that the course of the propionic acid concentration is a more useful indicator for process imbalance than the total VFA concentration.

As a result of the high ammonia nitrogen concentration occurring in digested piggery waste the system has a high buffer capacity; a dramatic pH-drop below the critical value of pH 6 hardly can occur. The digestion of piggery waste, therefore, is extremely stable with respect to the pH (Kroeker et al., 1976). Even in case of severe process failure as occurred in the experiment with 9% TS manure at a 10-days detention time the pH remained 7.55 in spite of the 200 meq l^{-1} VFA present in the mixed liquor.

4.4.4 Process rate limiting step

From the point of view of process optimalization it is important to get insight in the course of the separate biochemical steps of the digestion process. Therefore, the experimental results have been evaluated for the degree of hydrolysis, acid formation and methane formation according to the method described in Section 2.5.

The results of the calculations are shown graphically in relation to the organic load in Fig. 4.5 A for the pilot plant experiments with 6% TS manure and in Fig. 4.5 B for the laboratory experiments with 9% TS manure.

The results of the pilot plant experiments indicate a slight decrease in the rate of the distinguished steps at decreasing the detention time from 40 to 12 days. From this it can be concluded that the first step in the anaerobic conversion, i.e. the hydrolysis of undissolved organic material, is the rate limiting step in the overall process. This is in accordance with ideas of Maki (1954) and Hobson et al. (1974).

In the experiments with 90 g TS l^{-1} manure hydrolysis once again is the rate limiting step when the detention time is decreased from 20 to 15 days.

However, in decreasing the detention time from 15 days (organic loading rate 6.4 kg TS m^{-3} day^{-1}) to 10 days (organic loading rate 9.5 kg TS m^{-3} day^{-1}) the rate of methanogenesis drops off much stronger than that of hydrolysis and acid formation, indicating that methane formation is the rate limiting step under these circumstances. Acid formation was never found to be rate limiting in anaerobic digestion of piggery waste.

4.4.5 Practical aspects

Considering the practical application of anaerobic digestion for the stabilization of piggery waste, the most important requirements to achieve are:

1. a satisfactory reduction of the objectionable manure odours and
2. optimum process economics.

Optimum process economics is obtained when a high space load is combined with a maximum gas production. From the results presented in this Chapter it appears that a balanced digestion can be maintained at a loading rate of 6.4 kg TS m^{-3} day^{-1}, either by digesting 9% TS manure at a 15-days detention time (CH_4 production 0.180 m^3 kg^{-1} TS added) or by digesting 6% TS

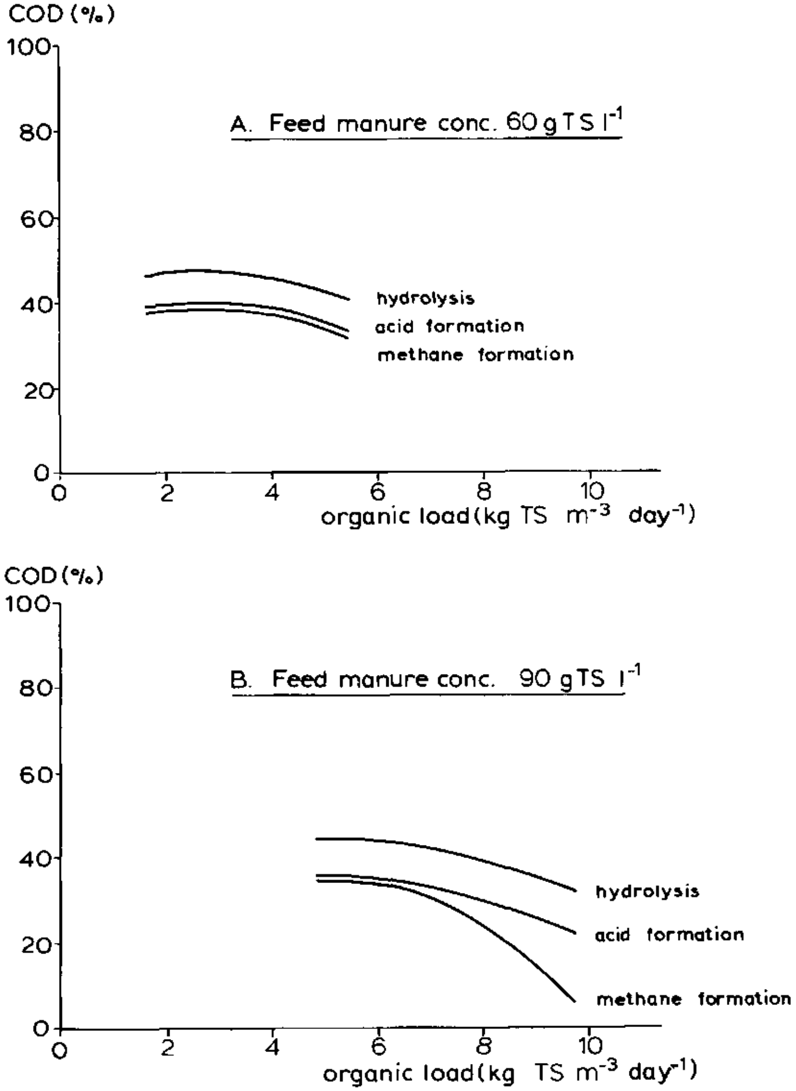

Fig. 4.5 Influence of the organic loading rate upon hydrolysis, acid formation and methane formation.

manure at a 10-days detention time (CH_4 production 0.166 m^3 kg^{-1} TS added). However, at a loading rate of 6.4 kg TS m^{-3} day^{-1} the reduction of malodorous compounds is much less satisfactory. In order to accomplish a situation in which both requirements are met, the optimum loading rate should be approx. 4 kg TS m^{-3} day^{-1}, provided this loading rate is obtained by digesting 6% TS piggery waste at a 15-days detention time. The methane production will be about 0.2 m^3 kg^{-1} TS added in that case.

5 INFLUENCE OF TEMPERATURE

5.1 INTRODUCTION

The potentials of anaerobic digestion to recover energy from organic matter largely depend on the methane production rate. However, apart from the production of methane gas, the digestion process consumes energy as well, viz. for pumping, mixing and generally also for heating the manure and maintaining the digester at the desired temperature. In temporate climates like in Western Europe the greater part of the energy consumption is used for heating purposes (Mills, 1979).

As in temporate climate areas the energy requirements of the process chiefly depend on the applied digestion temperature, knowledge of the influence of temperature on the gas production is indispensable for determining the optimum process temperature with respect to the net energy recovery.

Anaerobic digestion of animal wastes can be conducted at mesophilic temperatures (20 - 40°C) and at thermophilic temperatures (40 - 65°C). Although thermophilic digestion of manure may have benefits over mesophilic digestion, such as higher rates of digestion, greater conversion of waste organics to gas, faster solid-liquid separation and minimization of bacterial and viral pathogens, the mesophilic temperature range should be preferred for animal waste digestion at farm-scale application because of practical reasons.

For digestion in the mesophilic range commonly a temperature of 33 - 35°C is used. This temperature range originates from the practice of sewage sludge digestion. However, the optimization of the sewage sludge digestion process has been primarily directed to a maximum stabilization of the sludge, and therefore to a maximum gas production, rather than to a maximum net energy recovery.

The studies were performed to investigate the effects of temperature on the digestion of wet piggery manure. The main objectives of the study were

1. to assess the digestion temperature at which the maximum gas yield is obtained and
2. to collect sufficient information for optimizing the net energy recovery in the mesophilic temperature range (20 - 40°C).

5.2 PROCEDURES

The influence of temperature on the digestion of wet piggery manure was investigated in three series of experiments, designated as series A, B and C. In each series three different temperatures were tested. Table 5.1 shows the temperatures investigated together with the loads applied.

Table 5.1 Experimental scheme.

	Series of experiments		
	A	B	C
Load (kg TS m^{-3} day^{-1})	3.3	4.2	3.6
Detention time (days)	22.5	15	20
Temperatures investigated (°C)	20	27	13
	25	32	35
	30	40	55 (in duplicate)

The experiments of series A and B were performed in three 45-litre laboratory digesters (Section 2.3.2), whereas the experiments of series C were conducted in four 5-litre laboratory digesters equipped with a water jacket (Section 2.3.2).

All digestion units were seeded with well-digested sludge obtained from experimental digestion units fed with 6% TS piggery waste and operated at 30°C. The VFA concentration of the seed material was less than 200 mg l^{-1}. After seeding the digester temperatures were adjusted immediately to the desired value. The digesters were fed daily or bidaily (viz. in the weekends) by discharging a fixed amount of the digester contents. Then the same volume of fresh manure was added.

As the three series of experiments were not conducted simultaneously the piggery waste used in the various series differed with respect to the origin and the composition. The chemical manure characteristics are summarized in Table 5.2.

The manure used in series A originated from an experimental pig housing unit. The pigs were fed here ad libitum so that the manure may contain some spilled feed rests. The series B experiments were conducted with manure from a pig-fattening farm and those of series C with manure from an experimental farm where the manure solids and the manure liquid were discharged separately. Both fractions were combined in appropriate proportions before supplying it to the digester. The manure in this case partly consisted of saw dust and other litter material. The experiments were continued for a period of at least 25 days after the digestion process had reached a steady state with respect to the gas production and the VFA concentration.

Table 5.2 Chemical characteristics of piggery wastes used in the experiments on digestion temperature.

	Exp. A	Exp. B	Exp. C
pH	7.1	7.2	6.9
TS (g l^{-1})	74.0	63.3	71.6
VS (g l^{-1})	56.2	42.5	55.1
Ammonia nitrogen (mg N l^{-1})	2320	1840	1730
Total nitrogen (mg N l^{-1})	3850	n.d.	3810
COD supernatant (mg l^{-1})	22800	17500	16000
COD total (mg l^{-1})	67500	64500	65000
COD of the VFA (mg l^{-1})	15900	9600	10250
VFA (meq l^{-1})	176.2	107.2	115

5.3 RESULTS

The experimental results of series A, B and C are summarized in Tables 5.3, 5.4 and 5.5, respectively. The data presented in these Tables concern the average values of the results during the last 25 days of the experiments when the digestion process had reached a steady state. In Tables 5.3 - 5.5 the values of TS, VS and COD total are not presented as they are inadequate due to the incomplete mixing of the digester contents.

The gas production obtained in the reference experiments conducted at approx. 30°C of each series differed considerably from each other, which should be mainly attributed to the use of manure of different origin and composition. Therefore, but also because of the application of different loading rates in the three series (between 3.3 and 4.2 kg TS m^{-3} day^{-1}) the interpretation of the results should be primarily related to the data obtained within each separate series.

The results in Table 5.5 indicate that methane production is nihil at a process temperature of 13°C under the conditions applied. This can not be attributed to a lack of adequate substrates for the methane-forming bacteria because of the high VFA concentration in the digester mixed liquor. The VFA concentration even increased from 115 meq l^{-1} in the feed to 181.7 meq l^{-1} after being exposed to 13°C in the digestion experiment.

However, at 20°C an active methane fermentation takes place; this results in a sharp decrease in the VFA concentration of the digester contents relative to that of the fresh manure. The results presented in Table 5.3 also reveal that the activity of the bacterial sludge is considerably higher at 25°C (CH_4 production 0.246 m^3 kg^{-1} TS added) than at 20°C (CH_4 production 0.172 m^3 kg^{-1} TS added). However, an increase of the temperature from 25°C to 30°C does not further improve the methane production significantly, viz. only from 0.246 m^3 kg^{-1} TS added at 25°C to 0.262 m^3 kg^{-1} TS added at 30°C.

Table 5.3 Experimental results of series A. (Piggery waste concentration 74 g TS l^{-1}; detention time 22.5 days; load 3.3 kg TS m^{-3} day^{-1}).

	Exp. A_1	Exp. A_2	Exp. A_3
Working digester volume (litres)	45	45	45
Duration experiment (days)	95	95	95
Temperature (^oC)	20	25	30
Gas			
Gas production (l gas l_r^{-1} day^{-1})	0.795	1.155	1.248
(m^3 gas kg^{-1}TS added)	0.242	0.351	0.379
Gas composition (% CH_4)	71.1	70.1	69.0
Methane production (m^3 kg^{-1}TS added)	0.172	0.246	0.262
COD removed via gas (g COD l^{-1}manure)	32.3	46.3	49.3
Digester effluent			
pH	7.4	7.55	7.6
Ammonia nitrogen (mg N l^{-1})	2870	2990	2990
Total nitrogen (mg N l^{-1})	3950	3680	3650
COD supernatant (mg l^{-1})	7000	7200	5200
VFA (meq l^{-1})	18.9	3.2	2.7
Acetic acid (meq l^{-1})	14.7	3.2	2.6
Propionic acid (meq l^{-1})	3.8	0	0.1
COD total reduction (%)	54.8	78.6	83.5
COD_s reduction (%)	69.7	68.8	77.6
VFA reduction (%)	90.3	98.4	98.6

Table 5.4 Experimental results of series B. (Piggery waste concentration 63.3 g TS l^{-1}; *detention time* 15 days; load 4.2 kg TS m^{-3} day^{-1}).

	Exp. B_1	Exp. B_2	Exp. B_3
Working digester volume (litres)	45	45	45
Duration experiment (days)	90	90	90
Temperature (^oC)	27	32	40
Gas			
Gas production (l gas l_r^{-1} day)	0.989	1.076	1.129
(m^3 gas kg^{-1}TS added)	0.234	0.255	0.268
Gas composition (% CH_4)	74.0	72.8	73.5
Methane production (m^3 kg^{-1}TS added)	0.173	0.186	0.197
COD removed via gas (g COD l^{-1}manure)	27.9	29.9	31.6
Digester effluent			
pH	7.6	7.65	7.7
Ammonia nitrogen (mg N l^{-1})	2330	2255	2275
Total nitrogen (mg N l^{-1})	n.d.	n.d.	n.d.
COD supernatant (mg l^{-1})	7230	5760	7930
VFA (meq l^{-1})	3.4	1.1	6.5
Acetic acid (meq l^{-1})	3.1	1.1	4.8
Propionic acid (meq l^{-1})	0.3	0	1.5
COD total reduction (%)	43.4	46.4	49.2
COD_s reduction (%)	58.6	67.1	54.6
VFA reduction (%)	96.8	99.0	93.9

Correspondingly the results of series B (Table 5.4) show that the methane production increases only slightly and almost linearly with the digestion temperature in the range from 27 to 40°C. The results of the separate experiments within this series are very similar except that the VFA concentration and the COD of the supernatant solution in the 32°C experiment are clearly lower than that in the 27°C and 40°C experiments. From the experiments in the mesophilic temperature range the reduction of malodorous compounds was only determined at 20°C, 25°C and 30°C (series A). The analytical results, presented in Table 5.6, show that there is an evident reduction of these compounds at increasing temperatures.

The digestion process at 55°C was investigated in two parallel experiments, which showed very similar results (Table 5.5). A raise in temperature from 35°C to 55°C adversely affects the gas production because the methane production at 55°C, viz. 0.190 m^3 CH_4 kg^{-1} TS added, is only 75% of that at 35°C (0.249 m^3 CH_4 kg^{-1} TS added). Furthermore, the methane content of the gas produced under thermophilic conditions is somewhat lower.

Table 5.5 Experimental results of series C. (Piggery waste concentration 71.6 g TS l^{-1}; detention time 20 days; load 3.6 kg TS m^{-3} day^{-1}).

	Exp. C_1	Exp. C_2	Exp. C_3 (in duplicate)	
Working digester volume (litres)	5	5	5	5
Duration experiment (days)	73	73	80	80
Temperature (°C)	13	35	55	55
Gas				
Gas production (l gas l_r^{-1} day^{-1})	0	1.382	1.134	1.195
(m^3 gas kg^{-1} TS added)	0	0.384	0.315	0.332
Gas composition (% CH_4)	-	65.0	58.5	59.0
Methane production (m^3 kg^{-1} TS added)	0	0.249	0.184	0.196
COD removed via gas (g COD l^{-1} manure)	0	44.3	32.7	34.7
Digester effluent				
pH	6.7	7.4	7.8	7.7
Ammonia nitrogen (mg N l^{-1})	1820	2061	→2430	→2400
Total nitrogen (mg N l^{-1})	3790	3643	3740	3450
COD supernatant (mg l^{-1})	20600	5630	18100	17200
VFA (meq l^{-1})	181.7	1.6	84.5	81.0
Acetic acid (meq l^{-1})	113.1	1.5	19.7	18.9
Propionic acid (meq l^{-1})	38.1	0.1	41.6	36.4
COD total reduction (%)	-	68.1	50.3	53.3
COD_s reduction (%)	-28	64.9	-12.9	-7.1
VFA reduction (%)	-58	98.6	26.5	29.6

→ increasing to

In additional experiments various efforts were made to initiate thermophilic digestion at 55°C of piggery waste having an ammonia nitrogen concentration of 3000 mg l^{-1}. However, in no case methane production did start under these circumstances. As it was believed that the main reason for the poor results of the latter experiments was the stronger inhibitory action

Table 5.6 The concentration of aromatic malodorous compounds (in mg l^{-1}) in raw manure and after digestion at 20°C, 25°C and 30°C in experimental series A (Detention time 20 days; load 3.3 kg TS m^{-3} day^{-1}).

	Fresh manure	Digested manure		
		20°C	25°C	30°C
phenol	24.6	2.0	1.1	1.1
p-cresol	103.3	27.9	4.3	0.8
4-ethylphenol	25.4	3.5	1.5	1.0
indole	0.9	0.5	0.3	0.2
skatole	8.7	3.8	5.2	3.4

of ammonia nitrogen on the digestion process under thermophilic than under mesophilic conditions, two additional experiments were performed with the intention to get more information about the influence of ammonia nitrogen on thermophilic digestion.

1. After finishing series C one of the 55°C-digesters was continuously operated at 55°C and fed with 6% TS piggery waste at a 30-days detention time (reference digester).
2. The other 55°C-digester of series C was operated similarly as the reference digester except that 5.1 g urea per litre piggery waste was added in order to increase the ammonia nitrogen concentration in the digester contents to approx. 3800 mg l^{-1}.

The results of the additional thermophilic experiments are presented in Table 5.7. They indicate that gas production almost completely ceases at an ammonia nitrogen concentration of 3800 mg l^{-1} and does not recuperate within the experimental period of 50 days. The methane production in the reference digester is very similar to that obtained in the 55°C-experiments of series C (Table 5.5).

5.4 DISCUSSION

The most relevant digestion characteristic for evaluating the influence of temperature on anaerobic digestion is the methane production, especially when energy recovery is the main objective for applying the digestion process. However, the methane production only represents the final degree of conversion and does not necessarily reveal the rate limiting step in the process. To gain a better insight in the course of the digestion process, the fraction of organic material converted in the three separate steps,

Table 5.7 Thermophilic digestion at various ammonia nitrogen concentrations (temperature 55°C; detention time 30 days; manure concentration 65 g TS l^{-1}; load 2.2 kg TS m^{-3} day^{-1}).

	Manure	Reference	Ureum added
Duration of experiment (days)		50	50
Gas			
Gas production (m^3 gas kg^{-1} TS added)		0.276	0.038
Gas composition (% CH_4)		63	39
Methane production (m^3 kg^{-1} TS added)		0.174	0.015
Digester effluent			
pH	7.2	7.9	7.7
Ammonia nitrogen	1200	1500	3800
COD supernatant (mg l^{-1})	8000	n.d.	n.d.
COD of the VFA (mg l^{-1})	3870	5380	12900
VFA (meq l^{-1})	46.6	50.3	164.4
Acetic acid (meq l^{-1})	33.6	22.8	133
Propionic acid (meq l^{-1})	7.6	16.2	17.5

viz. hydrolysis, acid formation and methane formation, has been calculated according to the method explained in Section 2.5. The results of these calculations have been plotted versus the temperature in Fig. 5.1 for the experimental series A and B and are presented in Table 5.8 for series C.

Table 5.8 *Influence* of temperature on the degree of hydrolysis, acid formation and methane formation (in % of the manure-COD) in experimental series C.

	Manure	13°C	35°C	55°C
Hydrolysis	24.6	31.8	76.1	78.5
Acid formation	15.8	25.7	67.7	67.2
Methane formation	0	0	67.5	51.5

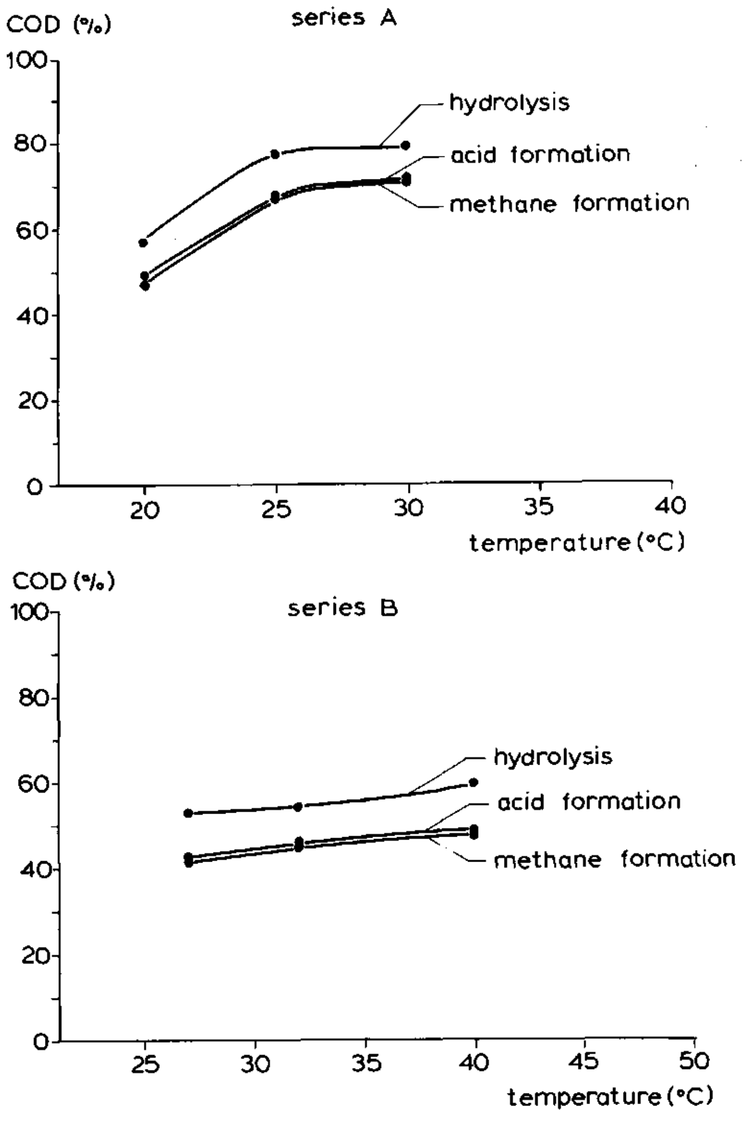

Fig. 5.1 Influence of temperature in the mesophilic range (20°C to 40°C) on the degree of hydrolysis, acid formation and methane formation.

5.4.1 Digestion at 13°C

Literature data (Hatfield et al., 1928; Rudolfs, 1927; Imhoff, 1936) concerning the digestion of solid wastes such as sewage sludge indicate that methane production still takes place at temperatures of approx. 10°C though at a very low rate. Contrary to these reports no gas is produced in the present 13°C-experiment under the process conditions applied. The absence of methane production in this experiment may indicate that environmental factors such as the high ammonia nitrogen concentration and the high pH levels in piggery manure together with the low temperature create conditions which are unfavourable for methanogenic organisms.

Contrary to the results of experiments conducted in our laboratory indicating that methane-forming organisms accomodate well to changes in temperature between 10 and 45°C (Lettinga, 1979) the gas production in a piggery manure digester evidently ceases at a fall in temperature from 30 to 13°C.

Heukelekian et al. (1948) and Speece and Kem (1970) also reported that, in the digestion of sewage sludge, the gas production did not continue immediately after a sudden decrease in temperature in this range.

The cessation of methane production in combination with the application of a 20-days detention time immediately after the start of the experiments may have caused a wash-out of methanogenic bacteria. Therefore, better results may be expected when more time is taken to acclimate the bacterial population to the change in temperature.

The results in Table 5.8 also indicate that hydrolysis is very incomplete at 13°C. This may be attributed (1) to the low activity of the hydrolytic enzyme complexes at this temperature and/or (2) to an inhibition of hydrolysis by the endproducts of the acid formation, such as VFA and hydrogen, which accumulate in the digester contents.

5.4.2 Digestion at mesophilic temperatures (20 - 40°C)

As appears from Fig. 5.1 all three separate steps of the digestion process (hydrolysis, acid formation and methane formation) are affected to about the same extent by the temperature in the range 20 - 40°C. Hence it can be concluded that changes in the methane production at the different temperatures investigated should be attributed mainly to changes in the rate of hydrolysis (liquefaction of undissolved material). Evidently the methane production in the underlying experiments reflects in fact the influence of temperature on the rate of hydrolysis. The fact that hydrolysis is the rate limiting step in the digestion of piggery waste at a temperature of 30°C was already observed in the experiments described in Chapter 4. These observations are in accordance with those of Pfeffer (1974) who investigated the effect of temperature on the digestion of shredded domestic refuse.

Fig. 5.1 further reveals that the rate of hydrolysis strongly increases when the temperature is raised from 20 to 25°C. The combined results of series A and B indicate that the rate of hydrolysis - and therefore the methane production - increases almost linearly with temperature in the range

25 - 40°C, although the average rate of increase in the gas production is only about 1% per °C in this temperature range!.

Fair and Moore (1934), in summarizing the results of batch experiments concerning the influence of temperature on the digestion of sewage solids, also approximated the relation between the relative rate of digestion and temperature in the range 25 - 40°C by a linear relationship and concluded that in fact little can be gained by raising the digestion temperature above 25°C. Similarly Golueke (1958) reported a linear relationship between temperature (30 - 40°C) and gas production at the digestion of raw sewage sludge at a load of 1.45 kg VS m^{-3} day^{-1}.

In assessing the effect of the temperature on the digestion process, important factors to be considered are the detention time and the ammonia nitrogen concentration. At a shorter, and therefore more critical detention time the differences in methane production at different temperatures presumably are more pronounced. Hobson et al. (1979) conducted experiments with pig slurry at a 10-days detention time and reported an increase in the gas production from 0.30 m^3 kg^{-1} TS added at 30°C to 0.42 m^3 kg^{-1} TS added at 40°C which corresponds to an average increase in the gas production of 4% per °C. The ammonia nitrogen concentration of the digester contents may also affect the relation between the temperature and the digestion, because an increase in the digestion temperature is, as we have seen before, accompanied with an increase in the concentration of undissociated ammonia. In our experiments (series B) the undissociated ammonia nitrogen concentration, as calculated by the equation of Section 2.5.2, increases from 59.1 mg l^{-1} at 27°C to 165.6 mg l^{-1} at 40°C.

5.4.3 Digestion at 55°C

The increase of the digestion temperature from 35°C to 55°C resulted in a decrease in the gas production of approx. 25%, despite a slight increase in the degree of hydrolysis (Table 5.8). This has to be attributed to an improved conversion of carbohydrates and nitrogenous substances such as proteins. Table 5.9 which contains a rough estimate of the relative breakdown of carbohydrates at digestion temperatures of 13, 35 and 55°C, indicates that the conversion of both cellulose and hemicellulose increases at raising temperatures.

The breakdown of nitrogenous components can be estimated from the ammonia nitrogen concentration before and after digestion. These analyses give only an impression of the overall process because apart from the release of ammonia nitrogen during the breakdown of nitrogenous substances, part of the ammonia nitrogen is incorporated in new cell material. Furthermore, some ammonia nitrogen may have been lost from the digestion liquor with the gas. The ammonia nitrogen concentration in the experimental series C in-

Table 5.9 Rough estimate of the reduction of hemicellulose and cellulose as observed in experimental series C (in % of the concentration in the manure). Lignin is used as an internal standard.

	13°C	35°C	55°C
Hemicellulose	38	43	53
Cellulose	34	39	52

creased significantly with increasing temperature, i.e. from 1820 mg l^{-1} at 13°C to 2400 mg l^{-1} at 55°C , which points to an increased conversion of nitrogenous components at 55°C. Both the higher VFA concentration and supernatant COD at thermophilic digestion as compared to mesophilic digestion has also been observed by Buhr et al. (1977), Golueke (1958), Garber et al. (1975) and Pohland and Bloodgood (1963). Apparently the observed decrease in the gas production in the underlying experiments is due to both a less complete acid and methane formation.

Earlier investigations on the effect of temperature on anaerobic digestion of wastes gave rather conflicting results. Heukelekian (1930) and Fair and Moore (1932) found in batch experiments with sewage sludge both a higher gas production and gas production rate at increasing temperatures up to 60°C, whereas in similar experiments Maly and Fadrus (1971) found that this only holds for the gas production rate. Continuous experiments at different temperatures were conducted with municipal sewage sludge (Golueke, 1958; Pohland et al., 1963; Malina, 1961; Garber et al., 1975), shredded domestic refuse (Pfeffer, 1974) and dairy manure (Converse et al., 1977). Garber et al. (1975) and Pfeffer (1974) reported an increase in the gas production when increasing the temperature from the mesophilic to the thermophilic range. On the other hand the reports of Golueke (1958), Pohland and Bloodgood (1963), Malina (1961) and Converse et al. (1977) showed a similar or somewhat lower gas production at thermophilic as compared with mesophilic temperatures. These discrepancies in the reports may be caused either by differences in the adaptation procedures applied or by the influence of some yet unidentified inhibitory agent for thermophilic methanogenic organisms.

Anyhow, the results obtained in the additional experiment conducted in the present study, where the ammonia nitrogen concentration was increased from 1500 mg l^{-1} to 3800 mg l^{-1}, clearly indicate that ammonia nitrogen severely inhibits the methane formation under thermophilic conditions. In fact such an inhibition also occurs under mesophilic conditions but the experimental results presented in Chapter 3 have shown that the digestion process can adapt in that case to ammonia nitrogen concentrations up to 5000 mg l^{-1}. Under these conditions the concentration of undissociated ammonia nitrogen is approx. 300 mg l^{-1}. The concentration of undissociated ammonia nitrogen in the 55°C-experiment at 3800 mg l^{-1}, however, is 619 mg l^{-1}. As it is presumed that undissociated ammonia is the inhibitory agent for methanogenesis, it is clear that the effect of ammonia nitrogen is considerably greater under thermophilic than under mesophilic conditions.

5.4.4 Determination of the optimum digestion temperature

The experimental results reveal that the mesophilic temperature range is most adequate for the digestion of wet piggery manure. At a submaximum load of about 4 kg TS m^{-3} day^{-1}, which is imperative when the abatement of odour nuisance is pursued (Chapter 4), the gas production increases almost linearly with temperature in the range 25 - 40°C with about 1% per °C.

Starting from the results of experimental series B a raise in temperature from 27°C to 40°C causes an increase in the methane production from 0.024 m^3 kg^{-1} TS (Table 5.4) which corresponds to an increase in energy yield of 0.865 MJ kg^{-1} TS. However, considering that heating of 6% TS manure feed from 27°C to 40°C theoretically requires 0.91 MJ kg^{-1} TS, the extra methane production is not sufficient to heat the manure from 27°C to 40°C, even not at a 100% efficiency of the energy conversion.

The energy balance might be more positive when more concentrated manure is digested. However, the gas production per kg TS levels off at increasing TS concentrations beyond 6% TS (Chapter 4). Therefore, when the heat requirements of a digester have to be supplied exclusively with high-grade fuel, e.g. with part of the biogas produced, the optimum temperature with respect to net energy recovery is at the lower temperature range of mesophilic digestion, i.e. between 25°C and 30°C. This confirms recent finding of Stevens et al. (1979) mentioning that low temperature (approx. 25°C) digestion is technically feasible for a swine manure management and energy producing strategy. Moreover, as can be deduced from our results an additional benefit of piggery waste digestion at low temperatures is the decrease in the concentration of undissociated ammonia in the lower mesophilic temperatures range making the process less sensible to high ammonia nitrogen concentrations. However, since the reduction of malodorous compounds improves at higher temperatures in the range 20 - 30°C (Table 5.6), a digestion temperature of approx. 30°C should be preferred if the abatement of odour nuisance is pursued as well.

When sufficient waste energy, f.e. cooling water of a gas motor/generator set, is available the optimum process temperature for energy recovery is at the upper part of the mesophilic range, i.e. approx. 40°C, because then the maximum conversion to methane is achieved.

6 GENERAL DISCUSSION

The main objectives of the application of anaerobic digestion of complex, chiefly solid substrates are energy recovery and waste stabilization. Additional benefits to be achieved in the application of the process include the conservation of the fertilizer value and the reduction of the TS content of the waste. The economical viability of the anaerobic digestion process is dependent upon factors such as the gas production, the total costs of the process (investment costs, interest, running costs and costs of the biomass), the prevailing retail prices for energy, the local availability of energy and the efficiency of the gas utilization. Moreover, other potential achievements of digestion, such as the malodour reduction, the reduction of pathogens and the value of the digested residues as a fertilizer may improve the feasibility of the process in specific cases, especially when the compulsory application of other treatment processes, e.g. aerobic stabilization, can be saved.

In anaerobic digestion, biodegradable organic material is converted to methane and bacterial cells. In treating substrates consisting mainly of easily biodegradable compounds anaerobic bacteria, grown on the substrate constitute main part of the digester solids. As a consequence these sludges will develop a high specific activity, which allows the application of high organic loading rates provided the sludge is sufficiently retained in the digester. This can be achieved by applying anaerobic treatment systems, such as the anaerobic filter (Young and McCarty, 1969) and the recently developed upflow anaerobic sludge blanket process (Lettinga et al., 1980). With both systems high loading rates can be applied with dissolved, but also with easily biodegradable solid substrates, e.g. even exceeding 30 kg COD m^{-3} day^{-1} (Lettinga et al., 1980).

However, many types of biomass and organic residues available for biological conversion to methane, e.g. crops, straw, manure, domestic refuse and sewage sludge, contain a relatively high fraction of poorly or nonbiodegradable components. The presence of refractory materials in the digester feed not only adversely affects the process efficiency, viz. the conversion degree and the TS reduction, but also reduces the specific activity of the sludge, due to the entrapment of biologically inert solids from the feedstock. As a consequence the maximum applicable organic loading rate with these feedstocks will be significantly lower than that with dissolved or easily biodegradable solid substrates. A conventional digester of the flow-through type is adequate to treat complex, partly biodegradable substrates. The digestion of these substrates as a rule is not completed within a period of 10 days, which can be regarded as the minimum sludge detention time under mesophilic conditions.

The major refractory component in these types of biomass is lignocellulosic material originating from plant cell walls. This material mainly consists of cellulose and hemicellulose incorporated in a lignic complex. As lignin is regarded as virtually undegradable by anaerobic processes (Hobson et al., 1974) and the cellulolytic enzymes cannot penetrate in the lignin matrix because of steric hindrance (de Wit, 1980), the incorporated polysaccharides are hardly available for biodegradation. As a consequence the digestibility of these substrates can only be improved to a greater or smaller extent by applying a proper pretreatment method, directed towards a disrupture of the lignin matrix. Such a method may consist of a physical, chemical, mechanical or combined treatment of the feedstock.

Gottschall et al. (1978) examined the effect of gamma-radiation on the biodegradability of domestic sewage sludge and reported that a radiation dose of approx. 0.4 Rad per hour with a cobalt 60 source for 24 hours increased the gas yield by approx. 50% provided the irradation was performed in the presence of excess of oxygen. Kelsey et al. (1980) investigated the effect of ball-milling and wet-milling with concurrent enzymatic hydrolysis on the saccharification of cellulosic materials, including 'pure' cellulose, newspaper, lignocellulose and wood. In all cases investigated a combined treatment of wet-milling with cellulase proved to be far more effective than ball-milling. For lignocellulose 93% could be saccharified by wet-milling with concurrent enzymatic attack for 24 hours, whereas ball-milled and untreated lignocellulose only yield a degree of saccharification of 30% and 9%, respectively. Chemical pretreatment methods, either with mineral acids or alkaline, have been reported to be effective in improving the biodegradability of straw (Ginnivan et al., 1977; Detroy et al., 1980), the fiber fraction of cattle manure (Nesse et al., 1977) and pig faeces (Ngian et al., 1977). Chemical treatment seems to be more effective at elevated temperatures. Studies on the thermochemical pretreatment of the fiber fraction of cattle manure (Nesse et al., 1977), domestic sewage sludge (Stuckey et al., 1978; Haug et al., 1978; McCarty et al., 1976), newspaper (McCarty et al., 1978), organic municipal refuse (Pfeffer et al., 1976) and pig faeces (de Wit, 1980) indicated that these methods effect a substantial increase of the biodegradability of these substrates, although the efficiency depends on the treatment temperature and on the type of chemical used. NaOH is the most effective chemical, both at ambient and at elevated temperatures. However, Stuckey et al. (1978) reported that thermochemical pretreatment causes the formation of toxic compounds and refractory nitrogen containing organic materials. This effect seems to be more pronounced as treatment conditions become more severe, e.g. at temperatures above 175°C.

Although the biodegradability of refractory materials can be improved by applying physical, chemical or mechanical pretreatments, it should be recognized that these contrivances are accompanied with the consumption of energy and/or chemicals.

An alternative, and potentially more attractive, pretreatment method might be the use of lignin-degrading organisms. A number of fungi, including actinomycetes, basidiomycetes and ascomycetes (Crawford et al., 1974; Ginnivan et al., 1977; de Wit, 1980; Dickinson and Pugh, 1974) and bacteria have been reported to be effective in the attack of lignified cellulose. Recently Akin (1980) isolated a facultative anaerobic micro-organism from rumen fluid, capable to attack lignified tissue in forage grasses under anaerobic conditions. However, thus far the application of biological pretreatment methods still is limited because the penetration of enzymes into the lignin-cellulose complex, and consequently the reaction rate, is slow. Nevertheless, in view of their potential importance biological methods have recently become one of the major areas of research.

6.1 BIODEGRADABILITY OF PIGGERY WASTE

The gas production from piggery wastes in well-established digestion units is in the range 0.24 - 0.49 m^3 kg^{-1} VS added. The gas yields obtained in the present investigation under various process conditions are summarized in Table 6.1 together with relevant literature data. Table 6.1 shows that the gas yields obtained in the present study are of the same order of magnitude as those reported in literature.

Table 6.1 Gas production from piggery wastes.

Temp. (^{o}C)	Detention time (days)	Organic load (kg VS m^{-3} day^{-1})	Gas production (m^3 kg^{-1} VS added)	Reference
35	10	1.4 - 4.5	0.43	Hobson et al. (1980)
35	15 - 30	1.05 - 2.1	0.62 - 0.82	Kroeker et al. (1975)
35	15	4.0	0.55	Fisher et al. (1977)
35	26.5	2.4	0.36 - 0.42	Haga et al. (1979)
33	10 - 15	1.92 - 3.85	0.26 - 0.45	Gramms et al. (1971)
35	10 - 50	0.32 - 3.20	0.49 - 0.64	Taiganides et al. (1963)
35	20	2.41 - 3.05	0.37 - 0.54	Jeffrey et al. (1964)
30	15	3.5	0.33 - 0.49	see chapter 3
32	12 - 40	1.1 - 3.6	0.31 - 0.37	see chapter 4
30	10 - 15	2.0 - 3.0	0.24 - 0.33	see chapter 4
30	10 - 20	2.3 - 4.5	0.28 - 0.39	see chapter 4
30	15 - 20	3.4 - 4.5	0.32 - 0.33	see chapter 4
20-30	22.5	2.5	0.32 - 0.50	see chapter 5
27-40	15	2.8	0.35 - 0.40	see chapter 5

However, considering the extreme values reported, the gas yield from piggery wastes varies in a wide range, viz. from 0.24 - 0.82 m^3 kg^{-1} VS added. This variation originates partly from differences in the process conditions, e.g. the digestion temperature and the organic and hydraulic loading rate. However, even under very similar process conditions the gas yields may differ considerably. This can be primarily attributed to differences in the composition and the origin of the feed manure. Important factors affecting the composition of animal slurries include the animal diet, the presence of spilled feed rests and of bedding materials, the manure collection and storage system applied, the storage temperature and the duration of the storage period. Moreover, it should be emphasized at this place, that the use of TS and VS values as parameters for describing the process efficiency is erratic because the standard methods applied in the measurement of these parameters frequently gives incorrect results, especially if the waste contains high concentrations of volatile compounds such as VFA, ammonia nitrogen and alcohols. These compounds are only partly included in the standard TS analysis and therefore the VS value does not represent the actual organic content of the waste. Both the standard TS and VS values have to be corrected for the ammonia nitrogen and VFA losses (Section 2.1.1). Doing so, the gas yield is 0.22 - 0.32 m^3 kg^{-1} VS added and the methane yield per kg VS destroyed is in the range 0.40 - 0.60 m^3 with an average value of about 0.54 m^3. From this it can be calculated that the COD:VS ratio of the converted organic material ranges from 0.99 - 1.47.

Fig. 6.1 shows the average fractional composition of raw piggery waste, in terms of the biodegradable COD-fraction (i.e. converted to methane and bacterial mass) and the refractory COD-fraction (dissolved and solid). The conversion of piggery waste-COD to methane varies from 30 to 80%, with a mean value of about 50%. Obviously, the biodegradable COD-fraction of piggery waste is slightly higher than this 50%, because part of the biodegradable constituents is converted to bacterial cells. Estimating the bacterial growth yield factor at 0.10 the biodegradability of piggery wastes is about 55%. Yet, considering that both the gas production and the elimination of malodorous compounds show little if any increase in the digestion of 6% TS manure at 30°C at detention times exceeding 15 days, the manure can be considered as well stabilized after a digestion time of 15 days.

This idea is supported by the results of storage experiments conducted with digested piggery waste at 4°C and 20°C. The results indicate that the formation of malodorous compounds is close to nihil under these circumstances. The remaining organic matter, therefore, can be regarded as highly refractory. As illustrated in Fig. 6.1 part of the refractory material (about 10% of the original manure-COD) occurs in the supernatant solution of the digester effluent. According to results of Hobson et al. (1973) this fraction is also hardly biodegradable under aerobic conditions, i.e. aerobic post-treatment of a supernatant solution of 5400 mg COD^{-1} results in a COD reduction of only 2%

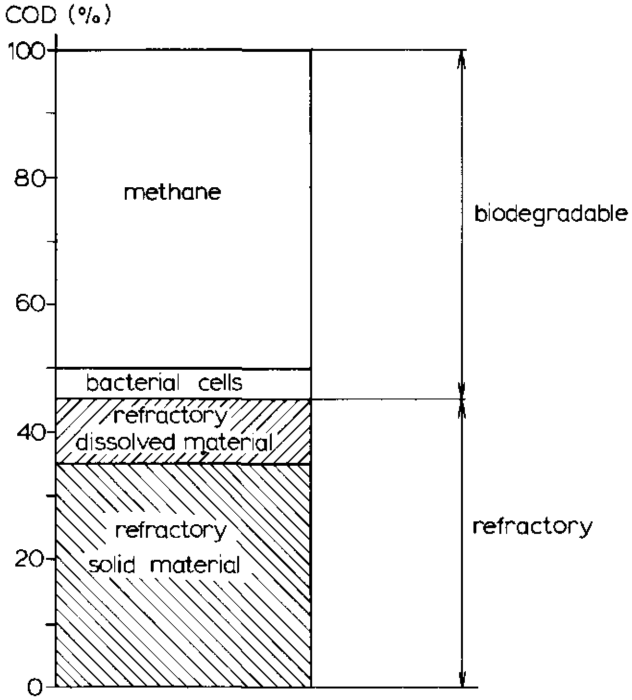

Fig. 6.1 The average fractional composition of piggery wastes in terms of the biodegradable COD-fraction and the refractory COD-fraction.

The remaining 30 - 40% of the original manure-COD even has not been hydrolysed and remains in the digester residue as solid material. This solid fraction presumably mainly consists of lignocellulosic material originating from plant cell walls. Because these feed ingredients are hardly digested by pigs with a maximum weight of 100 kg (Dammers, 1964) they find their way into the wastes. According to results of de Wit (1980) lignocellulosic material comprises 35 - 40 percent of the total solids in freshly voided pig faeces. The main part of this material, viz. 60 - 65 percent, consists of cellulose and hemicellulose, whereas the lignin content is 16 - 19 percent (de Wit, 1980). Obviously, the efficiency of piggery waste digestion can be increased only by improving the availability of the incorporated polysaccharides through a proper pretreatment of the waste.

Preliminary pretreatment experiments conducted in our laboratory concerning the liquefaction of piggery waste indicated that

- particle size reduction increases the rate but does not affect the ultimate degree of liquefaction
- chemical treatment with acid or alkali for 1 hour at pH 1 and 13, respectively increases the degree of liquefaction, especially in the case of the alkaline treatment which increased the liquefaction by about 30%
- thermal pretreatment during 1 hour at 100°C under atmospheric pressure improved the degree of liquefaction by about 80%
- a combined 1 hour alkaline-thermal treatment at pH 13 at 100°C under atmospheric pressure was the most effective pretreatment method investigated. The degree of liquefaction in that case was almost doubled.

These results together with those from literature, cited before, demonstrate that a proper pretreatment can increase the biodegradability of piggery wastes. However it is quite doubtful whether or not these methods will result in a net energy gain. Moreover, as they will be accompanied with high investment and running costs, the application of these methods at individual farms is not attractive.

The presence of refractory solids in piggery wastes and the fact that they as a rule are highly concentrated, viz. in the range 4-10% TS, suggest that a digester of the conventional flow-through type is adequate to handle these wastes. At present mixed digesters and plug-flow digesters are most common in the treatment of animal wastes. Mixing of the digester contents is indispensable when the manure feed easily forms deposits such as in poultry and piggery wastes, or floating layers such as in straw-containing dairy manure. Therefore, mixed digesters seem most appropiate for these types of animal slurries. On the other hand manure with a more homogeneous consistency can be succesfully treated in unmixed digesters of the plug-flow type. Such a horizontal plug-flow type digester was originally introduced by Reinhold and Noack (1955) and has been applied later by Fry (1971) and Hayes et al. (1980).

At the present retail energy prices and the total digestion running costs, energy production from animal manure is estimated as economically feasible in the Netherlands at a digester volume exceeding approx. 100 m^3, treating the wastes produced by 1300 fattening pigs or 100 dairy cows. Accounting for the present Dutch governmental subsidies on the investment costs of digesters, i.e. 30 - 35%, the process becomes economically feasible at digester volumes exceeding approx. 70 m^3 (1000 pig units or 70 dairy cows). The number of farms keeping such an amount of animals is rather limited. In order to make the process economically feasible for smaller farms there is a need for simple, cheap and safe digester systems with a minimum demand for energy. Moreover as the investment costs of gas storage systems are high (Dohne, 1980) it should be pursued that gas production coincides as much as possible with its production.

6.2 FACTORS AFFECTING THE DIGESTION OF BIODEGRADABLE MATERIAL IN PIGGERY WASTE

The digestion process can be considered as completed when the biodegradable fraction of the waste has been converted to methane. The biological degradation already starts during the storage of the waste: on this stage part of the solid material is converted to low-molecular weight compounds such as VFA, phenol, *p*-cresol, 4-ethylphenol, indole and skatole. As these compounds are hardly degraded further under the conditions prevailing in a holding tank they will accumulate in the waste, thus contributing to its malodour (Spoelstra, 1978). Upon subjecting stored piggery waste to methane fermentation, the low-molecular compounds are converted to methane. In the first instance this holds for those compounds, that can be utilized directly by the methanogenic organisms. The elimination of these compounds, including hydrogen, provides thermodynamically favourable conditions for the H_2-producing acetogenic and fermentative bacteria, resulting in an effective degradation of longer chain VFA and other fermentation products. However, dependent on the stage of the digestion process, at the same time low-molecular compounds may be formed from the complex biodegradable solids still present in the stored waste. Therefore, the actual concentration of these intermediates in the digester mixed liquor depends on their rate of formation and degradation. For this reason the concentration of some specific compounds in the digester effluent may be higher than in the influent. This was so for some of the aromatic compounds, e.g. 4-ethylphenol, indole and skatole, in the digestion of 9% TS piggery waste at detention times up to 20 days and also in the digestion of 6% TS piggery waste at a 10-days detention time. As VFA have been well eliminated under these conditions, the degradation of the concerning aromatic compounds obviously proceeds much less effective than that of the VFA. Nevertheless, microbial populations present in digested piggery waste are capable of converting phenol and *p*-cresol (Appendix) and presumably also other aromatic compounds to methane. All malodorous compounds are effectively eliminated from a 6% TS piggery waste when digested at a detention time of 15 days (loading rate 4 kg TS m^{-3} day^{-1}). As both the reduction of malodorous compounds and the gas production hardly increase with an increase in the detention time above 15 days, 6% TS piggery waste can be considered as well stabilized under these circumstances.

Except from factors such as temperature, an effective digestion of the biodegradable fraction depends on the environmental factors imposed by the feed composition, such as the presence of buffering agents and the occurrence of inhibitory or toxic compounds and process intermediates.

Piggery waste digesters are well buffered against changes in the pH mainly as a result of the high ammonia nitrogen concentrations, viz. ranging from 3000 - 5000 mg l^{-1} (215 - 360 meq l^{-1}). As ammonia nitrogen is present mainly as bicarbonate a dramatic pH-fall below the critical value of pH 6 can hardly occur. Obviously, measurements of the digester-pH is not a proper way of controlling piggery waste digesters. Unfortunately ammonia nitrogen affects the process stability also in a negative sense. Ammonia nitrogen at concentrations in excess of approx. 1700 mg l^{-1} is inhibitory except if the sludge has been allowed to acclimatize. During the adaptation of unacclimatized sludge to high ammonia nitrogen concentrations, which may last 8 - 10 weeks, the digestion process becomes slightly out of balance. But also with sludge being well adapted to ammonia nitrogen concentrations in excess of 1700 mg l^{-1}, the methane production from piggery wastes slightly decreases with increasing ammonia nitrogen concentrations in the range 1700 - 5000 mg l^{-1}. According to McCarty and McKinney (1961) the ammonia nitrogen inhibition is connected with the presence of undissociated ammonia. As the fraction of undissociated ammonia sharply increases with temperature, this may explain the poor results obtained in thermophilic digestion of piggery waste.

Other potentially inhibitory compounds for the anaerobic digestion process that may occur in piggery wastes include feed additives, e.g. $CuSO_4$, antibiotics and disinfectants. The toxicity of copper and other heavy metals on anaerobic digestion seems to be mainly concerned with the free metalions. As a consequence the degree of toxicity of these compounds mainly depends on the presence of complexing or precipitating anions. Investigations of Lawrence and McCarty (1965) revealed that heavy metals, including copper, are extremely toxic when added as chlorides, but not when added as sulphates. According to Mosey et al. (1971) copper does not inhibit anaerobic digestion processes at sulphide-ion concentrations in excess of $10^{-17.2}$ mol l^{-1}. Therefore, when the digester feed contains sulphuric compounds (sulphate, S-containing proteins) in excess, as is the case in piggery wastes, very high copper concentrations are admissible. Antibiotics used in animal production units may find their way into the wastes, but their effect on the anaerobic digestion has not yet been ascertained properly. Disinfectants in use for agricultural purposes generally contain compounds like sodium hydroxide, sodium hypochloride and formalin. As these means are used incidentally they will be greatly diluted in the manure collection and storage system. Because of this dilution and because of the rapid decomposition of chemically active compounds in anaerobic environments, disinfectants in general will not occur any more in significant amounts in the digester influent.

In the present study any incomprehensible process inhibition has never been observed, despite the fact that the manure used in the laboratory experiments originated from different farms and the 6-m^3 digester has been continuously fed with the wastes from one pig housing unit for more than 1.5 year. Nevertheless, antibiotics and/or disinfectants, if present in the wastes, might have influenced the process but never significantly.

6.3 USE OF DIGESTER RESIDUES AS A FERTILIZER

The value of organic slurries for fertilizing purposes depends mainly on their mineral content, especially that of N, P and K. Of these elements only nitrogen occurs in a form that it may be lost with the gas during anaerobic digestion, viz. as ammonia nitrogen. Analyses made in pilot-plant studies with piggery wastes (v. Velsen, 1979) have shown that the nitrogen content remains almost unchanged at ammonia nitrogen concentrations below 2100 mg l^{-1}. However, at a concentration of 5000 mg l^{-1} the N-losses were about 11%, mainly due to the higher pH of such solutions.

Field studies on the fertilizing properties of digested residues of intensive animal keeping units are scarce. Some investigations made with digested and undigested slurries on grassland demonstrated little differences in yield (Summers et al., 1980). Moreover, in this study indications were obtained that the use of digested slurry affects the soil structure in a positive way as compared to the use of untreated slurry.

6.4 APPLICATION OF ANAEROBIC DIGESTION IN PIGGERY WASTE PURIFICATION PLANTS

In view of the problems involved in the production of excess amounts of animal manure in certain regions of Western Europe, a complete purification of these wastes should be taken into consideration at present. Anaerobic digestion as a first step provides a means to achieve a substantial reduction of carboneous material. This can be even improved by the application of a proper (pre)treatment of the solid fraction, either before or after digestion. Therefore, in these cases a (pre)treatment of animal wastes may be attractive.

However, as anaerobic digestion is uncapable of producing effluents that can be discharged on the surface water, the method should be followed by a proper post-treatment. Since animal wastes are high in valuable constituents, such as N and P, such a post-treatment should aim at a recovery of these minerals.

A proper post-treatment system, as shown in Fig. 6.2 might include sludge dewatering, supply of lime to rise the pH (precipitation of P), followed by a combined strip/absorption process for removal and recovery of ammonia, and as a last step a physico/chemical treatment of the remaining liquid fraction. Because of the high investment costs and the complexicity of the technologies involved, such a complete treatment of animal wastes can be practised only in large-scale, centralized plants treating the wastes of 50 000 or more pig units.

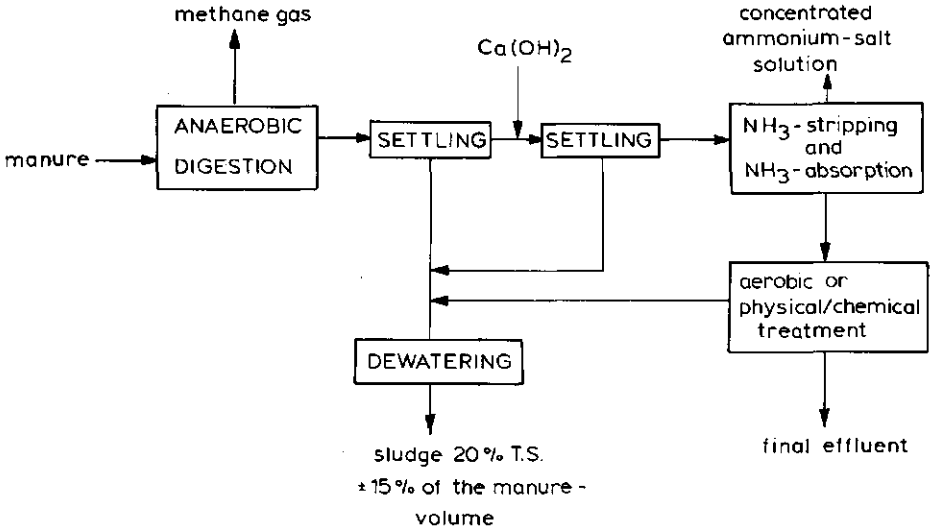

Fig. 6.2 Proposed scheme for complete purification of animal wastes.

APPENDIX

PRELIMINARY RESEARCH INTO THE ANAEROBIC DECOMPOSITION OF PHENOL AND p-CRESOL

A.1 INTRODUCTION

In the digestion of piggery waste at loading rates of 4 kg TS m^{-3} day^{-1} and lower, the concentration of the odorous aromatic compounds phenol, *p*-cresol, 4-ethylphenol, indole and skatole is considerably reduced as has been shown in Chapters 3, 4 and 5. Since the presence of these compounds in anaerobically stored pig slurries results from the microbial breakdown of proteins (Spoelstra, 1978), these aromatics will presumably be formed upon anaerobic storage of most other protein-rich wastes. Aromatics also occur in the effluents of coal gasinfication plants, oil refineries and other chemical industries.

In comparison to the aerobic degradation of aromatics relatively little is known about the degradation under anaerobic conditions. However, the fate of aromatic compounds in methane digestion gets more and more attention not only because of their possible inhibitory effects on the digestion process but also to assess the potential energy production from aromatic material such as lignin, which is the most abundant organic carbon source in the world after cellulose.

Lin Chou et al. (1978) investigated the toxicity of nine aromatic compounds (benzoic acid, phtalic acid, ethyl benzene, aniline, hydroquinone, phenol, catechol, nitrobenzene and resorcinol) to unacclimated methane fermentation systems. Except phtalic acid all these aromatics were found toxic to some degree. The inhibitory level of phenol appeared to be approx. 470 mg l^{-1}. Pearson et al. (1980) studied the toxicity of phenol under shock loading and reported a 50% reduction in sludge activity at a dose of 500 mg l^{-1}. According to Neufeld et al. (1980) the non-methanogenic anaerobic phenol decomposition is inhibited by phenol at concentration levels in excess of 686 mg l^{-1}.

The degradation of aromatics during methane fermentation has been investigated in detail only in the case of benzoic acid. Tarvin and Buswell (1934), Fina and Fiskin (1960), Nottingham and Hungate (1969), Keith (1972) and Ferry and Wolfe (1976) all demonstrated the conversion of benzoic acid to methane and carbon dioxide. Keith (1972) and Ferry and Wolfe (1976) suggested from sequential induction experiments that benzoate is degraded by a reductive pathway. A similar reductive pathway has been shown to occur in the anaerobic benzoate degradation during photosynthetic metabolism (Dutton and Evans, 1969) and nitrate respiration (Williams and Evans, 1973; Bakker, 1977). Ferry and Wolfe (1976) further presented evidence that benzoate is converted to methane by a microbial food chain in which the removal of intermediates, such as acetate and hydrogen, by the methanogenic bacteria provides thermodynamically favourable conditions for the benzoate degradation. Chmielowski et al. (1965, 1965, 1966) performed kinetic studies on the anaerobic decomposition of phenol, *p*-cresol and resorcinol in batch experiments with 100 mg l^{-1} of the respective phenolic compounds. Mixed bacterial populations gave complete conversion of these phenolics to methane and carbon dioxide. These investigators further reported that during fermentation some unidentified intermediates having the nature of satured compounds temporarily accumulated. The degradation of phenol by methanogenic populations has also been reported by Healy and Young (1978), Lin Chou et al. (1978) and Pearson et al. (1980).

Other aromatics reported to be degradable under methanogenic conditions include catechol (Healy and Young, 1978; Lin Chou et al., 1978), nitrobenzene, phtalic acid, hydroquinone, resorcinol (Lin Chou et al., 1978) and ferulic acid, a model lignin derivate (Healy et al., 1980).

The experiments described in this Appendix concern the elimination of phenol and *p*-cresol by mixed methanogenic populations. The study was performed to obtain a better understanding of the elimination mechanism of these aromatics in anaerobic digestion.

A.2 PROCEDURES

The degradation of phenol and *p*-cresol was investigated in four 5-litre glass fermentors (Section 2.3.1). The digestion units were seeded with 4 litres of a 1:1 mixture of well-digested piggery waste and tap water. The TS and VS content in the experimental units at the start of the experiments were 22.3 g l^{-1} and 15.1 g l^{-1}, respectively. After seeding nitrogen gas was passed through the digester contents to assure anaerobic conditions. Because of the poor solubility of phenol and *p*-cresol in water the following feeding procedure has been applied. The feed components were dissolved in as little water as possible. Immediately after adding the feed-solution to the digester and subsequent mixing of the digester contents a sample was taken to determine the initial feed concentration.

Four series of batch experiments, designated as A, B, C and D were performed. In series A the reactor was fed 5 successive times with about 400 mg l^{-1} phenol as the only carbon source. During the degradation of phenol in the fifth feeding 410 mg l^{-1} *p*-cresol was added.

In series B the reactor was fed 5 successive times with about 400 mg l^{-1} *p*-cresol. During the degradation of *p*-cresol in the fifth feeding 404 mg l^{-1} phenol was supplied.

In series C the reactor was fed 2 successive times with a mixture of phenol and *p*-cresol, each at a concentration of about 400 mg l^{-1}.

In series D the first feeding consisted of a mixture of phenol, *p*-cresol and acetic acid, each at a concentration of about 400 mg l^{-1}. In the second feeding only phenol and *p*-cresol were added.

A.3 RESULTS

The result of the series A experiments are shown graphically in Fig. A.1 viz. the concentration of phenol and acetic acid in relation to the time.

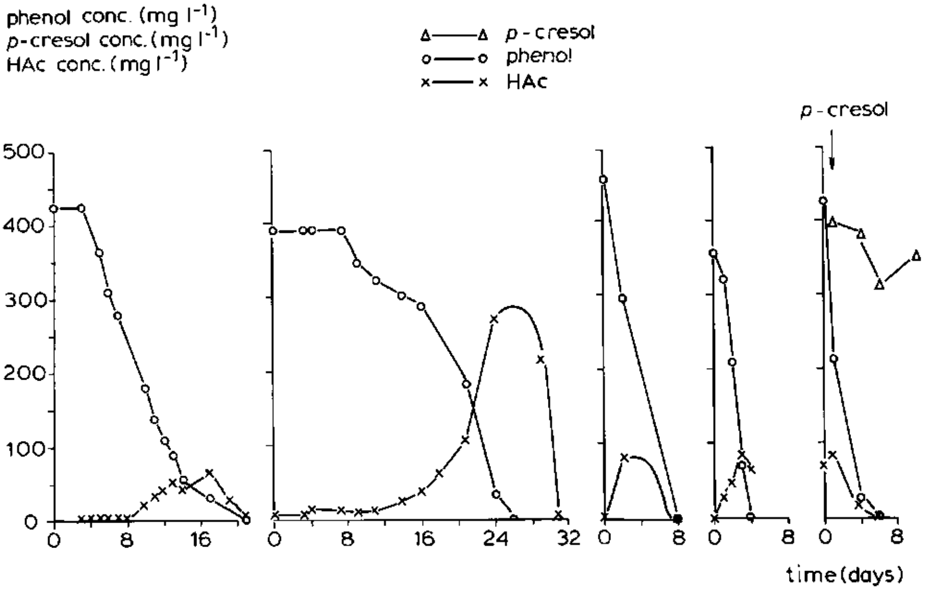

Fig. A.1 Course of the digestion process in experimental series A, fed 5 successive times with phenol as the only carbon source. During the last feeding p-cresol is added.

Due to a leakage in the gas system, the gas production in this experimental series has not been determined. Fig. A.1 shows that phenol is eliminated under the prevailing anaerobic conditions, although a lag phase of 4-8 days occurred in the first two feedings. In the course of the successive feedings the maximum elimination rate of phenol increased from 2.4 mg g^{-1} VS day^{-1} in the first feeding to 14.3 mg g^{-1} VS day^{-1} in the fifth feeding. However, this was not a gradual increase because the phenol elimination rate in the second feeding is significantly lower than that in the first feeding. In all feedings acetic acid appeared in the digester mixed liquor indicating that this compounds acts as an intermediate in the phenol degradation. The addition of 410 mg l^{-1} *p*-cresol during the degradation of phenol in the fifth feeding did not influence the phenol degradation. However, *p*-cresol was not eliminated in the first 7 days after feeding, indicating that the phenol adapted population is unable to metabolize *p*-cresol immediately.

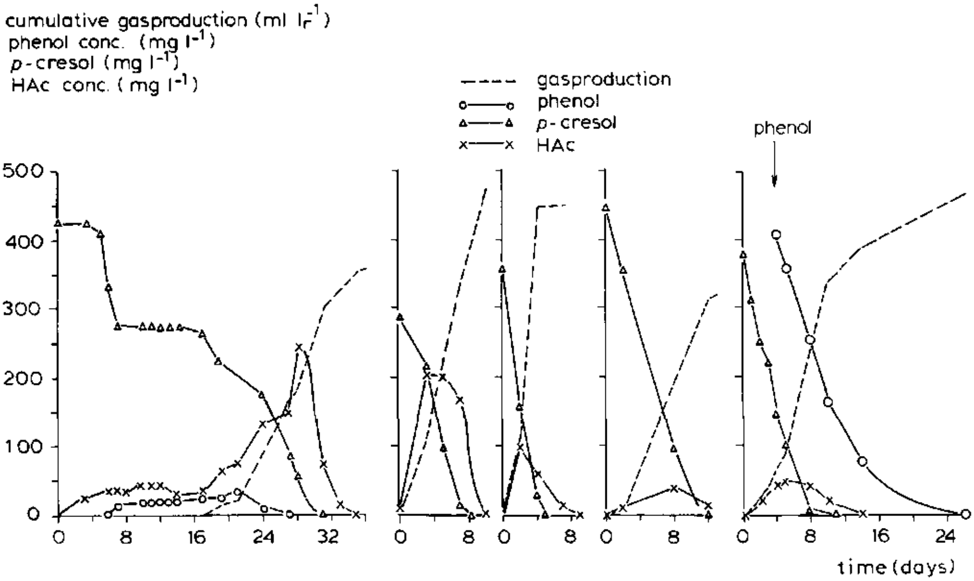

Fig. A.2 Course of the digestion process in experimental series B, fed 5 successive times with p-cresol as the only carbon source. During the fifth feeding phenol is added.

Fig. A.2 shows the results of experimental series B, viz. the course of the gas production and that of the *p*-cresol, phenol and acetic concentration. In the first feeding the *p*-cresol elimination started after a lag phase of 4 days, resulting in a sharp drop in the *p*-cresol concentration from 425 mg l^{-1} at day 4 to 280 mg l^{-1} at day 7. However, the *p*-cresol elimination during this period was not accompanied with a gas production but with a certain accumulation of acetic acid and phenol in the digester mixed liquor. The concentration of these intermediates is too low to account fully for the decrease in the *p*-cresol concentration. From day 7 to day 16 the concentration of *p*-cresol and that of the intermediates remained unchanged, but beyond day 16 *p*-cresol was degraded further and now it was accompanied with an evident gas production. At the five successive feedings the maximum elimination rate of *p*-cresol tended to increase from 1.9 mg g^{-1} VS day^{-1} in the first feeding to 3.7 mg g^{-1} VS day^{-1} in the fifth feeding. The results of the fifth feeding of series B, when 404 mg l^{-1} phenol was added during the degradation of *p*-cresol, indicate that phenol is degraded immediately and at a rate comparable to that of the *p*-cresol degradation. The methane content of the gas in this series ranged from 87% to 89%.

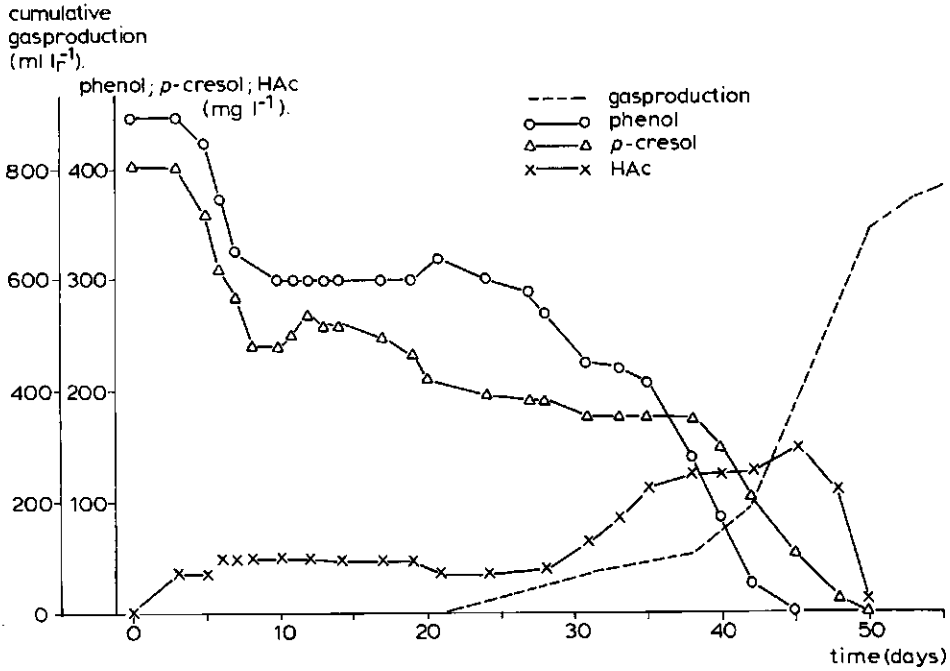

Fig. A.3 Course of the digestion process in the first feeding of experimental series C, fed with phenol and p-cresol.

The experimental results of the first feeding in series C, consisting of 443 mg l^{-1} phenol and 400 mg l^{-1} *p*-cresol are shown in Fig. A.3. In comparing the results with those in Fig. A.2, it appears that the elimination of both phenol and *p*-cresol resembles that of the *p*-cresol elimination found in the first feeding of experimental series B, fed exclusively with *p*-cresol. After an initial lag phase of 4 days the concentration of both compounds decreased from day 4 to day 7, then followed by a period of retarded digestion. Only approx. 35 days after incubation a further degradation of phenol and *p*-cresol occurred. In the second feeding, consisting of 455 mg l^{-1} phenol and 456 mg l^{-1} *p*-cresol, both compounds were eliminated within

17 days at an almost linear rate. The methane content of the gas was 92% and 94% respectively.

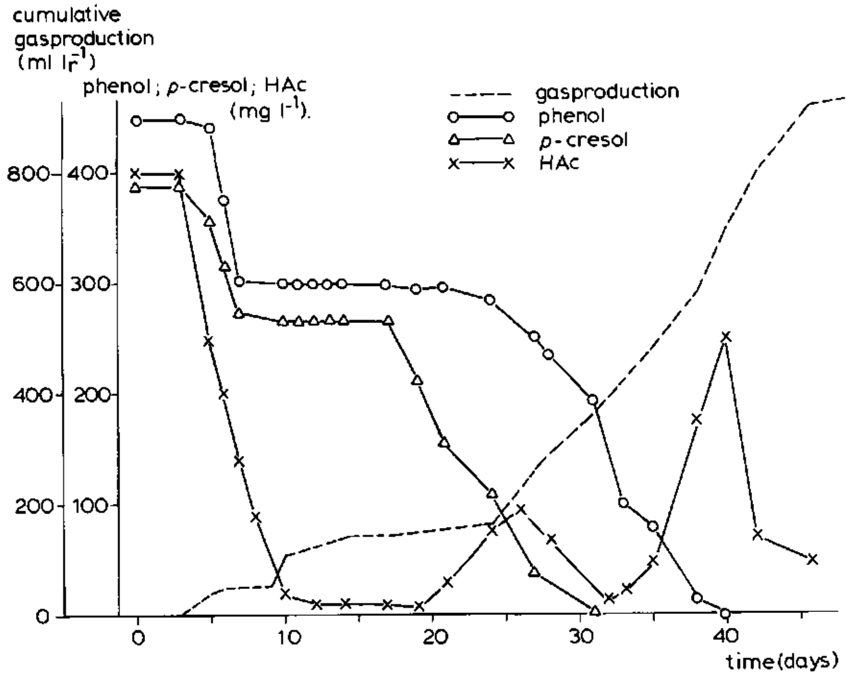

Fig. A.4 Course of the digestion process in the first feeding of experimental series D, fed with phenol, p-cresol and acetic acid.

The experimental results of the first feeding in series D, consisting of phenol, *p*-cresol and acetic acid each at a concentration of approx. 400 mg l^{-1} are shown in Fig. A.4. Fig. A.4 shows that after a 4-days initial lag phase acetic acid was rapidly converted into methane within 10 days whereas the elimination of both aromatic compounds resembled that observed in series C (Fig. A.3). However, *p*-cresol and phenol now were degraded within 40 days which is considerably faster than in series C, where the degradation took 50 days. Although the microbial population was well adapted to the degradation of acetic acid, this intermediate accumulated in the digester mixed liquor whenever the *p*-cresol or phenol degradation reached its maximum rate. In the second feeding, consisting of 375 mg l^{-1} phenol and 338 mg l^{-1} *p*-cresol, phenol and *p*-cresol were degraded within 9 days at an almost linear rate. The methane content of the gas amounted to 86% in both feedings.

In all experiments described in this Chapter the pH was in the range 6.8-7.2.

A.4 DISCUSSION

The reduction of phenol, *p*-cresol and other odorous compounds as found during the anaerobic digestion of piggery wastes (Chapters 3, 4 and 5) may be the result of one of the following mechanisms: (a) volatilization, (b) incorporation of these compounds in cell proteins or (c) microbial degradation.

Considering the experimental results presented in this Appendix, showing production of methane in experiments where phenol and *p*-cresol were supplied as the only carbon source, it is evident that microbial degradation is the main mechanism for the phenol and *p*-cresol elimination under the prevailing methanogenic conditions and not the incorporation of the compounds in cell proteins. Also the ability of the microbial populations to adapt to the aromatic compounds points to a microbial degradation.

In all experiments the degradation of phenol and *p*-cresol is accompanied with a temporary increase in the acetic acid concentration in the digester contents. Evidently acetic acid is an intermediate in the conversion of these aromatics to methane, which is conform the observations of Ferry and Wolfe (1976) who reported that acetic acid is an intermediate in the conversion of benzoate to methane. The appearance of phenol in the first feeding of series B, fed with *p*-cresol as the only carbon source, may indicate that phenol is an intermediate in the *p*-cresol degradation (Fig. A.2). This idea is supported by the observation that *p*-cresol adapted sludge is also adapted to phenol, as clearly appears from the results of the fifth feeding in series B. On the other hand the phenol adapted sludge is not capable to degrade *p*-cresol immediately (Fig. A.1). Apparently, a demethylation is the first step in the *p*-cresol degradation under methanogenic conditions. Such a degradation route of *p*-cresol deviates from the pathway suggested by Bakker (1977) for the microbial degradation of *p*-cresol in nitrate respiration, involving a decomposition route leading to 4-ethylcaproic acid. Nevertheless, it seems quite reasonable that under methanogenic conditions a demethylation of compounds like *p*-cresol can take place because methanogenic organisms are capable to convert methylgroups directly to methane. The experimental results, however, do not allow to draw any conclusion concerning the mechanism of the *p*-cresol demethylation and the fate of the methylgroup. For this purpose labelling experiments should be performed.

As the concentration of acetic acid and phenol during the period before stagnation in the first feeding of series B, C and D is too low to account fully for the decrease in the concentration of the feed compounds, the accumulation of other, unidentified intermediates is plausible. This corresponds with the findings of Chmielowski et al. (1965, 1965, 1966) who reported about an accumulation of some intermediates having the nature of saturated compounds, during the anaerobic fermentation of phenol, *p*-cresol and resorcinol. Ferry and Wolfe (1976) reported that apart from acetic acid also carbon dioxide, hydrogen and formic acid were intermediates in the benzoate degradation under methanogenic conditions. The appearance of process intermediates during the degradation of phenol and *p*-cresol indicates that these aromatics are degraded by a microbial consortium and not by a single organism as has been suggested by Nottingham and Hungate (1969) for the methanogenic fermentation of benzoate.

The course of the phenol and *p*-cresol elimination in the first feeding differs from one experimental series to another. In all cases the elimination, even that of acetic acid, starts after a lag phase of 4-5 days. This indicates that it takes some time before the conditions in the experimental units are suitable for anaerobic conversions.

After the lag phase the phenol concentration in series A decreased almost linearly (Fig. A.1), whereas in series B, C and D the elimination of phenol and *p*-cresol is characterized by the occurrence of a period of stagnated elimination. (Figs. A.2, A.3 and A.4). The reduction in the phenol and *p*-cresol concentration before the stagnation reveals the ability of the microbial population to assimilate these aromatics. Furthermore, phenol and *p*-cresol are partially degraded in this period as appears from the accumulation of process intermediates.

The stagnation in the elimination of phenol and *p*-cresol may be attributed to a retarded methanogenesis. As the methanogenic organisms serve as terminal organisms of a food chain producing methane from intermediates formed during the degradation of aromatic compounds (Ferry and Wolfe, 1976),the accumulation of intermediates may create thermodynamically unfavourable conditions for a further degradation of phenol and *p*-cresol. The lag phase in the methane formation occurring in the present experiments may be caused either by unfavourable environmental conditions, e.g. the redox potential and/or the presence of unidentified inhibitory intermediates, or by a disrupture of micro-environments in the methanogenic seed sludge during the start-up procedure. Micro-environments are supposed to play an important role in the interspecies H_2 transfer. De Zeeuw et al. (1980) recently suggested that the formation of micro-environments is part of the adaptation process of methanogenic populations to propionic acid. Ferry and Wolfe (1976) reported that the benzoate degradation under methanogenic conditions ceased if the bacterial floc was disrupted by vortexing, which also points to the importance of micro-environments in the degradation of aromatics.

Another explanation for the differences in the course of the phenol elimination between series A and that in series C and D might be found in a temporary inhibition of one of the metabolic stages in the phenol and *p*-cresol degradation by some intermediate accumulating during the *p*-cresol degradation, because a stagnation in the phenol and *p*-cresol elimination only occurs when the feed contains *p*-cresol (series B, C and D). However, *p*-cresol itself does not affect the phenol degradation by adapted sludge as appears from the results of the last experiment of series A.

The results of the present experiments do not allow to be certain of the reasons underlying the discrepancies between the results of the first feeding of experimental series A and those of the other series.

The addition of acetate, a substrate suitable for methanogenic bacteria, in series D does shorten the period of stagnated elimination occurring in the phenol and *p*-cresol degradation (compare with the results of series C). Evidently, the conversion of acetate at the start of the experiment creates more favourable conditions for the degradation of phenol and *p*-cresol.

SUMMARY

Anaerobic digestion is a biological process by which organic matter is converted to methane and carbon dioxide by microbes in the absence of air (oxygen). In nature, anaerobic conversions occur at all places where organic material accumulates and the supply of oxygen is deficient, e.g. in marshes and lake sediments. Microbial formation of methane also plays a role in the ruminant digestion.

In digestion units, the external conditions acting upon the process can be regulated to speed it up as compared with that occurring in nature. Moreover, the gas produced can be collected and used as a fuel. In this way, anaerobic digestion for waste treatment allows us to reduce the concentration of polluting organic substances and produce useful energy. So there is increasing interest in anaerobic digestion, especially as fossil energy threatens to give out and people are concerned about environmental pollution.

By the end of the 19th Century, anaerobic digestion was used to stabilize excess sewage sludge. The process was also utilized for energy production from agricultural wastes (manure, straw) whenever fossil energy was in short supply, e.g. during and shortly after World War II in France, Algeria and Germany. However, after the War energy from digestion of agricultural wastes could not compete with cheap fossil energy and direct interest in the process diminished. The main objectives of using digestion for disposal of animal manure are the abatement of malodour nuisance and the recovery of energy from wastes. The change-over from traditional animal husbandry to intensive systems has resulted in the production of large amounts of manure in certain regions of Western Europe. Disposal of the manure creates severe environmental pollution.

The wastes produced in intensive animal production often consist of combined droppings: faeces, urine and spillage water. During storage of the slurry, low-molecular malodorous compounds such as volatile fatty acids, phenol, *p*-cresol, 4-ethylphenol, indole and skatole are formed by microbial activity (Spoelstra, 1978). The spreading of these slurries often causes malodour nuisance, especially in more densely populated areas. Upon subjecting the wastes to anaerobic digestion under controlled conditions, most of the biologically degradable material is converted to methane, thus producing a stabilized residue without offensive odour. Any malodorous compounds given out during digestion form components of the digester gas and will not lead to malodour nuisance in the surroundings unless the gas be discharged unburned.

The gas produced is an useful fuel either for heating or for combustion engines, e.g. to generate electricity. Farms with a high energy consumption can largely cover their farm needs with the gas produced. Examples are pig and poultry breeding units and dairies. However, in spite of the favourable prospects on individual farms, manure digestion can play only a minor role

in the national energy supply, since the maximum production is about 1% of present energy consumption.

According to present knowledge of the microbiology and biochemistry of anaerobic digestion (Zeikus, 1980; McInerney et al., 1980) the conversion of complex organic material to methane results from the activity of at least 4 distinct trophic groups of bacteria: fermentative bacteria, hydrogen-producing acetogenic bacteria, methanogenic bacteria and hydrogen-consuming acetogenic bacteria. As a result of the interaction between the contributing groups, anaerobic digestion as a whole is a stable process.

However, the classification of the microbial population does not provide an adequate method of analysing the course of the digestion process in practice. So a simplified scheme was proposed, based on routine chemical analysis. In the scheme, anaerobic digestion was split up into three steps: (1) hydrolysis of undissolved compounds, (2) acid formation from dissolved organic compounds and (3) methane formation. The course of these separate steps of the digestion process can be estimated from routine data, such as the gas production, gas composition, Chemical Oxygen Demand and volatile fatty acids (Chapter 1).

The feasibility of anaerobic digestion processes depends on the availability of a suitable seed material. In Chapter 3 experiments are described on the influence of organic loading rate, in the range 0.07 - 0.125 kg COD kg^{-1} VS day^{-1}, on the adaptation of digested sewage sludge to piggery waste. Digested sewage sludge was a fairly suitable seed material, since methane formation started immediately after adding piggery waste. However, all experiments were characterized by a period of imbalance, which might be attributable to an inhibition of the methanogens by ammonia nitrogen at concentrations over about 1700 mg l^{-1}. In a period of 2-3 months, dependent on the loading rate, the methanogenic population was adapted to these concentrations of ammonia nitrogen. The adapted populations were also capable of forming methane at far higher ammonia nitrogen concentrations, viz. up to 5000 mg l^{-1}. The idea that methanogenesis was temporarily inhibited by ammonia nitrogen was supported by the results of two series of batch-type experiments, on the digestion of volatile fatty acids by two types of methanogenic sludge, viz. digested sewage sludge and digested piggery waste, at increasing ammonia nitrogen concentrations. In the experiment with digested sewage sludge adapted to 815 mg l^{-1} ammonia nitrogen, methane formation occurred at all ammonia nitrogen concentrations investigated (in the range 730 - 4990 mg l^{-1}), but an increasing lag phase was observed at increasing concentrations. This was not so for the experiment with digested piggery waste adapted to 2420 mg l^{-1} ammonia nitrogen. Here the methane formation started immediately without any significant lag phase for the propionic acid degradation. The maximum rate of methane formation slowly decreased with increasing ammonia nitrogen concentrations. Daily fed experiments with piggery waste (loading rate 4.1 kg TS m^{-3} day^{-1}) demonstrated successful digestion at all ammonia nitrogen concentrations imposed, viz. 2070, 3670 and 5290 mg l^{-1}. The gas production only slightly decreased with increasing ammonia nitrogen concentration.

From the combined results it appears that the presumed inhibitory effect of ammonia nitrogen concentrations in excess of approx. 1700 mg l^{-1} is only temporary. Consequently the use of anaerobic digestion may also be considered for wastes having a N-content far exceeding this level.

Chapter 4 deals with the influence of the piggery waste concentration and the detention time on the digestion at a temperature of 30°C. At all manure concentrations (4%, 6% and 9% TS) and detention times investigated (in the range 10 - 40 days) a stable digestion could be achieved and maintained except with 9% TS manure at a 10-days detention time (space load approx. 9 kg TS m^{-3} day^{-1}). At fixed detention times an increase in the manure concentration effected a decrease in both the gas yield per kg TS added and the reduction of malodorous compounds. The gas production improves significantly in increasing the detention time from 10 days to 15 days, but beyond a 15-days detention time there is only a slight further increase. Although a stable digestion could be maintained at space loads up to 6 kg TS m^{-3} day^{-1}, the maximum load for a satisfactory reduction of objectionable piggery waste odours was about 4 kg TS m^{-3} day^{-1}, provided this loading rate was achieved by digesting 6% TS piggery waste at a 15-days detention time and not by digesting 9% TS manure at a 22.5-days detention time. The experimental results further indicate that hydrolysis is the rate-limiting step in the digestion of piggery wastes.

Experiments concerning the effect of temperature in the range 13°C - 55°C on the digestion of piggery waste are described in Chapter 5. The experiments were performed in daily fed laboratory digesters at a loading rate of approx. 4 kg TS m^{-3} day^{-1}. No methane was produced at a digestion temperature of 13°C, but an active methane fermentation took place at 20°C. In the mesophilic temperature range (20°C - 40°C) the methane production increased sharply with temperature in the range 20°C - 25°C, but in the range 25 - 40°C there was only a slight further increase. Hydrolysis of undissolved manure components turned out to be the rate-limiting step of the digestion process.

Under thermophilic conditions (55°C) the methane production decreased by about 25% as compared to mesophilic digestion, in spite of a somewhat higher degree of hydrolysis. Digestion under thermophilic conditions seems to be more sensitive to high ammonia nitrogen concentrations than mesophilic digestion, which presumably is caused by an increase in the fraction of free ammonia with temperature (at constant pH and total ammoniacal nitrogen concentration).

From the results it can be concluded that digestion under mesophilic conditions is most adequate for piggery waste stabilization. Furthermore the results of the mesophilic experiments indicated that at a load of approx. 4 kg TS m^{-3} day^{-1} the optimum temperature with respect ot the net energy recovery is 27°C - 30°C when high-grade fuel is required to elevate the temperature. When sufficient waste energy is available the process preferentially should be conducted at a temperature of 35°C - 40°C.

Chapter 6 contains a general discussion on the application of the anaerobic digestion process and provides information about the features of piggery waste as a substrate for anaerobic digestion. Items discussed include the biodegradability, the degree of stabilization, the process stability and the economical feasibility of the process. Data are provided which demonstrate that the gas production from piggery wastes can be increased by petreating the wastes. However, in view of the accompanying consumption of energy and/or chemicals it is doubtful whether or not these pretreatment methods are effective in increasing the net energy production.

The Appendix deals with preliminary batch-type experiments on the elimination of two aromatic malodorous compounds, viz. phenol and *p*-cresol, in anaerobic digestion. The results demonstrate that both these compounds are converted into methane by the microbial population in piggery waste digesters. Furthermore indications were obtained that phenol is an intermediate in the *p*-cresol degradation and that the first step in the microbial breakdown of *p*-cresol apparently is a demethylation of *p*-cresol to phenol.

SAMENVATTING

Anaërobe gisting is een microbiologisch proces, waarbij dood organisch materiaal in een zuurstofloos milieu wordt omgezet in een gasmengsel, dat voornamelijk bestaat uit methaan (60 - 75%) en koolzuur, het z.g. biogas. In de natuur komt het anaërobe mineralisatieproces voor op plaatsen waar zich organisch materiaal ophoopt en de toevoer van luchtzuurstof stagneert, b.v. in moerassen en bodemsedimenten van meren en plassen. Ook bij de spijsvertering van herkauwers speelt methaangisting een belangrijke rol.

In gistingsinstallaties vindt het anaërobe proces plaats onder controleerbare omstandigheden, als gevolg waarvan de omzettingen sneller kunnen verlopen dan doorgaans in de natuur het geval is. Bovendien wordt het gevormde gas opgevangen en als brandstof gebruikt. Wanneer anaërobe gisting wordt toegepast voor de behandeling van afvalprodukten biedt het proces de mogelijkheid de verwerking van afval te combineren met de produktie van energie. Gezien de dreigende schaarste aan fossiele brandstoffen en de huidige zorg voor het milieu is het niet verwonderlijk, dat er een groeiende belangstelling is voor de toepassing van anaërobe gisting voor de behandeling van afval en afvalwater.

Methaangisting wordt reeds vanaf het eind van de vorige eeuw toegepast voor de stabilisatie (=stankvrij maken) van surplus-slib, gevormd bij de aërobe zuivering van huishoudelijk afvalwater. In landen als Duitsland, Frankrijk en Algerije is het proces ook toegepast voor de produktie van energie uit landbouwafval tijdens en vlak na de tweede wereldoorlog, toen de energievoorziening stagneerde. In de na-oorlogse periode is de belangstelling voor het anaërobe gistingsproces als energie-opwekkingsmethode echter weer afgenomen als gevolg van de lage prijzen van fossiele brandstoffen.

De belangrijkste argumenten voor de toepassing van mestvergisting zijn momenteel de reductie van meststank en de produktie van energie. Door de opkomst van de intensieve veehouderij worden er plaatselijk en regionaal grote hoeveelheden mest geproduceerd. In een aantal streken van ons land, waar veel intensieve veehouderij wordt bedreven, zoals de Veluwe en Oost-Brabant, heeft dit geleid tot de produktie van mestoverschotten. Het dumpen van deze mest betekent een ernstige aantasting van het milieu.

In de intensieve veehouderij wordt de geproduceerde mest meestal verzameld onder de stallen te zamen met de urine en het spoelwater, de z.g. drijfmest. Bij de opslag van drijfmest worden stankstoffen, zoals vluchtige vetzuren, fenol, *p*-cresol, ethylfenol, indol en skatol, gevormd als gevolg van microbiologische omzettingen (Spoelstra, 1978). Het uitrijden van de aangerotte mest geeft dikwijls aanleiding tot stankoverlast, vooral in dichtbevolkte gebieden. Bij vergisting van de mest worden de organische stoffen die aanleiding geven tot stank, of waaruit stankstoffen kunnen worden gevormd, grotendeels verwijderd en omgezet in gas.

Het gevormde gas is een uitstekende brandstof, zowel voor verwarming als voor het aandrijven van motoren, b.v. electriciteitsopwekking met warmtekrachtkoppeling. Afhankelijk van het bedrijfstype en de bedrijfsgrootte kunnen veehouderijbedrijven met het geproduceerde gas voor een belangrijk gedeelte in hun eigen energiebehoefte voorzien. Landelijk gezien is de potentiele energieproduktie uit mest echter gering, nl. ongeveer 1% van de nationale consumptie van primaire energie.

Naast de bovengenoemde aspecten wordt in hoofdstuk 1 verder ingegaan op de microbiologische en biochemische achtergronden van de methaangisting. Naar de meest recente inzichten (Zeikus, 1980; McInerney et al., 1980) spelen bij de omzetting van complexe organische stof tot methaan vier verschillende groepen micro-organismen een rol, nl. fermentatieve bacteriën, H_2-producerende acetogene bacteriën, methanogene bacteriën en H_2-consumerende acetogene bacteriën. De stofwisseling van deze groepen van bacteriën grijpt op elkaar in als gevolg waarvan anaërobe gisting als geheel een stabiel proces is. In de praktijk kan het verloop van het gistingsproces echter niet of slechts met zeer veel moeite worden beschreven aan de hand van de aanwezige microbiologische populatie. Daarom is een vereenvoudigd schema opgesteld, waarmee het procesverloop kwantitatief kan worden beschreven aan de hand van chemische routine analyses. In dit schema wordt de vergisting van complex organisch materiaal ingedeeld in 3 stappen, nl. (1) de omzetting van onopgelost materiaal in oplosbare bestanddelen, (2) de vorming van vluchtige vetzuren uit de opgeloste bestanddelen en (3) de vorming van methaan.

In hoofdstuk 2 is een overzicht gegeven van de experimentele opstellingen en de gebruikte methodes.

Een belangrijk aspect voor de toepassing van het proces is het opstarten van gistingsinstallaties. In hoofdstuk 3 is de invloed beschreven van de belasting op de adaptatie van rioolslijk aan varkensdrijfmest. Voor dit onderzoek werd een aantal vergisters gevuld met rioolslijk en dagelijks gevoed met varkensdrijfmest bij belastingen varierend van 0,07 tot 0,125 kg CZV kg^{-1} org. stof dag^{-1}. Rioolslijk bleek een geschikt entmateriaal. Onmiddellijk na voeding met varkensdrijfmest kwam de methaanproduktie op gang. Na verloop van tijd raakte het proces in alle vergisters echter uit balans. Dit kan worden toegeschreven aan een remming van de methaanvormende bacteriën door ammoniumstikstof, aangezien het proces in alle experimenten wordt verstoord wanneer de ammoniumstikstof-concentratie een waarde van ongeveer 1700 mg l^{-1} overschrijdt. Volgens McCarty en McKinney (1961) is de remmende werking van ammoniumstikstof bij deze en hogere concentraties het gevolg van een te hoge concentratie aan ongedissocieerd ammonia. Het slib adapteert evenwel na 2 tot 3 maand (afhankelijk van de toegepaste belasting) aan deze ammoniumstikstof-concentraties. Na de adaptatieperiode bleek het slib in staat tot methaanvorming bij aanzienlijke hogere ammoniumstikstof-concentraties, nl. tot ca. 5000 mg l^{-1}.

De aanwijzing dat de methaangisting gedurende de opstartperiode tijdelijk werd geremd door de toename van de ammoniumstikstof-concentratie werd bevestigd door de resultaten van twee series ladingsgewijze gevoede experimenten. In deze experimenten is de invloed onderzocht van toenemende ammoniumstikstof-concentraties op de methaanvorming uit vluchtige vetzuren door rioolslijk en vergiste varkensdrijfmest. Het bleek dat rioolslijk geadapteerd aan een ammoniumstikstof-concentratie van 815 mg l^{-1} methaan produceerde bij alle onderzochte ammoniumstikstof-concentraties (tussen 730 en 4990 mg l^{-1}), maar dat de methaanvorming pas begon na een lag-fase. De lagfase duurde langer bij toenemende ammoniumstikstof-concentraties. Vergiste varkensdrijfmest geadapteerd aan een ammoniumstikstof-concentratie van 2420 mg l^{-1} daarentegen was in staat onmiddellijk na incubatie methaan te vormen bij alle onderzochte ammoniumstikstof-concentraties (tussen 605 en 3075 mg l^{-1}). De maximale methaanvormingssnelheid nam af bij toenemende ammoniumstikstof-concentraties.

Uit de resultaten van dagelijks gevoede experimenten (belasting 4,1 kg DS m^{-3} dag^{-1}; temperatuur 30°C) naar de invloed van ammoniumstikstof in concentraties van 2070, 3760 en 5290 mg l^{-1} op de vergisting van varkensdrijfmest bleek, dat bij alle ammoniumstikstof-concentraties de vergisting goed verliep, maar dat de gasproduktie enigszins afnam bij toenemende ammoniumstikstof-concentraties.

De resultaten van de experimenten beschreven in hoofdstuk 3 tonen aan dat de vermeende remmende werking van ammoniumstikstof boven een concentratie van ca. 1700 mg l^{-1} slechts tijdelijk is en dat afvalstromen met aanzienlijk hogere stikstofgehaltes met succes kunnen worden vergist.

In hoofdstuk 4 zijn een aantal experimenten beschreven die tot doel hadden de invloed vast te stellen van de verblijftijd en de mestconcentratie op de vergisting bij 30°C. Bij alle onderzochte mestconcentraties, nl. 4%, 6% en 9% droge stof, en verblijftijden, tussen 10 en 40 dagen, stelde zich een stabiel gistingsproces in, behalve bij het experiment waarin mest met een droge stofgehalte van 9% werd gevoed bij een verblijftijd van 10 dagen (belasting 9 kg DS m^{-3} dag^{-1}). De methaanproduktie in deze experimenten varieerde van 0,141 tot 0,207 l kg^{-1} toegevoerde drogestof. In het algemeen nam bij gelijke verblijftijden de gasproduktie per kg toegevoerde drogestof en de reductie van stankcomponenten af bij een verhoging van de mestconcentratie. Bij eenzelfde mestconcentratie nam de gasproduktie sterk toe bij een verlenging van de verblijftijd van 10 tot 15 dagen. Bij verblijftijden boven 15 dagen nam de gasproduktie per kg toegevoerde droge stof en de reductie van stankcomponenten nauwelijks toe.

Alhoewel de vergisting nog goed verloopt bij een belasting van 6 kg DS m^{-3} dag^{-1} bleek de optimale belasting met betrekking tot de stankreductie en de energieproduktie ongeveer 4 kg DS m^{-3} dag^{-1} te zijn, althans wanneer deze belasting wordt verkregen door mest met een droge stof gehalte van 6% te vergisten bij een verblijftijd van 15 dagen. Uit de experimenten bleek verder, dat de hydrolyse van onopgeloste mestbestanddelen de beperkende stap is in het gistingsproces.

In hoofdstuk 5 wordt aandacht besteed aan de invloed van de temperatuur op het gistingsproces. Dagelijks gevoede vergisters werden bedreven bij een belasting van ca. 4 kg DS m^{-3} dag^{-1} en bij temperaturen varierend van 13°C tot 55°C. Bij een temperatuur van 13°C werd er geen gas gevormd. In het mesofiele temperatuurgebied (20 - 40°C) nam de gasproduktie toe met de temperatuur, vooral bij een verhoging van de temperatuur van 20°C naar 25°C. In het gebied van 25°C tot 40°C was de invloed van de temperatuur minder groot: de gasproduktie nam lineair toe met ongeveer 1% per graad temperatuurverhoging. Onder thermofiele omstandigheden (55°C) was de methaanproduktie ca 25% lager dan bij 35°C, alhoewel meer onopgeloste mestbestanddelen werden gehydrolyseerd. Het bleek dat thermofiele vergisting gevoeliger is voor hoge ammoniumstikstof-concentraties dan mesofiele vergisting, wat waarschijnlijk toegeschreven moet worden aan een toename van de concentratie aan ongedissocieerd ammonia bij toenemende temperatuur (bij constante pH en totale ammoniumstikstof-concentratie).

De resultaten van deze experimenten geven aan dat mesofiele vergisting het meest geschikt is voor de behandeling van varkensdrijfmest. De optimale vergistingstemperatuur met betrekking tot de produktie aan netto-energie (geproduceerde energie minus de energie gebruikt voor verwarming van de mest) is 27 - 30°C wanneer hoogwaardige brandstof, b.v. biogas, aardgas of olie, wordt gebruikt om de vergistingsinstallatie op de gewenste temperatuur te houden en 35 - 40°C wanneer voldoende afvalwarmte, b.v. koelwater, beschikbaar is.

In de algemene discussie (hoofdstuk 6) wordt een aantal aspecten van de vergisting van varkensdrijfmest besproken, zoals de variaties in de gasproduktie onder vergelijkbare procesomstandigheden, de mate van stabilisatie van de mest na vergisting en de stabiliteit van het proces. Verder wordt op basis van de verkregen resultaten aangegeven, dat de gasproduktie uit varkensdrijfmest kan worden verhoogd door een voorbehandeling van de mest. Gezien de energieconsumptie van dergelijke bewerkingen valt het echter te betwijfelen of de extra gasproduktie voldoende is om het energieverbruik van de voorbehandeling te compenseren.

In de appendix zijn een aantal voorlopige experimenten beschreven, die er op waren gericht inzicht te krijgen in de eliminatie van twee aromatische stankcomponenten, nl. fenol en *p*-cresol, tijdens het gistingsproces. De resultaten van deze experimenten geven aan dat fenol en *p*-cresol door vergiste varkensdrijfmest kunnen worden omgezet in methaan. Verder zijn er duidelijke aanwijzingen verkregen dat fenol een tussenprodukt is bij de vergisting van *p*-cresol. Hieruit kan worden afgeleid dat demethylering van *p*-cresol tot fenol waarschijnlijk de eerste stap is bij de vergisting van *p*-cresol.

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