MESOPHILIC AND PSYCHROPHILIC DIGESTION OF LIQUID MANURE

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MESOPHILIC AND PSYCHROPHILIC DIGESTION OF LIQUID MANURE

Proefschrift
ter verkrijging van de graad van
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STELLINGEN

1. Het niet slagen van de vergisting van koemest bij lage temperaturen (< 18°C) moet worden toegeschreven aan de toegepaste korte verblijftijden.
Yaldiz, O. (1987). Laboruntersuchungen zur Methanproduktion aus Rinder- und Hühnerflüssigmist bei verschiedenen Reaktionsbedingungen als Grundlage der Prozeßoptimierung von unbeheizten Biogasanlagen. Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften, Universität Hohenheim.

2. Toepassing van vergisting van mest in combinatie met opslag kan bijdragen tot de reductie van het gebruik van fossiele energie en van de ammoniak emissie en is uit dien hoofde sterk aan te bevelen.

3. De opstart van een accumulatie systeem voor de vergisting van mest bij 15°C, met niet 'geadapteerd' slib, dient bij 20°C plaats te vinden.
Dit proefschrift

4. Uit oogpunt van doelmatig gebruik van grondstoffen en energie is het noodzakelijk bij mestverwerking de grote hoeveelheid ammonium-stikstof, die in de mest aanwezig is, terug te winnen t.b.v. hergebruik en niet d.m.v. het nitrificatie/denitrificatie proces om te zetten in het 'waardeloze' stikstofgas.

5. Mestverwerking zou weliswaar het probleem van de mestoverschotten kunnen oplossen maar zal daarmee de intensieve veehouderij nooit tot een duurzaam systeem maken, aangezien niet wordt voldaan aan de voorwaarden voor werkelijke duurzaamheid.

6. Het grote verschil in gebruik van mestvergisting en rioolslibvergisting vindt z'n grond in de toekenning van verschillende belangen aan de effecten van vergisting.

7. De kritische houding t.a.v. het kweken van algen in mest, getuigt niet van conservatisme, zoals gesteld door Mur, maar van realisme.
Vink, S. (1991). 'Algen vormen gewoon een nieuw gewas'. Amsterdams hoogleraar ergert zich aan Wageningse conservatisme. WUB, 7-3-1991/ 5.

8. Door het maken van constructies om personeel langer in tijdelijke dienst te houden dan wettelijk toegestaan, wordt zowel misbruik gemaakt van de regels als van het personeel.

9. De keuze van 'duurzame armoedebestrijding' als hoofddoelstelling in het Nederlandse ontwikkelingsbeleid is een *contradictio in termine*.
Een wereld van verschil. Nieuwe kaders voor ontwikkelingssamenwerking in de jaren negentig. Tweede kamer, vergaderjaar 1990-1991, 21813, nrs. 3-4.

10. Niemand is verder van de waarheid, dan hij die denkt alle antwoorden te weten.
Brand, L. (1990). Helders Weekblad, 28-12-1990.

11. Vele wetenschappelijke onderzoeken zijn van dien aard dat de looptijd hiervan zich zou dienen uit te strekken over een dusdanig lange periode dat de uitvoering van het onderzoek uitstekend is te verenigen met de wensen van deeltijdwerkers.

G. Zeeman
Mesophilic and psychrophilic digestion of liquid manure
Wageningen, 3 mei 1991.

aan: mijn moeder en vader,
Ruben en Piet Hein

VOORWOORD

Bij de totstandkoming van dit proefschrift wil ik graag allen bedanken die hieraan een bijdrage hebben geleverd.
Het onderzoek is begeleid door Gatze Lettinga. De goede samenwerking, de vele ideeën en kritische opmerkingen die naar voren kwamen gedurende het onderzoek en bij het schrijven van het proefschrift hebben in belangrijke mate bijgedragen tot het uiteindelijk resultaat. De samenwerking met Lood v. Velsen, zowel tijdens zijn aanstelling bij de vakgroep als bij Haskoning was zeer stimulerend. De assistentie van analytisch personeel bij dit onderzoek was onontbeerlijk. Met Hennie Halm, Marianne Koster en Theo Vens is gedurende een lange periode op bijzonder prettige wijze samengewerkt. Toon Helmink, Paul de Jong en Paul van der Ven assisteerden gedurende kortere tijd bij de uitvoering van het onderzoek. Verscheidene studenten hebben in het kader van hun doctoraal studie meegewerkt aan het onderzoek. Met name de resultaten van het onderzoek van Jan Formsma hebben een bijdrage geleverd aan dit proefschrift. De heren van de Centrale Dienst van het Biotechnion waren altijd bereid technische assistentie te verlenen indien dat noodzakelijk was voor de voortgang van het onderzoek. Katja Grolle heeft veel tijd en aandacht besteed aan het corrigeren van dit proefschrift. De heren van de tekenkamer van het Biotechnion, in het bijzonder de heer Rijpma, hebben een aanzienlijke bijdrage geleverd aan dit proefschrift in de vorm van adviezen bij de lay-out en het uitvoeren van alle tekenwerk. Tijdens het onderzoek is op constructieve wijze samengewerkt met de vakgroep Microbiologie van de Landbouwuniversiteit, het IMAG en MT-TNO (Delft). De samenwerking met Art Wellinger, van het 'Swiss Federal Research Station for Farm Management and Agricultural Engineering', bij het schrijven van onze gemeenschappelijke publicatie werd zeer gewaardeerd. Het werken op de vakgroep Milieutechnologie werd bijzonder op prijs gesteld. De leden van de begeleidingscomissies onder voorzitterschap van Harm Kruijdenberg van NOVEM hebben zich veel inspanning getroost om het onderzoek naar anaërobe vergisting van mest tot een succes te maken.

Het onderzoek is gefinancierd in het kader van het NOH programma, beheerd door NOVEM en RIVM.

Ontwerp omslag: Anne Margreet Louwerse

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CHAPTER 1
GENERAL INTRODUCTION

1.1. LITERATURE REVIEW

1.1.1. History of sludge and slurry digestion.

The history of slurry digestion can be traced from 1808, when Davy (1814) collected methane gas from cattle manure in a retort under vacuum. Gayon, (Dubaquie, 1943) investigating manure digestion in 1883-1884, collected such large amounts of gas, that Pasteur suggested to utilize the gas produced from horse manure for improving street illumination in Paris (Le Figaro, 1884).

The first tank to separate and retain solids from sewage was designed in 1850. Mouras developed in 1860 the first unit to treat settled waste water. The first septic tank was built in Cameron in Exeter in England in 1895, where the gas was really used for street illumination (Liebmann, 1956). The first dual purpose tank, incorporating sedimentation and sludge digestion was installed in Hamston in England. Some months later the well known Imhoff tank, based on a similar principle was introduced in Germany. It is now over 60 years ago since the earliest heated and enclosed tanks with gas collection facilities were installed at sewage works in Europe for separate stabilization of raw sewage sludge. Since then anaerobic digestion plants have demonstrated their great value at many hundreds of sewage works (Bruce, 1985).
The main objective of sewage sludge digestion is the stabilization of the primary and secondary sludge produced at settling and aerobic treatment of sewage. The produced methane gas is mostly considered as a useful by-product. When further treatment of sewage sludge will become necessary, anaerobic digestion of sludge could also be aimed at improvement of the dewaterability of the sludge. While anaerobic digestion of sewage sludge was continuously applied from the start of the technique, the digestion of agricultural residues was only applied in periods of energy shortage, e.g. during and shortly after the World War II in France, Algeria and Germany (Tietjen, 1975). In the period after the war, the interest in anaerobic digestion of agricultural wastes diminished due to low prices of fossil energy. Only after the energy crisis in 1973, the interest in energy production by anaerobic digestion of agricultural residues increased, e.g. from animal slurry. Research in anaerobic digestion of animal manure in the Netherlands was started in 1976 (v. Velsen, 1981). The results obtained, led to the installation of the first full scale digester on a pig farm in 1979. The expected rise in energy prices stimulated research on the anaerobic digestion and also the building of several full scale on farm digesters. In a few years period of time, 25 digesters were build, both on pig and dairy cow farms. The aim for applying anaerobic digestion of animal slurry on farm was mainly energy production. A concomitant benefit of anaerobic digestion of manure is the prevention and reduction of odor (v. Velsen, 1981).
The development of the CSTR type digesters for the digestion of animal slurry was based on the sewage sludge digesters. However the on-farm application required a cheaper design and construction. The latter was one of the reasons that in the first instance many technical problems had to be solved. Higher investment costs and lower natural gas prices than originally supposed, are cause of the present break in the development and construction of the on-farm CSTR type digesters. Demuynck and Nyns (1984) report that somewhat over 500 biogas installations are in use on European farms. But throughout Europe, the rate of construction has slowed down remarkably for the last years (Wellinger, 1988). The increasing environmental problems, e.g. the greenhouse effect due to excessive use of fossil energy, could give a new impulse to the further development of 'alternative energy'. The, in this thesis described, technique of anaerobic digestion in combination with storage of animal slurry can contribute to the reduction of the use of fossil energy and the emission of ammonia during storage of animal manure.

1.1.2. Objectives of slurry digestion.

Anaerobic digestion of animal manure could aim at several aspects:

energy production and reduction of CO_2 emission by replacing fossil energy.
Anaerobic digestion of animal manure will lead to a partial conversion of the slurry-COD into methane-COD. In the digestion of pig slurry about 40% of the COD will be converted in CH_4-COD (v. Velsen, 1981), while in the digestion of cow slurry this is approximately 25% (this thesis). The major amount of slurry in the Netherlands is produced by pigs and cows. In 1988 a total amount of $^{+}83.5 * 10^9$ kg slurry was produced, from which $58.7 * 10^9$ by cows and $19.9 * 10^9$ by pigs (Landbouwcijfers, 1989). From this amount of manure approximately $616 * 10^6$ plus $261 * 10^6$ m^3= 877 $.10^6$ m^3 CH_4 gas can be produced, which accounts for 1.8 % of the annual natural gas consumption in the Netherlands.
By using this amount of gas and replacing fossil energy, reduction of the CO_2 emission is accomplished. The latter assumes that at spreading 'fresh' slurry on the fields, degradable organics are degraded to CO_2 and H_2O. At the increasing storage periods of animal slurry the amount of CH_4 gas spontaneously produced by anaerobic digestion in the storage will definitely increase (this thesis). Methane contributes to a greater extend to the 'greenhouse effect' than CO_2 (Goossensen and Meeuwissen, 1990). Controlled anaerobic digestion of animal slurry and subsequent use of the biogas will then become even more important.

reduction /prevention of the formation of malodorous compounds.
During storage of slurry, malodorous compounds are formed, which has to be attributed to the occurrence of an incomplete anaerobic fermentation. When subjected to anaerobic digestion under controlled conditions, most of the digestible organic matter is eliminated, resulting in the production of a stabilized material without offensive odors (v. Velsen, 1981). The results of the research of v. Velsen (1981) illustrate that the odorous compounds phenol, p-cresol, 4-ethyl-phenol, indole and skatole are removed in mesophilic anaerobic digestion of slurry provided the detention time is long enough. The reduction of offensive smell could be a reason for the application of digestion processes, especially when the farm is situated in a populated region.

improvement of the fertilizing value.
Van Nes *et al.* (1990) review the results of fertilizing experiments with digested and non-digested slurry. The results are varying. Application of digested slurry on grassland mostly results in higher TS recovery of the first cutting, the recovery of the second cutting however is mostly lower compared to fertilization with non digested slurry. Altogether both lower and higher yields are found for fertilization with digested in comparison wit non-digested slurry. From the results of the limited research on the application of digested cow slurry on arable land, a positive effect is shown compared to application with non digested cow slurry. No differences are found between digested and non digested pig slurry.

reductions of pathogens and seeds.
Demuynck *et al.* (1984a) review the effect of anaerobic digestion on disease survival in sludge and animal manure. They concluded that in theory the effect of pathogen reduction by anaerobic digestion is significant for bacteria, pathogens, viruses, parasitic cysts and plant pathogens, whereas it is poor for parasitic eggs. In practice, especially in completely mixed digesters, this effect is reduced by short-circuiting and by simultaneous drawing off and feeding the digester. Farrell *et al.* (1988) conclude from their research on the influence of the feeding procedure on microbial reduction during anaerobic digestion, that draw/fill operation produces much larger reduction in bacterial densities than fill/draw operation. The effect was similar but smaller for viruses.
Besson *et al.* (1987) researched the effect of digestion, in comparison with aeration and storage, of animal manure on the germinative faculty of weed-seeds in batch systems. The best results are found with anaerobic digestion. The germinative faculty of the seeds is reduced to zero after three weeks in an anaerobic digestion system for cow slurry at 33°C. The reduction

of the germinative faculty in pig slurry is much faster. Even after only two weeks storage, at 14°C, the germinative faculty is reduced to zero.

Temperature is an important parameter controlling pathogens in anaerobic digestion systems. Demuynck *et al.* (1984a) conclude that thermophilic digestion significantly improves the reduction of pathogens as compared to mesophilic digestion and produces an effluent almost free of pathogens. Last was also concluded by Shih (1988) from the results of his research on pathogen control by anaerobic digestion of poultry waste.
On the contrary, the inactivation rates are considerably reduced at temperatures $\leq$ 20°C. Pike (1982) reported that at temperature of 20-25°C, 90% reduction of respectively bacteria, parasite ova and viruses, require one month, 6 months and more than 2 months. At temperatures below 20°C, these periods are increased considerably (Demuynck *et al.* 1984a). However at low temperature digestion rather long detention times (at least 3 months) will be applied.

(in combination with storage) reduction of the NH3 emission
A considerable part of the nitrogen in animal slurry is available as NH_4^+-N. Depending on the pH and temperature, a part of the total ammonium is in the NH_3 form. During (open) storage of slurry, emission of NH_3 will occur. Digested slurry normally contains slightly more NH_3 than undigested slurry. Open storage of digested slurry can therefor result in more NH_3 emission than open storage of undigested slurry. However, when slurry is digested in an accumulation system, where digestion and storage are combined (Wellinger and Kaufmann, 1982, this thesis), ammonia emission during storage is prevented. The emission of ammonia at the subsequently spreading of the slurry on the fields could be reduced by slurry injection.

simplification of the further processing of the animal slurry.
Anaerobic digestion of animal manure on-farm is mainly aimed at producing energy. But slurry digestion can also be part of a slurry purification system. Such a slurry treatment system should aim at minimal energy consumption and maximal recovery of valuable constituents. Anaerobic digestion contributes to the first objective by the production of biogas. A proper system to recover a part of the nitrogen, is by stripping ammonia from the separated liquid fraction (v. Velsen, 1985, Drese, 1988). The NH_4^+-N concentration and the pH are increased by anaerobic digestion. Both are favorable for the application of the air stripping process.

The separation of the 'solid' and the 'liquid' fraction as a pretreatment step is often proposed for slurry processing systems (Rulkens, 1990). The results of research of van Veen (1983), show that anaerobicly digested slurry is easier to dewater than undigested slurry. The results of Lawler *et al.* (1986) demonstrate that the improved dewaterability of sewage sludge, after anaerobic digestion, is caused by a decrease of the specific surface area of the sludge particles. However, at an incomplete digestion, opposite results can be found (Lawler *et al.*, 1986).

1.1.3. Comparison between sludge and animal slurries

The difference between sludge and slurry digestion can be deduced from the influent characteristics of these two types of raw materials. The composition of sewage sludge and animal slurry, as used by several researchers is given in resp. Table 1 and 2.

Table 1. Composition (in % of the total solids) of sewage sludges used by several researchers.

	Fat	protein	carbo-hydrates	anorg. residue	reference
prim.+sec.	20	28	23	29	1
prim.+sec.	18	37	-	45	3
prim.+sec.	-	28	-	29	4
sewage sludge	16-44	19-28	12-31	20-40	2
prim.	14.5	22	38	25.5	1
prim.	-	20.5	-	32	4
prim.	13.8	25.3	23.9[+]	28	4
prim.	14.7	33.5	22.8[+]	30	4
sewage sludge	20.5	32.0	22.5	25	5
sewage sludge	14.8	27.5	30.5	27.3	5

[+] calculated by substraction

1. Temper *et al.* 1981; 2. Kotzé *et al.* (1969); 3. Wechs (1985); 4 Temper (1983); 5. Steiner (1983).

Table 2. Composition (in % of the total solids) of animal slurries, as used by several researchers.

	Fat	protein	carbo-hydrate	cellu-lose	hemi-cellulose	lignin	anorg. residue	refer.
cow slurry	6.1	13.7	59.9	-	-	-	20.3	1
cow slurry	6.1	15.0	62.1	-	-	-	16.9	1
cow slurry	7.5	15.6	-	14.5	19.3	8.2	29.0	2
cow slurry	3.5	15	-	17.0	19.0	6.8	28.0	3
cow slurry	4.0	15[+]	-	25.0	2·0	9.0	16.0	6
pig slurry	12.3	16.0	-	10.3	17.1	3.7	17.3	2
pig slurry	7.7	20.9	53.8[*]	22.9	20.8	10.1	17.6	4
pig slurry	7.0	28.9	-	-	-	-	27.0	5

[*] cellulose, hemi-cellulose,lignin; [+] {(total-N)-(NH_4^+-N)}*6.25.

1. Steiner (1983); 2. Wellinger, (1984); 3. Varel, Isaacson, and Bryant (1977); 4. Hobson, Bousfield and Summers (1974); 5. Temper (1983); 6. Robbins, Gerhardt and Kappel (1989).

Table 1 shows that the total solids of sewage sludges, generally consists of ± 15% fat, while proteins and carbohydrates both account for 20-30 % of the total solids. Table 2 shows that animal slurries contain just a very small amount of fat (± 5% of the total solids), while the protein content is somewhat higher, viz. ± 15-30%. Animal slurries mainly consist of carbohydrates (40-60%).

Apart from the composition of the organics, the TS concentration and concentration of nutrients of animal slurry and sewage sludge can differ considerably. Table 3 gives the mean concentrations nutrients, TS and VS of cow and pig slurry and sewage sludge as found for the Netherlands.

Table 3, Mean solids and nutrients concentrations of animal slurry (CAD, 1987) and sewage sludge (CBS, 1988).

	cow slurry	pig slurry	sewage sludge
TS (g/l)	95	75	45.2
VS (g/l)	75	50	25.5
N (g/l)	4.4	6.5	2.2
P_2O_5(g/l)	1.8	3.9	2.5
K_2O (g/l)	5.5	6.8	0.2
CaO (g/l)	2.1	3.5	3.9
MgO (g/l)	1.0	1.5	0.3
Na_2O(g/l)	1.0	1.0	-
Cl (g/l)	3.0	1.7	-
SO_3 (g/l)	1.8	1.6	-

The NH_4^+-N concentration of animal slurry is considerably higher than that of sewage sludge. The consequences of high NH_4^+-N concentration for the digestion process are described in Chapter 1.1.8.1 and Chapter 6.

Animal slurry is a rather inhomogeneous substrate. It contains a large amount of solids of several sizes. Table 2 shows that an important part of the total solids is made by fibrous materials, viz. cellulose, hemi-cellulose and lignin. Cellulose and hemi-cellulose are anaerobically degradable but the structure of the fiber will determine whether or not it will actually be digested. Lignin is generally recognized as anaerobically inert and to limit the extent of carbohydrate digestion. Chandler *et al.* (1980) found a linear decrease of TS degradation with lignin content for several plant materials and animal slurries.
Animal slurries also contain dissolved organic components. Altmann and Dittmer (1974) review ± 100 and 40 components occurring in resp. cattle and pig urine. An substantial part of the dissolved fraction of both pig and cow slurry consists of anaerobically refractory material. The amount of dissolved refractory organics in pig slurry amounts to ±10% of the influent-COD (v. Velsen, 1981) and in cow slurry this amounts to 15-20% of the influent-COD.(Chapter 4 of this thesis). The other part of the dissolved fraction is made up by VFA's. The concentration of the VFA's varies with the type of slurry but also with the storage conditions (Chapter 2). The VFA concentration in pig slurry mostly is higher than in cow slurry and generally also a higher gas production from pig slurry is found. The fact that cow slurry already is partially digested in the rumen represents an important reason for this difference.

1.1.4. Comparison between rumen digestion and slurry digestion.

The rumen very likely is the most investigated methane-producing ecosystem and has been subject to many reviews (Gijzen, 1987). Hobson and Wallace (1982) have reviewed results of the last ten years work in rumen microbiology and biochemistry. This review includes 750 references. Anaerobic digesters and rumens are similar habitats with similar microbes. This is demonstrated experimentally using the techniques for culturing extreme anaerobic bacteria (Hobson, 1983a). Hungate (1950) cultured cellulolytic bacteria from a rumen and a sewage digester. However, besides similarities the two systems show also important differences. Some of the characteristic resemblances and differences between the two systems are summarized below.

Similarities between a rumen and a slurry digester:

-The rumen and slurry digesters are inoculated with the same inoculum bacteria. The method of the development of the final population follows similar lines, in that both populations are

allowed to develop by gradually increasing the feed rate in a continuous culture system. In both systems the populations take many weeks or months to develop and stabilize (Hobson, 1983a).
-Both systems are mixed, the digester by mechanical or gas stirrers, the rumen by contraction and expansion of the rumen wall (Hobson, 1983a).

Differences between a rumen and a slurry digester:

-Although the principal substrate for both systems are vegetable fibers, the feed of the digester contains a relatively high concentration recalcitrant fibers, i.e. fibers, which could not be digested sufficiently well in the animal rumen or guts.

-The stirring in a CSTR only provides a homogeneous mixing and an equal detention time of bacteria, solids and liquid. The rumen contraction and expansion also provides favorable conditions for speeding-up the fibre degradation by exposing more surface of the solids to bacterial attack and colonization. Moreover solids are prevented from leaving the rumen, until they have reached at least millimeter size. So, unlike the slurry digester, the detention time of the solids in the rumen can be much longer (depending on the particle size and rate of degradation) than the liquid detention time (Hobson, 1983a).

-The stability of the anaerobic digestion of animal slurry in a CSTR is only controlled by the feed input. The stability of the rumen digestion however, is controlled by saliva, which washes out fermentation products and increases the buffering capacity. Moreover the absorbtion of VFA's and ammonia through the rumen wall prevent the occurrence of inhibitory concentrations of these compounds.

-The detention time in the rumen is much lower than in a anaerobic slurry digester, viz. 0.5-3 days and 10-20 days resp. The latter is the most important reason, that different reactions occur or reactions occur at different rates (Hobson, 1983a). The rumen optimizes the degradation of feed to the lower fatty acids needed by the animal tissues and minimizes the methanogenesis, which is useless for ruminants. The anaerobic digester was developed to maximize methane production and to minimize the residual fatty acid concentration.

-Protozoa play an important part in the rumen digestion. Demeyer (1981) concludes from a literature research, that protozoa account for ± 34% of the total rumen fibre digestion. No protozoa activity so far was reported in anaerobic slurry digesters.

1.1.5. Systems applied in practice.

Although the CSTR so far is the usually applied system for the slurry digestion in Western Europe, presently several other systems are implemented (Demuynck *et al.*, 1984).
Anaerobic slurry digesters can be classified as follows:

-batch systems.
-accumulation systems, which can be used for combined storage and digestion.
-continuous systems, i.e.
. CSTR
. plug flow
-high rate systems, like anaerobic filter systems or UASB reactors, which can be used for the digestion of the liquid fraction of animal slurry.

The application of the various digestion systems is discussed below.

1.1.5.1. Batch digestion systems.

A batch reactor is filled in one go with 85-90% fresh slurry and 10-15% inoculum. In a period of time of ±30 days the digestible material is gradually converted to methane gas, generally at mesophilic conditions. In order to overcome the great differences in gas production rate during the digestion period, two different batch reactors could be performed out of phase. Although a batch system is a rather simple system, on farm application needs, unlike the accumulation system (see 1.1.5.2), an additional influent and effluent tank. However, when continuous feeding and mixing becomes complicated, i.e. at the digestion of concentrated manure, the application of a batch system should be considered.

1.1.5.2. Accumulation systems.

Unlike the batch-system the accumulation system is continuously fed and characterized by an increasing effective reactor volume in time. The reactor is, like the batch-system, emptied in one go. The accumulation system actually is the most simple system for on-farm application of slurry digestion as it employs all the facilities normally available on a farm and it optimizes the processes, proceeding in a regular slurry storage. Extra facilities required as compared to a normal storage consist of equipment for the collection and the use of the produced biogas and equipment to optimize the process temperature, viz. isolation and/or heating. Moreover ± 10-15% of the stored, 'digested' slurry should be left in the system in order to optimize the digestion during the next storage period. Wellinger and Kaufmann (1982) were the first to publish about the successful full scale application of an accumulation system under the slats at two newly build pig farms. Based on the results of laboratory (Chapter 2, 3, 5 of this thesis) and pilot plant research (Hoeksma *et al.*, 1987), the first full scale accumulation system for the storage and digestion of pig slurry was build in the Netherlands at the Research Station for Pig Breeding in Rosmalen in 1988. In this case the storage/digestion is applied in a 700 m^3 silo, with heating facilities, next to the farm (v. Asseldonk and Voermans, 1990).

1.1.5.3. Plug flow systems.

The plug flow system is continuously fed and the feed passes through the reactor in a horizontal direction. No mixing is provided. All slurry remains in the reactor for the full detention time. The latter is especially important when digestion is also aimed at a maximal pathogen reduction. A plug flow system provides a constant gas production at a constant loading rate. At on-farm application an additional effluent tank is needed.
The results of the research of Hayes et al. (1979) showed that a plug flow system is appropriate for the digestion of slurry with a total solids concentration of 10-12%. At low TS concentrations problems with floating and settling layers will appear. In the Netherlands the application of a plug flow system for the digestion of cow slurry was not successful due to the occurrence of floating layers (Bruins, 1984).

1.1.5.4. CSTR systems

A CSTR system is characterized by a continuous feeding rate and a complete mixture of bacteria and substrate. Bacteria and substrates consequently have an equal detention time. At a constant loading rate, a constant gas production rate is provided. Anaerobic digestion of animal slurry in a CSTR is generally applied at mesophilic conditions (Demuynck *et al.*, 1984). In Denmark also thermophilic digestion of animal slurry is executed aiming at a maximal pathogen reduction (Van Diemen and Van Nes, 1989). Like a plug flow system an additional effluent tank is necessary at an on-farm situation.

1.1.5.5. High rate systems

As animal slurry contains a lot of suspended organics, high rate systems, like UASB or anaerobic filter, are unfit for the digestion of the raw slurry. The digestion of separated liquid pig slurry and also raw liquid calf manure in an anaerobic filter and UASB systems have been demonstrated by Kennedy and van den Berg (1982), Poels *et al.*, (1981), Colleran *et al.*, (1982) and Schomaker, (1987). Separation of liquid and solid fraction with subsequent digestion in, e.g. an UASB and a 'solid' digester should be considered at large scale application (Zeeman and Lettinga, 1990). For on-farm application the latter becomes too expensive.

1.1.6. The course of the anaerobic digestion process.

1.1.6.1. Hydrolysis

The hydrolysis step generally is considered as the rate limiting step in anaerobic slurry digestion systems (Hobson, 1983, v. Velsen, 1981, Bousfield *et al.*, 1979). As mentioned in Chapter 1.1.3. (hemi)-cellulose is the main component of the solid fraction of animal slurry. The rate of hydrolysis of this component is determined by both microbiological constraints such as generation time of the organisms involved and the rate of cellulase production and physical and chemical characteristics of the substrate such as, cristallinity of cellulose , degree of association with lignin (see chapter 1.1.3.) and surface area/ particle size ratio (Gijzen, 1987).

According to the literature review of Hobson and Wallace (1982), four species of cellulolytic bacteria can be considered as being the most important in the rumen, viz. *Bacteroides succinogenes, Ruminococcus albus, R. flavefaciens* and sometimes *Butyrivibrio fibrisolvens.* A greater variety of cellulolytic bacteria occurs in anaerobic sludge and slurry digesters (Hobson, 1983). Hungate (1950) isolated three different strains of cellulolytic rods from digested sewage sludge. Maki (1954) isolated 10 different species from digested sewage sludge and distinguished two groups of cellulolytic bacteria. The first group was characterized by a high cellulolytic activity and the second by a low cellulolytic activity. Hobson and Shaw (1974) isolated 11 types of cellulolytic bacteria from digested pig slurry while Sharma and Hobson (1985), showed that digested cattle waste consists of a heterogeneous cellulolytic bacterial population from which 390 isolations were made. They distinguished eleven groups which were investigated in detail. Five of these groups were similar to *Clostridium butyricum, beijerinckii, acetobutylicum, bifermantans* and *sporogenes.* Houwaard (1984) reported the isolation of approximately 100 cellulolytic bacteria from 'fresh' and digested cattle manure.

The practical applicability of the results of the different studies mentioned above, so far are very limited. Some researchers give temperature and or pH optima for some of the cellulolytic species (Houwaard, 1984; Sharma and Hobson, 1985) but it is unknown which organism plays which role in these extremely complex systems of slurry digestion (see also Chapter 1.1.9.1).

Cellulolytic bacteria have been shown to attach to the solids, both in the rumen and in anaerobic digesters (Hobson and Wallace, 1982; Houwaard, 1984). The physical coupling of the microorganisms to their substrate enables them to employ their cellulolytic enzyme activities in an more optimal way (Gijzen, 1987). Cellulase in fact consists of a mixture of cellulolytic enzymes, viz. exo-glucanases, endo-glucanases and cellobiases (Garcia-Martinez, 1980; Khan, 1980 and Ljungdahl and Eriksson, 1985). A scheme of the working mechanism of the different cellulolytic enzymes is given in Figure 1 (Beldman, 1986). Many differences have been reported between cellulases originating from the various bacterial species with respect to subunit composition, molecular weight, substrate specificity and activity of isolated enzymes (Ljungdahl and Eriksson, 1985). According to the present knowledge, cellulolytic enzyme systems are almost as diverse as cellulolytic microorganisms (Gijzen, 1987).

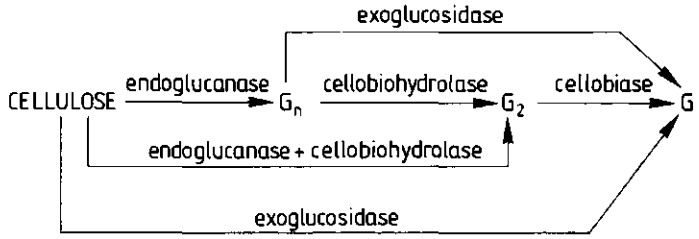

Figure 1. Model according to Klyosov for the breakdown of cellulose. G=glucose (Beldman, 1986).

The appearance of inhibition of the hydrolysis in anaerobic slurry digestion systems so far has rarely been reported in literature. V. Velsen concludes from his digestion experiments with pig manure that the decrease in methane production as a result of addition of urea cannot be attributed merely to the inhibition of the methanogenesis but is also caused by inhibition of the hydrolysis step. Recent results of experiments of Koster (1989) with chicken manure indicate that inhibition of hydrolysis may occur at high NH_4^+-N concentrations.
Chung (1976) reported that accumulated H_2 could inhibit hydrogen producing cellulolytic bacteria. Inhibition of cellulase by the soluble products cellobiose and glucose has been reported by Ladisch *et al.* (1983) and Ljungdahl and Eriksson (1985). Chesson *et al.* (1982) found that the plant phenolic acids, trans-p-coumaric and trans-ferulic suppressed completely the growth of the cellulolytic strains *Ruminococcus albus, Ruminococcus flavefaciens* and *Bacteroides succinogenes* when exposed to a simple sugar medium at concentrations >5 mM. Also the extent of cellulose digestion by *R. flavefaciens* and *B. succinogenes* was substantially reduced.
Hobson (1983) reported that dry solids of piggery waste and cattle waste contained resp. 0.14 and 1.14% of trans-p-coumaric plus trans-ferulic acid, which could possibly lead to inhibition in the digestion process.

1.1.6.2. Acidogenesis

The soluble products of the hydrolysis are metabolized intracellularly by hydrolytic and non-hydrolytic bacteria. Pure cultures of bacteria produce a variety of end products from carbohydrates (Wolin and Miller, 1983). The environmental conditions in an anaerobic digester will determine the actual products of the acidogenesis. The partial hydrogen pressure (pH_2) plays an important role in the control of the proportions of the various products. At a low pH_2 (< 10^{-3} atm.) the acidogenic bacteria produce more H_2, CO_2 and acetate, while at high pH_2 more reduced products, such as propionate, ethanol or lactate are generated (McInerney and Bryant (1981b). The latter could cause the development of different bacterial populations in e.g. an batch reactor, an accumulation system and a CSTR for the digestion of animal slurry. The results of the research of v. Velsen (1981) and the in this thesis published results of research of anaerobic digestion of resp. pig and cow slurry show that acidogenesis becomes not rate limiting in digestion of animal slurry.

1.1.6.3. Acetogenesis

Bryant *et al.* (1967) demonstrated for the first time that ethanol could not directly be used as a substrate for methanogenic bacteria, but was first oxidized to acetate and H_2 by acetogenic bacteria. From that time it became clear that acetogenic bacteria form an intermediate metabolic group which may deliver substrates for the methanogens. The evolved hydrogen has to be removed because it negatively affects the energy derived from the oxidation of propionate, butyrate and ethanol (Gujer and Zehnder, 1983). In a stable anaerobic system the hydrogen is removed by the hydrogen consuming methanogens or sulfate reducers. This process is referred to as interspecies hydrogen transfer (Ianotti *et al.*, 1973). Hydrogen partial pressure

should be kept below $2*10^{-3}$ and $9*10^{-5}$ atm. for the degradation of butyrate and propionate, respectively (Zeikus, 1980 and McInerney *et al.* , 1980). The acetogenesis can indirectly be disturbed by inhibition of hydrogen consuming methanogens as proposed by Wiegant and Zeeman (1986) for the NH_4^+-N inhibition in animal slurry digestion at thermophilic conditions (see also Chapter 1.1.8.1).
Recently, Blomgren *et al.* (1989) found strong evidence that at mesophilic conditions, in a batch as well as in a continuous enrichment culture on acetate, at a NH_4^+-N concentration of 7 g/l, acetate was converted to methane by means of a two step mechanism. This requires a syntrophic relationship between an acetate oxidizing bacterium and a hydrogenotrophic methanogen. The same batch experiment, but at a drastically lower NH_4^+-N concentration (0.3 g/l) resulted in acetoclastic methane formation, as indicated by the highly enriched *Methanosarcina* culture. Zinder and Koch (1984) reported the oxidation of acetate to CO_2 and H_2 to occur at thermophilic conditions.

1.1.6.4. Methanogenesis

The final step in the digestion of complex organic material to CO_2 and CH_4 is carried out by the methanogenic bacteria. Methanogenic bacteria can only use a few substrates. The possible methanogenic reactions are given in Table 4.
The conversion of acetate (acetoclastic methane formation) and H_2/CO_2 (hydrogenotrophic methane formation) into CH_4 are the two most important reactions. Although only a few species of the methanogens isolated up to now are capable of acetoclastic methane formation, approximately 70% of the methane production from complex organic material is derived from the methyl group of acetate (Jeris and McCarty, 1965; Smith and Mah, 1966, Mah *et al.*, 1977, Mackie and Bryant, 1981, Boone, 1982).

Table 4. Methanogenic reactions and the values of free energy changes (ΔG^0) (Zehnder *et al.*, 1982)

$CH_3COO^-+H_2O$	→	$CH_4+HCO_3^-$	$\Delta G^0_=$- 28.2kJ/mol CH_4
$4CH_3OH$	→	$3CH_4+CO_2+2H_2O$	$\Delta G^0_=$-102.5kJ/mol CH_4
$4HCOO^-+2H^+$	→	$CH_4+CO_2+2HCO_3^-$	$\Delta G^0_=$-126.8kJ/mol CH_4
$4H_2+CO_2$	→	CH_4+2H_2O	$\Delta G^0_=$-139.2kJ/mol CH_4
$4CO+2H_2O$	→	CH_4+3CO_2	$\Delta G^0_=$-185.1kJ/mol CH_4
$4CH_3NH_2+ 2H_2O+4H^+$	→	$3CH_4+CO_2+4NH_4^+$	$\Delta G^0_=$-101.6kJ/mol CH_4
$2(CH_3)_2NH+2H_2O+2H^+$	→	$3CH_4+CO_2+2NH_4^+$	$\Delta G^0_=$-86.3 kJ/mol CH_4
$4(CH_3)_3N+6H_2O+4H^+$	→	$9CH_4+3CO_2+4NH_4^+$	$\Delta G^0_=$-80.2 kJ/mol CH_4

The acetoclastic methanogens can be divided in the *Methanosarcina* and the *Methanothrix* group, distinguished by resp. a relatively high maximum specific growth rate (μ_m) together with a low substrate affinity (K_s) and a low μ_m together with a high K_s. Gujer and Zehnder (1983) illustrate the typical growth kinetics of the two different acetate cleaving methanogens. At low substrate concentrations, *Methanothrix* (μ_m=0.1 day^{-1}; k_s=30 mg/l) outcompetes *Methanosarcina* (μ_m=0.3 day^{-1}; k_s=200 mg/l). However at high substrate concentrations the *Methanosarcina* will predominate. Therefor in digestion at long detention times (>±15 days at 35°C) *Methanothrix sp.* will probably dominate (Gujer and Zehnder, 1983).
Boone (1982) reports that the major part of the methanogens in the digestion of cow slurry consists of rod-shaped bacteria, morphological similar to *Methanothrix soehngenii.*
Several micro-organisms have been isolated, which catalyze the hydrogenotrophic methane production (Zehnder *et al.*, 1982).
In rumen digestion *Methanobrevibacter ruminantium* and *Methanomicrobium mobile* are the predominant H_2-consuming methane bacteria (Hobson and Wallace, 1982). Hobson and Shaw, (1974) isolated the hydrogenotrophic *Methanobacterium formicicum* in pure culture from a pig slurry digester.

1.1.7. Effect of process parameters

1.1.7.1. Effect of the temperature

Methanogens have been found in several natural and man-made environments, where anoxic conditions prevail and organic matter is decomposed. Such environments are the intestinal tracts of animals, waterlogged soils, limnic and marine sediments, swamps and marshes, as well as sewage disposal tanks and solid waste landfills (Mah and Smith, 1981). Methanogens were isolated from hot springs (Stetter *et al.*, 1981) and Baross *et al.* (1982) presented evidence for the occurrence of biological methane production in submarine hydrothermal vents at temperatures above 300°C. Methane production in relation to temperature in different ecosystems was studied by several researchers (Koyama, 1964; Atkinson and Hall, 1976; Zeikus and Winfrey, 1976; Zinder and Brock, 1978; King *et al.*, 1981 and Kelly and Chynoweth ,1981). Although several of the habitats where methanogens have been studied, had temperatures below 10°C, the observed temperature optima for the production of methane were always between 30 and 40°C (Svensson, 1984). The only evidence for the existence of low temperature methanogens was established by Romesser *et al.* (1979) and Svensson (1984).
Romesser *et al.* (1979) were able to isolate two methanogenic species of the genus *Methanogenium* with temperature optima between 20 and 25°C from sediment of the Black sea and the Cariaco Trench. Svensson (1984) executed enrichment studies and provided evidence for the existence of two different methanogenic populations in peat: one unaffected by hydrogen and using acetate, with a temperature optimum at 20°C; the other oxidizing H_2 and having an optimum at about 28°C.

Animal slurry is mostly digested at mesophilic conditions in CSTR type reactors (Demuynck *et al.*, 1984). V. Velsen (1981) gives the results of an extended research in the anaerobic digestion of pig slurry at mesophilic conditions. The results of the digestion of cow slurry at mesophilic conditions in CSTR type digesters is presented in Chapter 4 of this thesis.

The digestion of pig slurry at temperatures of approximately 20°C was studied by Cross and Duran (1970) and Stevens and Schulte (1979). Cross and Duran could establish a stable digestion at 23°C at a loading rate of 0.8 g VS/l.d. Stevens and Schulte (1979) achieved stable digestion at a process temperature of 22.5°C and detention times of 20-55 days (0.6-1.8 g VS/l.d). Gas production however increased considerably at decreasing loading rates, viz. from 0.14 to 0.30 l/ g COD_{added}. V. Velsen (1981) could not detect any gas production at the digestion of pig slurry at 15°C and 20 days detention time, while the gas production at 20°C was 66% of that at 30°C. Hawkes *et al.* (1984) reported results of the research in the digestion of the 'liquid' fraction of separated dairy cow slurry at temperatures of 10-35°C and detention times varying from 5-20 days. At the highest detention time applied no and hardly any gas production was recorded at process temperatures of respectively 10°C and 15°C and gas production at 20°C is only 37% from that at 30-35°C. Recently Yaldiz (1987) published results of his research in anaerobic digestion of cow and chicken slurry (7% VS) at process temperatures of 10-30°C at detention times from 16-52 days. At the applied detention times it was not possible to achieve a stable process for the digestion of cow slurry and chicken slurry at temperatures of resp. 14°C and 18°C or lower. The results of the research presented in this thesis (Chapter 4) show that is possible to achieve a stable digestion process for cow slurry at temperatures as low as 15°C, when a sufficiently long detention time (100 days or longer) is provided. Wellinger and Kaufmann (1982) proved for the first time that it was possible to apply on-farm low temperature (<20°C) digestion in combination with storage at prolonged detention times (see Chapter 1.1.5.2.). The digestion of cow and pig slurry at low temperatures in combination with storage is described in detail in Chapter 2, 3 and 5 of this thesis.

Besides mesophilic methane bacteria, many thermophilic methane bacteria have been isolated (Zinder, 1988). The possibilities of the application of thermophilic digestion for waste and waste water was investigated in detail by Wiegant (1986).
Many researchers investigated the thermophilic digestion of animal slurry (Converse *et al.* (1977a), Varel *et al.*, 1977, Hashimoto et al. 1978, Hashimoto, 1982, Hashimoto, 1983, v. Velsen,

1981 Shelef, *et al.*, 1980, Chen *et al.*, 1980, Zeeman *et al.*, 1985). Zeeman *et al.* (1985) showed that the poor performance of thermophilic digestion of cow slurry was due to the high NH_4^+-N concentration and concluded that the thermophilic digestion with respect to energy production is not applicable for animal slurry, at least not in the Netherlands, where NH_4^+-N concentrations tend to be relatively high. Shih (1988) carried out a series of experiments to determine the effects of thermophilic and mesophilic anaerobic digestion of poultry waste on a variety of microbial pathogens. The results show that thermophilic digestion is much more effective in the reduction of pathogens than mesophilic digestion. Pathogen reduction is the main reason for the application of large scale thermophilic digestion in Denmark (v. Diemen and v. Nes, 1989).

1.1.7.2. Effect of the detention time and the slurry concentration.

Besides temperature, the detention time is an important parameter determining the process stability and the ultimate methane production achieved per g TS, added to the reactor.
V. Velsen (1981) showed already for the digestion of pig slurry that the detention time at which a certain gas production is reached is not only depending upon the process temperature but also upon the influent slurry concentration. Similar gas productions were found at digestion of pig slurry with 6 and 9% TS at resp. 12 and 20 days detention time. Summers and Bousfield (1980) also illustrate a decreasing specific gas production (l/g TS_{added}) at increasing the TS influent concentration from 5 to 9%. Baserga found similar results for the digestion of dairy cow slurry. Both v. Velsen (1981) and Summers and Bousfield (1980) attribute this phenomenon to the inhibition of the methanogenesis at an concomitant increased NH_4^+-N concentration (see Chapter 1.1.9.1.). The effect of detention time at different process temperatures (15-40°C) at the digestion of cow slurry is described in Chapter 4 of this thesis, the distinguished effects of VS and NH_4^+-N concentration in the digestion of cow slurry is described in Chapter 6 of this thesis.

1.1.8. Inhibiting compounds

1.1.8.1. NH_4^+-N toxicity in anaerobic digestion.

Inhibition of methanogenesis at high ammonia concentrations has been frequently reported in literature. McCarty and McKinney (1961) reported that ammonia inhibition occurs at total ammonia concentrations between 1.5 and 3.0 kg N/m^3 at pH levels exceeding 7.4, while at concentrations above 3.0 kg N/m^3 it occurs at all pH levels. Hashimoto (1986) reported about inhibition of the methanogenesis in cattle waste digestion. He added NH_4Cl to the manure to increase the NH_4+-N concentration. A threshold inhibition NH_4^+-N level of 2.5 g/l was found for mesophilic and thermophilic systems, corresponding to 0.03 g, 0.20 en 0.39 g NH_3-N /l for resp. unacclimated mesophilic, unacclimated thermophilic and acclimated thermophilic fermentors. The value for the unacclimated mesophilic system is considerably lower than the inhibition level of 0.15 g NH_3-N/l found by McCarty and McKinney (1961). According to the experimental results of Koster (1986), inhibition of NH_4^+-N starts at a concentration between 1.9-2.0 g/l at a pH of 7.6-7.8 , corresponding to NH_3-N concentrations of approximately 0.08 g/l. Latter value is in accordance with the observations of De Baere *et al.* (1984) and Anderson *et al.* (1982). According to various investigators, e.g. Parkin and Speece (1982) a considerable adaptation of the biomass to high NH_4^+-N can occur. Van Velsen (1981) showed that a total ammonia concentration up to 5.0 g/l could be tolerated after an adaptation period. Results of batch experiments with adapted granular sludge (Koster and Lettinga, 1988) reveal that even total ammonia concentrations up to 11.8 g/l (pH=7.4) can be sustained, although the maximum specific methanogenic activity of the sludge exponentially drops from 0.46 to 0.04 g COD/g VS.day at increasing the NH_4^+-N concentration from 2.3 to 11.8 g/l. Van Velsen (1981) found in batch experiments with adapted digested pig slurry a linear decrease of the specific gas production rate from about 0.3 to 0.25 ml gas/g VS.day, when increasing the NH_4^+-N concentrations from 0.605 to 3.075 g/l. He also observed a decreasing gas production in CSTR digestion experiments with pig slurry at 15 days detention time when increasing the NH_4^+-N

concentrations from 2.1 to 5.3 g/l. The increased concentrations of intermediates could not account for the observed decreased gas production. From this it can be concluded that apparently also the hydrolysis is affected by the NH_4+-N concentration.

A variety of processes may play a more or less significant role in the digestion of livestock wastes. On the level of methanogenesis, the ammonia inhibition may be associated with pH-inhibition, as well as with substrate and end product inhibition. A concise survey for both, mesophilic and thermophilic digestion is presented in Table 5 (modified version of Wiegant and Zeeman, 1986). Based on the available insights and results of research, Wiegant and Zeeman (1986) proposed a scheme (see Figure 2) for the NH_4^+-N inhibition in the thermophilic digestion of cow slurry. This scheme illustrates an inhibition of the hydrogen consuming methanogens by NH_4^+-N, while the acetate consuming bacteria are not directly inhibited by NH_4^+-N. The propionate accumulated via ammonia-promoted inhibition of the hydrogen utilizing bacteria may play an important role in the accumulation of acetate in stressed digesters (Wiegant and Zeeman, 1986).

Table 5. Some relevant conversions and their inhibitors, likely to occur in the anaerobic digestion of livestock wastes (modified version of Wiegant and Zeeman, 1986).

substrate	product	inhibitor	action	reference
hydrogen	methane	ammonia	strong	a, o
		ammonia	moderate	n
		propionate	moderate	a
acetate	methane	ammonia	moderate	b, c
		hydrogen	moderate	*d, e
		acetate	slight	f, g, o
		propionate	moderate	*h, i, j, *o
		propionate +NH_3	strong	*o
propionate	acetate	H_2	strong	k, l
		acetate	moderate	m

a. Hobson and Shaw (1976); b. van Velsen (1979); c. Koster and Lettinga (1984); d. Zinder and Mah (1979); e. McInerney and Bryant (1981); f. Andrews (1969); g. Bolle *et al.* (1983); h. Zinder *et al.* (1984); i, Märkl *et al.* (1984); j. Lin Chou *et al.* (1978); k. Heyes and Hall (1981); l. Gujer and Zehnder (1983); m. de Zeeuw (1984); n. Koster and Koomen (1988); o. Wiegant and Zeeman (1986); * concerns thermophilic digestion.

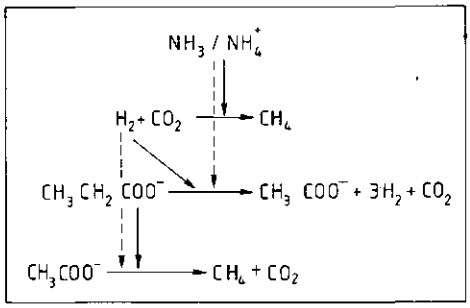

Figure 2. Proposed scheme for the inhibiting action of ammonia in thermophilic digestion of cow slurry. Horizontal arrows: inhibited reaction; vertical arrows: inhibiting action. Possible inhibiting actions are dotted (Wiegant and Zeeman, 1986).

Parkin and Miller (1982) also have shown that in enrichment cultures adapted to elevated ammonium concentrations which contained acetate as the only substrate, the degradation of acetate to methane proceeds unabatedly up to approximately 8 g NH_4^+-N/l and even some degradation occurred at concentrations as high as 17 g NH_4^+-N/l. Koster and Lettinga (1984) gathered some proof that acetate-consuming methanogenic bacteria in un-adapted mesophilic systems are more affected by high NH_4^+-N concentrations than H_2-consuming bacteria. From experiments conducted by Sprott and Patel (1986) with pure cultures it also seemed that acetoclastic methanogens are more sensitive than the hydrogenotrophic bacteria to increasing ammonium concentrations (Table 6).

Table 6. Eight methanogens classified as to their sensitivity/tolerance to ammonium (Sprott and Patel, 1986)

NH_4^+sensitive	NH_4^+tolerant
Methanospirillum hungatei	*Methanobrevibacter arboriphilus*
Methanosarcina barkeri	*Methanobrevibacter smithii*
Methanothrix concillii	*Methanobacterium strain G2R*
Methanobacterium bryantii	
Methanobacterium formicicum	

Blomgren *et al.* (1989) conducted experiments with enrichment cultures on acetate, one at 7 g/l NH_4^+-N and one at 0.3 g NH_4^+-N/l. The results of the experiments provide evidence that a shift in the acetate utilizing bacterial population may occur at high $NH4^+$-N concentrations, resulting in a syntrophic degradation of acetate into CH_4 and CO_2.
Wiegant and Zeeman (1986) report that it is highly unlikely that syntrophic acetate oxidation have played a role in their thermophilic digestion experiments, as the concomitant CH_4 formation from H_2/CO_2 would have been inhibited at the applied NH_4^+-N concentrations, exceeding 3.5 g/l.

1.1.9.2. Antibiotics and feed additives.

Visek (1978) reviewed the historical developments leading to the use of antibacterial agents in animal feeds and the generally accepted concepts about their growth promotion action. Nowadays many antibiotics and feed additives are used in factory farming systems for the control of diseases and the promotion of growth and moreover many disinfection agents are used in the stables. Winterhalder (1985) researched for his phD study, the effect of several of these compounds on the mesophilic anaerobic digestion of pig slurry in continuous flow experiments (detention time=16 days). He concludes that of the researched additives, tylosin, monensin, olaquindox, avoparcin, carbodox and animed, only monensin was inhibiting the anaerobic digestion process. The concentration applied in the influent slurry were resp. 8.6 and 17.2; 8.6 and 17.2; 10.8 and 21.5 and 43.0; 8.6 and 17.2; 10.8 and 21.5; 8.6 and 17.2 and 34.4. These concentrations could be expected when resp. 50 and 100% of the animals were fed with the normally applied dose. With respect to olaquindox and animed also a concentration of resp. 43.0 and 34.4 mg/l was applied, corresponding to application of an overdose to 100% of the animals. Camprubi *et al.* (1988) report that tylosin was even not affecting methanogenic activity in CSTR experiments (12 days detention time, 37°C) in concentrations of 200 mg/l. This was also found for chlortetracyclin and erythromycine in concentrations of 225 and 50 mg/l respectively. Carbadox in concentrations similar as applied by Winterhalder (1985), viz. 19 and 22 mg/l showed also no detrimental effect on the methanogenesis, however after a necessary period of acclimatization. The difference in the acclimatization period as found by Winterhalder (1985) and Camprubi (1988) is probably caused by the difference in rate of increase of the additive concentration. In the first case the concentration gradually increased, concomitant with the feeding of the slurry, containing the additive. In the second situation the additive concentration in the digester was rised directly to the desired value. Camprubi *et al.* (1988) found similar results for furazolidone at concentrations of 100-150 mg/l. Also Poels *et al.* (1984) did not find any inhibiting effect of chlortetracyclin, tylosin and erythromycine (resp. 33.3, 16.7 and 4 mg/l in anaerobic digestion of pig slurry (20 days detention time, 30°C).
Inhibition of the methanogenesis by monensin, as reported by Winterhalder (1985) was found by several other researchers (Hilpert *et al.*, 1983; Varel and Hashimoto, 1982; Zeeman *et al.*, 1984).
Hilpert *et al.* (1983) observed a recovery of the sewage sludge digestion process at monensin concentrations as low as 0.5 mg/l but at the higher concentrations (2-5 mg/l), the inhibition continued. In our digestion experiments with slurry of cows, fed with monensin, an initial inhibition of the methanogenesis was followed by recovery of the process. In both, batch and CSTR experiments however, less organics were hydrolysed (Zeeman *et al.*, 1984).
The inhibition of monensin could be of importance at the digestion of slurry of fattening cattle and chickens as monensin is regularly added to the feed of these animals. Also bacitracine, virginiamycine (Hilpert, 1983, Poels *et al.*, 1984) and chloramphenicol (Hilpert *et al.*, 1981, Camprubi, *et al.*, 1988) is reported to be toxic for anaerobic systems.
Of course many other additives are or will be used in animal husbandry and many of them could have a negative effect on anaerobic digestion of the produced slurry. Ianotti and Fischer (1981) have tested several antibiotics and feed additives in short term digestion experiments.
Copper is normally used as an additive in pig feed. Nowadays addition of maximal 175 ppm Cu in pig feed is permitted in the Netherlands (Produktschap Veevoeders, 1990). The (mean) concentration in pig slurry is consequently maximal 34 mg / liter slurry (pers. communication v.d. Peet, IKC, Rosmalen). Camprubi *et al.* (1988) executed batch and semi-continuous experiments with the digestion of pig slurry and added copper sulfate to concentrations of 15-94 mg/l (as Cu). The copper sulfate inhibition appeared to be progressive, not being significant in batch experiments but producing a decrease in methanogenesis, higher than 80%, when it was fed continuously at concentrations of 94 mg/l.

1.1.9. Mathematical description and modelling

1.1.9.1. Aim of modelling

From the foregoing it will be clear that anaerobic digestion of complex materials like animal slurry and sewage sludge is still not fully understood. Hobson (1983a) stated that the identification of the relevant bacteria comprises one of the major problems in investigating bacterial habitats such as digesters. Roughly the system can be described by a few general reactions which could be brought about by a few types of hydrolytic, fermentative and methanogenic bacteria. In such an approach the intermediate reactions are being ignored. Hobson (1983a) illustrated with an example concerning digestion in the rumen of lambs, that in fact we are dealing with very complex systems. The rumen of completely isolated and sterile lambs was inoculated with a few species of bacteria, isolated from a normal lamb rumen. These bacteria had the *in vitro* ability to carry out all the rumen functions.
However after some months the rumen metabolism failed and the animals ceased to gain weight. The malfunctioning was reversed by putting the animals into a normal animal house. In a short period of time the few inoculated species had been joined by all the many strains and species which normally make up the normal rumen flora. The bacteria which were originally inoculated were however still predominant. It is likely that all or most of the many types of bacteria of the rumen or digester are in some form of symbiotic association and are necessary for the stability and function of the mixed population (Hobson, 1983a). The composition of the bacterial population and the different reactions carried out in the conversion of the feed to products and residues will be determined by the composition of the feed and the conditions imposed on the system.
Obviously the composition of the slurry will vary for different types of animals (see Chapter 1.1.3.) but also taking slurry from the same sort of animal, considerable differences in the composition will be found. The composition of the slurry will vary with the animal feed, the storage conditions, such as time and temperature (Zeeman *et al.*, 1985 and 1988, Steiner, 1983, Williams and Hills, 1981), and the storage collection system, e.g. with or without urine, cleaning and rain water. Also the presence of toxic compounds could affect the composition of the bacterial population and consequently the intermediate reactions. In slurry digestion, NH_4^+-N is one the most important toxic agents. While so far it was presumed that anaerobic bacteria were able to adapt to high ammonia concentrations (v. Velsen, 1981, Koster, 1986), recently published results of Blomgren *et al.* (1989) produce evidence, that a shift in the acetate utilizing bacterial population may occur at high NH_4^+-N concentrations, resulting in a syntropic degradation of acetate into CH_4 and CO_2 (see Chapter 1.1.8.1.).

Despite all big uncertainties about the intermediate reactions and organisms involved in the conversion of complex organic material into CH_4 and CO_2, anaerobic digestion of sludges and slurries has inspired many researchers to attempt to model the process.

1.1.9.2. Different type of models

Two types of models have often been used for describing the anaerobic digestion of animal slurry.
1. Models based upon Monod kinetics (Hobson, 1983, Durand *et al.* 1988, Hill, 1982, Hill, 1983, Hill *et al.*, 1983, Hill, 1983, Hill ,1983)
2. Models based upon Contois kinetics (Chen and Hashimoto, 1978, Fischer *et al.*, 1984).

1. Monod kinetics

Hill and co-workers developed a model, aiming at the mathematical description of the anaerobic digestion of animal slurry, based on Monod kinetics. The finally developed model (Hill, 1982) describes the kinetics and interactions of four microbial groups, viz., the acidifiers, the hydrogen producing acetogens, the homo-acetogens and the methanogens (divided in H_2 utilizing and acetate converting). The effect of VFA's on growth and decay is

described by the following equations, developed by Hill *et al.* (1983) (the sequence of actual publication is not similar to the sequence of the development of the models):

$$\mu = \frac{\mu_{max}}{1+K_s/s + VFA/K_{ic}} \quad (1)$$

$$K_d = \frac{K_{dmax}}{1+K_{id}/VFA} \quad (2)$$

where, s=substrate concentration in the digester (g/l); μ= specific growth rate (day^{-1}); μ_{max}=maximum growth rate (day^{-1}); K_s= Monod 'half velocity' constant (g/l); K_{ic}='half velocity' growth inhibition constant (g VFA/l); VFA= VFA concentration (g/l); K_{id}='half velocity' decay constant (g VFA/l); K_d=specific decay rate (day^{-1}); K_{dmax}= maximum decay rate (day^{-1}).

The values assessed for the kinetic constants used in the model of Hill (1982) are given in Table 7.

Table 7. Values assessed for the kinetic constants used in the model of Hill (1982).

	μ_{max} day^{-1}	K_{dmax} day^{-1}	K_s g sub./l	K_{ic} g VFA/l	K_{id} g VFA/l	Y g org/g sub.
acidifiers	*	+	5.0	9.0	16.0	0.07
acetogenic	*	+	1.0	9.0	16.0	0.07
homo-aceto-genic	*	+	0.5@	9.0	16.0	0.125
H_2-methano-genic	*	+	0.01@	11.0	16.0	0.056
acetate-methanogenic	*	+	0.01	11.0	16.0	0.042

Y=Yield; @ 1H_2/l digester volume

* Temperature dependent (from Chen and Hashimoto, 1979)

\+ numerically equal to μ_{max}

Hill (1982) validated his model with data from literature, viz. from Burford *et al.* (1977), Hashimoto *et al.* (1979), Fischer *et al.* (1975, 1979), Steven and Schulte (1979), Lapp *et al.* (1975), Converse *et al.* (1977, 1977a, 1981), Coppinger *et al.* (1978), Bartlett *et al.* (1981). Of the 16 data from literature, 14 were within 10% and 11 showed errors of less than 5%, compared to the simulated data.

Durand *et al.* (1988) also checked Hill's model using the results of experiments carried out by Fischer *et al. (1984) and Ianotti et al.* (1983). They showed that the model was rather well in fitting the methane production and VFA concentration after stepwise increasing the influent concentration (Fischer *et al.* ,1984) from 60 (NH_4^+-N =2.9 g/l) to 97 g TS /l (NH_4^+-N=3.7 g/l) and predicting the failure after the TS concentration raised to 108 g/l (NH_4^+-N=6.1 g/l). The model appeared inadequate in predicting ammonia inhibition, because upon increasing the ammonia level (up to 9.0 g/l) the model does not predict any decrease in methane production, while experiments show significant decrease in methane production (Georcakis *et al.*, 1982). At increasing the ammonia concentration in the experimental digesters to 7.6 g/l, much higher levels of total acids could be sustained compared to those predicted by the model, i.e. because

it predicts a complete failure at total acid level exceeding 3 g/l. According to Durand *et al.* (1988) the inability of the model to account for ammonia inhibition should be regarded as a minor flaw. Regarding the results to be presented in Chapter 6 this is not correct, because NH_4^+-N inhibition plays an important role in anaerobic digestion, especially in slurry digestion. Hobson (1983) developed a model for the digestion of animal slurry based on Monod kinetics. Some major differences between the two approaches will be discussed here. Hill (1982) uses a set of constants, B_0 (Table 8), which determine the amount of solid organic material that is converted into soluble organics. Each type of animal slurry has a fixed B_0 value.

Table 8. Biodegradability constants for four animal waste types (Hill, 1982).

Waste Type	$B_0(gVS_{des}/gVS_{added}, \theta \rightarrow \infty)$
Beef(dirt)	0.60
Beef(confinement)	0.70
Dairy	0.36
Swine	0.90
Poultry(layer)	0.90
Poultry (broiler)	0.70

Hobson (1983) however divides the biodegradable solids in two categories, which concentration may differ in different samples of the same type of waste (e.g. pig slurry) and which character may differ for different animal slurries. It is obvious that most of the easily biodegradable components of the animal feed are degraded in the animal. What remains in the slurry is mainly fibers (see Chapter 1.1.3.). The fibrous materials are composed of cellulose, hemi-cellulose and lignin. The main substrates available in the manure for the hydrolysing bacteria are cellulose and hemi-cellulose. Results obtained by Summers and Bousfield ((1980) reveal a clear increase of lignin content in the digestion process but they found that the ratio between cellulose and hemi-cellulose did not alter significantly. Apparently any preferential digestion of the components does not occur. According to the views of Hobson (1983) the rate of hydrolysis is not related to the composition of the solids but mainly to the particle size and consequently in fact to the number of sites available for bacterial colonization and attack. There indeed exists some other experimental evidence in literature about the existence of a relation between particle size and the rate of degradation (Williams and Evans, 1981, Chang and Rible, 1975).

According to Hobson (1983) the degradation of solids could be described by Monod kinetics. The concentration term K_S, usually applied to substrate in solution, now refers to the weight of solids in relation to the surface area. The K_S value for the slowly degrading particles is expected to be larger than that for the rapidly degrading solids. The term μ_m is theoretically difficult to explain. However it seems that, in spite of the theoretical complexity, the breakdown of solids can be described by K_S an μ_m simulating that dissolved substrates were involved (Hobson, 1983).

While the inhibition kinetics of Hill (1982) were based on inhibition of VFA 's, the 'Hobson-model' only includes inhibition of the methanogenic bacteria at NH_4^+-N concentrations >1.8 g/l and inhibition of the hydrolysis step at TS concentrations exceeding 5.4% in the digestion of pig slurry. The K_S and μ_m values as found by Hobson (1983) are given in Table 9.

Table 9. Kinetic constants at hydrolysis and fermentation of solids (Hobson,1983)

	pig slurry*	cow slurry+
μ_{max} (day^{-1}) (slowly degradable)	0.139	0.08
μ_{max} (day^{-1}) (rapidly degradable)	0.36	0.32
k_s (g substr./l)(slowly degradable)	0.782	2.2
k_s (g substr./l) (rapidly degradable)	0.500	1.0

* Figures are obtained with the use of results of Bousfield and Summers (1980). According to Hobson (1983), the results of v. Velsen (1981) would require different values of the kinetic constants, due to the use of a different type of pig slurry. + Figures are obtained with the use of results of Singh *et al.* (1982).

2. Contois kinetics

Chen and Hashimoto (1978) developed a kinetic model for the digestion of animal manure based upon Contois (1959) kinetics, viz.:

$$B = B_0\{1 - K/(\theta.\mu_{max}-1+K)\}$$

and

$$r_v = \{B_0.S_0/\theta\}.\{1 - K/(\theta.\mu_{max}-1+K)\}$$

where

r_v= volumetric gas production (l CH_4/l.d)
S_0= influent VS concentration (g/l)
B_0= ultimate methane yield (l CH_4/g VS_{added}) as $\theta \rightarrow \infty$
B= methane yield (l CH_4/g VS_{addded})
θ= HRT (days)
μ_{max}= maximum specific growth rate of the bacteria (day^{-1})
K= kinetic parameter (dimensionless)

The kinetic parameter K is reported to increase with increasing S_0 concentrations (Chen and Hashimoto, 1978, Chen and Hashimoto, 1980, Hashimoto *et al.* 1981, Hashimoto, 1982, Fischer *et al.*, 1984). In 1983, Hashimoto proposed that K is not merely effected by S_0 but also by the ammonia concentration. The different relations between K and S_0, as reported by various research workers (Chen and Hashimoto, 1978, Chen and Hashimoto, 1980, Hashimoto *et al.* 1981, Hashimoto, 1982, Fischer *et al.*, 1984), could be caused by different NH_4^+-N /S_0 ratio's of the used slurries.

1.2. SCOPE OF THIS THESIS

This thesis presents results of two research projects, viz.:
1. 'Anaerobic digestion of dairy cow slurry for energy production on farm'.
2. 'Low temperature digestion of animal slurry'.

The aim of the first research project was to optimize the anaerobic digestion of dairy cow slurry in a Completely Stirred Tank Reactor (CSTR) with respect to energy production on farm.

The feasibility of further application of anaerobic digestion of animal manure in a CSTR on farm, did not prove to be very promising (Werkgroep perspectieven Biogasproductie, 1984).
The aim of the research project 'Low temperature digestion of animal slurry' was to unravel the processes at low temperature manure digestion in combination with storage in a so called Accumulation System (Wellinger and Kaufmann, 1982).

The combination of storage and digestion of manure could lead to a more simple process and enlarge the feasibility of energy production on farm. Apart of on-farm energy production, it is also possible, to apply low temperature digestion for energy production in combination with collective storage of manure.

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CHAPTER 2.
PSYCHROPHILIC DIGESTION OF DAIRY CATTLE AND PIG MANURE: START-UP PROCEDURES OF BATCH, FED-BATCH AND CSTR-TYPE DIGESTERS.

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CHAPTER 2.
PSYCHROPHILIC DIGESTION OF DAIRY CATTLE AND PIG MANURE: START-UP PROCEDURES OF BATCH, FED-BATCH AND CSTR-TYPE DIGESTERS.

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ABSTRACT

Procedures were evaluated to start up anaerobic digesters at psychrophilic temperatures (5-20°C) fermenting either pig or dairy cattle manure. Below 20°C no methane formation was initiated without inoculum. Using a temperature-adapted seed material (50%), batch cultures and accumulation systems could be started at temperatures as low as 5 and 10°C, respectively. Independent of the system, the time to start up cattle manure at 20-25°C took as long as 10-40 days, depending on whether an inoculum was used or not. The corresponding value for pig manure could be shortened by 20 days when stored cow manure was added. The addition of a 100% seed of mesophilically-treated sludge was a suitable means of initiating digestion of cattle manure in a continuous-flow system at 20°C as long as the retention time remained above 40 days.

2.1. INTRODUCTION

In nature, methane is formed over a wide temperature range, from 0 to 97°C, in such places as under glaciers (Berner *et al.*, 1975) and in ice fields (0°C; McGhee, 1968), in sediments and marshes (4-15°C), in rumen (39°C) and in hot springs (Stetter, 1985).

Until recently, biogas production at low temperatures (below 25°C) has been the subject of little research compared with mesophilic or thermophilic digestion, even though in Third World countries thousands if not millions of rural digesters are working at ambient temperatures. Most of these systems are operated in a batch-fed mode (e.g. Chinese dome digesters), whereas a smaller number are fed semi-continuously (e.g. KVIC and red mud-plastic digesters).

Results on the operation of two full-scale, unheated, batch-fed digesters (accumulation systems) in Switzerland have recently been reported by Wellinger & Kaufmann (1982). The systems proved to be an economically viable alternative to mesophilic continuous-flow digesters. Daily gas production approached net energy values known for fermenters operating at 35°C. In the observed temperature range, between 15 and 21°C, gas production increased linearly with temperature. The start-up procedure which was monitored in one of the plants was, however, long and tedious, taking as long as 6 months before stable operation could be established. Subsequently, specific gas production continued to increase during the whole monitoring period of nearly 2 years. This suggested that the adaption of the bacterial population to low temperatures was an extremely slow process.

In a recent paper by Cullimore *et al.* (1985) on the low-temperature digestion of lagooned hog manure, the above-mentioned data have been confirmed and extended in lab-scale experiments. The authors also found a fair amount of adaption still going on in their second year of experiments.

Since, in cold climates, unheated accumulation systems are restricted to constructions below buildings (heat transfer from the barn), a partially-heated system was constructed in Switzerland where the excess gas was used to heat up the digester. Despite the large seasonal

temperature variations from 16°C in winter to 35°C in summer, the fermentation process remained fairly stable during the 2-year monitoring program (Sutter *et al.*, 1987).

Lately, Grin *et al.* (1983), using a UASB-reactor, successfully demonstrated that raw sewage could be degraded at temperatures as low as 10°C. At a hydraulic retention time (HRT) of 8h, COD removal rates were hardly affected between 20 and 14°C. Again, the start-up was a difficult task. Using septic tank sludge as an inoculum, it took as long as 10 weeks to achieve reasonable COD removal rates, even though the digester was operated at 22°C and HRT between 24 and 18h. Without seed material the start-up period lasted as long as 6 months. Below 20°C it was not possible to start digestion at all without seeding.

Obviously, the anaerobic digestion of animal manure and sewage at low temperatures might represent a reasonable alternative to mesophilic or thermophilic processes. However, start-up is still a major constraint for the application of this technique. The present paper describes two possible ways to shorten the start-up period when digesting animal manure: either by using a suitable inoculum or by adapting a mesophilically-operated digester to psychrophilic temperatures.

2.2. METHODS

Individual experiments and analyses were carried out in two laboratories independently of each other. However, identical, or at least comparable, methods were used.

2.2.1. Analyses

The slurries were analyzed for total solids, volatile solids, total nitrogen and COD according to Standard Methods (1975). Ammonia nitrogen was measured photometrically using direct Nesslerization after a 10-fold dilution of centrifuged or membrane-filtered samples. Gas composition (GC with TCD, molecular sieve 5A and Poropak Q columns) and VFA (GLC with FID, Chromosorb 101 columns) were determined by gas chromatography. VFA COD was calculated from the measured VFA concentrations. Total COD was measured as described by Zeeman *et al.* (1983).

2.2.2. Substrate

Manure samples were collected at the research farms in Duiven, Holland (NL), and Tänikon, Switzerland (CH). Where necessary, the origin of the manure is indicated. If not stated otherwise, the complete manure including urine was used. Cow manure was either scraped from the floor as it dropped from the animal or collected from the storage tanks located below, or in front of, the sheds. Pig manure was collected from the storage tanks located along the side of the pig barn (CH). Samples were stored at 4°C before use except for the pilot-plant experiments where the slurry was stored at ambient temperatures in a 25-m^3 holding tank. The dry matter content of the pig manure was increased by about 1.5% using sedimentation with subsequent removal of the top liquid layer. Cow manure (CH) contained about 1% (w/w) of chopped straw (3-5 cm) from bedding.

Table 1 gives typical values for the composition of the manures utilized in the two laboratories. Where considered as necessary, more detailed information on the manure composition is given within the text. All animals were fed diets which were free of antibiotics and other feed additives.

Table 1. Typical Composition of the Influent Pig and Dairy Cattle Manure in Holland and in Switzerland (kg/m^3).

	Holland	Switzerland	
	Dairy cattle	Dairy cattle	Pig
Total solids	85.4	83	43
Volatile solids	74.7	73	74
NH_4-N	2.2	1.5	1.9
Total COD	101	-	-
Dissolved COD	27.6	-	-
VFA COD	11.1	2.6	7.4
pH	7.5	7.4	7.2

2.2.3. Inocula (other than manure)

Sewage sludge
Samples of anaerobically-treated sludge were taken from a nearby domestic-sewage treatment plant. The sludge had the following average composition: TS 3.5%, VS 40%, pH 7.3, NH_4^+-N 0.4 g/l, VFA 700 mg/l.

Swamp soil
Swamp samples were collected at a nearby lakeside. Activity tests in 1-litre anaerobic serum bottles at 20°C showed methane formation after 4 days when the 100-ml sample was flushed with N_2.

Sanofor
Sanofor is a brand name for concentrated peat bog with 9.9% TS and 52% VS. This natural product is rich in minerals, in iron-II-oxide as well as in organically bound trace elements. It is commonly used as a feed additive for pigs and calves to improve their digestion.

2.2.4. Design of laboratory digesters

Two-litre plastic bottles served as batch digesters. Double bottles filled with 1.5 liters of manure (50% inoculum) were placed in water baths or constant temperature rooms at 5, 10, 12, 15, 17, 18, 20, 22, 25 and 35°C. Digesters working at 18°C served as interserial standards.

The start-up of accumulation systems with low inoculation was simulated in the laboratory using 20-litre polyethylene digesters placed on a large rotary shaker in a constant temperature room. Starting with 4 kg of fresh manure and 0.4 kg of inoculum, 1/100 of the final volume of fresh manure was added daily (200 ml), independent of the actual gas production.

Six-litre acrylic glass vessels served as semi-continuously-fed digesters with a working volume of 5 liters. They were intermittently stirred (30 s/30 min) by a blade stirrer. Temperature was maintained by a water jacket. The same digesters were also used for some of the batch experiments and for the start-up of the accumulation system with high inoculation. Starting with 2.5 kg of inoculum, 1/200 of the final volume of fresh manure was added daily (25 ml/day).

Gas production was measured by a salt brine displacement system either daily or continuously with an electronic reading device built in our own workshops and corrected for the gas produced by the inoculum (CH). In the Dutch experiments the CO_2 was removed by passing the gas through a column of soda lime pellets prior to gas measurement with a wet test gas

meter. With the accumulation systems the gas was collected in gas bags and measured weekly (NL).

2.2.5. Design of a pilot-plant

An old railroad tank of a total volume of 35 m^3, formerly used to transport heavy oil, was converted into a horizontal, batch-fed stirred-tank reactor (accumulation system; Figure 1). The tank was insulated with 5 cm of polyurethane and was equipped with heating coils. A horizontal, slowly rotating (5 rpm) paddle stirrer was operated intermittently (one turn every hour). The fermenter was fed once a day through a 10-cm conduit (Figure 1). As for the lab-fermenters, 1/100 of the final volume of fresh manure was added daily (300 liters).

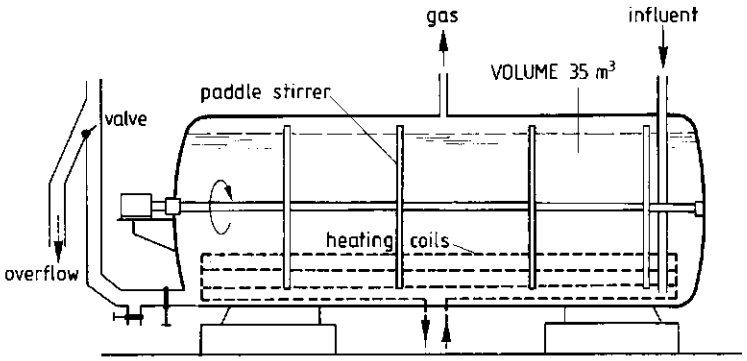


Figure 1. Horizontal steel digester constructed from a former heavy oil tank of the railroad which had the heating pipes already installed.

2.3. RESULTS

2.3.1. Start-up of batch digesters: dairy cattle manure

In a first experiment, 5-litre batch digesters were set up with fresh dairy cow manure at temperatures of 5, 10, 15, 25 and 30°C without inoculum. The rapid accumulation of VFA indicated that the first two steps of anaerobic degradation, hydrolysis and acidification, were initiated immediately after the start-up of the experiment. As depicted in Figure 2, the rate of acid formation increased with increasing temperatures. However, no methane was formed at the lower temperatures (5-15°C) within a digestion period of 5.5 months. Methane production at the higher temperatures increased rapidly after a lag-phase of 33 and 66 days at 30 and 25°C, respectively.

No such effect of low temperature on the initiation of gas production was encountered when a 50% inoculum of cow manure was added. This inoculum had been precultured in a continuous-flow system at 18°C for an extended period of time. Figure 3 gives the biogas yields as a function of temperature for batch cultures inoculated with an 18 or a 35°C seed.
In the cultures with the psychrophilic seed, gas yield increased linearly in the range from 10-22°C. The slope was the same for both 30- and 50-day fermentation times. This suggests that differences in gas production between the different temperature levels occur during the first 20-30 days only.

At 5°C a steady increase of gas accumulation was recorded. However, the gas yield remained very low (5 liters/kg of VS added), even after a fermentation period of 50 days.

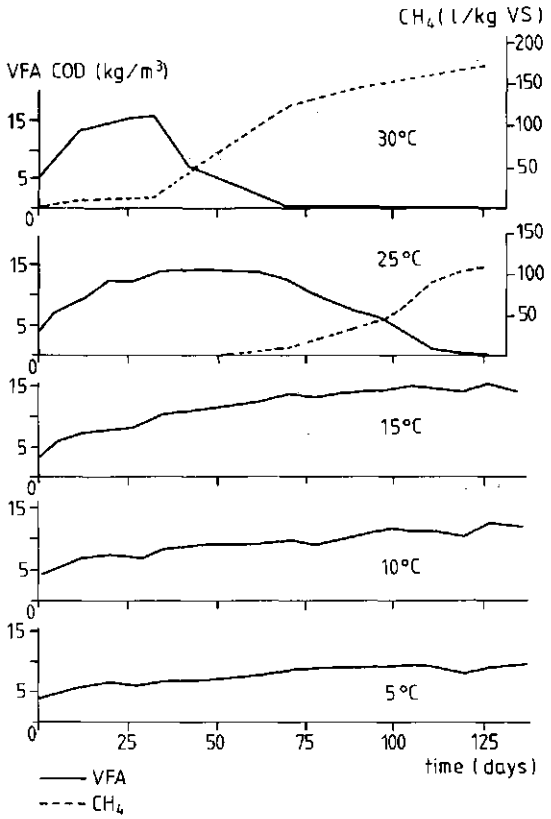


Figure 2. Start-up of cow manure digestion in batch systems. Development of VFA concentrations and gas production as a function of time and temperature.

It is interesting to note that at 10°C the final VFA levels after 50 days of fermentation were considerably higher than at 22°C, indicating that at the lower temperature the methane formation might have been inhibited (Table 2). The slower increase in gas yield at temperatures higher than 22°C might be explained by hydrolysis becoming rate-limiting.

Table 2. Influence of Fermentation Temperature on VFA Concentration during Batch Digestion.

	Initial	After 50 days	
		10°C	22°C
Acetate (ppm)	3385	1247	467
Propionate (ppm)	638	780	74
Butyrate (ppm)	236	42	10
Total COD (g/litre)	4.7	2.4	0.6

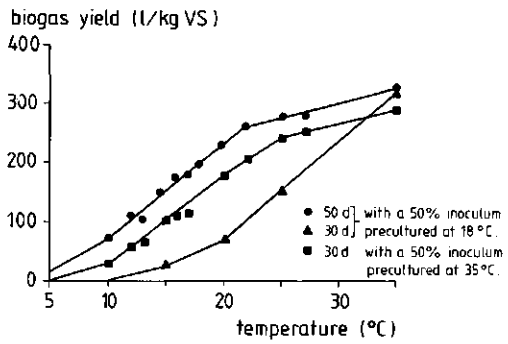


Figure 3. Batch digestion of cow manure with inocula precultured at two different temperatures. Gas yields after 30 and 50 days fermentation time as a function of the fermentation temperature.

Despite the large inoculum of 50%, no gas production was initiated below 15°C when mesophilically-adapted seed material was used (Figure 3). Unfortunately, the mesophilic inoculum contained a relatively high propionate concentration (3300 ppm) which might have slightly inhibited methane formation (Wiegant & Zeeman, 1986). Hence the difference in gas production between the mesophilic and the psychrophilic seed was emphasized.

However, the results were qualitatively confirmed by a further experiment where the influence of the fermentation temperature of the inoculum from a continuous-flow digester on the final gas yield of a batch culture was studied (Figure 4). Again, the psychrophilic seed precultured at 18°C demonstrated the highest gas formation at 20°C. The bacterial populations of the two other inocula (27 and 35°C) could not adapt to the lower temperature during the 50 days of batch digestion. At 25 and 35°C, on the other hand, approximately the same gas yields were achieved after 50 days by all three inocula. Only during the first 20 days the best adapted seeds (27°C at 25°C fermentation temperature and 35°C at 35°C fermentation temperature) demonstrated a slightly higher gas production.

This result pattern strongly suggests a psychrotrophic behavior of the entire system, where bacteria slowly adapt to low-temperature fermentation without losing their mesophilic capabilities.

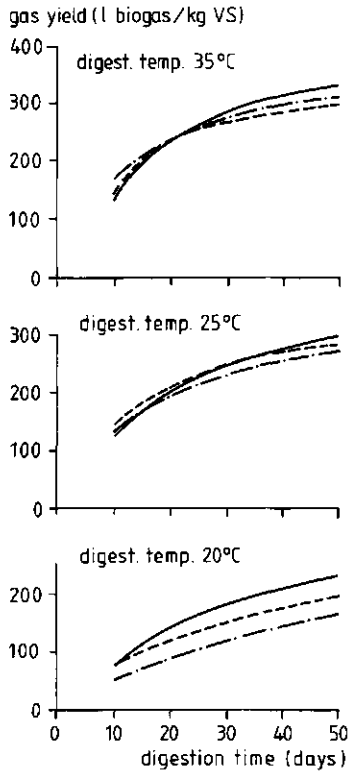


Figure 4. Influence of temperature adaption at 18°C (—), 27°C (---) and 35°C (-.-.-) of the inoculum on the gas yield from batch digestion of cow manure at mesophilic and psychrophilic temperatures.

2.3.2. Start-up of an accumulation system at 20°C: pig manure

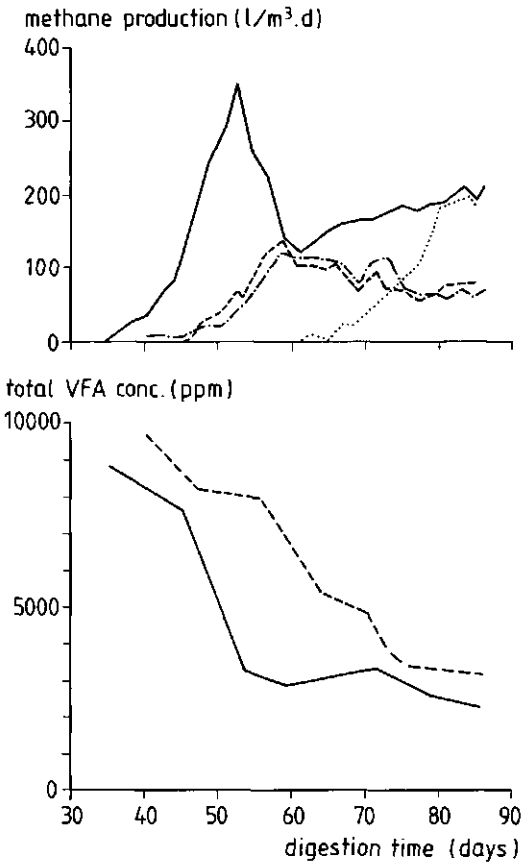
Lab-scale and pilot-plant results on the start-up of pig slurry digestion in accumulation systems at 20°C are shown in Figure 5. Addition of a 10% inoculum of either sewage sludge, swamp soil, Sanofor or psychrophilically-digested pig manure (13°C) did not shorten the start-up time when compared to samples without inoculum. It is surprising that even the sludge from a full-scale psychrophilic accumulation system had no effect. The material was probably not very active, as is shown by the high VFA concentrations which were only marginally lower than those of the fresh slurry (acetate 3230 ppm, propionate 370 ppm, butyrate 50 ppm).

Addition of 10% cow slurry or a 1:1 mixture of cow slurry and sewage sludge shortened the lag-phase by 20-45 days in the lab-scale experiment. The seeding with cow slurry in the pilot-plant experiment resulted in an even shorter lag-time of only 40 days. In the latter case, however, a relatively large inoculum was added ($2m^3$ for a daily load of 300 liters), as compared to the lab situation where 400 ml inoculum was applied for a daily feed of 200 ml. In addition, very diluted (TS 1.8%, VS 61%, VFA 3250 ppm), stored, cattle manure was used except in the laboratory, where fresh manure was applied (TS 13%, VS 73%, VFA 1580 ppm).

In both pilot-and lab-scale, the ammonia nitrogen content increased from 1.7 to 2.1 kg/m^3 during the experiments. During the lag-phase (no methanogenic activity), the concentrations of total VFA increased to 8800 ppm (11.1 kg COD/m^3) in the pilot-scale and to 9600 ppm (12.1 kg COD/m^3) in the lab-scale experiments, respectively, which corresponds to the behavior during batch digestion (Figure 2). When gas production started, the acetic acid level was reduced to 300 ppm within 20 days whereas propionate remained above 2000 ppm throughout the entire experiment of 85 days.

2.3.3. Start-up of an accumulation system at 20°C: dairy cow manure.

Low inoculation



Type	Scale	Inoculum
——	pilot	Sewage sludge/cow manure (1:1)
- - - - -	Lab	Sewage sludge/cow manure (1:1)
-.-.-	Lab	Cow manure
.....	Lab	None or sewage sludge or swamp or Sanophor or psychrophilically-digested pig manure

Figure 5. Methane production and VFA concentration during start-up of lab- and pilot-scale accumulation systems at 20°C with pig manure using different seed materials.

Neither the addition to 4 kg of fresh manure of 0.4 kg of Sanofor nor of 0.4 kg of mesophilically-digested sewage sludge, pig manure or cattle manure could significantly shorten the start-up time of laboratory accumulation systems (results not shown). Only actively fermenting pig manure from the pilot-plant at 20°C could slightly shorten the lag-time (Figure 6). But in any case the lag-phase with cattle manure was shorter than with pig manure.

Contrary to the finding with pig manure, the start-up with the cattle manure in the lab experiment was faster than in the pilot-plant, which might be explained by the somewhat lower TS content (7.8% vs 9.2%). The initial gas peak, which is typical for the start-up of a batch digestion, was far less pronounced in the experiment with cow manure than in that with pig manure.

At pilot- and laboratory-scale, ammonia levels remained below 1.5 mg N/litre.

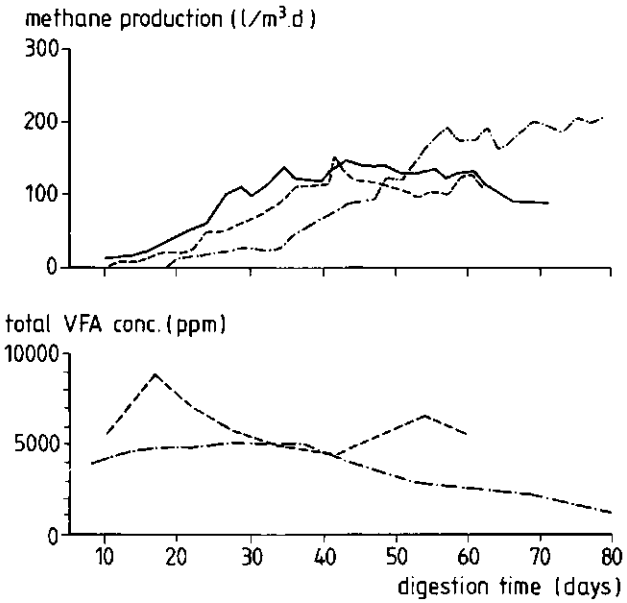


Figure 6. Methane production and total VFA concentration during start-up of accumulation systems with cow manure in lab-scale [(——) with a 10% inoculum of psychrophilically-digested pig manure, (---) without inoculum] and pilot-scale [(-.-.-) without inoculum] experiments at a fermentation temperature of 20°C.

2.3.4. Start-up of an accumulation system at temperatures of 10, 15 and 20°C: dairy cow manure

High inoculation

Figure 7 illustrates the methane production and the VFA concentrations as a function of time and fermentation temperature of a second accumulation experiment where the size of the inoculum (which was precultured in a CSTR either at 20 or 30°C was set to 50% of the final volume of the digester).

Unlike the accumulation experiment with the low inoculum but similar to the batch experiment with a 50% inoculum (20°C), the methane formation started without a lag-phase right from the

first day. The rate of gas formation at 10 and 15°C remained constant throughout the experiment, indicating that it was already at a maximum in the inoculum. A slight increase occurred during the initial phase at 20°C when the VFA was degraded.

The higher gas production at 20°C than at 15°C is most certainly caused by an increased hydrolysis. The lower acid level does not account for the whole difference. The difference in methane formation between 15 and 10°C, however, might be explained by the reduced VFA conversion at 10°C alone.

Using a mesophilically-adapted inoculum (30°C) still allowed digestion to start at 15°C. However, gas formation was slightly delayed when compared with the culture with the 20°C seed.

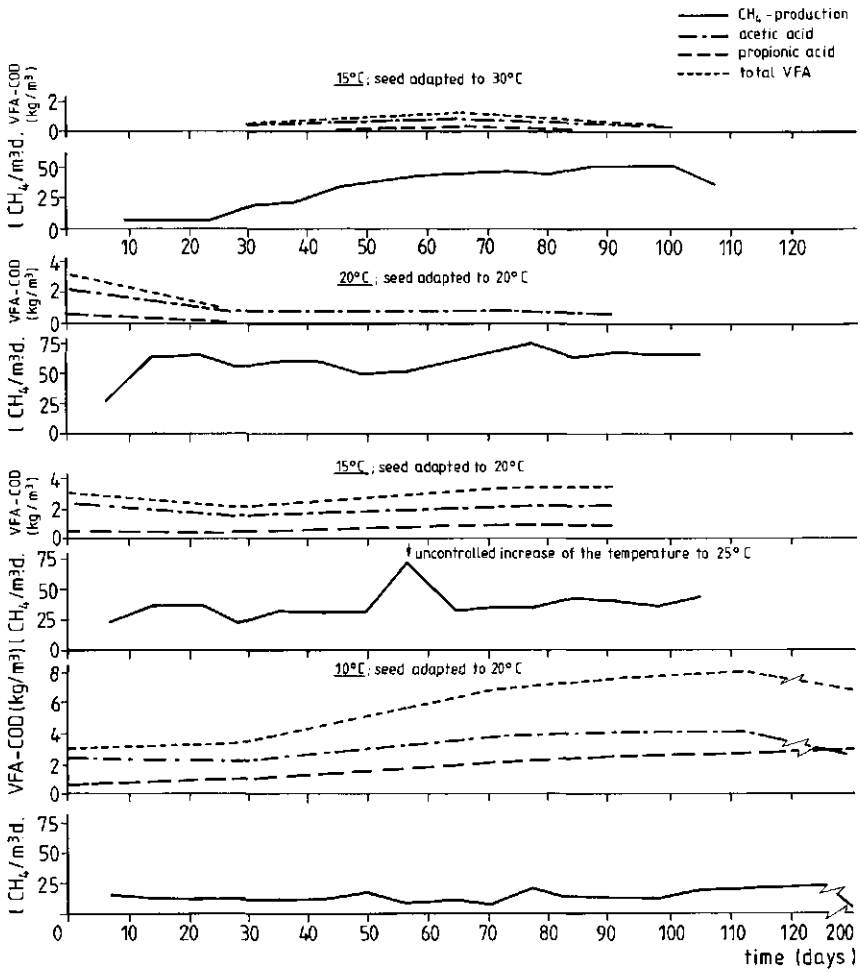


Figure 7. Development of methane production and VFA concentrations (expressed in terms of COD) during start-up of accumulation systems with cow manure at three different process temperatures (10, 15 and 20°C). The 50% inocula were adapted to either 20 or 30°C. The filling time of the digesters was 100 days.

2.3.5. Start-up of a CSTR at 20°C: dairy cow manure

A first digester working at 20°C was started with 100% stored pig manure. The data of the start-up period are not shown. At time equal to zero (Figure 8; digester A) it had already been operated for 5½ months at an HRT of 40 days. A later reduction to 30 days (at t=120 days) did not significantly change daily gas production nor acid levels. Digester B was started with t=0 with a 100% seed of mesophilically-digested cow slurry at an HRT of 30 days and a fermentation temperature of 20°C. Gas production adjusted to a low but constant level, whereas the VFA continued to increase. Hence steady-state conditions could not be established within 175 days of operation. When the HRT was increased to 50 days, VFA started to decrease and gas production almost instantly doubled.

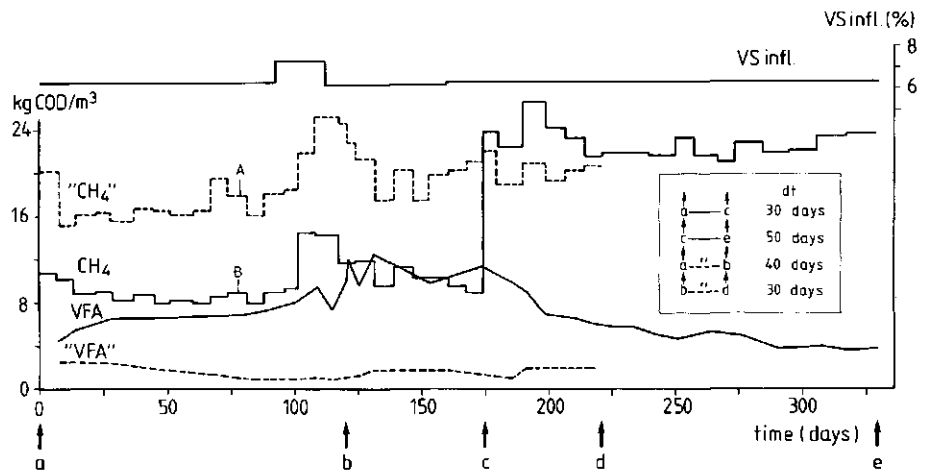


Figure 8. Development of methane and VFA COD during digestion of dairy cow slurry in a CSTR at 20°C and 30-day HRT when started A: with psychrophilically-digested manure at 20°C (---) and B: with mesophilically-digested manure at 30°C (—).

In a second series of experiments at 20°C (Figure 9) it was confirmed that the addition of a 100% seed of mesophilic sludge might be a good way of starting up continuous-flow digesters on the condition that the HRT is kept at 40 days or higher. Again, "overloading" at 30 days HRT led to a low gas production and an increase of the acid level.

A temporary increase in temperature from 20 to 25°C was allowed to correct the critical situation. When the temperature was readjusted after 20 days, the accumulated VFA's in the "overloaded" digester were converted to methane and steady-state conditions were achieved at a 30-day retention time with comparable production rates for both types of inoculation materials.

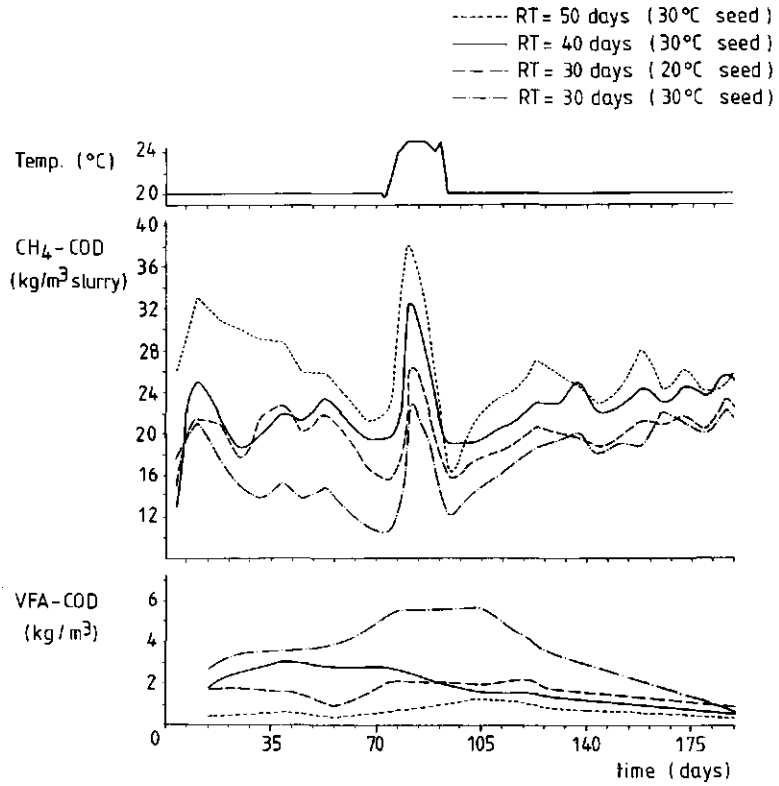


Figure 9. Methane production, VFA concentration and process temperature as a function of time during the digestion of dairy cow manure at 20°C and HRT between 30 and 50 days.

2.4. DISCUSSION

The work clearly indicated that without seed start-up of any type of psychrophilic digester is not possible at 15°C or lower. This finding is not surprising. It confirms observations with a full-scale installation, where digestion of cold pig slurry started only after the addition of stored cattle manure and warm sewage sludge.

The present data on accumulation systems show that at 20°C start-up without inoculation is possible. The lower temperature limit is probably around 18°C, as was indicated by the start-up of a full-scale low-temperature system at the research farm in Tänikon (Sutter & Wellinger, 1987a).

Using a large inoculum (50% or more) of mesophilically-digested sludge allows initiation of gas production at 15°C or higher in both batch and accumulation systems.

When an abundant amount of psychrophilically-adapted sludge is available, start-up at temperatures as low as 5 and 10°C are possible, as was shown in the batch and accumulation experiments. However, in practice there are limits to the inoculation volume in accumulation systems. A too large amount would reduce the storage capacity, leading ultimately to application to land of the digested manure during periods without vegetation, which is, or will be, forbidden by Swiss and by Dutch law. Anyway, gas production at temperatures around 10°C or lower is too low in practice to be exploited. Moreover, the risk of process failure is too

high considering the demonstrated increase of VFA. As demonstrated by full-scale installations (Wellinger & Kaufmann, 1982), 15°C might be a reasonable lower temperature limit for an accumulation system. Recent results by Zeeman *et al.* (1986) indicated that with a well-adapted 13% inoculum an accumulation system can be started within a reasonably short period.

For the time being, it is difficult to find the large volumes of psychrophilically-treated manure that are required for the start-up of full-scale installations. We therefore recommend the start-up of any of the systems with a mesophilic seed at temperatures around 20°C, even though the final operational temperature might be lower. If a proper retention time is chosen, bacteria adapt easily to lower temperatures, as we have shown in our CSTR experiments. Since in cold climates an initial manure temperature of 20°C is not attained naturally, at least an emergency heating system is recommended for low-temperature systems.

The increase of temperature from 20 to 25°C during the start-up experiment with continuous-flow digesters revealed an interesting phenomenon: a digestion process heading towards breakdown can be cured by temporarily increasing the temperature. This observation points again to the fact that low-temperature systems should be equipped with some sort of heating system.

Together with temperature and volume of seed, the TS-content seems to play a certain role during start-up, as is shown by the lab- and pilot-scale experiments with batch-fed systems: the higher the TS-content the longer the lag-phase. For mesophilic digestion Summers & Bousfield (1980) and van Velsen (1981) with pig manure and Baserga (1984) with cattle manure have already indicated the close relation between TS-concentration and gas yield independent of retention time.

The results of these experiments might be summarized as follows:

Anaerobic digestion of animal manure can be started at temperatures as low as 5°C if an adapted inoculum of an adequate size (50%) is available. Without seed it is not possible to initiate methane production below 20°C.

The start-up time of pig manure digestion at 20°C is significantly reduced by the addition of cow manure. All other additives tested gave no reduction of the lag-phase. Small additions of warm sewage can create a positive effect by temporarily raising the fermentation temperature.

Some evidence was presented that high TS concentrations in the inoculum could be inhibitory to the start-up process.

The activation of a continuous-flow digester at low temperatures initially requires long retention times. Best results were achieved at 50 days HRT.

Mesophilically-digested sludge is an excellent inoculum to start a continuous-flow digester as long as fermentation temperature is initially kept at 20°C or higher. If applied in large quantities (50% inoculum) it also initiates gas production in accumulation systems at temperatures as low as 15°C.

Despite the fact that psychrophilic digesters might work at ambient temperatures, it is proposed to equip them with some sort of heating device. It was shown that start-up time can be shortened considerably at higher temperatures. In addition, a short-term rise in temperature above 25°C can even cure a slightly inhibited process.

2.5. ACKNOWLEDGEMENTS

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Swiss study was made possible by a contribution of the Swiss Federal Office of Energy (BEW). Both contributions are gratefully acknowledged.

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CHAPTER 3.
START-UP OF LOW TEMPERATURE DIGESTION OF MANURE

(published in: Anaerobic Digestion 1988, Advances in Water Pollution Control)
(revised version)

Contents

Abstract

Keywords

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CHAPTER 3.
START-UP OF LOW TEMPERATURE DIGESTION OF MANURE

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ABSTRACT

The start-up of an AC-system for the digestion of cow and pig slurry at low temperatures (15-20°C) was tested with different percentages inoculum.
For start-up of an AC-system, digesting cow slurry at 15°C at low inoculation (≤ 15% of the total reactor volume) with digested slurry precultured in a CSTR-system, a minimal digestion time of 240 days is necessary.
The start-up time can be reduced considerably by increasing the process temperature to 20°C. Start-up with AC-seed is much faster than with CSTR-seed, due to a simultaneous removal of acetic and propionic acid. The start-up of an AC-system at 15°C with 15°C-AC-seed is faster than with 20°C-seed.

KEYWORDS

Cow slurry; pig slurry; start-up; accumulation system; low temperature digestion.

3.1. INTRODUCTION

So far, anaerobic digestion of cow and pig slurry is mainly performed in continuous-flow digesters (CSTR-systems) at mesophilic conditions. Results of digestion of manure at farm-scale are however often disappointing, due to low process efficiency, high investment costs and predigestion of manure during storage (Zeeman *et al.*, 1985).
Wellinger and Kaufmann published in 1982 results of full-scale digestion of pig manure at ambient temperatures in a so-called accumulation system (AC-system), where storage and digestion were combined. This low temperature digestion system proved to be an attractive alternative to a mesophilic CSTR-system. The research of low temperature digestion of manure at laboratory- and pilot-scale in the Netherlands started in 1984. The start-up experiments formed a main part of this research.

Wellinger and Kaufmann (1982) and Cullimore *et al.* (1985) observed an adaptation of the bacterial community to the new conditions during the digestion of pig slurry in resp. an AC-system and an anaerobic lagoon at ambient temperatures.
The effect of the quality of the seed, viz. the temperature and the system where it was precultured, on the start-up of a low temperature AC-system is described in this paper. The paper also presents a method to obtain an appropriate inoculum for the start-up of an AC-system at 15°C within a relatively short time.

3.2. MATERIAL AND METHODS

3.2.1. Analysis

Total COD was determined by weighing ± 1.5 g sample and adding 18.5 ml H_2O plus 20 ml $K_2Cr_2O_7$ (2N) plus 30 ml Ag_2SO_4/H_2SO_4 (30 g Ag_2SO_4/l). The mixture is boiled for two hours and partly titrated with $FeSO_4(NH_4)_2$. Samples for the determination of the concentrations of volatile fatty acids (VFA), total ammonia and dissolved COD, were prepared by membrane filtration (pore diameter, 0.45 μm), preceded by centrifugation (10-60 min, 27000 *g*). Concentrations of VFA of non acidified samples were determined on a Packard Becker model 417 gas

chromatograph with FID, equipped with a 2mx2mm (ID) glass column with either Chromosorb 101 (80-100 mesh) at 190°C, or Fluorad FC-431 on Supelcort (100-120) at 130°C. Detector temperature was 240°C, and nitrogen gas saturated with formic acid was used as the carrier gas (25 and 50 ml min^{-1}, respectively). The VFA concentrations are given in COD, as calculated from the acid concentrations determined. Total ammonia (NH_3+NH_4^+) was measured photometrically using direct Nesslerization of membrane-filtered samples. A correction was applied for the colour of the samples by measuring the extinction of the samples without reagents. Dissolved COD, VS and TS were measured according to Standard Methods (American Public Health Association (1975).

3.2.2. Substrate

Table 1 gives the values for the composition of the slurries utilized in the different experiments.

Table 1. Composition of the influent pig and dairy cattle slurries (g/l).

	cow slurry	pig slurry*	pig slurry+
Total COD	94.5	113.5	73.9
Dissolved COD	24.6	43.9	22.7
Total VFA-COD	11.2	32.4	14.8
C_2-COD	6.0	14.6	6.1
C3-COD	3.4	6.8	3.1
i-C4-COD	0.4	1.9	1.1
n-C4-COD	0.7	6.1	2.4
i-C_5-COD	0.6	2.6	1.7
n-C_5-COD	0.2	0.5	0.3
TS	87.9	82.8	56.0
VS	67.6	55.8	39.2
NH_4^+-N	1.9	5.1	3.7

*used for the start-up experiments with granular and sewage sludge

+used for the start-up experiments with 15°C-AC-seed, 15°CSTR-seed and 20°-AC-seed

3.2.3. Reactors

Experiments were made in reactors with an effective volume of 1.5 or 5 liters. Reactors were fed once a week. Digesters were only manually shaken, except for the mechanically stirred (30 sec. per 30 minutes) digesters with cow slurry at 50% inoculation. All digesters were filled in 100 days, except for the two reactors in the experiment described in Chapter 3.3.1.2. Cooling was performed by the use of a thermostated water bath. The gas produced, passed a column of soda lime pellets and was collected in a gas bag. The amount of gas was measured weekly by pumping the collected gas through a wet test gas meter.

3.3. RESULTS

3.3.1. Effect of the loading rate at 15° and 20°C

3.3.1.1. Percentage inoculation

In a first series of experiments, the start-up of an AC-system for the digestion of cow slurry was tested for different process temperatures and percentages of inoculation.
At 20°C, in a CSTR-system digested slurry (20°-CSTR-seed) was used for inoculation. Figure 1 and 2 present the daily gas production rates and VFA-concentrations during the start-up of an AC-system with 1, 7 and 13% (of the final slurry volume) inoculation at resp. a process temperature of 15 and 20°C. With the exception of the start-up with 1% inoculation the gas production at 20°C started without any lag-phase. The gas production rate strongly increased during the 100 days that the reactor was filled up. The VFA's could be converted efficiently into CH_4-gas within the filling time. At 1% inoculation a lag phase of ± 100 days was observed and a complete removal of VFA's was only achieved at a total digestion time of 180 days. The accumulated VFA's in the digester at 15°C, could only be broken down completely at a total digestion time of 240-310 days.

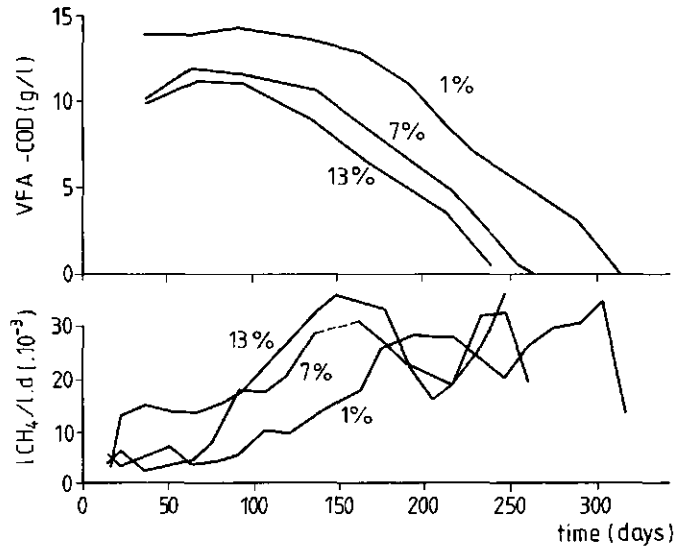


Figure 1. Experimental results of the start-up of an AC-system, conducted with cow slurry, viz., course of the volumetric methane gas production and total VFA concentration in relation to the digestion time.

digester temperature	:15°C
amount of inoculation	:1-13%
type of inoculum	:20°C-CSTR-seed
filling time	:100 days
applied influent slurry	:94.5 g COD/l
mixing	:no mixing

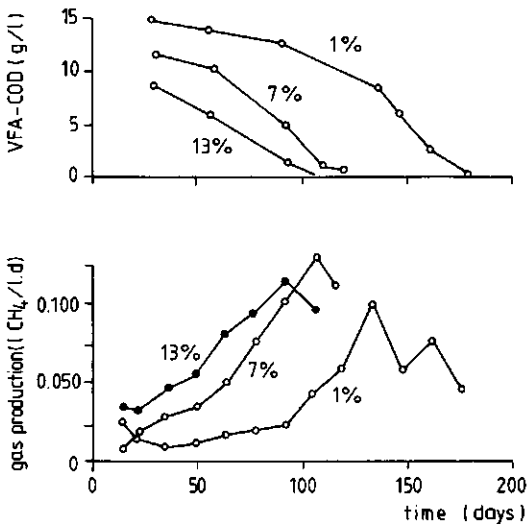


Figure 2. Experimental results of the start-up of an AC-system, conducted with cow slurry, viz., course of the volumetric methane gas production and total VFA concentration in relation to the digestion time.

digester temperature	:20°C
amount of inoculation	:1-13%
type of inoculum	:20°C-CSTR-seed
filling time	:100 days
applied influent slurry	:94.5 g COD/l
mixing	:no mixing

3.3.1.2. Filling time.

At the start-up of an AC-system for the digestion of cow slurry at 50% inoculation (20°C-CSTR-seed) a relatively constant gas production rate was shown during the 100 days that the reactor was filled at both 15 and 20°C (Figure 3). When a filling time of 70 instead of 100 days was applied at a process temperature of 20°C, a higher gas production rate was observed. At a process temperature of 15°C however, the highest gas production was found at the lowest loading rate (100 days filling time). The activity of the seed was not only insufficient for the applied loading rate at a filling time of 70 days, but the CH_4-production was apparently also inhibited by the accumulation of intermediates. The situation could be improved by imposing a temporary increase of the temperature to 25°C. After the temperature increase, the highest gas production was found at the highest loading rate (Figure 3.)

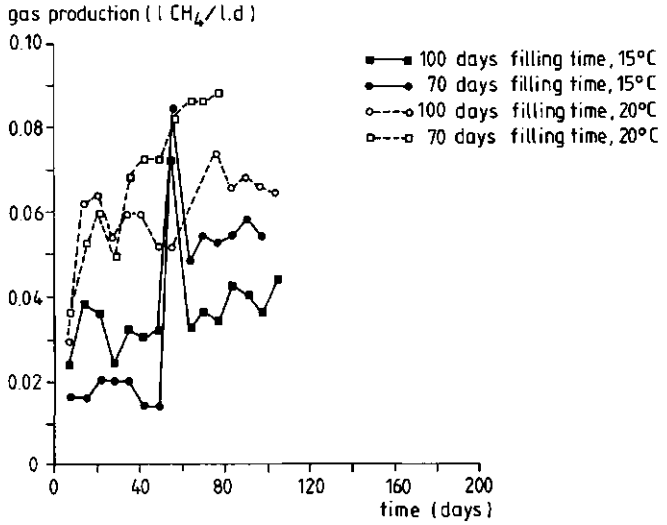


Figure 3. Experimental results of the start-up of an AC-system, conducted with cow slurry, viz., course of the volumetric methane gas production and total VFA concentration in relation to the digestion time.

digester temperature	:15 and 20°C
amount of inoculation	:50%
type of inoculum	:20°C-CSTR-seed
filling time	:70 and 100 days
applied influent slurry	:94.5 g COD/l
mixing	:mixing applied

3.3.2. Effect of the quality of the inoculum.

3.3.2.1. Digested slurry

An important aspect, influencing the quality of the inoculum, are the conditions under which the seed material was cultivated, especially the digestion system and the process temperature. The second start-up of the AC-system at 15°C and 13% inoculation was seeded with biomass from the first start-up. Figure 4 illustrates higher gas productions during this second start-up as compared with the first start-up, while also a complete removal of VFA's could be achieved in 140 instead of 240 days. Moreover, while C_2 and C_3 were sequentially removed during the first start-up, a simultaneous breakdown of these acids occurred during the second start-up with the 15°-AC -seed.

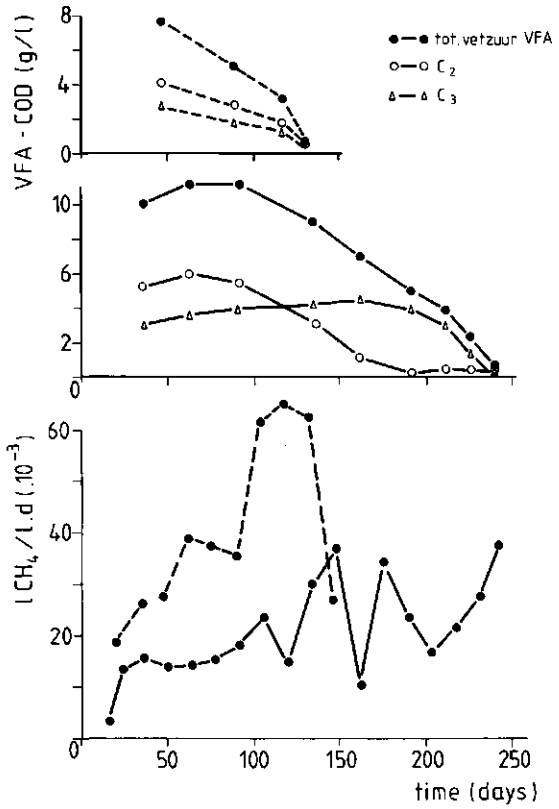
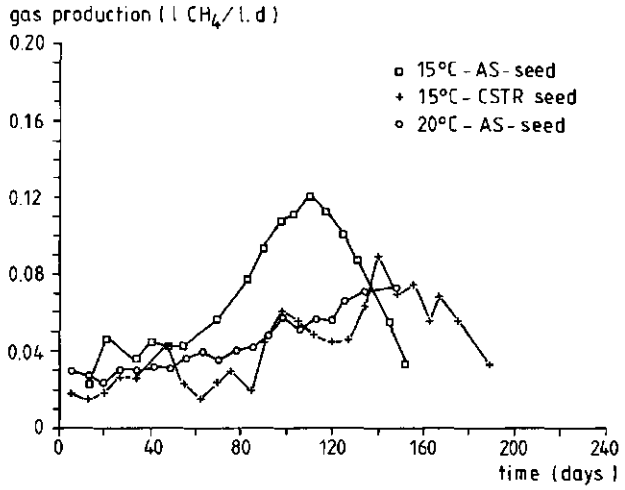


Figure 4. Experimental results of the start-up of an AC-system, conducted with cow slurry, viz., course of the volumetric methane gas production and total VFA concentration in relation to the digestion time.

digester temperature	:15°C
amount of inoculation	:13%
type of inoculum	:20°C-CSTR-seed for the first start-up (—)
	:15°C-AC-seed for the second start-up (---)
filling time	:100 days
applied influent slurry	:94.5 g COD/l
mixing	:no mixing

Results of batch experiments with VFA's, showed that 15°C-seed, precultured in a CSTR-system was unable to digest C_2 and C_3 simultaneously (Zeeman *et al.*, 1986). These results were confirmed by the experiments, in which the influence of the quality of the inoculum, viz., the digestion system and temperature where it was precultured, on the start-up of a pig slurry AC-system at 15°C was studied (Figure 5). Figure 5 illustrates a faster start-up with AC-seed than with CSTR-seed. In using CSTR-seed no C_3 removal occurred within the first 200 days of digestion. However, using 15°- and 20°-AC-seed a simultaneous removal of C_2 and C_3 could be achieved in resp. 140 and 200 days.



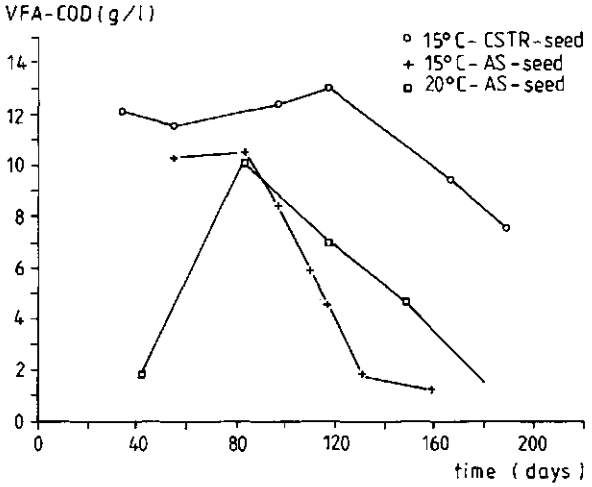


Figure 5. Experimental results of the start-up of an AC-system, conducted with pig slurry, viz., course of the volumetric methane gas production and total VFA concentration in relation to the digestion time.

digester temperature	:15°C
amount of inoculation	:15%
type of inoculum	:15°C-CSTR-seed
	:15°C-AC-seed
	:20°C-AC-seed
filling time	:100 days
applied influent slurry	:74 g COD/l
mixing	:no mixing

3.3.2.2. Granular and sewage sludge

In addition to start-up experiments with digested cow- and pig-slurry, research was carried out on the start-up of an AC-system with 30°C-granular sludge from a UASB and 30°C-sewage sludge from a CSTR-system. Figure 6 gives the gas production during the start-up of an AC-system for the digestion of pig and cow slurry at 15°C, using 50% granular sludge or sewage sludge as seed material. The gas production in the digestion of cow slurry started right from the beginning and remained relatively constant during the 100 days that the reactor was filled. The VFA concentration remained below 1 g COD/l. The gas peak at day 70 results from a temporary increase of the temperature.

In digesting pig slurry, the gas production rate dropped at day 80 and remained very low for the following 120 days. Accumulated VFA's were only converted into methane gas after a total digestion time of 200 days (Figure 6a). The stagnation in the conversion of VFA into CH_4-gas was caused by a strong increase of the NH_4^+-N-concentration (0.5 → 2.5 g/l), caused by the high NH_4^+-N concentration of the influent pig slurry (5.1 g/l). The NH_4^+-N-concentration of the cow slurry was only 1.9 g/l.

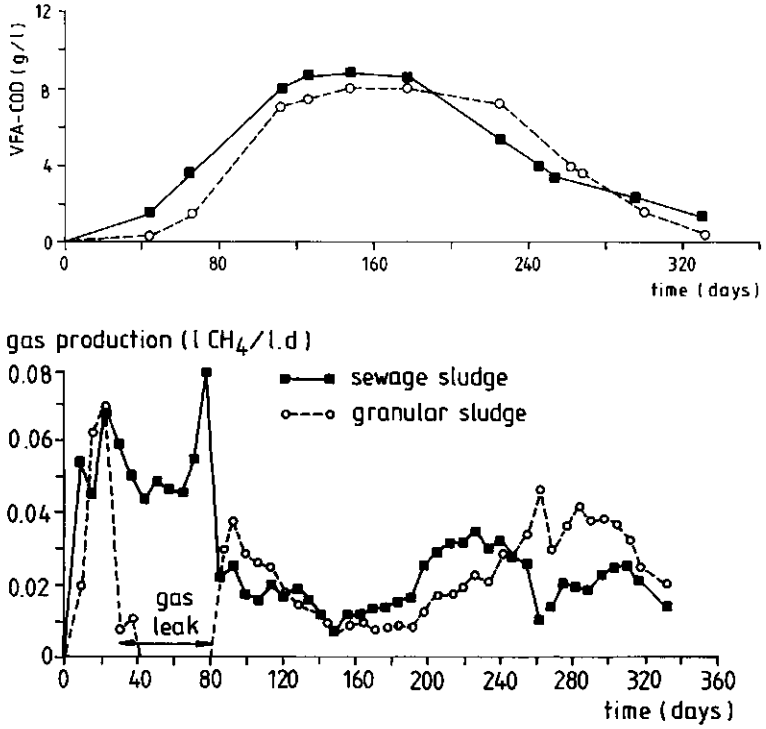


Figure 6a. Experimental results of the start-up of an AC-system, conducted with pig slurry, viz., course of the volumetric methane gas production and total VFA concentration in relation to the digestion time.

digester temperature	:15°C
amount of inoculation	:50%
type of inoculum	:30°C-UASB granular sludge
	:30°C-CSTR digested sewage sludge
filling time	:100 days
applied influent slurry	:114 g COD/l
mixing	:no mixing

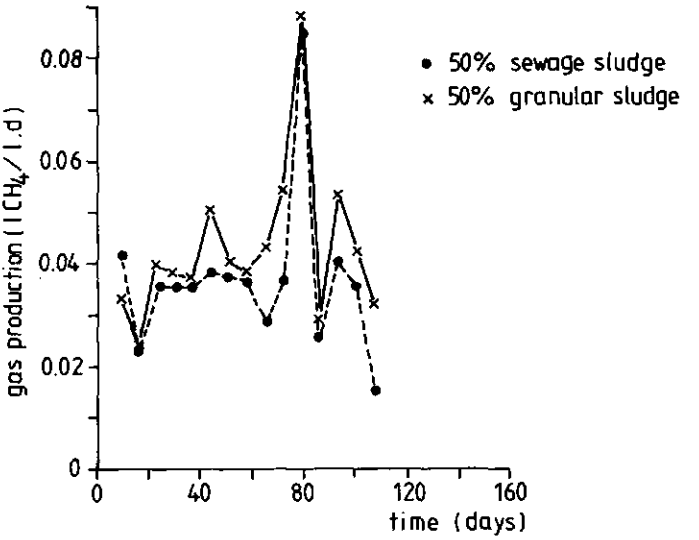


Figure 6b. Experimental results of the start-up of an AC-system, conducted with cow slurry, viz., course of the volumetric methane gas production in relation to the digestion time.

digester temperature	:15°C
amount of inoculation	:50%
type of inoculum	:30°C-UASB granular sludge
	:30°C-CSTR digested sewage sludge
filling time	:100 days
applied influent slurry	:114 g COD/l
mixing	:no mixing

3.4. DISCUSSION AND CONCLUSIONS

From the results of the research presented in Chapter 2, it became obvious that no gas production could be generated within 5 months of start-up of digestion of cow slurry without inoculation at temperatures ≤ 15°C.
The present data proved that the first start-up of an AC-system with 20°C-CSTR -seed at 15°C was not only possible at a high inoculation of 50% (Chapter 2), but also at low inoculation of 1-13%, both at a filling time of 100 days.
While the gas production at 50% inoculation started right from the beginning, at 1-13% inoculation a lag-phase of 100-25 days was observed. Moreover, at low inoculation a complete removal of accumulated VFA could only be accomplished in 240-300 days. Characteristic of the slow first start-up at 15°C, is a non-simultaneous removal of C_2 and C_3. The second start-up at low inoculation and temperature is much faster, C_2 and C_3 are simultaneously eliminated and daily gas productions are higher.

Wellinger and Kaufmann (1982) also observed an increasing daily gas production during the 2 years following upon the start-up of a low temperature AC-system for the on-farm digestion of pig slurry. Results of experiments with lagooned pig slurry made by Cullimore *et al.* (1985) indicated an adaptation of the system to lower temperatures in a period of two years. Results of Swiss laboratory research (Chapter 2) demonstrated that the best start-up of an AC-system at low temperature was obtained when the temperature of the seed corresponded with the process temperature. The results presented show that besides the temperature, the system where the seed was grown, strongly influences the start-up of an AC-system at low

temperature. While the start-up of an AC-system at 15°C for the digestion of pig slurry with 15°-AC- and 20°-AC-seed could be accomplished in resp. 140 or 200 days, no propionic acid was eliminated in 200 days of digestion when 15°C-CSTR-seed was applied.
In consequence of the relatively low loading rate applied in a CSTR, CSTR-seed is essentially unsuitable for the start-up of an AC-system at low inoculation (15%) at 15°C. At start-up of an AC-system with CSTR-seed, at low inoculation and at a process temperature of 20°C, the activity in the reactor is rapidly increasing and consequently start-up can be completed within the 100 days filling time.

AC-seed is as yet not, or scarcely, available. We recommend therefore to start-up an AC-system for low temperature digestion of manure at a process temperature of 20°C by inoculation with digested slurry, precultured in a CSTR-system. When no heating is installed, the summer season should be chosen for the first start-up. Sewage sludge or granular sludge, not adapted to NH_4^+-N, can only be used for inoculation when the NH_4^+-N concentration of the slurry is $\leq$ 2 g/l. With the start-up of AC-systems on pig manure, Hill et al. (1983) attributes the inhibition of the methane production to the high concentrations of accumulated fatty acids, when sewage sludge was used as inoculum. The results in this Chapter indicate that when unadapted inoculum (such as sewage sludge and granular sludge) is used in an AC-system for digesting manure with a relatively high $NH4^+$-N concentration, an adaptation period is required to $NH4^+$-N in which a stagnation of the fatty acid degradation and gas production will occur. The results of the starting-up experiments of AC-systems with CSTR sludge at a relatively high loading rate (filling time=70 days) indicate that also inhibition by fatty acids can appear. It is shown that inhibition of the digestion process caused by overloading during start-up can be cured by a temporary increase of the temperature to 25°C. This phenomenon was also seen at the start-up of a CSTR-system at 20°C and high loading rate (Chapter 2). As already stated in Chapter 2, some sort of (auxiliary) heating is advisable for low temperature systems, both for starting-up the system and for recovering of the process in an inhibited situation.

3.5. ACKNOWLEDGEMENT

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CHAPTER 4.
ANAEROBIC DIGESTION OF COW MANURE IN A CSTR-SYSTEM
Effect of temperature (15-40°C) and detention time (10-150 days)

Contents

4.1. Introduction

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CHAPTER 4.
ANAEROBIC DIGESTION OF COW MANURE IN A CSTR-SYSTEM
Effect of temperature (15-40°C) and detention time (10-150 days)

4.1. INTRODUCTION

Approximately $58.7*10^6$ m^3 cow slurry is produced yearly in the Netherlands, which represents about $616*10^6$ m^3 methane gas. The question to be answered is how to get control of this relatively large amount of potential energy. One possible option might be on farm production and use of biogas. However the use of biogas is only attractive when it can be produced at sufficiently low costs. For this purpose optimization of process parameters of the digestion process, such as particularly the temperature and detention time is of great importance. In this respect the last ten years much attention has been given to the digestion of cow manure. Steiner (1983) reviewed the literature dealing with this subject. The major part of the research reported by Steiner (1983) concerns the digestion of beef cattle slurry and not of dairy cattle. A comparison of the results of the research in the digestion of beef cattle slurry at different process temperatures has been made by Varel *et al.* (1980) and Chen *et al.* (1980). Hawkes *et al.* (1984) researched the digestion of the liquid fraction of separated dairy cow manure at temperatures of 30 to 35°C and detention times of 9 to 17 days. Both researchers found an increase of the gas production at increasing detention time in this range and Chen *et al.* reported an increase of the gas production when increasing the temperature in the range of 30 to 40°C. Baserga (1984) investigated the digestion of dairy cow slurry at 33°C and detention times ranging from 8-30 days. He found a strongly increasing gas production when increasing HRT from 8 to 17 days followed by a slowly increasing gas production up to HRT=30 days.

So far little attention was paid to the digestion of manure at low temperatures. Wellinger and Kaufmann (1982) published results of 2 full scale accumulation systems for the digestion of pig slurry at ambient temperatures. These results were very promising. Daily gas productions approached the net gas productions obtained in mesophilic continuous flow digesters. Sutter *et al.* (1985) found that a stable digestion for cow slurry can be achieved in a continuous flow digester at a temperature of 14°C and a detention time of 37 days, which is rather short for such a low temperature. Hawkes *et al.* (1984) also performed the digestion of separated cow slurry at a temperature of 15°C and at detention times as low as 20 days, but just very little gas was produced, viz. 0.067 l biogas/m^3. Cullimore *et al.* (1985) published about the low temperature digestion of lagooned pig manure and of additional laboratory experiments. They found that a fair amount of adaptation occurs during a research period of two years.

The main objective of our investigations was:
1. to determine the optimum temperature and detention time with respect to the gas production from dairy cow slurry in CSTR systems.
2. to assess the stability of the anaerobic process at the various conditions and
3. to achieve more information about the kinetics of cow slurry digestion. In addition we also investigated the possibility of adjusting the detention time for controlling the gas production in order to meet seasonal needs.

4.2. MATERIAL AND METHODS

4.2.1. Experiments

Three series of experiments, viz. series A, B and C were executed at mesophilic conditions. The low temperature experiments (15 and 20°C) were performed in the series D. The experimental set up of the four series of experiments is given in Table 1.

Table 1. Set up of the experiments performed with dairy cow slurry at various detention times and temperatures.

Experiment A

t=0-90		t=90-145		t=145-165		t=165-230	
Dt (d)	temp (°C)	Dt (d)	temp (°C)	Dt (d)	temp (°C)	Dt (d)	temp (°C)
30	30	30	35	10	35		
25	30	25	35	10	35		
20	30	20	35	10	35	10	35
15	30	15	35	10	35		
25	40	25	40	25	40		

Experiment B

t=0-20		t=20-60	
Dt (d)	temp (°C)	Dt (d)	temp (°C)
10	30	10	30
25	30	10	30
25	30	25	30+

+ sludge used in experiment C

Experiment C

t=0-100		t=100-270		t=270-290		t=290-325		t=325-400	
Dt (d)	temp (°C)	Dt (d)	temp (°C)	Dt (d)	temp (°C)	Dt (d)	temp (°C)	Dt (d)	temp (°C)
25	30	25	25	10	25	15	25	20	25

Experiment D

t=0-90	
Dt (d)	temp (°C)
50	15

t=0-631		t=631-708		t=708-923		t=923-1132*	
Dt (d)	temp (°C)	Dt (d)	temp (°C)	Dt (d)	temp (°C)	Dt (d)	temp (°C)
100	15	150	15	100	15	100	15

t=0-403		t=403-1150		t=1150-1406*	
Dt (d)	temp (°C)	dt (d)	temp (°C)	Dt (d)	temp (°C)
100	15	150	15	150	15

t=0-416	
Dt (d)	temp (°C)
50	20

t=0-420		t=420-510	
Dt (d)	temp (°C)	Dt (d)	temp (°C)
100	20	100	15

t=0-510	
Dt (d)	temp (°C)
150	20

t = digestion time (days), * concentrated slurry

Main characteristics of the different experimental series:
A. Effect of increasing the temperature from 30 to 35°C and effect of a subsequent decrease of the detention time from 15, 20, 25 and 30 to 10 days. In addition one experiment was made at 40°C and 25 days detention time.
B. Effect of a decrease of the detention time from 25 to 10 days at a digestion temperature of 30°C and comparison with continuous digestion at a temperature of 30°C at HRT=25 and at HRT=10 days.
C. Effect of decreasing the temperature from 30 to 25°C at HRT=25 days and effect of a subsequent decrease of the detention time to 10 days.
D. Digestion at 15 and 20°C at a detention time of 50, 100 and 150 days.

4.2.2. Slurry

Dairy slurry was collected from a storage pit situated under slatted floors of an experimental dairy farm of the Institute of Agricultural Engineering in Duiven. The collected slurry was mixed with an ultra-turrax and then stored in 5 liters buckets at 5°C.
Experiments A, B, C, and D were performed with slurry collected at different dates. The slurries were always analyzed for total COD, dissolved COD, VFA, TS, VS, and NH_4^+-N concentrations. The characteristics of influent slurries used in the experiments A, B, C and D are given in Table 2.
The experiments were performed in jacketed digesters through which thermostated water was pumped for maintaining the desired temperature. The working volume of the digesters was 5 liters and they were mechanically mixed for 30 seconds every 30 minutes. Feeding of the mesophilic digesters, operated at 30 to 40°C, was given daily, except during weekends when a double feeding was supplied. The digesters, operated at 15 and 20°C, were fed weekly. The mesophilic digesters were monitored via daily gas production measurement, while analyses for VFA, dissolved COD and NH_4^+-N concentrations were conducted once a week. In the low temperature digesters gas production was measured once a week while the analyses were made once a month. The methane gas was measured using a wet test gas meter or collected in a gas bag after passing the biogas through a column of soda lime pellets to absorb CO_2. The amount of gas, collected in the gas bag was measured by pumping it through a wet test gas meter.

Table 2. Chemical characteristics of influent slurries used in experiments A, B, C and D (g/l).

	A	B,C	C	C	C	C	D
Total COD	96.6	116.2	131	103	108	102	94.1
TS	77.5	91.8	-	84.1	84.5	81.1	85.5
VS	56.2	72.5	80.8	61.9	62.1	61.6	65.9
diss COD	35.2	26.6	34.1	29.4	30.7	27.2	24.6
VFA COD	13.6	9.9	12.9	13.6	15.8	12.5	12.2
C_2 COD	7.5	5.7	7.5	9.1	7.5	6.5	-
C_3 COD	3.6	2.3	3.6	3.9	2.8	3.7	-
i-C_4 COD	0.8	0.3	0.6	0.7	0.5	0.4	-
C_4 COD	0.7	0.8	0.9	0.9	0.7	0.7	-
i-C_5 COD	1.0	0.6	0.8	0.9	0.9	0.7	-
C_5 COD	0.2	0.1	0.2	0.3	0.2	0.2	-
NH_4^+-N	-	2.4	2.9	2.4	2.8	2.5	1.9

4.2.3. Analyses

Analyses were performed as described in Chapter 3.

4.2.4. Inocula

The A experiments were started with mesophilic digested cow manure obtained from the 110 m^3 experimental digester in Duiven, which was operated at 30°C and at 20 days detention time. At t=0 the digesters had already been operated for 100 days at the detention times to be researched. The B experiments were also started with mesophilic digested cow manure obtained from the 110 m^3 experimental digester in Duiven, which was operated at 30°C and at 20 days detention time. The C experiments were started with digested manure from experiments B. The digester working at 15°C and 100 days detention time and those at 20°C at HRT= 50, HRT=100 and HRT=150 days were started with digested slurry from the digesters working at 20°C and 30-50 days detention time (see Chapter 3). The digester working at 15°C and 150 days detention time was started with at 15°C, in a batch reactor, digested slurry.

4.3. RESULTS

The results of the series A experiments are presented in Figure 1. The volumetric gross gas production (CH_4-COD/ m^3.d.) is shown here versus time for the four different detention times investigated at 30, 35 and 40°C. The results of the 35°C experiments show that upon decreasing the detention time from resp. 30, 25, 20 and 15 to 10 days the volumetric gas production, rapidly increases, viz. up to 2 kg COD/m^3.d within a period of 10 days. The period of time required to reach this value is positively related to the detention time applied in the preceding period. The drop in the gas production occurring at day 162 merely can be attributed to a lower VS concentration of a new batch of influent manure.

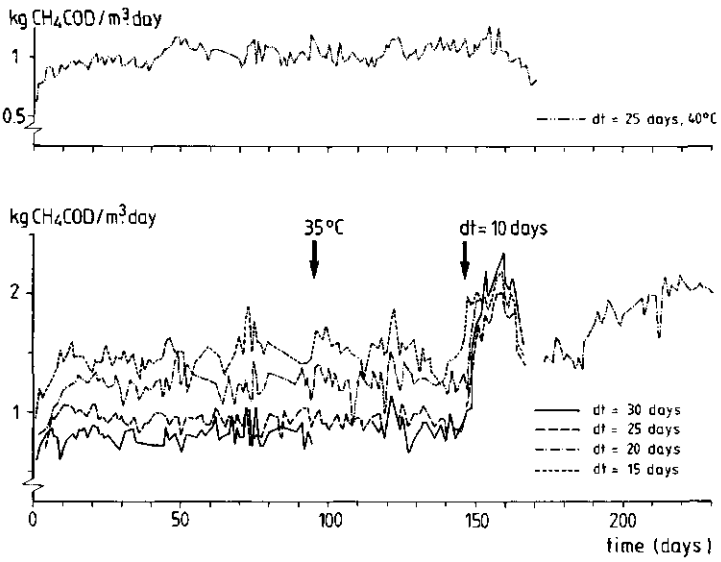

Figure 1. Course of the volumetric methane gas production rates (kg COD /m^3.d) at detention times of 15, 20, 25 and 30 days at a subsequent digestion temperature of 30°C and 35°C and at changing the detention time to 10 days at a digestion temperature of 35°C (experiments A).

The results of the series B experiments, presented in Figure 2, illustrate that it is also possible to achieve a stable digestion at 30°C and 10 days detention time. Even upon decreasing the detention time abruptly from 25 to 10 days, within a 10 days period of time, the same constant gas production rate is reached as in the experiments operated at 10 days detention time from the beginning. The VFA concentration only increases for a short period of time

after imposing the higher loading rate. The mean composition of the effluent and the specific gas production for the experimental series A an B are given in Table 3.
The differences shown in the specific gas productions between 20, 25 and 30 days detention time at 30°C did not prove to be significant. Except for 15 days detention time the differences in the specific gas production between 30, 35 and 40°C were also not significant.

Table 3. Composition of the effluent and the specific gas production found in the series A and B experiments.

series A

kg/m^3	dt=15 days 30°C	dt=15 days 35°C	dt=20 days 30°C	dt=20 days 35°C	dt=25 days 30°C	dt=25 days 35°C	dt=25 days 40°C	dt=30 days 30°C	dt=30 days 35°C
total VFA-COD	1.7	1.6	1.3	0.8	0.6	0.6	1.3	0.7	0.5
diss-COD	21.1	21.8	20.2	21.2	20.9	21.2	22.4	20.0	20.0
NH_4^+-N	3.4	3.4	3.7	3.7	3.5	3.5	3.4	3.5	3.5
CH_4-COD	22.4	23.4	26.1	25.2	24.1	23.9	25.1	24.5	26.8
(m^3/kg)									
CH_4/VS	0.140	0.146	0.163	0.158	0.151	0.149	0.157	0.153	0.168

series B

kg/m^3	dt=25 days 30°C	dt=10 days 30°C
total VFA-COD	0.24	0.5
diss-COD	17.6	19.1
NH_4^+-N	2.6	2.8
CH_4-COD	26.8	21.3
(m^3/kg)		
CH_4/VS	0.13	0.10

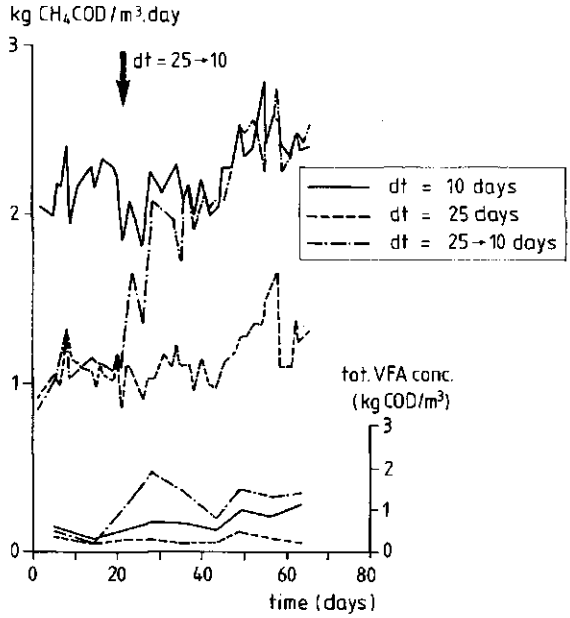

Figure 2a. Measured volumetric methane gas production (kg COD/m^3.d) and VFA concentration (kg COD /m^3) as a function of time at digestion at 10 and 25 days detention time and at a change of the detention time from 25 to 10 days at a digestion temperature of 30°C (experiments B).

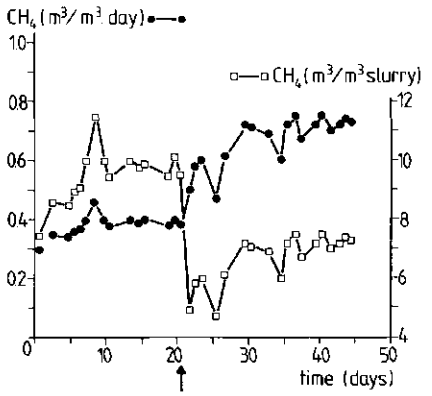

Figure 2b. Measured methane gas production rate (m^3/m^3.d) and specific methane gas production (m^3/m^3 slurry) as a function of time before and after changing the detention time from 25 to 10 days at a process temperature of 30°C (experiment B).

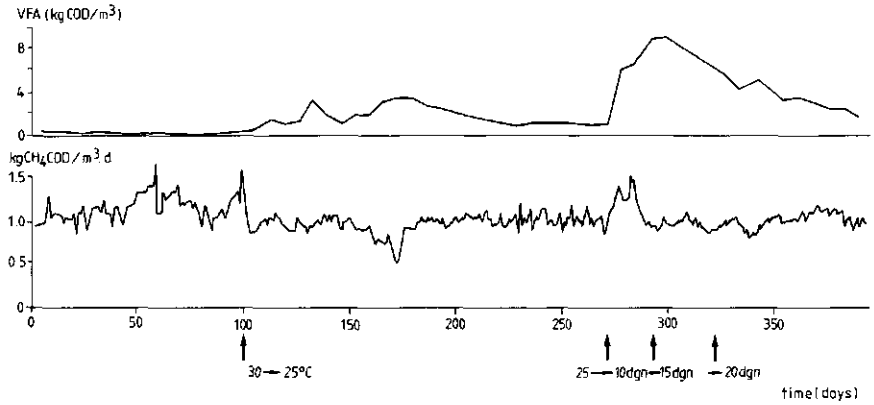

Figure 3. Volumetric methane gas production (kg COD/m^3.d) and VFA concentration (kg COD/m^3) as a function of time at 30°C and 25 days detention time and 25°C and successively 25, 10 15 and 20 days detention time (experiments C).

Figure 3 shows the volumetric gas production and VFA concentration found at the operational temperatures and detention times, applied in the series C experiments. The applied temperatures and imposed detention times are indicated in the Figure. Upon lowering the temperature at dt=25 days from 30 to 25°C, a slight but distinct decrease of the specific gas production is apparent, i.e. from 0.15 to 0.14 m^3/kg VS. Contrary to the experiments in series A and B, conducted at resp. 35 and 30°C, a reduction of the detention time from 25 to 10 days, but now at a process temperature of 25°C, results in a deterioration of the digestion process. The VFA concentration increases from about 1 kg COD/m^3 to a level of about 9 kg COD/m^3. The volumetric gas production increases for a short period of time but drops to the former level of about 1 kg CH_4 COD/m^3.d, corresponding to a drop of the specific gas production from 0.14 to 0.05 m^3/kg VS. Subsequently increasing the detention time to resp. 15 and 20 days results in a recovering of the digestion process, viz. the specific gas production increases to about 0.08 and 0.11 m^3 CH_4/ kg VS and the VFA concentrations decrease to resp. 5 and 2 kg COD/m^3. As a result of the increasing specific gas production the volumetric gas production is hardly effected by the increase in detention time.

The results in Figure 4 and Table 4 illustrate that it is quite well possible to digest cow manure at process temperatures of 15 and 20°C, provided the detention times are sufficiently long. The results in Figure 4 show the volumetric gas production at 15°C and 20°C at a detention time of 50, 100 and 150 days. It is evident, that at a digestion temperature of 15°C and at a 50 days detention time, the gas production drops rapidly to-wards zero. For that reason, the experiment at a 50 days detention time was terminated after 90 days of operation. The results in Figure 4d also illustrate that the gas production decreases when decreasing the process temperature from 20 to 15°C at a 100 days detention time. The gas production however remains somewhat higher compared with the gas production in the reactor all the time operated at 15°C and 100 days detention time.

Table 4, Established steady state[#] with respect to hydrolysis, acidification and methane formation at process temperatures of 15 and 20°C at detention times of 50, 100 and 150 days.

	15°C		20°C		
	1	2	3	4	5
detention time (days)	100	150	50	100	150
total hydrolysis (kg COD/m^3)	27.8	32.2	37.0	39.5	39.9
total acidification (kg COD/m^3)	16.0	17.3	25.9	28.8	29.0
hydrolysis reactor* (kg COD/m^3)	3.2	7.6	12.4	14.9	15.3
acidification react.+ (kg COD/m^3)	3.8	5.1	13.7	16.6	16.7
CH_4-COD (kg/m^3)	13.7	16.9	25.6	28.7	28.9

* total hydrolysis minus dissolved $COD_{influent}$

+ total acidification minus VFA $COD_{influent}$

mean values over the period: 1: t=812-930 days; 2: t=1048-1151 days; 3: t=349-412 days; 4: t=300-405 days; 5: t=372-503 days.

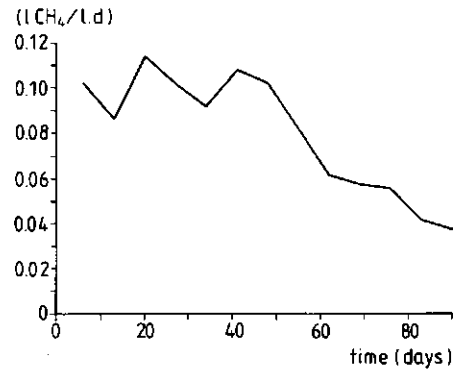

Figure 4a. The course of the volumetric methane gas production (m^3/m^3.d) at 50 days detention time and at a process temperature of 15°C.

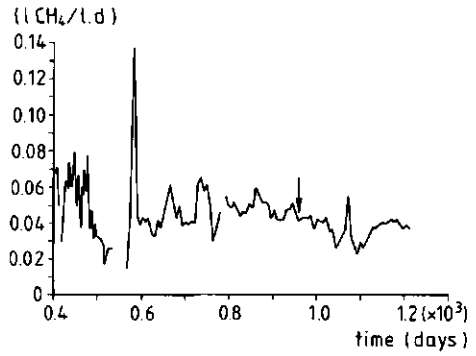

Figure 4b, The course of the volumetric methane gas production ($m^3/m^3.d$) as a function of time at 100 days detention time at a process temperature of 15°C. In the period of time: t=631-708 days, HRT= 150 days; at t=923 days more concentrated slurry is supplied.

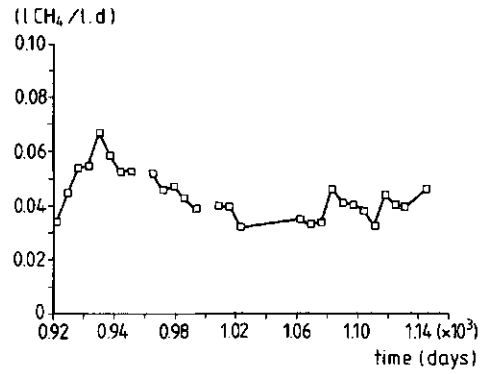

Figure 4c, The course of the volumetric methane gas production ($m^3/m^3.d$) at 150 days detention time and at a process temperature of 15°C.

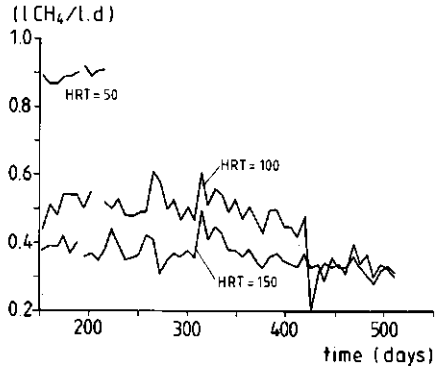

Figure 4d, The course of the volumetric methane gas production ($m^3/m^3.d$) at 50, 100 and 150 days detention time, at a process temperature of 20°C and at subsequently changing the temperature from 20 to 15°C (t=420 days) at 100 days detention time.

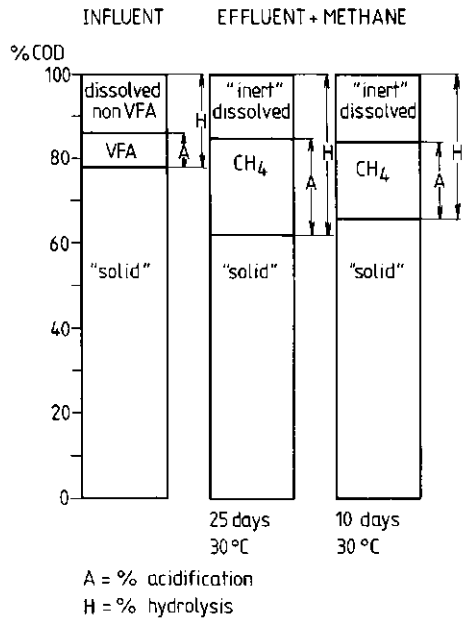

Figure 5. Results of experiments A: Fractional composition of dairy cow manure and the fractional methane formation, acidification and hydrolysis in the digestion of the manure at the different detention times and at process temperatures of 30°C to 35°C (as a percentage of the influent COD).

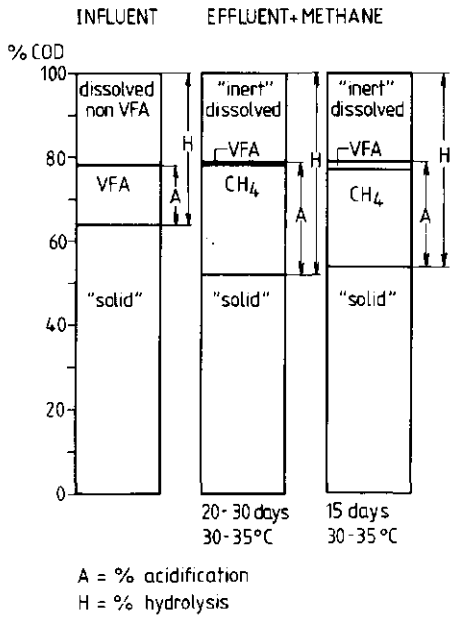

Figure 6. Results of experiments B: Fractional composition of dairy cow manure and fractional methane formation, acidification and hydrolysis at digestion at 10 and 25 days detention time and at a process temperature of 30°C (as a percentage of the influent COD).

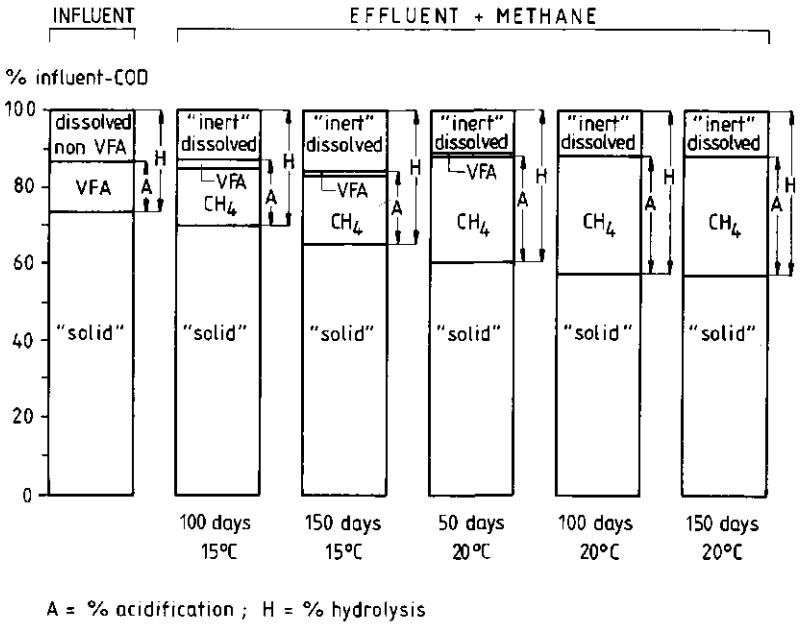

Figure 7. Results of experiments D: Fractional composition of dairy cow manure and the fractional methane formation, acidification and hydrolysis at 100 and 150 days detention time at a process temperature of 15°C and at 50, 100 and 150 days detention time at a process temperature of 20°C (as a percentage of the influent COD).

The first diagram in the Figures 5, 6 and 7 shows the measured fractional COD hydrolysed and acidified in the influent mixture.
The following diagrams, shown in Figure 5, 6 and 7 present the assessed mean fractional (in % of the influent COD) hydrolysis, acidification and conversion in methane in the digestion of the manure under the different conditions.

4.4. DISCUSSION OF THE RESULTS

The detention time and temperature are two essential factors influencing the gas production during the anaerobic digestion of manure. Figure 8 shows the observed COD reduction for the different conditions investigated. Some scatter is shown due to the use of different batches of influent manure. It is quite clear that a very similar relationship is found for the COD reduction with the detention time for the different batches of manure.

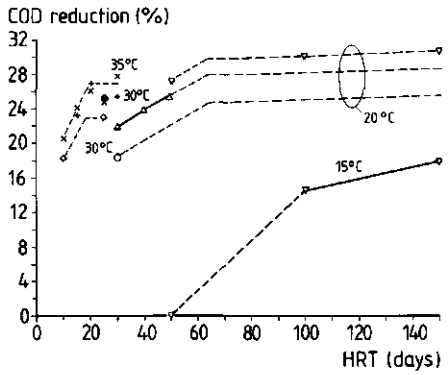

Figure 8. COD reduction in relation to the detention time at temperature from 15 to 35°C at using different batches of dairy cow manure.
▽94 g COD/l; + 97 g COD/l, 30°C; x 97 gCOD/l, 35°C; o 108 g COD/l; △84 g COD/l (results presented in Chapter 2); ◇116 g COD/l.

Compared to the gas production found at 20-25 days detention time and a process temperature of 30°C, the specific gas production is not significantly increased by increasing the detention time to 30 days, neither by increasing the temperature to 35°C and 40°C. Our results are in accordance with those of several other researchers, at least with respect to temperature increase in the range of 30-40°C at detention times of about 20 days (Table 5). However, according to results obtained by Chen *et al.* (1980) in the digestion of beef cattle slurry at detention times shorter than 20 days a distinct difference is found between digestion at 30 and 40°C. The incomplete hydrolysis, that we found in our experiments at 30°C and 10 days detention time, does sustain the expectation that an increase in temperature to 40°C will cause a significant increase in the specific gas production.

Table 5, Methane productions at various temperatures and detention times.

references	DT	30°C	33°C	35°C	37°C	40°C	slurry
Hawkes *et al.*	9-10.5			0.256			separated
(1984)	10-11.5	0.255					dairy
	16.5-20	0.314					cow
	15-17			0.314			slurry
Chen *et al.*	9	0.19		0.223		0.226	beef
and Varel *et al.*	12	0.217		0.231		0.274	cattle
(1980)	18	0.256		0.261		0.275	slurry
Wellinger (1984)	30		0.20*		0.21*	0.21*	-
Baserga (1984)	8		0.166				dairy
	10		+0.082-0.197				cow
	12		+0.079-0.228				slurry
	16		+0.090-0.240				
	20		+0.124				
	24		0.247				
	30		0.238				
own research	10	0.10					dairy
	15	0.140		0.146			cow
	20	0.163		0.158			slurry
	25	0.13-0.151		0.149		0.157	
	30	0.153		0.168			

* calculated from given figure, assuming: 65% CH_4 in biogas.

\+ it was not possible to achieve a stable digestion process at an influent concentration of 8.4-10.4 kg TS/m^3 (NH_4^+-N 2.4-2.8 kg/m_3).

The sharp increase in the specific gas production in the digestion at 30°C when increasing the detention time in the range of 10-20 days, corresponds well with observations made by Bousfield et al. (1979), Chen *et al.* (1980), Varel *et al.* (1980), Hawkes *et al.* (1984), Patelunas & Regan (1977), Baserga (1984) and Singh *et al.* (1982). Opposite to our experiences, Baserga (1984) was unable to achieve a stable digestion process with influent TS concentrations of 84 kg/m^3 at a detention time of 10 days, and with an influent concentration of 104 kg/m^3 at detention times of 10- 20 days. The high NH_4^+-N concentration (2.4-2.8 g/l) in the digesters with these concentrated slurries, in comparison with that in the other experiments (0.91-2.05 g/l) made by Baserga, might indicate that a poor adaptation to high NH_4^+-N concentrations is the main reason for this instability (see Chapter 6). Our results clearly show that it is possible to achieve a stable digestion process at a detention time of 10 days with TS concentrations of 77.5-91.8 g/l and NH_4^+-N concentration of 2.8-3.5 g/l. This is even the case after a sudden decrease of the detention time from 25 to 10 days. The results of the latter experiments, where the organic loading rate was suddenly increased as a result of the decreasing detention time show that it is possible to increase the volumetric gas production up to about 0.75 m^3 CH_4/m^3.day within a few days without disturbance of the digestion process. Apparently, in this way, stored slurry could be used as a potential energy source, in periods of energy shortage. Results of non-inoculated start-up experiments prove that it is possible to store fresh cow slurry at a temperature of 15°C or lower for at least 5,5 months, without producing methane (Chapter 2).

So far little research was made at detention times exceeding 20 days. Baserga (1984) shows a small increase of the gas production at increasing the detention time from 16 to 24 days or from 16 to 30 days, similarly as we found. Steiner (1983) and Maurer and Enssle (1981) observed an increasing gas production by increasing the detention time from 26 to 37 and 22 to 33 days at 35°C. The composition, the origin and the age of the slurry (beef cattle) might be the cause of these differences.

The data in Figure 8 show that a similar COD reduction at 20°C, at detention times ≥ 100 days and at 30°C at detention times ≥ 20 days can be expected when using the same batch of slurry. However at a process temperature of 15°C the specific gas production remains significantly lower even at a detention time as high as 150 days. It was not possible under these low temperature conditions (15°C) to achieve a stable digestion process at 50 days detention time.
The data in Table 3 and 4 show a slight increase in the VFA concentration when decreasing the detention time; in all situations except for the digestion at 15°C and at 50 days detention time a low VFA concentration was found (2.3 g COD/l). This observed increase in VFA concentration can be explained on the basis of Monod kinetics, similarly also proposed by Hobson and McDonald (1980) for the digestion of pig slurry. The lower VFA concentration found in the B series of experiments compared with the A series may be related to the significantly lower NH_4^+-N concentration in the B-experiments. Results with the mesophilic digestion of dairy cow slurry reveal a exponential increase of the effluent VFA concentration at increasing NH_4^+-N concentrations, independent of the TS concentration (see Chapter 6). We have found similar results for the thermophilic digestion of dairy cow slurry (Zeeman *et al.*, 1985).

As the fraction of dissolved organic matter in the effluent equals or almost equals that in the influent, the hydrolysis apparently is the main rate limiting step in the digestion of cow slurry under the conditions investigated. Only at a process temperature of 15°C and at 50 days detention time the VFA concentration increases up to 8 g COD/l, while the gas production drops towards zero, illustrating a washout of the methane bacteria. Latter results do not correspond with those of Sutter and Wellinger (1985), who observed an undisturbed digestion process for cow slurry at 14°C at a detention time of 37 days. Hawkes *et al.* (1984) could only achieve a limited amount of biogas (0.067 m^3 per kg VS) with the digestion of separated cow slurry at 20 days detention time and at 15°C. Differences in slurry concentration, especially the NH_4^+-N concentration (see Chapter 6) very likely cause these differences in reactor performance. Hawkes *et al.* (1984) used separated dairy cow slurry with a TS concentration of 37.8 kg/m^3 and a NH_4^+-N concentration of about 1 kg/m^3. The inhibiting effect of the slurry NH_4^+-N concentration is once more clearly demonstrated by the results in Figure 4b, which shows the effect of increasing the influent concentration from 94 to 113 kg COD/m^3 and the NH_4^+-N concentration from 1.9 to 3.5 kg /m^3 in the digestion of cow slurry at 15°C at a detention time of 100 days. Such a disturbance does not occur at a detention time of 150 days, when imposing the same change of conditions (results not shown).

From the results obtained it becomes clear that at higher digestion temperatures (≥20°C) and/or detention times a considerable part of the gas originates from the suspended organic material while at the lower temperatures mainly VFA 's are responsible for the methane production. The diagrams in Figure 5 and 6 show that at 30°C and 20-25 days detention time 12-15% of the influent COD is hydrolysed while at 20°C and 50-150 days detention time this amounts 13-16% (Figure 7). Liquid-solid separation of the raw manure prior to mesophilic or sub-mesophilic digestion would therefore considerably reduce the specific gas production. This was clearly demonstrated by results of Pain *et al.* (1984), who reported a 30 % lower gas production for the digestion of the liquid fraction as compared with the digestion of the raw manure.

At a process temperature of 15°C only 3-8% of the influent is hydrolysed in the reactor even at detention times as long as 100-150 days. At the digestion at 15°C and 150 days detention time the extent of hydrolysis exceeds slightly that at a detention time of 100 days, but part

of the dissolved organics are not acidified, which is clear from the increase of the fraction non-VFA dissolved organics. A retarded acidification is not found in any of the other presented experiments. In non-inoculated start-up experiments, conducted with freshly collected slurry (see Chapter 2), an increase of the non-VFA dissolved COD of 15 to 19 g COD/l (not published) was found when decreasing the temperature from 30 to 5°C in a batch digestion after a 5 months period. Latter results indicate that at low temperature not only the rate of hydrolysis but also the rate of acidification drops.
The low % of hydrolysis and acidification occurring at a process temperature of 15°C is conform the results of start-up batch experiments at 15°C (Zeeman *et al.*, 1988, Chapter 2). In the latter experiments, the total hydrolysis at 5, 10, 15, 25 and 30°C was resp. 11.6, 14.4, 18.1, 27.2 and 45.2 % (not published) at a digestion time of about 125 days.
According to Hobson (1983) the rate of hydrolysis of solids can be described by an equation similar as that of Monod. He distinguished between a fast and a slowly degradable solid fraction with a maximal growth rate at 35°C (μ_{m35}) of resp. 0.32/day and 0.08/day and a ks value of 1.0 g/l and 2.2 g/l. The model predicts a washout detention time of 33 days at a process temperature of 15°C. In our experiments, an important part of the biodegradable COD of the influent slurry was already acidified during the storage of the slurry. One can consider this acidified fraction as originating from the easily biodegradable solids. The fraction which is hydrolysed during the digestion at 30-35°C can be regarded as consisting of slowly biodegradable solids. The maximum amount of solids hydrolysed during the digestion in these experiments measured about 12 g COD/l. With the results of the 35°C experiments at 15 and 20 days detention time the μ_{m35} and k_s can be estimated at 0.08/day and 0.3 g VS/l. The μ_m value corresponds to that found by Hobson (1983), the k_s value is considerably lower than that found by Hobson (1983). Assuming there exist a relationship between μ_m and the temperature (T), corresponding to $\mu=b(T-T_0)$, where To is an intrinsic property of the bacteria (Ratowski *et al.* 1982) we can calculate that μ_m for the slowly degradable solids amounts to 0.03/day at a process temperature of 15°C and to 0.04/day at 20°C (T_0=275, according to Hobson, 1983). Using these figures and supposing that the solids hydrolysed during the 15 and 20°C experiments belong to the category 'slowly degradable' with an influent concentration of 15.5 g COD/l (maximal found value) the hydrolysis in the digestion at 15°C and 150 and 100 days detention time would amount to resp. 15.4 and 15.3 g COD/l. The experimental found values are only 7.6 and 3.2 g COD/l. At a process temperature of 20°C at 50, 100 and 150 days detention time, the experimentally found values, resp. 12.4, 14.9 and 15.3 g COD/l, are more close to the predicted values of resp. 15.1, 15.4 and 15.5 g COD/l.

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CHAPTER 5.
ANAEROBIC DIGESTION OF COW AND PIG MANURE IN AN ACCUMULATION SYSTEM AT TEMPERATURES OF 15-30°C

5.2. MATERIAL AND METHODS

5.2.1. Conducted experiments

The accumulation experiments were executed at temperatures varying from 15 to 30°C, using an amount of inoculum varying from 10 to 50 % of the reactor volume. The filling period was set at 100 days except in two series of experiments, where it was 90 and 270 days. The set-up of the various experiments is given in Table 2.
This Table also provides information about the type of inocula used in the experiments.

Table 2. Experimental set-up, applied in the various experiments.

			COW SLURRY				
exp (no)	temp (°C)	inoculation (%)	inoculum (sort)	filling time (days)	filling period (no)	mixing (+/-)	slurry (sort)
1	15	13	D.C.A.15	100	2	-	C,95
2	15	13	exp.1	100	3	-	C,95+112
3	15	13	exp.2	100	4	-	C,112
4	15	13	exp.2	100	4	+	C,112
5	15	15	exp.3	100	5	-	C,112
6	15	50	D.C.A.15	100	3	+	C,95
7	15	40	exp.6	100	1	+	C,112
8	15	50	D.C.A.15	100	3	+	C,95
9	15	40	exp.8	100	1	+	C,112
10	15+	20	exp.9	100	1	+	C,112
11	15	15	exp.10	100	1	+	C,112
12	15	15	exp.10	100	1	-	C,112
13	20	13	D.C.CSTR.20	100	1	-	C,95
13a	20	13	D.C.CSTR.30	100	1	-	C,95
14	20	13	exp.13	100	2	-	C,95
14a	20	13	exp.13a	100	2	-	C,95
15	20	13	exp.14	100	3	-	C,95
15a	20	13	exp.14a	100	3	-	C,95
16	20	7	D.C.CSTR.20	100	1	-	C,95
16a	20	7	D.C.CSTR.30	100	1	-	C,95
17	20	7	exp.16	100	2	-	C,95
17a	20	7	exp.16a	100	2	-	C,95
18	20	7	exp.17	100	3	-	C,95
18a	20	7	exp.17a	100	3	-	C,95
19	20	1	D.C.CSTR.20	100	1	-	C,95
19a	20	1	D.C.CSTR.30	100	1	-	C,95
20	20	1	exp.19	100	2	-	C,95
20a	20	1	exp.19a	100	2	-	C,95

continuation of Table 2

PIG SLURRY

exp (no)	temp (°C)	inoculation (%)	inoculum (sort)	filling time (days)	filling period (no)	mixing (+/-)	slurry (sort)
21	15	15	exp.5	100	1	-	P,74
22	15	15	exp.21	100	2	-	P,74
23	15	10	D.c.P.A.20	270	1	-	P,74
24	15	10	exp.25	270	2	-	P,74
25	16-27	10	$D.P.A._{pilot}$	270	1	-	P,'B'*
26	20	10	exp.35	100	1	-	P,74
28	20	10	exp.26	100	2	-	P,74
29	30	10	exp.38	100	1	-	P,74
31	15	40	D.c.P.A.15	100	1	-	P,118
32	15	40	exp.31	100	2	-	P,118
33	15	20	exp.31	100	1	-	P,118
34	20	10	D.c.P.A.20	90	1	+	P,118
35	20	10	D.c.P.A.20	90	1	-	P,118
36	20	10	exp.35	100	2	-	P,118
37	30	10	D.c.P.A.20	90	1	+	P,118
38	30	10	D.c.P.A.20	90	1	-	P,118
39	30	10	exp.38	100	2	-	P,118

*=pilot plant experiment; C=cow slurry; 95=influent COD 95 g/l; 112=influent COD 112 g/l; +=temporary temperature increase; D.C.A.15=at 15 °C,in a AS digested cow slurry; D.C.CSTR 20= at 20 °C in a CSTR digested cow slurry; D.C.CSTR 30= at 30 °C in a CSTR digested cow slurry; Amb.=ambient temperature; P= pig slurry; P,B= pig slurry, experimental farm Bantam; D.c.P.A.20 (15)=at 20 (15) °C in an AC digested concentrated pig slurry ; D.c.P.CSTR20= at 20 °C in a CSTR digested concentrated pig slurry; DPA_{pilot}=in a pilot plant accumulation system, digested pig slurry. The experiments 27 and 30 are omitted.

5.2.2. Influent slurry composition

The applied slurry was collected from the slurry storage pit situated under slatted floors at a fattening pig farm and at the experimental dairy cow farm in Duiven and stored according to the procedure described in Chapter 4. The composition of the slurry is given in Table 3.

5.2.3. Conducted analyses.

Analyses were performed according to the procedures described in Chapter 3.

Table 3. Composition of the pig and dairy cow slurry used in the different experiments (g/l).

slurry	pig*	cow	cow
total-COD	147.8	94.5	112.5
dissolved-COD	45.3	24.6	32.6
total VFA-COD	29.6	11.2	13.7
C_2-COD	12.2	6.0	6.1
C_3-COD	6.3	3.4	5.0
i-C_4-COD	2.3	0.4	0.7
n-C_4-COD	4.8	0.7	0.7
i-C_5-COD	3.5	0.6	1.1
n-C_5-COD	0.6	0.2	0.2
Total solids (TS)	111.9	87.9	95.0
Volatile Solids (VS)	78.3	67.6	71.2
NH_4^+-N	7.4	1.9	3.5

* resp. diluted to 50 and 80%.

5.2.4. Design of laboratory digesters

Laboratory experiments were made in reactors with a working volume of 1.5 to 5 liters. The 'non-stirred' digesters were shaken merely manually before and after the feeding. The 'stirred reactors' were stirred mechanically, at approximately 400 rpm during 30 seconds each 30 minutes. Feeding was carried out weekly. The 5 liters digesters had double walls through which water was pumped, in order to provide the desired temperature. The 1.5 liters digesters were placed in a thermostated water bath or in a 20°C climate room. The produced gas was collected in a gas bag, after passing a column of soda lime pellets, for removing CO_2.

5.2.5. Design of the pilot plant digester

The pilot plant research was made in a 6 m^3 digester located at the experimental farm in Duiven. The digester was weekly fed with pig slurry originated from the experimental farm the 'Bantam' in Maartensdijk. Heating was only provided at temperatures <15°C by recirculating electrically heated water through the digester bottom and internal heat exchanger. Mixing was only made at the time the reactor was fed.

5.2.6. Calculations.

The experimental results are presented in Figure 1 t/m 10.
Most of these results have been presented in terms of measured volumetric gas production ($l/l_{reactor\ volume}$·d) and VFA concentrations. The results of the experiments 22, 28, 29, 32, 36 and 39, have been elaborated into fractional hydrolysis (H), acidification (A) and methane formation (M), in all cases in relation to the influent COD and expressed in %, using equations 1, 2, 3, 4, 5:

$$COD_{diss} = \frac{(V.COD_{measured} - V_{inoculum}.COD_{inoculum})}{(V - V_{inoculum})} \qquad (1)$$

$$COD_{VFA} = \frac{(V.VFA_{measured})}{(V - V_{inoculum})} \qquad (2)$$

where,

COD_{diss}=	dissolved COD of the slurry added to the reactor (g/l)
$COD_{measured}$=	measured dissolved COD of total reactor content (g/l)
$COD_{inoculum}$=	dissolved COD of the inoculum (g/l)
COD_{VFA}=	VFA-COD of the slurry added to the reactor (g/l)
$VFA_{measured}$=	measured VFA-COD of the total reactor content (g/l)
V=	volume reactor content (liters)
$V_{inoculum}$=	volume inoculum (liters)

$$H= \frac{100\ (COD_{CH4} + COD_{diss.})}{COD_{infltot}} \qquad (3)$$

$$A= \frac{100\ (COD_{CH4} + COD_{VFA})}{COD_{infltot}} \qquad (4)$$

$$M= \frac{100\ (COD_{CH4})}{COD_{infltot}} \qquad (5)$$

where,

COD_{CH4}=	produced CH_4*, expressed in COD per liter slurry added to the reactor.
COD_{diss}=	dissolved COD of the slurry added to the reactor (g/l).
COD_{VFA}=	VFA-COD of the slurry added to the reactor (g/l)
$COD_{infltot}$=	total COD of the influent slurry (g/l).

* the methane production from the inoculum is supposed to be zero.

The potential specific cumulative gas production as illustrated in Figure 11 is calculated according to the following equation:

$$M_{cumpot}= \frac{M_{cumT}}{ft} .t \qquad (6)$$

where,

M_{cumpot}=	potential cumulative gas production at a certain digestion time (m^3/m^3 slurry).
M_{cumT}=	maximal measured specific gas production at $\tau \rightarrow \infty$, at a specific process temperature (m^3/m^3 slurry).
ft=	filling time (days) ($t \leq ft$).
t=	digestion time (days).

According to Powell (1983), the accumulation of gas from time=0, in batch cultures, is given by the equation:

$$p'=p_0\{e^{\mu(t-to)}-1\} \qquad (7)$$

where,

p'=	quantity of gas accumulated since t=0 (liters).

p_0 = quantity of gas accumulated at t=0, i.e. that quantity which has resulted from the synthesis of x_0 g of biomass (liters)
μ = specific growth rate (day^{-1}).
x_0= biomass at t=0 (g).

The quantity of gas accumulated since t=0, produced in a reactor (R") with an amount of inoculation of x_0" instead of x_0 can be calculated according to equation (8):

$$p" = p_0 \, x_0"/x_0 \, \{e^{\mu(t-to)}-1\} \qquad (8)$$

where,
p"= quantity of gas accumulated since t=0, at x" g of inoculum.
x"= biomass at t=0 in reactor R".

5.3. RESULTS AND PRELIMINARY DISCUSSION

5.3.1. Digestion of cow slurry, using 13-50% inoculation conducted at 100 days filling time and process temperatures of 15-20°C.

experiments at 13% inoculation, at 15°C, at 100 days filling time and effect of mixing.

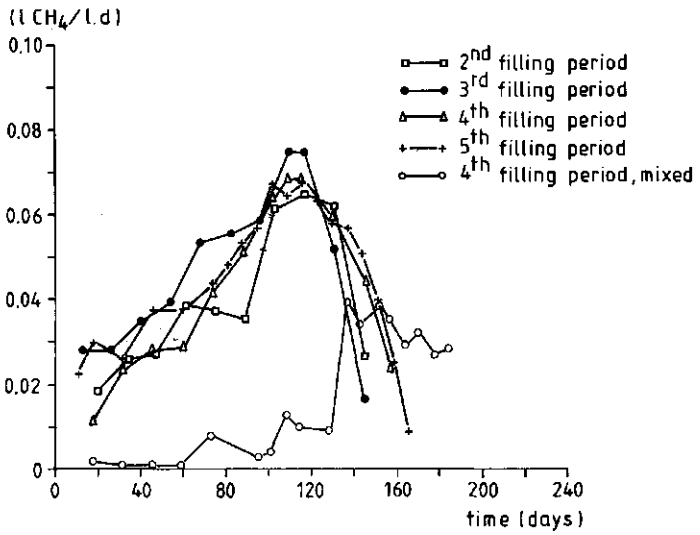

Figure 1a, Experimental results of the accumulation digestion experiments conducted with cow manure, viz. course of the volumetric methane gas production in relation to the digestion time (experiments no: 1 to 5).

digester temperature	:15°C
amount of inoculation	:13%-15%
filling time	:100 days
applied influent slurry	:94.5 g COD/l to day 26 of the 3th run and 112.5 g COD/l afterwards.
mixing	:no mixing except for the 4th digestion run which is executed in a non-mixed and a mixed reactor.

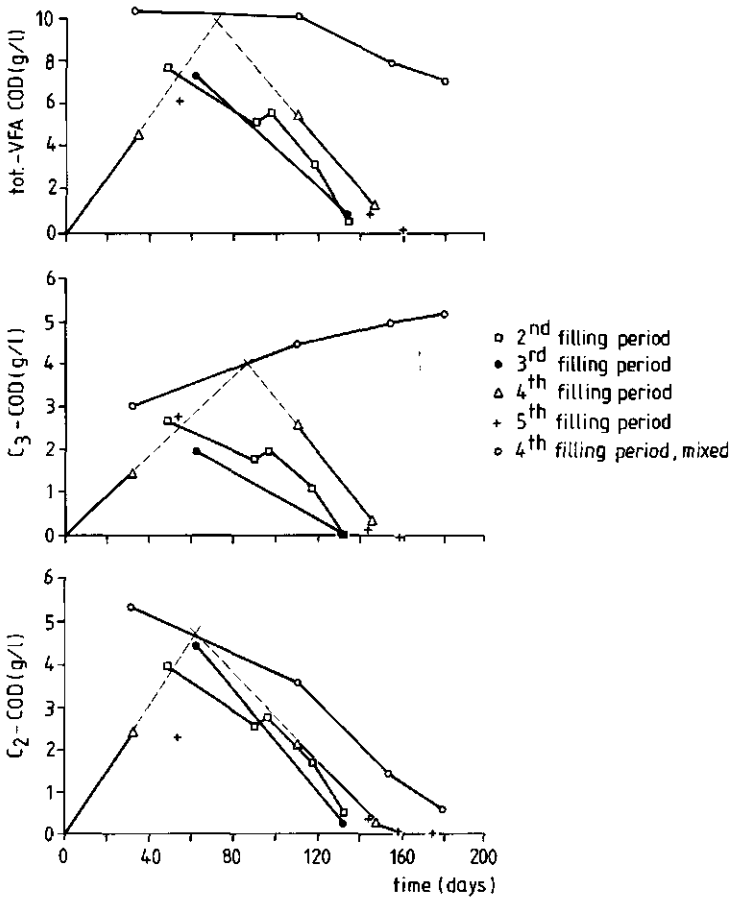

Figure 1b, Experimental results of the accumulation digestion experiments conducted with cow manure, viz. course of the VFA concentration in relation to the digestion time (experiments no: 1 to 5).

digester temperature	:15°C
amount of inoculation	:13%-15%
filling time	:100 days
applied influent slurry	:94.5 g COD/l to day 26 of the 3th run and 112.5 g COD/l afterwards.
mixing	:no mixing except for the 4th digestion run which is executed in a non-mixed and a mixed reactor.

The results in Figure 1a show the volumetric methane gas production as a function of the digestion time in an accumulation system using 13% inoculation for the second (also given in Figure 4, Chapter 3) to the fifth run. In all cases a run includes a filling period of 100 days and a period of varying length for completion of the digestion. From t=26 onwards in the third run, the feed influent COD was 112.5 instead of 94.5 g/l. This change in influent COD did not result in a significant change in gas production. The results of the VFA analyses are presented in Figure 1b. In all cases a distinct accumulation of VFA is notable during the filling period, indicating that insufficient methanogenic capacity is available in order to degrade the VFA within the filling period of 100 days. Compared to the 2nd and the 3rd run

the breakdown of VFA's, especially that of propionic acid, is slightly retarded in the 4th and the 5th run. Parallel to the fifth run in the non-mixed digester, we conducted an additional experiment in a mixed digester. The results in Figure 1a clearly show a much lower gas production in the mixed reactor compared to that in the non-mixed reactor. From the results in Figure 1b it is notable that a significant accumulation of propionic acid occurs in the mixed reactor. The results also reveal that the conversion of acetic acid in the mixed reactor is somewhat retarded, compared to the non-mixed reactor.

Experiments at 40-50% inoculum at 15°C under mixed and unmixed conditions.

The results in Figure 2 present the assessed methane production and VFA concentration in a mixed accumulation system again at 15°C, but now at a high inoculation, resp. 50 and 40%. Both experiments were conducted in duplicate. In spite of the fact that mixing was applied, in this case any accumulation of propionic acid did not occur. The results in Figure 2 reveal also that the volumetric gas production increases strongly upon increasing the space loading rate by:

1. using a lower % inoculation (40% instead of 50%)
2. increasing the influent COD from 94.5 g/l to 112.5 g/l

This increased load was not accompanied with any distinct increase in the VFA concentration.

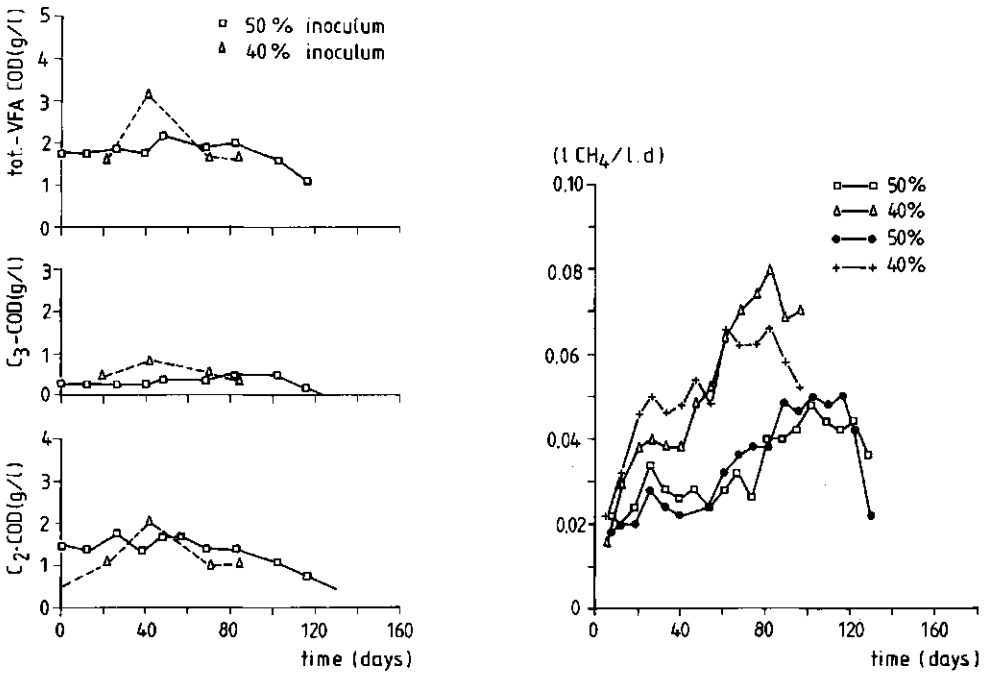

Figure 2, Experimental results of the accumulation digestion experiments conducted with cow manure, viz. the course of the volumetric methane gas production and VFA concentration in relation to the digestion time (experiments no: 6 to 9).

digester temperature	:15°C
amount of inoculation	:40% and 50%
filling time	:100 days
applied influent slurry	:94.5 g COD/l at 50% inoculation and 112.5 g COD/l at 40 % inoculation
mixing	:experiments are conducted in a mixed reactor.

Effect of mixing at 15°C, 15% inoculation and the effect of a temporary increase of the temperature.

Figure 3 presents the results of an additional experiment in which we studied the effect of mixing in two parallel accumulation reactors, operated at 15°C and 100 days filling time. One experiment was executed in a stirred reactor (experiment no 11), the other in an unmixed reactor (experiment no 12). Both reactors were seeded with 15% sludge from a preceding accumulation experiment (no 10), also conducted at 15°C (except during period 38-58 days, when the temperature was 23°C). The results of latter experiment are also shown in Figure 3. Like in the experiments already shown in Figure 1, the results once again reveal that a distinct accumulation of VFA occurs during the filling period in the reactor. Particularly notable is the clear stagnation in the propionic acid degradation under stirred digestion conditions in experiment no. 11. In the preceding experiment no 10, where stirring was also applied but the temperature was increased temporary from 15 to 23°C, the concentration of VFA's, particularly of propionic acid, decreased right after the imposed temperature rise. After the temperature was returned to 15°C, the propionic acid concentration remained low. Apparently, at that time, sufficient propionic acid degradation capacity and methanogenic capacity are available in the system to accommodate the imposed daily amount of substrate.

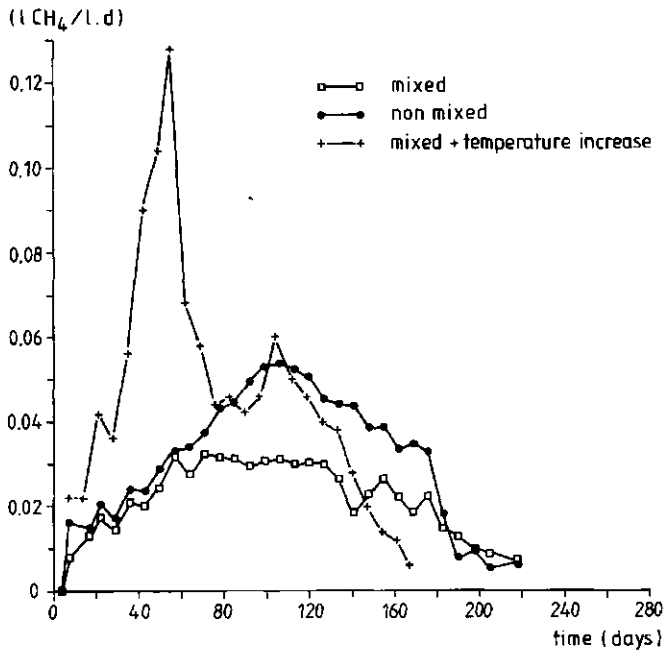

Figure 3a, Experimental results of the accumulation digestion experiments conducted with cow manure, viz. the course of the volumetric methane gas production in relation to the digestion time (experiments no: 10 to 12). (see also Figure 3b)

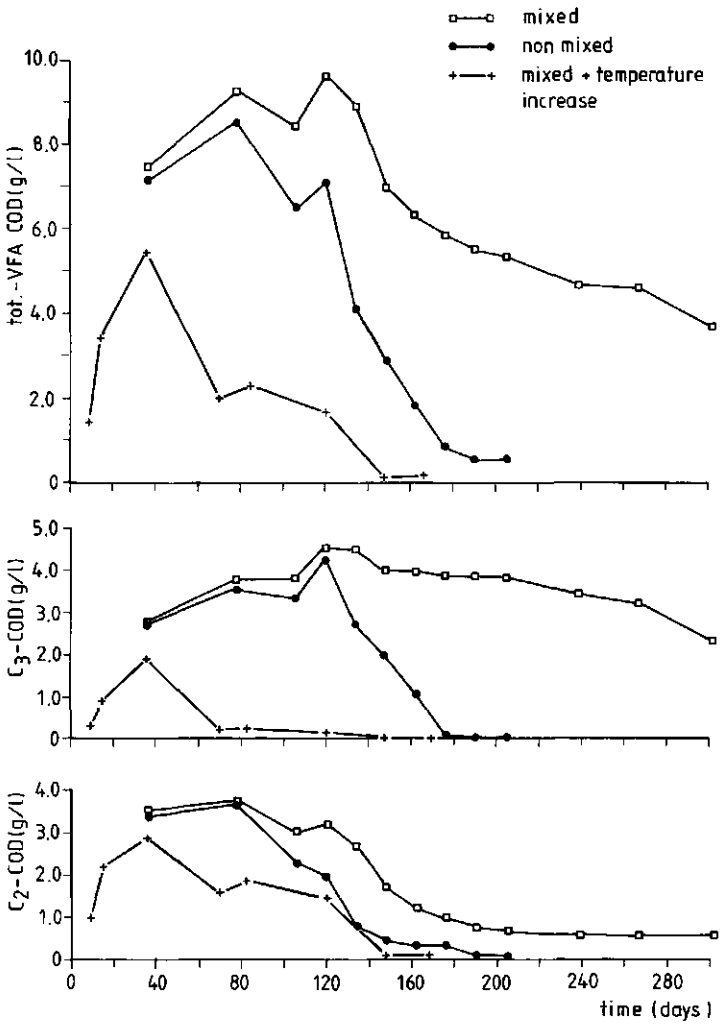

Figure 3 b. Experimental results of the accumulation digestion experiments conducted with cow manure, viz. VFA concentration in relation to the digestion time (experiments no: 10 to 12).

digester temperature	:
experiment 10 (+——+)	:15°C, except day 38-58: 23°C
experiment 11 (□——□)	:15°C
experiment 12 (●——●)	:15°C
amount of inoculation	:
experiment 10	:20%
experiment 11 and 12	:15% (seed from experiment 10)
filling time	:100 days
applied influent slurry	:112.5 g COD/l
mixing	:
experiment 10 (+——+)	:mixed
experiment 11 (□——□)	:mixed
experiment 12 (●——●)	:non-mixed

Experiments at 20°C and 1, 7 and 13% inoculation and 100 days filling time.

The results of the experiments with accumulation systems with cow manure operated at 20°C are presented in Figure 4. In these experiments different amounts of inoculum were used. The first run was executed with two types of inoculum, viz. digested cow manure from a CSTR operated at 20°C and from one operated at 30°C (see Chapter 3).
The results show that at 20°C digestion and 7-13 % inoculation the maximum volumetric gas production is already achieved after 30-50 days and that accordingly the VFA concentration, at the moment the digester is filled up, is rather low in these systems. However, when using 1% inoculation, the maximum gas production rate is reached only after the digester is completely filled.

The results in Figure 4 reveal that their exists very little difference in the course of the VFA concentration and the gas production between the second and third digestion run.

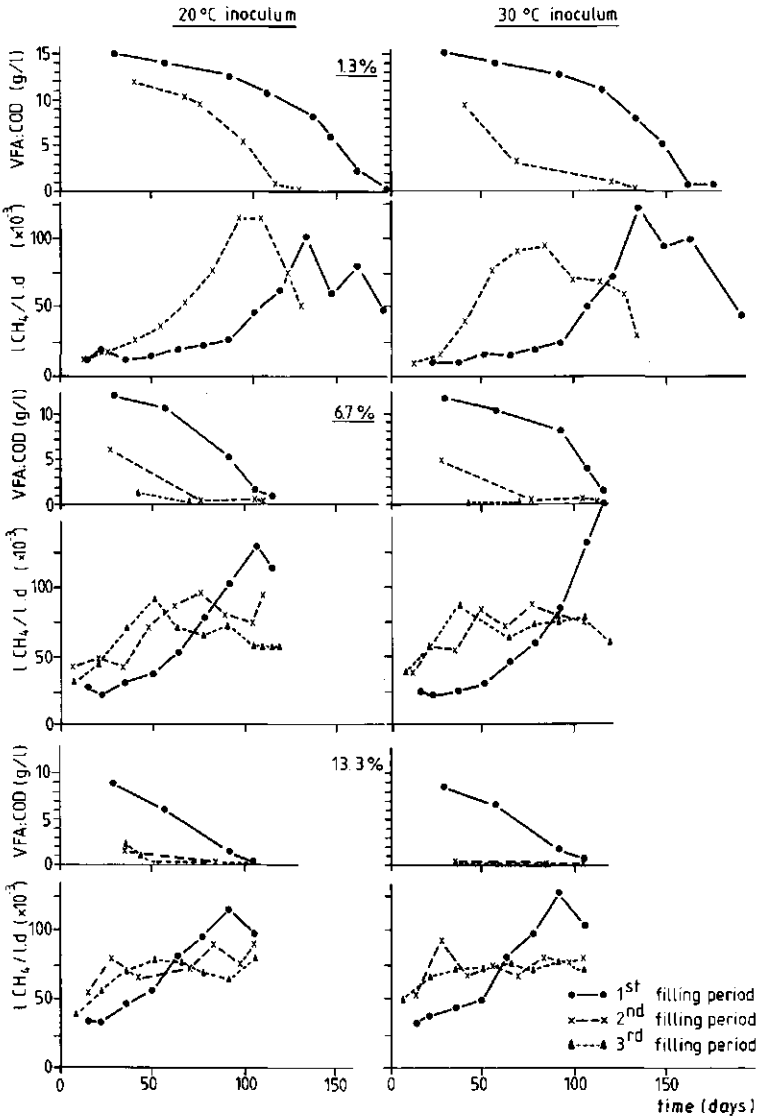

Figure 4, Experimental results of the accumulation digestion experiments conducted with cow manure, viz. the course of the volumetric methane gas production and VFA concentration in relation to the digestion time (experiments no: 13 to 20 and 13^a to 20^a).

digester temperature	:20°C
amount of inoculation	:1, 7 and 13%
filling time	:100 days
applied influent slurry	:94.5 g COD/l
mixing	:non-mixed

5.3.2. Accumulation digestion experiments of pig slurry at 10-20% inoculation, filling times of 100 and 270 days and process temperatures of 15-30°C

Experiments at 15°C at 15% and 10% inoculation and resp. 100 and 270 days filling time.

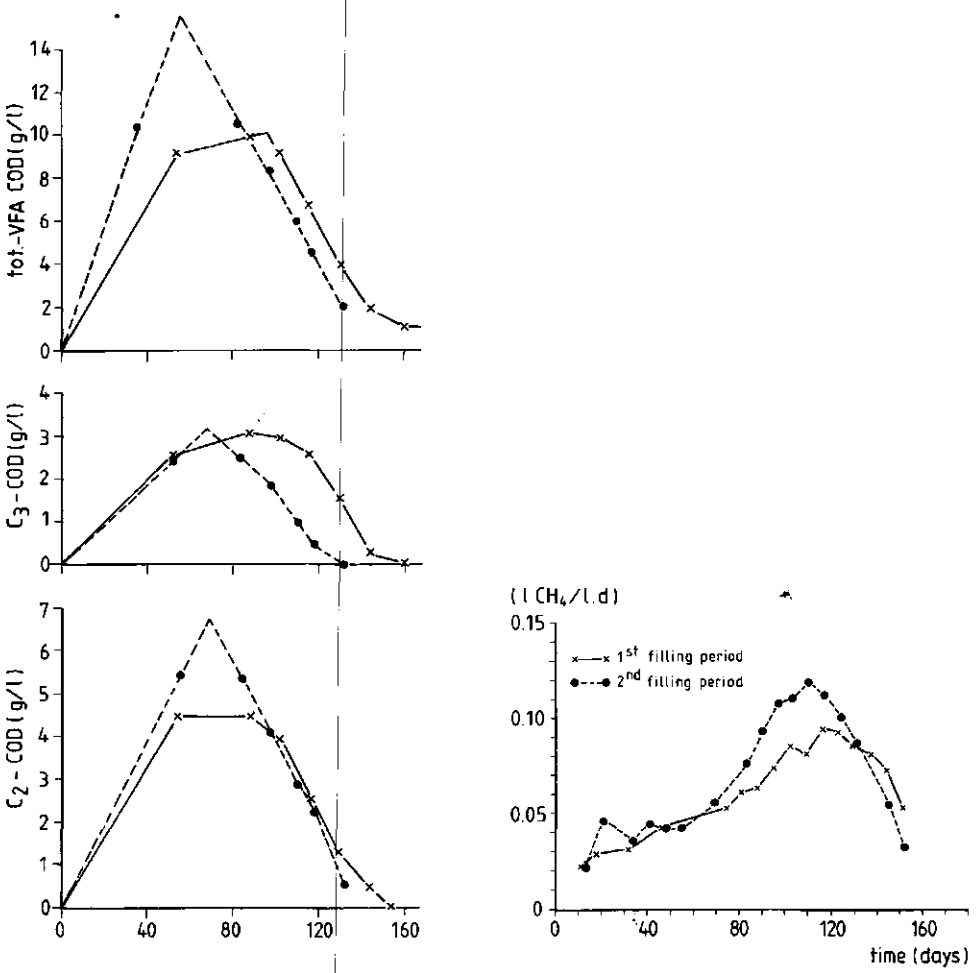

Figure 5. Experimental results of the accumulation digestion experiments conducted with pig manure, viz. course of the volumetric methane gas production and VFA concentration in relation to the digestion time (experiments no: 21 and 22).

digester temperature	:15°C
amount of inoculation	:15% (seed from experiment no 5)
filling time	:100 days
applied influent slurry	:74 g COD/l
mixing	:non-mixed

The digested cow slurry from experiment no. 5 (AC at 15°C) was used as the inoculum in the accumulation digestion experiments with pig slurry with approximately the same NH_4^+-N

concentration (3.7 instead of 3.5 g/l). The results of these experiments are given in Figure 5 for a filling time of 100 days and in Figure 6 for a filling time of 270 days.

These results do not deviate significantly from those obtained in the digestion of cow slurry at similar conditions, although higher volumetric gas productions are found for pig slurry. Except for iso-valeric acid, all the accumulated VFA's are being eliminated within 150 days digestion time. Even after 240 days digestion still about 1 g COD iso-valeric acid is present. Comparing the gas evolution data in Figure 5 and 6 it is clear that the rate of increase is significantly higher in the experiments conducted at a filling time of 100 days (0.02-0.11 l CH_4/l.d) than at a 270 days filling time (0.01-0.025 l CH_4/l.d). There exist very little differences between the first and the second run, conducted at a filling time of 270 days, despite the different inocula used. The VFA concentration remained below 4 g COD/l for the total digestion period of 270 days.

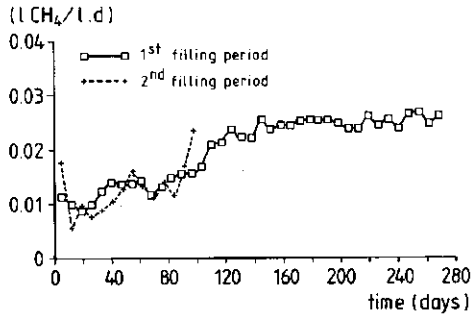

Figure 6, Experimental results of the accumulation digestion experiments conducted with pig manure, viz. course of the volumetric methane gas production in relation to the digestion time [experiments no: 23 and 24 (only the first 120 days are presented)]

digester temperature	:15°C
amount of inoculation	:10% (first run, with concentrated digested pig slurry, 20°C AS)
filling time	:270 days
applied influent slurry	:74 g COD/l
mixing	:non-mixed

Results of an experiment, conducted at 6 m^3 pilot plant scale at the experimental dairy cow farm in Duiven with pig slurry originating from the experimental pig farm 'The Bantam' in Maartensdijk, at temperatures of 17-20°C (except a short period at 24-27°C) are presented in Figure 7. Compared to the results of the laboratory experiments, shown in Figure 6, the initial gas production in the pilot plant experiment is significantly higher, viz. during the first 35 days. This can be attributed to the higher initial process temperatures of 17-27°C. The sharp decrease in gas production beyond day 140 in the pilot plant experiment is mainly caused by the decreased influent COD.

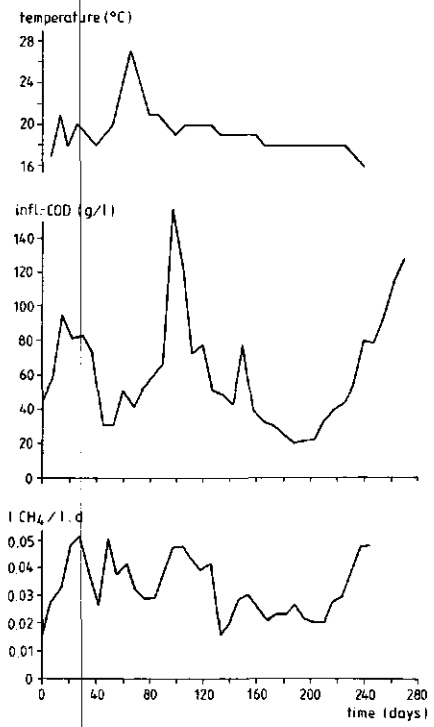

Figure 7, Experimental results of an accumulation digestion experiment, conducted with pig manure in a 6 m^3 pilot plant digester, viz. course of the volumetric methane gas production, process temperature and influent COD concentration in relation to the digestion time (experiment no: 25).

digester temperature	:see Figure
amount of inoculation	:10%
filling time	:270 days
applied influent slurry	:see Figure
mixing	:non-mixed

Experiments at 20°C and 30°C at a filling time of 100 days.

Additional accumulation digestion experiments were executed at a filling period of 100 days at process temperatures of 20 and 30°C. The results, viz. the course of the volumetric methane production and of the calculated fractional hydrolysis, acidification and methane formation are presented in Figure 8 a and b, together with the results obtained in an experiment at 15°C, at 15% inoculation (instead of 10% at 20 and 30°C).

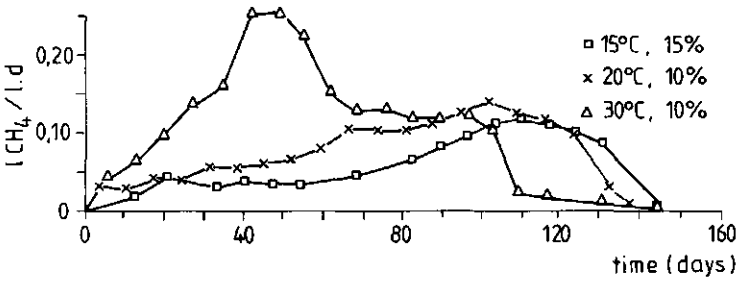

Figure 8a, Experimental results of the accumulation digestion experiments conducted with pig manure, viz. course of the volumetric methane production in relation to the digestion temperature (experiments no: 22, 26, 28 and 29).

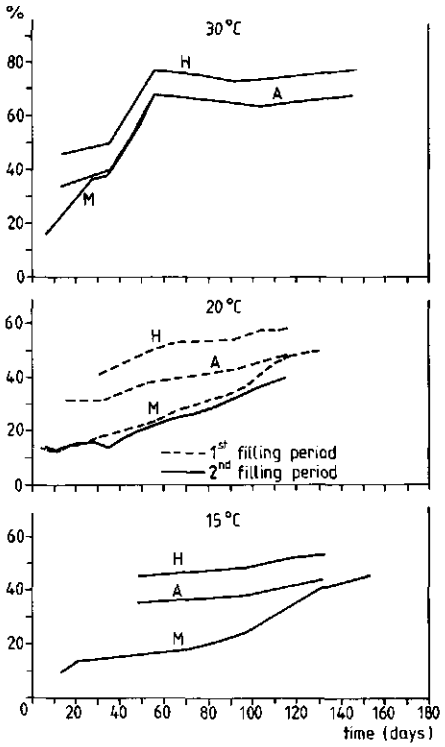

Figure 8b, Experimental results of the accumulation digestion experiments conducted with pig manure, viz. course of the volumetric methane production, % hydrolysis (H), acidification (A) and methane formation (M) in relation to the digestion temperature (experiments no: 22, 26, 28 and 29).

digester temperature	:15°C, 20°C and 30°C
amount of inoculation	:10 -15%
filling time	:100 days
applied influent slurry	:74 g COD/l
mixing	:non-mixed

The results reveal that an almost complete degradation of VFA's at the time the digester became filled up, occurred merely in the system conducted at 30°C. In latter system the increase in the percentage methane formation becomes limited by the % hydrolysis starting from day 45. The daily gas production rate (Figure 8a) in that system dropped to very low levels as soon as the feeding was terminated. In contrast, the VFA concentration in the '15 and 20°C reactors' was still rather high at the time feeding was terminated and the hydrolysis only became rate limiting after resp. 120 and 140 days digestion time. Figure 8b also clearly reveals that the percentage organics degraded, increases with the applied process temperature.

Experiments with concentrated pig slurry and effect of mixing at 20 and 30°C.

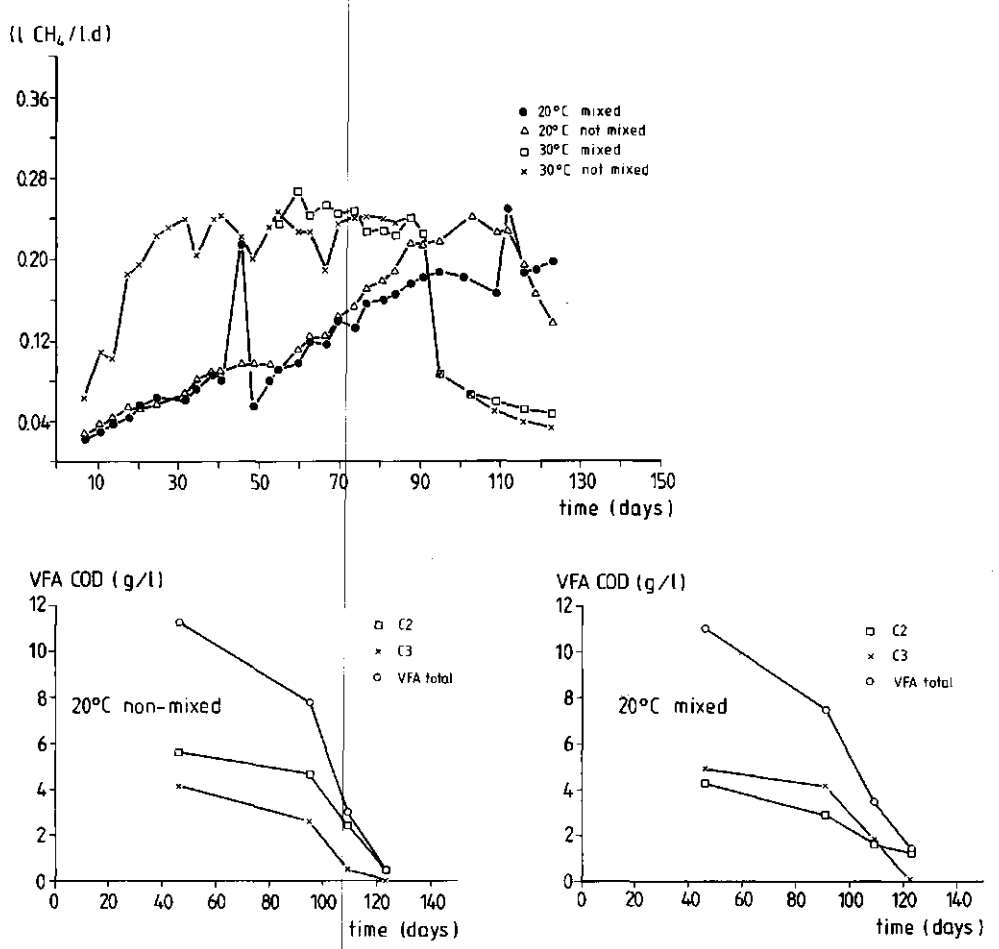

Figure 9, Experimental results of mixed and non-mixed accumulation digestion experiments conducted with pig manure, viz. course of the volumetric methane gas production and VFA concentration in relation to the digestion time (experiments no: 34, 35, 37 and 38).

digester temperature	:20°C and 30°C
amount of inoculation	:10%
filling time	:90 days
applied influent slurry	:118 g COD/l

Results of accumulation digestion experiments conducted with pig slurry with 118 instead of 74.5 g COD/l are presented in Figure 9 and 10.
The first run at 20 and 30°C, inoculated with 10% digested pig slurry (20°C, AC), was made under mixed and non-mixed conditions. As far as mixing is concerned the results show neither a positive nor a negative effect, at a process temperature of 30°C. However at 20°C once again a distinct, although rather slight, negative effect of mechanical agitation is found.
The results shown in Figure 10 a and b, refer to the second run conducted at 15°C using 20% and 40% inoculation and at 20°C and 30°C, both at 10 % inoculation under unmixed conditions. It is clear that a complete digestion of accumulated VFA's in concentrated pig slurry cannot be achieved within a period of 285 days at a process temperature of 15°C. This is even not the case when using 40% inoculation. Merely acetic acid is eliminated. Propionic acid accumulates. After 160 days digestion when all acetic acid is being removed and only propionic acid is left, the methane formation becomes limited by the hydrolysis rate. The gas production rate and the VFA concentrations, found at a process temperature of 20°C and 30°C, are very similar to those in the first run (Figure 9). It was not possible to accomplish at 20°C complete digestion of accumulated VFA's within the filling period of 100 days in the digestion of this high strength pig slurry. Figure 10 also illustrates that the hydrolysis becomes rate limiting after a digestion time of ± 130 days. At a process temperature of 30°C this already is the case after 50 days.

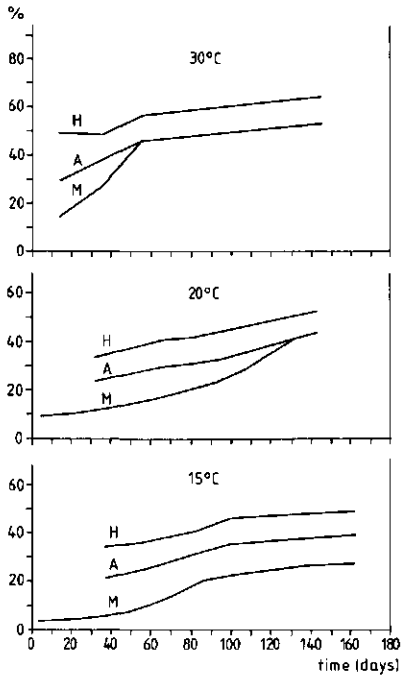

Figure 10a. Experimental results of the accumulation digestion experiments conducted with pig manure, viz. course of the % methane formation (M), acidification (A) and hydrolysis (H) in relation to the digestion time (experiments no: 33, 36 and 39).

digester temperature	:15°C, 20°C and 30°C
amount of inoculation	:10% (exp. 36, 39) and 20% (exp. 33)
filling time	:100
applied influent slurry	:118 g COD/l
mixing	:non-mixed

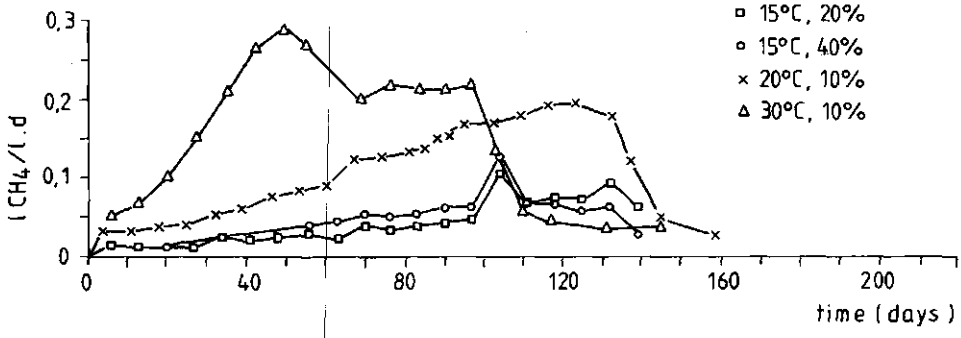

Figure 10b. Experimental results of the accumulation digestion experiments conducted with pig manure, viz. course of the volumetric methane gas production in relation to the digestion time (experiments no: 33, 36 and 39).

digester temperature	:15°C, 20°C and 30°C
amount of inoculation	:10% (exp. 36, 39) and 20% and 40% (exp. 33, 32)
filling time	:100
applied influent slurry	:118 g COD/l
mixing	:non-mixed

5.4. FINAL DISCUSSION

The presented results make clear that a stable digestion of slurry in an accumulation system, in principle is practically feasible, provided the % inoculation (or length of the filling time) is adjusted to the process temperature (or *vice versa*) and to the slurry concentration as well. We consider the digestion process by definition as stable when the accumulated VFA's are removed within the filling period. The conditions imposed to the system will determine whether the gas production rate will increase over the total filling period or remain rather constant for at least a part of that time, defined as a period of 'steady state'. In this period the VFA concentration of the mixed liquor is fairly low. Opposite to an AC-system, a CSTR-system is characterized by a continuously constant daily gas production and low VFA concentration, provided start-up has been completely accomplished.

AC- and CSTR-systems are both continuously fed systems, but they differ in one essential aspect. While the effluent in a CSTR-system is continuously removed, the effluent in an AC-system is only removed once, at the end of a filling period. A CSTR-system is characterized by a constant digestion volume, V_d (m^3), while that of an AC-system is increasing in time. The V_d of a CSTR-system is equal to the effective reactor volume, V_e (m^3), and is determined by the slurry flow rate, Q (m^3/day), and the detention time, dt (days). The V_d in an AC-system is $\leq$ the V_e.
The V_e of an AC-system is determined by the filling time, ft (days), the inoculation, e (%) and Q and can be described by the equation:

$$V_e = \frac{ft.Q}{1-e/100}$$

At an on-farm situation, ft and Q are normally fixed. Therefor, all other things being equal, V_e depends merely on the % inoculation.

Figure 11 gives the required effective reactor volume (V_e) as a function of the % inoculation at filling times of 100, 150 and 270 days at a fixed Q. (Q = 1 m^3/day)

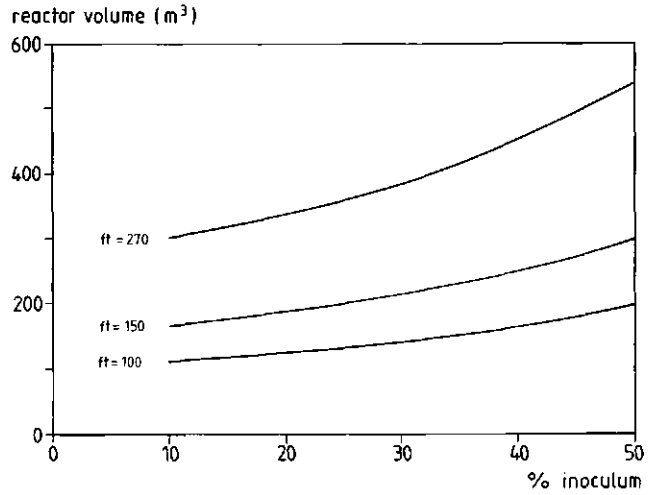

Figure 11. Calculated required effective reactor volume (V_e) in relation to the % inoculation, for an AC-system at different filling times.

Minimizing the reactor volume is of course desirable in terms of cost reduction, but the % inoculation should be sufficient, so the accumulated VFA's can be removed within the filling period. The Figures 12 a to f give the potential methane gas production (according to equation 6) and the experimental and calculated gas production (according to equation 8) in relation to the digestion time at resp. a filling time of 100, 150 and 270 days for different percentages of inoculation at both 15 and 20°C. The Figures illustrate that the minimum % inoculation at 15°C at 100 and 270 days detention time at the digestion of diluted pig slurry is resp. 25 and 10 %. Figure 11 illustrates that, at Q=1 m^3/day, consequently a reactor volume of resp. 133 and 300 m^3 is needed. At a process temperature of 20°C and 30°C the minimum inoculation at ft= 100 days is resp. about 13% and 10%, corresponding with reactor volumes of 115 and 111 m^3. At a higher inoculation, than the above-mentioned, a certain period of 'steady state', characterized by a constant daily gas production, can be achieved during the filling period as is shown in Figure 12.

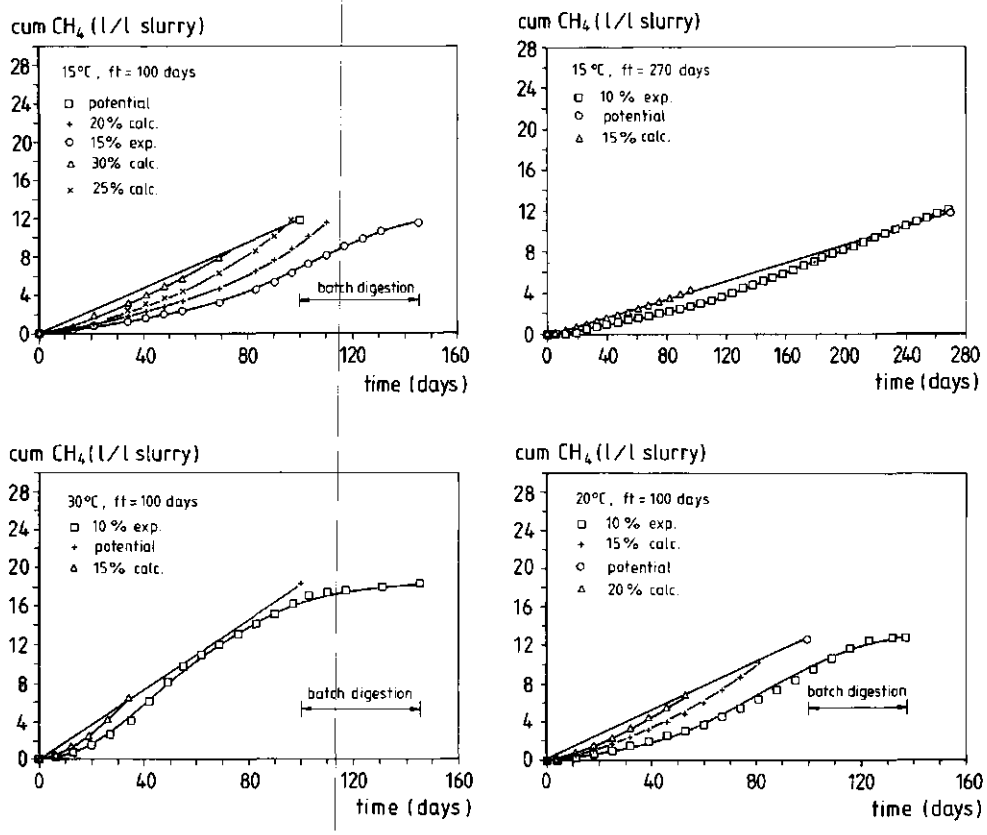

Figure 12 a t/m d. Experimentally assessed, calculated (equation 8) and potential (equation 6) cumulative methane gas production per liter final digestion volume ($V_f=V_e$-e/100.V_e) in the digestion of pig slurry at 15°C and 20°C at 100, 150 and 270 days filling time and different inoculation.

The gas production data from the accumulation systems (exp. no: 22, 28, 29, 33, 36, 39) operated on concentrated and diluted pig slurry at 15, 20 and 30°C and filling times of 100 days have been summarized in Figure 13.

For the sets of curves found for diluted and concentrated slurry it can be concluded that there exist little difference in the specific growth rate for both slurry concentrations at process temperatures of 20°C and 30°C. The results presented here deviate from those presented in Chapter 6, where we found a clear relation between NH_4^+-N concentration and the effluent VFA concentration in CSTR systems. As the accumulation system is completely different from the CSTR, the methanogenic population might also differ. Boon (1982) observed that the methanogens during the mesophilic CSTR digestion of cow slurry were rod shaped bacteria morphologically similar to *Methanothrix soehngenii*. Hulshoff Pol *et al.* (1983) and Zehnder *et al.* (1981) report that *Methanothrix* is the predominant acetate utilizing methanogen in mesophilic sludge digestion and in mesophilic treatment systems with a high solids retention time. The substrate saturation constant of the *Methanosarcina*, acetate utilizing, bacteria is higher compared to that of *Methanothrix*. Given the high VFA concentrations

prevailing during part of the digestion process in an AC, it is possible that *Methanosarcina's* become predominant in such a system. *Methanosarcina's* are reported to be less sensitive to high NH_4^+-N concentrations than *Methanothrix* (Hulshoff Pol 1983, Koster and Lettinga ,1984).

An other possible explanation for the phenomenon mentioned before this, is that the VFA concentration is not high enough to support maximum growth rate due to a high k_s value of the bacteria involved. The latter could also explain the increase in the volumetric gas production at changing the influent of an 15°C-AC-system (15% inoculation) from cow to pig slurry at a similar NH_4^+-N concentration.

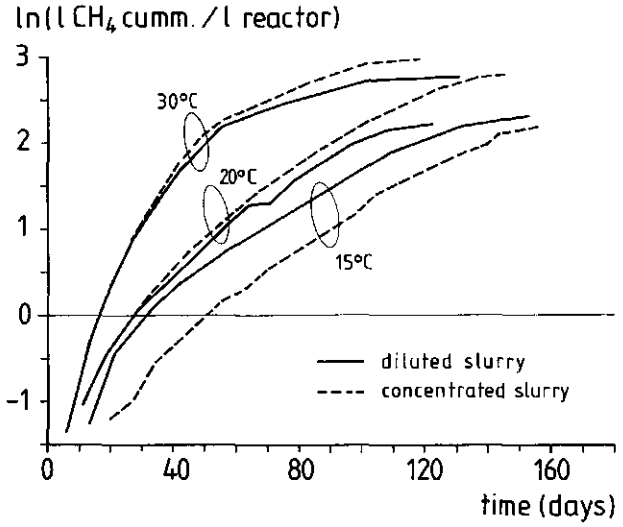

Figure 13, Course of the natural logarithm of the cumulative methane gas production, in the digestion of concentrated and diluted pig slurry at 15, 20 and 30°C in accumulation systems with a filling time of 100 days (exp. no: 22, 28, 29, 33, 36, 39).

Figure 13 shows beyond day 20 onwards, two parallel curves for the digestion of concentrated and diluted pig slurry at 15°C. The results of C_2-degradation with both types of manure at 15°C, indicate that apparently there is little difference in the effect of NH_4^+-N on the C_2-degradation at concentrations of 3.5 and 5.9. g/l. However for the C_3-degradation we observed severe inhibition due to the high NH_4^+-N concentration.

The results presented in the Figure 8b and 10b show that the percentage hydrolysis increases with the applied process temperature. Comparison between these two Figures show also, that the % hydrolysis from concentrated slurry, at a certain digestion time, is considerably lower than from diluted slurry.

The results of our investigations clearly show a strongly detrimental effect of mixing of the digestion process, particularly the breakdown of propionic acid, is negatively affected by mixing. This effect is more pronounced at low inoculation and at low temperature. Similar results were presented by de Zeeuw (1984) for batch experiments conducted with digested sewage sludge and with a mixture of VFA's as a feed. The most likely explanation for this phenomenon is the destruction of the adjacent structures between the H_2 consuming and propionic acid oxidizing bacteria, which will result in an increase of the H_2 concentration in the immediate vicinity of the latter bacteria. Due to this increased H_2 concentration, the degradation of propionic acid will be retarded. At a higher bacterial concentration (f.e. higher

inoculation), 'detached' bacteria are more likely to link up to 'new' bacteria. The fact that little if any detrimental effect is found for mixing at higher temperatures (≥20°C) can be attributed to the higher activity of the sludge, as a result of which, the bacterial concentrations in these systems will increase rapidly.

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CHAPTER 6
EFFECT OF NH_4^+-N AND TOTAL SOLIDS CONCENTRATION ON THE ANAEROBIC DIGESTION OF ANIMAL SLURRIES IN CSTR-SYSTEMS.

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CHAPTER 6
EFFECT OF NH_4^+-N AND TOTAL SOLIDS CONCENTRATION ON THE ANAEROBIC DIGESTION OF ANIMAL SLURRIES IN CSTR-SYSTEMS.

6.1. INTRODUCTION

The composition of animal manure depends on the type of animal, the feeding strategy, the animal housing and the slurry collection and storage system. As a consequence of these varying conditions in animal husbandry a lot of differences are found in the slurry composition. Table 1 gives a comparison of the composition of the cow slurry, used by different researchers in their anaerobic digestion experiments. This Table shows that Dutch and also German slurry is relatively high in NH_4^+-N concentration.

Table 1. Composition of cow slurry as used by different researchers.

		TS g/l	VS g/l	total-N g/l	NH_4^+-N g/l
CAD (1987)	(NETH)	95	75	4.4	n.d.
Chapter 4	(NETH)	77.5-91.8	56.2-80.8	n.d.	2.4-2.9
Hawkes *et al.* (1984)	(GB)	37.8$^+$	28.1	1.7-2.2	0.8-1.1
Hills & Kemmerle (1981)	(USA)	88.2	73.6	2.9	0.6
Singh *et al.* (1985)	(INDIA)	85.1	69.8	1.2	n.d.
Steiner (1983)#	(G)	93.9	75.0	4.7	2.6
Steiner (1983)	(G)	71.4	58.9	3.7	2.0
Wellinger (1984)	(Sw.)	n.d.	43.7-64.6	1.9-2.8	1.0-1.3
Yaldiz (1987)	(G)	85.0	69.6	2.8	0.6

fresh slurry; $^-$ stored slurry; $^+$ separated slurry

NETH= The Netherlands; GB= Great Britain; G= Germany; Sw= Switzerland; n.d.= not detected.

The great environmental problems, due to big amounts of excess manure in the Netherlands will force farmers to reduce the consumption of cleaning and spilling water. Apart from that, the necessary prevention of NH_3-emission will lead to covering of slurry storages. Both these measures will induce an increase of the NH_4^+-N concentration in the slurry. However, at the same time also research concerning the improvement of animal feed conversion has been initiated in the Netherlands (Beudeker *et al.*, 1990), which may lead to a decrease of the slurry NH_4^+-N concentration in future.

Several researchers observed clear effects of the slurry composition on the efficiency of the anaerobic digestion.
Summers and Bousfield (1980) and v. Velsen (1981) reported a decreasing gas production per kg pig slurry solids added to the digester at influent solid concentrations exceeding 6%. V. Velsen also showed a decrease of the methane production in the digestion of pig slurry, when urea was added to the manure, viz. at a NH_4^+-N concentration of resp. 2.1, 3.8 , and 5.3 g/l . This decrease in methane production can be attributed to inhibition of both methane production and hydrolysis. Hobson (1983) proposed an inhibition function of the methanogenesis according to the equation:

$$\mu_m^* = \mu_m - \mu_m \frac{(NH_4^+\text{-N} - 1800)}{1700}$$

where μ_m^* is the actual μ_m at an NH_4^+-N concentration between 1.8 and 3.5 g/l and μ_m is the maximum non-inhibited growth rate. Below or above these concentrations μ_m^* is either μ_m or zero (Hobson, 1983).
Hunnik *et al.* (1990) measured the maximum growth rate (μ_m) of acetoclastic methanogens at extremely high NH_4^+-N concentrations, viz. between 7.7 and 10.4 g/l and a pH between 7.8 and 7.93. According to their calculations, the best mathematical description for the inhibition of the growth rate is given by an equation which contains the total ammonia concentration as well as the pH .
Hashimoto (1982) showed that the gas production from cattle manure can be predicted using the Contois kinetics (see Chapter 1). The kinetic parameter K in this equation, is reported to increase with the VS concentration of the influent, according to the equation:

$$K = 0.8+0.016\ e^{0.06VS}$$ (Hashimoto, 1982)

As the NH_4^+-N concentration and the VS concentration are interdependent, it may be clear that K could also be correlated with the NH_4^+-N concentration.

The research presented here was made to assess the separate effect of the VS content and NH_4^+-N content on both the CH_4 production and the hydrolysis in the digestion of dairy cow manure. For this purpose, manure and urine were collected separately and then mixed to get a series of slurries with equal VS content and varying NH_4^+-N concentration and a series with a varying VS content and $NH4^+$-N concentration. The digestion was performed at a detention time of 10 days at 30°C. In addition to these experiments, we investigated if a temporary decrease of the loading rate could improve the reactor performance in an inhibited situation.

6.2. MATERIAL AND METHODS.

6.2.1. Experiments conducted

The experimental set-up is given in Table 2.

Table 2. Experimental set-up

experiments A									
Operational conditions at time=0 hours					Imposed change in conditions at time (hours) :				
					t=130		t=1600	t=2300	t=3100
no	HRT days	te °C	NH_4^+-N g/l	load g COD /l.d.	NH_4^+-N g/l	load g COD /l.d.			
1.	10	30	4.9	10.1	6.1	9.1	batch	end	
2.	10	30	3.6	8.3	4.4	8.9		HRT=15	-B-
3.	10	30	2.5	5.7	3.0	9.6		HRT=15	end
4.	10	30	1.2	3.0	2.2	9.6		HRT=15	end
5.	10	30	4.0	10.7	6.1	9.1		batch	end
6.	10	30	2.9	10.4	4.4	8.9		HRT=15	-B-
7.	10	30	1.7	10.5	3.0	9.6		HRT=15	end

experiments B (new time scale)

operational conditions at time=0 hours					Imposed change of conditions at time (hours) :		
no	HRT days	te °C	NH_4^+-N g/l	load g COD /l.d.	t=600	t=2400	t=3600
2.	15	30	4.5	8.9			end
6.	15	30	4.5	8.9	HRT=30	HRT=15	end

no=reactor number; HRT=Hydraulic retention time (days); te=temperature.

6.2.2. Influent slurry composition.

The various influent slurries were prepared by mixing faeces, urine and water in different proportions. The composition of the influent slurries is given in Table 3.

Table 3. The investigated experimental mixtures of faeces, urine and water (in ratio) used to prepare the influent for the seven reactors. Total COD, dissolved COD, volatile solids (VS) and NH_4^+-N concentration for the seven influent slurries.

reactor (no)	1	2	3	4	5	6	7
faeces	4	3	2	1	4	4	4
urine	4	3	2	1	3	2	1
water	0	2	4	6	1	2	3
COD_{tot} (g/l)	104.5	82.8	57.0	29.6	107.1	103.8	105.4
COD_{diss} (g/l)	26.8	21.7	14.9	7.4	25.0	22.4	21.3
VS (g/l)	68.2	51.2	34.0	18.0	67.5	65.3	66.2
*NH_4^+-N (g/l)	4.9	3.6	2.5	1.2	4.0	2.9	1.7

* digester content

6.2.3. Design of laboratory digesters

The experiments were performed in jacketed digesters through which thermostated water was pumped to maintain the desired temperature. The working volume of the digesters was 5 liters and they were all mechanically mixed for 30 seconds every 30 minutes. Feeding of the digesters operated at 30°C, was made daily. The digesters were monitored via daily gas production measurement, while analyses of VFA, dissolved COD and NH_4^+-N concentrations were conducted once a week. The methane gas was measured by a wet test meter or bottle of Mariotte, after passing the biogas through a column of soda lime pellets to absorb CO_2.

6.2.4. Conducted analyses

The applied analytical methods are described in Chapter 3

6.2.5. Inoculum used

The experiments were started with digested cow slurry from a 6 m^3 pilot plant digester at the experimental farm in Duiven. The characteristics of the digestion process at the time that the inoculum was taken, are given in Table 4.

Table 4. Relevant information concerning the digestion process, conducted in the 6 m^3 pilot plant cow slurry digester, used for inoculation of the present experiments.

process temperature (°C)	33
detention time (days)	10
loading rate (g COD/m^3.d)	>11.3
NH_4^+-N (g/l)	2.5
% methane formation	26
% acidification	27
% hydrolysis	43

6.2.6. Calculations

The total fractional hydrolysis (H), the fractional hydrolysis that occurs in the reactor (H_R), the fractional acidification (A) and the fractional methanogenesis (M), all in relation to the influent COD and expressed in %, were calculated using equation 1, 2, 3 and 4.

$$H = \frac{100\ (COD_{CH4} + COD_{diss})}{COD_{infltot}} \qquad (1)$$

$$H_R = H - \frac{100\ (COD_{infldiss})}{COD_{infltot}} \qquad (2)$$

$$A = \frac{100\ (COD_{CH4} + COD_{VFA})}{COD_{infltot}} \qquad (3)$$

$$M = \frac{100\ (COD_{CH4})}{COD_{infltot}} \qquad (4)$$

where,

COD_{CH4}= produced CH_4, expressed as COD per litre slurry.

COD_{diss}= dissolved COD in the reactor contents (g/l).

$COD_{infldiss}$= dissolved COD in the influent slurry (g/l).

COD_{VFA}= VFA-COD in the reactor contents (g/l).

$COD_{infltot}$= total COD of the influent slurry (g/l).

The free ammonia concentration was calculated with equation (5)

$$NH_3\text{-}N = NH_4^+\text{-}N \frac{10^{pH}}{kb/kw+10^{pH}} \quad (5)$$

where,

NH_3-N= concentration of undissociated ammonia nitrogen (mg/l)

NH_4^+-N= measured total ammonia concentration (mg/l)

k_b= dissociation constant of aqueous ammonia (Weast,1972)

k_w= ionization constant for water (Weast, 1972)

6.3. RESULTS

The experimental results are shown in Figure 1 to 3 and in Table 5 and 6. Figure 1 gives the calculated fractional hydrolysis, acidification and methane formation in relation to the NH_4^+-N and free NH3-N concentration for the seven separate experiments conducted.

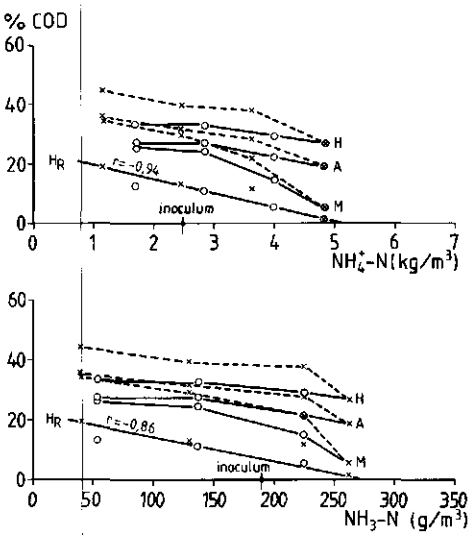

Figure 1. The course of the fractional hydrolysis (H), acidification (A), methane formation (M) and hydrolysis in the reactor (H_R) as a function of the NH_4^+-N and NH_3-N concentration in the digesting cow slurry at 30°C and 10 days detention time. ---- dilution series; —— series with equal influent COD and varying NH_4^+-N concentrations; r= correlation coefficient.

The results in Figure 1 reveal that an increasing NH_4^+-N concentration leads to a decreasing methane formation but also to a strongly decreasing hydrolysis, both in the dilution series and in the series with equal VS concentrations. The total hydrolysis in the experimental series conducted at constant influent COD, is lower compared to that in the dilution series. This is

due to the lower ratio of urine/faeces in the series conducted at a similar COD concentration (Table 3). The organics in urine are completely soluble.

With respect to the % hydrolysis taking place in the reactor (H_R) it appears that:
1. The same relation is found in both series of experiments
2. H_R is inversely proportional to the NH_4^+-N and NH_3-N concentration and can be described by:

$$H_R = 22.8\text{-}4.16\ [NH_4^+\text{-}N],\ \text{with } r=-0.93$$

and

$$H_R = 19.3\text{-}57.3\ [NH_3\text{-}N],\ \text{with } r=-0.86.$$

where, r= the correlation coefficient.

The effluent VFA concentration increases exponentially with the NH_4^+-N concentration (Figure 2).

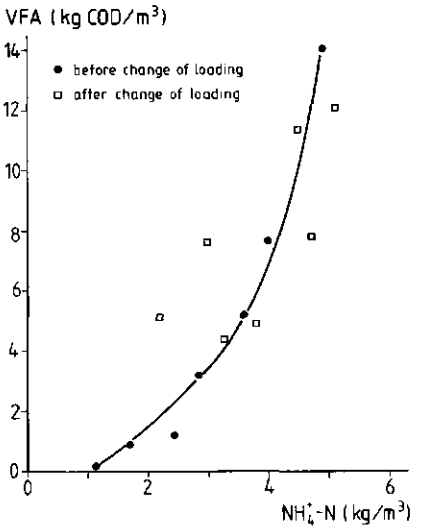

Figure 2. The course of the effluent VFA concentration as a function of the NH_4^+-N concentration in the digesting cow slurry at 30°C and 10 days detention time, before and after changing the influent concentration (see Table 2).

The results of the effluent analyses are summarized in Table 5.

Table 5. Composition of the effluent and the specific gas production found in the series A experiments.*

reactor no	1	2	3	4	5	6	7
tot VFA-COD	14.1	5.2	1.1	0.2	7.7	3.2	0.9
diss COD	22.3	13.2	5.6	2.9	15.1	8.9	7.5
NH_4^+-N	4.9	3.6	2.5	1.2	4.0	2.9	1.7
pH	7.8	7.9	7.8	7.6	7.9	7.8	7.6
CH_4 (m^3/m^3 slurry)	1.9	6.2	7.5	3.5	5.4	8.6	9.5

*Results are given as mean values of observations made at time t=998 hours, t=1142 hours and 1287 hours.

The slurry composition was changed after a digestion period of 1300 hours (see Table 2). The loading rate was increased only in reactor 2, 3 and 4, viz. to resp. 8.9, 9.6 and 9.6 g COD/l.d. The NH_4^+-N concentration however, was increased in each of the seven reactors. The imposed change in slurry composition results in an increase of the effluent VFA concentration, in all seven reactors. However, after three detention times, the relation between effluent VFA and NH_4^+-N concentration coincides with the relation found before the change of conditions (see Figure 2). There are two exceptions, viz. reactor 4 and 7, which both had an original NH_4^+-N concentration $\leq$ 1.7 g/l. Both these reactors showed a considerably higher VFA concentration.

In order to assess an improvement in the reactor performance, the detention time of digesters 2, 3, 4, 6 and 7 was increased from 10 to 15 days after 2300 hours of digestion. This resulted in a decreased effluent VFA concentration in reactor 3, 4 and 7, but not in reactor 2 and 6. The experiments in the former three reactors were terminated after 3100 hours, while the operation of the latter two was continued for another 3600 hours in experimental series B. The results are presented in Figure 3. At time t=460 hours, the VFA concentration in reactor 2 and 6 is 8.94 and 8.02 g COD/l, respectively (Fig 3). The increase in detention time of reactor 6, at time t=460, resulted in an increased specific gas production and a drop in the effluent VFA concentration. After decreasing the detention time back to 15 days, at time t=2490 hours, a new 'steady state' established at a stable VFA concentration of 4.4 g COD/l and a specific methane production of 21 g COD/l. The VFA concentration is lower and the specific gas production higher than in the digester operated continuously at a 15 days detention time. Table 6 summarizes the measured VFA concentrations at t=460 hours, at t=2330 and at t=3080 hours.

Table 6. Effluent VFA concentration (g COD/l) of reactor 2 and 6 at three different moments during the digestion process (see Figure 4).

		C_2	C_3	C_4	C_5	tot VFA
t=460 hours						
reactor 2	(HRT=15)	2.29	4.63	0.98	0.88	8.89
reactor 6	(HRT=15)	2.59	4.09	0.76	0.57	8.02
t=2330 hours						
reactor 2	(HRT=15)	3.14	3.95	0.28	0.45	7.80
reactor 6	(HRT=30)	0.81	0.14	0.02	0.02	0.98
t=3080 hours						
reactor 2	(HRT=15)	3.32	3.25	0.52	0.51	7.60
reactor 6	(HRT=15)	2.06	1.75	0.32	0.22	4.35

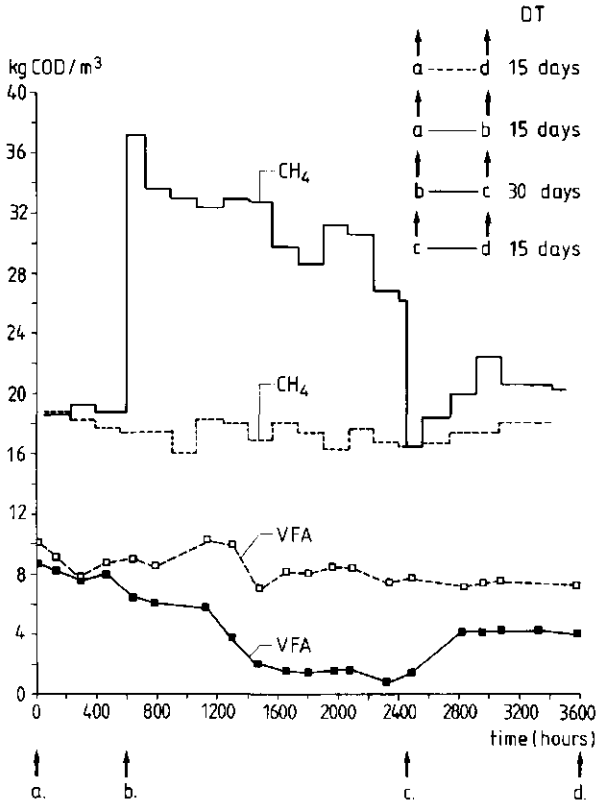

Figure 3. The course of the specific methane production and the effluent VFA-COD as found in the digestion of cow slurry in an experiment conducted at a constant detention time of 15 days (period a-d) and in an experiment in which the detention time was varied from 15 days (period a-b) to 30 days (period b-c) and again 15 days (period c-d). The digestion temperature was 30°C; Influent COD= 89 g/l; NH_4^+-N concentration= 4.5 g/l .

6.4. DISCUSSION

The results presented above clearly show that, at a fixed detention time, the specific gas production declines at an increasing NH_4^+-N concentration. This decrease in methane production is caused by inhibition of the methane production of acetic and propionic acid, but also by inhibition of the hydrolysis of suspended solids. The inhibition of the methane fermentation is demonstrated by the exponential increase of the VFA's in the effluent at increasing NH_4^+-N concentration. This was also observed for the thermophilic digestion of manure (Zeeman *et al.* 1985).

The mechanism of the NH_4^+-N inhibition is more complicated than the relation between residual VFA concentration and NH_4^+-N concentration might suggest. Recently we (Wiegant & Zeeman, 1986) proposed a possible explanation for the inhibition of ammonia in thermophilic digestion. We showed that particularly the specific growth rate of hydrogen consuming ther-

mophilic methanogens is inhibited at increasing NH_4^+-N concentrations. The energy of the propionate breakdown strongly depends on the hydrogen partial pressure. The breakdown of propionic acid therefore is indirectly affected by high NH_4^+-N concentrations. The accumulated propionic acid inhibits the thermophilic acetate utilizing methanogens. When both propionic acid and NH_4^+-N are present, the breakdown of acetic acid is even more inhibited.

The inhibition by intermediates could also explain the existence of two different 'steady state' situations as we observed in the mesophilic digestion of cow slurry at similar conditions, viz. at 30°C and 15 days detention time. A sudden increase of the VFA concentration as a result of a loading 'shock' and/or due to a sudden increase of the NH_4^+-N concentration could give rise to inhibition by intermediates and explain the observed high level of VFA's in the effluent. We (see Chapter 2) observed the same phenomenon in the digestion of cow manure at 20°C. The occurrence of such a VFA accumulation can be avoided by applying a gradual increase of the influent concentration, by temporary increasing the detention time or increasing the temperature.
Recently, Blomgren *et al.* (1989) provided evidence, that in mesophilic digestion a shift in the acetate utilizing methane bacterial population may occur at high NH_4^+-N concentrations (7 g/l), resulting in a syntrophic degradation of acetate into CH_4 and CO_2 (see Chapter 1). It is highly unlikely that syntrophic acetate oxidation has played a role in our thermophilic experiments (Wiegant & Zeeman, 1986) as the concomitant methane formation from H_2/CO_2 would have been inhibited at the applied NH_4^+-N concentrations, exceeding 3.5 g/l. The presented results here, can not clear up whether or not syntrophic acetate oxidation plays a role in the mesophilic anaerobic digestion of cow slurry at higher NH_4^+-N concentrations. As long as it not sure which bacterial population accounts for the conversion of acetic acid to methane gas is it not possible to relate the increase of the effluent VFA concentration at increasing NH_4^+-N concentrations to an inhibition of the μ_m. Contrary to the proposition of Hobson (1983), we also found an increasing VFA concentration in range of 1.2 to 1.7 g/l. This could be caused by the existence of a different bacterial population with both a different K_s and μ_m value.

The presented experimental results show that the VS concentration does not effect the digestion process up to 7% VS, which was the highest concentration tested. This is contrary to the kinetic model developed by Chen and Hashimoto (1980), which is based on the VS concentration of the influent manure. The kinetic parameter K (see Chapter 1) shows a sudden increase beyond a certain VS concentration, indicating a deterioration of the process performance. This sudden increase occurs at VS concentrations in the range 4 to 8 % (Hashimoto *et al.*, 1981, Hashimoto, 1982). The present results indicate that this variation can be explained by the fact that not the VS concentration but the NH_4^+-N concentration of the manure is affecting the process performance.

In addition to the observed inhibition of the methanogenesis, the present results also reveal a strong inhibition of the hydrolysis at increasing NH_4^+-N concentrations. This was also observed in the digestion of pig slurry (v. Velsen, 1981). The results of these different experiments are summarized in Figure 4. Figure 4 reveals a linear decrease of the factor H_R with the NH_4^+-N concentration both for pig and cow slurry. The difference in the extent of hydrolysis between pig and cow slurry is due to the difference in biodegradability.

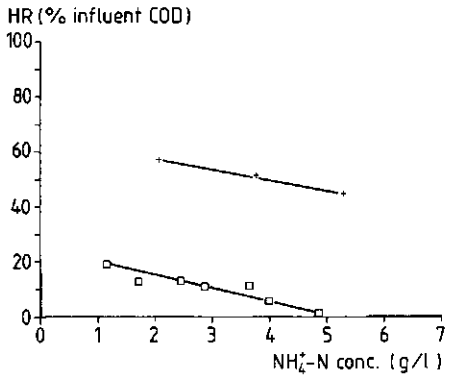

Figure 4. Percentage hydrolysis as a function of the NH_4^+-N concentration for the digestion of pig slurry (v Velsen, 1981) and the digestion of cow slurry (present research) in a CSTR at 30°C.

With respect to the observed effect of the NH_4^+-N on the hydrolysis, at least four possible explanations can be given, viz.:

1. NH_4^+-N causes the inhibition of the hydrolysis
2. Accumulated intermediates like VFA's and H_2 are inhibiting the hydrolysis.
3. The simultaneous presence of higher concentrations of NH_4^+-N and accumulated intermediates causes the inhibition of the hydrolysis.
4. Not NH_4^+-N but organic compounds available in urine inhibit the hydrolysis.

There is some evidence available in literature that accumulated H_2 could inhibit the growth of hydrogen producing cellulolytic bacteria (Chung, 1976). The results of the experiments in accumulation systems do not sustain this observation. No increase in hydrolysis was observed at the time that all VFA's and most likely also H_2 are being removed from the system (see Chapter 5). Chesson *et al.* (1982) found that the plant phenolic acids, trans-p-coumaric and trans-ferulic, completely inhibited growth of cellulolytic and some other rumen bacteria at concentrations exceeding 5 mmol, and repressed growth in some cases at 1 mmol. Hobson (1983) however reported that according to Chesson (unpublished) the liquid of pig and dairy cattle slurries did not contain such acids. In the present research the NH_4^+-N concentration was increased by addition of urine. V. Velsen increased the NH_4^+-N concentration by adding urea and also found an inhibition of the hydrolysis. The above observations lead to the conclusion that NH_4^+-N and no other compound in the urine plays the most important role in the inhibition of the hydrolysis. However, it is obvious that further research concerning the inhibition of the hydrolysis by NH_4^+-N and about the mechanism involved is required.

6.5. CONCLUSIONS

1. The effluent VFA concentration in the digestion of cow slurry in a CSTR at 30°C and 10 days detention time increases exponentially with the NH_4^+-N concentration, in the range 1.2 to 4.9 g/l, irrespective of the influent VS concentration (tested up to 7% VS).
2. The % hydrolysis in the reactor is inversely proportional to the NH_4^+-N concentration, irrespective of the influent VS concentration (tested up to VS =7%) and can be described by the equation: $H_R = 22.8 - 4.16\ [NH_4^+\text{-N}]$.

3. A high loading, applied right from the beginning, can lead to a 'steady state' at a relatively high effluent VFA concentration. However, when increasing gradually the loading rate to a similar level, a 'steady state' with a relatively low VFA concentration is achieved.

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CHAPTER 7
SUMMARY AND DISCUSSION

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CHAPTER 7
SUMMARY AND DISCUSSION

7.1. IN GENERAL

In this thesis the possibilities for digestion of cow and pig manure are described for a completely stirred tank reactor system (CSTR) and an accumulation system (AC-system).
For this purpose were researched:
1. Anaerobic digestion of cow manure. Optimization of the digestion process for energy production on dairy farms.
2. Digestion of manure at lower temperatures.

The goal of the first mentioned research was optimization of anaerobic digestion of cow manure in a mesophilic CSTR-system. The results of this research as well as practice experience show that without considerable problems a stable anaerobic digestion process is obtained. Further application of anaerobic digestion of animal slurries on farm-scale in CSTR-systems under mesophilic conditions is at the present energy prices economically not remunerative. This was an important reason for starting research on the applicability of simpler and cheaper systems for digesting manure, such as an accumulation system (AC-system) at lower temperatures.

A major part of research on manure digestion is carried out in mesophilic conditions and also in practice the application is mostly in the mesophilic temperature range (Demuynck *et al.*, 1984). Results of laboratory research (v. Velsen, 1981; Hawkes *et al.*, 1984; Yaldiz, 1987; Hill *et al.*, 1983) offered little perspective for application of low temperature digestion in practice. Results in this thesis show that the retention times in the above mentioned research were to short to reach a stable digestion at lower temperatures.

The goal of the research on digestion of manure at lower temperatures was to obtain insight into processes concerning anaerobic digestion of manure at low temperatures and to investigate the applicability of digestion and storage in a so-called AC-system.

A CSTR-system is characterized by a constant supply of fresh manure as well as by a constant removal of digested manure. The sludge load is therefore constant. An AC-system is also characterized by a constant supply of fresh manure, but the removal of digested manure only takes place once per filling (storage) period. The small inoculation at the start of the filling period, typical for an AC-system, results in a high initial sludge load. Through the growth of the biomass during the filling time the sludge load will decrease.

7.2. START-UP OF SLURRY DIGESTION SYSTEMS.

It is clear that the first start-up should be carried out with inoculation material as much as possible adapted to the digestion conditions. However such inoculation material is hardly ever available. It is therefore important to know the factors affecting start-up and the stability of the ultimate digestion process, such as:
- process temperature.
- the quality of the inoculation material.
- the amount available inoculation material.
- sludge load.

Chapter 2 and 3 of this thesis describe the start-up of manure digestion in several systems. The results presented in Chapter 2 show that with batch wise digestion of fresh manure without inoculation at 5, 10 and 15°C no methane is produced within a 5 months period. Methane production at these low temperatures is possible when high inoculation percentages are applied. A 50% inoculation shows even at a process temperature as low as 5°C methane production.

Systems, also without inoculation, at process temperatures of 20°C and higher will obtain in a relatively short time sufficient methanogenic activity. With batch wise digestion of fresh cow manure at 25 and 30°C the lag-phase is respectively ± 60 and 20 days, while the accumulated fatty acids are converted into methane in 125 en 75 days. With a non-inoculated manure digestion start-up the quality of the manure to be digested is of considerable importance. When manure has been stored for some time a sort of pre-start-up has taken place. This explains the relative short lag-phase (respectively ± 20 and 60 days) at the start-up of an AC-system for digesting stored cow and pig manure at 20°C.
The results in Chapter 2 also show that a non-inoculated start-up at temperatures of 15°C and lower, will cover an extended period of time. High inoculation provides a fast start-up, even at low temperatures, but is mostly not feasible in practice.

Chapter 3 describes the realization of start-up of an AC-system at 15°C with low inoculation (1-13%). The quality of the inoculation material appears to be of great importance for the proceeding of the start-up. This quality is highly dependable on the cultivation conditions, viz.:
- The type of digestion system used.
- The applied process temperature.

The used digestion system
Research results show that digested manure from a CSTR-system is not suitable for starting-up an AC-system at 15°C, 1-13% inoculation and 100 days filling time. In order to remove accumulated fatty acids when starting-up an AC-system on cow manure, extreme long digestion times are required (240-310 days), under the above mentioned conditions and inoculated with at 20°C digested CSTR-system manure (20°C -CSTR-sludge). At a following 'second start-up', 13% inoculated with the 'freshly' digested material, the start-up period is only 150 instead of 240 days. The 'first start-up' is characterized by a successive degradation of acetic and propionic acid, whereas in the 'second start-up' with AC-sludge these fatty acids are removed simultaneously. Similar results can be achieved with the start-up of an AC-system at 15°C for the digestion of pig manure with 15°C-CSTR-sludge as inoculation.

The applied process temperature
The quality of the inoculation material is not only influenced by the system in which it was cultivated but also by the temperature it was liable to. The starting-up of an AC-system at 15°C will proceed faster with a 15% inoculation of 15°C-AC-sludge than with 20°C-AC-sludge. Similar results have been found with batch digestion of manure. Inoculation with sludge cultivated at 18°C will give a higher gas production in batch digestion at 20°C, than inoculum cultivated at 27 and 35°C. At process temperatures of 27 and 35°C the mentioned inocula show little difference in gas production. Wellinger and Kaufmann (1982) and Cullimore *et al.* (1985) also found this acclimatization of the sludge to lower temperatures.

When using the same amount and type of inoculum at a digestion temperature of 20°C the starting-up period in an AC-system is considerable shorter than at 15°C. At 20°C the accumulated fatty acids can be converted within the filling time of 100 days with a 7-13% inoculum, whereas at 15°C a start-up period of at least 240 days was required.

In practice non or little adapted inoculum is available. It is than recommendable to operate the AC-system during the first start-up at 20°C. The following filling can be conducted at 15°C. In practice this means AC-systems should be started-up preferably in summer or additional heating should be installed. When it is impossible to raise the process temperature temporarily, additional storage capacity should be available, in order to convert the accumulated fatty acids and to obtain a sludge with sufficient C2 and C3 degradation capacity.

NH_4^+-N is a major inhibition component in the digestion of manure. When sludge unadapted for high NH_4^+-N concentrations, is used for starting-up an AC-system, an adaptation period should be taken into calculation, in which the fatty acid degradation and gas production will stagnate (v. Velsen, 1981). With the start-up of AC-systems on pig manure, Hill *et al.* (1983) attribute the inhibition of the methane production to the high concentrations of accumulated

fatty acids, when sewage sludge was used as inoculum. The results in Chapter 3 indicate that when unadapted inoculum (such as sewage sludge and granular sludge) is used in an AC-system for digesting manure with a relatively high NH_4^+-N concentration, an adaptation period is required to NH_4^+-N in which a stagnation of the fatty acid degradation and gas production will occur. The results of starting-up experiments of both CSTR- as AC-systems with CSTR sludge (presented respectively in Chapter 2 and 3) indicate that also inhibition by fatty acids can appear, viz.:
- When a CSTR-system at 20°C is started-up with mesophilic sludge operated at a high sludge loading, the 'steady state' is characterized by a high fatty acid concentration in the effluent and low gas production. However when the sludge loading is increased step wise, other conditions unchanged, the 'steady state' is characterized by a low fatty acid concentration in the effluent and a high gas production.
- When 50% 20°C -CSTR-sludge is used as inoculum for starting-up an AC-system for treating cow manure at 15°C, the lowest gas production was observed with the highest sludge loading (ft= 70 days instead of 100 days). The results of the experiments with as well the CSTR- as the AC-system show that the inhibition can be terminated by a temporary raise of the process temperature.

7.3. PROCESS MANAGEMENT OF CSTR- AND AC-SYSTEMS.

Results concerning continuous experiments with CSTR- and AC-systems are described in Chapter 4 and 5. The methane production in the digestion of manure depends on the course of hydrolysis, acidification and methanogenesis. Temperature and applied loading are the two most important process parameters .

The research concerning the digestion in completely mixed systems is carried out with cow manure in a temperature range from 15 to 40°C. It is indeed possible to obtain a stable digestion at 15°C, but in this case very long retention times are required (≥ 100 days). Also the gas production is then considerably lower compared with a digestion at 30°C and 20 days retention time. This lower gas production is caused by a reduced hydrolysis.
Digestion at 20°C and a retention time of 100 days results in a similar gas production as a digestion at 30°C and 20 days retention time. Under these conditions approximately 25% of the influent COD is converted to methane gas. About half of this origins from fatty acids already present in the influent and the rest is derived through hydrolysis of suspended solids. At longer retention times no significant increase of the specific gas production is found. With an increase to 35°C only at a retention time of 15 days a small significant increase of the gas production is observed. With process temperatures of 30-35°C the specific gas production (m3/m3 manure) increases with increasing retention times ranging from 10-20 days. The hydrolysis is the rate limiting step. The non-VFA-dissolved-COD fraction is inert to anaerobic treatment. For non of the investigated process conditions a reduction of this non-fatty acid dissolved COD is found.

Digestion at 30-35°C in a CSTR-system is well capable of dealing with sudden increases of the loading as a result of decreases of retention time. A one step reduction of the retention time of 15, 20, 25 or 30 to 10 leads to a direct increase of the gas production and only a minor raise of the effluent fatty acid concentration. In this way the volumetric gas production (m3/m3.day) can be more than doubled within a few days. In practice the methane production can be adapted, in this way, to the energy demand at that moment.

In Chapter 5 results of cow and pig manure digestion in AC-systems are presented. The course of the digestion is dependent on process temperature, filling time, percentage inoculation and the composition and concentration of the manure.

The results of experiments with cow manure give an insight in the stability of the process during several successive filling periods at process temperatures of 15 and 20°C. When digesting cow manure (influent COD= 95-113 g/l) at 15°C in an AC-system with a 15% inoculation, a 'steady state' can not be reached within the filling time of 100 days. An extra digestion

period of 40-50 days is necessary for a complete conversion of the accumulated fatty acids. The course of the following filling periods is more or less identical. A change of manure composition (95 → 113 g/l COD; 1.9 → 3.5 g/l NH_4^+-N) results in an increase of the digestion time in order to degrade the accumulated fatty acids. The gas production rate however, remains almost the same.
The AC-digestion of cow manure (95 g COD/l) at 20°C has been conducted with inoculation percentages of 1, 7 and 13% at a filling period of 100 days. Even with the lowest inoculation the accumulated fatty acids are nearly completely converted during the filling time of 100 days. When an inoculation of 7 and 13% is applied, a considerable part of the filling period is in a 'steady state'.

Considering the better perspectives for practical application of AC-systems for pig manure instead of cow manure, more extended research was conducted with pig manure.
When applying digested cow manure (15°C-AC-sludge) as inoculation for digesting pig manure (74 g COD/l; 3.7 g NH_4^+-N/l) at 15°C, higher gas production rates are found than for digesting cow manure (113 g COD/l; 3.5 NH_4^+-N/l) under the same process conditions. However also with the digestion of pig manure it is not possible to covert the accumulated fatty acids within the filling period, a post digestion of ± 50 days is required. Theoretically a minimal inoculation of 25% is necessary in order to convert the accumulated fatty acids within the filling period. With filling periods of 270 days a 10% inoculation will be sufficient. At a manure production of 1 m3/day reactor volumes of respectively 133 and 300 m^3 should be installed.

When digesting more concentrated pig manure (118 g COD/l; 5.9 g NH_4^+-N/l) in an AC-system at 15°C, this results in an accumulation of propionic acid. Even after extreme long digesting periods (100+180 days post digestion) no degradation of propionic acid is shown. Probably the high concentration of NH_4^+-N (5.9 g/l) in the manure is the cause of stagnation of the propionic acid breakdown.

An increase of process temperature from 15 to 30°C, with a similar filling time, results in an increase of as well the volumetric as the specific gas production. Both the hydrolysis and the methane generation increase with a raise of temperature from 15 to 30°C.

In contrast with a process temperature of 15°C, higher temperatures (20 and 30°C) induce hardly any difference in gas production and fatty acid course when the manure concentration is lowered from 118 to 74 g COD/l. The accumulated fatty acids are degraded into methane respectively in 140 and 120 days, at 20°C; at 30°C this is respectively 50 and 40 days, after which a steady state occurs. The specific growth rate of the methanogens as well for 20 as for 30°C does not differ with concentrated or diluted manure.

Results of the executed research show, that intensive stirring in the AC-systems with a high initial loading (e.g. a 15% inoculation and a 100 day filling period at a process temperature of 15°C) has a strong negative effect on the degradation of propionic acid and to a lesser extent on acetic acid. In an AC-system with a high initial activity (e.g. a 10% inoculation and a 100 days filling time at process temperatures of 20 and 30°C) hardly or no negative effect of stirring was found. It should be concluded that intensive mixing is process technologically unfavorably. Only for reasons of management some stirring can be useful, e.g. when removing the digested manure or when heating is applied.

7.4. TOXICITY

In Chapter 6 the results are discussed of the research dealing with the effect of organic matter and the NH_4^+-N concentration on the digestion of cow manure in CSTR-systems, at a process temperature of 30°C and a retention time of 10 days. The organic matter concentration of the manure, within a range of 2-7%, has no effect on the digestion. The NH_4^+-N concentration however has a very distinct effect, as well on the hydrolysis as on the methanogenesis. The effluent fatty acid concentration increases exponentially and the

hydrolysis declines linear with NH_4^+-N concentrations in the range of 1.2-4.9 g/l. The relation between the percentage hydrolysis in the reactor (H_R) and the NH_4^+-N concentration can be empirically described with the equation:

$$H_R = 22.8\text{-}4.16\ [NH_4^+\text{-N}].$$

When digesting manure with a high NH_4^+-N concentration (4.9 g/l), at relative short retention times, two different equilibrium effluent fatty acid concentrations can establish. The lowest concentration can be achieved through a temporarily lowering of the loading. Similar results were found with starting up digesters at lower temperatures as described in Chapter 2 and probably can be attributed to the inhibitory effect of fatty acids.
Results of research on CSTR-systems show a clear negative effect of NH_4^+-N, this is however not the case with AC-systems at 20 and 30°C.

7.5. APPLICABILITY OF AC- AND CSTR-SYSTEMS.

The digestion of manure in CSTR-systems for gas production on farm-scale is applied in the Netherlands since 1979. Between 1979-1982 ± 25 installations have come into operation on pig and dairy farms. The development of the energy prices is the main factor causing no further growth of biogas installations after 1982.

The earning-capacity of biogas installations was apart from the drop of energy prices, negatively influenced by the considerable lower efficiencies of the installations (especially on piggeries) found in comparison with laboratory and semi-technical investigations. The cause of this is the occurrence of 'pre-digestion' in the manure storage. In fact, every farmer has got already a digester, however the process is not optimized and the gas is not collected and used. This finding gave the main rise to investigate the possibilities of combined storage and digestion. The application of an AC-system, in which the storage and digestion is combined, is in principle also much more suitable for manure digestion on farm-scale than the CSTR-system, for no extra reactor and/or effluent storage is required. Present developments on manure handling, viz., the required extension of manure storage and the requirement to cover the storage gas-tight for eliminating emission of NH3, make that the storage starts to resemble a digester.
The results presented in this thesis show clearly that an increase of the storage time can result in an important raise of the gas production, even at low temperatures (15°C). Since it is known that CH_4 contributes by far more severe to the greenhouse effect than CO_2 (Goossensen & Meeuwissen, 1990), the biogas production during storage should be avoided or optimized and used in order to save fossil fuel. In the latter way a contribution can be made to fight the greenhouse effect.

The obtained results also show that avoiding methane production is very difficult. When a suitable bacterial population has established the methanogenesis will develop irrevocably, unless inhibitory substances are used. The latter is from an environmental point of view not recommendable. At a manure temperature of 20°C, as it can occur in summer, 1% inoculation is enough to start the digestion process within the filling period. Since practice shows it is impossible to empty manure storages completely, approximately 10-15% of the manure in general remains, it can be concluded from the obtained results that with the extension of storage time to 5 months or longer also at temperatures of 15°C, considerable gas production will appear. At the test accommodation for piggery at Rosmalen a 700 m3 manure silo was isolated, covered gas-tight and equipped with a simple heating system. During two years research was done on the combination of storage and digestion of manure at ± 18°C. When it becomes compulsory to cover manure storages gas-tight, the combination of manure storage and digestion will certainly becomes an attractive alternative.

The use of an AC-system is in principle suitable when long term storage is compulsory. In the present situation in the Netherlands, where much organic manure is produced, a major part of this manure will have to be processed. When processing manure a maximal regain and re-use

of valuable products and a minimal use of energy should be aimed. Anaerobic digestion as a part of such a system is to be seen as a method to remove and convert a major part of the solid and soluble organic fraction of the manure into methane. The methane produced can be used as energy for further process steps. When there is no need for longer storage if manure is processed on a large scale, the AC-system is less attractive than the CSTR-system.

By anaerobic digestion only a part of the organic compounds can be converted into methane. A further removal of solids can be accomplished by mechanical separation after or if possible before digestion. Mechanical separation of the 'fresh' slurry, into a solid and soluble fraction, has the advantage of a possible separate digestion of the fractions, the soluble fraction in a high loaded reactor, e.g. an UASB and the solid fraction in a low loaded reactor. In this way the total required reactor volume can be considerably reduced.

The soluble fraction contains the major part of the nitrogen, while the solid fraction contains most of the phosphor. The nitrogen in the soluble fraction is mainly present as NH_4^+-N. An in principle very attractive system for removal of NH_4^+-N (and phosphate) in the soluble fraction is the stripping/absorption process. A diagram of this process is given in Figure 1 (v. Velsen, 1985).

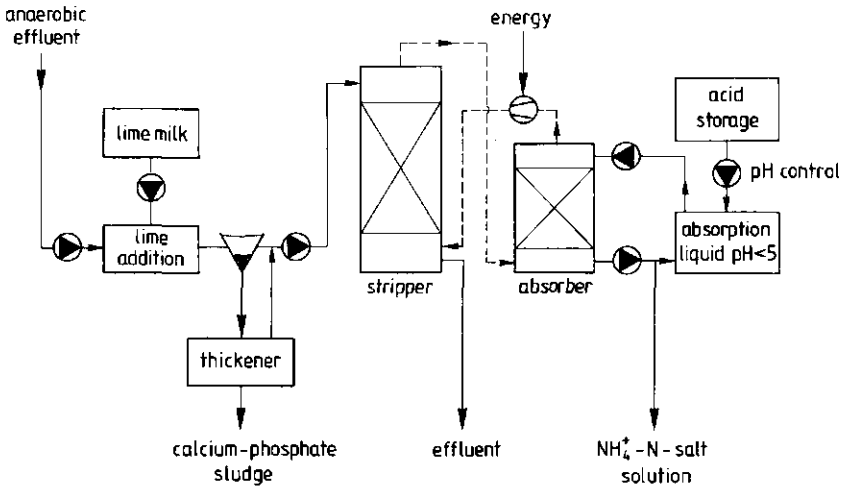

Figure 1. Scheme of the stripping/absorption process for the recovery of NH_4^+-N from anaerobically digested liquid manure (v. Velsen, 1985).

It is incomprehensible that this system or other modified systems of this (Drese, 1988) are not yet tested on large scale.
From the point of view of appropriate use of resources it is necessary to regain and recycle the large amounts of nitrogen from the manure and not as proposed sometimes (Koster, 1990) to convert this into nitrogen gas by means of nitrification/denitrification, especially because this requires vast amounts of high grade energy. Complete recovery of NH_4^+-N is not always necessary or economically justified. A combination of the stripping/absorption with the nitrification/denitrification process should at least be considered.

7.6. REFERENCES

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HOOFDSTUK 8
SAMENVATTING EN DISCUSSIE

Inhoud

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8.6. Referenties

Hoofdstuk 8
SAMENVATTING EN DISCUSSIE

8.1. ALGEMEEN

In dit proefschrift worden de mogelijkheden beschreven van de vergisting van koe- en varkensmest in een volledig gemengd doorstroomsysteem (VGD-systeem) en in een accumulatie systeem (AC-systeem).
Hiertoe zijn twee praktijkgerichte onderzoeken uitgevoerd nl.
1. Anaërobe vergisting van koemest. Optimalisering van het gistingsproces t.b.v. energieproduktie op melkveebedrijven.
2. Vergisting van mest bij lagere temperaturen.

Het doel van het eerstgenoemde onderzoek was het optimaliseren van de anaërobe vergisting van koemest in een mesofiel VGD-systeem. Zowel uit de resultaten van dit onderzoek als uit praktijkervaring blijkt dat zonder noemenswaardige problemen een stabiel anaëroob vergistingsproces kan worden verkregen. Verdere toepassing van anaërobe vergisting van dierlijke mest op boerderijschaal in volledig gemengde doorstroomsystemen onder mesofiele omstandigheden, blijkt bij de huidige energieprijzen economisch echter niet haalbaar. Een en ander vormde een belangrijke rede om onderzoek te starten naar de toepassingsmogelijkheden van eenvoudigere en goedkopere systemen voor de vergisting van mest, zoals het AC-systeem bij lagere temperaturen.

Het overgrote deel van de onderzoekingen naar mestvergisting is uitgevoerd onder mesofiele condities en ook de praktijktoepassing vindt voornamelijk plaats bij mesofiele temperaturen (Demuynck *et al.*, 1984). De resultaten van laboratoriumonderzoek (v. Velsen, 1981, Hawkes *et al.* 1984, Yaldiz, 1987, Hill *et al*, 1983) boden weinig perspectief voor de praktijktoepassing van mestvergisting bij lagere temperaturen ($\leq$ 20°C). Uit de resultaten beschreven in dit proefschrift blijkt, dat de toegepaste verblijftijden in bovengenoemde onderzoeken, veelal te kort waren voor het verkrijgen van een stabiele vergisting bij lagere temperaturen.

Het doel van het onderzoek naar de vergisting van mest bij lagere temperaturen was het verkrijgen van inzicht in de processen die een rol spelen bij de anaërobe vergisting van mest onder koude omstandigheden en de mogelijkheden van toepassing van vergisting van mest in combinatie met opslag in een zogenaamd AC-systeem te onderzoeken.

Een VGD-systeem wordt gekenmerkt door zowel een constante toevoer van verse mest als een constante afvoer van vergiste mest. De slibbelasting is onder die condities danook constant. Een AC-systeem wordt weliswaar ook gekenmerkt door een constante toevoer van verse mest, maar de afvoer van de vergiste mest vindt slechts eenmaal per opvul-(=opslag)periode plaats. De geringe enting aan het begin van de opvulperiode, kenmerkend voor een AC-systeem, resulteert in een hoge initiële slibbelasting. Als gevolg van ingroei van de biomassa zal de slibbelasting afnemen gedurende de opvulperiode.

8.2. OPSTART VAN MESTVERGISTINGSSYSTEMEN

Het is duidelijk dat een eerste opstart het best kan worden uitgevoerd met entmateriaal dat zoveel mogelijk is aangepast aan de condities tijdens de vergisting. Een dergelijk entmateriaal is echter lang niet altijd beschikbaar. Het is danook van belang de faktoren te kennen die de snelheid van de opstart en de stabiliteit van het uiteindelijke vergistingssysteem beïnvloeden, zoals:

- procestemperatuur.
- de 'kwaliteit' van het entmateriaal.
- de beschikbare hoeveelheid entmateriaal.
- belasting

In Hoofdstuk 2 en 3 van dit proefschrift is de opstart van mestvergisting in verschillende systemen beschreven.
Uit de resultaten beschreven in Hoofdstuk 2 blijkt dat bij ladingsgewijze vergisting van verse mest bij procestemperaturen van 5, 10 en 15°C binnen een periode van 5 maanden geen methaanvorming optreedt, wanneer geen entmateriaal wordt gebruikt. Methaanvorming bij deze lage temperaturen is wel mogelijk, indien hoge ent percentages worden toegepast. Bij 50% enting wordt zelfs bij een procestemperatuur van 5°C nog methaanvorming waargenomen.

Bij procestemperaturen van 20°C en hoger kan zich, ook in ongeënte systemen, in een betrekkelijk korte periode voldoende methanogene aktiviteit ontwikkelen. Bij de ladingsgewijze vergisting van 'verse' koemest bij procestemperaturen van 25 en 30°C bijvoorbeeld, bedraagt de lag-fase resp. ± 60 en 20 dagen terwijl de geaccumuleerde vetzuren in resp. 125 en 75 dagen worden omgezet in methaangas. Bij de ongeënte opstart van mestvergisting is de 'kwaliteit' van de te vergisten mest van groot belang. Wanneer de mest gedurende kortere of langere tijd is bewaard in een opslagkelder, heeft reeds een soort vóór-opstart plaatsgevonden. Dit verklaart de betrekkelijk korte lag-fase (resp.± 20 en 60 dagen) bij de opstart van een AC-systeem voor de vergisting van bewaarde koe- en varkensmest bij 20°C.
Uit de resultaten in Hoofdstuk 2 blijkt duidelijk dat met een ongeënte opstart bij temperaturen van 15°C of lager een zeer lange periode gemoeid is. Door toepassing van een hoge enting is een snelle opstart ook bij lagere temperaturen mogelijk. Het gebruik van grote hoeveelheid entmateriaal zal in de praktijk in het algemeen echter niet realiseerbaar zijn.

In Hoofdstuk 3 wordt beschreven op welke wijze, opstart van een AC-systeem bij 15°C, met lage enting (1-13%) kan worden gerealiseerd. De 'kwaliteit' van het entmateriaal blijkt van groot belang te zijn voor het verloop van de opstart. Deze kwaliteit wordt in hoge mate bepaald door de condities waaronder het entmateriaal is gekweekt, nl.:
- het gebruikte vergistingssysteem
- de toegepaste procestemperatuur

Het gebruikte vergistingssysteem
Uit de verkregen resultaten blijkt, dat vergiste mest uit een VGD-systeem, in principe ongeschikt is voor de opstart van een AC-systeem bij 15°C, bij 1-13% enting en 100 dagen opvultijd. Bij de opstart van AC-systemen voor de vergisting van koemest onder de hiervoor genoemde omstandigheden bij gebruik van, bij 20°C in een VGD-systeem vergiste mest ('20°C-VGD-slib'), zijn extreem lange gistingstijden (240-310 dagen) nodig, teneinde geaccumuleerde vetzuren uit het systeem te kunnen verwijderen. Bij een daaropvolgende 'tweede opstart' bij 13% enting met het 'nieuw vergiste' materiaal, bedraagt deze opstartperiode nog slechts 150 i.p.v. 240 dagen.
De 'eerste opstart' met 'VGD-slib' wordt gekenmerkt door een opeenvolgende afbraak van azijnzuur en propionzuur terwijl deze vetzuren bij de 'tweede opstart' (met AC-slib) gelijktijdig worden verwijderd. Overeenkomstige resultaten worden bereikt bij de opstart van een AC-systeem bij 15°C voor de vergisting van varkensmest met 15°C-VGD-slib als entmateriaal.

De toegepaste procestemperatuur
Behalve het gebruikte vergistingssysteem bepaalt tevens de toegepaste vergistingstemperatuur de 'kwaliteit' van het entmateriaal. De opstart van een AC-systeem bij 15°C voor de vergisting van varkensmest met 15% '20°C-AC-slib' verloopt minder snel dan die met eenzelfde hoeveelheid '15°C-AC-slib'. Soortgelijke resultaten zijn gevonden bij de ladingsgewijze vergisting van mest. Enting met een bij lage temperatuur (18°C) gekweekt slib, geeft bij ladingsgewijze vergisting bij 20°C, een hogere gasproduktie dan enting met een bij 27°C en 35°C gekweekt slib. Bij procestemperaturen van 25°C en 35°C worden bij de genoemde entingen nauwelijks verschillen in gasproduktie gevonden. Wellinger & Kaufmann (1982) en Cullimore *et al.* (1985) vonden tevens dat 'acclimatisatie' van het slib aan lagere temperaturen optreedt.

Vergeleken bij 15°C is bij een procestemperatuur van 20°C een veel kortere periode nodig voor de opstart van een AC-systeem. Bij 7-13% enting kunnen in dat geval de geaccumuleerde

vetzuren vrijwel volledig worden omgezet binnen een opvultijd van 100 dagen, terwijl bij 15°C met 'niet aangepast slib' een opstartperiode van minimaal 240 dagen vereist is.

In de praktijk is in het algemeen geen of onvoldoende 'aangepast' entmateriaal beschikbaar. Het is aan te bevelen, de eerste opstart van een AC-systeem dan uit te voeren bij een procestemperatuur van 20°C. Bij de daaropvolgende opvulperiode kan de procestemperatuur worden verlaagd tot 15°C. Voor de praktijk betekent dit, dat onverwarmde AC-systemen bij voorkeur in de zomer dienen te worden opgestart of moeten worden voorzien van bijverwarming. Indien het niet mogelijk is een hogere procestemperatuur toe te passen zal tijdelijk extra opslagcapaciteit moeten worden gerealiseerd, zodat in het systeem geaccumuleerde vetzuren, volledig kunnen worden omgezet en een slib wordt verkregen met voldoende C_2 en C_3 afbrekende capaciteit.

NH_4^+-N is een belangrijke remmende component bij de vergisting van mest. Wanneer voor de opstart van een accumulatie mestvergistingssysteem, slib wordt gebruikt dat niet is geadapteerd aan hoge NH_4^+-N concentraties moet rekening worden gehouden met een 'adaptatieperiode', waarin stagnatie van de vetzuurafbraak en gasproduktie optreedt (v. Velsen, 1981). Hill *et al.* (1983) daarentegen schrijft de remming van de methaanproduktie, bij de opstart van AC-varkensmestvergistingssystemen, bij gebruik van slijkgistingsslib als entmateriaal, volledig toe aan de hoge concentraties aan geaccumuleerde vetzuren. De resultaten in Hoofdstuk 3 geven aan dat bij toepassing van 'niet geadapteerd slib' (b.v. slijkgistingsslib of korrelslib), als entmateriaal voor een AC-systeem voor de vergisting van mest met een betrekkelijk hoge $NH4^+$-N concentratie, rekening moet worden gehouden met een adaptatie periode aan NH_4^+-N. Gedurende deze periode stagneert de afbraak van vetzuren en daalt de gasproduktie. Ook resultaten in Hoofdstuk 2 en 3 van opstartexperimenten met VGD-systemen en een AC-systeem met gebruik van VGD-slib, wijzen uit dat tevens remming van de methaanproduktie kan optreden als gevolg van hoge vetzuurconcentraties,. nl.
-Wanneer een VGD-systeem bij 20°C wordt opgestart met mesofiel slib bij een betrekkelijk hoge belasting, stelt zich een 'steady state' in met een hoge effluentvetzuurconcentratie en lage gasproduktie. Indien de belasting langzaam wordt opgevoerd (door verkorten van de verblijftijd) stelt zich onder deze omstandigheden echter een 'steady state' in met een lage effluentvetzuurconcentratie en een hoge gasproduktie.
-Wanneer 50% 20°C-VGD-slib wordt gebruikt als ent voor de opstart van een AC-koemestvergistingssysteem bij 15°C, wordt de laagste gasproduktie waargenomen bij de hoogste belasting (ft=70 dagen, i.p.v. 100 dagen).
De resultaten van zowel het experiment met het VGD-systeem als het experiment met het AC-systeem, laten zien dat de remming kan worden opgeheven door een tijdelijke verhoging van de procestemperatuur.

8.3. BEDRIJFSVOERING VAN VGD- EN AC- SYSTEMEN.

De resultaten van langdurige bedrijfsvoering van VGD- en AC-systemen onder verschillende omstandigheden zijn beschreven in Hoofdstuk 4 en 5. De optredende methaanproduktie in de vergisting van mest is afhankelijk van het verloop van de hydrolyse, verzuring en methaanvorming. De temperatuur en de toegepaste belasting zijn de belangrijkste procesparameters.

Het onderzoek naar de vergisting in volledig gemengde systemen is uitgevoerd met koemest, bij procestemperaturen variërend van 15 tot 40°C. Het is weliswaar mogelijk zelfs bij 15°C een stabiele vergisting te verkrijgen, maar er zijn in dat geval wel zeer lange verblijftijden noodzakelijk ($\geq$ 100 dagen). De gasproduktie is ook dan nog aanzienlijk lager, vergeleken met vergisting bij 30°C en 20 dagen verblijftijd. Deze lagere gasproduktie bij 15°C wordt veroorzaakt door een verminderde hydrolyse.
In geval van vergisting bij 20°C, wordt bij een verblijftijd van 100 dagen vrijwel dezelfde gasproduktie gevonden als bij 30°C en 20 dagen verblijftijd. Onder die condities wordt ongeveer 25% van de influent CZV omgezet tot CH_4-gas. Ongeveer de helft is afkomstig van vetzuren die reeds in het influent aanwezig waren en de rest wordt gevormd via hydrolyse van gesuspendeerd materiaal. Bij langere verblijftijden wordt geen significante toename van de

specifieke gasproduktie waargenomen. Bij verhoging van de temperatuur tot 35°C wordt alleen bij een verblijftijd van 15 dagen een geringe maar significante toename van de gasproduktie waargenomen. Bij procestemperaturen van 30-35°C neemt de specifieke gasproduktie (m^3/m^3 mest) toe met de verblijftijd in de range van 10 tot 20 dagen. De hydrolyse vormt dan de snelheidsbeperkende stap. De 'opgelost-niet-vetzuur-CZV' fractie van koemest is inert voor anaërobe vergisting. Onder geen der onderzochte procescondities wordt enige reduktie van het niet vetzuur-opgelost-CZV waargenomen.

Vergisting in een VGD-systeem bij temperaturen ≥ 30°C is zeer goed bestand tegen plotselinge verhogingen van de belasting als gevolg van verkorten van de verblijftijd. Het in eenmaal verkorten van de verblijftijd van 15, 20, 25 of 30 dagen tot 10 dagen resulteert in een direkte verhoging van de gasproduktie en slechts een geringe toename van de effluentvetzuurconcentratie. Op deze wijze kan binnen enkele dagen de volumetrische gasproduktie (m^3/m^3.dag) meer dan worden verdubbeld. In een praktijksituatie kan aldus de methaanproduktie worden aangepast aan de energievraag op het bedrijf.

In Hoofdstuk 5 worden de resultaten van vergisting van koemest en varkensmest in AC-systemen beschreven. Het verloop van de vergisting is afhankelijk van de procestemperatuur, de opvultijd, het percentage entmateriaal en de samenstelling en concentratie van de mest.

De resultaten van de experimenten met koemest geven inzicht in de stabiliteit van het proces gedurende meerdere opeenvolgende opvulperiodes bij temperaturen van 15 en 20°C.
Met koemest (influent CZV= 95-113 g/l) kan bij 15°C, in een AC-systeem met 15% enting binnen de opvultijd van 100 dagen, geen 'steady state', d.w.z. een periode met een continue dagelijkse gasproduktie, worden bereikt. Een extra periode van 40-50 dagen vergisting is nodig voor de volledige omzetting van de geaccumuleerde vetzuren. Het verloop van de vergisting gedurende de opeenvolgende opvulperiodes is vrijwel identiek. Bij verandering van de samenstelling van de mest (95 → 113 g CZV/l; 1.9 → 3.5 g NH_4^+-N/l) neemt de noodzakelijk vergistingstijd (voor de volledige afbraak van vetzuren) enigszins toe, de gasproduktiesnelheden blijven echter vrijwel gelijk.

De AC-vergisting van koemest (95 g CZV/l) bij een procestemperatuur van 20°C is uitgevoerd bij entingen van 1, 7 en 13% en opvulperiodes van 100 dagen. Zelfs bij de laagst toegepaste enting kunnen de geaccumuleerde vetzuren vrijwel volledig worden omgezet. Bij toepassing van 7 en 13 % enting wordt tevens een aanzienlijke periode van 'steady state' bereikt.

Gezien de betere praktijk perspectieven voor de toepassing van AC-systemen voor vergisting van varkensmest in vergelijking met koemest is AC-vergisting van varkensmest uitgebreider onderzocht.

Bij toepassing van vergiste koemest (15°C-AC-slib) als entmateriaal voor de vergisting van varkensmest (74 g CZV/l; 3.7 g NH_4^+-N/l) bij 15°C, worden bij gelijke procescondities aanzienlijk hogere gasproduktiesnelheden gevonden dan bij de vergisting van koemest (113 g CZV/l; 3.5 g NH_4^+-N/l). Anderzijds kunnen ook bij de vergisting van varkensmest de geaccumuleerde vetzuren niet binnen de opvultijd worden verwijderd en is een nágistingsperiode van ± 50 dagen nodig. Theoretisch is een minimale enting van 25% noodzakelijk, teneinde geaccumuleerde vetzuren, binnen een opvultijd van 100 dagen, te kunnen verwijderen. Bij opvultijden van 270 dagen kan worden volstaan met slechts ±10% enting. Bij een mestproduktie van 1 m^3/dag moet dan een totaal reactor volume van resp. 133 en 300 m^3 worden geïnstalleerd.

De AC-vergisting van meer geconcentreerde varkensmest (118 g CZV/l; 5.9 g NH_4^+-N/l) bij 20% enting en 100 dagen opvultijd, bij 15°C, resulteert in accumulatie van propionzuur. Zelfs na lange gistingsperiodes (100+180 dagen nágisting) wordt geen propionzuurafbraak waargenomen. De hoge NH_4^+-N concentratie (5.9 g/l) van de mest is hiervan waarschijnlijk de oorzaak.

Verhoging van de procestemperatuur van 15 tot 30°C, bij een zelfde opvultijd, resulteert in een stijging van zowel de volumetrische ($m^3/m^3.d$) als de specifieke gasproduktie. De hydrolyse- en de methaanproduktiesnelheid nemen beiden toe met stijging van de temperatuur van 15 tot 30°C.

In tegenstelling tot een procestemperatuur van 15°C, worden bij de hogere procestemperaturen (20 en 30°C) nauwelijks verschillen gevonden in gasproduktie en vetzuurverloop, bij verlaging van de mestconcentratie van 118 tot 74 g CZV/l. De geaccumuleerde vetzuren zijn bij 20°C na resp. 140 en 120 dagen omgezet tot methaangas. Bij 30°C zijn geaccumuleerde vetzuren reeds na resp. 50 en 40 dagen verwijderd en treedt er vervolgens een 'steady state' periode op. De specifieke groeisnelheid van de methanogene bacteriën bij 20°C en 30°C met verdunde mest, verschilt niet van die met geconcentreerde mest.

Uit de resultaten van het uitgevoerde onderzoek blijkt, dat intensieve menging in AC-systemen met een hoge initiële belasting, (b.v. 15% enting, 100 dagen opvultijd en bij 15°C) een sterk negatief effect heeft op de afbraak van propionzuur en, zij het in mindere mate, op die van azijnzuur. In AC-systemen met een hogere aanvangs-activiteit (b.v. bij 10% enting, 100 dagen opvultijd en 20 en 30°C) wordt nauwelijks of geen negatief effect van intensieve menging waargenomen. Geconcludeerd kan worden dat intensieve menging nadelig is. Alleen wanneer dat voor de bedrijfsvoering nodig is, b.v. bij het verwijderen van vergiste mest of t.b.v. de warmteoverdracht in verwarmde systemen, is het zinvol enige (niet intensieve) menging toe te passen.

8.4. TOXICITEIT

In Hoofdstuk 6 worden de resultaten besproken van het onderzoek naar het effect van het organische stof (OS) gehalte en de NH_4^+-N concentratie op de VGD-vergisting van koemest, bij 30°C en een verblijftijd van 10 dagen. Het organische stof gehalte van de mest heeft geen effect op het vergistingsproces in de range van 2-7 %. De NH_4^+-N concentratie heeft daarentegen een zeer sterk nadelig effect, zowel op de hydrolyse als op de methaanvorming. De effluentvetzuurconcentratie neemt exponentieel toe en de hydrolyse lineair af met de NH_4^+-N concentratie in de range van 1.2 tot 4.9 g/l. De relatie tussen het % hydrolyse (H_R) en de NH_4^+-N concentratie kan empirisch worden beschreven met de vergelijking:

$$H_R = 22.8 - 4.16\ [NH4^+\text{-}N].$$

Bij vergisting van mest met een hoge NH_4^+-N concentratie (4.5 g/l) kunnen bij een betrekkelijk lage verblijftijd twee verschillende 'evenwichtseffluentvetzuurconcentraties' optreden. De laagste concentratie wordt bereikt door een tijdelijke verlaging van de belasting. Soortgelijke resultaten werden gevonden bij de opstart van vergistingssystemen bij lagere temperaturen zoals beschreven in Hoofdstuk 2 en moeten waarschijnlijk worden toegeschreven aan de remmende werking van vetzuren. In tegenstelling tot VGD-systemen wordt in AC-systemen bij 20 en 30°C geen duidelijk negatief effect van NH_4^+-N geconstateerd.

8.5. TOEPASSINGSMOGELIJKHEDEN VAN AC- EN VGD-SYSTEMEN IN DE PRAKTIJK.

De vergisting van mest in VGD-systemen t.b.v. energieproduktie op boerderijschaal wordt in Nederland toegepast sinds 1979. In de periode van 1979-1982 zijn ±25 installaties in bedrijf genomen op varkens- en melkveehouderijbedrijven. De ontwikkeling van de energieprijzen is de belangrijkste factor die er toe heeft geleid, dat na 1982 geen nieuwe biogasinstallaties op de boerderij zijn geïnstalleerd.

De rentabiliteit van biogasinstallaties werd behalve door de dalende aardgasprijzen daarnaast nadelig beïnvloed doordat, met name op varkensbedrijven, veelal aanzienlijk lagere gasproduktie gevonden werden dan op grond van voorafgaand laboratorium en semi-technisch onderzoek mocht worden verwacht. De oorzaak hiervan is het optreden van 'voor'-vergisting van de

mest tijdens de opslag onder de roosters. In feite heeft iedere boer reeds de beschikking over een vergister, zij het dat deze niet is geoptimaliseerd en het gas niet wordt opgevangen en gebruikt. Deze constatering vormde een belangrijke aanleiding om de mogelijkheden van een combinatie van vergisting en opslag verder te onderzoeken.
De toepassing van een AC-systeem, voor gecombineerde opslag en vergisting van mest is bovendien in principe een veel geschikter systeem voor mestvergisting op boerderijschaal dan een VGD-systeem, omdat geen extra reactor en/of effluentopslag behoeft te worden gebouwd. Huidige ontwikkelingen t.a.v. de mestopslag, nl. de vereiste verlenging van de mestopslagperiode en de eis om de mestopslagtank gasdicht af te dekken teneinde NH_3-emissie naar de omgeving tijdens opslag te voorkomen, maken bovendien dat een mestopslag steeds meer op een mestvergister gaat lijken.
De resultaten beschreven in dit proefschrift laten duidelijk zien dat verlenging van de opslagtijd een belangrijke toename van de gasproduktie tot gevolg kan hebben, zelfs bij lage temperaturen (15°C). Aangezien bekend is dat CH_4 een aanzienlijk belangrijker bijdrage aan het broeikaseffect levert dan CO_2 (Goossensen en Meeuwissen, 1990), zal de biogasproduktie tijdens de opslag van mest óf moeten worden voorkomen, danwel moeten worden geoptimaliseerd t.b.v. een nuttig gebruik, opdat bespaard wordt op het gebruik van fossiele brandstof. Aldus wordt een nuttige bijdrage geleverd aan de bestrijding van het broeikaseffect.

Overigens blijkt uit de verkregen resultaten ook, dat het bijzonder moeilijk is de methaanvorming tegen te gaan. Wanneer zich eenmaal een geschikte bacteriepopulatie heeft ontwikkeld komt de methanogenese onherroepelijk opgang, tenzij men zou overgaan tot het gebruik van remmende stoffen. Dit laatste is uit milieuhygiënische overwegingen niet verantwoord. Bij een mesttemperatuur van 20°C, zoals in de zomer kan optreden, is 1% entmateriaal reeds voldoende om een aktieve gisting binnen de opslagperiode op gang te brengen.
Aangezien uit de praktijk bekend is dat het vrijwel onmogelijk is mestopslagen volledig te legen, ongeveer 10-15% van de mest blijft in het algemeen achter, kan op basis van de verkregen resultaten worden geconcludeerd dat bij verlenging van de opslagperiode tot 5 maanden of langer ook bij temperaturen van 15°C aanzienlijke gasprodukties zullen optreden. Op het proefstation voor de varkenshouderij (PV) te Rosmalen is een 700 m^3 mestopslagsilo geïsoleerd, gasdicht afgedekt en voorzien van een eenvoudig verwarmingssysteem. Gedurende twee jaar is onderzoek uitgevoerd naar de combinatie van vergisting en opslag van mest bij temperaturen van ± 18°C. Uit de resultaten blijkt, dat als mestsilo's gasdicht moeten worden afgedekt combinatie van vergisting en opslag van mest zeker een aantrekkelijk alternatief wordt.

Toepassing van een AC-systeem is in principe geschikt wanneer langdurige opslag noodzakelijk is, zoals bij gebruik van organische mest in de landbouw. In de huidige situatie in Nederland, waar landelijk gezien te veel organische mest wordt geproduceerd, zal een aanzienlijk gedeelte van de geproduceerde mest moeten worden verwerkt. Bij een dergelijke procesmatige verwerking van mest zou moeten worden gestreefd naar een maximale terugwinning en hergebruik van waardevolle produkten en een minimaal verbruik van energie. Als onderdeel van een dergelijk systeem kan anaërobe vergisting een belangrijk deel van de vaste- en opgeloste organische stof uit de mest verwijderen en omzetten in methaangas. Het geproduceerde CH_4-gas kan worden gebruikt als energie voor de overige verwerkingsstappen. Het ontbreken van de noodzaak van langdurige opslag bij de grootschalige verwerking van mest maakt toepassing van een AC-systeem in dat geval minder geschikt dan toepassing van een VGD-systeem.

Bij de anaërobe vergisting kan slechts een deel van de organische componenten worden omgezet in CH_4-gas. Verdergaande verwijdering van vaste stof kan worden verkregen door toepassing van mechanisch afscheiding ná of mogelijk vóór de vergisting. Mechanische scheiding in een vloeibare en vaste fractie vóór de vergisting biedt als mogelijk voordeel dat beide fracties afzonderlijk kunnen worden vergist, de 'vloeibare fractie' in een hoog belaste reactor, b.v. een UASB en de 'vaste fractie' in een laag belaste reactor. Op deze wijze kan het totaal benodigde vergistingsvolume aanzienlijk worden gereduceerd.

De vloeibare fractie bevat het grootste gedeelte van de stikstof terwijl de vaste fractie het grootste gedeelte van de fosfor bevat. De stikstof in de vloeibare fractie is aanwezig als

NH_4^+-N. Een principieel zeer aantrekkelijk systeem voor de verwijdering en de terugwinning van NH_4^+-N (en fosfaat) uit de vloeibare fase is het gecombineerde strip/absorbtie proces. Een schema van dit proces is weergegeven in Figuur 1 (v. Velsen, 1985).

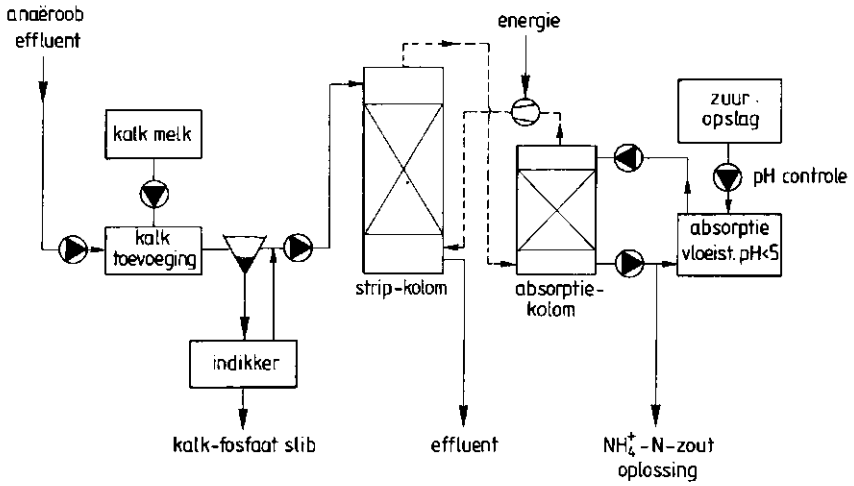

Figure 1. Schema van het strip/absorptie proces voor de terugwinning van NH_4^+-N uit anaëroob vergiste mest (v. Velsen, 1985).

Het is moeilijk te begrijpen waarom dit systeem of gemodificeerde systemen hiervan (Drese, 1988) nog steeds niet op grote schaal zijn uitgetest. Uit oogpunt van doelmatig gebruik van grondstoffen is het noodzakelijk de grote hoeveelheden stikstof uit de mest terug te winnen en her te gebruiken en niet zoals soms wordt voorgesteld (Koster, 1990) volledig om te zetten in stikstofgas d.m.v. nitrificatie/denitrificatie, temeer omdat hiervoor een grote hoeveelheid hoogwaardige energie nodig is. Volledige terugwinning van NH_4^+-N zal niet altijd economisch verantwoord zijn. Een combinatie van het strip/absorptie met het nitrificatie-/denitrificatie proces zou op z'n minst moeten worden overwogen.

8.6. REFERENTIES

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LIST OF ABBREVIATIONS

AC-system	=accumulation system.
COD	=chemical oxygen demand
CZV	=chemisch zuurstof verbruik
CSTR-system	=completely mixed tank reactor system
d	=days
DT(t)	=detention time
e (%)	=percentage inoculation of the effective reactor volume (V_e) in an AC-system
FID	=flame ionization detector
ft (days)	=filling time
GC	=gas chromatograph
GLC	=gas liquid chromatograph
HRT	=hydrolic rentention time
ID	=inner diameter
OS	=organische stof
Q (m^3/day)	=slurry flow rate
t (days)	=digestion time
TCD	=thermal conductivity detector
TS	=total solids
V_d (m^3)	=digestion volume, which is that part of the reactor which is actually used for the digestion process.
V_e (m^3)	=effective reactor volume, which is the volume which can be used for digestion
V_f (m^3)	=final digestion volume in an accumulation system equal to V_e-e.V_e and reached at t=ft
VFA	=volatile fatty acids
VGD-systeem	=volledig gemengd doorstroom systeem
VS	=volatile solids
UASB	=upflow anaerobic sludge blanket

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