THERMOPHILIC ANAEROBIC DIGESTION FOR WASTE AND WASTEWATER TREATMENT

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W M Wiegant

THERMOPHILIC ANAEROBIC DIGESTION FOR WASTE AND WASTEWATER TREATMENT

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr C C Oosterlee,
in het openbaar te verdedigen
op vrijdag 28 februari 1986
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen.

6. Dat de specifieke groeisnelheid van een methanogene ko-kultuur afhankelijk is van de methaanbakterie, is net zo juist als de stelling dat die groeisnelheid van de acetaat-vormende bakterie afhankelijk is.

   Archer D B & G E Powell (1985). Dependence of the specific growth rate of methanogenic mutualistic cocultures on the methanogen. Arch Microbiol 141: 133-137.

7. Alles te willen onderzoeken wat een gek maar wil betalen is een vorm van intellektuele prostitutie.

8. Het ontbreken van de Siberische Boompieper *Anthus hodgsoni* op de Nederlandse avifaunistische lijst wordt veeleer veroorzaakt door het lage aantal gekwalificeerde vogelkenners in Nederland dan door de vogel zelf.

   IJzendoorn E J & P de Heer (1985). Herziening van de Nederlandse Avifaunistische Lijst. Limosa 58: 65-72.

9. Het hoge aantal waarnemingen van het Klein Waterhoen *Porzana parva* in de Ooypolders kan worden toegeschreven aan een gebrekkige kennis van de geluiden van de Waterral *Rallus aquaticus*.

   Vogelwerkgroep Rijk van Nijmegen en omstreken (1985) Vogels van de Ooypolders. O M van Hoorn, Nijmegen.

10. Dat de sluiting van homo-bars zou leiden tot het terugdringen van de ziekte AIDS is ongeveer net zo illusoir als het idee dat men het totale geslachtsverkeer in een regio zou kunnen beinvloeden door de verkoop van tweepersoonsbedden aan banden te leggen.

    NRC-Handelsblad, 8 nov. 1985.

11. Het feit dat de ambtenaar zich behoort "te onthouden van het bezigen van vloeken en van ruwe of onzedelijke taal" maakt het formuleren van een lijst van voor ambtenaren verboden woorden gewenst: de snelle evolutie van het Nederlandse spraakgebruik geeft daar reden toe. Als voorbeeld kan de inburgering van het woord "lullig" dienen.

    Algemeen Rijksambtenaren Reglement, officieuze uitgave, Staatsuitgeverij, Den Haag, 1971.

W M Wiegant
Thermophilic anaerobic digestion for waste and wastewater treatment
Wageningen, 28 februari 1986.

STELLINGEN

1. Voor het verkrijgen van een zo hoog mogelijke volumetrische aktiviteit in UASB reaktoren die verzuurde afvalwaters behandelen, is het van belang dat gedurende de eerste opstart een zeer lage koncentratie van acetaat in het effluent wordt nagestreefd.

   Dit proefschrift.

2. Het verdient aanbeveling om studies naar *interspecies hydrogen transfer* uit te voeren met thermofiele kultures, omdat de waterstof-koncentratie bij de afbraak van acetogene substraten onder thermofiele kondities veel hoger is dan onder mesofiele kondities.

   Dit proefschrift.

3. De geschatte evenwichtswaarde van de waterstofspanning in het gas, vrijkomend bij gekombineerde acetaat-oxidatie en methaanproduktie bij 60 °C, is tenminste een faktor 5 te laag.

   Zinder S H & M Koch (1984). Non-aceticlastic methanogenesis from acetate: acetate oxidation by a thermophilic syntrophic coculture. Arch Microbiol 138: 263-272.

4. Een niet eerder gekonstateerd voordeel van een tweetrapssysteem voor de anaerobe behandeling van niet verzuurde afvalwaters boven een eentrapssysteem is de aanzienlijk lagere slibproduktie van de acidogene bakteriepopulatie als gevolg van de lagere pH, die in de verzuringsreaktor wordt gehandhaafd. Voorwaarde is wel dat dan een hoge celverblijftijd in de verzuringsreaktor wordt gehanteerd.

   Dit proefschrift.

5. De stelling dat de toevoeging van korrelslib de adaptatie-periode van slijkgistingsslib verkort bij het opstarten van UASB reaktoren, wordt niet gestaafd door de uitkomsten van de verrichte experimenten.

   Zeeuw W J de (1984). Acclimatization of anaerobic sludge for UASB-reactor start-up. Proefschrift, Landbouwhogeschool, Wageningen.

Dankwoord

Bij het verrichten van het onderzoek dat uiteindelijk tot dit proefschrift heeft geleid, heb ik zeer veel steun ondervonden van de studenten die in het kader van hun studie meewerkten aan het onderzoek. Zij zijn het, die in de allereerste plaats bedankt dienen te worden.

In de tweede plaats dank ik Gatze Lettinga, die het onderzoek in alle fasen begeleidde. Hij voorzag me van diskussies, kritiek, onenigheden en ontelbare korrekties, maar meestal was de samenwerking prettig en uiteindelijk hielp hij me altijd vooruit.

In de derde plaats zou ik Grietje Zeeman en Marianne Koster willen bedanken voor de prettige samenwerking tijdens onze gezamenlijke onderzoeksinspanningen.

Ook wil ik Bert Lijklema danken voor zijn bereidheid als promotor op te treden.

Verder zou ik iedereen willen bedanken, die aan de totstandkoming van dit proefschrift hebben meegewerkt, met name de technische dienst, de fotografische afdeling en de tekenkamer van het Biotechnion en het technisch personeel van de vakgroep Waterzuivering.

Een speciaal woord van dank gaat uit naar Eveline en Michael Kimman, Math Geurts en Jim Field, die bereid waren het manuskript van tekstuele en typografische ongerechtigheden te ontdoen. Met name Eveline Kimman verdient voor haar inspanningen de hoogste lof.

Abstract

This thesis deals with thermophilic anaerobic waste and wastewater treatment. A literature survey is presented, in which the thermophilic treatment processes are evaluated with respect to the loading rates and treatment efficiencies, and some relevant theoretical considerations concerning thermophilic anaerobic processes are discussed.

Thermophilic anaerobic treatment of livestock wastes with a high total ammonia concentration cannot be recommended, due to the toxic effect of the ammonia. The volatile solids concentration turns out to be of minor importance in determining the efficiency of the thermophilic digestion of livestock wastes. The toxic effect of ammonia is exerted on the level of methanogenesis from H_2/CO_2, resulting in a buildup of the partial pressure of H_2, which inhibits propionate degradation. The latter compound is shown to be toxic for the methanogenesis from acetate.

The major part of this thesis deals with the processes in thermophilic upflow anaerobic sludge blanket (UASB) reactors. Solutions of sugars can be treated effectively in UASB reactors operated at 55 °C. With the granular sludge cultivated on sugars, other wastewaters can be treated effectively, with loading rates up to 103 kg COD/m^3 and treatment efficiencies exceeding 77 %. Vinasse, a high strength wastewater, could be treated also at high loading rates, but the efficiencies were rather low, due to the high concentrations of toxic compounds in the vinasse. The treatment efficiency appeared to be determined by the concentration of the vinasse applied, rather than by the loading rate, which was in the range of 17-86 kg COD/m^{3}d.

The decrease in the treatment efficiency at very high loading rates is mainly caused by a deterioration of the propionate degradation. The hydrogen concentration plays a very important role in the conversion of propionate. A two-step methanogenic UASB system was developed, in which the propionate degradation was delegated to the second step. The two-step system operated with appreciably higher efficiency than a one-step system with a similar total volume: at a loading rate of 52 kg COD/m^{3}d, the treatment efficiencies were 92 and 82 % for the two and one stage system, respectively. As with mesophilic sludge, the unfed storage of thermophilic sludge at low temperatures results in a very slow decrease in its capacities. Food shortages at the operating temperature of 55 °C, however, result in a rapid decay of the sludge.

The granulation of methanogenic sludge was studied with acetate as substrate. Granulation occurred only after approximately three months of operation when using mesophilic seed materials. This process could be speeded up by the use of adapted seed materials. The addition of inert particles to the seed material or the nature of the un-adapted seed materials did not have any influence on the ultimate granulation. By using different criteria for the operation of UASB reactors, different granules could be cultivated. Granules consisting of filamentous methanogenic bacteria are to be preferred above those consisting of sarcina-type methanogenic bacteria. With the granular sludge consisting of filamentous bacteria, loading rates of 162 kg COD/m^{3}d could be treated with over 89 % efficiency.

The thermophilic UASB process is ready for application in practice.

Contents

1. Introduction

At higher temperatures, reaction rates increase. This is also true for biological reactions, which, however, are limited by the upper temperature that the organism performing the reaction can resist. For most organisms this upper temperature lies around 45 °C. For some eukaryotes the temperature maximum is 62 °C (Brock 1978). Adaptation to even higher temperatures has long been shown to exist in prokaryotes. One may assume that bacterial life is possible in water of any temperature, as long as it doesn't boil (Baross & Deming 1983).

Thermophilic organisms - here to be defined as organisms exerting optimum growth at temperatures exceeding 45 °C - may be useful in the bacteriological processes used industrially: fermentations, the microbiological manufacturing of specialized products, as well as biological wastewater treatment processes. To date, only the research on thermophilic wastewater treatment has a history of more than five decades. The research performed in this field is mainly focused on anaerobic waste and wastewater treatment.

Anaerobic wastewater treatment methods have some very attractive advantages: a low energy input, an energy-rich end product, methane, and a low production of sludge. Besides the still relatively polluted effluent stream, the main disadvantage is the extremely slow growth of the anaerobic bacteria involved in the production of methane. Low growth rates of bacteria require either huge reactor volumes, methods to increase the bacterial concentrations, or ways to increase this low growth rate. Principally, thermophilic anaerobic digestion may be regarded as a method for the optimization of anaerobic treatment by means of an increase of the growth rates of the bacteria performing the rate limiting reactions. With respect to the thermophilic anaerobic wastewater treatment in completely mixed reactors, a considerable amount of research has already been carried out. This will be dealt with in more detail in chapter 2.

When using methods to increase the retention of the bacterial mass to reach biomass levels far beyond the steady state levels in completely mixed reactors, even low strength wastewaters can be treated anaerobically. The increased retention of the biomass may be realized either by attachment of the

Table I. Comparison of reactor types for anaerobic wastewater treatment in the mesophilic temperature range (slightly modified from de Zeeuw 1984).

REACTOR TYPE	METHOD OF SLUDGE RETENTION	ACHIEVABLE[a] LOADING RATES (kg COD/m^3d)
conventional (completely stirred)	none	*c* 1
anaerobic contact process	separate settling tank with sludge return	*c* 5
anaerobic filter	fixed film on filter particles and sludge retention in filter interstices	10-15
upflow anaerobic sludge blanket	granulation of bacterial biomass and an internal settling compartment	20-50
(partially) fluidized bed processes	fixed film on fluidized particles	20-50

a: treating wastewaters with a moderate strength (5-10 kg COD/m^3) at removal efficiencies of more than 80 %.

bacteria to some support material, by gravity settling, or by a combination of both. In recent decades a number of systems, which are summarized in Table I, has been developed. Of these systems, the upflow anaerobic sludge blanket (UASB) reactor will be explained in more detail. The UASB process is characterized by the absence of externally supplied support material, the absence of mixing devices, and an internal settling compartment (Fig. 1). The UASB reactor is used in an upflow mode. Bacterial aggregates with a higher settling velocity than the upflow velocity of the wastewater will be retained in the reactor. The UASB process is described in full detail by Lettinga *et al* (1980). The success of the UASB concept in the treatment of various wastewaters (Lettinga *et al* 1984) can be found in its feature to form and maintain a highly settleable and active granular type of sludge, consisting largely of viable biomass. Very high biomass concentrations can be maintained with such a granular sludge.

As support material essentially is lacking in UASB reactors, the void volume available for sludge retention will be the highest of all high rate reactor types developed so far. So it may contain the highest biomass concentrations.

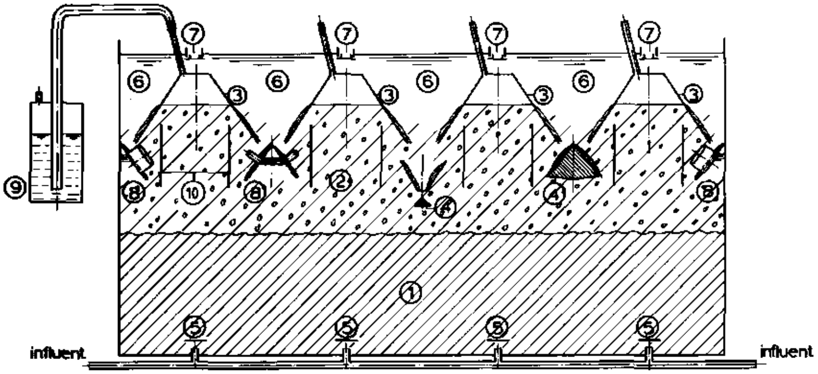

Fig. 1. Diagram of a full-scale UASB plant with vertical baffles installed beneath the gas collector. 1: sludge bed; 2: bulk of the liquid with dispersed sludge forming a "blanket"; 3: gas bowl; 4: gas seal; 5: feed-inlet distribution system; 6: settler; 7: effluent launder; 8: gas collector with exhaust pipe to (3); 9: water seal (Lettinga *et al* 1984).

The performance of UASB reactors may be improved when operated in the thermophilic temperature range. In this case a profitable use is sought of the high maintenance energy of thermophilic bacteria (Sonnleitner 1983), because generally the bacterial growth rate in UASB reactors is not very high.

The main objective of this study was to investigate the feasibility of thermophilic anaerobic digestion for the generation of methane from wastes and wastewaters, as originally formulated in a three-years project granted by the European Community. It mainly comprised research on the start-up and operation of the thermophilic UASB process, and research to improve the understanding of the process. Also, the thermophilic anaerobic digestion of cow manures was studied, in close cooperation with Grietje Zeeman, who made a comprehensive study of psychrophilic, mesophilic and thermophilic digestion of dairy cow slurry.

The organization of this thesis is as follows. In chapter 2 a literature review on thermophilic anaerobic digestion is presented. In chapter 3 the thermophilic digestion of cow manure is described, followed by a more detailed investigation on the mechanism of the observed ammonia inhibition in chapter 4. Chapters 5 to 9 deal with the thermophilic UASB process. In chapter 5 the results of thermophilic anaerobic digestion of solutions of sugars is reported. Thermophilic treatment may become attractive for vinasses, the effluents of alcohol distilleries. Considerable attention was paid to the applicability of the process to this type of waste. Its digestion was studied at mesophilic and thermophilic temperatures in completely mixed systems, and its thermophilic treatment in UASB reactors was investigated. The results are presented in chapter 6.

Propionate accounts for up to 15 % of the COD of an acidified wastewater (Gujer & Zehnder 1983). It was found that considerable difficulties may be involved in the degradation of this compound, even more than under mesophilic conditions. Therefore a separate study was made of the thermophilic treatment of wastewaters containing high concentrations of this compound. In chapter 7 the investigations on thermophilic propionate degradation are described. A modified reactor set-up is tested for improving the propionate degradation under thermophilic conditions.

The possibility to store thermophilic sludge under unfed conditions, and the behaviour of thermophilic sludge under sub-optimal feeding conditions and temperatures is described in chapter 8.

In this study much attention was paid to granulation phenomena. The sludges, used for the investigations presented in chapters 5 to 8, all were cultivated on sugars. The granulation with an acidified wastewater was investigated with the use of acetate, the main methanogenic substrate. A survey of these investigations is given in chapter 9.

In chapter 10 the summary and the conclusions of the investigations are presented.

Part of the investigations presented in this thesis has been published already, as posters (Zeeman *et al* 1983), as a conference paper (Wiegant *et al* 1983) and as yearly reports for the contractors meetings of those working in the group "Solar Energy Research and Development in the European Community" (Wiegant & Lettinga 1981,1982,1983a), and as a report for the project mentioned before (Wiegant & Lettinga 1983b). In this thesis this material has been corrected, extended, completed and reorganized. Chapters 3 to 8 have been published already in appropriate journals, or have been accepted for publication. Chapter 9 has been submitted to such a journal.

This thesis may be regarded as the definitive version of the report on the project mentioned before.

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2. The potentials of thermophilic waste and wastewater treatment

W M Wiegant

Literature review

INTRODUCTION

At higher temperatures, reaction rates tend to become higher. This also applies for biological reactions, but as there are upper limits to the temperatures at which biological reactions occur, reaction rates will not ever increase. The upper temperature for biological reactions to take place seems to be determined by the boiling point of water. There is evidence for biological activity, and growth, at temperatures up to 250 °C, at high pressures (Baross *et al* 1982, Baross & Deming 1983). Trying to make use of the increased reaction rates at increased temperatures is the principal aim of the cultivation of thermophilic organisms.

Thermophilic organisms perform optimally at temperatures exceeding 45 °C. For anaerobic wastewater treatment processes it is not necessary to differentiate between facultative and obligate thermophiles (see Sonnleitner 1983). For the purpose of wastewater treatment, thermophily may best be defined by "performing net growth at temperatures exceeding 45 °C". In this way the distinction between mesophilic and thermophilic anaerobic wastewater treatment processes coincides more or less with the temperature ranges for net growth of the bacteria the most critical for anaerobic processes, namely, the acetate utilizing methanogens (Mah *et al* 1978, Zinder & Mah 1979, Mah 1980, Huser *et al* 1982, Zinder *et al* 1984a,1984b).

Apart from the reaction rates, various physical properties of the environment of the organisms will change at increasing temperatures. They include the viscosity, which will become lower at increasing temperatures, as will the solubility of gases (Wilhelm *et al* 1977). The dissociation constant for many compounds, for instance, volatile fatty acids, hydrogen sulfide and ammonia, (*cf* Fig. 2), changes considerably with increasing temperatures. These physicochemical properties will, of course, influence the performance of the bacteria involved in wastewater treatment.

Thermophilic anaerobic waste and wastewater treatment processes, and the bacteria involved in it, have repeatedly been reviewed previously (Cooney & Wise 1975, Buhr & Andrews 1977, Sonnleitner 1983, Varel 1983). In this review the attention will be focussed mainly on the retention times applied and the methane generation rates and removal efficiencies achieved in the thermophilic anaerobic treatment processes, and the bacteria performing the rate limiting steps in it. In these respects, thermophilic digestion processes will be compared with mesophilic processes.

EARLY INVESTIGATIONS

As early as 1875, methane evolution from sewage solids was studied at temperatures of 40-55 °C (Popoff 1875). It was concluded that fermentation was not possible at temperatures exceeding 45-50 °C and that the optimal temperature was 40 °C. It was not until 50 years later that Coolhaas (1928) demonstrated that thermophilic methane generating bacteria - defined as exerting growth at 55 °C - truly existed: fatty acid salts and carbohydrates were converted to methane and carbon dioxide at 45-60 °C. Rudolfs and Heukelekian (1930) were the first to recognize the possible advantage of the thermophilic anaerobic digestion of sewage solids. After experiments with different - even thermophilic - inocula they concluded that thermophilic digestion of sewage solids gives a considerable reduction in the digestion time needed, but that the economics, however, would depend on several factors and local conditions. Some years later, thermophilic digestion already received considerable attention. These studies mainly concerned experiments in which sewage solids were digested batch-wise and they were focused on the optimal temperature for thermophilic digestion, and the distinction between the temperature ranges of mesophilic and thermophilic digestion (Heukelekian 1930,1933, Fair & Moore 1932,1934,1937). They found a clear-cut minimum activity, or a maximum in the time needed to achieve 90 % of the ultimate biogas yield, which is also found by others (Malina 1964), at 43 to 47 °C. Apparently this temperature range is the upper limit for mesophiles or the lower limit for thermophiles, or both. This will be discussed later.

The decomposition of pure substrates was also investigated by Tarvin & Buswell (1934) at various temperatures including 55 °C. From their results a higher decomposition rate in the thermophilic temperature range was inferred (Buswell & Hatfield 1939). The ultimate biogas yield from sewage sludge was concluded to be independent of the temperature in the range 25-57 °C (Fair & Moore 1932, Viehl 1941).

For practical purposes, batch experiments have only a limited use. Heukelekian (1931) conducted semi-continuously fed digestion experiments, leading to the conclusion that the retention time can be reduced significantly when adopting thermophilic digestion. Rudolfs & Miles (1935) did not find significant differences between 30 and 55 °C, but they "did not obtain a true thermophilic digestion". Continuous and semi-continuous digestion was also carried out by Tarvin & Buswell (1934). From their experiments with fatty acid salts and carbohydrates it was suggested that the thermophilic decomposition rate was higher than the mesophilic (Buswell & Hatfield 1939).

COMPLETELY MIXED SYSTEMS

Sludge digestion

The experimental results gathered up to 1940 apparently led to further exploration: from 1942 to 1944 the first full-scale tests were carried out. Fischer & Greene (1945) noted a higher volatile solids reduction at 12-13 days retention time at 54, as compared to 32 °C. Later investigations led to similar conclusions: when digesting sewage sludge or activated sludge thermophilically, either a higher volatile solids reduction at equal retention times (the differences becoming smaller with increasing retention times), or lower retention times with equal volatile solids destructions are achieved. Obviously, these are both manifestations of the same bacterial kinetic relationship (Lawrence & McCarty 1970, Chen & Hashimoto 1980). Some of the investigations comparing thermophilic with mesophilic sludge digestion are summarized in Table I. It may be concluded that the reduction in retention time is the most important feature of thermophilic sludge digestion. This is confirmed by the results of Kandler *et al* (1980), who investigated retention times down to three days, at 30, 56 and 60 °C.

Table I. Comparisons of mesophilic and thermophilic digestion of sewage and activated sludge.

mesophilic					thermophilic					
temp °C	RT days	load $\frac{kg\ VS}{m^3 d}$	destr[a] %	biogas $\frac{m^3}{kg\ VS}$[c]	temp °C	RT days	load $\frac{kg\ VS}{m^3 d}$	destr[a] %	biogas $\frac{m^3}{kg\ VS}$[c]	ref[b]
32.6	18	2.4	50	0.43	51.2	9	3.6	50	0.39	1
32	12.5	0.45	50.5	1.26	54	12.9	0.45	56.4	1.07	2
29	27	0.53	38	1.00	52	27	0.53	44.2	1.08	2
29.4	43.5	1.4	56.9	1.14	48.9	35.3	1.2	55.6	1.12	3
29.4	12.5	3.9	48.6	1.09	48.9	12.5	3.8	54.4	1.00	3
35	30	1.5	52.8	1.12	55	30	1.5	49.9	1.18	4
32.5	12	1.6	46.3	1.05	52.5	12	1.6	48.8	0.91	5
32.5	12	3.2	47.1	0.98	52.5	12	3.2	49.9	0.84	5
32.5	12	4.8	42.2	0.94	52.5	12	4.8	45.8	0.80	5
32.5	6	1.6	39.1	0.95	52.5	6	1.6	42.3	0.86	5
32.5	6	3.2	40.7	0.95	52.5	6	3.2	44.1	0.81	5
32.5	6	4.8	33.2	0.85	52.5	6	4.8	35.9	0.71	5
36.1	15.8	2.4	nd[e]	0.41[d]	52.2	15.3	4.6	nd[e]	0.33[d]	6
35.6	20	1.6	68	1.06	48.9	20	1.6	65	1.19	7
34.4	17.0	2.1	31.3	1.00	52.7	11.3	3.2	34.0	1.20	8

a: volatile solids destruction; b: references: 1, Popova & Bolotina 1963; 2, Fischer & Greene 1945; 3, Garber 1954; 4, Golueke 1958; 5, Malina 1961; 6, Pohland & Bloodgood 1963; 7, Garber 1977,1982, Garber *et al* 1975; 8, Rimkus *et al* 1982; c: values refer to VS destroyed, except for d: VS added; e: not determined.

To date thermophilic sludge digestion is adopted for two reasons. It may be adopted in places where the land prices are exceedingly high, thus making a shift into the thermophilic temperature region favourable compared with extension of the existing mesophilic installation (Popova & Bolotina 1963, Rimkus *et al* 1982). It may also be adopted to lower the costs of waste sludge disposal (Garber *et al* 1975). For this reason it was included in a multiple digestion system, consisting of a mesophilic sludge digestion and a thermophilic secondary step in the sludge digestion (Torpey *et al* 1984).

Thermophilic digestion has some very important additional features. Pathogen reduction is very effective in the thermophilic temperature range (Garber 1982, Temper *et al* 1981, Torpey *et al* 1984). In a thermophilic aerobic digester at 45 to 60 °C, the number of pathogenic bacteria in the effluent was one to three orders of magnitude lower than in the effluent of an anaerobic digester at 35 °C (Kabrick & Jewell 1982). Effluents after digestion at 50 and 36 °C

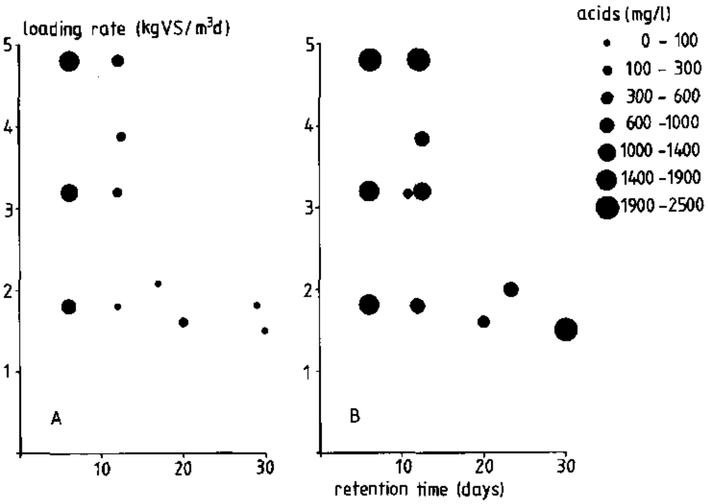

Fig. 1. Volatile fatty acid concentrations (as acetic) in the effluents of sludge digesters as a function of the loading rate and the retention time for mesophilic (32.5-35.6 °C)(A) and thermophilic (48.9-55 °C)(B) temperatures. The size of the dots refer to the acid concentrations. Data were obtained from refs. 3,4,5,7, and 8 as in Table I.

differ two orders of magnitude in their content of pathogenic bacteria (Torpey *et al* 1984). The reduction of parasites is also much better at higher temperatures, and viral inactivation is essentially complete at temperatures exceeding 40 °C (Kabrick & Jewell 1982). Another important advantage of thermophilic sludge digestion is the improved dewatering characteristics of the digested sludge, due to the larger particle size (Garber 1954,1977,1982, Garber *et al* 1975, Rimkus *et al* 1982). On the other hand, the drainability of the sludge was experienced to be somewhat inferior in thermophilic sludge as compared to mesophilic sludge (Rudolfs & Miles 1935).

Summarizing full-scale as well as laboratory experience, it can be concluded that thermophilic sludge digestion permits a reduction of the retention time of 40-60 % at equal volatile solids reductions, compared to mesophilic digestion. Additional advantages are the better kill-off of pathogens and the improved solids dewatering. The drawbacks are lying in the costs of heating and insulation. Another drawback is the higher concentration of volatile fatty acids in the effluent of thermophilic digesters (Fig. 1), leading to a somewhat more odorous sludge (Rimkus *et al* 1982).

The higher concentrations of volatile fatty acids in the thermophilic effluents have generated the opinion that thermophilic digestion is a highly unstable process. Indeed, thermophilic digestion is apparently disturbed more easily than mesophilic digestion (Pohland & Bloodgood 1963). On the other hand, Rimkus *et al* (1982) conclude that thermophilic operation "did not require any greater knowledge or skills by the operating personnel than that required for the mesophilic process".

The effects of temperature drops in thermophilic digesters were investigated by Heukelekian & Kaplovski (1948) and Speece & Kem (1970). They concluded that no lasting detrimental effects are obtained when the temperature is restored at the original level after a temperature drop. The reaction of the digestion on a temperature drop is very fast, in the order of minutes (Speece & Kem 1970). Garber *et al* (1975) state that a temperature drop of only 1.7 °C causes upset in a full-scale digester operated at 49 °C. Contrary to Garber, Rimkus *et al* (1982) conclude that changes in the temperature of 3 °C, as experienced in a full-scale digester in a 24-hour period, do not exert any adverse effects.

The clear-cut minimum in activity in the temperature range of 43-47 °C, as found in batch experiments, is not found in semi-continuously fed digestion experiments at 42.5 °C (Malina 1961) or 45 °C (Golueke 1958), even at retention times down to six days. This also applies for the digestion of manure (Varel *et al* 1980) and domestic refuse (Pfeffer 1974). Apparently it is difficult to promote an actively digesting bacterial population in this temperature range, but once established, its activity will fit into a continuous increase in activity over the temperature range of 10-55 °C.

Livestock wastes

Livestock waste digestion essentially is not different from that of sludges, with respect to the bacterial processes and kinetics. The differences between the wastes, however, justify a separate review. A comparison between the composition of the volatile solids of sewage sludge and livestock wastes is presented in Table II. Despite the wide scatter in the values given for each constituent, it is clear from the Table that in both wastes the biodegradability is rather low and that the hydrolysis of macromolecular compounds will be the rate limiting step in the methane digestion. There is, however, a large difference in ionic strength. Particularly the total ammonia concentration is much higher in livestock wastes. This implies a higher buffer capacity

Table II. Characterization of the dry solids of sewage sludge (Kotzé *et al* 1969) and of pig and beef cattle manure (Hobson *et al* 1974, Shuler 1980). Values are given as the percentage of the dry solids.

	sewage solids	manure
volatile solids	60-80	74-84
ether soluble	6-44	3-11
cellulose	3-22	30
hemicellulose	3.2	26
lignin	5.8	9.4-10.1
protein	19-28	12-30
amino acids	1.3	4.7-15
free carbohydrates	0.3	nd[a]

a: nd, not determined

of the digestion, but also a much greater risk of ammonia inhibition. In mesophilic digestion, the total ammonia concentration can be toxic at levels above 1.7 kg N/m^3 (McCarty & McKinney 1961), but adaptation to higher levels can occur (van Velsen 1979, Parkin & Speece 1982). The lower level of dissociation at thermophilic temperatures, as illustrated in Fig. 2, is a factor of great importance, since the free ammonia is considered to be much more toxic than ionic NH_4^+ (McCarty & McKinney 1961).

In recent years a lively interest in the thermophilic digestion of livestock wastes has developed. It was preceded by investigations into the thermophilic digestion of night soil, which is comparable with livestock wastes. From batch experiments, night soil digestion was considered recommendable only when cheap surplus heat is available (Iwai *et al* 1962). The optimum temperature proved to

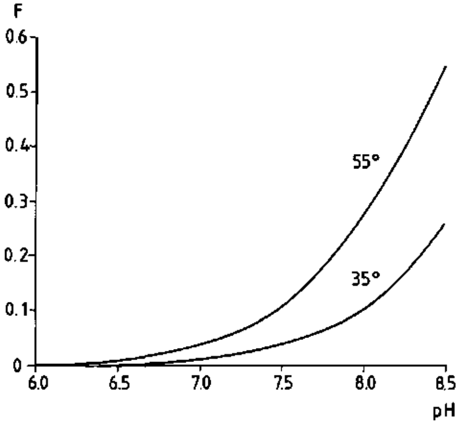

Fig. 2. The fraction F of the total ammonia (NH_3 + NH_4^+) concentration which is in the unionized form, as a function of the pH for 35 and 55 °C. Data were obtained from Weast (1976).

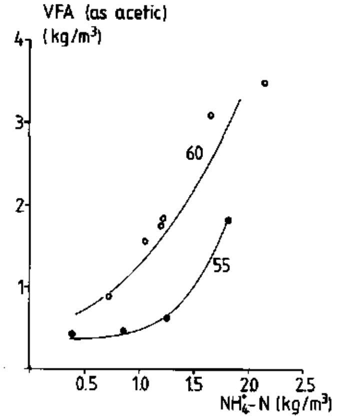

Fig. 3. Effluent VFA concentrations (as acetic) as a function of the total ammonia concentration in the thermophilic digestion of manures and mixtures of manures and molasses, at 6-6.2 days retention time and at 55 (●) and 60 (o) °C. The data presented were obtained from Varel *et al* 1977, Converse *et al* 1977, Hashimoto *et al* 1978, and Hashimoto 1981.

be 33 °C after semi-continuously fed digestion experiments (Matsumoto & Endo 1964), probably because of the high total ammonia concentrations in night soil (Iwai *et al* 1962), man being the only carnivorous livestock.

Many investigations into the digestion of livestock wastes at thermophilic temperatures have been published since 1977. Comparative investigations over a wide range of temperatures have been carried out by Varel *et al* (1980). The Some relevant parameters of the thermophilic digestion of wastes of beef cattle, dairy cows, pigs, and swine are presented in Table III. The digestion seems to be influenced strongly by the total ammonia concentration, as reflected in the concentrations of volatile fatty acids in the effluents (Fig. 3, Varel *et al* 1980). From this Figure, 60 °C seems less effective than 55 °C . The VS concentration in the manure is also considered to be of importance for predicting digester performance. This will be dealt with in another section.

Very high volumetric loading rates can be attained at retention times of 3-5 days with low ammonia wastes; the maximum methane production rates are 6 m^3/m^3d for beef cattle waste at 55 °C and 4 days retention time (Hashimoto 1982) and *c* 3.5 m^3/m^3d for dairy cow and pig wastes (Shelef *et al* 1980, Hashimoto 1983, Mathisen *et al* 1983). For chicken wastes the production rates are considerably lower, even although an unintentional pretreatment method - autoclaving - was applied (Shih & Huang 1980). It should be noted that in the thermophilic temperature range one should not expect a more complete conversion of livestock wastes than in the mesophilic range (Hashimoto *et al* 1981).

Although there is a considerable kinetic advantage in digesting beef and pig wastes at thermophilic temperatures and low retention times (Chen *et al* 1980, Chen 1983), which does not seem to apply for dairy cattle waste (Hill 1983), its feasibility still has to be demonstrated on a scale, larger than in the laboratory. Economic feasibility may be achieved by application of the hygienically reliable effluents. Feeding of effluents to livestock can take place (Hashimoto *et al* 1978, Marchaim *et al* 1981), and the effluents can be used as soil fertilizer (*cf* Dzekshenaliev *et al* 1984). Reviews on effluent utilization are given by Marchaim (1983) and Hashimoto *et al* (1983).

A variety of wastes can be added to livestock wastes in the digestion process. Firstly the C/N ratio can be increased in this way, and secondly there can be a significant increase in the gas production. The reports on thermophilic digestion include addition of straw (Shelef *et al* 1980, Hashimoto 1983a), corn stover (Fujita *et al* 1980), cabbage (Matsumoto & Endo 1964), cotton plants (Shelef *et al* 1980) and molasses (Hashimoto 1981). In general, additions do not alter the features of the digestion process.

Additions to the feed of animals may alter the methane digestion of the wastes: for instance, some dietary antibiotics significantly lower the methane production. Monensin may even lead to reactor failure at 55 °C, due to accumulation of acids, whereas chlortetracyclin was shown to lead to a 20 % lower methane production rate without accumulation of acids (Varel & Hashimoto 1981). Monensin was concluded to have a detrimental effect, but adaptation was considered possible.

Nutrient deficiencies can hardly be expected in the digestion of livestock wastes. Yet, addition of cobalt was shown to lead to higher gas production rates at low retention times (Shelef *et al* 1980).

Other wastes

One can hardly expect thermophilic digestion of domestic refuse or shredded newsprint to differ much of that of sewage sludge. Still, a remarkably large difference in the methane production per unit volatile solids fed was experienced at high retention times: at 65 °C, domestic refuse produced 76 % more

Table III. Relevant parameters of thermophilic digestion of livestock wastes.

temp °C	RT days	loading kg VS/m^3d	concn kg VS/m^3	VS-red[a] %	biogas m^3/kg VS	methane m^3/m^3d	biogas m^3/m^3d	methane %	pH -	NH_3/NH_4^+ kg N/m^3	VFA[b] kg/m^3	stock	ref[c]
55	18	3.76	67.6	68.3	0.55	1.13	2.08	54.2	7.6	1.40	0.59	beef	1
55	9	7.51	67.6	68.0	0.53	2.10	4.00	52.5	7.7	1.28	0.35		
55	6	11.27	67.6	65.0	0.54	3.00	5.99	50.1	7.8	1.24	0.63		
65	6	6.67	40	38.9	0.35	1.24	2.34	52.9	7.2			beef	2
60	6	6.67	40	43.6	0.40	1.51	2.66	56.7	7.3				
55	6	6.67	40	44.6	0.38	1.45	2.52	57.5	7.1				
60	9	13.00	117	48.5	0.33	2.29	4.35	52.6	5.4	2.11	4.23	beef	2
60	6	21.67	117	40.8	0.29	3.32	6.29	52.8	7.6	2.14	3.49		
60	3	39.00	117	7.4		-		-	7.6		9.80		
60	9	10.79	97.1	49.2	0.42	2.38	4.48	53.1	7.4	1.71	2.39		
60	6	16.18	97.1	37.9	0.32	2.67	5.14	51.9	7.6	1.65	3.09		
60	3	32.4	97.1	31.0	0.13	4.19	8.20	51.1	7.6	1.89	4.86		
60	9	8.67	78	47.4	0.43	2.12	3.73	56.9	7.5	1.41	1.49		
60	6	13.0	78	47.6	0.40	2.94	5.16	57.0	7.5	1.19	1.75		
60	3	26.0	78	35.8	0.31	4.15	7.98	52.0	7.5	0.97	1.90		
60	9	6.56	59	50.0	0.42	1.54	2.75	56.1	7.3	0.91	0.68		
60	6	9.83	59	44.7	0.41	2.25	4.01	56.1	7.3	1.04	1.57		
60	3	19.67	59		0.33	3.46	6.41	54.0	7.4	0.77	1.52		
55	20	3.43	68.5	44.2	0.38	0.75	1.21	58	8.2	1.97	1.95	beef	3
55	12	5.15	61.8	52.8	0.56	1.59	2.89	55	7.9	1.89	1.15		
55	6	11.45	68.7	46.1	0.43	2.73	4.96	53	7.9	1.82	1.82		
55	4	14.88	59.5	39.8	0.42	3.14	6.28	50	7.9	1.90	2.55		
55	4	25	100			6.11						beef	4
55	5	20	100			5.07							
60	15	3.6	54	35	0.36	0.72	1.31	55				cattle	5
60	10	7.6	76	19	0.25	0.91	1.90	48					
60	7	8.5	60	18	0.16	0.67	1.40	48					
60	7	7.5	53	11	0.17	0.62	1.27	49					

Table III (continued)

temp °C	RT days	loading kg VS/m^{3}d	concn kg VS/m^3	VS-red %	biogas m^3/kg VS	methane m^3/m^{3}d	biogas m^3/m^{3}d	methane %	pH -	NH_3/NH_4^+ kg N/m^3	VFA kg/m^3	stock	ref
60	15	4.23	63.5	40.8	0.27	0.56	1.14	49.2	7.1			dairy	6
60	15	44.28	64.2	33.5	0.25	0.53	1.06	50.2	7.3				
60	6.2	9.9	61.4	26.3	0.21	1.07	2.11	50.6	7.5	0.71	0.87		
60	6.2	10.51	65.1	28.9	0.25	1.41	2.59	54.5	7.5	1.21	1.83		
60	6	6.77	40.6	37		1.13				1.18	0.60	dairy	7
60	3	13.53	40.6	35		2.30				1.30	0.68		
55	6		120^{d}		0.23^{d}		5.5					dairy	8
55	10		120^{d}		0.23^{d}		2.8						
55	8		120^{d}		0.23^{d}		3.5						
55	6		120^{d}		0.21^{d}		4.3						
55	10		150^{d}		0.26^{d}		3.9						
55	8		150^{d}		0.23^{d}		4.25						
55	25	2.55	62.5	51.4	0.10	0.26	0.62	41.7	7.6	2.94	8.74	pig	9
55	15	3.36	50.4	56.9	0.43	1.45	2.38	61.0	8.0	2.06	1.31		
55	10	5.04	50.4	61.9	0.36	1.80	2.94	61.3	7.8	1.86	0.97		
55	5	10.08	50.4	50.2	0.31	3.12	5.11	61.1	7.7	1.77	1.54		
55	20	2.77	55.1		0.42	0.68	1.16	58.8	7.8	2.42	4.97	pig	10
55	30	1.7	50		0.23			63	7.9	1.5	3.02		
55	30	1.7	50		0.05			39	7.7	3.0	9.86		
55	7	6.9-8.4					2.6-3.0			1.3-2.1		pig	11
55	5	9.6-11.8					3.1-3.9			1.3-2.1			
55	3	19.2-19.9					4.5-6.0			1.0-1.3			

a: VS-reduction

b: *Volatile fatty acids as acetic*

c: references: 1, Hashimoto 1981; 2, Varel *et al* 1977; 3, Hashimoto *et al* 1978; 4, Hashimoto 1982; 5, Thyselius 1981; 6, Converse *et al* 1977; 7, Rorick *et al* 1980; 8, Shelef *et al* 1980; 9, Hashimoto 1983b; 10, van Velsen *et al* 1979; 11, Mathisen *et al* 1983.

d: (based upon) total solids.

biogas than at 37 °C (Cooney & Wise 1975), and at 60 °C 59 % more than at 35 °C (Pfeffer 1974), both at a retention time of 30 days. Low solids removals were obtained in the digestion at 55 °C of shredded newsprint, which had an optimum volumetric gas production at 5 days retention time (Cooney & Ackerman 1975). Apparently the hydrolysis of newsprint or refuse, which contains much paper, is only partially completed, even at high retention times. These huge differences, however, were not found in the digestion of cellulosic waste, comparable with the domestic refuse as above. At a 12-day retention time and at 25 to 60 °C, 35 °C was found to be the optimum (Ghosh *et al* 1977). Thermophilic digestion of various crops was not always beneficial as compared with mesophilic digestion. For instance, kelp digestion proved disadvantageous (Ghosh *et al* 1980), as did the digestion of grass (Dhavises *et al* 1985), in the thermophilic temperature range.

Slaughterhouse wastes, which seem well comparable with livestock wastes, could be treated at 9 days retention time at 55 °C with the same results as at 18 days and at 30 °C (Maurer & Pollack 1983).

Little research has been performed on the thermophilic digestion of industrial wastewaters in completely mixed systems. Effluents of alcohol distilleries have been investigated repeatedly. From batch experiments thermophilic digestion of these wastewaters was concluded to have no benefits (Sen & Bhaskaran 1962), which was confirmed in continuous experiments for beet molasses distillery waste (Basu & Leclerc 1975). On the other hand, Ono (1965) reported that thermophilic full-scale installations could accommodate loading rates of 6 kg VS/m^{3}d, as opposed to only 2.5 kg VS/m^{3}d under mesophilic conditions. Sulfate is considered to be toxic at levels of 5 kg/m^3 when applied for longer than one week. In the thermophilic digestion of distillery waste the continuous addition of 10 % (v/v) night soil was shown to improve the digestion strongly (Ono 1965). In laboratory experiments a maximum methane generation rate of 3.2-3.8 m^3/m^{3}d was achieved at a 2.9-day retention time.

Effluents of a butanol-acetone fermentation plant could be successfully treated in a 250 m^3 pilot plant at a 2.4-day retention time at 56-57 °C, but unfortunately, no further details of this process are given (Babayants *et al* 1971). Brune *et al* (1982) reported that experiments with a cellulose factory wastewater, which contained 6-24 kg/m^3 of acetic acid, 0.5-3.0 kg/m^3 of furfural and 0.64-1.28 kg/m^3 of sulfur (sulfite and sulfate), could be digested successfully at both 37 and 60 °C, at a retention time of 14 days, with over 90 % conversion of COD into methane. By applying solids recycle, retention times down to three days could be obtained.

Cheese whey could be digested at 55 °C with moderate success, but presumably the rather poor results can be attributed to the fact that the digestion was carried out with emphasis on the research on automatic control, rather than on the digestion process itself (Follman & Märkl 1979, Märkl 1981).

Thermophilic digestion at 44-52 °C is reported to be feasible for the treatment of palm oil mill effluents. Methane production rates of 1.5 m^3/m^3d were obtained in full-scale digesters of 3700 m^3, at a 10-day retention time and loading rates of 2.8-4.2 kg VS/m^3d (Quah & Gillies 1981). In a pilot plant study 95 % BOD reduction was achieved at 52 °C and a mean BOD load of 2.78 kg/m^3d, with a maximum of approximately 3.6 kg/m^3d. This figure compares favourably with the results of mesophilic digestion, where the effluent BOD was much higher than in the thermophilic experiments, at loading rates of 2.0 kg BOD/m^3d (Bidin & Raj 1982). Very effective thermophilic treatment of palm oil mill effluents is reported by Chin & Wong (1983). With a 67,000 mg COD/l influent, removals of 72 % at a retention time of 5 days, and over 90 % at retention times of 15 days and higher were obtained.

HIGH RATE TREATMENT SYSTEMS

Anaerobic digestion as a treatment process for low-strength wastes and wastewaters has become increasingly interesting after the introduction of systems with a high biomass retention. This high biomass retention is a prerequisite when one wants to impose liquid retention times considerably shorter than the residence time of the bacteria. The more biomass retained in a reactor treating a given wastewater, the higher the bacterial retention time will be under normal conditions (Lawrence & McCarty 1970), and as a consequence of this, the better the performance of the system will be.

In recent years a number of systems with a high biomass retention time has been developed for the treatment of wastes with low concentrations of suspended solids. Of these, the anaerobic filter (Young & McCarty 1969), the downflow stationary fixed film reactor (van den Berg & Lentz 1979), the anaerobic attached film expanded bed (Switzenbaum & Jewell 1980) and the fluidized bed (Jeris *et al* 1974, Jeris 1983) use support material, either to prevent biomass washout or to give the bacteria the opportunity to attach. The anaerobic contact process (Dague *et al* 1970) and the upflow anaerobic sludge blanket process (Lettinga *et al* 1980) don't make use of externally supplied support material. All these processes have their advantages and disadvantages, which have repeatedly been discussed (see McCarty 1981).

As yet, little experience exists with thermophilic digestion in these high rate systems, although it is now rapidly increasing. Anaerobic filters proved to be very efficient in the treatment of distillery effluents. Organic loading rates of 38 kg VS/m^3d could be handled with a COD-treatment efficiency of 40-50 % (Braun & Huss 1982). However, at the working temperature of 42 °C used in this study, it is questionable whether thermophilic organisms will prevail. In downflow stationary fixed film (DSFF) reactors, there seems to be little, if any, advantage in treating bean blanching waste at 55 °C as compared with 35 °C. The maximum methane generation rates were similar at 35 and 55 °C, 4.7 m^3 CH_4(STP)/m^3d, when red draintile clay was used as support material. The biofilm in the thermophilic reactor was more evenly distributed over the reactor than in the mesophilic reactor (Kennedy & van den Berg 1982). With the same wastewater the resistance of this type of reactor against overloading was tested. Thermophilic DSFF reactors became unstable already at 30 to 40 kg COD/m^3d, whether organically or hydraulically overloaded. Mesophilic DSFF reactors could handle shock loads up to 90 kg COD/m^3d. It was suggested that in thermophilic reactors the methanogenic segment of the bacterial population was less "film associated" than in mesophilic reactors (Duff & Kennedy 1982).

Upflow fixed film reactors treating stillage of wood hydrolysate, with a COD of 22.5 kg/m^3, performed similarly at 55 and 35 °C, with COD reductions of 84.4 and 86.6 % at organic loading rates of 10.7 and 10.0 kg COD/m^3d, respectively. The reactors could handle 4.2-4.5 times the loading rate of a completely mixed reactor with a 9.5-day retention time with similar treatment efficiencies (Good *et al* 1982).

AAFEB process

More extensive research has been carried out into the thermophilic anaerobic attached film expanded bed (AAFEB) process. This process, a kind of hybrid between the upflow filter and the fluidized bed, was used for the digestion of sucrose containing solutions at 55 °C. Loading rates of 40 kg COD/m^3d were treated with a soluble COD removal of over 70 % (Schraa & Jewell 1984). With 90 % of the biomass attached to the support particles, no such biomass wash-out or inactivation occurred at a loading rate of 150 kg COD/m^{3}d, as observed for the DSFF process (Duff & Kennedy 1982). The high buffer capacity of the substrate, 4.9 Mole $NaHCO_3$ per Mole of sucrose, may account for this (Schraa & Jewell 1984). The activity of the biomass in the thermophilic AAFEB process ranged from 0.3-1.5 kg $COD_{converted}$ /kg VS.d in the reactor, depending on the biomass retention time (Fig. 4, Schraa 1983). These activities compare favourably with activities of 0.43-0.49 kg $COD_{converted}$/kg VS.d reported for an upflow anaerobic sludge blanket reactor treating glucose solutions at 30 °C (Cohen *et al* 1980). The maximum methanogenic activity of 0.67 kg CH_4-COD/kg VSS.d compares also favourably with a maximum of 0.5 kg CH_4-COD/kg VSS.d in a UASB reactor fed with sucrose and VFA (5 % of the influent COD) at 30 °C (Hulshoff Pol *et al* 1984). This activity may be somewhat higher than that for a pure sucrose solution, as the addition of low percentages of VFA to sugar solutions leads to a strong increase in the maximum methanogenic activity (Cohen 1982).

The sludge from the thermophilic AAFEB process, grown on sucrose, had maximum activities (55 °C) for acetate, propionate and butyrate of 0.19, 0.03 and 0.21 kg COD/kg VS.d, respectively. The sludge was quite resistant against a three-day decrease in the temperature from 55 to 25 °C, during which the feed supply

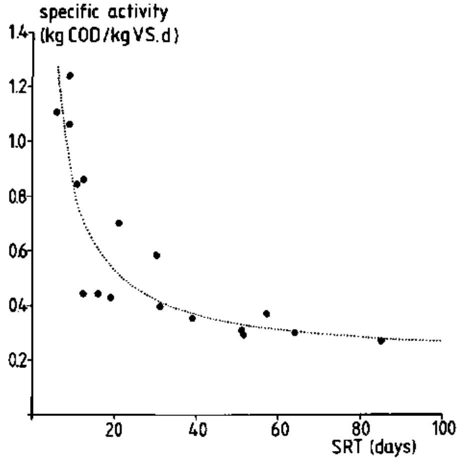

Fig. 4. The specific activity (kg $COD_{converted}$/kg VS.d) as a function of the solids retention time for the biomass in the thermophilic AAFEB process during the digestion of sucrose solutions. Data were obtained from Schraa 1983. The dashed line represents $A = (\Theta_c^{-1} + b)/Y_o$, with Y_o = 0.152 kg VS/kg COD and $b = 0.031\ d^{-1}$. See p.29 for further explanation.

was continued at 12-18 kg COD/m^{3}d. The original activity was restored in one day, after the temperature was brought to 55 °C. An increase of the temperature to 65 °C led to a severe deterioration of the process with only 6 % COD removal after 20 days of operation at 65 °C. The original activity was not restored after 19 days of operation at 55 °C (Schraa 1983).

MICROBIOLOGY OF THERMOPHILIC DIGESTION

Recently, a review on the bacteriology of thermophilic digestion was presented by Varel (1983). From this review, it is clear that of the bacteria involved in the hydrolysis step, only cellulose degrading bacteria are isolated and described as yet. Bacteria degrading other carbohydrates are investigated less extensively, whereas no references are available on bacteria degrading non-carbohydrate macromolecular compounds under thermophilic conditions.

Acidogenesis

As in mesophilic digestion, the degradation of monomeric carbohydrates, amino acids and other small compounds is virtually never rate-limiting in thermophilic digestion processes. For this reason thermophilic acid formation is only sparsely investigated. Glucose acidification was shown to have two distinct temperature optima, one at 36-38 °C, where a minimum retention time of 2.0 hrs could be employed in a completely mixed reactor, and one at 51-53 °C, where a minimum retention time of 1.4 hrs could be employed (Zoetemeyer *et al* 1982). The product distribution appears to be a strong function of the temperature, but as no hydrogen was removed, the results are not indicative for methanogenic systems (Zoetemeyer *et al* 1982). A preliminary study on the fermentative bacteria from a thermophilic digester showed a low species diversity. In the influent slurry, fresh cow manure, high numbers of thermophilic bacteria were found (over 10^6/g VS), which may explain the rapid digester start-up in the thermophilic temperature range (Varel 1984). This observation is corroborated by M Chen (1983), who showed that 9 % of the bacteria in a 35 °C-digester is capable of growth at 50 °C, whereas for 60 °C this value is only 1 %. This may explain the difficulties experienced in setting up a digestion at temperatures exceeding 60 °C (*cf* Cooney & Wise 1975).

Generally, fermentative bacteria have no difficulties in keeping up with the retention times applied in methane digestion. So, from the engineering point of view there is no urge to evaluate this bacterial group more closely.

Acetogenesis

This definitely is not true for the acetogenic bacteria. These bacteria convert volatile fatty acids and alcohols into acetate, hydrogen and carbon dioxide. The evolved hydrogen has to be removed, because it negatively affects the energy that can be derived from the conversion (see also Chapter 7). In stable methane digestion systems the bulk of the hydrogen is removed via consumption by methane bacteria, but also sulfate reducers may be responsible for the consumption of hydrogen. This process is generally referred to as interspecies hydrogen transfer (Ianotti *et al* 1973). Some bacteria of this group were isolated in coculture and have been described, e.g. those degrading ethanol (Bryant *et al* 1967), short-chain fatty acids from butyric up to larger (McInerney *et al* 1981, Stieb & Schink 1985), and propionate (Boone & Bryant 1981). Recently, a thermophilic syntrophic butyrate oxidizing bacterium was isolated in coculture with *Methanobacterium thermoautotrophicum* (Henson & Smith 1985). It appears to have a rather low specific growth rate, like the propionate oxidizing thermophilic enrichment, described by Zinder *et al* (1984a). Only few kinetic data are available: zero order kinetics can be applied for the degradation of propionate and butyrate by sucrose-grown sludge in an AAFEB reactor (Schraa 1983), which indicates that the substrate half-saturation constants for these compounds must be low (below *c* 20 mg/l). This was also observed under mesophilic conditions at temperatures exceeding 25 °C (Lawrence & McCarty 1969). No data are available on the specific growth rates of syntrophic acetogens, except for a specific growth rate of 0.0072 hr^{-1} presented for a propionate converting bacterium at 58 °C (Zinder *et al* 1984a). This value, however, is probably too low, as propionate degradation was demonstrated at a three days biomass retention time (Varel *et al* 1980), corresponding with a μ_{max} of at least 0.0097 hr^{-1}.

The conversion of CO_2 and H_2 into acetate has been demonstrated to play a distinct, but limited, role in mesophilic semi-continuously fed digesters (Balch *et al* 1977, Boone 1982). Under thermophilic conditions this conversion also occurs (Wiegel *et al* 1981). Under continuous feeding conditions the bacteria performing this reaction will be fully outcompeted, as the affinity of hydrogen utilizing methanogens for their substrate is much higher than that of hydrogen consuming acetogens (Wiegel *et al* 1981).

The alleged methane bacterium *Methanobacillus kuzneceovii*, which was reported to have an optimum temperature of 52-57 °C, consists of a consortium of bacteria, as it forms methane from acetate, and acetate and methane from methanol, formaldehyde, formate and carbonate. The photograph presented, and the observation of spores (Pantskhava & Pchelkina 1968,1969, Pantskhava 1969) suggest an association of organisms resembling *Methanobacterium thermoautotrophicum* (Zeikus & Wolfe 1972) and *Clostridium thermoautotrophicum* (Wiegel *et al* 1981). The methanogen in this association is stated to differ slightly in ultrastructure from *Methanobacterium thermoautotrophicum* (Zhilina *et al* 1983).

Recently, the reverse of autotrophic acetogenesis, namely, formation of H_2 and CO_2 from acetate, was found to occur under thermophilic conditions. The oxidation of acetate is concomitantly performed with methanogenesis, to provide interspecies hydrogen transfer. The acetate oxidizer has a specific growth rate of 0.017-0.023 hr^{-1} (Zinder & Koch 1984). It seems unlikely that this acetate oxidation plays a significant role in methane digestion systems, because the hydrogen pressure has to be extremely low to make the oxidation thermodynamically feasible, for instance, about three times as low as for propionate oxidation (Zinder & Koch 1984). Acetate conversion via interspecies hydrogen transfer would definitely lead to acetate concentrations higher than propionate concentrations in the effluents of digesters. This normally is not observed.

Methanogenesis

Half a century ago, Coolhaas (1927) already reported about an enrichment culture forming methane from calcium acetate as the sole carbon source at 60 °C. The one photograph available shows bacteria that definitely do not belong to the genus *Methanosarcina*.

Presently, a rapidly increasing number of thermophilic methanogens have been isolated or obtained in enrichment cultures reaching purity. The best known species is *Methanobacterium thermoautotrophicum* (Zeikus & Wolfe 1972). It has been and is being used in numerous studies on the biochemistry of methanogenesis. This organism is an extreme thermophile, growing at temperatures from 40 to 75 °C. Its temperature optimum lies around 65 °C, where it exhibits a theoretical maximum specific growth rate of 0.69 hr^{-1}, at extrapolated H_2 and CO_2 concentrations of 100 % each (Schönheit *et al* 1980). The substrate

Table IV. Comparison of characteristics of thermophilic methanogenic bacteria. Adapted and extended from Blotevogel *et al* (1985).

species	morphology	growth substrates	temp optimum (°C)	pH optimum	μ_{max} (hr^{-1})	G + C (mol%)	ref[a]
Methanobacterium thermoautotrophicum	long rod to filaments	H_2/CO_2	65-70	7.2-7.6	0.69[b]	49.7 48.6	1 2
Methanobacterium thermoalcaliphilum	rod-shaped	H_2/CO_2	58-62	8.0-8.5	0.17	38.8	3
Methanobacterium wolfei	rod-shaped	H_2/CO_2	55-65	7.0-7.5	0.20	61	4
Methanobacterium thermoformicicum	rod-shaped	H_2/CO_2,formate	55	7.0-8.0	nd[c]	nd	5
Methanococcus thermolithotrophicus	coccoid	H_2/CO_2,formate	65	6.5-7.5	0.76	31.3	6
Methanococcus jannaschii	coccoid	H_2/CO_2	85	5.0	1.60	31	7
Methanogenium thermophilicum	coccoid	H_2/CO_2,formate	55	7.0	0.28	59	8
Methanogenium frittonii	coccoid	H_2/CO_2,formate	57	7.0-7.5	0.66	49.2	9
Methanothrix sp	rod-shaped	acetate	60	nd	0.023[d]	nd	10
Methanothrix thermoacetophila	rod-shaped	acetate	65	nd	nd	nd	11
Methanosarcina TM-1	clumps of coccoids	acetate,methanol methylamines	50	6.0	0.058[d]	nd	12
Methanosarcina CHTI 55	clumps of coccoids	acetate,methanol methylamines	57	6.8	0.085[d]	39.3	13
TAM organism	filaments	H_2/CO_2,formate acetate	60	7.3	0.010[d]	nd	14

a: references: 1, Balch *et al* 1979; 2, Brandis *et al* 1981; 3, Blotevogel *et al* 1985; 4, Winter *et al* 1984; 5, Zhilina & Ilarionov 1984; 6, Huber *et al* 1982; 7, Jones *et al* 1983; 8, Rivard & Smith 1982; 9, Harris *et al* 1984; 10, Zinder *et al* 1984ab; 11, Nozhevnikova & Chudina 1984; 12, Zinder & Mah 1979; 13, Touzel *et al* 1985; 14, Ahring & Westermann 1984,1985.
b: theoretical value (see text); c: nd, not determined; d: with acetate.

half saturation constant is in the range of 80-120 μM (Zehnder *et al* 1981, Schönheit *et al* 1980). This is appreciably higher than the 1-6.6 μM reported for mesophilic hydrogen consuming methanogens (Robinson & Tiedje 1984). The K_S compares well with the 90 μM found in a digester operated at 55 °C (Whitmore *et al* 1985). A number of hydrogen-utilizing methanogenic isolates show characteristics similar to those of *Methanobacterium thermoautotrophicum* (Marty & Bianchi 1981, Rönnow & Gunnarsson 1981, Le Ruyet *et al* 1984, Zhilina *et al* 1983, Zinder *et al* 1984b, Zinder & Koch 1984). In Table IV some characteristics of a number of thermophilic methanogens are summarized. Rönnow & Gunnarsson (1981) describe an isolate with an extreme sulfide requirement: it exhibited no growth at sulfide levels below 0.1 M. Halophilic and halotolerant hydrogen utilizing methanogens are described by Rivard & Smith (1982) and Ferguson & Mah (1983), having maximum specific growth rates of 0.28 and 0.36 hr^{-1} at their optimum growth temperature, 55 °C. Super-extreme thermophilic methanogens were obtained from superheated submarine hydrothermal vents. They exhibit very high growth rates, but their significance for thermophilic digestion will be nil, as growth does not occur below 80 °C (Baross *et al* 1982, Baross & Deming 1983, Jones *et al* 1983).

Generally, the major part of the evolving methane in digestion processes originates from the methyl moiety of acetate. This accounts for 75-86 % of the methane in thermophilic cattle waste digesters, whereas this is 72-75 % in a mesophilic digester at 40 °C (Mackie & Bryant 1981). Methane bacteria using acetate were described, presumably all belonging to the genera *Methanosarcina* and *Methanothrix*. Those belonging to the genus *Methanosarcina* have a relatively high growth rate, of up to 0.085 hr^{-1}, and a relatively low maximum temperature for growth, of 60-65 °C (Zinder & Mah 1979, Zinder *et al* 1984b, Touzel *et al* 1985). The substrate saturation constant, K_s, for *Methanosarcina* grown on acetate is 5 mM at 50 °C (Zinder & Mah 1979), but at 60 °C a K_s of 15.9 mM is presented (Brune *et al* 1982). This latter value may be an overestimation due to diffusion limitation, because *Methanosarcina* clumps can reach diameters as high as 3 mm (Brune *et al* 1982). Other bacteria are present in these granules, which may provide growth factors (Bochem *et al* 1982).

Thermophilic *Methanosarcina* are enriched quite easily. At 35 °C, they exert growth rates comparable to mesophilic strains of *Methanosarcina*. This led to the assumption that thermophilic strains can compete with obligately mesophilic ones at mesophilic temperatures (Zinder & Mah 1979). The situation for the methanogens converting hydrogen to methane is less clear in this respect (Wise *et al* 1978, Tracy & Ashare 1983). Thermophilic *Methanosarcina* strains differ from mesophilic ones since they are unable to use hydrogen for methanogenesis (Zinder & Mah 1979, Smith *et al* 1980, Touzel *et al* 1985). Thermophilic strains have maximum specific growth rates 2-4 times as high as mesophilic strains. In both temperature ranges, the growth rate on acetate is greatly improved upon addition of methanol (Zinder & Mah 1979, Krzycki *et al* 1982).

Methanosarcina were estimated to be the predominant acetate utilizing methanogens in anaerobic digesters operated at a 10-day retention time at 58 °C (Zinder *et al* 1984a) and at 60 °C (Zinder *et al* 1984b), and also in a full-scale thermophilic sludge digestion plant, operated at a 20-day retention time at 51 °C (Zinder & Mah 1979). After four months of operation the *Methanosarcina* was outcompeted by another methanogen in a digester at 58 °C and a 10-day retention time, but apparently this was not the case in the sewage digestion plant, which was operated for several years at a 20-day retention time, before the bacterial count was made (Garber 1982).

The other methanogen just mentioned, outcompeting *Methanosarcina* at long retention times, is believed to belong to the genus *Methanothrix* (Zinder *et al* 1984a,b). It is a long, rod-shaped methanogen, often growing in filaments. Contrary to the mesophilic *Methanothrix soehngenii* (Huser *et al* 1982), it contains gas vacuoles, but otherwise they greatly resemble each other (Zinder *et al* 1984a, Nozhevnikova & Yagodina 1982). The thermophilic acetate utilizing methanogen, described by Ahring & Westermann (1984,1985), presumably also is a *Methanothrix*, although this organism uses H_2/CO_2 and formate for growth. All these bacteria have a low K_s, of 0.3-0.8 mM, and relatively low specific growth rates, of 0.010-0.023 hr^{-1}. The temperature range for growth of these bacteria is 45-70 °C. One isolate has an optimum temperature of 50-55 °C (Nozhevnikova & Yagodina 1982), the other have optima at 60-65 °C. Above 60 °C the growth rate of *Methanothrix* exceeds that of *Methanosarcina* (Zinder *et al* 1984a).

The thermophilic *Methanothrix* grows much faster than the mesophilic, which has a μ_{max} of 0.005 hr^{-1} (Huser *et al* 1982) and a comparably low K_s. However, recently a new mesophilic *Methanothrix* was described, exhibiting a μ_{max} of 0.029 hr^{-1} at 40 °C (Patel 1984). This high growth rate makes it hard to believe that this bacterium plays a significant role in anaerobic treatment systems with a high biomass retention time; the biomass activity would have to be very high. This applies also for another strain of this genus (Fathepure 1983): in both cases maximum activities of *c* 11 kg CH_4-COD/kg VS.d should be expected. In Fig. 5 the growth rate for a few acetate-utilizing methanogens is given. The curves indicate that the acetate concentration where the specific growth rate of *Methanothrix* equals that of *Methanosarcina*, will be somewhat higher at thermophilic than at mesophilic temperatures. Below these acetate concentrations *Methanothrix* grows faster than *Methanosarcina*. This higher "critical"

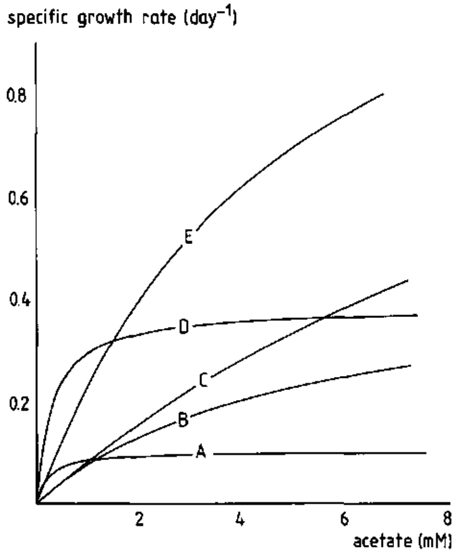

Fig. 5. Specific growth rates of acetate utilizing methanogens as a function of the acetate concentration for mesophilic *Methanothrix* (A) and *Methanosarcina* (B)(data from de Zeeuw 1984), and for thermophilic *Methanothrix* with μ_{max}= 0.38 d^{-1} and K_s= 0.3 mM (Zinder *et al* 1984a,b)(D) and thermophilic *Methanosarcina* with μ_{max}= 1.38 d^{-1} (Zinder *et al* 1984b) and K_s= 5 mM (Zinder & Mah 1979)(E), or 15.9 mM (Bochem *et al* 1982)(C).

concentration implies that selection for *Methanothrix* is more easily carried out in the thermophilic than in the mesophilic temperature range. This may have implications for the start-up of UASB reactors (see Chapter 9).

theoretical considerations

From this review it will be clear that thermophilic digestion has definite advantages over mesophilic digestion. Lower retention times are possible, to obtain equal effluent qualities and treatment efficiencies, accompanied with an effective pathogen kill-off and a readily dewaterable solid fraction of the effluent.

Low biomass retention times, however, should only be aimed at in the treatment of wastes when developing biomass cannot possibly be retained. With manures and slurries, sewage sludge and solid wastes a high biomass retention time often is impossible, so thermophilic digestion of these types of wastes offers the above advantages. Whether or not the advantages will balance the disadvantages of heating costs and higher concentrations of VFA in the effluents, will still depend on several factors and local conditions, as concluded by Rudolfs & Heukelekian in 1930.

In systems treating wastewaters with low concentrations of suspended solids, often a high biomass retention time is possible. In these systems the higher specific growth rates of the thermophilic bacteria are not being used. Still, thermophilic systems will exhibit higher activities per unit of biomass, in comparison with mesophilic systems. This can be understood from a formulation of the specific activity using the simple model of Lawrence & McCarty (1970). With

$$\frac{dX}{dt} = Y_o \frac{dF}{dt} - bX \quad (1)$$

and

$$\Theta_c = \frac{X_t}{(\Delta X/\Delta t)_t} \quad (2)$$

which in this model defines the net growth and removal of active bacteria, with X is the concentration of microorganisms (kg VSS/m^3), Y_o the growth yield coefficient (kg VSS/kg substrate), dF/dt the rate of substrate utilization (kg substrate/m^{3}d), b the decay rate (d^{-1}), and $\Delta X/\Delta t$ the total quantity of of active bacterial mass withdrawn daily from X_t, the total bacterial mass, we can define the activity per unit of biomass, the specific activity, as:

$$A = \frac{dF/dt}{X} = (\frac{dX/dt}{X} + b)/Y \quad (3)$$

and with the following substitution of the cell residence time

$$\frac{dX/dt}{X} = \Theta_c^{-1} \quad (4),$$

we get

$$A = \frac{\Theta_c^{-1} + b}{Y_o} \quad (5)$$

In Fig. 4 the specific activity is depicted for the biomass in an AAFEB reactor at 55 °C. Although neither fixed film processes, nor the UASB process can be regarded as completely mixed with respect to their biomass, Fig. 4 gives an idea of how the "decay rate" is determining the specific activity of the biomass. In thermophilic bacteria one may expect little differences in the biomass yield Y_o, but a far higher decay rate b, in comparison with mesophiles. Likewise, a higher decay rate results in a lower net sludge yield. Defining the sludge yield as:

$$Y = \frac{dX/dt}{dF/dt} \quad (6)$$

it follows logically from eq. 1 that

$$Y = Y_o - \frac{bX}{dF/dt} \quad (7).$$

Combining eqs. 7 with 3 and 5 leads to the conclusion:

$$Y = Y_o \left(\frac{\Theta_c^{-1}}{\Theta_c^{-1} + b} \right) \quad (8).$$

An extreme example of the biomass yield as a function of the cell residence time is presented in Fig. 6 for the sludge in acidification reactors at pH = 5.9 (Zoetemeyer *et al* 1981).

Theoretically, the higher decay rate under thermophilic conditions may influence the effluent quality. Applying Monod kinetics yields the following equation for the effluent substrate concentration S (Lawrence & McCarty 1970):

$$S = \frac{K_s\ (1 + b\Theta_c)}{\Theta_c(Y_o k - b) - 1} \quad (9)$$

in which k is maximum substrate utilization rate (kg substrate/kg VSS.d). With

$$\mu_{max} = \Theta_{c_{min}}^{-1} = Y_o k - b,$$

and transformation to a dimensionless form using $B = b\Theta_{c_{min}}$ and $\hat{\Theta} = \Theta_c/\Theta_{c_{min}}$ we obtain:

$$S/K_s = \frac{1 + B\hat{\Theta}}{\hat{\Theta} - 1} \quad (10)$$

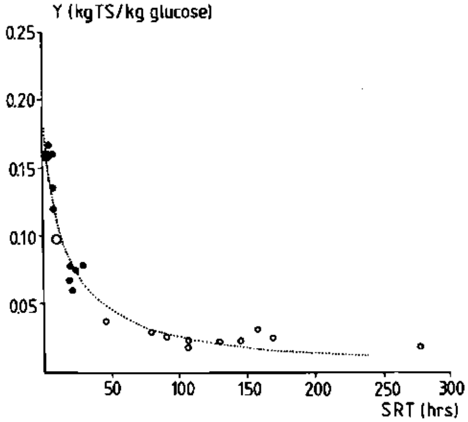

Fig 6. The net sludge yield as a function of the solids retention time for the mesophilic acidification of 10 (●) and 50 (o) kg/m³ of glucose at pH = 5.9 in UASB (small symbols) and CSTR (large symbols) reactors. Data were obtained from Zoetemeyer *et al* (1981). The dashed line represents

$$Y = Y_o\Theta_c^{-1}/(\Theta_c^{-1} + b)$$

with Y_o= 0.18 kg TS/kg glucose and b = 0.06 hr^{-1}. See text for further explanation.

in which B is the relative decay rate and $\hat{\theta}$ is the relative cell residence time. So, according to the model of Lawrence & McCarty (1970), the higher decay rate at higher temperatures will only lead to lower effluent qualities when the ratio between decay and maximum specific growth rate increases.

Summarizing these theoretical considerations, it can be concluded that in thermophilic high rate systems a higher volumetric loading rate can be applied than in mesophilic systems, provided high biomass concentrations can be retained in the reactor. The higher decay rate, that is, the higher coefficient for maintenance and decay, is the primary factor responsible for the better performance of thermophilic systems with a high biomass retention.

Finally, some remarks can be made on the impact of some physical factors on thermophilic high rate systems. In reactors without externally supplied support material, the settling of the sludge may be hindered by the higher methane production rates. On the other hand, the lower viscosity of water at 55 °C as compared with 35 °C will result in a better release of the biogas from the sludge particles, and in an increased settling rate. The diffusion of substrates and products will proceed faster in thermophilic systems, so that in systems with biofilms or large particles diffusion limitation may be expected to play a less pronounced role than in their mesophilic counterparts.

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3. The influence of the total ammonia concentration on the thermophilic digestion of cow manure

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ABSTRACT

Thermophilic digestion at 50 °C of cow manure with a very high total ammonia concentration (above 3 kg N/m^3*) was found to be very difficult. The experiments showed that the total ammonia concentration, and not the volatile solids concentration of the manure, is responsible for the poor performance of the thermophilic process. Some implications of this for the application of the kinetic model based on Contois' kinetics are discussed.*

INTRODUCTION

Thermophilic digestion of livestock wastes is receiving increasing interest. Thermophilic digestion, i.e. digestion in the temperature range of 45-65 °C, has several advantages over mesophilic digestion. First, the higher reaction rates at higher temperatures permit lower retention times to obtain the same amount of biogas from a given volume of waste. As a result, a smaller digester size will give the same biogas yield at thermophilic in comparison with mesophilic temperatures. Comparisons of mesophilic and thermophilic digestion of livestock wastes are presented by Varel *et al* (1980) and Shelef *et al* (1980). Another advantage of thermophilic digestion is the increased pathogen reduction (Garber 1982, Temper *et al* 1981) making a nutritional use of the digester effluents possible (Hashimoto *et al* 1978, Marchaim *et al* 1981).

Livestock wastes generally contain high amounts of total ammonia (NH_3 + NH_4^+), or compounds that readily release total ammonia, like urea and proteins. The total ammonia concentration strongly affects the methanogenic activity in digesters. For mesophilic digestion, a threshold value of about 1.7 kg NH_4^+-N/m^3 has been observed, above which process performance deteriorated rapidly (McCarty & McKinney 1961), but a considerable adaptation can take place (Parkin & Speece 1982). After thorough adaptation, total ammonia levels up to 5.0 kg N/m^3 will be tolerated in the mesophilic digestion of piggery manure (van Velsen 1979).

Until now, few investigations have been devoted to the influence of high total ammonia concentrations on thermophilic anaerobic digestion. Van Velsen (1979) concluded that the low methane production in the thermophilic digestion of pig slurry is due to high NH_4^+-N concentrations. A combined effect of the high VS and NH_4^+-N concentrations is supposed to cause the unstable thermophilic digestion process for pig slurry as found by Hashimoto (1983) at NH_4^+-N concentrations of 2.9 kg/m^3 and VS concentrations of 62.5 kg/m^3. Several researchers have, however, reported satisfactory results of the thermophilic digestion of cow manure, but total ammonia concentrations higher than 2.5 kg N/m^3 were not covered in these studies (Varel *et al* 1977, 1980, Shelef *et al* 1980, Hashimoto 1983).

The inhibitory effect of total ammonia in the digestion of livestock wastes is obscured by the fact that high total ammonia levels generally coincide with high concentrations of VS. Manure containing high VS concentrations has been investigated repeatedly in both the mesophilic and the thermophilic temperature range (Varel *et al* 1980, Hashimoto 1982, 1983). Some reports deal with the influence of the VS concentration on the parameter K, the product of the biomass yield factor (in kg bacterial mass formed per kg VS destroyed) and the biodegradability constant B (dimensionless) in the growth function

$$\mu = \mu_m S/(BM + S)$$

in which μ represents the specific bacterial growth rate, μ_m the maximal specific bacterial growth rate, M the biomass concentration and S the substrate concentration, as defined by Chen & Hashimoto (1980). The efficiency of the digestion is then given by:

$$E = (1 - \hat{\Theta})/(1 - \hat{\Theta} - K)$$

in which E is efficiency (dimensionless) and $\hat{\Theta}$ is the relative retention time (retention time divided by minimum retention time, dimensionless), and K is a kinetic parameter (Chen & Hashimoto 1980). This parameter K indicates the performance of the digester. It is reported to increase with increasing VS concentrations (Hashimoto *et al* 1981, Hashimoto 1982).

In the Netherlands, cow manure often contains total ammonia concentrations up to 4 kg N/m^3, more or less dependent on the feed composition and the way the manure is collected. The unclear role of ammonia in the digestion of livestock wastes prompted us to investigate the thermophilic digestion of cow manure containing high levels of total ammonia. The objective of this study was primarily to determine the feasibility of the thermophilic anaerobic digestion of cow manure with a high total ammonia content. This paper, however, deals more with the problems encountered in the thermophilic digestion of Dutch cow manures.

METHODS

Temperature

All experiments were carried out at 50 °C, unless stated otherwise.

Reactors

A semi-continuously fed digester of 120 litres volume was used in the main experiment. Feeding was carried out each day, except for the weekend when a double feed was supplied. The reactor (height 1.0 m; diameter 0.19 m) had a stirring blade which was used intermittently (30 s every 10 min). External water heating was applied by using flat hollow tubing connected with a thermostat. The outcoming biogas passed a column of soda lime pellets and a wet test gas meter. It was accounted for as (wet) methane.

For batch experiments and subsidiary experiments with semi-continuous feeding, 6-litre gas-tight digesters were used, of working volume 5.5 litres. They were equipped with a stirring blade which was used intermittently (5 s every 5 min). The digesters had double walls through which water was pumped to give the desired temperature. Batch experiments with diluted manure and additions of NH_4Cl were performed in 0.5 litre serum bottles of 0.32 litre working volume, placed in a thermostatted water bath. Methane production was measured by passing the biogas through a column of soda lime pellets and a liquid (1.5 % NaOH) displacement system.

Substrates

The manure used throughout the study originated from dairy cows from an experimental farm at Duiven, The Netherlands. The cows were fed a diet free of antibiotics, and 300-500 litre samples of the manure were stored at 4 °C before use. Important parameters of the manure are given in Table I. There was some variation in the VS content of the manure as it was impossible to obtain

Table I. The composition of the influent slurry.

Composition (kg/m^3)	Period (days)				
	0-70	150^a-224	224-284	284-306	306-354
Total COD	97	104	82.3	106	115
Dissolved COD	35	27	29.2	nd	29.2
VFA-COD	13.6	14.0	9.7	nd	10.1
NH_4^+-N	nd	1.5	3.0	3.0	3.0
Total Solids	77.5	82.5	66.2	87.2	99.5
Volatile Solids	56.2	57.6	48.6	65.7	74.6

a: day 154 is day 0 of the experiment given in Fig. 3.
nd, not determined.

enough manure to carry out all the experiments.

In batch experiments with effluent samples from semi-continuously fed digesters, with or without NH_4Cl addition and dilution, no additions were made to the dilution water, except that the pH was set at its original value. During the batch digestion, no pH corrections were made.

In a subsidiary experiment, in which a digester was fed semi-continuously (once a day) with diluted cow manure (about 2.5 % VS) and increasing additions of NH_4Cl, the following basal nutrients were added (mg/litre): NH_4Cl, 170; NaH_2PO_4. 37; $MgCl_2.4H_2O$, 9; KCl, 25; and 1 ml/litre of a trace element solution containing (mg/litre): H_3BO_3, 50; $FeCl_2.4H_2O$, 2000; $ZnCl_2$, 50; $MnCl_2.4H_2O$, 500; $CuCl_2.2H_2O$, 30; $(NH_4)_6Mo_7O_{24}.4H_2O$, 90; $CoCl_2.6H_2O$, 2000; $NiCl_2.6H_2O$, 50; $Na_2SeO_3.5H_2O$, 100; EDTA, 1000; Resazurin, 500, and 1 ml/litre 36 % HCl. A second subsidiary experiment in which acetate (9.4 kg COD/m^3) was the primary substrate, with only a small addition of manure (0.24 % VS final concentration), was performed in an identical way. These last two experiments were started up with fresh cow manure five times diluted in oxygen-free tap water, with basal nutrients as above, and incubated for 14 days. Thereafter, feeding was started at a 5.5 day retention time. The reactors were operated for at least 18 days before the experiments were started.

Analyses

Samples for the determination of the concentration of volatile fatty acids (VFA), total ammonia and dissolved COD, were prepared by membrane filtration (pore diameter, 0.45 µm), when necessary 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 2 m x 2 mm (ID) glass column with either Chromosorb 101 (80-100 Mesh) at 190 °C, or Fluorad FC-431 on Supelcoport (100-120 Mesh) 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, 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 Messlerization of membrane-filtered samples. A correction was applied for the colour of the samples by measuring the extinction of the samples without the reagents. Dissolved COD was determined according to standard methods (American Public Health Association, 1975). Total COD was determined as described by Zeeman *et al* (1983). Volatile and Total Solids were determined according to standard methods (American Public Health Association 1975).

RESULTS

To investigate the possibility of thermophilic manure digestion a 120-litre reactor at 50 °C was seeded with diluted, fresh, cow manure (about 0.5 % VS). After 2 weeks, feeding was started with a 40-day retention time. The course of the methane production, the VFA concentration and the total ammonia concentra-

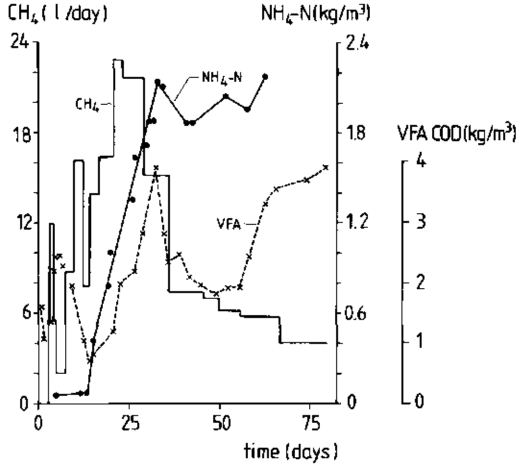

Fig. 1. The process performance of a thermophilic digester fed with cow manure, during start-up. Methane production rate, (——); total ammonia concentration, (●-●); effluent VFA concentrations, (x-x-x).

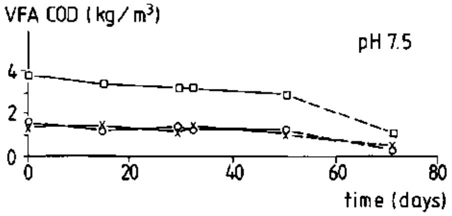

Fig. 2. The course of the VFA concentrations as a function of time during a prolonged interruption of the feeding of a thermophilic digester fed cow manure manure. Day 0 corresponds with day 80 in Fig. 1. (o), acetate; (x), propionate; (□), total volatile fatty acids. The corresponding methane production is given in Fig. 4.

tion is presented in Fig. 1. Both the methane production rate and the VFA concentrations increased during the first days of operation, until the ammonia concentration reached 1.7 kg N/m^3. A feed interruption from day 33 resulted in a decrease in the VFA concentrations. However, when the feeding was recommenced, the methane production rate did not increase and the VFA concentrations rose sharply (Fig. 1). After a new feed interruption, starting from day 65, the methane production rate remained low, but rather constant, and it decreased after the VFA were degraded. The course of the VFA concentrations during this feed stop is shown in Fig. 2.

Feeding was resumed 154 days after the start of the experiment for 38 days at a 40 day retention time; thereafter, a 30 day retention time was employed. The methane production rate remained constant at 0.17 m^3/m^3day, corresponding to

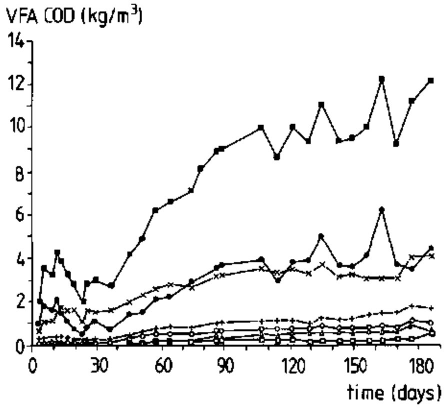

Fig. 3. The course of the VFA concentrations as a function of time during operation of a thermophilic digester fed cow manure, after resumption of the feeding. The retention time was 40 days from 0 to 38 days and 30 days thereafter. (●), acetate; (x), propionate; (o), isobutyrate; (Δ), butyrate; (+), isovalerate; (□), valerate; (■), total VFA. The total ammonia concentration during the experiment was 3.3 ± 0.4 kg NH_4^+-N/m^3 and the pH was 7.5.

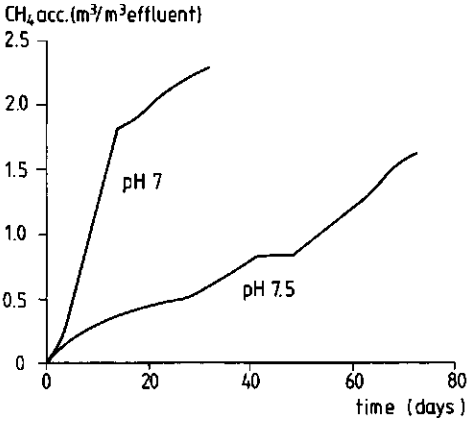

Fig. 4. Accumulated methane production as a function of time during the batch digestion of the content of a digester fed cow manure, with pH adjustment to pH 7.0, and at its original pH, 7.5. The experiment was started at day 80 of the times given in Fig. 1.

5 m^3/m^3 of slurry, for some 150 days. During this period the VFA concentrations reached very high levels, as shown in Fig. 3. The total ammonia concentration during this experiment was 3.3 ± 0.4 kg NH_4^+-N/m^3.

To investigate the possible inhibitory role of ammonia, the methane production during batch digestion of the effluent of the reactor just described was followed at its original pH (7.5) and at pH 7.0. The batch digestion was started at the 80th day of operation of the 120-litre digester shown in Fig. 1. The methane production at pH 7.0 was about four times as high as the production at pH 7.5 (Fig. 4). The difference in methane production corresponds with the difference in decrease in the VFA concentrations, indicating that the pH change did not affect the rate of hydrolysis (results not shown).

An additional set of experiments was conducted to determine whether the ammonia or the VS concentration had the major responsibility for the poor performance of the thermophilic digestion. The VS concentration was varied by using different dilutions of the effluent of the reactor just described. Dilutions were performed with and without addition of NH_4Cl. Dilution of the effluent did not improve the methane production when the total ammonia concentration was brought to the original level (Fig. 5). However, dilution without addition of NH_4Cl resulted in a clear improvement of the methane production and the VFA levels (Fig. 6). The VFA concentrations in the undiluted effluent of the experiment shown in Fig. 6 remained at the same level, of about 7 kg COD/m^3,

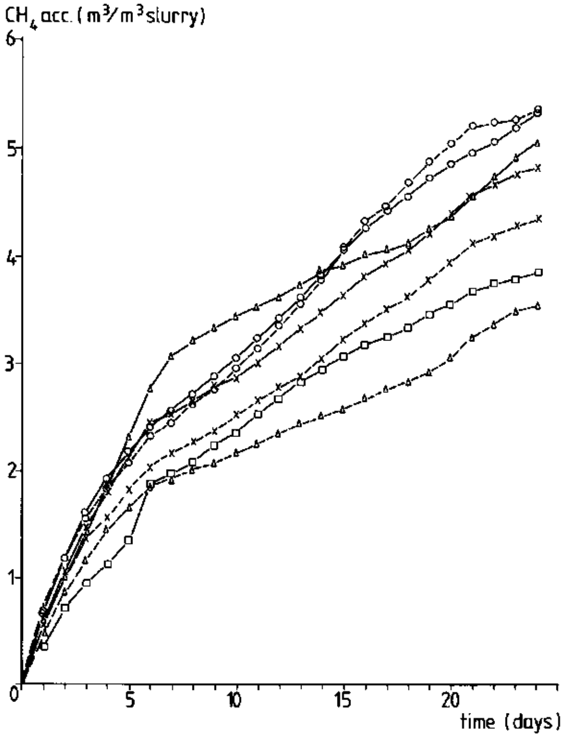

Fig. 5. Accumulated methane production as a function of time during batch digestion of the effluent of the main digester fed cow manure, taken at the end of the experiment shown in Fig. 3. The methane production was followed after dilution, with adjustment of the ammonia concentration to its original level of 3.0 kg N/m^3. (o), not diluted; (□), 1:3 diluted, final VS concentration 25 % of the original effluent; (Δ), 1:1 diluted, final concentration 50 %; (x), 3:1 diluted, final concentration 75 %. All experiments were performed in duplicate (—— and - - -); the duplicate of the 1:3 diluted slurry is not shown because of erroneous results due to gas leakage.

for over 100 days after the start of the experiment. The methane production was very low. Via the addition of VFA, it was attempted to find out whether the methane originated from H_2/CO_2 or acetate. Addition of 1 kg/m^3 of acetate and propionate each had only a minor effect, but the addition of another 3 kg/m^3 resulted in an increase in the methane production, with a concomitant decrease in the acetate concentration to the level before the acetate addition was made. Propionate, however, was not degraded (Fig. 7).

The question of whether this ammonia effect could be provoked was investigated separately in two experiments with a low retention time, as long retention times were not needed for this part of the investigations. High effluent VFA

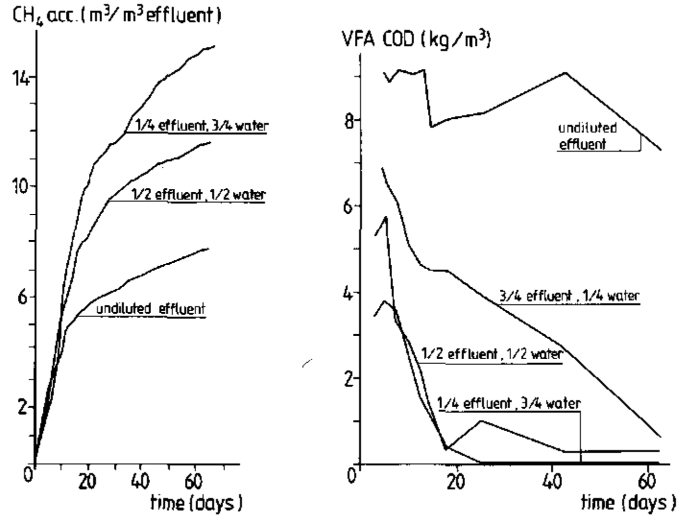

Fig. 6. Accumulated methane production (left) and the course of the VFA concentrations (right) as a function of time, during batch digestion of the effluent of the main digester fed cow manure, taken at the end of the experiment shown in Fig. 3. The methane production was followed after dilution without adjustment of the total ammonia concentration. The various dilutions are indicated in in the figure.

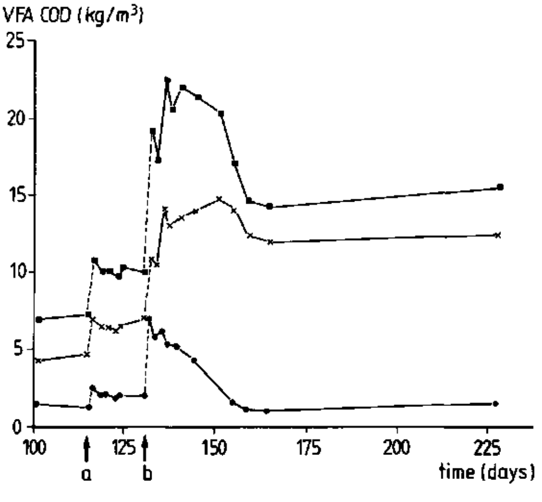

Fig. 7. The course of the VFA concentrations in the severely retarded thermophilic batch digestion of the undiluted effluent shown in Fig. 6 after addition of 1 kg/m^3 of acetate and propionate (arrow a) and 3 kg/m^3 of acetate and propionate (arrow b). (●), acetate; (x), propionate; (■), total VFA. The time scale is a continuation of that in Fig. 6.

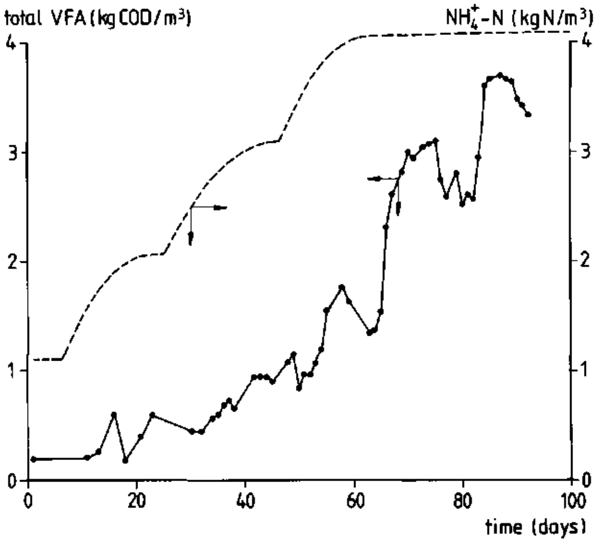

Fig. 8. Course of the VFA concentrations (●-●-●) in an experiment with increasing additions of NH_4Cl (- - -) to the substrate of a digester fed dilute cow manure (2.5 % VS) at 55 °C. The retention time was 5.5 days until day 58, no feed was supplied from day 58 to 63. Thereafter the retention time was 8.3 days.

levels could be generated by addition of NH_4Cl to a digester fed cow manure at pH 7.3 and 55 °C (Fig. 8). Adaptation to high total ammonia levels was investigated by imposing an instant increase in the total ammonia concentration from 2.5 to 4.0 kg N/m³ in a digester fed with acetate (9.4 kg COD/m³) and diluted cow manure (0.024 % VS) at a retention time of 5.5 days. Only after 40 days did the effluent acetate levels reach a stable value (Fig. 9).

DISCUSSION

From the results presented in Figs 1 to 3 it becomes clear that thermophilic anaerobic digestion of the cow manure used in this study is very difficult. This is somewhat surprising as the results of Varel *et al* (1977,1980) showed the opposite. In the range of 5-8 % VS, Varel *et al* observed volatile fatty acid concentrations below 2 kg/m³ (as acetic acid), whereas the authors found much higher levels at similar VS concentrations. As a consequence, the methane conversion efficiencies here are rather low. A possible reason for the poor performance of the thermophilic process is the composition of the organic matter in the manure; as feeding strategies in cattle breeding in America are quite different from those in Europe, the manures may differ considerably,

Fig. 9. Adaptation of a digester, fed acetate (9.4 kg COD/m^3) and dilute cow manure (0.24 % VS), to an increase in the total ammonia concentration from 2.5 to 4.0 kg N/m^3 (in both reactor and influent). The moment of the increase is indicated with an arrow. pH 7.3; digestion temperature 55 °C.

resulting in a different course of the efficiency in relation to the influent VS percentage. Moreover, the composition of the organic matter is influenced by the collection and storage system and the duration of the storage of the manure. The total ammonia concentration, the presence of other, yet unidentified, inhibitory compounds in the manure or a combination of these factors might also influence the digestion.

The pH effect on the rate of methanogenesis in the effluent samples and the increasing accumulation of VFA when the total ammonia concentration is increased, strongly suggest that the concentration of free ammonia is the main factor responsible for the poor performance of the process. However, a remarkable adaptation to high ammonia levels can take place. Whether the adaptation is the result of internal changes in the predominant species of methanogenic bacteria, or of a shift in the methanogenic population, is not clear. The recovery pattern shown in Fig. 9 is similar to the pattern observed in mesophilic digesters, where the adaptation was clearly shown not to be the result of a population shift (Parkin & Speece 1982).

It should be noted that, although the total ammonia concentrations in the two subsidiary experiments were much higher than in the main experiment shown in Fig. 3, the calculated free ammonia concentrations were much the same (290 and 275 mg NH_3-N/litre, respectively, at the end of the experiments). However, in the main experiment the VFA concentrations were appreciably higher than in the subsidiary experiments. This may be caused by the fact that no more propionate can be accumulated than about 15 % of the degradable COD (Kaspar & Wuhrmann 1978), which amounts to about 4.3 kg propionate-COD/m^3 in the main experiment, assuming 25 % of the COD is available as substrate. This value corresponds reasonably with the propionate concentration, given in Fig. 3. With the diluted manure used in the experiment shown in Fig. 8, this value is much lower. The low acetate concentration in the experiment shown in Fig. 9 may be the result of the relatively good resistance of acetate consuming methanogens to high total ammonia concentrations, as opposed to hydrogen utilizing methanogens (Wiegant, pers comm; *cf* Hobson & Shaw 1976).

Dilution did not improve the digestion of effluent samples from continuously fed digesters when the total ammonia concentration was restored to its original level. However, when no ammonia was added, dilution had a very significant positive effect on the digestion. Hence, it can be concluded that the VS concentration of the manure has only a minor effect on its digestion, and that the total ammonia concentration, rather than the concentration of the manure, is the predominant factor in determining the digestability in the thermophilic temperature range.

Contrary to Chen & Hashimoto (1980), who developed a kinetic model based on the VS concentration of the manure, we are of opinion that the total ammonia concentration is a more important parameter for predicting digester failure than the VS content. As the total ammonia and VS concentrations are directly linked to each other, it may be clear that the correlation between the total ammonia concentration and the process performance is as convincing as the correlation between the VS content and the process performance, when using one batch of slurry.

With increasing manure concentrations, the kinetic parameter K (Chen & Hashimoto 1980) shows a sudden increase, indicating a rapid deterioration of the process performance. The VS concentrations at which this sudden increase occurs vary from 4 to 8 % VS (Hashimoto *et al* 1981, Hashimoto 1982). The results presented here show that the deterioration of the process performance can be predicted from the total ammonia concentration. The results of mesophilic experiments, in which the VS and NH_4^+-N concentrations were varied

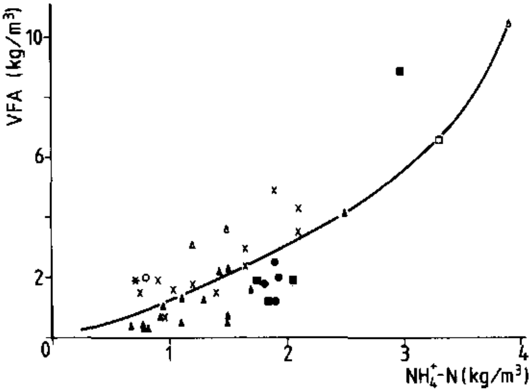

Fig. 10. Effluent VFA concentrations as a function of the total ammonia concentration in the thermophilic digestion of manure. Results of different investigations. (o), Shelef *et al* 1980; (★), Converse *et al* 1977; (x), Varel *et al* 1977; (●), Hashimoto *et al* 1978; (▲), Hashimoto 1982; (■), Hashimoto 1983; (Δ), van Velsen *et al* 1979; (□), this investigation.

independently, showed that the kinetic parameter K was exponentially increasing with the total ammonia concentration, and not with the VS concentration of the manure (Zeeman *et al* 1984). In contrast to Hashimoto, who considers the VS percentage in the manure or the combination of the VS percentage and the total ammonia concentration the cause of the deterioration of the thermophilic digestion of slurry (Hashimoto 1982,1983), we suggest that in this case the total ammonia concentration alone causes the poor performance.

From the kinetic point of view, there are some very confusing results, showing that the inhibition by ammonia might be more complex than hitherto supposed. It is clear that a severely retarded digestion still has a remarkable methane-forming capacity (Fig. 7), which apparently originated mainly from the conversion of acetate; propionate degradation seemed to be completely inhibited. As far as acetate is concerned, the experiment suggests an increase of its half saturation constant to extremely high levels. On the other hand, Monod kinetics do not seem to apply at all in cases of severe inhibition. Investigations on this matter are in progress.

The total ammonia concentrations at which process failure occurs in the mesophilic temperature range are much higher for pig manure than for cow manure (Zeeman *et al* 1983). This indicates that cow manure apparently contains compounds having an additional effect on the inhibition by ammonia. These compounds have yet to be identified. Possibly, compounds formed during the acidification step exert an inhibiting effect on the methanogenesis.

ACKNOWLEDGEMENT

This study was carried out as a part of studies on the feasibility of on-farm digestion of cow manure, supported by the Energy Research Project Office of the Dutch Institute of Applied Technological Research, and on the feasibility of thermophilic anaerobic digestion, supported by the Energy from Biomass Research and Development Programme of the European Community.

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4. The mechanism of ammonia inhibition in the thermophilic digestion of livestock wastes

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ABSTRACT

This paper proposes a scheme for the inhibition of thermophilic methane digestion processes by high ammonia concentrations. Ammonia acts as a strong inhibitor on the formation of methane from H_2 *and* CO_2*. It has only a minor effect on the formation of methane from acetate, as indicated by the independence of the specific growth rate of acetate consuming methanogens from the ammonia concentration (up to 4500 mg* NH_4^+*-N/litre). On the basis of thermodynamic considerations it is shown that the inhibiton of the hydrogen consumption leads to an inhibition of the propionate breakdown. Accumulated propionate in turn acts as an inhibitor on the acetate consuming methanogens.*
This scheme explains the discrepancy between the observed acetate accumulation in thermophilic methane digestion systems with high ammonia levels, and the independence of acetate mediated methanogenesis from high ammonia concentrations under laboratory conditions.

INTRODUCTION

Ammonia is generally considered as one of the major compounds, toxic for the methane generation from agricultural wastes. The nature of its toxicity is not fully understood. Under mesophilic conditions, normally total ammonia concentrations exceeding 1700 mg/litre are inhibitory (Kugelman & McCarty 1965, Koster & Lettinga 1984). Above this concentration a striking adaptation may occur (Melbinger & Donellon 1971, van Velsen 1979), which finally may result in total ammonia concentrations of 5000 mg N/litre being tolerated (van Velsen 1979). In the digestion of acetate even 8000 mg N/litre will be tolerated (Parkin & Miller 1982).

Several inhibition processes may play a significant role in the methane digestion of livestock wastes. On the level of methanogenesis, inhibition by the total ammonia concentration may be associated with pH-inhibition, substrate and end product inhibition, and other inhibition processes. A concise survey of these processes is presented in Table I.

Table I. Some relevant conversions and their inhibitors, likely to occur in the anaerobic digestion of livestock wastes.

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

References: a, Hobson & Shaw 1976; b, van Velsen 1979; c, Koster & Lettinga 1984; d, Zinder & Mah 1979; e, McInerney & 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 & Hall 1981; l, Gujer & Zehnder 1983; m, de Zeeuw 1984.

In a previous paper (Zeeman *et al* 1985), we reported on the influence of the total ammonia concentration on the thermophilic digestion of livestock manures. The objective of the research presented in this paper was to study the inhibition by ammonia in more detail. This was done by investigating the influence of inhibitors, and possible inhibitors, on the specific growth rates of methanogens. Measurements of the specific growth rates of methanogens may provide useful information on the nature of ammonia inhibition.

METHODS

Temperature

All experiments were carried out at 55 °C.

Inocula

The inocula were taken from digesters with an operating temperature of 55 °C, in which fresh cow manure was the primary source of bacteria.

Media

The media were made up in tap water (*c* 30 mg/litre Ca^{++}), containing the following additions (mg/litre): NH_4Cl, 170; NaH_2PO_4, 37; $MgCl_2.4H_2O$, 9; KCl, 25, and 1 ml/litre of a trace element solution containing (mg/litre): H_3BO_3, 50; $FeCl_2.4H_2O$, 2000; $ZnCl_2$, 50; $MnCl_2.4H_2O$, 500; $CuCl_2.2H_2O$, 30;

$(NH_4)_6Mo_7O_{24}.4H_2O$, 90; $CoCl_2.6H_2O$, 2000; $NiCl_2.6H_2O$, 50; $NaSeO_3.5H_2O$, 100; EDTA, 1000; Resazurin, 500, and 1 ml/litre 36 % HCl. These basal nutrients were supplemented with yeast extract (100 mg/litre).

In the growth rate determinations with acetate as substrate, 25 mM K^+-phosphate buffer (final concentration), pH 7.5 was added, and various concentrations of sodium acetate, propionate and chloride, and ammonium chloride. In the growth rate determinations with H_2/CO_2 as substrate, the medium was supplemented with 200 mg/litre $NaHCO_3$ and 200 mg/litre KH_2PO_4, and no buffer was added.

Oxygen was removed from the media by flushing N_2-gas through the solution for 5 min, addition of 0.5 ml/litre of a 1 M Na_2S solution, and again 5 min of N_2 flushing. The inoculum was always added shortly thereafter.

Growth rate determinations with acetate as substrate

320 ml serum bottles were filled with the media just described, to which 2.0 g/litre of acetate was added, and a series of NH_4Cl concentrations (100, and 500 to 4500 mg N/litre, with 500 mg/litre increments). 20 ml inocula were taken from a digester fed with dilute cow manure (2.5 % VS) to which NH_4Cl was added (digester concentration *c* 3000 mg NH_4^+-N/litre) at a retention time of 5.5 days. After the serum bottles were made free of oxygen and had reached 55 °C by placing them in a thermostatted water bath, the inocula were injected through the rubber caps. The liquid volume after inoculation was 250 ml. The methane production was measured by using a liquid displacement system, consisting of 320 ml serum bottles placed upside down and filled with a 1 M NaOH solution. These bottles were connected with the culture bottles through PVC tubing, connected with 2 ml syringes and needles with 0.75 mm internal diameter. The outcoming NaOH solution was measured by weight, and a correction for evaporation was applied.

The growth rate on acetate in the presence of high concentrations of acetate, propionate, NH_4Cl and NaCl was measured in 6.0 l gas-tight digesters with a 5.5 l working volume. 200 ml inocula were taken from digesters with a high retention time (over 40 days), and basically no propionate degrading activity (digester total ammonia concentration *c* 3500 mg N/litre). The evolved biogas passed a column of soda lime pellets and a liquid (0.4 M NaOH) displacement system, and was accounted for as methane. The outcoming solution was collected in 15.5 ml siphons, of which each time they were emptied the exact time was registered automatically. Calculation of the specific growth rate was performed by a least square minimalization of the equation for the accumulated gas production according to Powell (1983) for the data points.

Growth rate determinations with H_2/CO_2 as substrate

The growth rates of hydrogen consuming methanogens were determined using a 470 ml reactor with a working volume of 300 ml. Through the medium, to which 0 to 4250 mg NH_4^+-N/litre was added, a mixture of 80 % H_2 and 20 % CO_2 was blown, via a needle with 0.25 mm internal diameter (*c* 80 l/day). The final pH was 6.7-6.9. The reactor was stirred at maximum allowable speed (*c* 800 rpm) on a magnetic stirrer. 10 ml inocula were used from a digester fed with vinasse (*c* 50,000 mg COD/litre; digester total ammonia concentration 2310 mg N/litre) and a retention time of 18.2 days.

Measurements were started about 16 hrs after inoculation. The specific growth rate was determined by measuring the slope of the natural logarithm of the methane concentration in the gas which had passed the reactor. As the methane concentrations were low, no correction for the consumption of H_2/CO_2 was applied.

Analyses

Methane concentrations in the gas phase were measured with a Packard Becker Model 409 gas chromatograph with FID, equipped with a 1.5 m x 2 mm (ID) stainless steel column with Molecular Sieve 5A (60-80 Mesh). N_2 was used as the carrier gas (30 ml/min). Oven temperature was 170 °C and detector temperature was 220 °C. All other analyses were performed as previously described (Zeeman *et al* 1985).

RESULTS

The accumulated methane production from acetate, with various concentrations of NH_4Cl, is shown in Fig. 1. The Figure shows a number of parallel curves, indicating that the specific growth rates are virtually equal. A separate measurement of the growth rate on acetate with addition of 5000 mg NH_4^+-N/litre was made in a 6.0 l digester. The calculated specific growth rates of

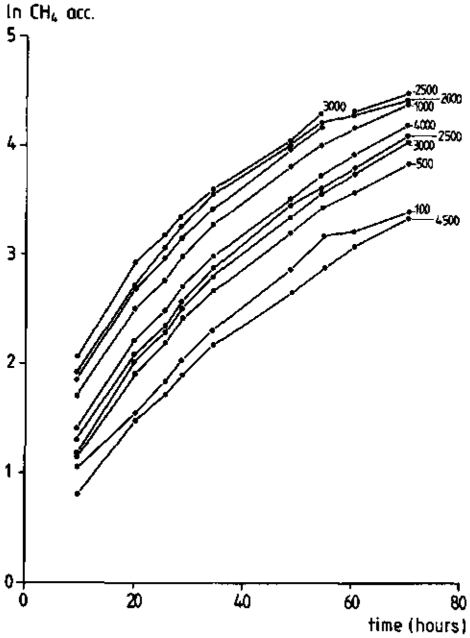

Fig. 1. The accumulated methane production at 55 °C from acetate at various total ammonia concentrations, indicated in the Figure in mg N/litre. Note the logarithmical scale of the methane production.

these experiments are presented in Fig. 2, together with those measured with H_2/CO_2 as substrate. The results of the experiments with various acetate concentrations and additions of propionate, NH_4Cl and NaCl are presented in Table II.

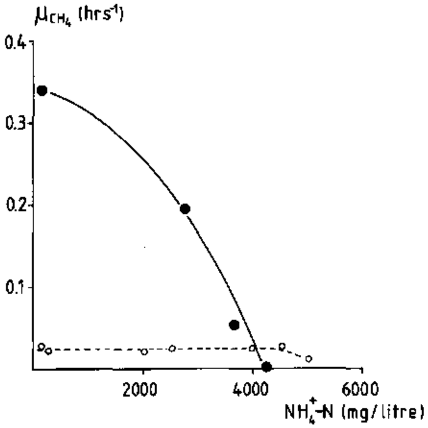

Fig. 2. The specific growth rates of hydrogen (●) and acetate (o) utilizing methanogens at 55 °C, as a function of the total ammonia concentration (in mg N/litre).

Table II. Specific growth rates of acetate consuming methanogens at 55 °C under various conditions.

media composition								growth rate
acetate		propionate		NaCl		NH_4Cl		
mg/litre	(mM)	mg/litre	(mM)	mg/litre	(mM)	mg N/litre	(mM)	hr^{-1}
1,000	(17)					45	(3)	0.030
10,000	(167)					45	(3)	0.030
2,500	(42)					45	(3)	0.040
2,500	(42)	5,000	(68)			45	(3)	0.023
2,500	(42)			4,000	(68)	45	(3)	0.030
2,500	(42)	5,000	(68)			2,000	(143)	0.014

DISCUSSION

Apparently the acetate utilizing methanogens, promoted in the above experiments, are very resistant against inhibition by ammonia. This may be the result of adaptation to the high total ammonia concentrations (*c* 3000 mg N/litre) in the digesters from which the inocula were taken. On the basis of their specific growth rate, it can be assumed that these acetate utilizing methanogens belong to the genus *Methanosarcina* (Zinder & Mah 1979, Zinder *et al* 1984). The other methanogen utilizing acetate, a *Methanothrix*, has a much lower specific growth rate, of 0.017 hr^{-1} at 55 °C (Zinder *et al* 1984). Syntrophic acetate oxidation (Zinder & Koch 1984) may be considered highly unlikely to have played a role in our experiments, as it occurs with concomitant methane formation from H_2/CO_2, which would be inhibited by total ammonia concentrations exceeding 3500 mg/litre.

In mesophilic sewage sludge digestion and in mesophilic anaerobic treatment systems with a high solids retention time, *Methanothrix* methanogens are the predominant acetate utilizing methanogens (Zehnder *et al* 1981, Hulshoff Pol *et al* 1983). These bacteria seem to have a lower tolerance for high total ammonia concentrations (*cf* Hulshoff Pol *et al* 1983, Koster & Lettinga 1984). Generally, the non-ionized form of ammonia is considered to be the toxic agens, at least it is far more toxic than the ionized form (McCarty & McKinney 1961). Therefore it seems that the inhibiting effect of ammonia is exerted after diffusion of the free ammonia into the bacterial cell. As *Methanosarcina* consists of large spherical cells with a much higher volume-to-surface ratio than the smaller, rod-shaped *Methanothrix*, the diffusion of free ammonia into the cell will be less, on a basis of kg NH_3 entered per kg cell mass per hour, for *Methanosarcina* than for *Methanothrix*. Thus, the removal of NH_3 would cost less energy for *Methanosarcina*. It seems appealing to attribute the higher resistance of *Methanosarcina* against ammonia to its higher volume-to-surface ratio. Likewise, this feature may also explain the higher substrate saturation constant of *Methanosarcina*, as compared with *Methanothrix*: here also the free acetic acid is considered to be the true substrate (Andrews 1969).

The total ammonia concentration, and also the free ammonia concentration, at which reactor failure occurs, is much lower than the concentration of total ammonia apparently tolerated by the acetate utilizing methanogens. In a previous paper (Zeeman *et al* 1985), we reported on concentrations of 3500 mg N/litre being too high for a satisfactory livestock waste digestion at 50 °C. However, the total ammonia concentration severely affects the growth rates of hydrogen utilizing methanogens. As in digestion processes acetate appears quite fast upon increasing total ammonia concentrations, one may wonder whether an intermediate compound accumulating during inhibition of the hydrogen consumption is affecting the conversion of acetate into methane. This compound may either be hydrogen or propionate. The energetics of the propionate breakdown are strongly depending on the maintenance of a low hydrogen partial pressure. This is illustrated in Fig. 3, from which it will be clear that the ammonia concentration will have a distinct but indirect influence on the propionate degradation.

The inhibition of the methanogenesis from H_2/CO_2 does, in fact, not only inhibit the degradation of propionate. It will also lead to a shift in the product formation of the acidogenic bacteria towards the production of more propionate, at the expense of acetate production. Propionate, and higher volatile fatty acids, may then act as an electron sink, instead of the hydrogen mediated methanogenesis (Wolin 1979). This is also observed during overloading (Cohen *et al* 1980). The accumulating propionate itself may also be toxic for the methanogenesis from H_2/CO_2 (Hobson & Shaw 1976).

From Table II it is clear that the propionate accumulated via the inhibition of hydrogen utilizing methanogens, will significantly inhibit the acetate utilizing methanogens. There seems to be also an inhibitory effect on the level of substrate inhibition at acetate concentrations of 10,000 mg/litre. It must be noted, though, that the decrease in the specific growth rate of the acetate utilizing methanogens may partially be attributed to the sodium used to

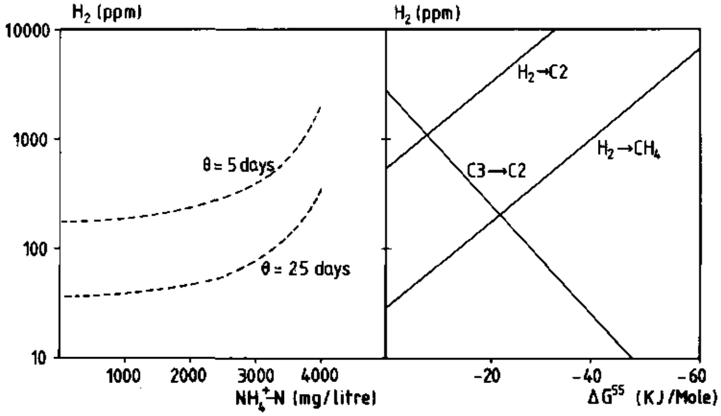

Fig. 3. The partial pressure of hydrogen, expressed as ppm H_2 in the gas phase at 1 atm., as a function of the total ammonia concentration (in mg N/litre), under assumption of a growth rate as in Fig. 2, and a K_s for hydrogen of 100 µM (Schönheit *et al* 1980, Zehnder *et al* 1981), for retention times Θ of 5 and 25 days (left). In the right part of the Figure the ΔG^{55} of the conversion of propionate into acetate, hydrogen and carbon dioxide ($C3 \rightarrow C2$), that of CO_2 and H_2 into acetate ($H_2 \rightarrow C2$), and that of H_2 and CO_2 into methane ($H_2 \rightarrow CH_4$) is shown, under assumption of pH = 7.2, 20 % CO_2 in the dry biogas, and a molar acetate/propionate ratio of 1.0 (Wiegant *et al* 1986), as a function of the partial pressure of hydrogen.

neutralize the acids. NaCl also has an inhibiting effect. Still, the growth rate with 10,000 mg/litre of acetate is much higher than that with 2500 mg/litre of acetate and 5000 mg/litre of propionate. The extremely low growth rate, measured with a combination of propionate and ammonia, may be due to an antagonistic effect of both propionate, ammonia and sodium.

Whether hydrogen itself plays an inhibitory role in the methanogenesis from acetate, is somewhat unclear. In the reports on inhibition of methanogenesis from acetate in *Methanosarcina* strains, hydrogen concentrations are applied that are far beyond those normally observed in operating digesters (*cf* Ferguson & Mah 1983). Acetate consumption by *Methanosarcina barkeri* was resumed when the hydrogen pressure dropped below 10,000 ppm (McInerney & Bryant 1981).

In experiments with continuously fed reactors at a 5.5 day retention time, we failed to demonstrate any effect of a continuous hydrogen supply. However, at a 1.1 day retention time there seemed to be some effect (results not shown). A 1.1 day retention time is not very realistic in livestock waste digestion, though.

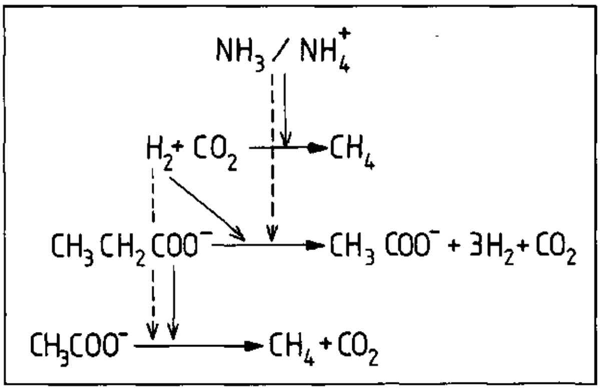

Fig. 4. Proposed scheme for the inhibiting action of ammonia. Horizontal arrows: inhibited reactions; vertical arrows: inhibiting action. Possible inhibiting actions are dotted.

It can be concluded that the propionate accumulated via ammonia-promoted inhibition of hydrogen utilizing methanogens may play an important role in the accumulation of acetate in stressed digesters. It may trigger inhibitory effects on the level of substrate inhibition in acetate utilizing methanogens. A scheme of the discussed action of ammonia is presented in Fig. 4.

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5. Thermophilic anaerobic digestion of sugars in upflow anaerobic sludge blanket reactors

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INTRODUCTION

In recent years, numerous designs have been developed for the high rate anaerobic treatment of wastewater (1-6). All these processes have their advantages and drawbacks, which have been discussed repeatedly (see, for instance, 7). Of these processes the upflow anaerobic sludge blanket (UASB) process is currently the most widely applied, at least in Western Europe. It is successfully applied in the treatment of wastewaters from maize and potato starch industries, and sugar beet and potato processing industries (8).

Perhaps the main advantage of the UASB process is that no specific support material is required. However, this main advantage may sometimes be a serious drawback: the absence of support material necessitates the availability of a sludge with very good settling properties. Whenever such a sludge is obtained, or can be maintained, successful operation of the UASB process is readily achieved.

Under certain circumstances the settleability of the sludge in UASB reactors is greatly improved because bacterial growth is in the form of spherical flocks with a quite consistent structure, normally referred to as granular sludge (6). Although already considerable research has been performed on factors, considered to be important in the phenomenon of granulation (9-11), the knowledge on this matter is still relatively poor.

Thermophilic anaerobic digestion may become an attractive alternative for mesophilic digestion because of the higher growth rates of the bacteria involved, and consequently the higher maximum activities per unit of biomass. As the specific growth rates of the methane-forming bacteria of the genus *Methanothrix*, which are believed to be the predominant acetoclastic methanogens in methane digestion processes with a high biomass retention time, is about 2.5 times as high at 55 °C as at 30 °C (12,13), also a 2.5 times higher methanogenic activity per unit of biomass may be expected. In thermophilic sewage sludge also a *Methanosarcina* was found to be the predominant acetoclastic methanogen, which even has a higher growth rate than the thermophilic *Methanothrix* (14).

The successful operation of high rate thermophilic systems requires a high activity per unit of volume, and therefore a high biomass retention is a prerequisite. In view of the successful use under mesophilic conditions, we investigated the thermophilic UASB process for this purpose. Glucose was chosen as the substrate, because granulation is reported to occur in UASB reactors when it is the substrate for an acid-formation process (15).

MATERIALS AND METHODS

Temperature

All experiments were carried out at 55 °C.

Media

All media and feed solutions were made up in tap water (ca. 30 mg/l Ca^{++}), to which the following basal nutrients were added (in mg/l): NH_4Cl, 170; NaH_2PO_4, 37; $MgCl_2.4H_2O$, 9; KCl, 25; and 1 ml/l of a trace element solution containing (in mg/l): H_3BO_3, 50; $FeCl_2.4H_2O$, 2000; $ZnCl_2$, 50; $MnCl_2.4H_2O$, 500; $CuCl_2.2H_2O$, 30; $(NH_4)_6Mo_7O_{24}.4H_2O$, 90; $NiCl_2.6H_2O$, 50; $Na_2SeO_3.5H_2O$, 100; EDTA, 1000; Resazurin, 500, and 1 ml/l 36 % HCl. Whenever the C/N ratio in the feed exceeded 35, the basal nutrient concentration was doubled.

Feed solutions consisted of basal nutrients and 1) volatile fatty acids (acetic, propionic and butyric in a 1:1:1 ratio (w/v)), partly neutralized with NaOH, or 2) glucose and sucrose with or without addition of volatile fatty acids. Sulfur was added as $Na_2S.9H_2O$ (100 mg/l) during initial start-up, and as Na_2SO_4 (70 mg/l) otherwise. To substrates consisting of volatile fatty acids, yeast extract was added (0.1-0.3 g/l); to sugar solutions $NaHCO_3$ was added (5-10 g/l).

Apparatus

Start-up experiments were carried out in 6 1 gas-tight digesters with a working volume of 5.5 1, equipped with a stirring blade which was used intermittently (2 s every 3 min at 140 rpm). The evolving biogas passed a column of soda lime pellets and a liquid (1.5 % NaOH) displacement system. The outcoming gas was accounted for as (wet) methane. A scheme of the digesters used is presented in Fig. 1.

The UASB experiments were carried out in 5.75 1 (ID = 9 cm) UASB reactors. They were equipped with a central axis with a stirring blade (used 0.5 s every 3 min at 100 rpm) and a gas-solids separating device. Evolving biogas passed a 5 M NaOH solution and a column of soda lime pellets, and was measured by a wet test gas meter. It was accounted for as (wet) methane. A scheme of these laboratory UASB reactors is presented in Fig. 1. In one case a 0.70 1 (ID = 5 cm) non-stirred UASB reactor was used.

Procedures

All start-up procedures were performed in an identical way. The reactors were filled with the desired solution, containing basal nutrients and sometimes carbon sources, yeast extract and $NaHCO_3$, but no seed sludge and no sulfide. Then N_2 gas was passed through the solution for 5 min, to remove oxygen. There-

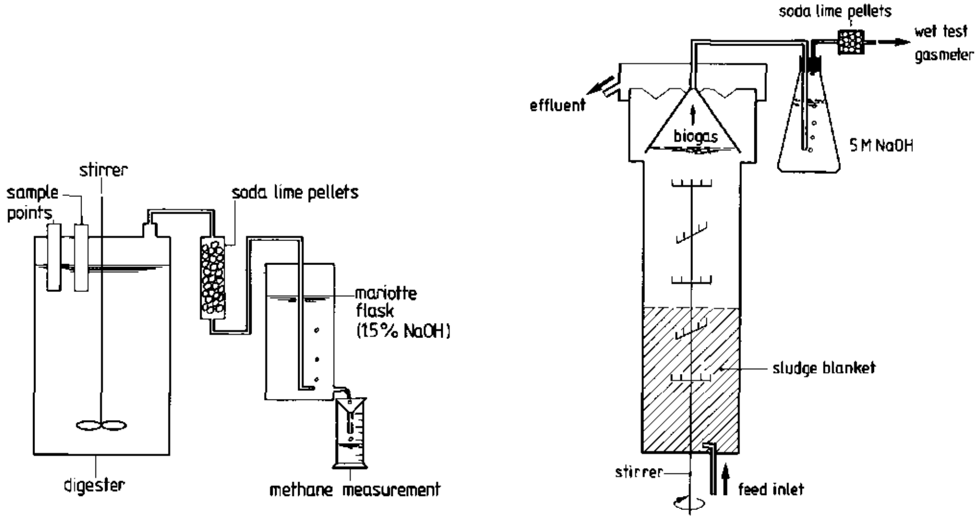

Fig. 1. Schemes of the digesters (left) and UASB reactors (right) used. The schemes are not to scale.

after the sulfide was added and passage of nitrogen went on until the Resazurin indicator turned colorless, indicating a redox potential below approx. -90 mV (16). Thereafter the seed sludge was added.

Analyses

Concentrations of volatile fatty acids were determined on a Packard Becker Model 417 gas chromatograph with FID, equipped with a 2 m x 2 mm (ID) glass column with either Chromosorb 101 (80-100 Mesh) at 190 °C, or 10 % Fluorad FC-431 on Supelcoport (100-120 Mesh) 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, respectively). The COD of the volatile fatty acids was calculated from their concentrations. The composition of biogas (CO_2, CH_4, N_2) was determined on a Packard Becker Model 409 gas chromatograph with TCD, equipped with a 2 m x 2 mm (ID) stainless steel column with Porapak Q (80-100 Mesh). Helium was used as the carrier gas. Oven and detector were at room temperature.
All other analyses were carried out according to Standard Methods (17).

RESULTS

Start-up experiments

Four inocula, namely, cow manure, mesophilic granular sludge, digested sewage sludge and a mixture of these materials, were used to identify what bacterial source would be appropriate to start-up a thermophilic digestion system. This experiment revealed that any bacterial source exerting a reasonable mesophilic methanogenic activity showed thermophilic methane production, indicating these materials would suffice for the start-up of a thermophilic digestion process (Fig. 2).

UASB experiments

Diluted fresh cow manure (approx. 1.5 % VS) was used as the seed material for the start-up of a UASB reactor, since in previous experiments the use of mesophilic granular sludge had shown no benefits over fresh cow manure. The feeding was started three weeks after inoculation, long after the methane production had peaked (see 18). As shown in Fig. 3, a stepwise increase in the loading rate from 4.7 to 33.2 kg COD/m^3day was accommodated in only 48 days, with

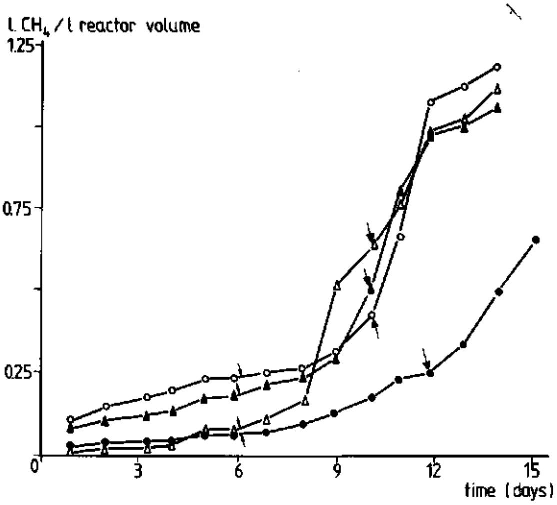

Fig. 2. Cumulative methane production as a function of time for different seed materials, as measured in 6.0 l digesters. The seed materials were: cow manure (Δ), digested sewage sludge (●), mesophilic granular sludge (o), and a mixture of the former three (▲). The VS percentages were 0.47, 0.48, 0.68, and 0.50 %, respectively. Arrows indicate addition of acetate, propionate and butyrate: (↑), 100 mg/l and (↓), 320 mg/l each.

over 85 % conversion of glucose to methane, on a COD basis.

Overloading occurred at day 52, which resulted in a temporary deterioration of the treatment efficiency, but the process recovered quite quickly. Thereafter a further stepwise increase of the loading rate was imposed (days 75 to 92), from 29.4 to 49.3 kg COD/m^{3}day. This led to a slow decrease in the conversion efficiency, indicating the process was near its maximum capacity. At its maximum capacity (day 87) the reactor produced 14.1 m^3 CH_4(STP)/m^{3}day, corresponding with a superficial biogas loading rate of 26.9 m/day (57 % methane in the biogas).

The substrate was shifted from glucose to sucrose from day 85 to 88. No essential changes in the process performance were observed. The decrease in the efficiency, seen from day 92 to 98 was the result of a feed stop, after which the process apparently deteriorated somewhat. At the end of the experiment the substrate concentration was lowered and the efficiency increased again.

Small sludge granules were clearly visible from approximately four weeks after the start of the feeding. At that time the superficial loading rates were approx. 0.54 and 10.0 m/day for the liquid and the biogas, respectively. Starting from approximately day 48 till the termination of the experiment, all

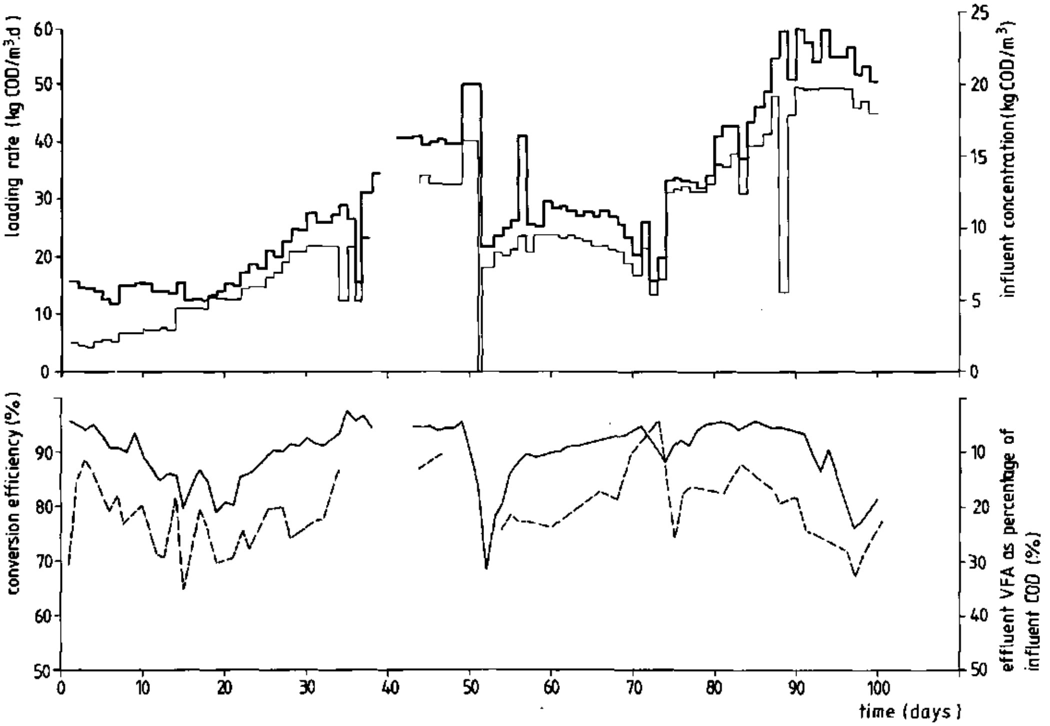

Fig. 3. Performance of a UASB reactor fed with glucose. Upper Figure: influent concentration (———) and organic loading rate (———); lower Figure: efficiency of the conversion of substrate to methane, on a COD basis (- - -), and the concentration of volatile fatty acids (in COD) in the effluent, expressed as percentage of the influent COD (———). On day 85 to 88 the substrate was shifted from glucose to sucrose.

the sludge was present in the form of granules. As indicated by fluorescence microscopy, the granules consisted mainly of non-fluorescent bacteria, and clumps of large fluorescent bacteria, presumably *Methanosarcina*. Also small, rod-shaped fluorescent bacteria were present, not unlike the hydrogen utilizing methanogen, isolated recently (19). Identical granules were obtained via the same procedure with the use of sucrose as substrate.

The granules obtained could be used for the conversion of other substrates into methane, with excellent results. The results of the experiments with volatile fatty acids as the main substrates are summarized in Table I. The decrease in the treatment efficiency with increasing loading rates is mainly due to the decreased degradation of propionate. No significant deterioration of the granular sludge was observed in using volatile fatty acids as the main substrate

Table I. Process performance of UASB reactors containing thermophilic granular sludge cultivated on glucose or sucrose, with substrates consisting mainly of volatile fatty acids.

substrate	acetate + yeast extr.	acids[a] + sucrose	acids[a] + yeast extr.	
concentration	1.41	8.02	14.65[d]	kg COD/m³
VFA	1.35	6.48	13.32	kg COD/m³
non-VFA	0.06	1.54	1.33	kg COD/m³
hydraulic retention time	2.1	5.7	3.2	hrs
organic loading rate	16.0	35	104	kg COD/m³day
treatment efficiency[b]	98.9	84.5	77.6	%
duration[c]	31	105	182	days

a: The acids were acetate, propionate and butyrate in a 1:1:1 (w/v) ratio.
b: (1 - effluent VFA-COD/influent COD) x 100 %
c: With duration the period of continuous feeding with the substrate mentioned is ment, not the period of maintenance of the process parameters given.
d: These parameters were measured in a 0.70 l UASB reactor.

for prolonged periods of time, although the granules significantly decreased in size, and their color changed from yellowish to grey.

Simultaneously, experiments were carried out to cultivate a granular sludge in thermophilic UASB reactors using a 1:1:1 (w/v) mixture of acetate, propionate and butyrate as substrate, with mesophilic granular sludge as seed sludge. Although loading rates of 10 kg COD/m³day could be accommodated satisfactorily, no granulation of the biomass was observed (results not shown).

DISCUSSION

From the results presented, it is clear that thermophilic anaerobic digestion of sugars in UASB reactors is very well possible: the start-up and operation are simple and the treatment efficiencies and concomitant methane generation rates are high. They are comparable with those found with the thermophilic anaerobic attached film expanded bed process (20). The granulation of a mixed acidogenic and methanogenic biomass may be held responsible for the satisfactory results with the UASB process. Mixed acidogenic and methanogenic granular sludge is also found in UASB reactors under mesophilic conditions in UASB reactors with digested sewage sludge as seed sludge (10). Acidogenic sludge is found in experiments concerning the acidification of glucose with aerobic activated sludge as seed sludge (15).

These three types of granular sludge have some remarkable differences and similarities, which will be discussed below. There is a clearly visible macroscopic resemblance: the granules cultivated in thermophilic and mesophilic methane digestion and mesophilic acidification all were 1-3 mm in diameter and had a yellowish color and a gleamy appearance. The inocula for the three experiments, however, were completely different: aerobic cultivated sludge in the experiment with mesophilic acidification, digested sewage sludge in the mesophilic, and fresh cow manure in the thermophilic methane digestion of sugars.

In the granulation process many factors are considered to be important. Among these are the start-up procedure (21), the selection of sludge particles with good settling characteristics by washout of lighter particles, and, of course, the maintenance of good conditions for bacterial growth (9). In the two experiments mentioned (10,15) and the one reported here, there is a resemblance in the values of the superficial biogas loading rates, which lie between 6.9 and 11.5 m/day, at the approximate moment that granulation becomes apparent. Other parameters vary much more, namely, the liquid superficial velocity between 1.1 and 6.1 m/day and the organic loading rate between 4 and 233 kg COD/m^{3}day - because of the different characters of methane digestion and acidification. Considering these figures, it must be borne in mind that the moment of appearance of the first granules is hard to establish. There are no objective criteria and moreover, granulation is a more or less continuous process without a clear demarkation between flocks and granules (9). Nevertheless, these figures would suggest an important role of the superficial biogas loading rate in governing the granulation process. This parameter certainly deserves more attention in investigations on the UASB process.

Acid-forming bacteria have much higher yield coefficients than methanogenic and acetogenic bacteria (10,22,23). Thus acid-forming bacteria will prevail numerically in mixed acidogenic and methanogenic granules, cultivated on sugars. Together with the observed lack of granulation on volatile fatty acids as substrate in thermophilic UASB reactors, this justifies the conclusions that sugar-fermenting acidogens have a strong tendency to granulate. The granular sludge cultivated on sugars, however, can be maintained for prolonged periods of time with substrates containing mainly volatile fatty acids, as given in Table I. Three explanations for this phenomenon seem to be possible: 1) the acid-forming bacteria responsible for the formation of the sludge granules have a low decay rate, 2) the structure of the granules is retained in spite of the decay of the organisms that built it, and 3) the ratio between the different bacterial groups in a granule can change without affecting the character of the granule. As yet it is not clear which of the explanations is right.

The wastewaters treated successfully in mesophilic full-scale UASB reactors are normally not fully acidified (6). All these wastewaters contain sugars or compounds which are readily hydrolyzed into sugars, like starch. So acid-forming bacteria will grow in these reactors, where they may play a significant role in the granulation process. However, granulation is fairly readily achieved in mesophilic UASB reactors with substrates consisting of volatile fatty acids (9). The granules cultivated in this way normally are different from those observed in full-scale reactors (21), but crushed granules obtained from full-scale reactors readily grow out to new granules, virtually identical to the original ones, with these substrates. In both the methanogen *Methanothrix soehngenii* outnumbers other bacteria (11). It seems that the acidifying bacteria are not necessary in the mesophilic granulation process. The question whether this is the case in the thermophilic granulation process is a very important one. If so, the use of thermophilic UASB processes can only be very restricted. At present the promotion of granulation of methanogenic biomass is studied intensively in our laboratory.

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6. Thermophilic anaerobic digestion of high strength wastewaters

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ABSTRACT

Investigations on the thermophilic anaerobic treatment of high-strength wastewaters (14-65 kg COD/m^3) are presented. Vinasse, the wastewater of alcohol distilleries, was used as an example of such wastewaters. Semi-continuously fed digestion experiments at high retention times revealed that the effluent quality of digestion at 55 °C is comparable with that at 30 °C at similar loading rates. The amount of methane formed per kilogram of vinasse drops almost linearly with increasing vinasse concentrations. This can be attributed to increasing concentrations of inhibitory compounds, resulting in increasing volatile fatty acid (VFA) concentrations in the effluent. The treatment of vinasse was also investigated using upflow anaerobic sludge blanket (UASB) reactors. Thermophilic granular sludge, cultivated on sucrose, was used as seed material. The sludge required a 4-month adaptation period, during which the size of the sludge granules decreased significantly. However, the settling characteristics remained satisfactory. After adaptation, high loading and methane generation rates could be accommodated at satisfactory treatment efficiencies, namely, 86.4 kg COD/m^{3}day and 26 m^3 CH_4(STP)/m^{3}day, respectively. As in the semicontinuously fed digesters, the effluent VFA concentrations were virtually independent of the loading rates applied, indicating that the toxicity of the vinasse is more important than the loading rate in determining the efficiency of the conversion of vinasse to methane.

INTRODUCTION

A number of medium and high-strength wastewaters are in theory particularly suited for thermophilic anaerobic digestion because they are discharged at high temperatures, for instance, wastewaters from canning industries, alcohol distilleries, and certain waste streams from paper and potato processing industries. The temperatures of these wastewaters vary between 60 °C and 90 °C, depending on whether or not heat is recovered from the wastewater stream, and on other factors.

Frequently these wastewaters are high in COD but also in inhibitory compounds, depending on the origin of the waste and the manufacturing process applied. Vinasse, the effluent from alcohol distilleries, is such a wastewater. It invariably contains high concentrations of COD, in the range of 40-120 kg/m^3. Depending on the source, it can contain considerable concentrations of heavy metals, cations such as ammonia and potassium, and sulfate. It is produced in tremendous quantities, especially in Brazil, where alcohol is being used as a liquid fuel.

Therefore, we chose vinasse as wastewater to assess the feasibility of thermophilic anaerobic digestion in treating high strength wastewaters. There has already been some experience both with mesophilic and thermophilic digestion of this type of wastewater and other comparable wastewaters. According to Japanese studies, the loading rates applicable at 54 °C are 2.4 times as high as those at 38 °C (1). On the other hand, Basu and Leclerc (2) concluded that thermophilic digestion of molasses distillery wastes offers little if any advantage over mesophilic digestion.

There is little experience with the thermophilic treatment of high-strength wastewaters in systems with a high biomass retention. Fixed film reactors treating stillage of wood hydrolysate, with a COD of 22.5 kg/m^3, performed similarly at 55 °C and 35 °C, with COD reductions of 84.4 % and 86.6 %, respectively, at organic loading rates 10.7 and 10 kg COD/m^{3}day, respectively (3). Similar observations were made in downflow stationary fixed film reactors in the treatment of bean blanching wastewater, which has a COD of ca. 9 kg/m^3. The maximum steady-state methane production rates were 4.7 m^3 CH_4(STP)/m^{3}day for operation at 35 °C and 55 °C (4). Biofilm washout occurred at 55 °C at lower hydraulic and organic loading rates than at 35 °C (5). Anaerobic filters were shown to be fairly efficient in the treatment of vinasse under mesophilic conditions. Loading rates up to 37 kg COD/m^{3}day were achieved with concomitant methane generation rates up to 14.2 m^3/m^{3}day. However, upflow anaerobic sludge blanket (UASB) reactors did not show a satisfactory performance. In the treatment of vinasse originating from potatoes and corn, anaerobic filters were not very successful, due to the high content of suspended solids in these wastewaters (6).

The objective of the present investigations was to study the thermophilic digestion of vinasse as an example of a high-strength wastewater in reactors with a high biomass retention. The approach was to investigate the performance of mixed digesters with a high liquid and biomass retention time, and of reactors with a low liquid, but a high biomass retention time. Experiments with (semi)continuously fed digesters can provide information about the effluent quality at high liquid and biomass retention times as a function of the influent concentration and the digestion temperature. This information will be indicative for the effluent quality of reactors with a high biomass and a low liquid retention time.

The investigations with high-rate systems were focussed on the upflow anaerobic sludge blanket (UASB) process for the following reasons: 1) At the present state of technology, we consider the UASB process as the most appropriate for treating a large variety of wastewaters, provided a sludge with good settling properties can be cultivated and maintained. Once these conditions are met, the UASB process undoubtedly offers the best potential because almost the complete reactor volume can be effectively used for the retention of the sludge, as no space is lost for any support material. 2) In our laboratory, there is a wide experience with this process in both the mesophilic and psychrophilic temperature range (7,8) Moreover, the results of UASB experiments under thermophilic conditions with sugar solutions were also very promising (9). 3) The UASB process is at present the most widely applied high-rate system for anaerobic treatment; already considerable full-scale experience exists with this process under mesophilic conditions (10).

Although the results of UASB experiments with vinasse under mesophilic conditions do not look very promising (6), we think this may be the result of an inaccurate start-up procedure. We still believe that the UASB process is feasible, provided the start-up of the process is made properly.

MATERIALS AND METHODS

Apparatus

The digesters, with a 5.5 l working volume, and the 5.75 l UASB reactors used were the same as those described elsewhere (9).

Vinasse

The vinasse used in the experiments was obtained as a so-called thick vinasse, a fivefold concentrate of the bottom slops from the ZNSF alcohol distillery at Bergen op Zoom, Holland. This thick vinasse is sold as a food additive for fattening pigs. For convenience the five times diluted concentrate (w/w) will

Table I. Relevant parameters of the vinasse used. The concentrated beet vinasse used was diluted five times to its original volume. The values given refer to this diluted vinasse. Data were supplied in part by the manufacturer.

Parameter	Concentration (kg/m^3)
Total solids	136
Suspended solids	0
Ash	46
COD	119
Biodegradable COD[a]	93
Total Nitrogen	6.4
Potential NH_4^{+}[b]	6.0
Potassium	16.4
Sulfate	4

a: Values determined in an anaerobic, 65-day batch digestion of 50 times diluted vinasse. b: This was the maximum NH_4^+-concentration possible, calculated from reactor concentrations.

be further indicated as vinasse. Some relevant characteristics of the vinasse, which in this case originated from beet molasses, are given in Table I.

Semicontinuously fed digesters

The semicontinuously fed digesters were seeded with fresh cow manure, which was diluted five times (final concentration). The addition of the cow manure was made after the dilution water in the digesters was made oxygen-free (9). In the mesophilic digesters, a small amount of mesophilic granular sludge (10) was added to accelerate the start-up. At both temperatures, the feeding was started after 2 weeks. The start-up method is comparable with that of Varel and co-workers (11).

The substrates used were made up with 0, 25, 125, and 250 g vinasse, diluted with tap water to 500 ml, and 170 g vinasse diluted to 250 ml. These feed solutions were supplied with basal nutrients (9), 10 g/1 $NaHCO_3$, and 0.1 g/1 $Na_2S.9H_2O$ (final concentrations). To these solutions 50 ml of fresh cow manure was added, giving final vinasse concentrations of 0, 9.1, 22.7, 36.4, and 56.7 % vinasse, as related to the (100 %) vinasse stock solution. Addition of the manure to the feed solution was made 1) to supply a vitamin source, 2) to minimize the effect of accidently introduced oxygen during the feeding, and 3) to guarantee a continuous inoculation.

From 2 weeks after seeding, feeding of 9.1 % and 22.7 % vinasse solutions at 30 °C and 0 %, 9.1 %, 22.7 %, and 36.4 % vinasse solutions at 55 °C were used at a retention time of 18.2 days. A 56.7 % vinasse solution was used at a 33.3-day retention time, and an additional digester was run with a 9.1 % vinasse solution at 9.1-day retention time, both at 55 °C. Feeding was performed once per two days, except for the 9.1 days retention time, which was fed daily. The feeding was carried out in the following way: first N_2 gas was blown over the liquid surface in the digester; then, the effluent was removed by siphoning; thereafter, new feed was added. During the feeding N_2 replaced biogas in the headspace of the digester; as the nitrogen gas replaced CO_2 in the headspace, a correction was applied to allow assessment of the correct methane production in the system used.

In the semicontinuously fed digesters samples were taken after three volume turnovers, except for the 0 and 56.7 % reactors, which were sampled after two volume turnovers, as the effluent quality was then considered constant. The samples were analyzed for COD after membrane filtration (0.2 μm pore diameter), VFA, and the total ammonia concentration.

UASB experiments

In the UASB experiments, thermophilic granular sludge, cultivated on sucrose from cow manure as seed material (9), was used as seed material. The thermophilic sludge was initially fed with a 2 % sucrose solution, at a loading rate of 18 kg COD/m^{3}day. Then, the sludge was adapted to propionate by replacing ca. 9 % of the influent COD by propionate. Next, the substrate was gradually shifted to a 20 % (w/v) vinasse solution. Thereafter, mere vinasse solutions were supplied. To all solutions used in the UASB experiments, basal nutrients (9), and 5 g/l $NaHCO_3$ were added. Sulfur was added as Na_2SO_4 (70 mg/l).

During the gradual shift in the substrate from sucrose to vinasse, as outlined above, the methane generation rate as well as the hydraulic retention time were kept within narrow limits. Adaptation was considered to be sufficient when the effluent VFA concentrations did not further decrease for a period of 3 days. After the adaptation was completed, different hydraulic loading rates and vinasse concentrations were applied.

In the UASB experiments, methane production rates and effluent VFA concentrations were measured daily. Samples for COD analyses were taken after at least three volume turnovers after applying a new loading rate or concentration of the vinasse. Samples were taken for 3-5 consecutive days, when the effluent VFA concentrations were considered constant.

Analyses

Measurements of the concentrations of VFA and COD and of the methane production are described elsewhere (9). The total ammonia concentration ($NH_3 + NH_4^+$) was measured photometrically using direct Nesslerization. A correction was applied for the color of the sample by measuring the extinction without the reagents.

RESULTS

Semicontinuously fed digesters

The methane production of the digester fed with 9.1 % vinasse and the production of the control digester reached stable values within one volume turnover. With higher vinasse concentrations, the methane production reached a maximum shortly after the beginning of the feeding. After having reached a second peak shortly thereafter, the methane production fell off slowly to a constant level. A typical example of the course of the methane production from the digesters receiving higher vinasse concentrations is given in Fig. 1.

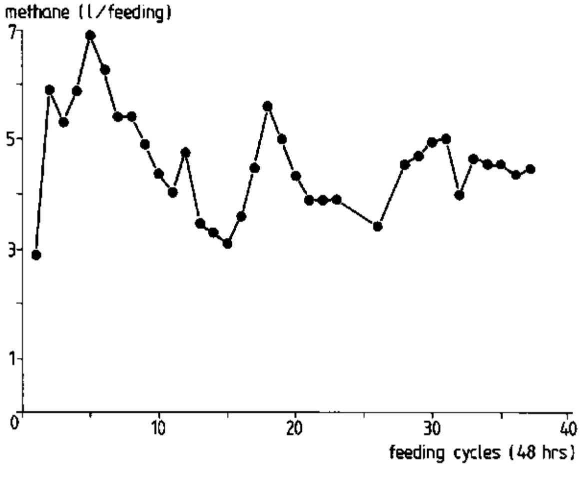

Fig. 1. The methane production from the digester receiving 22.7 % vinasse at 55 °C.

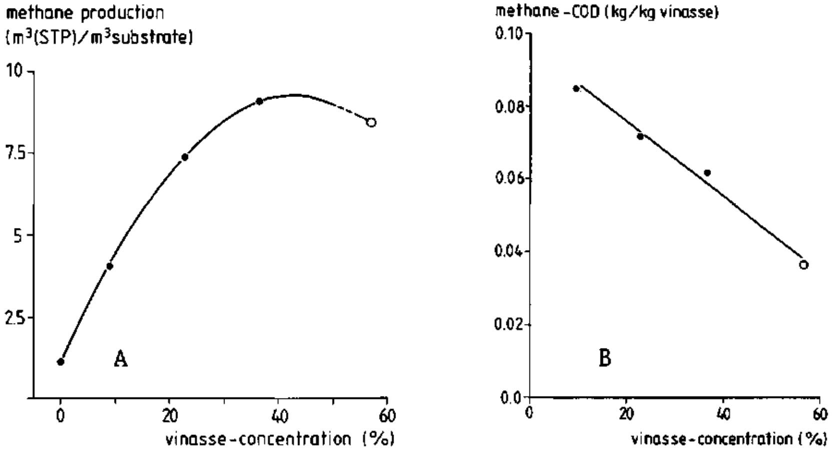

Fig. 2. Methane production at 55 °C as a function of the vinasse concentration at RT = (●) 18.2 days and (○) 33.3 days. The methane production is given (A) in m³ CH_4(STP)/m³ substrate (left) and, after correction for the methane production in the control digester, as (B) kg COD per kg vinasse (right).

The steady-state methane productions of the thermophilic digesters at 18.2 and 33.3 days retention time is given in Fig. 2A. After correction for the methane production from the control digester, an almost linear decrease in the methane production per kilogram vinasse fed, with increasing vinasse concentrations is apparent (Fig. 2B). To obtain information on the possible advantage of operating at increased retention times, the methane production after the last feeding of the digester receiving 36.4 % vinasse was followed for a longer period of time (Fig. 3).

The effluents of all the digesters were sampled for three to five consecutive feeding cycles after three volume turnovers. For the digesters receiving 0 and 56.7 % vinasse concentrations, this was made after two volume turnovers, because the values obtained in these cases are less important. The results of the effluent analyses are presented in Table II.

UASB experiments

The thermophilic granular seed sludge was first adapted to propionate, by replacing 1.7 kg/m³ of the influent COD of sucrose by propionate. The adaptation proceeded rapidly (Fig. 4, between arrows). Thereafter, the substrate was gradually shifted from a 2 % (w/v) sucrose to a 20 % vinasse solution. A higher vinasse concentration was applied when the VFA concentration in the

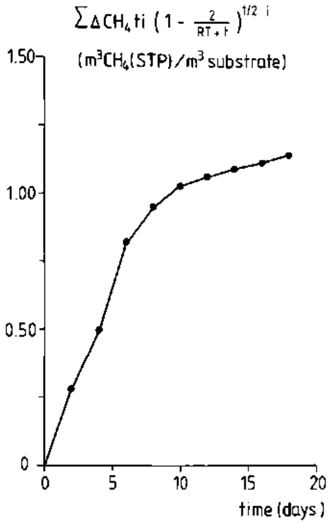

Fig. 3. Methane production from a digester fed with 36.4 % vinasse, after the last feeding cycle. The methane production is expressed in the form of

$$E = \sum_{i=1}^{i=t} \Delta CH_{4_{t_i}} \left(1 - \frac{2}{RT + t}\right)^{\frac{1}{2}i} ,$$

where E is the expected increase in the methane production (m^3 CH_4(STP)/m^3 substrate; $\Delta CH_{4_{t_i}}$ is the methane production at time interval t_i (m^3 CH_4(STP)/ m^3 reactor volume; and t the number of days with which the retention time would be increased. The factors 2 and ½ are introduced because feeding occurred only once per two days.

effluent remained constant for 3 days. During this shift, the methane generation rate and the hydraulic retention time were kept within the range 6.0-7.2 m^3(STP)/m^3day and 23.8-27.4 h, respectively. The adaptation took fairly long,

Table II. Some relevant parameters of the effluents from the semicontinuously fed digesters. The values represent the mean of three to five consecutive samplings after three volume turnovers (two for the 0 and 56.7 % vinasse concentrations.

Concentration (%)	RT (days)	Temperature (°C)	CODcent[a] (kg/m³)	CODvfa (kg/m³)	CODprop[b] (kg/m³)	pH	NH_4^+ (kg N/m³)
0	18.2	55	1.70	0.05	0.02	7.4	
9.1	18.2	55	4.78	0.64	0.15	7.6	0.98
9.1	18.2	30	4.67	1.62	0.59		
22.7	18.2	55	14.48	6.89	4.54	7.6	1.97
22.7	9.1	55	16.59	8.95	5.71		
22.7	18.2	30	12.16	6.34	2.20		
36.4	18.2	55	22.77	14.39	7.93	7.7	2.31
56.7	33.3	55	35.11	19.51	9.13	7.5	

a: centrifuged samples; b: propionate.

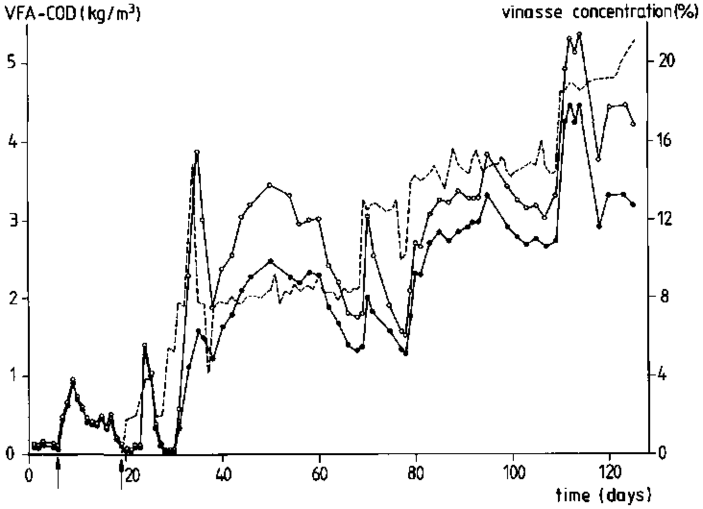

Fig. 4. Adaptation of thermophilic granular sludge to vinasse. Before starting the shift from sucrose to vinasse a propionate concentration of 1.7 kg COD/m^3 was imposed (between arrows). The (●) propionate and (o) total VFA-COD are given (left scale) as well as the (- - -, right scale) vinasse concentration, as the percentage vinasse used in the substrate. The methane generation rate and the hydraulic retention time were kept within the range 6.0-7.2 m^3 CH_4 (STP)/m^3day and 23.8-27.4 h, respectively.

as shown in Fig. 4. From Fig. 4, a clear relationship emerges between the vinasse concentration applied and the concentrations of VFA (mainly propionate) in the effluent.

During the shift in the substrate from sucrose to vinasse the granular appearnace of the sludge was gradually lost. The sludge aggregates decreased in diameter from 1-3 mm to less than 0.5 mm. Still, the settling characteristics remained satisfactory.

After completion of the adaptation, a series of hydraulic retention times (2.5-49 h) and vinasse concentrations (14-25 %) was imposed, resulting in loading rates varying from 17 to 98 kg COD/m^3day (Fig. 5). The effluent quality did not deteriorate with increasing loading rates, as is shown in Table III. The highest loading rate applied, namely, 98.3 kg COD/m^3day, at a hydraulic retention time of 2.5 h, could be handled for only a short period. In the first hours it seemed to be efficiently treated, accompanied by a methane generation rate of ca. 26 m^3(STP)/m^3day. A drastic sludge washout, however, occurred within two volume turnovers, resulting in a rapid deterioration of the process performance.

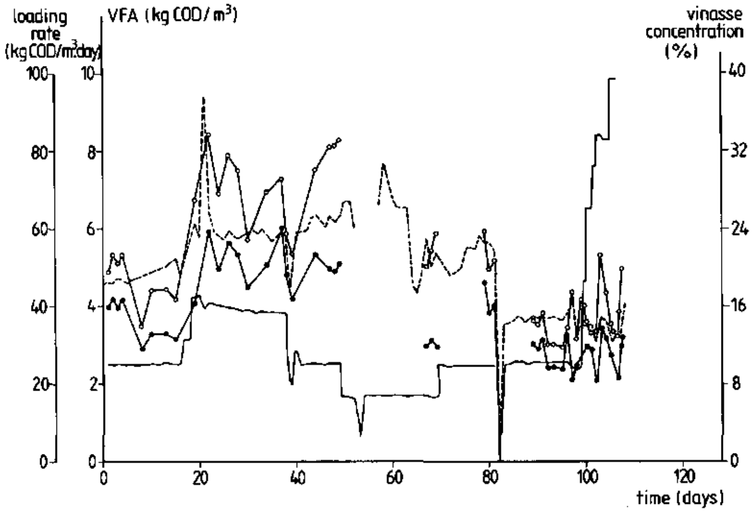

Fig. 5. Thermophilic anaerobic treatment of vinasse in a UASB reactor. The (●) propionate and (o) total VFA concentrations are given on the left-hand scale, the (——) space loading rate on the extreme left-hand scale, and the (- - -) vinasse concentration on the right-hand scale. The vinasse concentration is given as the percentage vinasse in the feed used.

Propionate formed the major VFA in the effluent, accounting for 70-90 % of the COD of the VFA (see Fig. 5), just as during the adaptation. A remarkable relation between the effluent VFA concentrations and the vinasse concentration in the feed, which was virtually independent of the organic loading rate applied, was experienced (Fig. 6).

Although the methane generation rates were high, the volume of the sludge bed did not exceed 50 % of the reactor volume. On the other hand, sludge retention was rather poor. This is illustrated by the fact that the difference between centrifuged and not centrifuged effluent samples averaged 8.3 ± 0.3 % of the influent COD; this value was 10.5 ± 0.4 % in a completely mixed, semicontinuously fed digester operated at a 12-day retention time with a 8 % vinasse solution without manure addition as feed.

DISCUSSION

The thermophilic digestion of vinasse in semicontinuously fed digesters produces a similar effluent quality as mesophilic digestion. However, propionate was the predominant VFA in the effluents of the thermophilic digesters, where-

Table III. Process performance of a thermophilic UASB reactor treating vinasse.

Loading rate (kg COD/m^{3}day)	Concentration (% dilution) (%)	Treatment[a] efficiency (%)	$\frac{\text{VFA-COD}_{out}}{\text{COD}_{in}}$ (%)	Potential treatment efficiency[b] (%)
17.2	21.0	61.9	21.2	83.1
25.6	14.8	59.0	19.7	78.7
25.5	19.6	64.9	17.4	82.3
24.8	22.3	56.8	20.1	76.9
25.3	24.9	52.6	27.1	79.7
38.6	23.6	58.8	23.5	82.3
83.6	14.6	59.6	22.5	82.1
98.3[c]	12.9	58.9[e]	ND[d]	ND[d]

a: (1 - filtered effluent COD/influent COD) x 100 %
b: (1 - filtered effluent COD - VFA COD)/influent COD) x 100 %
c: At this loading rate, sludge washout occurred a few hours after the loading rate was set at this value.
d: ND means not determined
e: This was determined after two volume turnovers.

as this was not so in the digesters operated at 30 °C. Apparently, propionate degradation is more seriously inhibited in the thermophilic temperature range, assuming the distribution of acidification products is very similar at mesophilic and thermophilic temperatures (12).

In the thermophilic digesters a strong influence of the vinasse concentration on the methane production rate was experienced. Considering the moderate loading rates applied, this at first sight looks surprising: the highest loading rate applied was ca. 4.6 kg COD/m^{3}day. This is well in the range of loading rates known to be feasible in the thermophilic digestion of livestock wastes (13,14). Apparently, the concentration of the vinasse, or the concentration of the toxic compounds in the vinasse, represents the primary factor determining the VFA concentrations in the effluent and , consequently, the overall performance of the process.

It must be noted that propionate-degrading bacteria apparently were active under all vinasse concentrations applied in the UASB experiments. This is clear from the results during the adaptation phase where, during days 50-60, the maximum effluent propionate/influent vinasse concentration is reached (Fig. 4). This ratio is appreciably lower during the rest of the experiments.

According to two basically different descriptive models for anaerobic treatment, the effluent quality from a completely mixed system without biomass retention is not strongly influenced by the retention time at retention times exceeding five

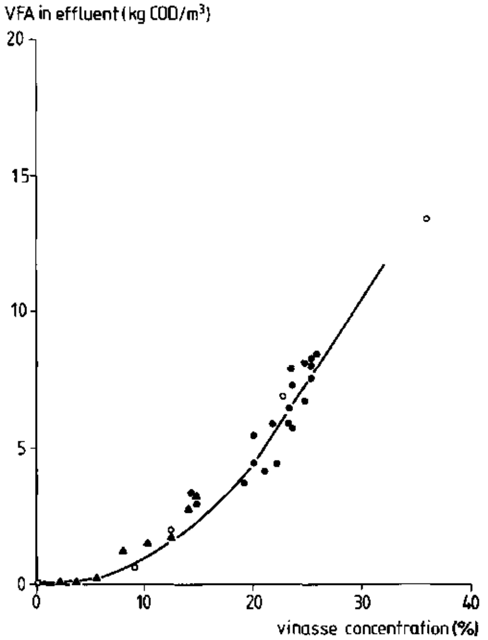

Fig. 6. Effluent VFA concentrations (expressed in kg COD/m^3) as a function of the vinasse concentration applied in the thermophilic treatment of vinasse. The values given refer to (▲) the situation immediately before an increase in the vinasse concentration during the shift from sucrose to vinasse as given in Fig. 4, and (●) before a change in the loading rate or the vinasse concentration in the experiments with vinasse as the sole substrate, as given in Fig. 5. Open symbols refer to values from the semicontinuously fed digesters (see Table II). The vinasse concentration is given as the percentage vinasse used in the feed.

times the minimum retention time (15,16). Varel and co-workers (14) observed in the digestion of cow manure at 55 °C only 13 % higher methane production per kilogram VS added at a retention time of 18 days, as compared with a 6-day retention time. From the minor difference observed in the effluent COD values in our experiments at 9.1 and 18.2 days retention time, respectively (Table II), it can safely be assumed that a retention time of 18.2 days is sufficiently long to allow extrapolation of the results to systems with a high biomass retention, with respect to the effluent quality. This can also be concluded by comparing the results shown in Figs. 2A and 3. The methane production (per m^3 substrate) for the 36.4 % vinasse feed at 18.2 days retention time is 8.13 m^3(STP)/m^3 substrate. The extra methane production that can be expected from an increase in the retention time with another 18.2 days is only 1.14 m^3(STP)/m^3 substrate, i.e, representing a 14 % increase.

As a consequence, the effluent quality of systems with a high biomass retention should be in the range of those found in the semicontinuous digestion experiments. The results in Fig. 6 indeed show a good fit between the effluent VFA concentrations measured in the digesters and those in the UASB experiments.

The adaptation of the seed sludge proceeds only very slowly. Even at the lowest vinasse concentration applied, there is an inhibitory effect. Although cation concentrations are quite high in the undiluted vinasse, the inhibition is already exerted at vinasse concentrations so low that any inhibiting effect from cations is virtually impossible (at 8 % vinasse, NH_4^+ and K^+ concentrations are 0.48 and 1.31 kg/m³, respectively). Therefore, presumably other compounds present in the vinasse, possibly those giving it its dark brown color, may exert an inhibiting action. Several phenolic compounds, present in the original material, are oxidized upon contact with oxygen, yielding a dark brown color. These oxidized phenolic compounds are toxic for methanogenic systems, as was recently shown (17).

With higher vinasse concentrations, however, cations such as NH_4^+ and K^+ will also contribute significantly to the toxicity of the substrate. For instance, thermophilic digestion of cow manure with a total ammonia concentration exceeding 3.0 kg N/m³ can be considered impossible (18). In the UASB experiments, the NH_4^+-concentration were in the range 0.65-1.25 kg N/m³. Although these values are not exceedingly high, mesophilic granulation is reported to proceed very poorly with total ammonia concentrations of 1.0 kg N/m³ (19).

Granular sludge has superior settling characteristics as compared with flocculent sludge (7). Thus, the observed "disintegration" of the granules during the adaptation phase would be a matter of considerable concern, unless it would proceed only to a limited extent. The results obtained show that the sludge retains good settling characteristics, despite the distinct decrease in the size of the particles. Still, exceptionally high loading rates could be satisfactorily accommodated, with sludge particles < 0.5 mm and < 50 % of the reactor volume occupied by the sludge bed. The lower liquid viscosity at thermophilic temperatures may also play an important role in this. The lower viscosity permits not only the sludge particles to settle more easily, but also the separation of the produced biogas to proceed more readily. As a consequence, smaller particles can be retained in the reactor at 55 °C as compared with 30 °C.

Considering the results obtained with granular seed sludge, the question rises whether or not it would be possible to cultivate a sludge with equal or better qualities on vinasse as substrate, using cow manure as inoculum. Previous attempts to cultivate a thermophilic granular sludge using VFA solutions as feed were not successful (9). Presumably a rather delicate start-up procedure should be applied. On the other hand, we observed a somewhat similar granule "disintegration" upon feeding sucrose-cultivated granular sludge with VFA solutions for prolonged periods of time, i.e., longer than 6 months (9). As yet, the reason for this disintegration is unclear. It seems reasonable to assume that the sugar fermenting organisms, presumably responsible for the structure of the granules (9,20), will decay and that the granule structure falls more or less apart with their decay.

Finally, it must be emphasized that for other types of vinasse, i.e., with different concentrations of toxic compounds, the situation may be quite different. Sugarcane vinasse, for instance, generally has much lower cation contents (21). Consequently, it is likely that this type of vinasse can be treated with a higher efficiency, or at higher concentrations.

Although there seems to be no lower threshold concentration for the toxicity of the vinasse, it will be evident that the lower strength concentrations will be handled with the highest efficiency. When dealing with high strength wastewaters, one has to find the optimum between the treatment efficiency in terms of methane evolved per unit of substrate, and the methane generation rate in terms of m^3 CH_4/m^3 reactor volume. These two factors will ultimately determine the concentration that can be applied in practice.

To summarize the results of this study, it may be concluded that thermophilic treatment of high-strength wastewaters in UASB reactors is much more attractive than treatment in reactors with little if any biomass retention, and that it compares favorably with treatment in mesophilic UASB systems. The degradation of propionate is the limiting factor; apparently, it is more seriously affected by inhibition than under mesophilic conditions. High loading rates can be handled, although dilution may be necessary, depending on the toxicity of the wastewater, to obtain high treatment efficiencies. High vinasse concentrations negatively affect the size of the sludge granules, but the effect is not strongly interacting with the process performance.

APPENDIX

calculation of the expected increase of the methane production

For a semicontinuously fed reactor with a retention time of RT days and a feeding cycle of one day, the replacement of the reactor content is the following fuction of time:

$$F = \left(1 - \frac{1}{RT} \right)^{i}$$

in which F is the fraction of the original content at $t = 0$ remaining in the reactor, and i is the number of days passed. With the aid of this equation, an estimate of the increase in the methane production per unit of substrate, when increasing the retention time, can be produced, using the methane production of a semicontinuously fed digester of which the feeding has been stopped.

Name i the number of days passed after finishing the last feeding cycle. Upon an increase in the retention time with t days to RT + t days, we should account for the fact that the fraction of the reactor content that has been replaced by new influent cannot produce methane inside the reactor. So, the methane production has to be corrected for the loss by replacement. With $\Delta CH4,i$ as the methane production during day i (m^3/m^3 reactor volume), we obtain as an estimate for a 1-day increase in the retention time:

$$E = \Delta CH_4,1 \left(1 - \frac{1}{RT + 1} \right)^{1}$$

With E in m^3/m^3 substrate, and similarly, for a 2-day increase:

$$E = \Delta CH_4,1 \left(1 - \frac{1}{RT + 2} \right)^{1} + \Delta CH_4,2 \left(1 - \frac{1}{RT + 2} \right)^{2}$$

This can be combined in the following function:

$$E = \sum_{i=1}^{i=t} \Delta CH_4,i \left(1 - \frac{1}{RT + t} \right)^{i}$$

which can be considered correct only if no major changes will occur in the microbial population, when the retention time is increased. With a feeding cycle of t_i days, the expected increase becomes

$$E = \sum_{i=1}^{i=t} \Delta CH_4,\ t_i \left(1 - \frac{t_i}{RT + t} \right)^{i/t_i}$$

in which $\Delta CH_4,t_i$ is the methane production during the time intervals t_i (m^3/m^3 reactor volume).

ACKNOWLEDGEMENT

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7. Separation of the propionate degradation to improve the efficiency in the thermophilic anaerobic treatment of acidified wastewaters

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ABSTRACT

The degradation of propionate generally is limiting in the thermophilic methane digestion of acidified wastewaters. In this paper the propionate degradation in thermophilic systems is studied. A two-stage process is developed, in which the propionate degradation is spatially separated from other conversions in which hydrogen is involved. Significantly better results were obtained with the two-stage system as compared with a one-stage system with an equal total volume: loading rates of 20-50 kg COD/m^{3}d showed a 10-13 % better treatment efficiency in the two-stage system.

It is concluded that the removal of the biogas evolved in the first degradation processes is the cause of the superior performance of the two-stage process.

INTRODUCTION

In high-rate anaerobic treatment systems propionate usually is the first compound of which the breakdown deteriorates. Apparently the breakdown of propionate is very delicate. In its complete degradation three different bacteria are involved, of which the first converts propionate into acetate, hydrogen and carbonate. The other two are methanogens, converting acetate and carbonate plus hydrogen to methane. Specimens of all three bacterial groups, relevant for mesophilic high-rate systems with a high biomass retention, have been isolated and described (Boone & Bryant 1980, Huser *et al* 1982, Mah & Smith 1981).

Propionate belongs to a group of compounds which for thermodynamic reasons can only be degraded anaerobically when the hydrogen concentration is low. The hydrogen concentration is kept low by hydrogen consuming methanogens, or by sulfate reducing bacteria. The exchange between hydrogen producing and hydrogen consuming bacteria is known as interspecies hydrogen transfer (Ianotti *et al* 1973). Of the compounds depending on interspecies hydrogen transfer for their degradation propionate requires almost the lowest hydrogen concentration. This is made clear in Table I, in which the thermodynamic properties of the various relevant reactions are compared at 25 and 55 °C. Only acetate, when

Table I. ΔG values of some relevant reactions. Conditions were as follows: acid concentrations (ionized and non-ionized), 0.01 M; pH = 7.2; 20 % CO_2 in the dry biogas; ionic strength, I, 0.087 M.
Values were calculated from ΔGf_0^{25} and ΔHf_0^{25} values for organic (*a*) and inorganic (*b*) compounds, and an approximation for the calculation of ΔGf_0^{55} values (*c*). The HCO_3^- concentration was calculated to be 57 mM at 25 °C and 31 mM at 55 °C (*d,e,f*). The concentrations of the volatile fatty acids were calculated to be 28, 36 and 32 µM at 25 °C, and 30, 40 and 39 µM at 55 °C for acetic, propionic and butyric acid, respectively (*g,h*). pH_2 is the partial pressure of H_2. 1 µM of H_2 corresponds with 1270 ppm at 25 °C and 1370 ppm at 55 °C, in the gas phase (*i*).

REACTION		ΔG (kJ/Mole)	
substrates	products	25 °C	55 °C
acetic + H_2O	methane + HCO_3^- + H_2	-26.90	-32.80
4 H_2 + H^+ + HCO_3^-	methane + HCO_3^-	-127.74 - 22.84 log(pH_2)	-114.98 - 25.15 log(pH_2)
acetic + 4 H_2O	2 HCO_3^- + 4 H_2 + 2 H^+	+100.83 + 22.84 log(pH_2)	+82.17 + 25.15 log(pH_2)
propionic + 3 H_2O	acetic + HCO_3^- + 3 H_2 + H^+	+62.55 + 17.11 log(pH_2)	+48.12 + 18.87 log(pH_2)
butyric + 2 H_2O	2 acetic + 2 H_2	+32.55 + 11.42 log(pH_2)	+22.84 + 12.55 log(pH_2)

a: Dean (1979) pp. 9-65 - 9-94; b: Weast (1979) pp. D-78 - D-79; c: Chang (1981) p. 149; d: Stumm & Morgan (1970) Ch. 3-4, pp. 79-85; e: idem, Ch. 4-3 pp. 124-129 and Ch. 4-7, pp. 148-152; f: Schumpe *et al* (1982) p.14;
g: Weast (1979) p. D-168; h: Stumm & Morgan (1970) p. 149; i: Wilhelm *et al* (1977) p. 226.

degraded via interspecies hydrogen transfer, demands for a lower hydrogen concentration. This conversion of acetate, however, does not seem to have much practical significance, although it has been demonstrated recently to exist under thermophilic circumstances (Zinder & Koch 1984). The need for a low hydrogen concentration, which for 55 °C is shown in Fig. 1, and the apparent extreme sensitivity of the propionate converting organism (Boone & Bryant 1980) may explain the often observed increased levels of propionate in the effluents of anaerobic reactors.

Thermophilic anaerobic digestion systems offer attractive kinetic advantages in comparison with mesophilic systems (Varel *et al* 1980, Schraa & Jewell 1984, Wiegant & Lettinga, 1985, Wiegant *et al* 1985). These systems, however, have a stronger tendency towards a retarded degradation of propionate. The effluents of semi-continuously fed digesters receiving vinasse - the bottom slops of alcohol distilleries - diluted to 28 kg COD/m^3, and operated at a retention time of 18.2 days, contained similar concentrations of volatile fatty acids (VFA) at 30 and 55 °C, 6.89 and 6.34 kg COD/m^3, respectively.

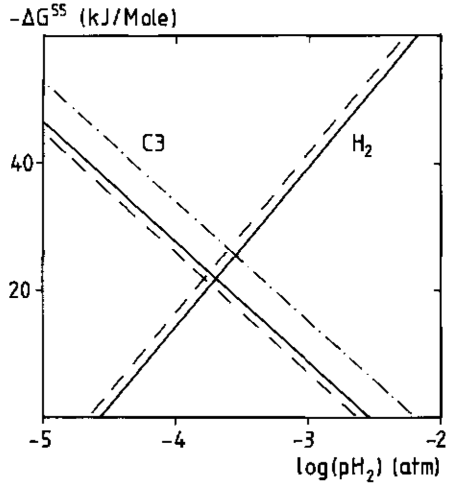

Fig. 1. - ΔG^{55} of the conversion of propionate to acetate, hydrogen and bicarbonate and of the conversion of hydrogen and bicarbonate to methane, as a function of the partial pressure of hydrogen in the gas phase. In the Figure the following conditions are assumed: pH = 7.2, a molar acetate/propionate ratio of 1.0 and 20 % CO_2 in the biogas (——). Different conditions are given with dotted lines: (-· - - -), a molar propionate/acetate ratio of 10; (- - -), 40 % CO_2 in the dry biogas.

Propionate contributed for 35 % at 30 °C, but for 66 % at 55 °C to the COD of the VFA (Wiegant *et al* 1985). In thermophilic upflow anaerobic sludge blanket (UASB) reactors (Lettinga *et al* 1980), the decrease in the treatment efficiency of substrates consisting of a mixture of volatile fatty acids at increased loading rates, is caused by a deterioration of the propionate degradation (Wiegant & Lettinga 1985).

The dislocation of the propionate degradation into a second methanogenic reactor, or a specific part of the reactor, might be a solution to this problem. In this way the extremely sensitive propionate converting bacteria are mainly exposed to propionate as substrate, and consequently the hydrogen concentration will be mainly generated by the propionate degradation itself. In a sense propionate degradation will become a self regulatory process, under such conditions, with respect to the hydrogen concentration. To a much lesser extent, this will also be the case for the acetate concentration. Acetate exerts less influence on the thermodynamics of the conversion of propionate (*cf* Table I).

The objective of this study is to investigate the advantage of a two-stage methane digestion system as outlined above, and to provide insight in the underlying principles. The approach was to obtain data on the specific growth rates of the bacteria performing relevant conversions. These values can provide information about the limiting processes. A continuous flow stirred tank reactor (CSTR) was set up with propionate as substrate, to verify the measurements of the specific growth rate on propionate, and to obtain information about the efficiency of the digestion of influents with a high propionate concentration. The content of the CSTR was also used for the determination of the substrate half-saturation constant for propionate.

A two-stage methanogenic system, consisting of two upflow anaerobic sludge blanket (UASB) reactors connected in line, was investigated using a mixture of VFA as substrate. Propionate degradation was also performed in a single UASB reactor, with propionate as the sole substrate, thus providing information about the performance of the second stage in a two-stage system.

The research was carried out at 55 °C, well within the thermophilic temperature range. We believe, however, that the results can also provide insight in anaerobic digestion processes in other temperature ranges, and that they are not specific for the UASB process, but for other high-rate processes as well.

MATERIALS AND METHODS

Temperature

All experiments were carried out at 55 °C.

Media

The composition of the media for growth rate measurements and of the feed solutions is given in Table II. The media for growth rate measurements and the content of reactors were made oxygen-free via a procedure previously described (Wiegant & Lettinga 1985).

Inocula and seed sludge

In all experiments cow manure was the primary source of bacteria. Growth rate measurements and CSTRs were inoculated with either fresh cow manure or the content of thermophilic digesters fed with dilute manure. In the UASB experiments thermophilic granular sludge was used, cultivated with glucose as substrate, using cow manure as inoculum, in a way similar to that previously

Table II. Media constituents and characteristics of the experiments performed.

type of experiment	specific growth rate				CSTR	UASB	UASB	
principal substrate	H_2/CO_2	C2	C3	C4	C3	mixture	C3	
food constituents:								
H_2/CO_2	80							1/day
acetate		2.0				1.0-3.0		g/l
propionate			2.75		1.0-5.0	1.0-3.0	1.0-3.0	g/l
butyrate				1.9		1.0-3.0		g/l
cow manure			0.71	0.12				% VS
yeast extract	0.3	0.1		0.1				g/l
idem					0.1	0.1	0.1	g/g COD
K-PO_4; pH 7.5		25						mM
$NaHCO_3$	0.2						0.0-6.0	g/l
Na_2HPO_4	0.2							g/l
nutrients[a]	+	+	+	+	+	+	+	

a: nutrients as described previously
Sulfur was added as Na_2S. $9H_2O$ (100 mg/l) in growth rate determinations and as Na_2SO_4 (70 mg/l) in CSTR and UASB experiments.

described (Wiegant & Lettinga 1985). The reactors were seeded with this sludge for 60 % of the reactor volume. The sludge was kindly provided by J. Voetberg, IBVL, Wageningen, Holland.

Growth rate measurements

The measurement of the growth rate of hydrogen consuming methanogens was performed by percolating a 80:20 H_2/CO_2 mixture through a narrow tube (0.25 mm internal diameter) in the liquid of a 470 ml vessel with a liquid volume of 300 ml. The liquid was stirred with a magnetic stirrer at maximum allowable speed. The methane production rate was periodically measured by determining the methane concentration in the gas released from the vessel after inoculation. The slope of the line fitting the natural logarithm of the methane production versus time gives the specific growth rate.

Growth rates on VFA were determined in batch experiments. 315 ml serum bottles with a 250 ml liquid volume were used for the determination of the growth rate of acetate utilizing methanogens. 20 ml of the contents of a digester fed with diluted manure (2.5 % VS) at a retention time of 5.5 days and at 55 °C was used as inoculum. The accumulated methane production was followed after the addition of the inoculum. Mathematical details on this method are presented by

Powell (1983).

The growth rates of propionate and butyrate degrading bacteria were determined in 6 l reactors with a 5.5 l working volume. For the determination of the specific growth rate of propionate degrading organisms, diluted cow manure (0.7 % VS) was digested for one month. Thereafter propionate was added, and the accumulated methane production was measured. For the determination of the specific growth rate of bacteria converting butyrate, an acetate-adapted population was cultivated by feeding acetate batch-wise for three weeks to an inoculum of diluted cow manure (0.1 % VS). Butyric acid was added thereafter, without supplying any extra inoculum. In the latter two experiments, exponential methane production for over three doubling times was assumed to be the result of growth of the bacteria converting the VFA. The evolved biogas passed a column of soda lime pellets and a liquid (0.4 M NaOH) displacement system, and was accounted for as methane. In the case of growth on butyrate, the outcoming solution was collected in siphons of *c* 15.5 ml, of which each time they were emptied the exact time was registered automatically. The media composition is summarized in Table II.

CSTR experiments

A 2.2 l CSTR was set up as schematically shown in Fig. 2. It was fed with propionate, yeast extract and minerals (Table II). A determination of the half-saturation constant for propionate was carried out with the content of the CSTR, after an interruption of the feed supply.
Curve fitting was performed by a least square minimalization of a numerical integration of the Monod equation for the data points. The intervals for μ_{max}

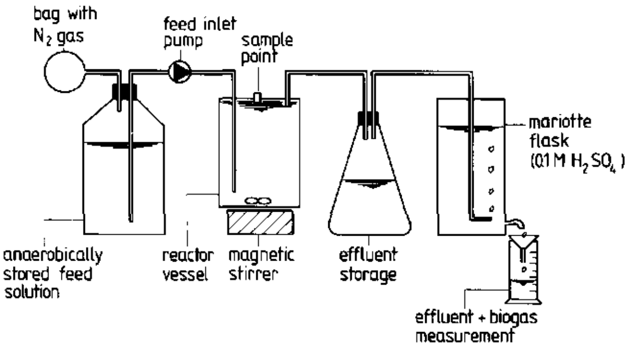

Fig 2. A scheme of the CSTR used. The scheme is not to scale.

and K_S that were obtained were visually checked for their fit of the data points in the lower concentration region.

UASB experiments

Three non-stirred UASB reactors with an internal diameter of 5 cm were used. Two of them, each having a volume of 595 ml, were connected in line, thus providing a two-stage digestion system. Over 85 % of the effluent of the first reactor was pumped directly into the second. This two-stage system was fed with a 1:1:1 (w/v) mixture of acetate, propionate and butyrate (Table II). A third UASB reactor, with a volume of 710 ml, received only propionate.

Analyses

The methane content in the biogas from the CSTR and the UASB reactors was measured with a Packard Becker 407 gas chromatograph with a thermal conductivity detector, equipped with a 2 m x 2 mm (ID) stainless steel column with Porapak Q (80-100 Mesh). Helium was used as the carrier gas (23 ml/min). Both column and detector were at room temperature.

During measurement of the growth rate on hydrogen and carbon dioxide, methane in the gas phase was determined on a Packard Becker 407 gas chromatograph with FID, equipped with a 1.5 m x 2 mm (ID) stainless steel column with Molecular

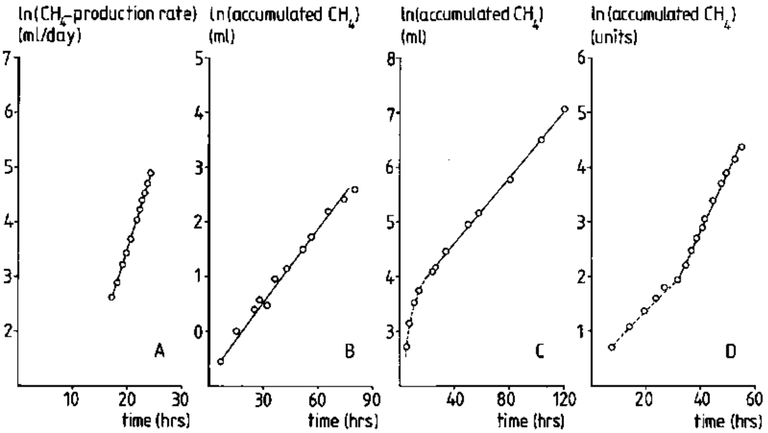

Fig. 3. Plots of the natural logarithm of the methane production rate from H_2/CO_2 (A) and of the accumulated methane production rate from acetate (B), propionate (C), and butyrate (D) as a function of time. In D the methane production is given in units of c 15.5 ml.

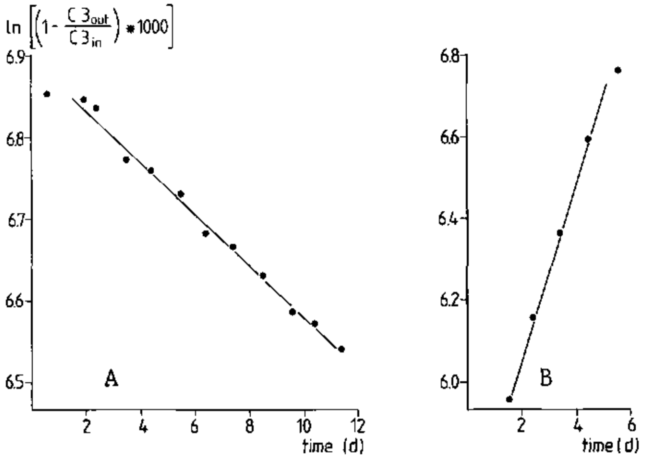

Fig. 4. The efficiency of the propionate degradation as a function of time at two different retention times. In Fig. 4A retention time was 45.3 hrs (D = 0.0221 hr^{-1}) and the specific growth rate is $\mu = 0.0221 - 0.0015 = 0.0206\ hr^{-1}$. In Fig. 4B the retention time was 108.4 hrs (D = 0.0092 hr^{-1}), and $\mu = 0.0092 + 0.0089 = 0.0181\ hr^{-1}$.

Sieve 5A (60-80 Mesh). N_2 was used as the carrier gas (30 ml/min). Oven temperature was 170 °C and detector temperature 220 °C.
All other analyses were carried out as described elsewhere (Wiegant & Lettinga 1985).

RESULTS

Growth rate determinations

The methane production rate with H_2/CO_2 as substrate, and the accumulated methane production from acetate, propionate and butyrate is shown in Fig. 3. The lines in Fig. 3 represent specific growth rates of 0.33 hr^{-1} for H_2/CO_2, 0.040 hr^{-1} for acetate, 0.030 hr^{-1} for propionate and 0.109 hr^{-1} for butyrate.

CSTR experiments

The maximum specific growth rate of the propionate degrading bacteria in the CSTR appeared to be lower than measured in the batch-experiments. At a retention time of 45.3 hrs a slow decrease in the efficiency of the propionate degradation occurred, whereas at $\Theta = 108.4$ hrs a clear increase in the efficiency was observed. From both loss and increase of the efficiency the maximum specific growth rate could be determined, which averaged 0.019 hr^{-1} (Fig. 4).

Table III. Steady state data of the CSTR with propionate as the substrate, taken during five consecutive days. Values in parentheses refer to standard deviations.

retention time (hrs)	influent propionate concn (mg/l)	effluent propionate concn (mg/l)	treatment[a] efficiency (%)
81.4 (3.4)	5000	54 (16)	98.3 (0.4)
68.4 (0.7)	2500	470 (54)	81.7 (2.2)

a: (1 - effluent VFA-COD/influent COD) x 100 %

The adaptation of the system to new influent concentrations and retention times proceeded only very slowly, with adaptation times exceeding seven volume turnovers. Two sets of steady state data were obtained, which are presented in Table III.

K_s determination

After an interruption of the medium supply, a slug addition of propionate was made and the propionate concentration was followed. The results of this experiment are shown in Fig. 5. The data are fitted well by using a μ_{max} of 0.0000-0.0018 hr^{-1} and a K_s of 6-18 mg COD/l of propionate. With growth rates higher than 0.004 hr^{-1}, no reasonable fits were obtained.

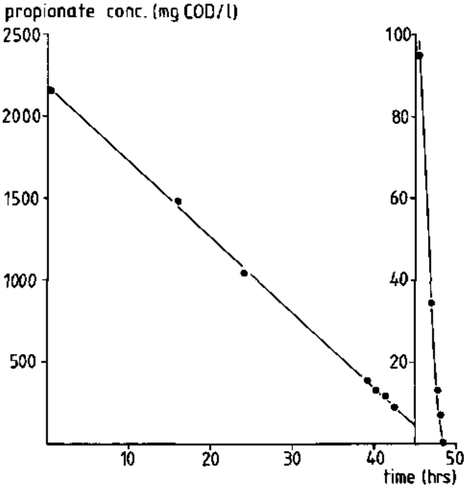

Fig. 5. Course of the propionate degradation after batch addition of propionate.

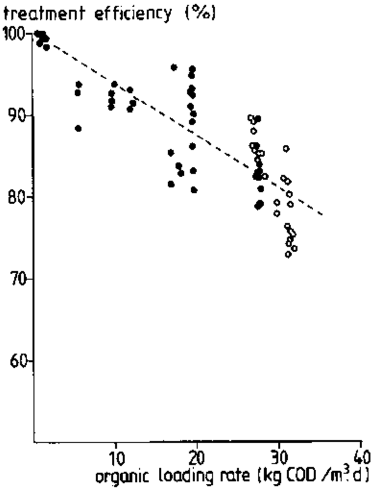

Fig. 6. The treatment efficiency, $(1 - \text{VFA-COD}_{out}/\text{COD}_{in}) \times 100\ \%$, of a UASB reactor with propionate as substrate, as a function of the organic loading rate. (●), without and (o), with addition of 6.0 kg/m³ $NaHCO_3$.

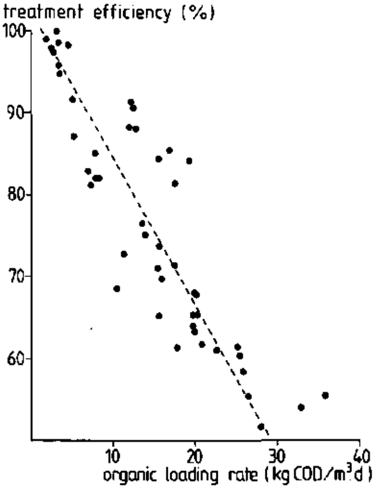

Fig. 7. The treatment efficiency of the second stage reactor of a two-stage UASB system, as a function of the volumetric loading rate.

UASB experiments

The degradation of propionate was also investigated in a one-step UASB process, using a thermophilic seed sludge. The reactor was operated for 180 days. The first 30 days were needed to accommodate to an organic loading rate of 10 kg COD/m³d. After this period the reactor adapted considerably faster to increased loading rates. To allow a comparison with the two-stage system treating a VFA mixture, $NaHCO_3$ was added to the influent from 115 days after the start of the experiment, to equalize the Na^+ and HCO_3^- concentrations in both experiments. The addition of 6 g/l $NaHCO_3$ had to be made stepwise, in a period of 12 days, because a slug addition of 6 g/l $NaHCO_3$ led to process failure.

In Fig. 6 the treatment efficiency of this reactor is presented as a function of the organic loading rate. In the Figure values are presented of days during which no obvious overloading occurred, from 5 days after an increase in the loading rate. The addition of $NaHCO_3$ to the influent led to increased concentrations of acetate in the effluent, from 34 ± 10 to 282 ± 17 mg/l, but propionate concentrations did not change. The sludge activity in the reactor, determined at the end of the experiment, was 1.14 kg CH_4-COD/kg VSS.d.

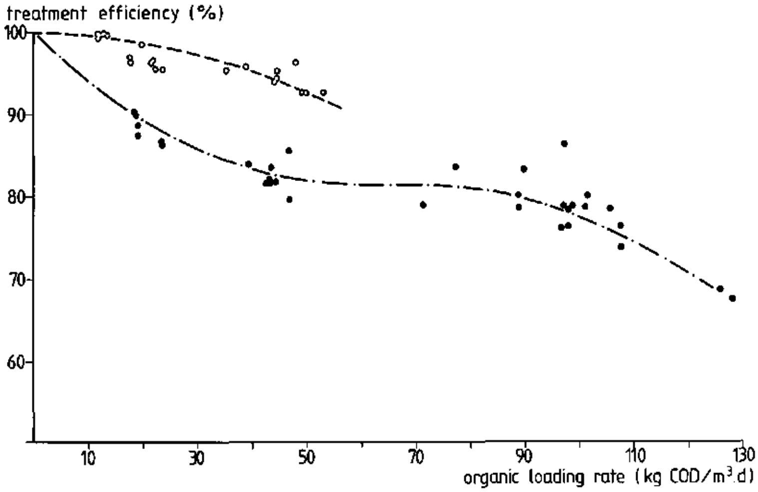

Fig. 8. The treatment efficiency of a two-stage UASB system. (o), first stage and (●), total system. The efficiency of the two-stage system is related to the total volume of the system.

Two-stage methanogenic treatment system

Initially, both reactors of the two-stage system were individually adapted to a mixture of acetate, propionate and butyrate during one month, after which they could handle a loading rate of 14 kg COD/m^{3}d at a treatment efficiency, (1 - eff.VFA-COD/inf.COD)x 100 %, exceeding 95 %. Thereafter the reactors were connected in line by pumping the effluent from the first reactor directly into the second reactor. The propionate-COD accounted for 80 ± 9 % of the VFA-COD in the effluent of the first stage reactor, except during overloading, technical casualties etc. After disturbances, the recovery of the propionate degradation required very much time. The recovery from a moderate pH-shock (pH down to 5.8 - 6.0 for c 4 hrs), before which the first stage reactor had a propionate converting activity of 9-10 kg COD/m^{3}d, lasted 12 days, during which the propionate degrading activity increased steadily from 0.7 to 10.5 kg COD/m^{3}d.

The performance of the reactors is presented in Figs. 7 and 8. Out of 210 days of operation data have been selected of days during which no obvious overloading occurred, i.e. data of days shortly after disturbances or during adaptation to an increased loading rate are not shown. The data are presented in Fig. 7 for the second stage reactor. Fig. 8 shows the treatment efficiency of the two-stage system as a whole, related to the total volume of the two reactors, together with the efficiency of the first stage reactor.

The maximum activities of the propionate conversion measured were 15-16 kg COD/m^3d of propionate converted for the first stage reactor, at effluent acetate levels of 370 ± 160 mg COD/1. For the second stage reactor the maximum propionate degrading activity was 8.5-9.5 kg COD/m^3d, at acetate levels of 84 ± 12 mg COD/1. The reactor fed with only propionate had considerably higher maximum values of the propionate converting activity of 25-26 kg COD/m^3d (Fig. 6). The effluent acetate levels during maximum activity in the latter experiment averaged 280 ± 20 mg COD/1.

At the end of the experiment, the methanogenic activities (methane production rate divided by VS content) of the sludges in the first and second stage reactor were 3.51 and 0.74 kg COD/kg VS.d for their respective substrates.

DISCUSSION

Propionate degrading bacteria apparently exert the lowest specific growth rates of the organisms relevant for the methanogenesis from acidified wastewaters (Fig. 3). However, the specific growth rate of the acetate degrading population is very high; presumably this high growth rate is exerted by a *Methanosarcina* (Zinder & Mah 1979). In anaerobic reactors with a high cell residence time generally an acetoclastic methanogen of the genus *Methanothrix* is predominant, in both the mesophilic and thermophilic temperature range (Huser *et al* 1982, Zinder *et al* 1984a). The thermophilic *Methanothrix* has a specific growth rate at 55 °C of 0.017 hr^{-1} (Zinder *et al* 1984b), which is even lower than the specific growth rate of the thermophilic propionate degrading bacteria.

Despite the fact that in systems with a high cell residence time propionate converting bacteria do not have the lowest maximum specific growth rate, propionate degradation is nevertheless the limiting step in the methanogenesis from acidified wastewaters. Presumably the intermediate formation of H_2 is responsible for that. The specific growth rate of propionate converting bacteria is strongly dependent on the partial H_2-pressure. This is evidenced by the fact that *Syntrophobacter wolinii*, the only propionate converting organism isolated as yet, exhibits a far higher growth rate in coculture with a hydrogen consuming sulfate reducer than in coculture with a hydrogen consuming methanogen((Boone & Bryant 1980). The reason for this is the higher affinity for hydrogen of the sulfate reducing bacteria in comparison with methane-forming bacteria (Lovley *et al* 1982, Kristjansson *et al* 1982), resulting in a lower partial pressure of hydrogen during the conversion of propionate.

The large difference in the growth rates of the propionate converting bacteria as found in the batch- and CSTR-experiments may also be explained by differences in the partial hydrogen pressure. Moreover, the steady state data from the CSTR experiments (Table III) are not fully consistent with the measured growth rate. Ignoring the decay rate and solving the equation $S/K_s = D/(\mu_{max}-D)$ with S is substrate concentration, K_s is half saturation constant (both in mg/1), D is dilution rate (reciprocal of the retention time) and μ_{max} is maximum specific growth rate (both in hr^{-1}), for the two sets of data, yields a maximum specific growth rate of 0.0146-0.0154 hr^{-1}, and a K_s of 5-19 mg/1. The K_s, however, compares well with the 6-18 mg COD/1 (4-12 mg/1), found in the separate determination, during which apparently no growth occurred.

In a culture degrading propionate or butyrate as the single substrate, the acetogens will exhibit a growth rate virtually equal to that of the hydrogen consuming methanogens. During the degradation of butyrate or propionate, the hydrogen will reach an optimal concentration, which permits the highest degradation rate. The hydrogen concentration can be assumed to limit the growth rate of both bacteria, as in the mesophilic temperature range, when hydrogen is removed by methanogens (Boone & Bryant 1980, McInerney *et al* 1981). Under assumption of $S/K_s = \mu/(\mu_{max}-\mu)$, with μ is the specific growth rate of the hydrogen consuming methanogen (equal to the growth rate of the propionate and butyrate converting bacteria during propionate and butyrate degradation, respectively), one can calculate that the hydrogen concentration during maximum growth or activity of the butyrate converting bacteria is 4.9 times as high as that during maximum propionate conversion. As a consequence, propionate degradation will be highly inhibited during periods of high activity of the butyrate converting bacteria.

This lower tolerance for a high hydrogen partial pressure would implicate that only a limited part of a UASB reactor is available for propionate degradation, viz. that part where the H_2 pressure is maintained at a sufficiently low level. This would especially be the case shortly after an increase in the loading rate, but even in a balanced reactor one can assume the hydrogen pressure to be too high for propionate degradation in the lower parts, because the butyrate degradation will proceed at high activity near the feed inlet point.

The paramount difference between the two-stage methanogenic system and a one-stage methanogenic process treating a mixture of VFA, seems to lie in the fact that the more rapid acetogenic degradation processes, of e.g. butyrate, are restricted to the first stage. The biogas evolved in this first stage reactor will have high H_2 levels. As a consequence, a new equilibrium of the biogas with the liquid in the second phase can develop. In the second stage reactor hydrogen is mainly generated from the propionate degradation itself. Apparently this lower hydrogen pressure permits a more effective propionate degradation. This hypothesis is speculative, as there are no measurements of the hydrogen pressure in the biogas from both reactors. It is in accordance with our finding that in the two-stage digestion of vinasse a reasonable effective propionate degradation in the second reactor was accompanied by lower partial pressures of hydrogen in the biogas, viz. 184 ppm in the biogas from the first, and 161 ppm in the biogas of the second reactor.

The activity of the sludge in the second stage reactor, as determined "on site", is significantly lower than the activity of the sludge in the UASB reactor fed with propionate. This indicates that the hydrogen concentration in the influent for the second stage reactor still influences the propionate degradation in the second stage reactor. It must be noted that the role of acetate is somewhat similar as that of hydrogen. Acetate exerts a less strong influence on the thermodynamics of the conversion of propionate (*cf* Table I), but it is known to be inhibitory for the degradation of propionate (de Zeeuw 1984). In our case the acetate concentrations were rather low.

Comparing the effectiveness of the second stage reactor with the reactor fed with propionate shows that the latter is far more effective. Even when 6 kg/m^3 of $NaHCO_3$ was added, which increased the acetate concentration to levels comparable with those in the influent of the second stage reactor, the difference is striking (see Figs. 6 and 7). So it seems that acetate did not play an important role in the phenomena observed.

The question rises whether a staging of the methanogenic process would be appropriate in the case of a retarded propionate degradation caused by some inhibiting compound. It seems impossible to meet the problems, caused by a severe inhibition, by changing the process design only, except in the case of gaseous compounds like H_2S.

So, in principle, two situations can be distinguished when an effective propionate degradation is lacking. In the case of toxification it looks hardly, if at all, possible to modify the process in such a way that this problem can be solved. However, in the case of an inhibition by H_2, staging of the methanogenic process may be very profitable. As it seems that the separation of the biogas evolved in the first stage degradation processes is the reason for the two-stage process being more effective, it may be possible to construct a kind of internal gas separator in a one-stage UASB reactor.

Although all the results presented here were obtained in experiments in the thermophilic temperature range, it is well known that the breakdown of propionate often offers serious problems under mesophilic conditions. A spatial separation of the propionate degradation in these cases might also be an effective way to deal with these problems.

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8. Behaviour of thermophilic methanogenic sludge under suboptimal feeding conditions and temperatures

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ABSTRACT

The behaviour of thermophilic anaerobic sludge under suboptimal conditions was investigated. The decay rate during storage at 20 and 30 °C was found to be comparable with that of mesophilic sludge. At 55 °C the decay rate is much higher. Feed interruptions of one to four days result in an increasingly poor performance upon resuming the feeding. Particularly the degradation of propionate deteriorates in this case.

Lowering of the operation temperature from 55 to 30 °C at continued feeding results in an overloading of the acetate degrading population. However, the recovery after restoring the temperature is very rapid. The results indicate that thermophilic sludge is more versatile than previously thought, and consequently, that thermophilic treatment can be successfully applied for discontinuously discharged wastewaters.

INTRODUCTION

In the past few years, several investigations have been carried out concerning the development and the performance of thermophilic high-rate anaerobic wastewater treatment systems (Kennedy & van den Berg 1982, Schraa & Jewell 1984, Wiegant & Lettinga 1985, Wiegant *et al* 1985). These investigations have led to interesting conclusions. Reactor start-up is far less time consuming than previously assumed (Garber 1982): within weeks a fully operative bacterial population can be established, as was shown for conventional systems (Varel *et al* 1977), the anaerobic attached film expanded bed (Schraa & Jewell 1984), the downflow stationary fixed film (Kennedy & van den Berg 1982) and the upflow anaerobic sludge blanket (UASB) process (Wiegant & Lettinga 1985). Also the maintenance of a thermophilic high-rate system is less difficult, and the resistance against overloading is better than supposed so far (Schraa & Jewell 1984). The kinetic advantages of thermophilic systems are becoming more and more clear: extremely high loading rates, of 50-120 kg COD/m^{3}d can be handled with good treatment efficiencies.

This paper deals with the effect of suboptimal conditions on the thermophilic UASB process. Latter process, described by Lettinga *et al* (1980) in detail, can be considered as very effective in the treatment of wastewaters with a low suspended solids content, and moderate COD levels. As support material is lacking in the UASB reactor, the UASB process offers the highest possible void volume. As a consequence, it will yield the best results on a methane production rate to volume basis, provided a sludge with superior settling qualities can be obtained and maintained. Frequently, such a sludge will develop after some time of operation without significant difficulties, provided the right conditions are met (Hulshoff Pol *et al* 1983,1984). This was shown also to be true under thermophilic conditions (Wiegant & Lettinga 1985, Wiegant & de Man 1986).

Campaign wastewaters from the sugar industry are treated at mesophilic temperatures with excellent results in UASB reactors (Pette *et al* 1980). As the anaerobic reactors are only operative during *c* 10 weeks, the sludge has to be preserved for 9-10 months. During this period no heating is supplied, resulting in a moderate storage temperature (the temperatures in the Netherlands average 9.3 °C, July being the warmest month with an average of 16.6 °C).

The objective of the present investigations was to assess the possibility of thermophilic treatment of wastewaters discharged seasonally, or otherwise discontinuously, and consequently, to assess the survival of thermophilic sludge in periods of severe underloading at various temperatures, and the behaviour of thermophilic systems during lowering of the operation temperature. Information on the specific growth rates of the relevant bacteria is presented elsewhere (Wiegant *et al* 1986).

MATERIALS AND METHODS

Thermophilic granular sludge

Thermophilic sludge was cultivated in the following way. 200 ml portions of thermophilic granular sludge, cultivated on sucrose as described previously (Wiegant & Lettinga 1985), were brought in a 5.75 l UASB reactor, together with 1.0 l of fresh cow manure. Three of these reactors were fed with a solution of sucrose and a 1:1:1 mixture of acetate, propionate and butyrate (w/v). The volatile fatty acids (VFA) contributed for 17 % of the influent COD, which amounted 7-10 kg/m^3. One reactor was fed merely with the VFA mixture, to which 0.1 kg/m^3 of yeast extract was added. The influent COD of this reactor amounted *c* 8 kg/m^3. After two months of operation at loading rates up to 15-20 kg COD/m^3d, enough sludge was available to carry out the present experiments.

Storage experiments

The thermophilic sludge cultivated on sucrose + VFA was cooled to 30 °C, and divided into a number of equal portions of *c* 100 ml which were stored anaerobically in 300 ml serum bottles. The serum bottles were stored at 20 and 30 °C. Periodically the content of a stored serum bottle was emptied in a 6 l gas-tight digester, containing oxygen-free water, basal nutrients and Na_2S (Wiegant & Lettinga 1985). Then 1.0 kg/m^3 of acetate and butyrate and 0.5 kg/m^3 of propionate was added (final concentrations) to the mixed liquor.

Experiments with feed interruptions at 55 °C

An experiment with feed interruptions for various periods of time was carried out with the sludge grown on sucrose + VFA and the sludge grown on the VFA medium as well. These experiments were carried out in the reactors in which the sludges were cultivated, thus avoiding any manipulation with the sludges. The same type of experiment was carried out with a completely mixed stirred tank reactor (CSTR) fed with acetate (2.5 kg/m^3), yeast extract (0.1 kg/m^3), basal nutrients (Wiegant & Lettinga 1985), and Na_2SO_4 (0.07 kg/m^3). The CSTR was operated at a temperature of 55 °C and at a retention time of 5.5 days. In this reactor the feed supply was interrupted several times for various periods of time, after which the feeding was resumed. The next feed interruption was made only after the system had reached "nearby steady state" again.

Experiments with a decreased temperature

Experiments in which the operating temperature was lowered from 55 to 30 °C were carried out with the sludge cultivated on sucrose + VFA. In the experiments the same feed was used as during the cultivation of the sludge. The loading rate was not changed during the period of decreased temperature.

Analyses

In the batch experiments for assessing the methanogenic activity, the methane production was measured by liquid (1.5 % NaOH) displacement, after passage of the biogas through a column of soda lime pellets. The displaced liquid was measured and registered via a small vessel which emptied itself by siphoning. For this purpose the vessel formed part of a small balance, connected with a microswitch. Each time the siphoning device emptied itself the balance turned and the microswitch signal was registered automatically. In the UASB reactors the biogas passed a 5 M NaOH solution, a column with soda lime pellets and a wet test gas meter. It was accounted for as (wet) methane. All other analyses were performed as previously described (Wiegant & Lettinga 1985).

RESULTS

The loss of activity as a result of unfed storage was investigated with the sludge grown on sucrose + VFA. The activity was determined at 55 °C using a batch feed of acetate, propionate and butyrate. As appears from the results shown in Fig. 1A, the maximum activity, i.e. the maximum methane production rate during a single batch feed, drops markedly upon storage at 20 and 30 °C. From the data presented in Fig. 1A a decay rate of 0.012 d^{-1} at 20 °C and 0.011 d^{-1} at 30 °C can be estimated. However, after 50 days of unfed storage at 20 °C, the decay rate dropped to 0.0014 d^{-1} (Fig. 1B).

As a considerably higher decay rate can be expected at 55 °C, we also investigated unfed storage at this temperature, in an acetate-fed CSTR. The longer the unfed period, the higher acetate concentrations were reached in the

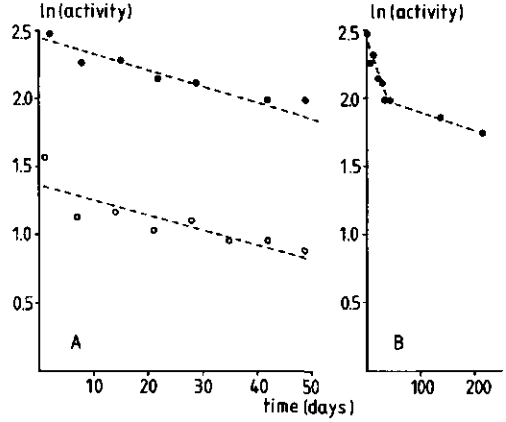

Fig. 1. The natural logarithm of the maximum methanogenic activity as a function of the storage time after storage at 20 (●) and 30 (o) °C. In Fig. 1A the first 50 days, and in Fig. 1B 215 days of storage are shown (for 20 °C only).

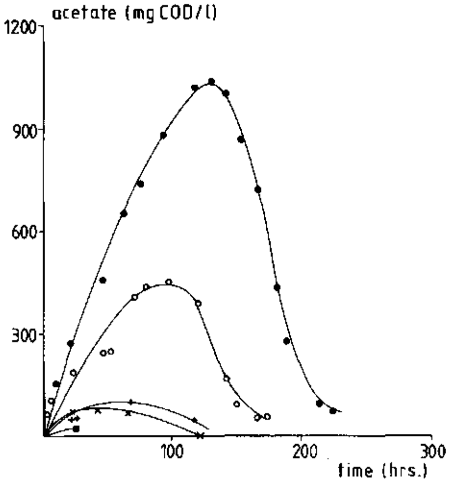

Fig. 2. The acetate concentration in a CSTR, as a function of time, after feed interruptions of 24 (■), 70 (+), 125 (x), 184 (o) and 384 (●) hrs. The curves are fitted by eye.

effluent after resuming the feeding (Fig. 2). After 16 days of unfed storage, nearly all the activity is lost, as is clear from the slow recovery. The effect of feed interruptions at the working temperature was also investigated with the two different types of sludge in UASB reactors. The sludge fed with sucrose + VFA apparently is fairly resistant to feedless conditions. Feed interruptions up to 96 hrs led to increased concentrations of VFA in the effluent, of which propionate contributed for 69 ± 12 % on a COD basis (Fig. 3). With the sludge fed with VFA, at a loading rate and influent concentration similar as in the

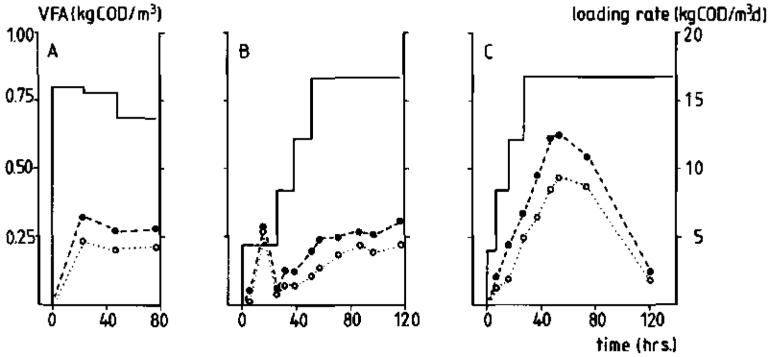

Fig. 3. Performance of a UASB reactor fed with sucrose + VFA after a feed interruption of 24 (A), 46 (B) and 96 (C) hrs. The total VFA (●) and propionate (o) concentrations are given on the left scale, the volumetric loading rate (——) on the right scale. The influent concentration was 9.3 kg COD/m³ and the loading rates applied were 16.4, 16.3 and 17.0 kg COD/m³d, respectively, before the experiments.

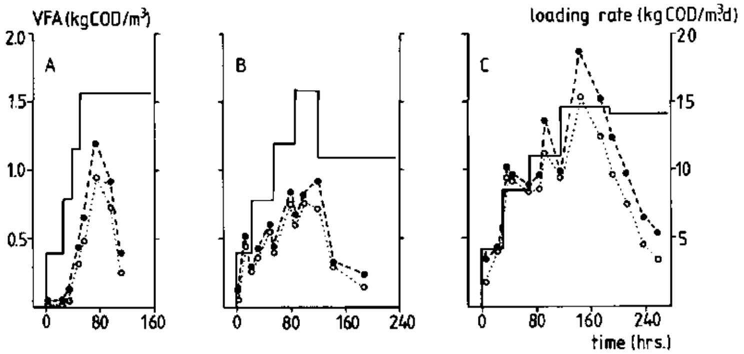

Fig. 4. Performance of a UASB reactor fed with mainly VFA after feed interruptions of 24 (A), 47 (B) and 97 (C) hrs. Legend as in Fig. 3. The influent concentration was 8.0 kg COD/m³ and the loading rates were 17.6, 16.4 and 17.7 kg COD/m³d, respectively, before the experiments.

previous experiment, the result of a feed interruption was much more drastic. Much higher VFA concentrations were observed in the effluent; 79 ± 14 % of the effluent VFA-COD consisted of propionate. Moreover, also a longer period of time was needed for recovery (Fig. 4).

The effect of decreasing the operation temperature from 55 to 30 °C under continued feeding was investigated with the sludge fed with sucrose + VFA. The lower temperature led to increased VFA concentrations, but now acetate was predominant. Propionate accounted only for 23 ± 7 % of the COD of the VFA (Fig. 5). The increased VFA concentrations led to a drop in the pH. However,

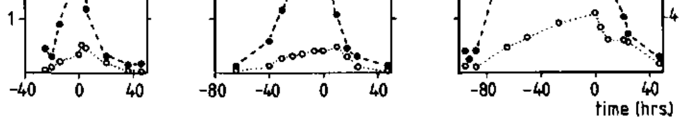

Fig. 5. Performance of a UASB reactor fed with sucrose + VFA during and after a lowering of the working temperature from 55 to 30 °C, for 24 (A), 47 (B) and 96 (C) hrs. The total VFA (●) and propionate (o) concentrations are given on the left scale, the pH (——) on the right scale. The influent concentration was 9.5 kg COD/m³ and the loading rate during the experiments were 12.9, 13.1 and 16.2 kg COD/m³d, respectively. Time is zero at the moment the temperature is restored at 55 °C.

after temperature drops applied for 24, 47 and 96 hrs, the recovery in all cases was complete within 30 hrs. The results of these experiments are summarized in Fig. 5.

DISCUSSION

The decay rate of thermophilic sludge under mesophilic conditions apparently is very similar to that of mesophilic sludges (*cf* Lawrence & McCarty 1970). The estimated decay rates at 20 and 30 °C are almost equal, although one would expect a lower decay rate at a lower temperature. This may be the result of experimental error: the growth rate of the methanogenic bacteria at 55 °C is *c* 80 times as high as their decay rate at 30 °C (*cf* Zinder & Mah 1979, Wiegant *et al* 1986), so the methanogenic activity may increase during the batch activity tests. As the maximum methane production rate was not always reached after similar periods of time, growth of the sludge may have masked its decay.

The maximum methanogenic activity of thermophilic sludge mainly reflects the activity of the acetate utilizing population. The occurrence of a sudden change in the decrease in the methanogenic activity (Fig. 1B) is rather puzzling. It suggests an acetate utilizing methanogenic population divided into two sub-populations each having different decay rates. These sub-populations may exist of *Methanosarcina* and *Methanothrix* bacteria, which can coexist for prolonged periods of time (Schraa 1983, Zinder *et al* 1984a). No data on the decay rates of thermophilic, nor mesophilic, species of both genera are available to date. However, the huge differences in the specific growth rates of these organisms (Huser *et al* 1982, Krzycki *et al* 1982, Zinder & Mah 1979, Zinder *et al* 1984b), may suggest quite different decay rates.

Unfed conditions at 55 °C may be expected to be very drastic, Indeed, the experiment shown in Fig. 2 suggests a high decay rate. As a computer simulation failed to produce consistent parameters for growth and substrate saturation for all the curves, no precise value for the decay rate can be presented. Tentatively, a value of 0.08 d^{-1} may be assumed. It must be noted though, that for mesophilic, dispersed sludge the decay rate is much higher than for mesophilic granular sludge (de Zeeuw 1984). Similar observations have been made for an aerobic nitrifying bacterium, of which the storage stability was much better when immobilized, as compared with free cell suspensions (van Ginkel *et al* 1983).

The experiments shown in Figs. 3 and 4 indicate a considerable loss in activity during feedless conditions at 55 °C. Particularly the strong decrease in the propionate degrading capacity is apparent. This may be the result of the high decay rate of propionate degrading bacteria, but it can also be caused by the activity of butyrate degrading bacteria, which have very high growth rates at 55 °C (Wiegant *et al* 1986). A high activity of butyrate degrading organisms may cause the hydrogen concentration to rise above the level where the degradation of propionate is still thermodynamically feasible (Gujer & Zehnder 1983, Wiegant *et al* 1986).

Applying a lowering of the operation temperature leads to a quite different situation. At 30 °C the sludge is still fairly active, although the VFA reach high concentrations, due to overloading. Apparently the propionate degrading symbiontic association, including a hydrogen utilizing methanogen, is not affected as severely as the acetate utilizing methanogens. On the other hand, there exists a serious risk of pH-inhibition, due to the reduced treatment efficiency. The recovery of the system from a temperature drop with concomitant overloading is very rapid, which is in accordance with the results presented in Fig. 1. Therefore, it can be concluded that, apart from the risk of VFA or pH inhibition, a short term decrease in temperature will cause no problems. Consequently, it seems justified to recommend a drop in the working temperature from the thermophilic into the mesophilic temperature region, whenever very low loading rates have to be accommodated, as may be the case during weekends. It also can be concluded that the acticity of thermophilic sludge under mesophilic conditions is close to that of mesophilic sludge. This is in accordance with data on the specific growth rate at 35 °C of thermophilic and mesophilic strains of *Methanosarcina*, which are in the same range (Zinder & Mah 1979).

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9. Granulation of biomass in thermophilic upflow anaerobic sludge blanket reactors treating acidified wastewaters

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ABSTRACT

The development of granular sludge in thermophilic (55 °C) upflow anaerobic sludge blanket reactors was investigated. Acetate and a mixture of acetate and butyrate were used as substrates, serving as models for acidified wastewaters. Granular sludge with either Methanothrix *or* Methanosarcina *as the predominant acetate utilizing methanogen was cultivated by allowing the loading rate to increase whenever the acetate concentration in the effluent dropped below 200 and 700 mg COD/l, respectively. The highest methane generation rates, up to 162 kg CH_4-COD/m^3.day, or 2.53 moles CH_4/litre.day, were achieved at hydraulic retention times down to 21 minutes, with granules consisting of* Methanothrix*. The formation of* Methanothrix *granules was not depending on the type of seed material, nor on the addition of inert support particles. The growth of granules proceeded rapidly with adapted seed materials, even when the reactors were inoculated at low concentrations. With mesophilic seed materials growth of granules costed much more time. Thermophilic* Methanothrix *granules strongly resemble mesophilic granules of the "filamentous" type. Some factors governing the thermophilic granulation process are discussed.*

INTRODUCTION

The operation in the thermophilic temperature range - from 45 to 65 °C - can be an attractive alternative for the operation in the mesophilic temperature range of high rate systems for anaerobic treatment of wastewater. For instance, thermophilic methane digestion from sugars as substrates has been shown to be successful. The start-up and the operation of the system is relatively simple at a working temperature of 55 °C (1,2).

Under most circumstances upflow anaerobic sludge blanket (UASB) reactors combine the advantage of a high biomass retention with a high void volume, because no support material is externally supplied. This has led to the installation of over 50 full-scale UASB reactors in the past few years (3). A prerequisite for successful operation, however, is the development and maintenance of a sludge with good settling characteristics (4).

Good to excellent results can be obtained with the UASB system in laboratory reactors as well as full-scale installations, when the sludge grows in the form of granules (3-7). Granulation can occur in the mesophilic anaerobic treatment of both non-acidified (5), and acidified substrates consisting mainly of volatile fatty acids (6,7). The occurrence of granulation is not yet fully understood, and neither are the factors responsible or the conditions necessary for granulation. Many factors are considered important by different investigators: the right conditions for growth of the methanogenic bacteria, the concentration of bivalent cations (4,8), the hydraulic retention time or liquid velocity (5), the influent concentration (5), the gravitational compression of the sludge particles (8,9), and the superficial loading rate of the evolved biogas (1). A selection pressure for bacterial aggregates with good settling properties by wash-out of smaller aggregates is considered favorable (5-7). The methanogenic activity of the seed material and the nature and amount of inert particles in the seed material also play an important role in the granulation process (5-7).

Under thermophilic conditions, granulation is readily achieved with the use of sugars as substrates. This granular sludge enables very high loading rates when acidified substrates are used - up to 105 kg COD/m^3.day - with a high treatment efficiency. A clear similarity in the superficial biogas loading rates at the moment granulation was ascertained in different experiments at 30 and 55 °C was noticed (1).

Generally, however, the COD of wastewaters does not consist solely of sugars. Thus, the question whether granulation will occur under thermophilic conditions when applying volatile fatty acids as substrates is a very important one for the application of the thermophilic UASB process. In the mesophilic temperature range granulation is very well possible with volatile fatty acids as substrates. The predominant bacterium in these granules is presumed to be *Methanothrix soehngenii* (5,6), a filamentous methanogen converting acetate into methane (10). At both mesophilic and thermophilic temperatures granules consisting of *Methanosarcina sp.* can develop in mixed cultures (11,12), but as yet they are rarely found in UASB reactors.

In the current investigations an answer was sought to the question whether granulation will occur in UASB reactors under thermophilic conditions with acetate as methanogenic substrate. The investigations aimed to obtain granules of the *Methanothrix*-type, as it was believed that they are more effective than those of the *Methanosarcina*-type. Thermophilic *Methanothrix*-bacteria were recently described, and they look much like the mesophilic *Methanothrix*, although the filaments are shorter (13-15). It was attempted to obtain also *Methanosarcina*-type granules for comparative purposes, which are described only in stirred cultures (11). Selection for the two types of acetate-splitting methanogens was imposed by control of the effluent acetate concentration. Because of the low substrate affinity and the high specific growth rate of *Methanosarcina* (16), they can be selected by maintaining a high effluent concentration. On the other hand, with low effluent acetate concentrations, *Methanothrix* will become the predominant acetate converting methanogen, because of its higher substrate affinity (15). By employing different criteria for the start-up of UASB reactors, one can simply promote the acetate-splitting methanogen of one's choice.

Some factors governing the granulation process were also studied: the use of different seed materials, the addition of inert particles to the seed sludge and the application of butyrate in the feed. Another variable, introduced in the experiments, was the percentage with which the loading rate was increased after the effluent acetate concentration had reached its critical value.

MATERIALS AND METHODS

Temperature

All experiments were carried out at 55 °C.

Media

In most of the experiments acetic acid was the principal source of carbon and energy (3.05 kg COD/m^3, 90 % neutralized with NaOH), supplied with 0.15 kg/m^3 of yeast extract, and basal nutrients as described previously (1). Sulfate was added as sulfur source (0.08 kg/m^3 of $Na_2SO_4.10H_2O$). Whenever the acetate concentration was raised, other media components were raised proportionally in concentration. In two experiments 1.07 kg/m^3 of acetate-COD and 3.33 kg/m^3 of butyrate-COD was used as feed, with additions as above.

Most of the oxygen was removed from the media by sparging nitrogen gas for 5 min. and connecting the media containers with gas bags filled with nitrogen, to maintain an oxygen-free headspace above the media. When over 10 l/day of medium was used, oxygen was no longer removed from the influent. The media were made up daily then, and stored at 30 °C. The influent was heated to 50 °C only when the hydraulic retention time dropped below 1.4 hrs. Influent pH was 5.7.

Seed materials

The seed materials used were the following: 1) digested sewage sludge from a municipal wastewater treatment plant in Ede, Holland. Sludge digestion was operated at 32 °C and at a 30 days retention time. 2) Fresh cow manure from dairy cows at an experimental farm in Duiven, Holland, stored at 4 °C before use. 3) The contents of a 5.5 l reactor, operated continuously at 55 °C and at a 2.2 day retention time with 12-15 kg/m^3 of acetate as feed (*cf.* 12). In this reactor granules of *c* 1 mm diameter had developed after six weeks of operation from fresh cow manure (*c* 0.2 % VS) as inoculum. 4) The contents of a completely mixed 5.5 l reactor, operated at 55 °C and at a 3.8-4.1 day retention time, fed with the acetate and butyrate medium described above. The reactor was inoculated with fresh cow manure (0.08 % VS) and operated for seven weeks before samples for inoculation of UASB reactors were taken. In this reactor filamentous bacteria resembling *Methanothrix soehngenii* were predominant, suggesting a thermophilic *Methanothrix* was the principal acetate-splitting methanogen.

Apparatus

Four of the UASB reactors used had a 5.75 l volume and 9 cm internal diameter, and were equipped with a stirring blade which was used intermittently (1 s every 3 min). Three UASB reactors had a 0.68 l volume and 5 cm internal diameter and were not equipped with a stirring device. These seven reactors all were made of plexiglass. One UASB reactor with a 16.7 l volume and 28 cm internal diameter was made of glass and had no stirring device. The biogas evolved in the 5.75 l reactors passed a column of soda lime pellets prior to measurement in a wet test gas meter. It was accounted for as (wet) methane. In the other reactors the biogas production was measured by liquid (0.1 M H_2SO_4) displacement or by a wet test gas meter. The methane concentration in the biogas was determined by gas chromatography.

Table I. Relevant data of the experiments performed.

reactor number	reactor volume	seed^a material	seed concn	variation	input C2 concn	output C2^b concn	%-age^c
	l.		$\frac{\text{kg VS}}{\text{m}^3}$		$\frac{\text{kg COD}}{\text{m}^3}$	$\frac{\text{kg COD}}{\text{m}^3}$	%
1	5.75	DSS	7.8		3.05	0.200	30
2	5.75	DSS	5.6	gravel addition	3.05	0.200	30
3	5.75	FCM	1.5	gravel addition	3.05	0.200	30
4	0.68	DCM	10		3.05	0.200	100
5	0.70	CSTR	0.025	butyrate medium	-^{e}	0.200	100
6	5.75	FCM	22		3.05	0.200	100
7	16.7	CSTR	0.041	butyrate medium	-^{e}	0.200	100
8	5.75	CSTR	ND^d		5.09	0.700	30

a: seed material: DSS, digested sewage sludge; FCM, fresh cow manure; DCM, digested (at 55 °C) cow manure; CSTR, content of mixed reactors fed with acetate and butyrate (1.06 and 3.33 kg COD/m^3) at RT = 3.8-4.1 days (reactors 5 and 7), or with 12-15 kg COD/m^3 of acetate at RT = 2.2 days (reactor 8, 100 % inoculum)

b: Output concentration at which the loading rate was increased.

c: Percentage by which the loading rate was incrementally increased.

d: ND, not determined.

e: acetate 1.06 and butyrate 3.33 kg COD/m^3.

Operation criteria

As can be estimated from literature data on specific growth rates and substrate saturation constants for *Methanosarcina* and *Methanothrix* in the thermophilic temperature range (11,14-16), the latter will have a higher growth rate at acetate concentrations below 0.12-0.4 kg/m^3, depending on the K_S-values used for *Methanosarcina* (11,16). In all reactors except for two, a selection pressure for *Methanothrix* was imposed by allowing the loading rate to increase only if the effluent acetate concentration had dropped below 0.2 kg COD/m^3. In the 16.7 l glass reactor this concentration was set at 0.35 kg COD/m^3 for experimental reasons. In one reactor, where a selection for *Methanosarcina* was imposed, this concentration was set at 0.7 kg COD/m^3. As soon as the effluent acetate concentrations dropped below these values, the loading rates were increased by 30 % in four reactors and by 100 % in the other four.

A summary of the seed materials, the extra additions, the influent concentrations and critical effluent concentrations as well as the increments in the loading rate is given in Table I.

Microscopical classification

The use of a single substrate leaves only a few species of bacteria to grow in mass numbers. In this case we searched for methanogens of the genera *Methanothrix* and *Methanosarcina* only. Clumps of large, spherical bacteria were identified as the latter; these clumps were visible as large blue-green spheres when seen at 1000 x magnification under an epifluorescence microscope. Small to long filaments, often grouped together in bundles, were identified as *Methanothrix*. These filamentous bacteria showed no fluorescence. Apparently syntrophic acetate oxidation (17) did not prevail, since these two bacteria were virtually the only ones seen in the acetate-fed reactors after two months of operation. No attempts were made to verify these identifications, but the simplicity of the media, and the fluorescence of the bacteria, which is in accordance with known specimens of both genera (10-16), seem to justify the classification. It must be noted though, that the gas-vacuoles described in thermophilic *Methanothrix* (13,14) were only rarely observed.

Analyses

Determinations of the particle size distribution were made by pouring a sludge sample into a petri dish, after which the size of the individual particles was measured by use of a stereomicroscope (10-40 x magnification) with a micrometer eyepiece. All other analyses were carried out as described previously (1).

RESULTS

Two reactors, seeded with digested sewage sludge, were used to investigate the influence of the addition of inert particles. Reactor 1 (reactor numbers refer to those in Table I) served as control. In reactor 2 0.4 kg/m^3 of fine gravel particles (50-100 μm) was added to the seed material at the start of the experiment, and another 16.2 kg/m^3 after 52 days of operation. The effluent acetate concentration dropped below 0.2 kg COD/m^3 after some 67 days of operation at a hydraulic retention time of 3.0-3.4 days. Then the loading rate was increased by 30 % by imposing a higher influent flow rate. This cycle of waiting for the effluent acetate concentration to drop below 0.2 kg COD/m^3 and consequently increasing the loading rate was repeated many times, until retention times even down to 21 min (for reactor 1) were reached. The course of the

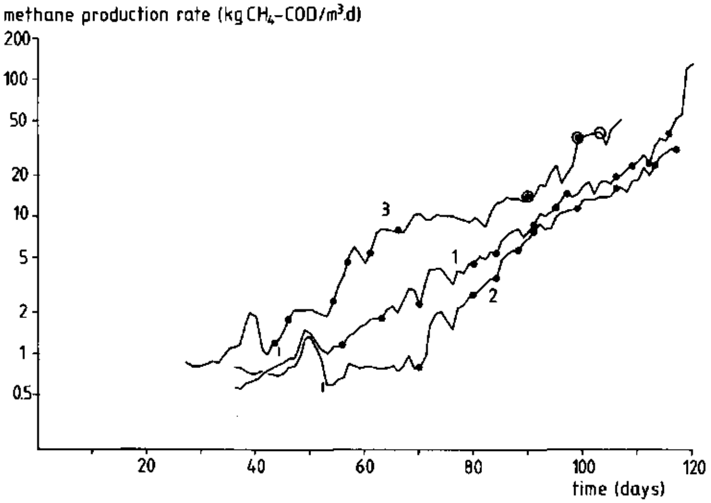

Fig. 1. Course of the methane production rate in the experiments with a 30 % increase in the loading rate at acetate concentrations below 0.2 kg COD/m^3 (●). Seed materials were digested sewage sludge without (1) and with (2) addition of gravel, and fresh cow manure with gravel addition (3). Gravel addition is indicated (|) in the Figure. In reactor 3 the last increases in the loading rate were made by increasing the acetate concentration to 4.2, 6.8 and 9.2 kg COD/m^3, as indicated by the open circles. Numbers refer to the reactor numbers in Table I.

methane production rate during these experiments is presented in Fig. 1, together with that of reactor 3, in which a low concentration of fresh cow manure was used as seed material. The last three increases in the loading rate in reactor 3 were not imposed hydraulically, but by increasing the influent concentration. At the end of the experiment, an influent concentration of 9.2 kg COD/m^3 of acetate resulted in effluent acetate concentrations below 0.35 kg COD/m^3, at a loading rate of 55 kg COD/m^{3}day.

Two other reactors received seed materials from completely mixed reactors. In reactor 4 digested manure from a thermophilic digester fed with diluted cow manure (ca. 1 % VS) was used. Reactor 5 was seeded with the contents of a mixed reactor fed with acetate and butyrate (1.06 and 3.33 kg COD/m^3, respectively). Reactor 5 was also fed with acetate and butyrate during the experiment. These 0.7 l reactors were operated in a different mode than the ones described before, in that the loading rate was increased by 100 % in stead of 30 %, once the effluent acetate levels dropped below 0.2 kg COD/m^3. The higher increments in the loading rate led to a higher rate of increase in the methane production rate (Fig. 2 and Table II). In both these reactors exceptionally

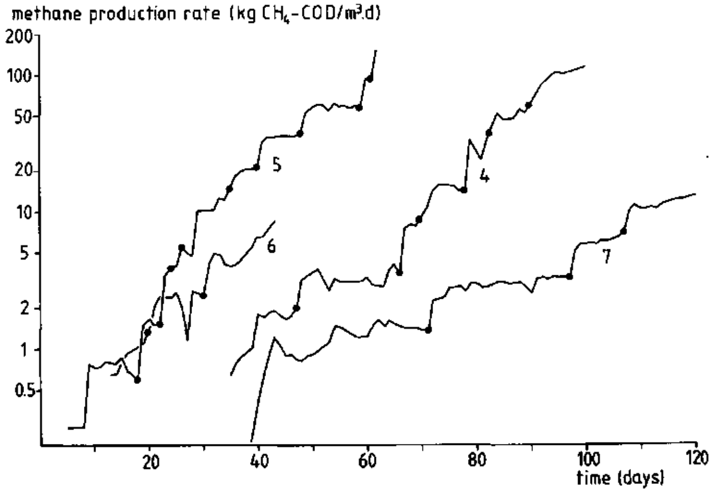

Fig. 2. Course of the methane production rate in the experiments with a 100 % increase in the loading rate at acetate concentrations below 0.2 (4,5,6) and 0.35 (7) kg COD/m³, indicated in the Figure (●). Seed materials were digested cow manure (4), fresh cow manure (6), and the contents of a CSTR fed with acetate and butyrate, which was also used as feed (5,7). For the sake of visual convenience, the curve for reactor 7 is shifted 35 days along the x-axis. Numbers refer to those in Table I.

high methane production rates were observed, of 113 kg CH_4-COD/m³day for reactor 4, fed with acetate, and even 162 kg CH_4-COD/m³day for reactor 5, fed with acetate and butyrate.

The mode of operation applied in the above experiments was repeated in a 5.75 l reactor (reactor 6), using fresh cow manure as seed material. A fairly similar increase rate in the methane production rate was observed (Fig. 2 and Table II). The foregoing experiments all were carried out in plexiglass reactors. In these reactors it was observed that part of the biomass was attached to the walls. In order to minimize the wall attachment, experiment 5 was repeated in reactor 7, a 16.7 l reactor made of glass, which had a much higher volume to wall surface ratio. In reactor 7 it was not possible to achieve effluent acetate concentrations below 0.2 kg COD/m³ within three weeks after start-up. It was decided to increase the loading rate once the effluent acetate concentrations had dropped below 0.35 kg COD/m³. The course of the methane production rate in this reactor is also shown in Fig. 2.

Reactor 8 was seeded with the contents of a mixed digester receiving high acetate concentrations. In this digester small *Methanosarcina* aggregates was observed. A methane production rate of 17 kg CH_4-COD/m^3day was the maximum under the conditions applied. An increase in the influent concentration from 5 to 14 kg COD/m^3 at days 88 and 89 only led to a 34 % increase in the methane production rate. The course of the methane production rate in the experiment is shown in Fig. 3. The increase rates of the methane production rates are given in Table II.

Sludge characteristics

Sludge samples from all reactors were examined regularly: the abundance of the relevant methane bacteria and the growth of bacterial aggregates was followed. Reactors 1 to 4, seeded with digested sewage sludge and fresh or digested cow manure, all showed the same pattern. After ca. 40 days of operation the first *Methanothrix* filaments were observed. They became more and more abundant, until after ca. 60-80 days of operation virtually only *Methanothrix* were observed, forming small aggregates. In reactor 5 and 7, which were fed with acetate and butyrate, the *Methanothrix* bacteria were accompanied by small, rod-shaped, fluorescent bacteria.

The first time granules larger than ca. 0.2 mm were observed was after 84 days of operation in reactor 1 and 2, seeded with digested sewage sludge, and after 69 days in reactor 3, seeded with fresh cow manure. The superficial biogas loading rates at that time ranged from 1.6 to 3.9 m/day. After 109 days of operation, clear sludge granules, with diameters up to 3 mm, were sampled from the bottom of these reactors. In the reactors containing gravel particles, the granules showed included gravel particles.

In reactor 3, seeded with fresh cow manure, two distinct types of *Methanothrix* granules were present at the end of the experiment. The first type had a whitish color and consisted of long filaments, which were packed rather loosely. This type was very similar to the so-called filamentous granules (6). The second type had a dark, greenish color, presumably resulting from the precipitation of metal sulfides, and this type also consisted of long filaments, which, however, were packed extremely dense. Macroscopically, they strongly resembled the so-called rod-type granules. Apart from these types a number of intermediate types, both in color as in density of the bacterial growth, were present.

In the other reactors only *Methanothrix* granules of the second type were

Table II. The rate of increase of the methane production rate for all reactors. Reactor numbers refer to those in Table I.

reactor number	increase rate (day^{-1})	r^2	time interval (days)	mean acetate concentration (kg COD/m^3)
1	0.053	0.97	42 - 104	0.32 ± 0.18
2	0.067	0.95	75 - 110	0.38 ± 0.15
3	0.044	0.73	50 - 90	0.27 ± 0.09
4	0.080	0.92	36 - 96	0.51 ± 0.36
5	0.109	0.95	5 - 62	insufficient data
6	0.070	0.82	19 - 43	0.66 ± 0.62
7	0.035	0.87	12 - 85	0.47 ± 0.10
8	0.057	0.96	10 - 50	1.12 ± 0.37

(ca. 0.1 mm in diameter) were visible. Reactor 8 was operated at high acetate levels by increasing the loading rate by 30 % once the acetate concentrations in the effluent dropped below 0.7 kg COD/m^3. In this reactor a severe washout occurred immediately after seeding, indicated by a sharp decrease in the methane production rate, at a hydraulic retention time of 1.8 days. This was also observed after increases in the loading rate: first the methane production increased for two or three days, then it decreased for three to five days to reach a minimum, after which a slow increase in the methane production rate

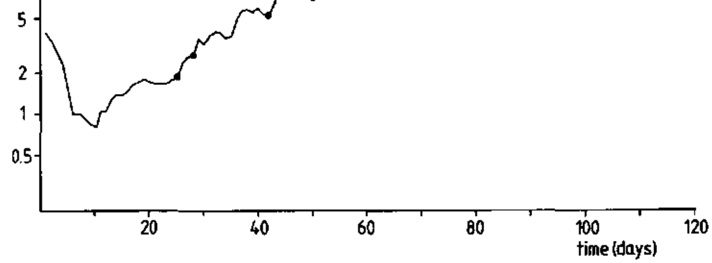

Fig. 3. Course of the methane production rate in the experiment with a 30 % increase in the loading rate at acetate concentrations below 0.7 kg COD/m^3 (●). (|) indicates days with an influent acetate concentration of 14.2 kg COD/m^3.

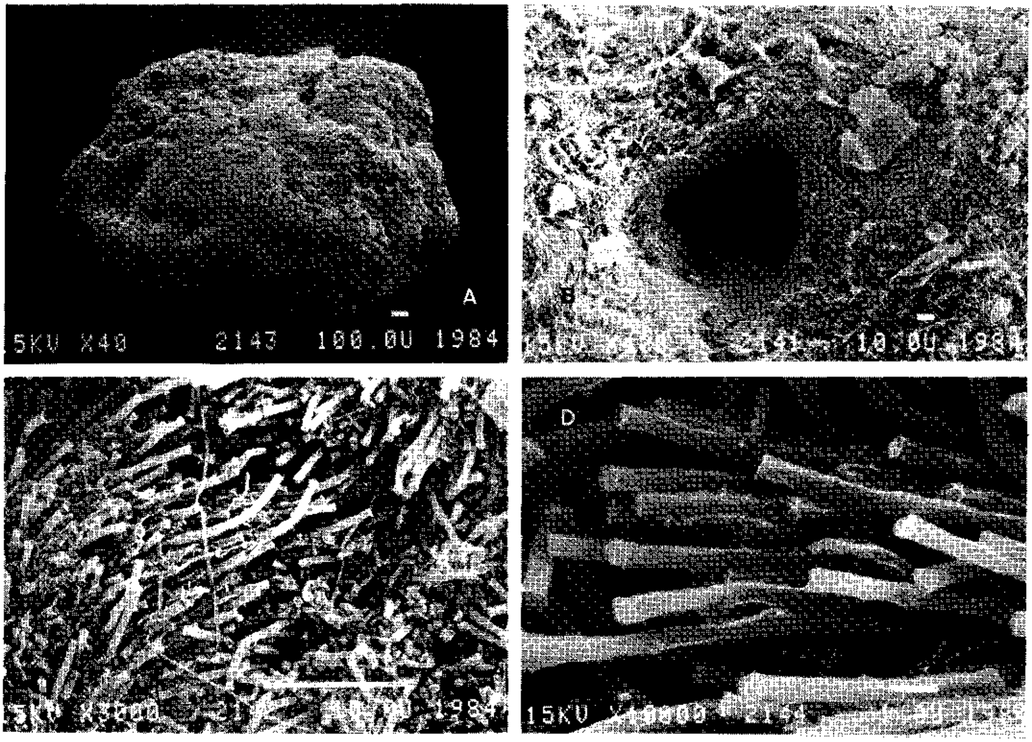

Fig. 4. Scanning electron micrographs of thermophilic granular sludge grown on acetate, from digested sewage sludge as inoculum. Fig. 4A: a large granule showing several cavities through which the biogas is released; Fig. 4B: detail of 4A, showing one such cavity; Fig. 4C: large magnification showing the dense structure of the granule, and 4D: detail, showing blunt-ended filaments, typical for *Methanothrix*. Bars indicate 100, 10, 10 and 1 µm, respectively.

present. In Fig. 4 some scanning electron microscopical photographs are presented of a granule of the second type described above. They show a large granule with typical cavities, through which the biogas is released (Fig. 4A). One of these is shown in detail in Fig. 4B. The very dense structure of the granule is shown in Fig. 4C, and the bacteria are shown in more detail in Fig. 4D. The photograph shows rather blunt-ended filaments, typical for *Methanothrix*.

In reactor 7, the one made of glass, no attached growth was observed. At the end of the experiment, there was a shallow but dense sludge bed consisting of small granular aggregates, sized 0.1 to 1.0 mm. By then the biogas superficial biogas loading rate was 1.20 m/day.

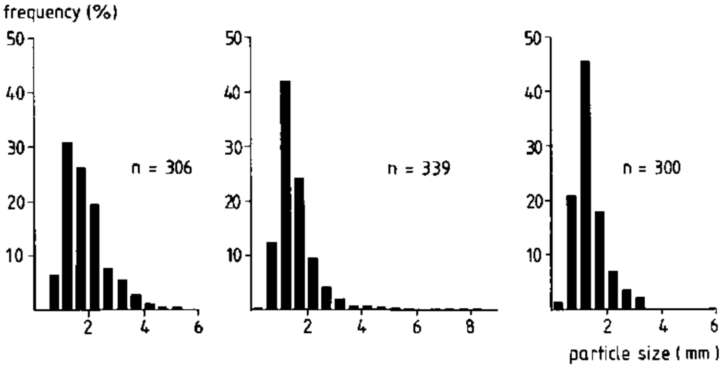

Fig. 5A. Particle size distribution in sludge samples taken from the sludge bed at the end of the experiments. The values in parentheses give the liquid upflow velocity (m/day) and the methane production rate (kg CH_4-COD/m^{3}day), respectively, by the time of sampling. From left to right: reactor 2, seeded with digested sewage sludge with gravel addition (0.97, 30.5); reactor 3, seeded with fresh cow manure with gravel addition (0.51, 29.3); and reactor 8, containing *Methanosarcina* granules (0.38, 19.6). Reactor numbers refer to those in Table I.

At the end of the experiments sludge samples were taken from the sludge blanket of reactors 2,3 and 8. The particle size distribution in these samples is presented in Fig. 5A. In Fig. 5B a logarithmic-normal relationship between the cumulative frequency and the particle size is shown.

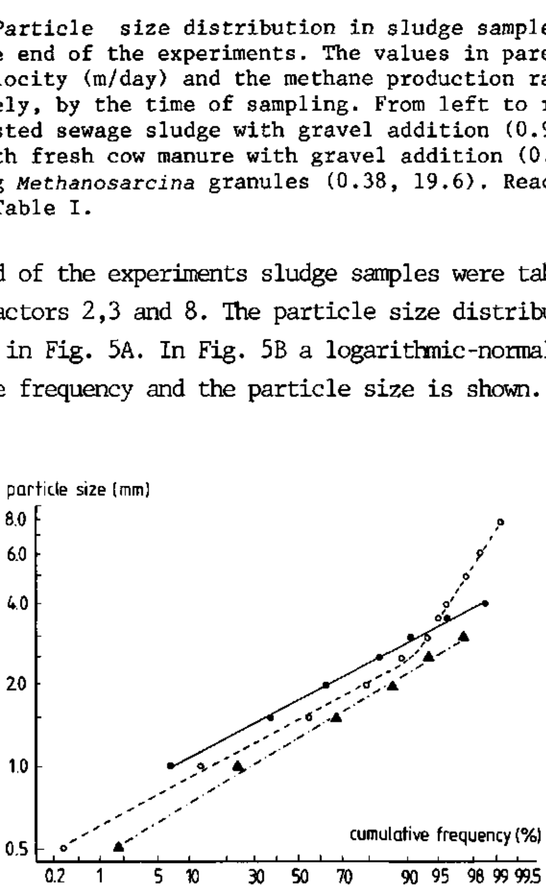

Fig. 5B. Plots of the logarithm of the particle size against the cumulative frequency, corresponding with the distributions given in Fig. 5A, for reactor 2 (●), 3 (o) and 8 (▲). Reactor numbers refer to those in Table I.

Table III. Sludge activities determined at the end of the experiments. Reactor numbers refer to those in Table I.

reactor number	methane production rate	sludge concn	sludge activity	acetate[a] concn	hydraulic retention time
	$\frac{\text{kg } CH_4\text{-COD}}{m^3\text{day}}$[b]	$\frac{\text{kg VSS}}{m^3}$	$\frac{\text{kg } CH_4\text{-COD}}{\text{kg VSS.day}}$	$\frac{\text{kg COD}}{m^3}$	hrs.
2	31	5.6	5.4	0.12	2.2
3	51	7.0	7.3	0.30	4.2
4	113	24.8	4.6	0.50	0.6
5	162	26.1	6.2	0.33	0.5
7	14	3.2	4.2	0.57	7.0
8	20	2.8	7.0	1.30	5.6

a: at the moment the experiment was terminated.
b: 64 kg CH_4-COD/m^3day corresponds with 1 Mole CH_4/1.day.

The sludge activities, measured as reactor activities (methane production rate divided by the amount of VSS) varied quite strongly. They are presented in Table III. The combination of the rather low sludge activity, the low biomass concentration, and the high acetate concentration in the effluent of reactor 7, is a strong indication that serious short-circuiting may have taken place, leading to the poor results obtained with this reactor.

DISCUSSION

From the results presented above, first of all it is clear that excellent results can be obtained with the thermophilic anaerobic digestion of acetate in UASB reactors. Although the experiments were not specificially aimed to reach the upper limits, methane production rates as high as 162 kg CH_4-COD/m^3day (2.53 moles CH_4/1.day) were established, at hydraulic retention times down to 21 minutes, and with good treatment efficiencies. As the biomass concentrations in the reactors were not exceedingly high, even higher maximum methane production rates may be expected.

Sludges, consisting of *Methanosarcina* , obviously perform inferiorly to those consisting of *Methanothrix*. Although *Methanosarcina* bacteria have a strong tendency to grow in the form of granules, these granules are easily washed out of the reactor. So it may be concluded that a selection pressure for *Methanothrix* should be pursued, rather than for *Methanosarcina*, to achieve optimal results. The activity of the *Methanothrix* granules, i.e. 4.2-7.3

kg CH_4-COD/kg VSS.day, is appreciably higher than that reported for *Methanothrix* granules, which, at 30 °C, is 2.2-2.4 kg CH_4-COD/kg VSS.day (6, G Lettinga, pers.comm.).

In several reports concerning mesophilic granulation, much emphasis has been laid on both the concentration and the methanogenic activity of the seed material, as well as the nature and quantity of the inert particles in the seed material. In the present investigations we observed granulation with digested sewage sludge, digested and fresh cow manure as seed materials, in concentrations ranging from 1.5 to 10 kg VS/m^3, with or without the addition of inert particles. Adapted seed materials could even be applied in a concentration of 0.025 kg VS/m^3, and still lead to granulation. It can be concluded that neither the seed material, nor the addition of inert support material is of particular interest in the occurrence of thermophilic granulation. Both may have an influence on the time of the onset and the speed of the granulation process, though. Apparently the selection for *Methanothrix* is a factor of importance. The results shown in Figs. 1 and 2 show a rapid increase in the methane production rate, but the onset of this increase differs largely in the various experiments. Only when the seed material has a high *Methanothrix* content already (reactor 5), this onset falls within 8 days. With the other materials apparently more time is needed. A methane production rate of 2 kg CH_4-COD/m^3day with effluent concentrations lower than 0.2 kg COD/m^3, was reached in 22 days in the reactor (5) with the *Methanothrix*-rich seed material, whereas it was 47-74 days in reactors 1 to 4. Reactor 6, started up with a high concentration of fresh cow manure, cannot be compared with reactors 1 to 5 in this respect, for it was unclear what acetate-splitting methanogen was predominant at the time this reactor was terminated.

The apparently very low numbers of *Methanothrix* in the seed material may explain the lack of effect of the addition of inert particles. In experiments with mesophilic digestion in which hydroanthracite particles (0.25-0.42 mm particle size) were added to digested sewage sludge, a significant reduction of the time needed for granulation was observed (Hulshoff Pol, pers.comm.) Such an effect may be the result of either a better attachment of the filamentous bacteria to the particles, which would lead to a better retention and thus to a higher rate of increase in the methane production rate, or a sort of "collection" of dispersed filamentous bacteria by the particles, catalyzing the aggregate formation, but not leading to a better retention. No clear conclusion about these phenomena can be drawn from the results presented in Fig. 1 and Table II. There seems to be a negative effect from the addition of gravel particles in the first half of the experiment. The retention, however, is somewhat better as can be seen in Table II: reactor 2 shows a higher rate of increase of the methane production rate than does reactor 1. It must be noted that the mean acetate concentration in reactor 2 also was higher than in reactor 1. The increase in the methane production rate does not show a spectacular difference, thus rendering a positive effect of the addition of gravel particles highly unlikely in the thermophilic UASB process.

The granulation process of thermophilic methanogenic bacteria does not seem to differ substantially from that under mesophilic conditions. The granules observed in both temperature ranges are similar: those consisting of *Methanosarcina* develop when a high effluent acetate concentration is maintained, whereas with low effluent acetate concentrations *Methanothrix* are found. Different types of *Methanothrix* granules can develop, but contrary to findings in the mesophilic temperature range (5-7,18), they both consist of long filaments. The two types of granules macroscopically strongly resemble the "rod-type" and "filamentous" granules, as described by Hulshoff Pol *et al* (6), though.

In the thermophilic case the second type of *Methanothrix* granules, having a very dense structure, apparently develops from the first type, indicated by the presence of many intermediate forms. A development from a dense structure to one with a low density of the bacterial growth is highly unlikely, as in the beginning only flocculent aggregates were observed. The presence of the intermediate forms suggests that the development of "dense structure" granules may be a matter of age of the granules. It is not known whether the manifestation of *Methanothrix* in the mesophilic temperature range is age-dependent, but the growth of "rod-type" *Methanothrix* granules from disintegrated granules of the same type (5,19) seems to suggest that this is not so. The dense "rod-type" granules, however, develop from aggregates with also a very dense structure. "Dense structure" granules may therefore develop in both temperature ranges, either from granules with a less dense structure, or from smaller aggregates with already a very dense structure. The growth from the second type out of the first may cost very much time under laboratory conditions at 30 °C.

The superficial biogas loading rates at the time granulation becomes apparent vary from 1.6-3.9 m/day. These values compare reasonably with those found for mesophilic granulation, which are in the range of 2.0-8.0 m/day (Hulshoff Pol, pers.comm.). The values of the superficial biogas loading rates for the granulation on sugars seem to be somewhat higher (1). In one experiment, not mentioned in the results section, we tried to demonstrate a positive effect of imposing a moderate gas turbulence by blowing nitrogen gas through the reactor. This experiment failed, probably because of short-circuiting as a result of the gas supply. The sludge activity was very low, 2.4 kg CH_4-COD/kg VSS.day, whereas low effluent acetate concentrations were not achieved within 60 days of operation at low hydraulic retention times.

In the majority of the reactors used, a significant part of the biomass was attached to the walls. This obviously is the result of the small diameter of the reactors used and the plexiglass used. In these reactors, the attached growth may have had an important role in the granulation process: the erosion of bacterial aggregates from the wall of the reactor into the bulk may give rise to a rapid granulation. Granulation, however, was also observed in the reactor made of glass, in which no attached growth was seen.

As shown in Fig. 5B, the particle size distribution seems to be similar for the three sludges sampled. The logarithm of the particle size shows a normal distribution, demonstrated by the fact that the points in Fig. 5B are fitted well by straight lines, except for the last part of one of the curves, which apparently is due to large particles consisting of biomass attached to the wall. The lines are nearly parallel, indicating nearly identical relative standard deviations, but have different medians. The order of the median particle size coincides with that of the liquid velocity and the methane production rate. These observations, together with those presented in Figs. 1 to 3, suggest that granulation is a continuous process: each increase in the loading rate causes a higher liquid velocity and biogas superficial loading rate, thereby increasing the turbulence. Due to the increased turbulence small particles will leave the reactor, so that growth of new biomass occurs on the remaining particles. Apparently this process is not necessarily preceded by a mass washout of sludge, as normally observed in mesophilic granulation processes started from digested sewage sludge (5-7,18,19). As shown in Fig. 2, the same process also occurs starting from very low concentrations of adapted biomass. The above described "mechanism" of granulation does not explain the preference granule formation above floc formation, nor the spherical shape of the granules. There is, however, a similarity of the superficial biogas loading rates at the moment the first granules are observed, in mesophilic as well as thermophilic digestion of sugars on one hand (1), and volatile fatty acids on the other. This strongly suggests that the evolving biogas is responsible for both the preference of granular growth and the spherical shape of the granules.

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10A. Summary and conclusions

In the past decades, anaerobic treatment of wastes and wastewaters has become a good alternative for conventional aerobic treatment methods, although a post-treatment of anaerobically treated wastewaters generally still is necessary. One of the major drawbacks of anaerobic treatment is the low growth rate of the bacteria involved, requiring a high retention time of the bacteria. In view of the increased rate of chemical reactions at elevated temperatures, thermophilic anaerobic wastewater treatment, between 45 and 65 °C, may be an attractive alternative for mesophilic treatment. Contrary to chemical reactions, biological reactions are limited to certain temperatures, and they are influenced by the changes in many equilibria, which occur at increasing temperatures and can produce adverse effects.

This thesis focuses on the question whether, and under what conditions and circumstances, thermophilic methane digestion in practice can be favourable in comparison with mesophilic digestion. The time hasn't come yet for an economical evaluation of the advantages of thermophilic digestion; this thesis mainly focuses on the "biological feasibility" and on the advantages that may be realized.

The literature on thermophilic digestion is evaluated in chapter 2. There exists a considerable interest in thermophilic digestion since the late twenties of this century. The research performed prior to 1960 mainly concerns the digestion of sewage sludge. Thermophilic sludge digestion can be operated at appreciably lower retention times in comparison with mesophilic digestion, with similar methane productions and treatment efficiencies. It is practised only rarely. In general, thermophilic operation yields a better elimination of pathogens, but also a slightly higher concentration of volatile fatty acids, yielding a somewhat more odorous sludge.

Since 1977, there is a strong increase in the interest in thermophilic digestion of livestock wastes. Essentially, it has the same advantages: shorter retention times are possible, and the pathogen elimination is better. Thermophilic anaerobic digestion of wastewaters in completely mixed reactors is not often used. Generally, the same advantages apply for these systems.

In modern systems for the treatment of wastewaters it is attempted to keep as much biomass as possible in the reactor. So the reactor can be operated with a high biomass retention time, but also with a short liquid retention time. There is little experience with these systems in the thermophilic temperature range. An important difference with the systems mentioned before is that these systems generally do not aim to reach the shortest bacterial residence times possible. So the high growth rates of the thermophilic bacteria is not utilized, but advantage is sought in their high decay rate and maintenance energy.

Generally, these high rate systems can handle much higher loading rates than similar mesophilic systems. A few exceptions are discussed. Some theoretical aspects of bacterial growth in high rate systems are discussed, and a survey is presented of parameters of a number of relevant thermophilic bacteria.

One of the major problems in livestock waste digestion is the high total ammonia concentration of the waste. Ammonia becomes very toxic for methanogenic bacteria at high concentrations. The un-ionized fraction of the ammonia, which is considered to be the toxic agent, increases with increasing temperatures. Thermophilic digestion of manures with a high total ammonia concen tration , exceeding 3.0 kg N/m^3, performs poorly. In chapter 3 we investigated whether the total ammonia concentration, or the volatile solids content of the manure, may be held responsible for the poor performance of the thermophilic process. It turned out that the VS concentration does not play an important role. Some implications for the use of a popular model describing the digestion process are discussed.

Some work was devoted to the mechanism of ammonia inhibition. Surprisingly enough, ammonia has little influence on the conversion of acetate into methane. Apparently the inhibition takes place via an intermediate compound. It is very likely that this compound is hydrogen. Hydrogen inhibits - at higher concentrations - the conversion of propionate, which in turn is inhibitory for the acetate conversion.

Chapters 5 to 9 deal with investigations on the thermophilic upflow sludge blanket (UASB) process. Firstly the digestion of a readily digestible substrate, glucose, was investigated. This compound is known to lead easily to granulation in the mesophilic temperature range. In the thermophilic range, at 55 °C, this was also the case. High loading rates, up to 45 kg COD/m^{3}d, were achieved, with over 70 % efficiency of the conversion of COD into methane. With the granular sludge cultivated on sugars, solutions of volatile fatty acids could be treated with loading rates up to 104 kg COD/m^{3}d with a treatment efficiency exceeding 77 %. In chapter 5 also attention is given to factors governing the granulation process. It seems likely that the biogas superficial loading rate, the amount of biogas evolved per unit of area and time, plays an important role in this process, among the other factors known to be important.

Vinasse, the wastewater of alcohol distilleries, is produced in enormous quantities and at high temperatures. It is well suited to serve as an example of a high strength wastewater. In chapter 6 the results of the investigations on the digestion of this wastewater are presented. In completely mixed systems with a high retention time and at moderate loading rates, thermophilic digestion of vinasse is about as efficient as mesophilic. So one can expect the effluent quality after treatment in UASB reactors to be independent of the temperature between 30 and 55 °C, when no overloading is occurring.

The treatment of vinasse in thermophilic UASB reactors was carried out with sludge cultivated on sugars. A long adaptation period was necessary. Although very high loading rates, up to 86 kg COD/m^{3}d, were accommodated after adaptation, the treatment efficiency was rather poor. The efficiency was, however, more affected by the concentration of the vinasse than by the loading rates applied, which were in the range of 17-86 kg COD/m^{3}d.

The degradation of propionate is even a larger problem in the thermophilic temperature range than in the mesophilic. Therefore, the propionate degradation was investigated in more detail. The results are presented in chapter 7. Kinetic parameters of the propionate degradation were determined, and the degradation of propionate was studied in UASB reactors, fed with both mixtures of volatile fatty acids and mere propionate solutions. Based on theoretical considerations and on the results obtained, a two-step methane digestion system is proposed, in which the propionate conversion is separated from other conversions. The two-step system turned out to be far more effective than a one-step system of a similar total reactor volume. At a loading rate of 52 kg COD/m^{3}d, the treatment efficiencies were 92 and 82 %, respectively.

When adopting thermophilic digestion, the question becomes relevant how well the sludge can resist sub-optimal conditions. This matter is dealt with in chapter 8. The deterioration of thermophilic sludge upon storage at low temperatures is comparable with that of mesophilic sludge. Lowering of the temperature during operation causes no problem, unless the lower activity of the sludge leads to an accumulation of volatile fatty acids an a concomitant drop in the pH. Much more serious is a shortage of substrate at a temperature of 55 °C. From the results obtained it is concluded that a four-day substrate shortage may be considered fatal. Consequently, when one expects a substrate shortage, it is recommended to lower the operation temperature.

In chapters 5 to 8 the granular sludge used was pre-cultivated on sugars. For the application of thermophilic digestion in practice, it is very important to know whether granulation will occur when using acidified substrates. Generally, 70-80 % of the evolved methane originates from acetate, and consequently, special attention should be paid to organisms performing this conversion in the granulation process on acidified wastewaters.

Formation of sludge granules in using merely acetate solutions as feed occurs after some three months of operation. It is shown that by applying the right criteria, a selection can be made for the acetate utilizing methanogens. Under conditions of high acetate concentrations in the effluent, *Methanosarcina* is the predominant methanogen, whereas at low concentrations *Methanothrix* is predominant. Granules consisting of *Methanothrix* are preferable above those consisting of *Methanosarcina*.

Apparently, the low numbers of *Methanothrix* in the seed material used plays an important role in the duration of the period before granulation was observed. Using a seed material with a high content of *Methanothrix* bacteria, granulation proceeds considerably faster. None of the parameters tested, namely, the type of seed material, the addition of inert particles to the seed material, and the use of nitrogen gas in the UASB reactor to achieve a fixed gas upflow velocity, proved to have a clear effect on the granulation. With the granules consisting of *Methanothrix*, extremely high loading rates, up to 162 kg COD/m^{3}d could be accommodated with 89 % treatment efficiency.

CONCLUSIONS

The following conclusions can be drawn from the investigations presented in this thesis.

Thermophilic anaerobic digestion,as a process for the treatment of wastes and wastewaters,can be started up easily. Any material exerting a reasonable methanogenic activity in the mesophilic temperature range, will suffice as a seed material.

Thermophilic digestion is not well suited for the digestion of livestock wastes with a total ammonia concentration exceeding 3.0 kg N/m^3. This can be attributed to the inhibitory effect of ammonia on the hydrogen utilizing methanogenic bacteria.

The performance of thermophilic UASB reactors in treating solutions of sugars is good. Using the granular sludge cultivated on sugars, other substrates, like acidified (model) wastewaters and high strength wastewaters, can be treated as well. Very high loading rates can be accommodated, but the treatment efficiencies depend on the concentration of inhibitory compounds in the wastewater to be treated.

A two-stage methanogenic system is recommended for treating acidified wastewaters containing propionate, because a better efficiency of the propionate degradation can be achieved as compared to one-step methanogenic processes.

Thermophilic sludge can be preserved satisfactorily under unfed conditions, provided the temperature is decreased. Under conditions of severe underloading a rapid deterioration of the sludge occurs. Therefore, a lowering of the operating temperature is recommended in that case.

Granulation in UASB reactors will also occur with the use of acidified wastewaters. Extremely high loading rates can be accommodated with the granular sludge developed on acidified substrates. The ultimate granulation seems to b be independent of the addition of inert particles to the seed material, or th the nature of the seed material. However, they do have an impact on the rate of the granulation process: the granulation could be speeded up by using an adapted seed material.

Considering the results of the investigations presented in this thesis, it can be concluded that little, if any, hampers the practical application of thermophilic anaerobic treatment of wastewaters, which are essentially free of inhibiting compounds. Of course, economical factors will ultimately determine the applicability of thermophilic anaerobic wastewater treatment.

10B. Samenvatting en konklusies

Anaerobe zuivering van afval en afvalwater is gedurende de laatste decennia een goed alternatief gebleken voor de meer gangbare aerobe zuivering, zij het dat een nabehandeling van het anaeroob gezuiverde afvalwater in het algemeen nog altijd noodzakelijk is. Een van de grootste nadelen van de anaerobe methode is de lage groeisnelheid van de anaerobe bakterien, zodat een hoge verblijftijd van de betrokken bakterien nodig is. Op grond van het feit dat chemische reakties sneller verlopen naarmate de temperatuur hoger is, zou thermofiele anaerobe afvalwaterzuivering - tussen 45 en 65 °C - een gunstig alternatief kunnen zijn voor mesofiele anaerobe zuivering. Wat voor chemische reakties geldt, hoeft nog niet op te gaan voor biologische reakties. Deze worden gelimiteerd door de grenzen van het biologisch mogelijke, en de verschuiving van vele evenwichten onder invloed van de temperatuur, die soms ongunstige effekten kan hebben.

Dit proefschrift houdt zich bezig met de vraag of thermofiele methaangisting ook in de praktijk gunstiger kan zijn dan mesofiele, en onder welke voorwaarden en omstandigheden dat het geval is. De tijd lijkt nog niet rijp voor een ekonomische evaluatie van de voordelen van de thermofiele gisting; in dit proefschrift wordt voornamelijk ingegaan op de "biologische haalbaarheid" en de voordelen die bij gebleken haalbaarheid te realiseren zijn.

In hoofdstuk 2 wordt de literatuur over thermofiele methaangisting geevalueerd. Het blijkt dat er sinds de twintiger jaren van deze eeuw belangstelling voor thermofiele gisting bestaat. Het onderzoek uit de tijd voor 1960 beperkt zich vooral tot de slijkgisting. Deze kan bedreven worden bij aanzienlijk lagere verblijftijden met eenzelfde rendement en zuiveringseffekt als bij mesofiele slijkgisting mogelijk is. Thermofiele slijkgisting wordt op het moment slechts op enkele plaatsen toegepast. Over het algemeen geeft een thermofiele bedrijfsvoering een betere eliminatie van pathogenen, maar ook een geringe verhoging van de koncentraties van vluchtige vetzuren, die een iets ongunstiger reuk tot gevolg heeft.

Sinds 1977 is er een sterk gestegen belangstelling voor thermofiele mestgisting. Deze heeft ongeveer dezelfde voordelen: kortere verblijftijden zijn mogelijk en de eliminatie van pathogenen is beter. Thermofiele zuivering van afvalwater in volledig gemengde systemen wordt weinig toegepast. In het algemeen gelden voor deze systemen dezelfde voordelen als de zojuist genoemde.

Modernere systemen voor de anaerobe behandeling van afvalwater zijn er meestal op gericht zoveel mogelijk bakteriemateriaal in de reaktor te houden. Dan kan worden gewerkt met een hoge verblijftijd van de bakterien, maar ook met een lage verblijftijd van het afvalwater in de reaktor. Er is nog niet veel ervaring met dergelijke systemen in het thermofiele gebied. Een belangrijk verschil met de boven besproken systemen is, dat in dit soort systemen niet gestreefd wordt naar een zo laag mogelijke verblijftijd van de bakterien. Dus er wordt niet zozeer gebruik gemaakt van de hoge groeisnelheid van thermofiele bakterien, als wel van hun hoge onderhoudsenergie en afstervingssnelheid.

In het algemeen blijken dit soort thermofiele systemen veel hogere belastingen te kunnen verwerken dan overeenkomstige mesofiele systemen. Een aantal uitzonderingen wordt besproken. Enige theoretische aspekten van de thermofiele methaangisting worden besproken, en er wordt een overzicht gegeven van de kinetische parameters van een aantal relevante bakterien.

Een van de grootste problemen van de mestgisting is het hoge ammoniakgehalte van de mest. Ammoniak is in hoge koncentraties zeer giftig voor methaanbakterien. Het dissociatie-evenwicht verschuift bij hogere temperaturen in de richting van het vrije ammoniak, dat als het giftige agens bekend staat. Inderdaad blijkt thermofiele methaangisting van mest met een hoog ammoniakgehalte, meer dan 3.0 kg N/m^3, zeer slecht te verlopen. In hoofdstuk 3 wordt onderzocht of het inderdaad het hoge ammoniakgehalte, of het hoge gehalte aan organische stof is, dat verantwoordelijk gesteld kan worden voor het falen van het thermofiele proces. De rol van het gehalte aan organische stof blijkt onbetekenend te zijn. Enige implikaties voor een veel gebruikt beschrijvend model worden aan de orde gesteld.

Enige aandacht is besteed aan de vraag hoe de remming van de thermofiele gisting door ammoniak in zijn werk gaat. Vreemd genoeg blijkt ammoniak bijna geen invloed te hebben op de omzetting van acetaat in methaan. Kennelijk vindt de vergiftiging via een intermediaire verbinding plaats. Het is zeer aannemelijk dat waterstof deze verbinding is. Deze remt, bij hoge koncentraties, de omzetting van propionaat, dat op zijn beurt weer een remmende werking heeft op de omzetting van acetaat in methaan.

De hoofdstukken 5 tot en met 9 houden zich bezig met het onderzoek naar het funktioneren van het thermofiele upflow anaerobic sludge blanket (UASB) proces (in nederlands: het opwaarts doorstroomde slibdeken proces). Allereerst werd de vergisting van een makkelijk vergistbaar materiaal onderzocht, te weten van druivesuiker. Daarvan is bekend dat het in het mesofiele temperatuurgebied gemakkelijk aanleiding geeft tot de vorming van slibkorrels, die een zeer gunstig effekt hebben op het funktioneren van een UASB reaktor. Dit bleek ook bij 55 °C het geval te zijn. Er werden hoge belastingen behaald, tot 45 kg COD/m^{3}d, met een omzetting in methaan van meer dan 70 %. Met het op suikers gekultiveerde slib konden oplossingen van vluchtige vetzuren worden behandeld bij belastingen tot 104 kg COD/m^{3}d, met een zuiveringseffekt van meer dan 77 %. In hoofdstuk 5 wordt verder nog enige aandacht geschonken aan de faktoren die aan de korrelvorming ten grondslag liggen. Een belangrijke rol van de biogas-oppervlakte-belasting - de hoeveelheid ontwikkeld biogas per eenheid van oppervlakte en tijd - wordt aannemelijk gemaakt, naast de al bekende faktoren.

Vinasse, het afvalwater van alkohol-destilleerderijen, wordt in grote hoeveelheden geproduceerd en komt bij hoge temperatuur vrij. Het is een geschikt materiaal om te dienen als voorbeeld voor een sterk gekoncentreerd afvalwater. In hoofdstuk 6 wordt het onderzoek met betrekking tot de vergisting van dit afvalwater gepresenteerd. Bij een hoge verblijftijd in volledig gemengde reaktoren is thermofiele gisting ongeveer net zo efficient als mesofiele gisting. Dit betekent dat men mag verwachten dat de kwaliteit van het effluent bij thermofiele behandeling in een UASB reaktor gelijk geacht mag worden aan dat na mesofiele gisting, vooropgesteld dat er geen overbelasting plaats vindt.

De behandeling van vinasse in een thermofiele UASB reaktor werd uitgevoerd met korrelslib dat op suikers was gekweekt. Hiervoor bleek een aanzienlijke adaptatie-periode benodigd te zijn. Vinasse bleek weliswaar tot zeer hoge belastingen, tot 86 kg COD/m^{3}d, behandeld te kunnen worden, maar het zuiveringseffekt was maar matig. De koncentratie van de vinasse bleek uiteindelijk een grotere invloed te hebben op het zuiveringseffekt dan de toegepaste belasting, die varieerde van 17-86 kg COD/m^{3}d.

De afbraak van propionaat blijkt in het thermofiele temperatuurgebied een groter probleem te zijn dan in het mesofiele. In hoofdstuk 7 wordt hier nader op ingegaan. Er werden kinetische parameters verzameld van de bakterien die het propionaat omzetten. De afbraak van propionaat werd bestudeerd in UASB reaktoren die gevoed werden met zowel mengsels van vluchtige vetzuren, als alleen propionaat. Op grond van theoretische overwegingen en de gedane waarnemingen wordt een tweetrapssysteem voorgesteld, waarin de afbraak van propionaat wordt gelokaliseerd in een aparte reaktor. Toepassing van het voorgestelde systeem bevestigde de vermoedens: het werkt veel effektiever dan eenééntrapssysteem met eenzelfde totale reaktorvolume. Bij een belasting van 52 kg COD/m^3d waren de zuiveringseffekten respektievelijk 82 en 92 % voor één- en tweetrapssysteem.

Bij een eventuele toepassing van thermofiele methaangisting is de vraag relevant hoe goed het gekultiveerde slib bestand is tegen minder gunstige omstandigheden. Hoofdstuk 8 is aan deze materie gewijd. De verslechtering van de kwaliteit van het slib tijdens het bewaren bij lage temperatuur is te vergelijken met die van mesofiel slib. Ook het dalen van de temperatuur tijdens het bedrijf levert geen problemen op, tenzij er door de lagere aktiviteit van het slib ongunstige milieu-omstandigheden ontstaan, als een te hoge koncentratie van vluchtige vetzuren of als gevolg daarvan een te lage pH. Uit de verrichte experimenten bleek dat het ontbreken van substraat bij 55 °C na vier dagen funest geacht mag worden. Ziet men dus een substraat-tekort aankomen, dan is het nuttig de temperatuur te verlagen, zodat het slib niet te snel zal afsterven.

Werd in de hoofdstukken 5 tot en met 8 gebruik gemaakt van korrelslib, dan betrof het altijd slib dat werd gekultiveerd met druive- of rietsuiker als substraat. Toch is het erg belangrijk voor een eventuele toepassing van het thermofiele UASB proces te weten of korrelvorming ook optreedt bij gebruik van verzuurde substraten. Normaliter wordt ongeveer 70 tot 80 % van het gevormde methaan via acetaat gevormd, dus de bakterien die deze omzetting uitvoeren verdienen speciale aandacht in het proces van de korrelvorming bij het gebruik van verzuurde substraten.

De vorming van korrelslib bij gebruik van acetaat als substraat bleek na ongeveer drie maanden inderdaad op te treden. Door het aanleggen van de juiste kriteria kon geselekteerd worden op de acetaat-omzettende bakterien. Bij een hoge koncentratie van acetaat in het effluent is *Methanosarcina* de dominante acetaat-omzettende methaanbakterie, bij lage koncentraties is dat *Methanothrix*. Korrels, bestaande uit *Methanothrix* bakterien blijken te prefereren boven *Methanosarcina* korrels.

Kennelijk speelde de lage dichtheid van de thermofiele *Methanothrix* bakterien in de toegepaste entmaterialen een belangrijke rol in de duur van de periode voordat korrelvorming werd vastgesteld. Bij gebruik van een meer geschikt entmateriaal, waar *Methanothrix* reeds in hoge aantallen in voorkwam, blijkt het korrelvormingsproces aanzienlijk te kunnen worden versneld. Van de onderzochte variabelen,- de toevoeging van hechtmateriaal aan het entmateriaal en de aard van het entmateriaal - kon geen invloed op de uiteindelijke korrelvorming worden vastgesteld. Met het uit *Methanothrix* bestaande korrelslib werden extreem hoge belastingen, tot 162 kg COD/m^{3}d, verwerkt met een zuiveringseffekt van 89 %.

KONKLUSIES

De konklusies van het in dit proefschrift gepresenteerde onderzoek kunnen de volgende zijn.

Thermofiele anaerobe methaangisting is als proces zeer eenvoudig op te starten. Ieder entmateriaal met een redelijke mesofiele aktiviteit volstaat.

Thermofiele gisting is echter niet erg geschikt voor de vergisting van mest met een hoog ammoniak-gehalte. Dit kan geweten worden aan de remmende invloed die ammoniak op de waterstof omzettende methaanbakterien heeft.

De vergisting van oplossingen van suikers in thermofiele UASB reaktoren verloopt suksesvol. Met het op suikers gekultiveerde korrelslib kunnen ook andere substraten behandeld worden, als verzuurde (model)afvalwaters en gekoncentreerde afvalwaters. De belastingen die verwerkt kunnen worden zijn zeer hoog, maar de zuiveringseffekten hangen af van de koncentraties van giftige stoffen in het te behandelen afvalwater.

Een tweetrapssysteem wordt aanbevolen voor de behandeling van propionaat bevattende afvalwaters, omdat een beter rendement van de propionaat-afbraak kan worden bereikt dan in een ééntrapssysteem.

Thermofiel slib kan goed bewaard worden onder ongevoede omstandigheden, bij een lage temperatuur. Bij ernstige onderbelasting bij 55 °C treedt een snelle verslechtering van de kwaliteit van het slib op. Daarom is verlaging van de temperatuur aan te raden bij een gebrek aan voeding.

Ook op verzuurde afvalwaters kan in thermofiele UASB reaktoren korrelvorming optreden. Met dergelijk korrelslib zijn extreem hoge methaanprodukties en belastingen haalbaar.

De korrelvorming op verzuurde substraten lijkt onafhankelijk te zijn van de toevoeging van inert hechtmateriaal, of de aard van het entmateriaal. Deze hebben wel invloed op de snelheid waarmee het korrelvormingsproces verloopt: bij gebruik van een geadapteerd entmateriaal kon de korrelvorming aanzienlijk worden versneld.

Uit de resultaten van de in dit proefschrift gepresenteerde onderzoekingen kan worden gekonkludeerd dat weinig de praktische toepassing van thermofiele anaerobe zuivering van een niet al te giftig afvalwater in de weg staat. Natuurlijk zullen ekonomische overwegingen de doorslag geven bij het in praktijk brengen van de thermofiele afvalwaterzuivering.

Curriculum vitae

De auteur van dit proefschrift werd te Utrecht geboren op 31 mei 1955. Na de kleuter-, lagere en middelbare school suksesvol doorlopen te hebben, ving hij in 1973 de studie Milieuhygiene aan de Landbouwhogeschool aan, die hij met specialisatie Waterzuivering, met Waterzuivering en Mikrobiologie als hoofdvakken, afrondde in 1980. In datzelfde jaar werd hij door de vakgroep Waterzuivering van bovengenoemde hogeschool aangetrokken voor het verrichten van een deels door de EEG betaalde studie naar de thermofiele anaerobe behandeling van afval en afvalwater. Na beeindiging van het projekt werd de auteur gelegenheid geboden om van zijn werkzaamheden verslag te leggen in het proefschrift, dat nu voor U ligt.