Willem de Zeeuw

acclimatization of
sludge
-reactor start-up

Promotor : dr. L. Lijklema, hoogleraar in de waterzuivering en
de waterkwaliteit

Co-promotor : dr. G. Lettinga, wetenschappelijk hoofdmedewerker

W.J. de Zeeuw

ACCLIMATIZATION OF ANAEROBIC SLUDGE FOR UASB-REACTOR START-UP

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 7 september 1984
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen.

STELLINGEN

1. In het UASB-proces zal licht slib geen struktureel probleem vormen, zoals het dat tot op heden in het aërobe aktief slib proces wel doet.

F. Wagner (1982) Study of the causes and prevention of sludge bulking in Germany. In: Bulking of activated sludge (Chambers, B. and Tomlinson, E.J., eds.), Ellis Horwood Ltd. Chichester.

2. De door Baresi en Wolfe voor Methanosarcina gerapporteerde F420-gehalten zijn dermate laag, dat aan de juistheid moet worden getwijfeld.

L. Baresi and R.S. Wolfe (1981) Levels of coenzyme F420, coenzyme M, hydrogenase, and methylcoenzyme M methylreductase in acetate-grown Methanosarcina. Appl.Env.Microbiol. 41, 388-391.

3. Een schatting van de hoeveelheid methanogene bacteriën in anaëroob slib op grond van het F420-gehalte van één daaruit geïsoleerde reinculture is dubieus.

P. van Beelen, A.C. Dijkstra en G.D. Vogels (1983) Quantitation of coenzyme F420 in methanogenic sludge by the use of reversed-phase high-performance liquid chromatography and a fluorescence detector. Eur.J.Appl.Microbiol.Biotechnol. 18, 67-69.

4. De konklusie uit het kinetische model van Van den Heuvel en Zoetemeyer, dat volledige slibuitspoeling uit een UASB-reactor mogelijk is bij influentconcentraties hoger dan 1,6 kgCZV.m^{-3} is onlogisch.

J.C. van den Heuvel en R.J. Zoetemeyer (1982) Stability of the methane reactor: a simple model including substrate inhibition and cell recycle. Process Biochemistry 17 (3), 14-19.

5. Het feit dat door anderen, bij hoge koncentraties propionzuur als enig substraat, geen propionzuur-afbrekende populatie is opgehoopt met een μ_{max} groter dan 0,15-0,4 d^{-1}, doet twijfel rijzen aan het bestaan van een door Heyes en Hall op basis van proeven met mengsubstraten gepostuleerde propionzuur-vergistende subgroep met een μ_{max} van 1,2 d^{-1}.

R.H. Heyes en R.J. Hall (1983) Kinetics of two subgroups of propionate-using organisms in anaerobic digestion. Appl.Env.Microbiol. 46, 710-715.

D.R. Boone en M.P. Bryant (1980) Propionate-degrading bacterium, Syntrophobacter wolinii sp.nov.gen.nov., from methanogenic ecosystems. Appl.Env.Microbiol. 40, 626-632.

6. Het paranormale wordt steeds normaler.

7. Varkensmestvergisting wordt door de Chinese boer terecht verdedigd als een technologie, die zijn welvaart verhoogt. Dat zijn Nederlandse kollega dit tot nu toe niet of nauwelijks doet, is meer een uitvloeisel van ons welvaartspeil, dan een onzorgvuldig omgaan met energiebronnen.

Gao Xiaosheng (1984) Een allereenvoudigst verhaal. (Vertaling: Koos Kuiper). Meulenhoff, Amsterdam.

8. Schaalvergroting in de dienstverlening verlaagt de welvaart.

9. Dat er onder ekonomen geen overeenstemming bestaat over maatregelen om uit de crisis te geraken, is een gevolg van het ontbreken in economische denkpatronen van het inzicht, dat natuurlijke omstandigheden de economische ontwikkelingen beïnvloeden.

Ekonomen over crisis. Uitgeverij Intermediair, Amsterdam/Brussel, 1982.

J.W. de Zeeuw (1980) Commoditeitenkunde. Mededeling Vakgroep Cultuurtechniek no. 34, Landbouwhogeschool, Wageningen.

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Acclimatization of anaerobic sludge for UASB-reactor start-up.
Wageningen, 7 september 1984.

VOORWOORD

De inbreng van velen heeft het mogelijk gemaakt dat dit proefschrift er is gekomen.
Een aantal mensen wil ik hier met name noemen.
Gatze Lettinga heeft mij met zijn niet aflatende enthousiasme uitstekend begeleid. Bedankt voor de mogelijkheden die je me hebt geboden en voor de prettige samenwerking.
Van Bert Lijklema, die pas in een laat stadium als promotor bij dit proefschrift betrokken raakte, heb ik de kritiek op het manuscript zeer gewaardeerd.
Jan Dolfing, Dook Noy, Henk van de Honing, Leo Habets, Jan Boon, Saskia Wanders, Jos Lubbers en Karin van Straten droegen met het werk in het kader van hun doctoraalstudie het merendeel van de gegevens aan.
De medewerksters en medewerkers van de vakgroep waterzuivering zorgden voor een uitstekende werksfeer. Met name de inspirerende gedachtenwisselingen met Look Hulshoff Pol en Wim Wiegant heb ik zeer op prijs gesteld.
De heer Adamse (vakgroep Microbiologie) en de heren Mayhew en Haaker (vakgroep Biochemie) hebben in de beginfase in de begeleiding van het onderzoek geparticipeerd en mij gebruik laten maken van de F420-onderzoeksfaciliteiten van hun vakgroep.
De centrale dienst van het Biotechnion dank ik voor de technische assistentie; de heren Rijpma en Schimmel voor het tekenwerk en Alfred van Baaren voor het reproduktiewerk.
Helen Cooper heeft de grootste fouten uit mijn versie van de Engelse taal gehaald.
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ABSTRACT

The Upflow Anaerobic Sludge Bed (UASB) reactor represents a high rate anaerobic wastewater treatment system. The majority of the active biomass in the reactor is present in the form of sludge granules which possess excellent settling properties.

If no acclimatized (granular) sludge is available, the first start-up is performed using unadapted seed material.

The subject of this study is the start-up of UASB-reactors using digested sewage sludge as seed material and volatile fatty acids (VFA) as substrate.

A relation was demonstrated between the dry suspended solids content of digested sewage sludge and its settleability and methanogenic activity.

The analysis of coenzyme F420 proved a reliable method to assess the potential methanogenic activity of anaerobic sludge.

Several environmental conditions were found to influence the length of the gas production lag phase of digested sewage sludge following the supply of a VFA-feed, e.g. the biomass concentration and the mixing intensity.

Exceptionally high sludge growth yields were determined with acetate as single substrate.

The rate of UASB-reactor start-up has been examined in relation to the type and quantity of seed sludge and the composition and strength of the wastewater. Two types of sludge wash-out are distinguished, i.e. sludge bed expansion wash-out and sludge bed erosion wash-out. The latter type represents a key feature of the UASB-reactor, i.e. the selection pressure exerted on the sludge particles, which is essential in the development of granular sludge from the digested sewage sludge seed. Indications were obtained that the use of different types of digested sewage sludge leads to the development of different types of granular sludge, as a result of differences in the average biomass retention time during the early stages of start-up. The role of Methanothrix and Methanosarcina is discussed.

The development of bulking anaerobic sludge is reinforced by seeding with a dilute digested sewage sludge type ($\leq$40 kgDSS.m^{-3}). This will prolong the start-up time. Guidelines are provided with respect to the choice of a seed sludge depending upon the strength and composition of the wastewater.

Keywords: anaerobic wastewater treatment, UASB-process, start-up, digested sewage sludge, coenzyme F420, lag phase, growth yield, granular sludge.

CONTENTS

CHAPTER 1 INTRODUCTION

1.1 ANAEROBIC WASTEWATER TREATMENT PROCESSES

1.1.1 GENERAL

Volta in 1776 is considered to be the first to realize the relation between decaying vegetation and the occurrence of inflammable gas. Systematic investigations of anaerobic digestion started in the second half of the nineteenth century, and at the turn of the twentieth century the first methane digesters were installed (van Brakel 1980, McCarty 1981).

For a long time anaerobic digestion was thought feasible only for the digestion of concentrated wastes such as manure and sewage sludge at long retention times. Around 1950 also the anaerobic treatment of wastewaters was attempted. Special reactor types for wastewater treatment were developed, i.e. the anaerobic contact process (Schroepfer et al. 1955) and the anaerobic filter (Young and McCarty 1969).

The energy crisis of 1973 strongly augmented the interest in anaerobic digestion for all biodegradable wastewaters. Advanced methods were introduced such as the Upflow Anaerobic Sludge Blanket (UASB) process (Lettinga et al. 1980), and various fixed film reactor types (Switzenbaum and Jewell 1980, van den Berg and Lentz 1979, Heijnen 1983).

Nowadays anaerobic digestion is rapidly becoming a full grown alternative for other wastewater treatment methods; albeit, that it essentially represents a pretreatment method, because the effluent quality usually necessitates some sort of posttreatment before the water can be discharged to surface water.

The main advantages of anaerobic digestion in comparison with aerobic wastewater treatment are:

- the lower energy requirements combined with the production of biogas, which represents an excellent fuel, and
- the much lower production of excess sludge, which in addition is well stabilized and therefore easier to dispose of.

In a time of increasing energy costs these characteristics of anaerobic digestion make the application of this treatment method increasingly attractive, even in combination with an aerobic posttreatment, which then

will need only a fraction of the oxygen input of a completely aerobic treatment.

1.1.2 TREATMENT METHODS

The maximal growth rate of the rate limiting bacteria in the methane digestion of soluble substrates is in the order of 0.08 to 0.15 d^{-1} (acetate degradation by Methanotrix söhngenii (Huser 1981) and propionate degradation (Koch et al. 1983)). This implies minimal sludge retention times of 7 to 12 days.
Hence high organic loading rates with low strength wastewater are only possible when the sludge retention time exceeds the hydraulic retention time.

TABLE 1 COMPARISON OF REACTOR TYPES FOR ANAEROBIC WASTEWATER TREATMENT

reactor type	sludge retention method	achievable loading rates (1) ($kgCOD.m^{-3}.d^{-1}$)	ref. (2)
conventional (completely stirred)	none	approx. 1	1
anaerobic contact process	separate settling tank with sludge return	approx. 5	1
anaerobic filter	bacterial immobilization on filter material combined with sludge particle retention in filter interstices	10 - 15	2
UASB	granulation of bacterial mass and an internal settling compartment	20 - 50	3,4
fixed film processes	bacterial immobilization on static surfaces (upflow or downflow mode) or	20 - 40	5
	on particles (expanded or fluidized bed mode)	20 - 50	6,7

(1) Treating wastewater with a moderate strength (5-10 $kgCOD.m^{-3}$) at removal efficiencies of more than 80%.
(2) 1: van den Berg et al. 1980, 2: Lettinga et al. 1982, 3: Lettinga et al. 1980, 4: Hulshoff Pol et al. 1983a, 5: Hall and Jovanovic 1982, 6: Switzenbaum and Jewell 1980, 7: Heijnen 1983.

The loading rates that can be applied to an anaerobic reactor, therefore, are primarily determined by the biomass retention of the system.
Methods to improve the sludge retention in the treatment systems are summarized in Table 1.

The UASB reactor is one of the reactor types with a high loading capacity. It differs from other high rate processes by the simplicity of its design. No separate settler with sludge return pumps is required, as in the anaerobic contact process. There is no loss of reactor volume through filter or carrier material, as is the case with the anaerobic filter and the fixed film reactor types. Also, there is no need for high rate effluent recirculation and concomitant pumping energy, as is the case with a fluidized bed reactor.

1.1.3 THE UASB REACTOR AND ITS APPLICABILITY

UASB reactor design

Figure 1 shows a schematic diagram of a full scale UASB plant.
The most important features of the Upflow Anaerobic Sludge Bed concept are the influent distribution system at the bottom of the reactor, and the gas/solids separator, which is installed in the upper part of the reactor (Lettinga et al. 1980). The gas is removed by gas collectors in which a gas/water interface is maintained by a water lock in the gas outlet. The gas liquid interface area should be large enough to avoid severe foaming and clogging of the gas outlet pipes. The interface must also be kept well stirred by the gas production to prevent the formation of a scum layer. Floating sludge particles must be able to release attached gas bubbles at the interface.
In the reactor space above the gas collectors a gas bubble free zone permits sludge particles to settle out and to slide back into the digesting compartment along the inclined walls of the gas/solids separator (with a minimum inclination of approximately 50^{o}) (Lettinga et al. 1980).

Generally no mechanical mixing device is installed in UASB reactors. At high organic loading rates the biogas production guarantees sufficient contact between substrate and biomass. Regarding the dynamic behaviour of the water phase, an UASB reactor approaches a completely mixed reactor. This is not the case for the bulk of the sludge particles retained in the

reactor. The sludge constitutes a dense sludge bed in the lower part of the reactor, which is relatively poorly mixed as far as the sludge particles are concerned. A much less concentrated, fairly well mixed sludge blanket fills up the space above the sludge bed and beneath the gas collector system.

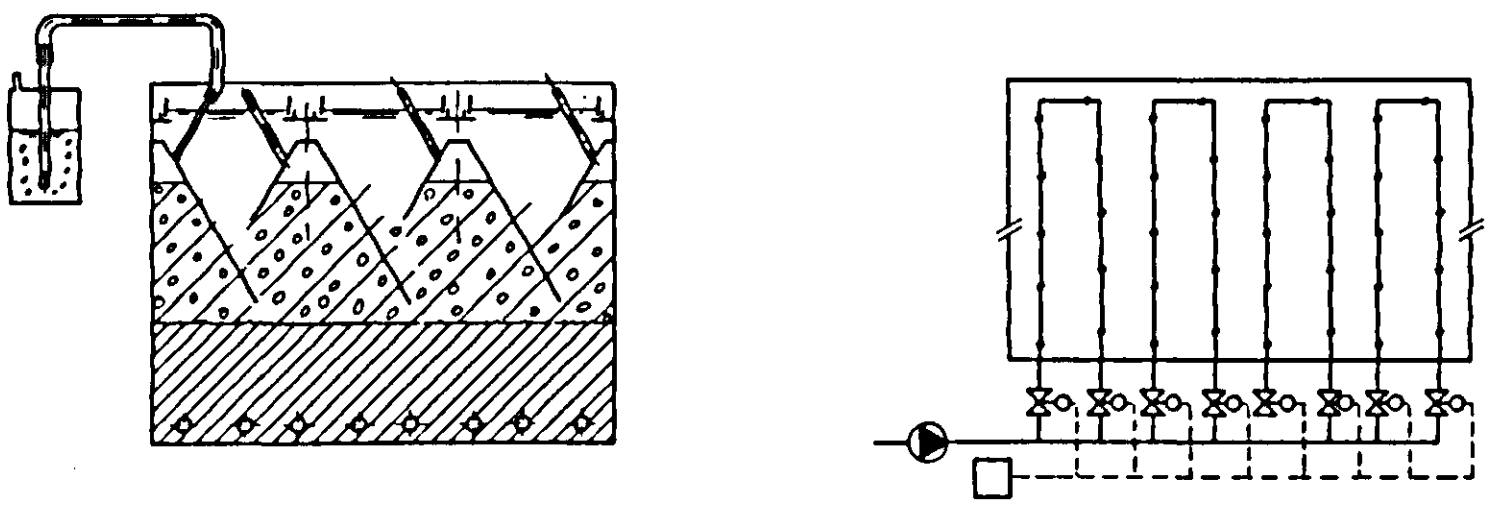


FIGURE 1 Schematic diagram of a full scale UASB plant (left) and of its wastewater inlet system (right).

A properly functioning UASB reactor needs an even distribution of the wastewater at the bottom of the reactor. This is especially true for reactor start-up or during restart after a period of standstill in which the sludge has settled out to a dense mass. Good results have been obtained in full scale reactors with one inlet point per 2 to 5 m^2.

U(A)SB reactor applications

The feasability of the U(A)SB concept has been proven for 4 different applications (Table 2).

Klapwijk et al. (1981) have shown that an Upflow Sludge Bed reactor can be used for denitrification of domestic sewage. A well settling, granular sludge was formed with an average concentration of 20 $kg.m^{-3}$, which permitted a surface loading rate of 8.5 m/h.

As shown by Zoetemeijer (1982), granular sludge also develops when using an UASB reactor for the anaerobic acidification of glucose solutions. A maximum loading rate of 440 $kgCOD.m^{-3}.d^{-1}$ was achieved at a hydraulic retention time of 0.43 hrs and at a biomass concentration of 25 $kg.m^{-3}$.

Today the most important application of the U(A)SB reactor undoubtedly is in the methane digestion of wastewater. Tables 3 and 4 summarize results

obtained with various types of wastewater. Dissolved wastes can be treated at very high organic loading rates (Table 4). Wastes which contain a relatively high percentage of suspended solids, like domestic sewage and slaughterhouse wastewater, can be treated profitably in a moderately loaded UASB reactor (Table 3).

TABLE 2 DIFFERENT APPLICATIONS OF THE U(A)SB CONCEPT

Application	Type of sludge
Denitrification	granular
Anaerobic acidification	granular
Methane digestion - at low loading rate - at high loading rate	 flocculent granular

The specific activity of the sludge developing under such conditions in the UASB reactor will not exceed 0.3 $kgCH_4$-COD.$kgVSS^{-1}$.d^{-1}. A considerable part of the suspended solids from the wastewater is retained in the flocculent sludge bed and will be stabilized there slowly. As the biodegradability of this matter is generally poor, the sludge yield will be larger than in the case of the treatment of completely soluble wastes.

TABLE 3 EXPERIENCES WITH METHANE DIGESTION IN PILOT SCALE UASB REACTORS USING FLOCCULENT SLUDGE.

type of wastewater	COD conc. ($kg.m^{-3}$)	suspended solids (% of COD)	loading rate ($kgCOD.m^{-3}.d^{-1}$)	COD removal (%)	ref. (1)
domestic sewage (20 °C)	0.65	35-40	2	65-70	1
slaughterhouse wastewater (20-30 °C)	2	50	3	70	2
liquid calf manure (30 °C)	9.5	67	4	90	3

(1) 1: Grin et al. 1983, 2: Sayed 1982, 3: van Velsen et al. 1979a.

When operating a granular sludge UASB reactor at high loading rates, poorly settling suspended solids present in the wastewater will not be removed to a significant extent because of the high gas and hydraulic surface loads.

This is reflected in the overall COD removal efficiency which was found for different medium strength wastewaters (Table 4). The specific activity of the granular sludge cultivated in these experiments normally exceeds 0.6 $kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$.
Loading rates of 30-60 $kgCOD.m^{-3}.d^{-1}$ can be treated satisfactorily with these dilute wastewaters (Table 4).
Presently, most full scale UASB reactors have a design capacity of only 8 to 15 $kgCOD.m^{-3}.d^{-1}$. Treatment efficiencies are high under these conditions and shock loading rates can easily be accomodated. In 1983 over 25 full scale UASB plants were in operation treating a variety of wastewaters (Pette and Versprille 1981).

TABLE 4 SOME EXPERIENCES WITH METHANE DIGESTION IN PILOT SCALE UASB REACTORS USING GRANULAR SLUDGE.

wastewater origin	temp. (°C)	COD conc. ($kg.m^{-3}$)	suspended solids (% of COD)	COD loading rate ($kg.m^{-3}.d^{-1}$)	COD removal (%)	ref. (1)
slaughterhouse	30	2	50	10	55	1
rendering plant	30	3.5	25	25 - 60	63	2
sugar factory	32	3.6	20	32	75	3
potato processing	30	2 - 5	10 - 15	40	84	4
VFA-mixture (2)	30	2.85	0	36	93	5

(1) 1: Sayed 1982, 2: de Zeeuw 1982, 3: Pette et al. 1980, 4: Versprille 1978, 5: Hulshoff Pol et al. 1982.
(2) Consisting of 20 mM acetate and 13.5 mM propionate.

1.2 SCOPE OF THIS STUDY

1.2.1 BACKGROUND

After the successful introduction in 1977 of the first industrial UASB reactor at the CSM sugar factory at Halfweg, the Netherlands, one of the main problems, still encountered in the application of the process, was the reactor start-up when using unadapted seed material. Although since that time an increasing amount of excess granular sludge from operating UASB reactors has become available for starting up new reactors, a large number of reactors, especially outside the Netherlands, still have to be started up with digested sewage sludge or other available anaerobic sludges as seed material.

In 1977 little knowledge was available on the microbiological and technological process parameters relevant for UASB reactor start-up.
For this reason a research program was set up to obtain a better understanding of the processes controlling UASB reactor operation, and more particularly to provide guidelines for UASB reactor start-up using unadapted seed sludge.
The results of this study are presented here. Part of the results have been reported previously (de Zeeuw and Lettinga 1980a, 1980b, 1983a, 1983b, de Zeeuw et al. 1981).

1.2.2 DEFINITION OF UASB REACTOR START-UP

Unless stated otherwise in this report reactor start-up is defined as the first start-up of a reactor seeded with unadapted seed material. The first start-up is meant, as opposed to the start-up after a standstill. The latter is referred to as restart. Unadapted seed material in this respect is considered to be anaerobic sludge with the characteristics mentioned in Table 5.

TABLE 5 CHARACTERISTICS OF UNADAPTED SEED SLUDGE FOR UASB REACTOR START-UP

a. The seed material is obtained from an anaerobic treatment systen without biomass retention, such as conventional mixed digesters, ruminants, septic tanks, or fresh water sediments.

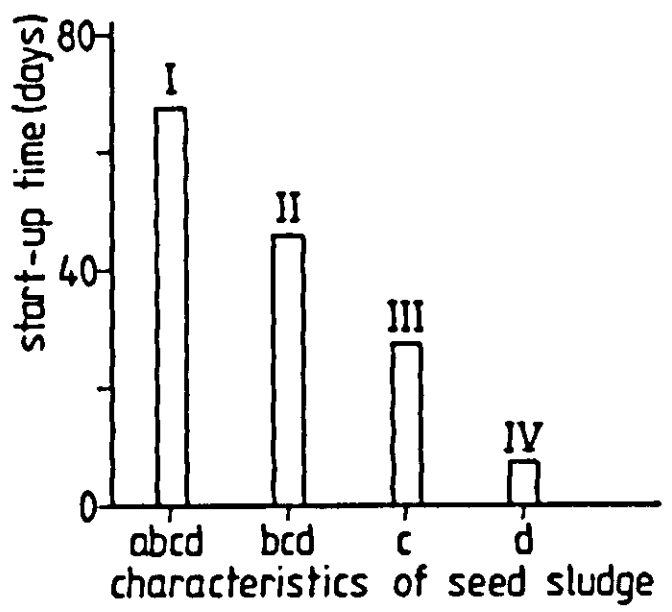
b. The sludge exerts a specific methanogenic activity of less than 0.2 $kgCH_4$-COD/kgVSS.d.

c. The settleability of the organic fraction of the sludge is poor.

d. The bacterial population of the sludge is unbalanced with respect to the composition needed for the simultaneous degradation of all waste ingredients.

e. The sludge is not adapted to specific inhibitors in the wastewater (such as sulphide, ammonia, etc.).

In general UASB reactor start-up will proceed more easily and more quickly when the quality of the seed sludge in one or more aspects exceeds that of unadapted seed material as defined in Table 5 (see Figure 2).
Obviously the fastest reactor start-up will be attained when using an appropriate amount of granular sludge as a seed sludge, obtained from another, well functioning UASB reactor treating the same type of waste.



I. Digested sewage sludge; treating a VFA mixture (this research).
II. Digested sewage sludge enriched with 5 % highly active flocculent UASB reactor sludge; treating a VFA mixture (this research).
III. Flocculent sludge from a full scale UASB reactor used as seed sludge for a laboratory UASB reactor, both treating acidified potato starch factory wastewater (van Campen et al. 1984).
IV. Treatment of rendering wastewater with sugar beet wastewater adapted granular sludge (de Zeeuw 1982).

FIGURE 2 Some representative case histories illustrating start-up times of UASB reactors as related to the type of seed material characterized by conditions 'a', 'b', 'c' and 'd' of Table 5. After completion of the start-up in all 4 cases granular sludge was present in the reactor.
Start-up time is defined here as the time needed to reach a methane production rate of 10 $kgCH_4$-COD.m^{-3}.d^{-1}.

A practical definition of UASB reactor start-up time would be the time required to meet the design criteria of the system. For research purposes obviously another definition is required.
A more scientific definition of the start-up period is the time after which the sludge characteristics do not change any more, when treating a wastewater of a constant composition under fixed conditions.
Once a small amount of well adapted sludge has been formed, changes in reactor performance (e.g. the possibility to apply higher space loading rates) will merely depend upon changes in the quantity of adapted sludge (as a result of growth and wash-out), and will no longer be due to changes in the nature of the sludge.
However, a complication that arises in adopting the above mentioned definition of the start-up period, is that frequently still two types of anaerobic sludge are present in UASB reactors at the end of the start-up; i.e. granular sludge constituting the bulk of sludge present in the sludge bed and flocculent sludge which predominates in the sludge blanket above the sludge bed. The relative amounts of both types of sludge change as a result of growth and selective wash-out. This means that the nature of the sludge as a whole is still changing. At higher hydraulic loading rates and higher gas production rates the flocculent sludge which has inferior settling qualities, will be washed out from the reactor preferentially, while at lower loading rates flocculent sludge may grow in again. These

changes in the fractional contribution of granular sludge to the total amount of sludge are not considered to constitute a part of the initial start-up period.

The start-up period, therefore, is defined as the time required for the development of the first macroscopic sludge granules.

To allow comparison with start-up experiments to which this definition cannot be applied (e.g. when granules are present in the seed sludge, or when referring to reactor types different from the UASB reactor) the time needed to reach a methane production rate of 10 $kgCH_4$-COD.m^{-3}.d^{-1} will be used as a practical definition of the start-up period (Figure 2).

1.2.3 PROBLEMS ENCOUNTERED IN UASB REACTOR START-UP AS DISCUSSED IN SUBSEQUENT CHAPTERS

In the first start-up of UASB reactors using unadapted seed sludge a number of problems may be encountered.

Duration

Because of the much lower growth rates of anaerobic bacteria as compared to aerobic bacteria, the first start-up of an anaerobic treatment plant always takes more time, than that of an aerobic one.

The slow growth rate of anaerobes also has a magnifying effect upon disturbing factors. If an inhibiting compound in the waste diminishes the net growth, the start-up time will increase accordingly, e.g. from 70 to at least 140 days in case the growth rate is decreased by 50 %. In an aerobic treatment plant a comparable increase would be in the order of days or weeks instead of months.

The growth rate and other kinetic parameters will be reviewed in Chapter 2. Start-up experiments are reported in Chapter 7.

Methanogenic activity of the sludge

To select a seed sludge the methanogenic activity of the sludge should be known. Different methods of measuring the specific methanogenic activity particularly for relatively inactive sludges, are discussed in Chapter 3.

Nature of the seed sludge

Theoretically any medium containing the proper bacterial flora can be used as seed sludge for an UASB reactor. Examples of possible seed materials are

(digested) manure, fresh water sediments, septic tank sludge, (digested) sewage sludge and surplus sludge from anaerobic treatment plants.
Apart from its availability and its cost, the quality of a particular seed material can be judged in terms of ash content, the specific methanogenic activity, and the settleability.
These factors are discussed in Chapter 4 for different digested sewage sludges. In many countries digested sewage sludge is a readily available source of anaerobic sludge, and it has already been used in a large number of pilot and full scale start-up situations.

Lag phases

The response of an unadapted seed sludge to a new waste may include a lag phase. Among the factors influencing the occurrence and the length of a lag phase are the concentration of active biomass, the mixing intensity and the initial substrate concentration.
These factors were examined in batch fed stirred tank digesters with digested sewage sludge. The results are presented in Chapter 5.

Growth yield

The magnitude of the growth yield influences the length of the start-up period. The sludge growth yield, i.e. the amount of new sludge formed per unit of COD converted, depends on the nature of the substrate, as well as on environmental factors.
In Chapter 6 results of growth yield measurements under different conditions in mixed batch fed digesters are presented. Similar measurements in UASB reactors are discussed in Chapter 7.

Sludge wash-out and sludge retention

During the start-up of an UASB reactor the upward velocity of both water and gas causes finely dispersed and poorly settling sludge particles to wash out from the reactor. This wash-out is not necessarily a problem, as sludge wash-out during start-up represents a key feature of the UASB concept, i.e. the selection pressure exerted upon the sludge particles. This selection ultimately leads to the development of a highly settleable, granular sludge.

Both in full scale, as well as in laboratory scale reactors a 'bulking' anaerobic sludge sometimes develops in the early phases of start-up. The bulking sludge has a poor settleability and washes out from the reactor in later stages of the start-up at higher gas and hydraulic loads. For a prolonged period of time sludge growth and wash-out of bulking sludge may compensate each other, and little progress towards a higher gas production rate is achieved. When large quantities of poorly settling sludge are washed out in a short period of time, only a small amount of well settling sludge will be left in the reactor. The shallowness of the retained sludge bed may then create problems of channelling and insufficient contact between biomass and substrate.

Sludge wash-out and sludge retention problems are dealt with in Chapter 7.

TABLE 1 FREE ENERGY CHANGES OF SOME METHANOGENIC AND ACETOGENIC *CATABOLIC REACTIONS*

substrate	reaction			$\Delta G^{o'}$ (kJ)
METHANOGENIC				
acetate	CH_3COOH	$\rightarrow$	$CH_4 + CO_2$	- 31
hydrogen	$4H_2 + CO_2$	$\rightarrow$	$CH_4 + 2H_2O$	-131
methanol	$4CH_3OH$	$\rightarrow$	$3CH_4 + CO_2 + 2H_2O$	-312
ACETOGENIC				
ethanol	$CH_3CH_2OH + H_2O$	$\rightarrow$	$CH_3COOH + 2H_2$	+ 9.7
propionate	$CH_3CH_2COOH + 2H_2O$	$\rightarrow$	$CH_3COOH + 3H_2 + CO_2$	+76
butyrate	$CH_3CH_2CH_2COOH + 2H_2O$	$\rightarrow$	$2CH_3COOH + 2H_2$	+48
$CO_2 + H_2$	$2CO_2 + 4H_2$	$\rightarrow$	$CH_3COOH + 2H_2O$	-95

The known acetotrophic methanogens in the mesophilic temperature range are mentioned in Table 2 together with literature values of some kinetic parameters.

Methanothrix spp. differ from acetate converting Methanosarcina-type bacteria in their much lower half saturation concentration K_s, and a lower maximum conversion rate V_{max} and maximum growth rate μ_{max}. This implies, that Methanothrix will outcompete Methanosarcina in situations where low acetate concentrations prevail (see Figure 2). Kaspar (1977) reported a K_s-value of 0.32 mM in digesting sludge in which no Methanosarcinas were detected. On the other hand in acetate enrichment cultures containing both Methanothrix and Methanosarcina type bacteria, a K_s-value of 2.9 mM was found (Lawrence and McCarty 1969).

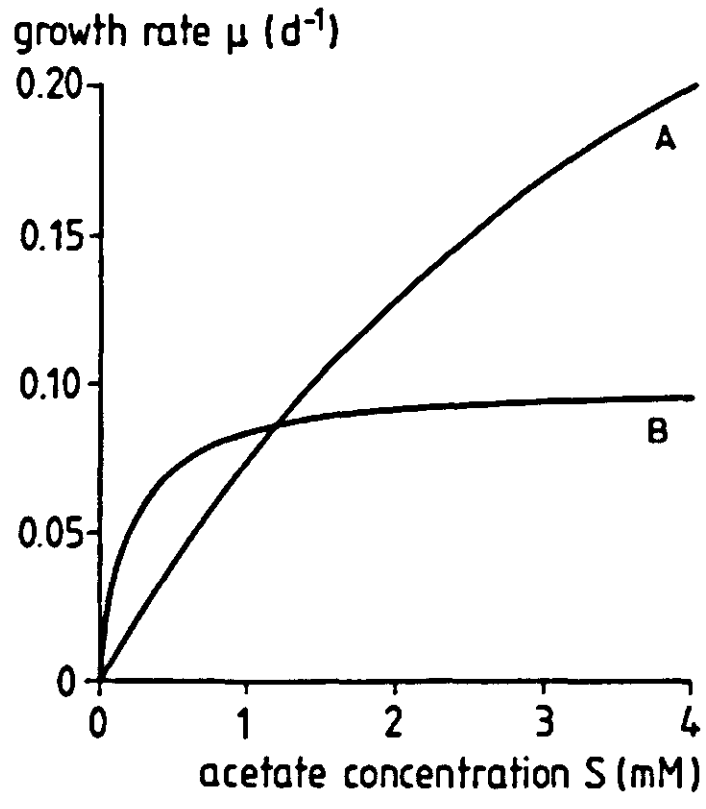


FIGURE 2
Relation between the acetate concentration and the growth rate of Methanosarcina barkeri (A) and Methanothrix söhngenii (B) as predicted by the Monod equation using μ_{max} values of 0.45 and 0.10 d^{-1} and K_s values of 5 and 0.2 mM resp.

In addition to the kinetic differences the morphological difference is of importance. Methanothrix grows as filaments that can be hundreds of cells long (Huser 1981). These filaments are often strongly intertwined to form flocs or even granules. The Methanosarcina organisms form different types of more or less round clumps (Zhilina 1976) ranging from a few cells to macroscopic granules.

TABLE 2 KINETIC PARAMETERS OF MESOPHILIC ACETOTROPHIC AND HYDROGENOTROPHIC METHANOGENS

species (1)	K_s (mMol)	T_{min} (days)	Y^* (2)	V_{max}^* (3)	ref. (4)
ACETOTROPHIC					
M.thrix söhngenii	0.46	8.7	0.019	3.8	1
M.thrix söhngenii	0.2	7	0.015	2.9-6.1	2,3
"fat rod"	0.17	2.7	0.4	0.4(5)	4
"fat rod"	-	1.1	-	-	5
M.sarcina barkeri	5	1.4	0.03-0.05	12	6
M.sarcina barkeri	-	-	0.015	-	7
M.sarcina barkeri	-	2.06	0.053	5.9	8
M.sarcina barkeri	-	1.25	0.05-0.07	8	9
M.sarcina barkeri	-	-	0.025	8.6	10
M.sarcina barkeri	-	1.0-3.0	-	-	11
M.coccus mazei	-	0.69	-	-	12
HYDROGENOTROPHIC					
M.brevibacter arboriphilus	0.005	-	-	-	13
M.brevibacter arboriphilus	-	0.5	0.04	50	14
M.sarcina barkeri	-	-	0.14	-	15
M.sarcina barkeri	-	0.50	0.09	-	16
M.bacterium formicicum	0.002	0.35	0.05	40	17

(1) M.= Methano- (2) as $gVSS.gCOD^{-1}$ (3) as $gCH_4\text{-}COD.gVSS^{-1}.day^{-1}$
(4) 1: Huser 1981, 2: van den Berg et al. 1976, 3: van den Berg 1977, 4: Cappenberg 1975, 5: Fathepure 1983, 6: Smith and Mah 1980, 7: Hutten et al. 1980, 8: Krzycki et al. 1982, 9: Scherer and Sahm 1981, 10: Weimer and Zeikus 1978b, 11: Mah et al. 1978, 12: Mah 1980, 13: Zehnder et al. 1981, 14: Zehnder and Wuhrmann 1977, 15: Smith and Mah 1978, 16: Weimer and Zeikus 1978a, 17: Schauer et al. 1980.
(5) Calculated from Figure 4 and Table 3 of reference 4.
(*) For pure cultures DSS was transferred to VSS assuming 1 gDSS = 0.872 gVSS (Luria 1960).

Mah (1980) described a new mesophilic species capable of methanogenesis from acetate called Methanococcus mazei. It is now believed to belong to the genus Methanosarcina.

2.1.2 ACETOGENESIS

Table 1 shows that the breakdown of volatile fatty acids into acetate and hydrogen does not yield energy under standard conditions. The acetogenic bacteria only perform those reactions when the concentration of acetate, and more specifically, the partial pressure of hydrogen in the liquid are kept sufficiently low by methanogens or sulphate reducing bacteria (McInerney et al. 1981). The acetogens are therefore obligate syntrophic bacteria and depend upon an effective interspecies hydrogen transfer (Wolin 1976).

Of the acetogenic substrates commonly observed in anaerobic digestion, propionate oxidation is thermodynamically the most unfavourable reaction (Zehnder and Koch 1983). Yet propionate represents an important intermediate. It is formed from the breakdown of odd-numbered fatty acids, from certain amino acids, as well as from carbohydrates.

The mean turnover time of hydrogen is in the order of fractions of a second at its normal concentration in anaerobic digestion of about 10^{-8} molar (Zehnder and Koch 1983). Accumulation of hydrogen caused, for instance, by an unbalance between the acidification and the methanogenesis can rapidly inhibit the turn-over of volatile fatty acids higher than acetate. Propionate degradation will be affected first.

Also higher acetate concentrations can adversely affect the rate of propionate breakdown (see paragraph 6.3.4.1).

Kinetic parameters of mesophilic acetogenic bacteria are given in Table 3. The minimum doubling time of butyrate acetogens is in the order of 2 to 3 days, as compared to 4 to 5 days for propionate decomposing acetogens. A recent kinetic study on a mixed culture (Heyes and Hall 1983) suggested the existence of a second group of propionate degrading bacteria (in the absence of sulphate) with a doubling time of only 0.6 days, but a much higher K_s value of 4.5 mM compared with about 0.1-0.4 mM for the first group.

In the presence of sulphate the breakdown of propionate into acetate without the release of hydrogen can be performed by Desulfobulbus propionicus, which exhibits a comparably short doubling time of 0.4 days (Widdel and Pfennig 1982). In the absence of sulphate this bacterium cannot perform this reaction. In the case of ethanol, another acetogenic

TABLE 3 KINETIC PARAMETERS OF MESOPHILIC ACETOGENIC BACTERIA

species	K_s (mM)	T_{min} (days)	Y (1) ($gVSS.gCOD^{-1}$)	ref. (2)
PROPIONATE				
-Syntropho-bacter wolinii	-	6.7(3) 3.6(4)	-	1
-enrichment culture	0.43	2.2	0.037	2
	-	-	0.027	3
-enrichment culture	-	4.6-5.8	-	4
-enrichment culture	2.2	4.5	0.022	5
-Desulfobulbus propionicus	-	0.42	- (5)	6
BUTYRATE				
-Syntropho-monas wolfei	-	3.5(3) 2.3(4)	-	7
-enrichment culture	0.06	1.8	0.041	2

(1) Including the growth yield of the methanogens involved.
(2) 1: Boone and Bryant 1980, 2: Lawrence and McCarty 1969, 3: McCarty 1966, 4: Koch et al. 1983, 5: Gujer and Zehnder 1983, 6: Widdel and Pfennig 1982, 7: McInerney et al. 1981.
(3) In coculture with Methanospirillum hungatei.
(4) In coculture with Desulfovibrio sp.
(5) 4.9 g dry weight/mol propionate oxidized.

substrate, some sulphate reducing bacteria can compete with other acetogens (e.g. the S-organism of the Methanobacillus omelianskii consortium) by using interspecies hydrogen transfer (Laanbroek et al. 1982).

In the methane digestion of volatile fatty acids the methanogens represent the bulk of bacteria present, viz. more than 90 % in a propionate enrichment culture (Koch et al. 1983).
This is in accordance with the fact that the biomass yield factor for the mixed population of acetogenic and methanogenic bacteria involved in propionate and butyrate digestion, is not significantly higher than can be expected from the methanogenic bacteria alone (compare Tables 2 and 3).

2.2 ONE STAGE AND TWO STAGE PROCESS CONFIGURATIONS

The anaerobic digestion of wastes, which need hydrolysis and/or acidification, can be divided into two separate processes. In the first

stage reactor the conditions for hydrolysis and acidification can be optimized and the products of this reactor then are treated further in a second stage reactor, in which the processes of acetogenesis and methanogenesis take place.

Both the number of end products of the acidification step as well as their relative amounts are directly influenced by phase separation. By rendering interspecies hydrogen transfer impossible between the acidifying bacteria and the hydrogen consuming methanogens, the hydrogen partial pressure in a separate acidification reactor is relatively high, and induces the formation of more reduced end products (Cohen et al. 1979). As opposed to the acetogenic bacteria which totally depend upon an effective interspecies hydrogen transfer, the acidifying bacteria have a choice of using H_2 or organic acids or alcohols as electron sink products depending on which of these possibilities is energetically most favourable.

The potential benefits of a two stage concept as mentioned in the literature are listed in Table 4.

TABLE 4 POTENTIAL BENEFITS OF A TWO-STAGE ANAEROBIC DIGESTION PROCESS

PROCESS OPTIMIZATION
- The first stage reactor can be operated at pH 5.5-6.0 which is the optimal pH for carbohydrate acidification (Witty and Märkl 1983), whereas in the methanogenic reactor a pH of 7.0-7.5 should be maintained.
- The high growth rates of acidifying bacteria enable very high conversion rates in the first stage acidogenic reactor (Zoetemeyer 1982) which consequently can be much smaller than the methane reactor.
- Excess acidifying biomass can be wasted without loosing the slower growing methanogenic biomass.

PROCESS STABILITY
- A proper working acidification reactor prevents overloading of the methane reactor by acidifiable substrates which may otherwise lead to a fatal pH-drop in poorly buffered systems.
- Some substances that inhibit methanogens can be removed or converted in a first stage reactor.
- The first stage reactor may act as an equalizing tank.

The drawbacks of phase separation are basically that two smaller reactors need to be built, instead of one big reactor, and the need for more control apparatus, because of a more intricate process, which may include the addition of chemicals for pH-control; particularly if optimal environmental conditions are pursued in the first and the second reactor.

TABLE 5 EXAMPLES OF TWO-STAGE ANAEROBIC DIGESTION PROCESSES IN WHICH THE SECOND STAGE REACTOR IS OR CAN BE A HIGH RATE UASB REACTOR

type of waste	main function of first reactor	one stage alternative	ref. (1)
A agricultural solid wastes	leaching of acidification products	conventional mixed digester	1,2
B wastewater containing biodegradable suspended solids	retention and liquifaction of suspended solids	anaerobic contact process or low rate UASB reactor	3
C wastewater containing toxics for methanogenesis	detoxification	none (or very low rate systems)	4
D wastewater with strong variations in composition, pH, etc.	equalization	none (or medium rate, very well controlled systems)	
E wastewater containing easily acidifying COD (e.g. simple carbohydrates)	acidification	high rate UASB reactor or other high rate systems	5

(1) 1: Rijkens 1981, 2: Colleran et al. 1982, 3: Norrman and Frostell 1977, 4: Welander and Hansson 1983, 5: Cohen et al. 1980.

In the anaerobic digestion (in conventional digesters) of slurries consisting predominantly of particulate organic matter, the hydrolysis step is rate limiting. No build-up of acidification products occurs. Some examples of two stage digestion of slurries, therefore, showed no increase in the overall digestion rate (Therkelsen and Carlson 1979, Witty and Märkl 1983), when compared with a one-stage approach.

Two stage digestion processes in which the second stage reactor is or can be a high rate UASB reactor, are listed in Table 5. The choice of a one or a two stage approach depends mainly on the characteristics of the waste. Only in the case of a wastewater containing easily acidifying COD, does a real choice exist between one or two stage operation. In practice partial acidification of such wastes occurs already in pipelines, sewers, etc. The one stage treatment of partially acidified carbohydrate solutions has been shown to yield results comparable to those of a two stage digestion (Cohen

1982, Hulshoff Pol et al. 1983b).
In the other examples of two-stage anaerobic digestion mentioned in Table 5, a one-stage treatment in a high rate UASB reactor offers no realistic alternative. For a wastewater containing slowly degrading suspended solids as a fraction of the COD (Table 5; example B), the one-stage treatment in a high rate UASB reactor is altogether feasible, but the suspended solids will not be removed to any appreciable extent at high loading rates; only the soluble COD will be converted into methane. A low rate UASB reactor offers a better alternative under those circumstances (compare Chapter 1, Table 3 and 4; the treatment of slaughterhouse wastewater).

2.3 KINETICS

Michaelis-Menten kinetics are normally applied to measurements of enzymatic reactions to characterize enzymes with regard to their substrate affinities (K_m) and maximal reaction rates (k_{max}).
Similarly Michaelis-Menten equations can often be used to estimate kinetic parameters for microbially mediated processes.

$$-\frac{dS}{dt} = k = \frac{k_{max}.S}{K_m + S} \qquad \text{(Michaelis-Menten)} \qquad (1)$$

in which: S = substrate concentration ($kgCOD.m^{-3}$),
t = time (d) and
k = substrate utilization rate ($kgCOD.m^{-3}.d^{-1}$).

However, equation 1 only describes bacterial substrate consumption when no growth occurs, or when the amount of growth occurring during the measuring period is insignificant. The value of k_{max} should not change during substrate consumption.

When substrate consumption is linked to growth, an increase in activity concomitant with substrate consumption yields an S-shaped (sigmoidal) substrate depletion curve in batch experiments consistent with Monod kinetics (equation 2) (Robinson and Tiedje 1983).

$$\mu = \mu_{max} \cdot \frac{S}{K_s + S} \qquad \text{(Monod)} \qquad (2)$$

in which: K_s = saturation constant ($kgCOD.m^{-3}$),
μ, μ_{max} = (maximum) specific growth rate (d^{-1}).

$$\text{Because } \mu = V.Y = \frac{k.Y}{X} = -\frac{dS}{dt} \cdot \frac{Y}{X} \qquad (3)$$

in which Y = growth yield factor ($kgVSS.kgCOD^{-1}$),
X = microbial mass concentration ($kgVSS.m^{-3}$) and
V = specific substrate utilization rate ($kgCOD.kgVSS^{-1}.d^{-1}$)

equation 2 can be rewritten as:

$$-\frac{dS}{dt} = \frac{\mu_{max}.S}{K_s + S} \cdot \frac{X}{Y} \quad \text{or} \quad k = \frac{k_{max}.S}{K_s + S} \quad \text{at constant X and Y.}$$

This is again equivalent to the Michaelis-Menten equation.

Presently the Monod equation is the most widely used for modelling the anaerobic digestion process.

A basically different approach, using Contois' kinetic equation has been proposed by Chen and Hashimoto (1980):

$$\mu = \mu_{max} \cdot \frac{S}{B.X + S} \qquad \text{(Contois)} \qquad (4)$$

in which B is a kinetic parameter (constant).
In this equation no saturation constant K_s appears. It is stated to describe the kinetics of mass transfer-limited microbial growth systems, such as the sludge and manure digestion process.

Mathematical modelling of the anaerobic digestion process

Most models developed to describe the anaerobic treatment process, apply to the conventional completely mixed reactor with or without biological solids recycle (Andrews 1968, Lawrence et al. 1970, Ghosh and Pohland 1974, Carr and O'Donnell 1977, Chen and Hashimoto 1980).

The complexity of the anaerobic digestion process and the multitude of micro-organisms involved make a detailed conceptual model virtually impossible (Barford and Hall 1978). The result has been the predominant application of the Monod equation as such (Lawrence et al. 1970, Ghosh and Pohland 1974, van der Meer 1979), or including different correction terms (Andrews 1968, Heyes and Hall 1981, van de Heuvel and Zoetemeijer 1982, Duarte and Anderson 1982, Mosey 1983).

Andrews (1968) introduced the concept of substrate inhibition. His model assumes that the inhibitory effect of volatile acids at high concentrations is due to the unionised fraction. The concentration of unionised acids strongly depends upon the pH. The inhibition function (equation 5) must therefore incorporate the effect of pH. This is accomplished by considering the unionised acid as the limiting substrate and expressing K_s and K_i as concentrations of unionised substrate (HS).

$$\mu = \frac{\mu_{max}}{1 + K_s/HS + HS/K_i} \qquad (5)$$

in which: HS = the unionized substrate concentration and
K_i = the inhibition constant.

The model developed by Andrews (1968) has been adopted by several other researchers (Carr and O'Donnell 1977, van den Heuvel and Zoetemeijer 1982, Eis and Rantala 1983, Bolle et al. 1983b), but was criticised by Heyes and Hall (1981).

They argued, that inhibition is not a function of the unionised volatile acid concentration, and that high volatile acid concentrations are the result, not the cause of digester instability. In their view the hydrogen concentration is the critical variable. An increase in the hydrogen partial pressure is the cause of high VFA concentrations. It renders the breakdown of propionate and butyrate thermodynamically unfavourable and at the same time enhances the production of VFA by the acidogenic bacteria. In their model the Monod equation is modified by multiplying the acetogen growth rates with free energy availability factors.

Mosey (1983) also adopted the hydrogen partial pressure as a controlling variable. In his view the ratio between the reduced (NADH) and the oxidized

(NAD^+) forms of the intracellular carrier molecule Nicotinamide Adenine Dinucleotide controls both the conversion rates and the composition of the mixture of acids formed during acidogenesis, as well as the conversion rates of propionic and butyric acids into acetic acid.
A direct relation is assumed between the $NADH/NAD^+$ ratio and the hydrogen partial pressure in the biogas, and this relationship is substituted into a Monod-type equation.

Although Bachman et al. (1983) use Monod kinetic coefficients, the Monod equation is not used as the basis of their model. The performance of their Anaerobic Baffled Reactor which can be regarded as a number of upflow reactors in series, could be described better with a fixed film model than with a suspended growth model. The apparent reason is, that the sludge particles within the reactor sludge blanket act as fluidised spheres with a surface area through which the substrate must diffuse prior to consumption. The fixed film model used is:

$$r_a = C.S^q \qquad (7)$$

in which r_a = specific substrate consumption rate per unit surface area of biofilm,
C = variable reaction rate coefficient and
q = variable reaction order.

The values C and q are functions of biofilm parameters, such as diffusion layer thickness, substrate diffusion coefficients in the liquid and biofilm, and the Monod rate (k) and half velocity (K_s) coefficients. The specific surface area was used as a fitting parameter.

Concluding remarks

Most models referred to in this chapter were used by the authors to demonstrate a good fit with their own experimental data, but little if any general guidelines with respect to digester instability which were not already well known, could be extracted from them.

Including a correction term in the Monod equation seems to be warranted. In a comparative study (Bolle et al. 1983b) Andrews' model yielded slightly better results than the simple Monod equation in 'substrate inhibition' experiments with an industrial wastewater.

As far as the conceptual basis for the models is concerned Andrews'

unionised acid toxicity hypothesis is much less supported by experimental data than the hydrogen partial pressure regulatory system (Heyes and Hall 1981, Mosey 1983).

Whether or not a fixed film model has definite advantages over a suspended growth model (Bachman et al. 1983) (i.e. a model neglecting possible substrate diffusion limitation) remains doubtful. Several researchers showed that substrate transport limitation is not significant in granular sludge. The efficiency factor always exceeded 0.85 (Bolle et al. 1983a) or even 0.94 at 1 mM (Tramper et al. 1983).

Van den Heuvel and Zoetemeyer (1982) used Andrews' substrate inhibition equation to construct a theoretical model including cell recycle, from which they derived certain conclusions with respect to UASB reactor start-up.

However, this model cannot be applied to the kind of reactor start-up experiments that were performed in the present work in which digested sewage sludge was used as seed material.

Their model does not take into account, that reactor start-up is normally done by flexible response to reactor performance, rather than applying the ultimate loading rate right from the beginning. Also the sludge characteristics (which also determine the sludge residence time) are taken as being constant, whereas great changes may be expected during UASB reactor start-up with digested sewage sludge as seed.

The fluid flow in UASB reactors has been modelled as well (Heertjes and van der Meer 1978, Bolle et al. 1983a). Again the models apply to well operating granular sludge bed reactors and not to reactor start-up using unadapted seed sludge.

CHAPTER 3 SLUDGE ACTIVITY MEASUREMENTS

3.1 INTRODUCTION

The specific activity of anaerobic sludge expressed as $kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$ is an important parameter in several respects.
At the beginning of the start-up of a new reactor the specific activity of the seed sludge together with the amount of sludge present determines the permissible initial organic loading rate.
During subsequent stages of UASB reactor start-up a regular determination of the sludge activity provides information about the development of the sludge from a digested sewage sludge with a large fraction of inert organic material, towards a sludge type which consists almost exclusively of viable biomass (when treating a completely dissolved waste).
Changes in sludge activity may indicate inhibition (e.g. caused by NH_3-toxicity), or the accumulation in the sludge bed of non or slowly degradable organic matter originating from the wastewater (e.g. coagulated proteins) (Wilkie et al. 1983, de Zeeuw et al. 1981).

A sludge activity test is commonly performed as a batch experiment in which a fixed amount of substrate is fed to a predetermined amount of sludge-solids. The specific sludge activity is calculated from the methane production rate or the substrate depletion rate and the amount of sludge present. Care should be taken, that the inevitable sludge growth during the experiment is negligible in comparison with the total amount of viable biomass present.
Under well defined conditions the sludge activity can also be deduced from in situ measurements of UASB reactor performance. To calculate the specific sludge activity the methane production rate or COD removal rate and the amount of sludge present in the reactor, as well as the fraction of the sludge actively participating in the digestion process must be known. The organic loading rate applied to the reactor determines to what extent the overall sludge activity approaches the maximum sludge activity.
Microbiological and biochemical assays aiming at the determination of the viable biomass present in the sludge may offer an alternative for assessing the specific methanogenic sludge activity. The assay should preferably provide information about the number of viable methanogens, as only these bacteria perform the final step in the anaerobic digestion process. Hence

the determination of e.g. ATP, DNA, or dehydrogenase activity will not be specific enough in mixed cultures. The estimation of the numbers of methanogens in mixed populations by counting, or isolation and culturing, is unreliable and time consuming. As methanogens belong to a group of bacteria (Archaebacteria), which differ considerably from the other bacteria, they possess certain unique compounds, like coenzyme-M and cofactor F420. A simple assay of coenzyme-M is not yet available. The analysis of F420 is relatively simple, and its application as an alternative for the assessment of the specific methanogenic sludge activity has been extensively studied in connection with the present work (see paragraph 3.3).

3.2 STANDARDISED BATCH ACTIVITY TEST

A standardised batch sludge activity test has been used throughout the work reported here. Its merits will be discussed in this section.

3.2.1 MATERIALS AND METHODS

All experiments were conducted at 30 °C.
In this test the specific sludge activity is measured as the methane production rate or substrate COD removal rate per unit of sludge-VSS, using a mixture of volatile fatty acids as substrate.

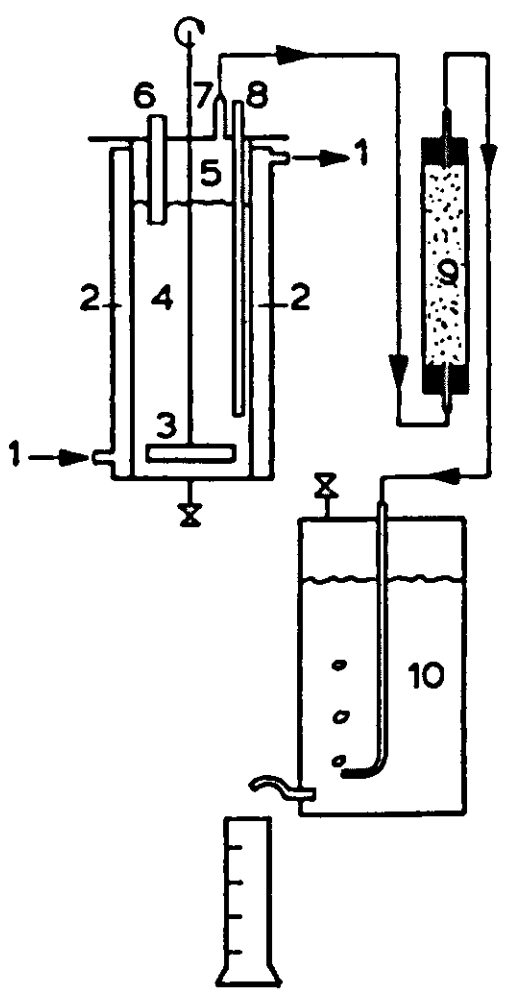


FIGURE 1
Batch reactor used for sludge activity tests.
1: warm water flow,
2: water jacket (30 °C),
3: stirring device,
4: sludge mixed liquor,
5: headspace,
6: inlet point for pH electrode,
7: gas outlet,
8: sampling port,
9: granular soda lime for CO_2 removal,
10: liquid displacement system for gas measurement.

Digesters

Most batch-fed activity tests were performed in intermittently stirred (15 sec at 140 rpm every 5-10 min) digesters with a wet volume of 2.5 or 5 liters (Figure 1). Smaller vessels like 0.1 liter serum flasks were also used occasionally. The size of the digester predominantly depends upon the amount of sludge available and/or the number and volume of samples required for different analyses. The reactors were connected to a liquid displacement system (flask of Mariotte) for gas production measurement. CO_2 was removed from the gas stream by using an alkaline solution (1% w/v NaOH) and/or by a granular chalk column placed before the liquid displacement system.

Sludge

A sludge concentration of 2.5 $kgVSS.m^{-3}$ was aimed at. The actual concentration ranged from 2 to 3 $kgVSS.m^{-3}$.

After dilution with deoxygenated tap water the sludge mixed liquor was flushed with nitrogen gas to remove remaining oxygen from the liquid as well as the headspace.

Substrate and nutrients

The substrate used was a mixture of acetate, propionate and butyrate, or a mixture of acetate and propionate only, with an initial digester concentration of each individual volatile fatty acid of 0.6 $kg.m^{-3}$. This is equivalent to a total COD of 2.64 or 1.55 $kg.m^{-3}$ respectively.

The VFA were supplied partly as their sodium salts in order to obtain a pH of 7.0-7.1. After complete digestion of the standardised VFA feed the pH had increased to 7.3-7.5. Subsequent VFA-feeds were supplied in the acid form.

Per liter of mixed liquor the following nutrients were added: 13.6 mg $(NH_4)_2SO_4$, 73.6 mg NH_4Cl, and 13.6 mg KH_2PO_4, as well as 1 ml of a trace element solution according to Zehnder (1976).

Analyses

DSS and VSS determinations were performed according to Standard Methods.

VFA analyses were made gaschromatographically using a Packard Becker 417; 2 m glass column, 60-80 mesh chromosorb 101, formic acid saturated N_2 as carrier gas, injection 180 °C, oven 190 °C, FID detector 200 °C, with a HP 7672 A Automatic Sampler and SP 4100 Computing Integrator.

3.2.2 RESULTS AND DISCUSSION

In this study, activity tests of anaerobic sludge samples had two objectives: a) the prediction of the behaviour of digested sewage sludge, when employed as seed sludge for UASB reactor start-up, and b) the assessment of the maximum specific activity of sludge samples from operating UASB reactors.

The choice of a VFA mixture as substrate logically followed from the substrate used in the UASB reactor start-up experiments (Chapter 7). Using a single substrate, preferably acetate (Valcke and Verstraete 1981) simplifies the method, but leaves out the contribution of the acetogenic bacteria and the hydrogenotrophic methanogens.

UASB-reactor sludge

The breakdown of the individual volatile fatty acids in a batch activity test performed on UASB reactor sludge generally reflected well the relative conversion rates observed in the upflow reactor itself. In the course of reactor start-up the specific activity of UASB reactor sludge rapidly exceeds that of the digested sewage sludge seed and will be in the order of 0.5 to 2.0 $kgCH_4$-COD.$kgVSS^{-1}$.d^{-1}.

The conversion of a standard feed of 2.64 kgVFA-COD.m^{-3} theoretically yields 2.64 x 0.024 = 0.063 kgVSS.m^{-3} (the yield factor of 0.024 gVSS.$gCOD^{-1}$ is taken from Table 1 of Chapter 6). The amount of viable biomass present in the sludge sample can be estimated from the maximum biomass activity of about 2.85 $kgCH_4$-COD.$kgVSS^{-1}$.d^{-1} (taken from Table 5 of Chapter 6) and the maximum methanogenic activity of the sludge sample itself. For instance at a specific sludge activity of 1.0 $kgCH_4$-COD.$kgVSS^{-1}$.d^{-1} and a sludge concentration of 2.5 kgVSS.m^{-3} the viable biomass concentration amounts to (1.0/2.85) x 2.5 = 0.88 kgVSS.m^{-3} and the maximum increase during the activity test is 0.063/0.88 x 100 = 7 %. The maximum gas production rate in the sludge activity test is already attained, when only part of the feed has been digested. This further reduces the possible error caused by growth.

Consequently any growth occurring during a standardised batch activity test with UASB reactor sludge samples can be neglected. This is in accordance with the observation, that the maximum sludge activity during digestion of a second feed was not significantly higher, than during digestion of the

first feed.
The period of time required for an activity test with adapted UASB reactor sludge ranged from 0.5 to 2 days.

Digested sewage sludge

Digested sewage sludges contain a large fraction of refractory organic matter. Consequently they exhibit a low methanogenic activity based on total volatile suspended solids (VSS). Standardised batch activity tests with these sludges take from 10 to 25 days (Figure 3). The specific sludge

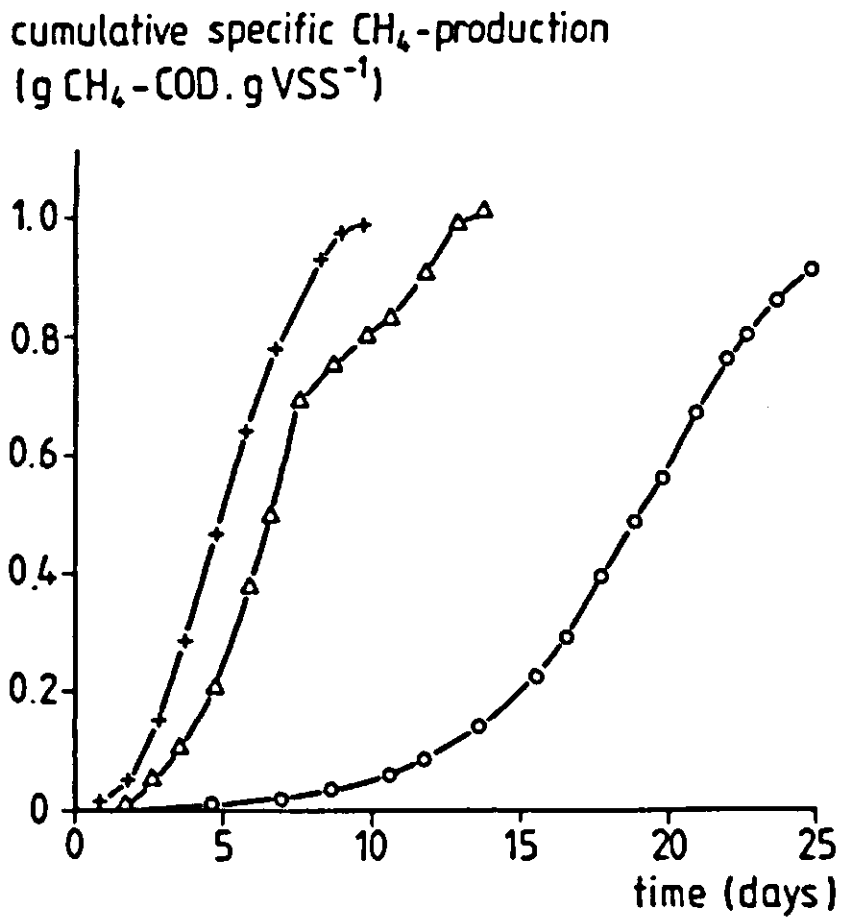


FIGURE 2
Cumulative specific methane production in standardised batch activity tests with 3 different digested sewage sludge types.

activity is calculated from the maximum slope of the cumulative methane production curve (see also Chapter 4). As appears from Figure 2 active gas production is preceded by a lag phase of varying length (see also Chapter 5).
Typical results with digested sewage sludge samples from two different sewage sludge digesters are shown in Figures 3 and 4.
From the breakdown of the individual volatile fatty acids a good estimation of their maximum conversion rates can be obtained. Sometimes long lag phases occur as with butyrate digestion in the experiment of Figure 3. Once butyrate breakdown starts, acetate often accumulates, because it is produced faster, than it is converted. Figure 4 shows the results of a standardised activity test with a digested sewage sludge sample of a

relatively high specific activity (total digestion time only 10 days). In both experiments the propionate breakdown slowed down during maximum butyrate conversion (Figure 3, day 15; Figure 4, day 5). This is probably caused by a temporarily higher hydrogen partial pressure. Also the synthesis of propionate from acetate, carbon dioxide and hydrogen may have played a role (Zehnder and Koch 1983).

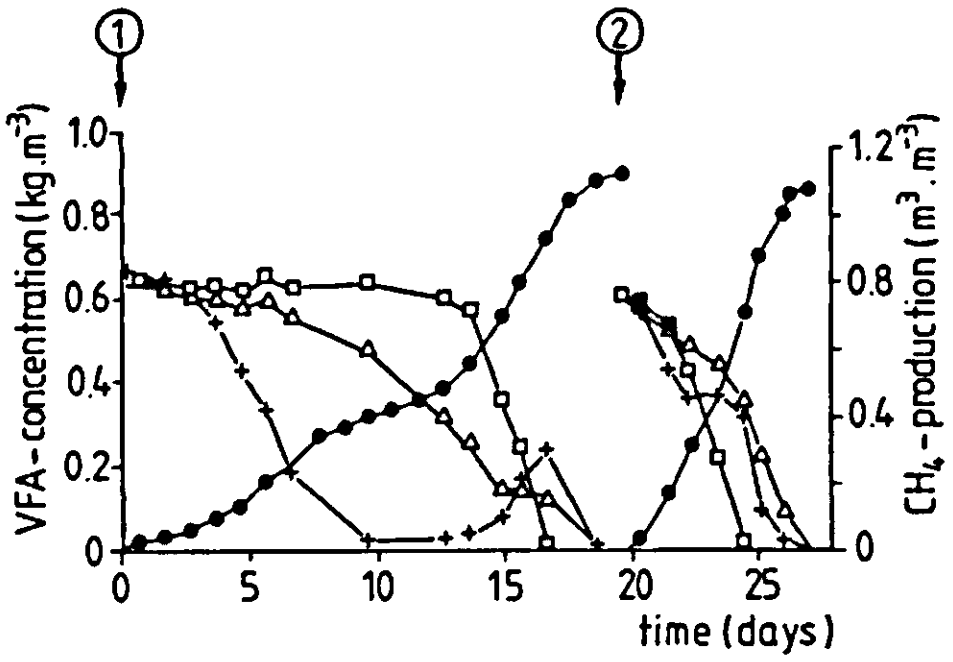


FIGURE 3
Example of standardised batch activity test of digested sewage sludge. 1: first feed, 2: second feed, (●): methane, (+): acetate, (Δ): propionate, (□): butyrate. Experimental conditions as described in paragraph 3.2.1. Sludge type 'F5'; see Table 1 of Chapter 4.

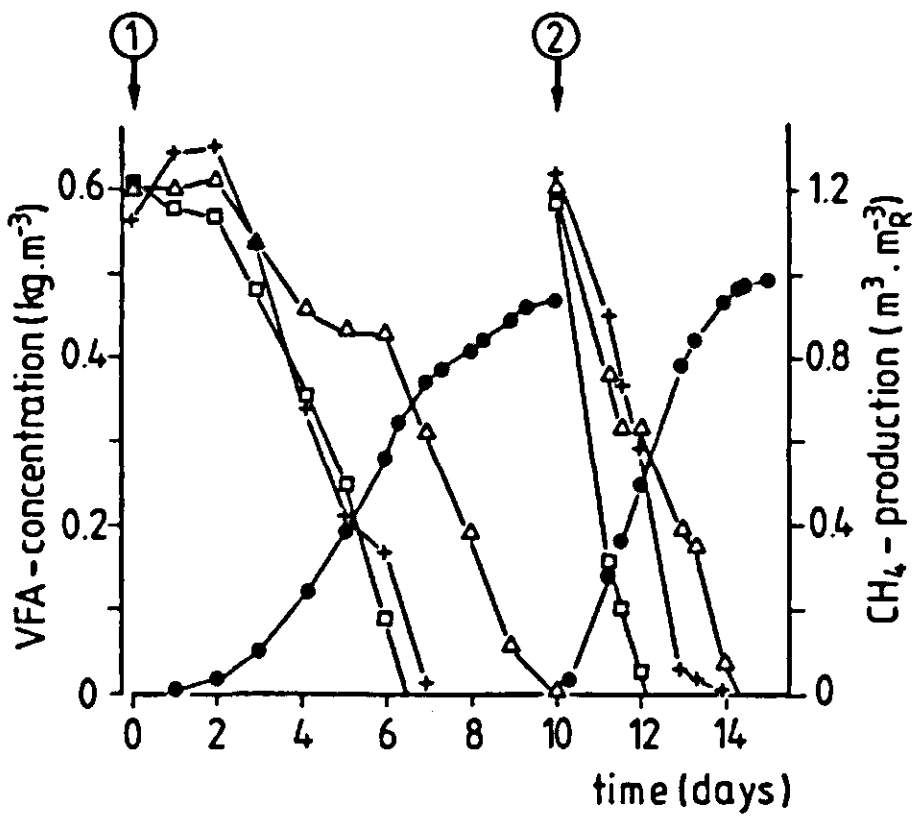


FIGURE 4
Example of standardised batch activity test of digested sewage sludge. 1: first feed, 2: second feed, (●): methane, (+): acetate, (Δ): propionate, (□): butyrate. Experimental conditions as described in para. 3.2.1. Sludge type 'E5'; see Table 1 of Chapter 4.

In all experiments with digested sewage sludge samples, a higher maximum methane production rate was observed during the digestion of a second and subsequent feeds, compared with the one measured during digestion of the first feed. Obviously this is at least partially due to growth of new

biomass. The theoretical growth on a standardised VFA feed is 0.063 $kgVSS.m^{-3}$. This is high in comparison with the initial concentration of active biomass present in the batch reactor. At a specific activity of digested sewage sludge of 0.1 to 0.2 $kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$ (see Figure 4 of Chapter 4) the active biomass concentration ranges from 0.088 to 0.175 $kgVSS.m^{-3}$ (see calculation above). A potentially large error may thus be introduced in the standardised batch activity test of digested sewage sludge samples. To prevent this, a higher sludge concentration (than 2.5 kgVSS.m-3) should be employed. However, comparison of measurements at different digested sewage sludge concentrations showed, that significantly lower specific sludge activities were measured at sludge concentrations above 3 $kgVSS.m^{-3}$, whereas between 1 and 3 $kgVSS.m^{-3}$ the specific sludge activity measurements yielded a reasonably constant value (Figure 5).

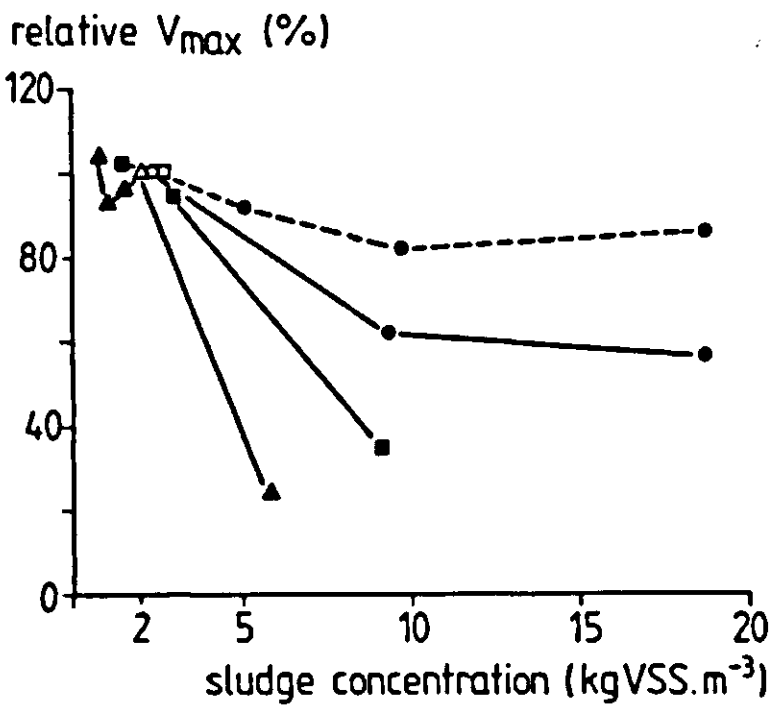


FIGURE 5
Maximum specific gas production rates as measured in standardised batch activity tests in relation to the sludge concentration for 3 digested sewage sludge types (●, ▲, ■). Open symbols (○, △, □) refer to tests at the standardised sludge concentration of 2-3 $kgVSS.m^{-3}$ and are set at 100 %. The dashed line refers to parallel experiments in which the digested sewage sludge was diluted with sewage sludge digester supernatant instead of tap water.

TABLE 1 EFFECT OF DILUTION MEDIUM ON THE MEASURED SPECIFIC ACTIVITY OF DIGESTED SEWAGE SLUDGE SAMPLES

dilution medium	specific sludge activity (1)	NH_4^+-concentration ($gN.m^{-3}$)
sewage sludge digester supernatant	0.11	750
tap water	0.17	100

(1) Expressed as $kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$.
Measured in standardised batch activity tests (2.5 $kgVSS.m^{-3}$).

A partial explanation for the lower activities measured at higher sludge concentrations can be found in the dilution of inhibiting compounds present in the digested sewage sludge mixed liquor, e.g. ammonia. Dilution of the sludge with sewage sludge digester supernatant instead of tap water yielded much more constant, but lower, sludge activity values (Table 1 and Figure 5; dashed line).

Further it has been observed that digested sewage sludge exhibits a gas production lag phase; the length of which strongly depends upon the sludge concentration applied (Figure 6).

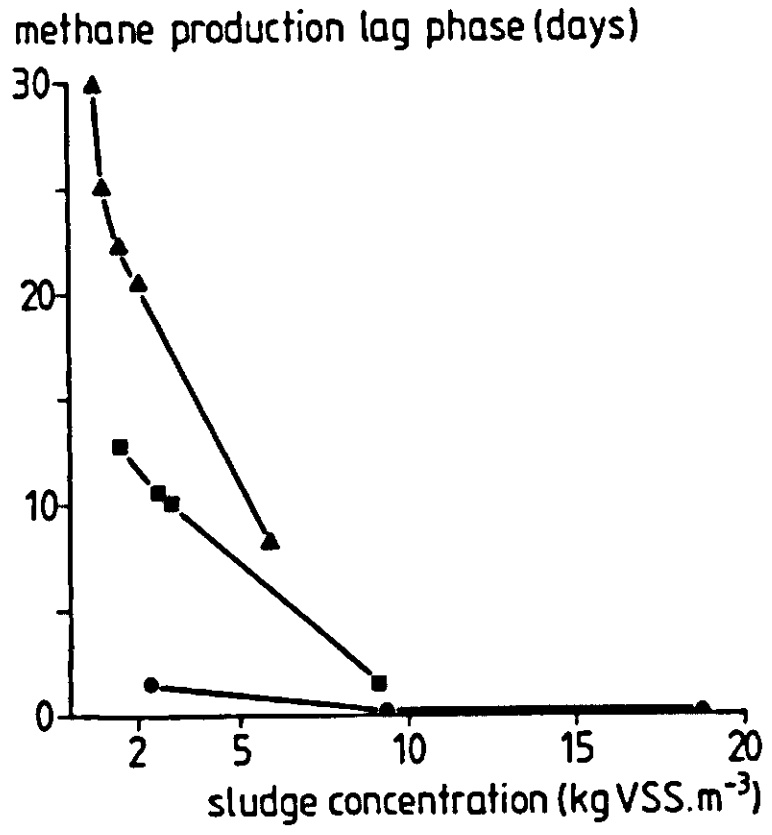


FIGURE 6
Methane production lag phases in batch sludge activity tests in relation to the sludge concentration for 3 different digested sewage sludge types (●, ▲, ■; symbols refer to the same experiments as those in Figure 5).

Indications were obtained (see Table 12 of Chapter 5), that during the digestion of the first feed a significant amount of COD is used is to restore the dissimilatory and assimilatory systems of the bacterial cells, that were starved during the storage period. A long gas production lag phase at a low sludge concentration would thus provide sufficient time for all viable biomass to become operative again. At higher sludge concentrations the lag phase is too short, and complete digestion of the feed is accomplished in such a short period of time, that part of the biomass may not yet have regained its activity.

Because of the need to first restore the depleted cellular system, the actual growth of new biomass may be limited during the digestion of the first standardised VFA feed. This would imply, that the error made in the sludge activity measurement caused by growth is probably much less than the

theoretical maximum calculated before.
The specific activity deduced from a second standardised VFA feed will be influenced significantly by growth of new biomass.

The above observations led to the adoption of a standardised sludge concentration of 2.5 $kgVSS.m^{-3}$ in the batch activity test of digested sewage sludge.
Valcke and Verstraete (1981), in their sludge activity test, used a suspended solids concentration of 5 $kgSS.m^{-3}$, which is equivalent to 2 to 3 $kgVSS.m^{-3}$ for digested sewage sludge. They compared the methane production of the first 24 hours of incubation at acetate saturation concentrations to an assumed maximum activity of 1.85 $kgCH_4\text{-}COD.kgVSS^{-1}.d^{-1}$ for a pure culture of acetoclastic methanogens. This method as well as other methods (van den Berg et al. 1974) are used in the testing of sludge samples from actively digesting reactors. In the case of digested sewage sludge the occurrence of a gas production lag phase would yield erroneous results with these methods.

Conclusions

The measurement of the specific sludge activity of digested sewage sludge is complicated by the low methanogenic activity of this sludge and the occurrence of a gas production lag phase. The best results are obtained during the first VFA-feed after diluting the sludge to 2.5 $kgVSS.m^{-3}$ with tap water. Thus inhibition by compounds present in the sludge mixed liquor is prevented and the experimental period is long enough for starved bacteria to regain their activity. The disturbing influence of growth of new biomass seems to be limited.

3.3 F420 ASSAY AS SLUDGE ACTIVITY TEST

3.3.1 INTRODUCTION

In anaerobic sludge the fluorescent coenzyme F420 is found exclusively in methanogens. Yet it is not unique to methanogens, because it has been detected in one or two micro-organisms occupying a quite different habitat (Vogels et al. 1982).
The fluorescence can be used for the microscopic identification of methanogens in pure and mixed cultures (Doddema and Vogels 1978). This does

not however, apply to acetoclastic methanogens, which form the majority of methanogens present in most digesters, and which contain too little F420 to make them visible by fluorescence (Zehnder et al. 1980).

The suitability of F420 for the estimation of the methanogenic activity of anaerobic sludge (Delafontaine et al. 1979, van Beelen et al. 1983) will be discussed in this paragraph.

Cofactor F420 is a low molecular weight (M = 840 as ammonium salt) deazaflavin derivative, which in its oxidised form fluoresces with a strong absorption at 420 nm (Eirich et al. 1979).
It is a major electron/hydrogen carrier involved in both catabolic and anabolic oxidation/reduction reactions. The compound is a primary acceptor of electrons from hydrogen, as well as the electron acceptor in formate dehydrogenase, and it is involved in F420-$NADP^+$ oxido-reductases. Coenzyme F420 is also the electron donor in two important reactions in cell carbon synthesis, viz. pyruvate dehydrogenase and alpha-keto-glutarate dehydrogenase (Keltjens 1982).

TABLE 2 REVIEW OF THE F420 CONTENT OF PURE CULTURES OF METHANOGENS

species	F420 content (*) (mmol/kg dry weight)	ref. (1)
HYDROGEN		
Methanobact. bryantii	2.35	1
Methanobact. bryantii	1.35	1
Methanobact. thermoautotr.	1.9	1
Methanobact. thermoautotr.	1.5	2
Methanobact. thermoautotr.	0.45-0.6	3
Methanobrevib. arboriphilus	1.8	1
Methanobrevib. ruminantium	0.04	1
FORMATE		
Methanococcus vannielii	2.4	4
ACETATE		
Methanosarcina barkeri	0.09	1
Methanothrix söhngenii	0.32	5

(*) Data was converted to uniform dimensions using a F420 molecular weight of 840, a protein content of 0.5 g/g dry weight, and a dry weight : wet weight ratio of 0.2.
(1) 1: Eirich et al. 1979, 2: Schönheit et al. 1981, 3: van Beelen et al. 1983, 4: Yamazaki and Tsai 1980, 5: Zehnder et al. 1980.

The cellular F420-content varies among the different methanogenic species, as shown in Table 2.
Also a considerable variation exists in data on the same species converting the same substrate, viz. 0.45 to 1.9 mmol F420/kg dry weight reported for Methanobacterium thermoautotrophicum (Eirich et al. 1979, Schönheit et al. 1981, van Beelen et al. 1983). Perhaps these variations may be attributed to differences in the specific substrate removal rates, which were not reported.
Furthermore the lack of uniformity, both in analytical methods (using different ways of extracting F420 from the cells, and quantification by either photometry or enzymatic methods), and in the ways of expressing the analytical results (based on g of dry or wet weight, or g of protein) may have contributed to the wide scatter in the reported values. In some cases this also led to erroneous quotations of literature data (van Beelen et al. 1983, Cohen 1982, Baresi and Wolfe 1981).
In spite of the variations mentioned above, the F420 content of methanogens which convert hydrogen is significantly higher, than that of acetoclastic methanogens (Table 2). Baresi and Wolfe (1981), who reported equal F420 concentrations in Methanosarcina barkeri grown on hydrogen, methanol or acetate, presumably made some error in their analysis, because the reported value of 0.005 mmol F420/kg dry weight is almost an order of magnitude lower than the lowest value reported by others.

The quantitative determination of F420 in anaerobic sludge mixed liquors by extraction and fluorometry has been reported first by Delafontaine et al. (1979). Their extraction method using 2-propanol was also adopted with some modifications in the present work (see Materials and Methods).
Other methods reported later were based on the use of acetone (Schönheit et al. 1981, van Beelen et al. 1983), and on a combination of ultrasonification, French press rupture and 2-methyl-2-propanol (Cohen 1982). These latter methods were reported to be equivalent to the extraction with 2-propanol (van Beelen et al. 1983).

3.3.2 MATERIALS AND METHODS

Sludge mixed liquor samples were stored at -20 °C before extraction. Daylight exposure was kept to a minimum throughout the analysis.
If necessary sludge samples were diluted to a concentration of

approximately 10 kgVSS.m^{-3} with a glycine buffer solution prior to homogenization in a Potter tube. Two ml homogenized sludge (or 3 ml in case of very dilute sludge samples) were diluted in a test tube to 10 ml with glycine buffer solution and placed in a boiling water bath.

A glycine buffer solution (10 mM glycine + 5 mM Mg-EDTA; pH 7) as medium for boiling released more F420, than distilled water. Ultrasonification and/or French Press rupture did not increase the F420 yield. The optimum boiling time was determined to be 14 min (Figure 7).

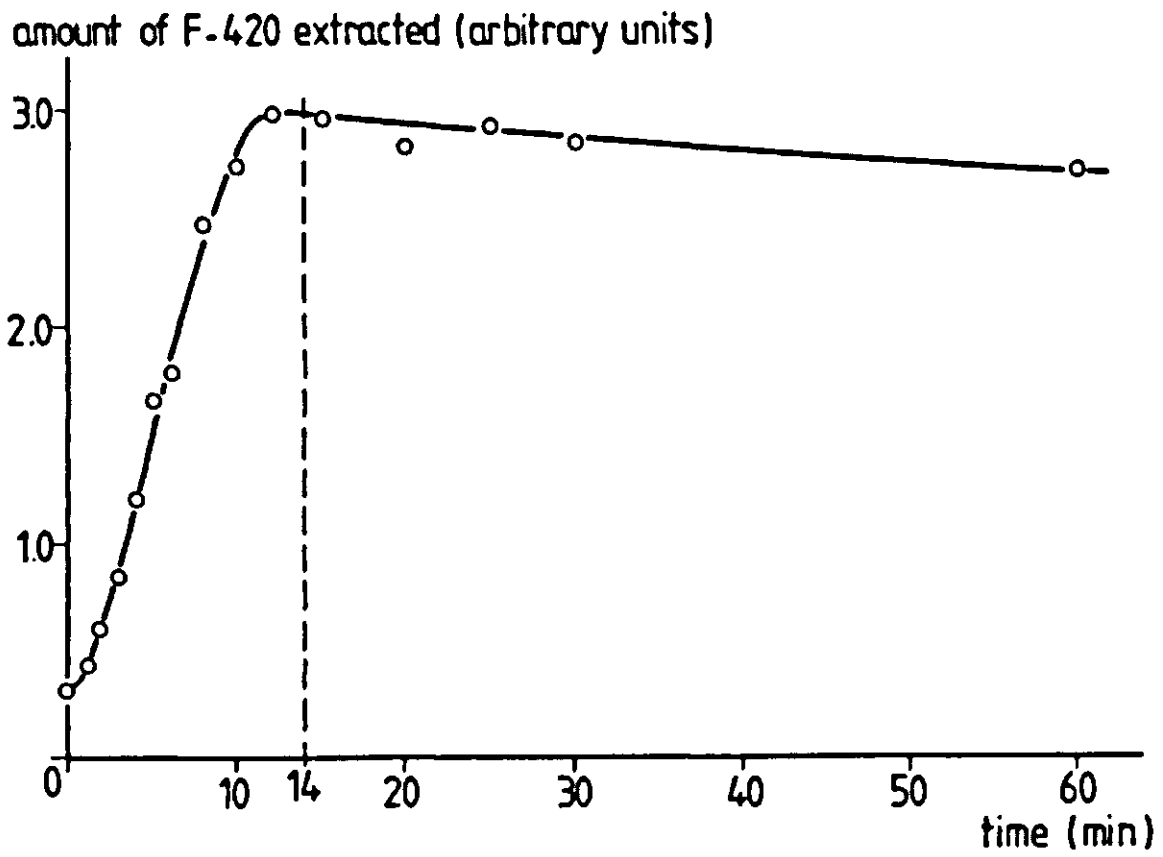


FIGURE 7
F420 yield at different extraction times in glycine buffer solution.

The use of other buffer solutions has not been investigated. Van Beelen et al. (1983) reported that a tris HCl buffer solution as employed by Cohen (1982) yielded similar results.

After cooling to room temperature on ice, samples were centrifuged for 10 min. Subsequently 5 ml of supernatant were vigorously mixed with 10 ml 2-propanol, and centrifuged again. The pH of the supernatant was adjusted to 8.6-8.8 in small glass bottles with a minimal amount of concentrated KOH (no volume correction needed). A pH of 8.8 in the samples enables working in daylight for some hours without appreciable loss of F420-fluorescence (Figure 8). If the fluorescence was not measured directly, samples were stored at -20 °C in the dark.

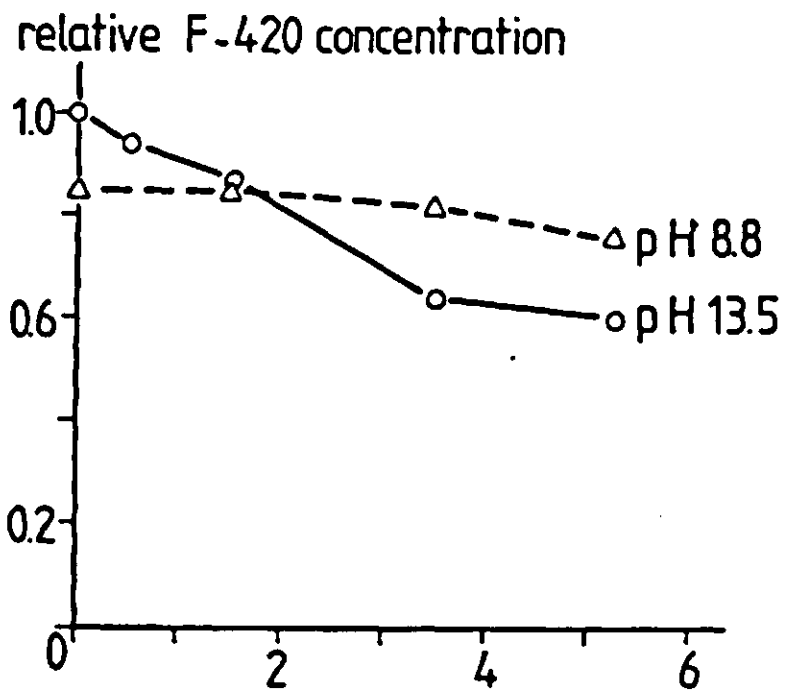


FIGURE 8
Influence of daylight exposure on F420 fluorescence

Standard solutions of pure F420 (which were kindly provided by professor Vogels, Department of Microbiology, University of Nijmegen, The Netherlands) were similarly diluted with buffer solution and 2-propanol, and the pH was also adjusted to 8.8.

In early F420 extractions in our laboratory the pH of the glycine buffer was set at 4.0. However, due to the low buffering capacity of the glycine solution the extraction always was at pH 5 or higher. Later the optimal pH for the boiling step was determined to be 8.0 to 8.5 (Hutschemakers et al. 1982, own results). The maximum error introduced by a too low pH in early measurements was estimated to be 15 %.
During boiling an increase in the pH occurs. Accordingly a glycine buffer solution of pH 7.0 was found to yield optimum results.

The calcium concentration in the sludge sample strongly influences the recovery of the F420 extraction (Figure 9), as has been confirmed recently by Archer (1983). F420 extracts from a mixed liquor originally containing

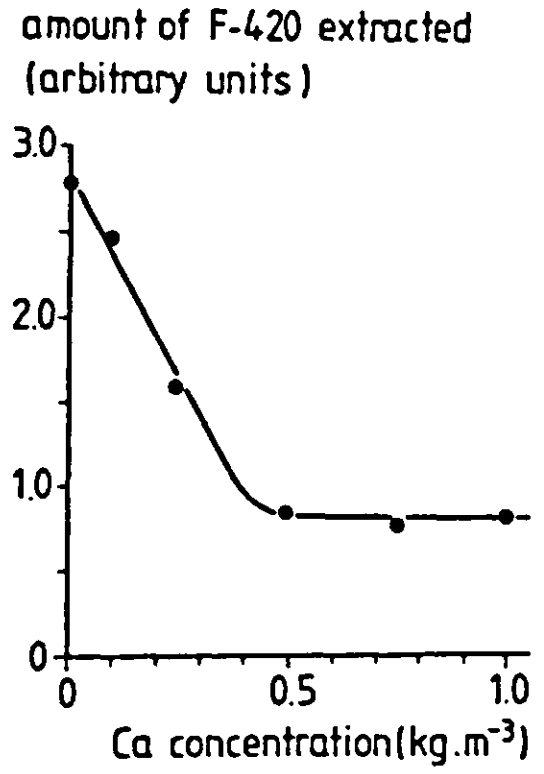


FIGURE 9
Influence of the calcium concentration in the sludge mixed liquor sample upon the F420 recovery.

300 mg Ca/l lost their fluorescing capacity after 4 months of storage at -20 °C. Identical samples from a mixed liquor containing 30 mg Ca/l fully retained their fluorescence during storage. Figure 9 shows, that the calcium concentration should be less than 100 mg Ca/l to keep the error within reasonable limits. Washing the sludge with a calcium-free buffer solution before extraction resolves the interference of calcium.

The amount of F420 in the sample was determined from the excitation spectra (Figure 10) by comparing either the peak height at 430 nm, or the peak surface area from 430 to 460 nm with that of pure F420. An Aminco SPF-500 ratio spectrofluorimeter was used. Excitation spectra were used instead of emission spectra, because a better separation between F420 and another cofactor, presumably F342 (Gunsalus and Wolfe 1978) is obtained.

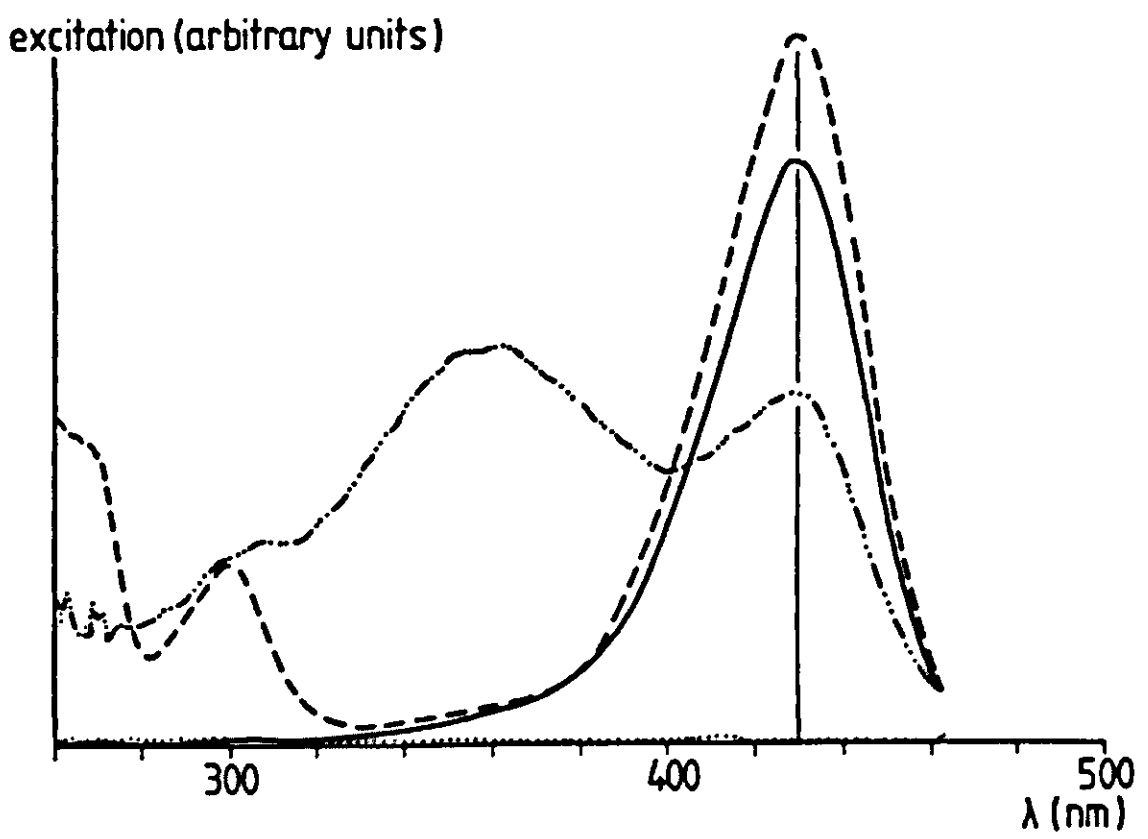


FIGURE 10
Characteristic F420 excitation spectra of pure F420 (---), of an extract of granular sludge (——) and of an extract of digested manure or digested sewage sludge (-·-·-).

In F420 extracts of sludges with a low methanogenic activity the F420 peak in the excitation spectrum is superimposed partly upon the peak of F342 (Figure 10). In that case a correction was estimated from spectra of known mixtures of F420 and F342. The F342 peak increases only slightly with increasing F420 concentrations in anaerobic sludge. At higher F420 levels (greater than approximately 100 nmol F420/gVSS), therefore, the interference by the F342 peak can be neglected.

For the measurement of F420 in sludge containing very little F420, but high concentrations of interfering substances, additional steps in the

extraction procedure were proposed (Binot et al. 1981, Hutschemackers et al. 1982). By centrifuging the sludge sample before extraction extra-cellular and therefore inactive, F420 can be removed. In the present study only mixed liquor samples were extracted.
In digested sewage sludge samples - which originate from digesters with long liquid retention times - 10 to 40 nM F420 is found in solution constituting 7 to 25 % of the total F420 concentration. Sludge samples from UASB reactors with short liquid retention times contained less than 5 nM F420 in the liquid compared to a total F420 concentration of 200 to 10.000 nM.

3.3.3 RESULTS AND DISCUSSION

Digested sewage sludge

The F420 content of digested sewage sludges has been measured in order to predict their specific methanogenic activity. A reasonably good correlation (a correlation coefficient of 0.80) was obtained between the F420 concentration of the sludge and the maximum methanogenic activity as determined in a standardised batch activity test for sludge samples from 9 different sewage sludge digesters (see Figure 5 of Chapter 4). This means that for each individual sludge sample the predictive value of the F420 content is rather poor. This is partly due to the inaccuracy of measuring close to the detection limit of the applied method, and partly to the presence of extra-cellular F420.

The concentration of F420 in digested sewage sludge mixed liquor increased rapidly during digestion of a standardised VFA feed in a batch activity test. The average increase (n=4) during digestion of the first VFA feed was 35.5 ± 7.5 nM F420, as compared to 15 ± 8.5 nM F420 during digestion of a second identical feed. This indicates, that during digestion of the first feed besides an increase in the F420 concentration due to growth, also some replenishment will have occurred of diminished cellular F420 levels of cells starved during storage. Exposure of the sludge to oxygen during transport and handling may also have caused some F420 degradation (Schönheit et al. 1981). Photolysis of F420 in digested sewage sludge can be ruled out, because no significant daylight exposure occurred.

At very long unfed storage times at 4 °C a decrease in the mixed liquor F420 concentration occurred, i.e. about 50 % in 200 days. This confirms,

that under anaerobic conditions the degradation of F420 is very slow.

UASB reactor sludge

A good correlation was obtained between the F420 content and the specific activity of anaerobic sludge in UASB reactor experiments. Figure 11 shows typical results of the first month of an UASB reactor start-up experiment using digested sewage sludge as seed material and a mixture of acetate and propionate as feed. At comparable low specific sludge activities the correlation coefficient between the F420 concentration and the methanogenic activity was far better in sludge samples from the UASB reactor with a relatively short liquid retention time (r = 0.98; Figure 11), than in the digested sewage sludge samples from sludge digesters with relatively long liquid retention times (r = 0.80; Figure 5 of Chapter 4).

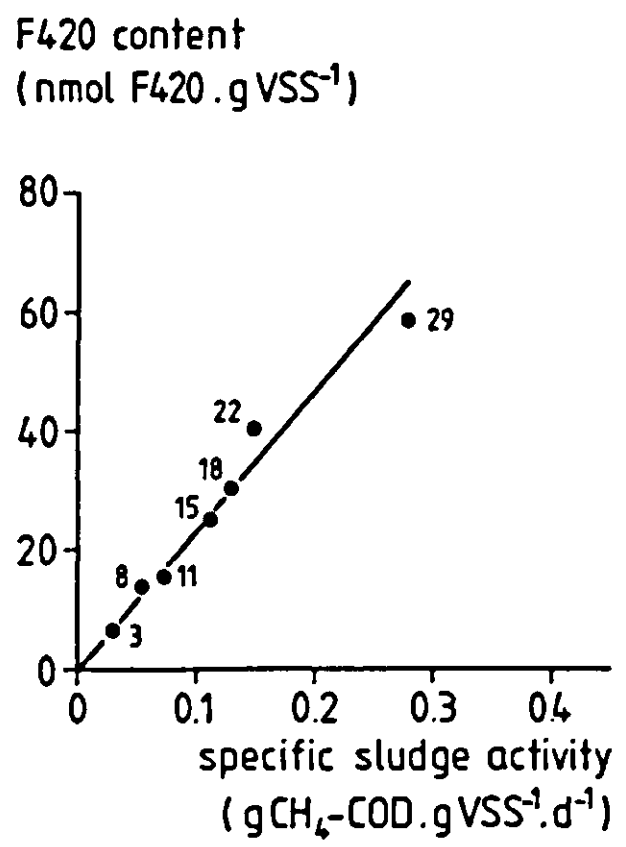


FIGURE 11
Relation between the F420 content of the sludge and the specific sludge activity during the initial stages of an UASB reactor start-up experiment.
Seed: digested sewage sludge.
Substrate: a mixture of acetate and propionate.
Numbers refer to the number of days elapsed since the start of the experiment.
Data refer to experiment 'b'; see Table 2 of Chapter 7.

The specific methane production rate based on F420 is called Q_{F420} (expressed in $mgCH_4\text{-}COD.nmolF420^{-1}.d^{-1}$). In order to predict the methanogenic activity of the sludge from the measured F420 concentration, the Q_{F420} has to be constant or if not, the variation should be known. In comparing the Q-F420 values obtained in UASB reactor experiments treating different volatile fatty acids a relation was found between Q_{F420} and the ratio of methane formed from hydrogen and methane originating from acetate splitting (Figure 12).

A large fraction of methane formed from acetate corresponds with a high Q_{F420}. The digestion of a pure acetate feed is one extreme showing a Q_{F420}

of 43 $mgCH_4$-COD.$nmolF420^{-1}.d^{-1}$, and the digestion of pure formic acid is the other extreme with a Q_{F420} of 1.6 $mgCH_4$-COD.$nmolF420^{-1}.d^{-1}$.
The data in Figure 12 all refer to sludges in which Methanothrix was the prevailing acetotrophic methanogen.

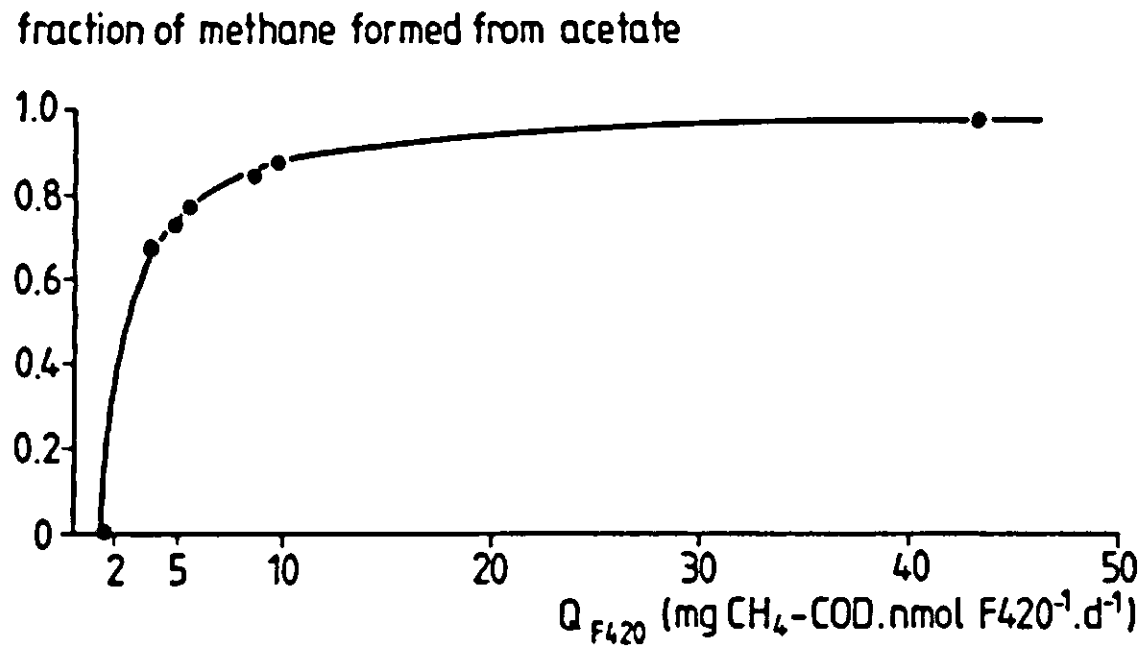


FIGURE 12 Relation between the fraction of methane formed from acetate and the Q_{F420}.

An equation that fits the data shown in Figure 12 very well is:

$$Q_{F420} = 1.3 / (1 - A) \qquad \text{(equation 1)}$$

in which A = the fraction of methane formed from acetate.

Equation 1 reflects that the amount of F420 present in acetoclastic methanogens is negligible in comparison with the F420 level in hydrogenotrophic methanogens (Table 2).
Another way of expressing the relation between the methanogenic activity of the sludge and the F420 content is to say that the Q'_{F420} is constant. Q'_{F420} is defined as the production rate of methane derived from hydrogen and carbon dioxide per unit of F420. The Q'_{F420} value belonging to the experimental data shown in Figure 12 is 1.31 $mgCH_4$-COD.$nmolF420^{-1}.d^{-1}$ (standard deviation 0.13; n=7).

The composition of the waste controls the Q_{F420} and the fraction of methane derived from acetate must be known to be able to predict the methanogenic activity of the sludge from the F420 content. For VFA mixtures this fraction can easily be calculated (see Chapter 2, Table 1). For simple

carbohydrates the fraction of methane derived from acetate is 0.67 (Jeris and McCarty 1965, Cohen 1982), whereas in the digestion of complex wastes about 72 % of all methane originates from acetate splitting (McCarty 1981).

The removal efficiency of the different constituents of the feed should be considered when calculating the fraction of methane derived from acetate. Especially when treating dilute wastewaters at low treatment efficiencies a considerable difference may arise between the theoretical acetate derived methane fraction A (based on substrate composition), and the actual A (Table 3).

TABLE 3 INFLUENCE OF COD REMOVAL EFFICIENCY ON Q_{F420}

type of wastewater	COD conc. ($kg.m^{-3}$)	removal efficiency (%)	theor. A (1) (%)	actual A (2) (%)	Q_{F420} (3)
acetate +	5.0	80-90	77	77	5.4
propionate	1.0	40-50	77	84-88	8.5-9.6
rendering	2.5(4)	93.5	78	78	4.7
wastewater	2.5(4)	48	78	81.5	7.5

(1) Acetate derived fraction of methane calculated from substrate composition.
(2) Actual acetate derived fraction of methane.
(3) Expressed as $mgCH_4$-COD/nmolF420.d.
(4) VFA fraction only; the suspended solids fraction was not removed significantly (data from de Zeeuw 1982).

After changing the substrate from a mixture of acetate and propionate to glucose in an UASB reactor experiment the Q_{F420} dropped from 5 to 3.5 $mgCH_4$-COD.nmolF420^{-1}.d^{-1} as predicted by equation 1.
In an one-stage glucose digestion Cohen (1982) found, that the fraction of methane derived from acetate is 67 %, as compared to 76 % in the methane reactor of a two-stage treatment of glucose. He reported a ratio of Q_{F420} values of 1 : 1.6. A ratio of 1 : 1.4 is predicted by equation 1.

Q_{F420} can also be used as a measure of inhibition of the sludge activity. The potential maximum methanogenic activity can be calculated from the F420 content of the sludge using the Q_{F420} derived from equation 1. Combining this figure with the actually measured Q_{F420} yields a measure of the inhibition of the sludge activity. In this way an inhibition of the methanogenic activity of the sludge due to high ammonia concentrations and

due to a lack of available phosphate was demonstrated in the anaerobic treatment of rendering wastewater (de Zeeuw 1982).

Conclusions

The F420 analysis can be used for a rapid assessment of the potential methanogenic activity of anaerobic sludge, and it can indicate possible inhibition of the methanogenesis.

The fraction of methane derived from acetate determines the Q_{F420}, i.e. the specific methane production rate based on F420. In comparing Q_{F420} values from different experiments the composition of the waste as well as the removal efficiency should be taken into account.

CHAPTER 4 CHARACTERIZATION OF DIGESTED SEWAGE SLUDGES WITH RESPECT TO THE START-UP OF UASB REACTORS

4.1 INTRODUCTION

Theoretically any medium containing the right fermenting and methanogenic bacteria can be used as seed sludge for the start-up of an UASB reactor. If the wastewater - through contamination in sewers, etc. - already contains all the necessary bacteria no seed sludge is needed at all. This has been demonstrated with raw sewage (Grin et al. 1983). The start-up time will be longer in that case, than with a proper seed sludge.

Many sewage treatment plants have one or more sludge digesters for the stabilization of primary sludge and excess sludge from the aerobic treatment facilities. The sludge from these digesters forms a cheap, and often the only readily available source of anaerobic sludge.
The use of digested sewage sludge for starting up new pilot and full scale UASB reactors is common practise, whenever adapted surplus sludge from existing UASB reactors is not available (Pette et al. 1979, Versprille 1978, Zeevalkink 1982).

Sludge samples from a number of sewage sludge digesters were characterised with respect to DSS, VSS, settleability, and methanogenic activity. The results are presented in this chapter, and discussed in the light of the potential use as seed sludge for the start-up of UASB reactors.

Both satisfactory and poor results were experienced with digested sewage sludge as seed sludge. Besides difficulties originating from the nature of the wastewater to be treated and from technological obstacles, also differences in the quality of digested sewage sludges may have caused the varying results in start-up. The major problems, that were encountered, were the development of bulking sludge and excessive sludge wash-out.

Apart from the concentration of dry suspended solids and the ash content little information is available about the quality of digested sewage sludge with respect to the use as seed sludge. Sewage sludge digesters are fully mixed reactors with a low loading rate of about 2 $kgVS.m^{-3}.d^{-1}$ and a

retention time of 30 to 12 days (Mitchell 1975). The reduction of volatile solids (VS) is about 40 % (Chynoweth and Mah 1971). Digested sewage sludge, therefore, contains a large fraction of refractory organic matter. Due to the complexity of the input of a sewage sludge digester the bacterial population will be very diverse. Hence digested sewage sludge is an excellent seed material for the digestion of all kinds of wastes. Total counts of anaerobic bacteria in digested sewage sludge vary from 10^8 to 10^{10} per ml (Toerien and Hattingh 1969, Hayes and Theis 1978). Approximately equal numbers of fermenting and methanogenic bacteria are present. Only 1 % of the total number are facultative anaerobes (Kirsch and Sykes 1971).

4.2 MATERIALS AND METHODS

Sludges

Digested sewage sludges were obtained from 10 sewage treatment plants throughout the Netherlands (Table 1).

TABLE 1 ORIGIN, DSS, AND ASH-CONTENT OF THE DIGESTED SEWAGE SLUDGE SAMPLES USED IN THE PRESENT WORK

code	location of treatment plant	sampling date	DSS ($kg.m^{-3}$)	ash content (% of DSS)
A	Amsterdam-Noord	14-09-79	16.2	34.3
B	Assen	10-81	64.7	47.9
C	Beverwijk	14-09-79	25.8	34.9
D	Doetinchem	18-11-78	64.2	47.3
E1	Ede-1	08-05-78	29.4	34.5
E2	Ede-1	27-11-79	38.3	38.7
E3	Ede-1	10-04-80	28.7	35.2
E4	Ede-1	19-05-80	34.85	35.6
E5	Ede-1	25-06-80	28.8	34.2
E6	Ede-1	02-09-80	40.4	40.0
E7	Ede-1	02-04-81	29.9	33.0
E8	Ede-1	15-10-81	40.25	35.2
E9	Ede-1	08-02-82	28.6	30.1
E10	Ede-1	24-08-82	43.5	37.9
E11	Ede-1	05-10-82	50.7	38.4
F1	Ede-2	04-75	68.8	44.0
F2	Ede-2	21-03-79	50	37
F3	Ede-2	21-03-79	79	46.8
F4	Ede-2	12-01-80	95.7	46.7
F5	Ede-2	25-06-80	61.0	42.0
G	Renkum	02-09-80	46.6	40.8
H	St.Willebrord	13-10-77	79.5	52.2
I	Veenendaal	02-09-80	64.2	36.1
J	Zaandam-Oost	14-09-79	74.6	37.3

Analyses

- DSS and VSS were determined according to Standard Methods.
- The Sludge Volume Index (SVI) was determined in one-liter volumetric cylinders (height 30 cm). The sludge was diluted with tap water to a concentration ranging from 2.5 to 5.0 kgDSS.m^{-3}, allowing for a settled volume after 30 minutes of 0.2 to 0.3 liter. The SVI value was based on the settled fraction of the sludge. Sludge solids remaining in suspension after 30 minutes were determined separately and are referred to in the text as "non-settleable" solids.
- The standardised sludge methanogenic activity test was performed according to paragraph 3.2.
- The analysis of F420 is described in paragraph 3.3.

4.3 RESULTS AND DISCUSSION

4.3.1 DSS AND VSS CONTENT

The DSS content of the examined digested sewage sludge samples from different digesters varied between 16 and 96 kgDSS.m^{-3}. Sludge samples taken at intervals from the same sewage sludge digester varied much less in DSS content (Table 1).

The VSS fraction (VSS/DSS) of the samples generally decreased with increasing DSS concentrations, i.e. from 65 % at 25 kgDSS.m^{-3} to 45 % at 100 kgDSS.m^{-3} (Figure 1). This trend was observed both for sludge samples obtained from different digesters, as for samples taken from the same digester at different times. Literature data confirm this relationship (see Figure 1), which can be explained by differences in the ash content and in the biodegradability of the digesters input.

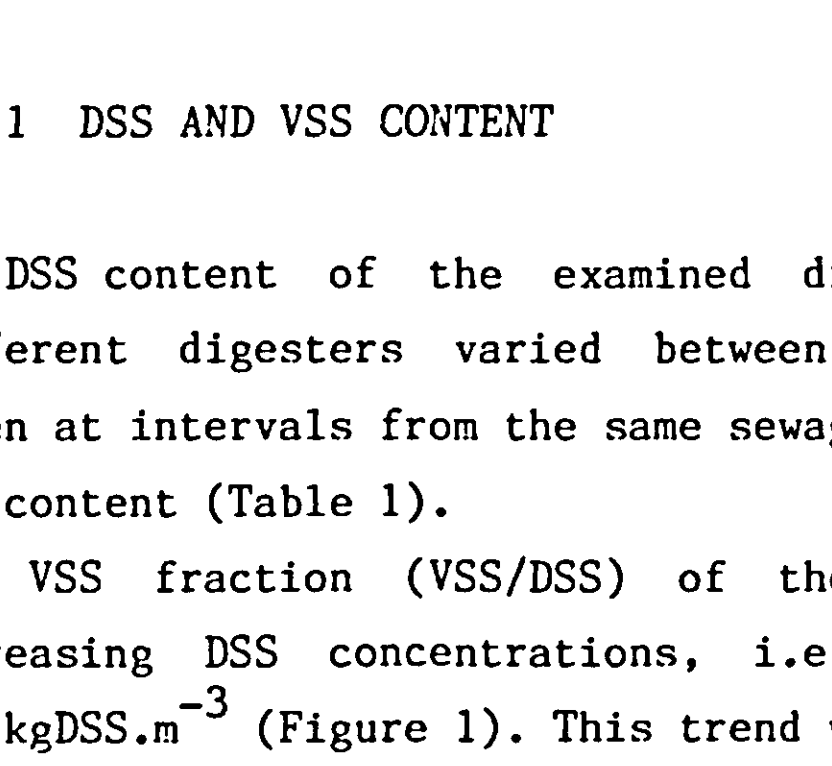


FIGURE 1
Relation between the organic fraction (VSS/DSS) and the DSS concentration of digested sewage sludge samples from different digesters (o), samples from one digester taken at different times (+ and x), and literature data (•); a: Hattingh et al. 1967, b: Kaspar 1977, c: van Velsen 1981, d: Pohland and Bloodgood 1963, e: Versprille 1978.

4.3.2 SETTLING CHARACTERISTICS

The sludge volume index (SVI) has also been determined in relation to the sludge concentration for sludges from 6 different sewage sludge digesters. The SVI ranged from 120 ml/gDSS for a sludge with 20 kgDSS.m^{-3}, to about 50 ml/gDSS for thicker sludges of 40 to 80 kgDSS.m^{-3} (Figure 2).

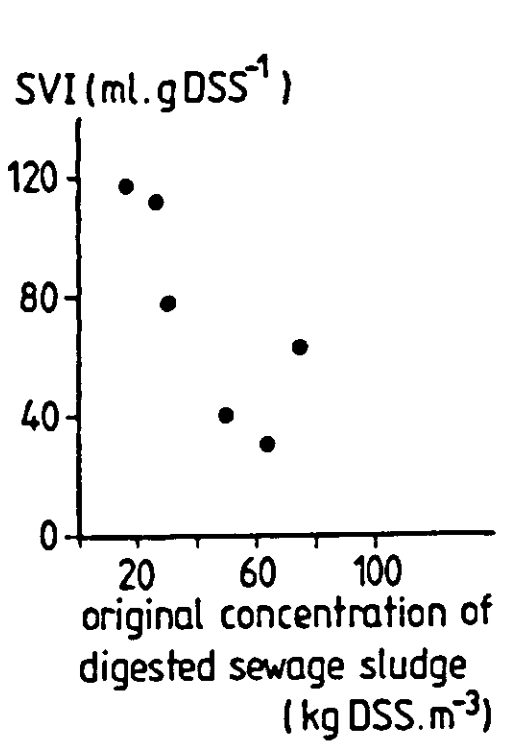


FIGURE 2
Relation between the Sludge Volume Index and the DSS concentration of digested sewage sludge from different digesters.

As only a part of the sludge solids settled out within a 30 min settling period, showing a distinct interface between sludge blanket and supernatant, the SVI values concern merely the heavier part of the sludge. This flatters the picture of the sludge settling characteristics.

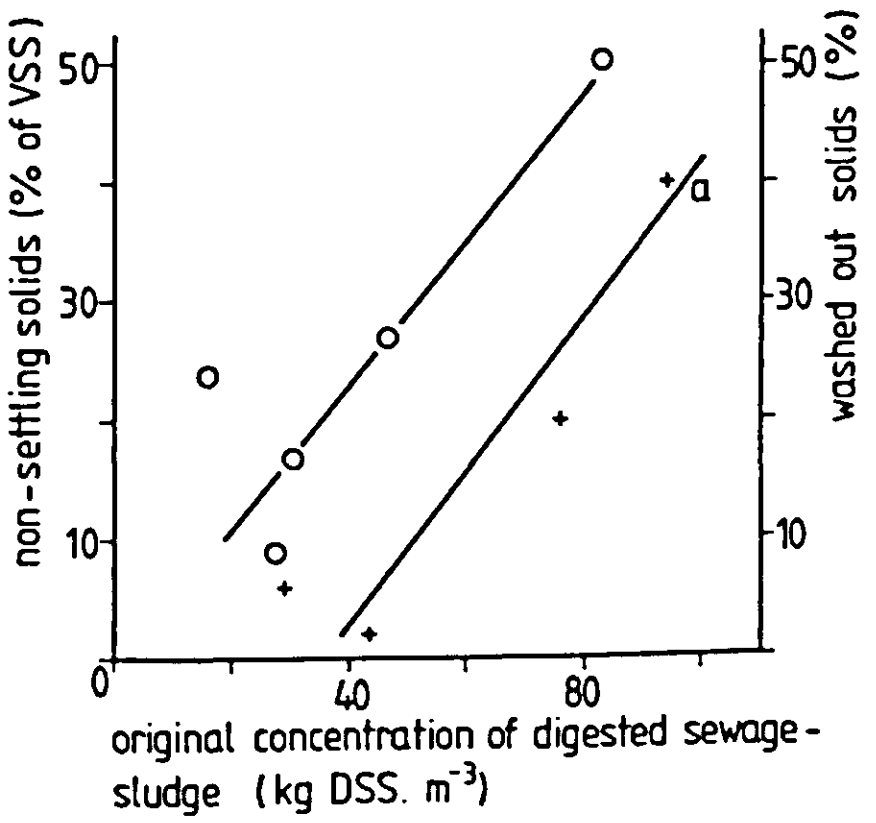


FIGURE 3
The fraction of the VSS not settled in a SVI measurement (o) and the fraction of the VSS washed out during the first 10 days of UASB reactor start-up (+) as a function of the original seed sludge concentration (a: Pette et al. 1979). See also Table 2.

Therefore, in addition to the SVI also the amount of finely dispersed suspended solids of the supernatant fraction has been determined. Figure 3 shows, that the fraction of these 'non-settleable' suspended solids is greater in more concentrated sludges, where it may even constitute as much as 50 % of the total VSS.

TABLE 2 SLUDGE WASH-OUT DURING UASB REACTOR START-UP AS A FUNCTION OF THE TYPE OF DIGESTED SEWAGE SLUDGE USED AS SEED MATERIAL

digested sewage sludge concentration			percentage of suspended solids washed out from the reactor within 10 days after start-up (1) (%)		ref. (2)
of the original sample from the sludge digester ($kgDSS.m^{-3}$)	per unit UASB reactor volume prior to start-up ($kg.m^{-3}$)				
	DSS	VSS	of DSS	of VSS	
29	23	15	4.5	6	1
43	16	10	1.5	2	2
79	22	11.5	11	20	3
94	42	16	44	40	4

(1) After replacing the reactor volume about 4 times with wastewater at loading rates between 0.5 and 2.0 $kgCOD.m^{-3}.day^{-1}$.
(2) 1, 2, and 3 refer to UASB reactor start-up experiments "g2", "t1" and "b" resp. of Table 2 of Chapter 7, 4: Pette et al. 1979.

These figures concerning the 'non-settleable' fraction of digested sludge, as determined in the SVI measurement, correspond quite well with the amount of sludge washed out in UASB reactor start-up experiments with digested sewage sludge of varying initial DSS-concentration (Table 2; Figure 3).

TABLE 3 ASH CONTENT OF THE SUSPENDED SOLIDS FRACTION IN THE EFFLUENT OF UASB REACTORS DURING THE FIRST DAYS OF START-UP

digested sewage sludge as obtained from the sewage sludge digester		washed out sludge (1)	ref (2)
concentration ($kgDSS.m^{-3}$)	VSS content (% of DSS)	VSS content (% of DSS)	
29	64.8	85.3	1
43	62.1	79.9	2
79	53.2	75.8	3

(1) Average of effluent during first 4 detention times.
(2) See Table 2.

At the same low initial loading rates a considerably larger fraction (up to 40 %) of the VSS is rapidly washed out from the reactor when using a very concentrated digested sewage sludge, than with a less concentrated digested sewage sludge as seed.

The wash-out of volatile suspended solids from UASB reactors often considerably exceeded the wash-out of non-volatile suspended solids (Tables 2 and 3).

Furthermore, this poorly settling, and therefore easily washed out VSS fraction of the digested sewage sludge showed a 2 to 3 times higher specific methanogenic sludge activity, than the digested sewage sludge as a whole (Table 4).

TABLE 4 RELATIVE SPECIFIC METHANOGENIC SLUDGE ACTIVITY OF THE 'NON-SETTLEABLE' (1) VSS FRACTION OF DIGESTED SEWAGE SLUDGE

sludge concentration as obtained from the sewage sludge digester ($kgVSS.m^{-3}$)	relative specific methanogenic sludge activity of the "non-settleable" VSS, as compared to the original seed sludge VSS (2)	ref. (3)
20	2.95 (4)	1
34	2.8 (5)	2
42	1.45-3.3 (4)	3

(1) For definition see paragraph 4.2; Materials and Methods.
(2) The specific activity of the seed sludge-VSS is set at 1.0.
(3) 1, 2, and 3 refer to sludge types E4, B, and F3 of Table 1.
(4) Based on F420 determination in terms of molF420/kgVSS.
(5) Based on standardised VFA fed batch activity tests.

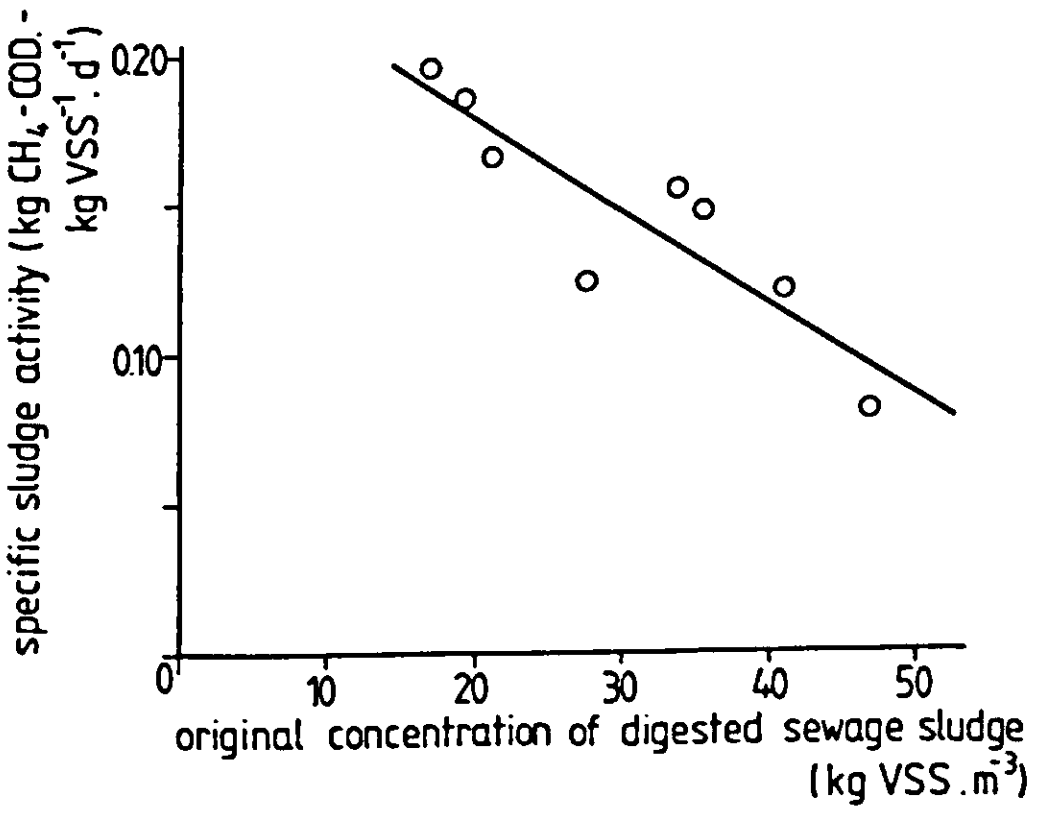


FIGURE 4
Maximum specific sludge activity of digested sewage sludge samples from 8 sewage sludge digesters as a function of the original VSS content of the sludge.

4.3.3 METHANOGENIC ACTIVITY

The methanogenic activity of different digested sewage sludges has been determined, both by the standardised test procedure in VFA fed batch experiments (see Chapter 3), and by analysing the F420 content of the sludge.

The maximum specific sludge activities ranged from as low as 0.08 $kgCH_4$-COD.$kgVSS^{-1}$.day^{-1} for concentrated (about 5 % VSS) digested sewage sludges, to 0.2 $kgCH_4$-COD.$kgVSS^{-1}$.day^{-1} for less concentrated ones (Figure 4).

Digested sewage sludge types with a sludge concentration of 30 to 40 $kgVSS.m^{-3}$ exhibit the highest methanogenic activity per cubic meter of sludge (Figure 5). The F420 concentration reaches a maximum at the same sludge concentration.

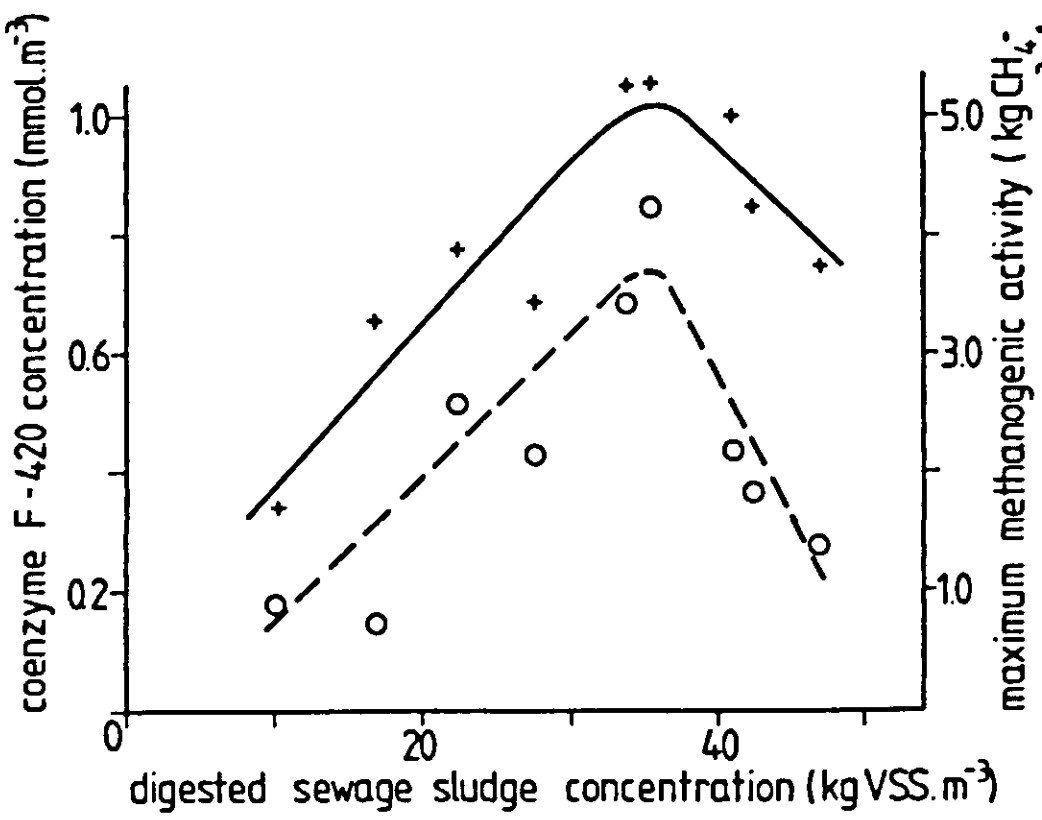
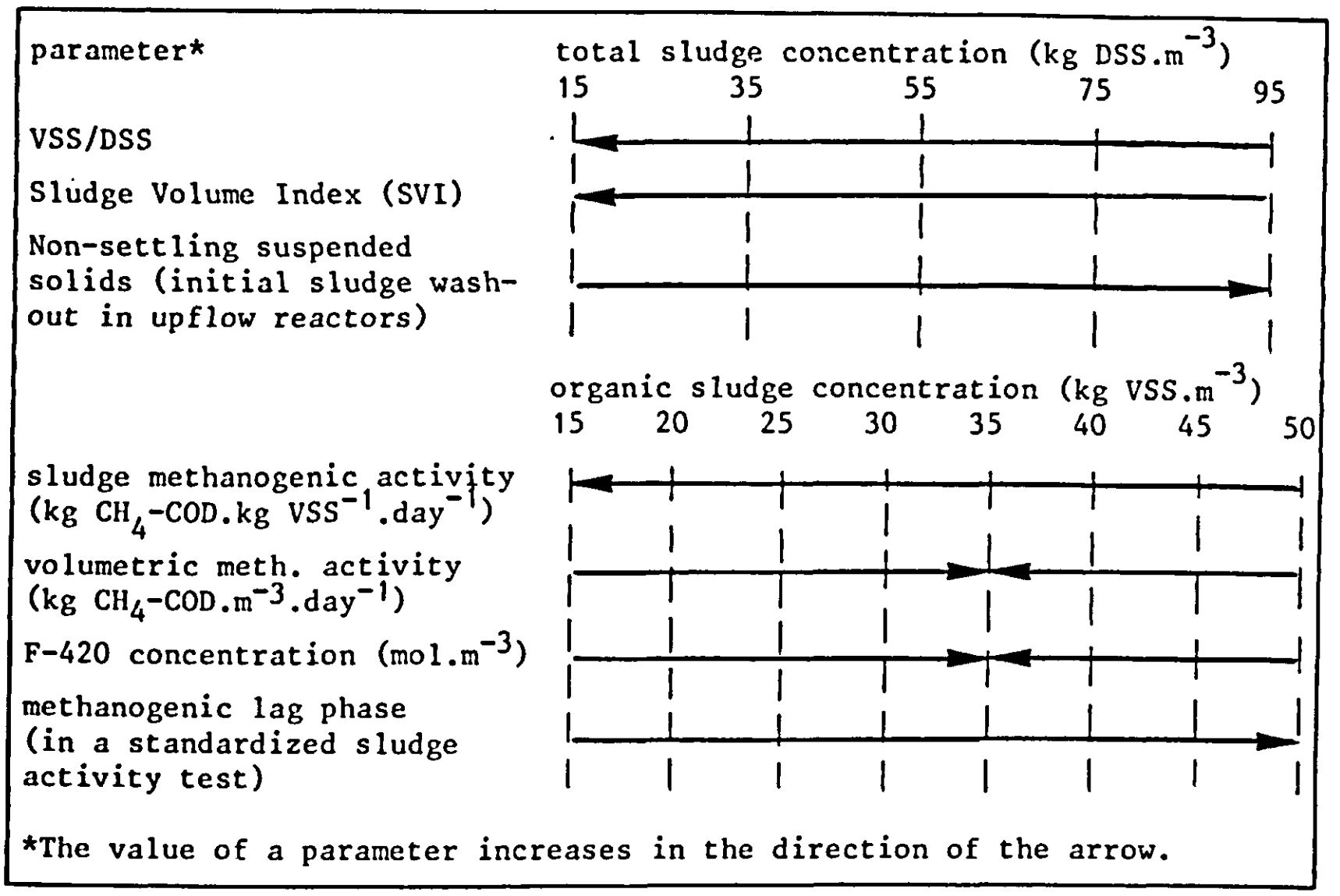


FIGURE 5
The maximum methanogenic activity (+) and the F420 concentration (o) of digested sewage sludge samples from nine different sewage sludge digesters as a function of the original digested sewage sludge concentration.

4.3.4 IMPLICATIONS FOR UASB-REACTOR START-UP

The trends observed with respect to the characteristics of digested sewage sludges as a function of the sludge concentration (Table 5) have implications for the use of these sludges as seed sludge for the start-up of UASB reactors.

TABLE 5 TRENDS OBSERVED IN TESTING DIGESTED SEWAGE SLUDGE SAMPLES FROM DIFFERENT SEWAGE SLUDGE DIGESTERS



The two extremes can be described as follows:

a. Digested sewage sludge with a sludge concentration of 30 kgDSS.m^{-3} or less is relatively homogeneous with respect to the settleability. Less than 6 % of the VSS remains dispersed in the supernatant after settling in an SVI determination. This fraction will rapidly wash out from an upflow reactor during start-up. The SVI of the settled sludge is relatively high, i.e. about 100 ml.gDSS^{-1}. As a result of the poor settleability in combination with the relatively high specific methanogenic activity of this sludge a rapid expansion of the sludge bed may be expected in an UASB reactor during start-up.

b. Digested sewage sludge with a sludge concentration of 75 kgDSS.m^{-3} or more is much less homogeneous. A large part of the VSS (more than 20 %) settles very poorly if at all, and consequently will be washed out rapidly from an upflow reactor. This also means that a considerable part (in the order of 50 %) of the original methanogenic activity is lost from the system (Tables 2 and 4). The remaining sludge has a substantially better settleability (SVI of about 40 ml.gDSS^{-1}), as compared to that of less concentrated digested sewage sludges. The relatively good settleability in combination with the low methanogenic activity of the retained sludge will cause relatively little sludge bed

CHAPTER 5 LAG PHASE PHENOMENA

5.1 INTRODUCTION

Lag phase phenomena, i.e. the temporary absence of the degradation of a compound, or the temporary absence of methane production, have received little specific attention in the literature.

Lag phases often occur after a major change in the environment of the bacteria. Seeding, which is equivalent to dilution of the biomass, may lead to an inoculum-size dependent lag phase (Kulkarni 1979). Borchardt (1971) and Blanc and Molof (1973) suggest that the length of the lag phase is controlled by the accumulation of an active substance in the extracellular medium.

A change in the substrate composition may also induce a lag phase, whenever there is a need to synthesize adequate levels of the necessary enzymes (Pamment et al. 1978) or adequate numbers of the necessary bacteria (Chynoweth and Mah 1977). A Methanosarcina culture growing on hydrogen and carbon dioxide frequently exhibits a lag phase, when the substrate is switched to acetate (Winter and Wolfe 1979).

Inhibiting compounds may also induce a lag phase until the culture is adapted. This has been shown in the case of high ammonia concentrations (van Velsen 1979).

Abandoning an experiment before the lag phase is ended, may lead to erroneous conclusions as to the feasibility of the process.

As lag phases occur after major changes in the environment they can be observed well in batch fed systems. In many respects the first start-up of an UASB reactor after seeding with digested sewage sludge, resembles the situation in a batch reactor after feeding for the first time.

In order to improve the insight into the reasons for the existence of a lag phase, several factors affecting its duration have been investigated:

- the initial methanogenic activity of the sludge mixed liquor,
- the length of the preceeding unfed storage period,
- the mixing intensity,
- the VFA substrate concentration and composition,
- the pH and redox potential, and
- the presence of inhibitory substances such as oxygen and sulphide.

5.2 MATERIALS AND METHODS

Defining the end of the lag phase

The duration of a lag phase is difficult to quantify. A period without any methane production may be succeeded by a period with only insignificant methanogenesis until suddenly a rather active digestion starts (see Figures 2 and 3 and Chapter 3; Figure 2). A definition of lag phase as the period of time without any methane production is unpractical. Lag phase can better be defined as the extra time required to digest the feed, when compared with the time it would take if the digestion rate would have been at its maximum from the start. As lag phase should be distinghuished from retarded digestion only the first part of the methane production is taken into account to calculate the duration of the lag phase. For the purpose of this study, therefore, the lag phase has been defined as the extra time required to produce a fixed small amount of methane per m^3 of digester volume (i.e. 0.2 $kgCH_4$-$COD.m^{-3}$), unless stated otherwise.

Sludges

Sludge types used are referred to by the codes of Table 1 of Chapter 4.

Experimental set-up

Batch experiments were conducted as described in paragraph 3.2.1.

Analyses

-DSS and VSS were determined according to Standard Methods.

-VFA were analysed as described in paragraph 3.2.1.

-Redox potential measurements were performed with a platinum electrode and an Ag/AgCl (3M KCl) reference electrode. The accuracy of the measurements was tested using a commercially available redox buffer solution. The time required for equilibration of the redox electrode in anaerobic sludge mixed liquors was found to depend upon the sludge concentration; from 4.5 hours in digested manure of about 4 % DSS to 15 and 40 hrs in digested sewage sludge with a concentration of 13.4 and 2.2 $kgVSS.m^{-3}$ respectively. Redox potential measurements were transformed into E_h values according to $E_h = E_{measured} + 204$ mV, and corrected for pH 7.0 using $rH = (E_h / 30) + 2pH$ (Jacob 1970).

-Sulphide analysis was performed using an ion-specific electrode (Orion) in a buffer solution (Standard Methods).

-Sulphate was determined turbidometrically using an EEL nephelometer (Standard Methods).

5.3 RESULTS

5.3.1 EFFECT OF THE METHANOGENIC ACTIVITY

As shown in Figure 1 (symbol +), the volumetric methanogenic activity of digested sewage sludge samples from 9 different sludge digesters ranged from 0.2 to 0.5 $kgCH_4$-$COD.m^{-3}.d^{-1}$ (when diluted to a sludge concentration of 2.5 $kgVSS.m^{-3}$ in standard sludge activity tests; see Chapter 3).
The lag phase decreased at increasing mixed liquor methanogenic activity, i.e. at increasing concentrations of active biomass.
In order to extend the measurements to higher methanogenic sludge activities, an additional experiment was conducted for one type of digested sewage sludge with higher sludge concentrations corresponding to mixed liquor methanogenic activities of 0.43, 0.55, 0.95 and 1.85 $kgCH_4$-$COD.m^{-3}.day^{-1}$. The data in Figure 1 (symbol x) reveal, that the gas production lag phase practically disappears at a methanogenic activity exceeding 0.5 $kgCH_4$-$COD.m^{-3}.day^{-1}$.

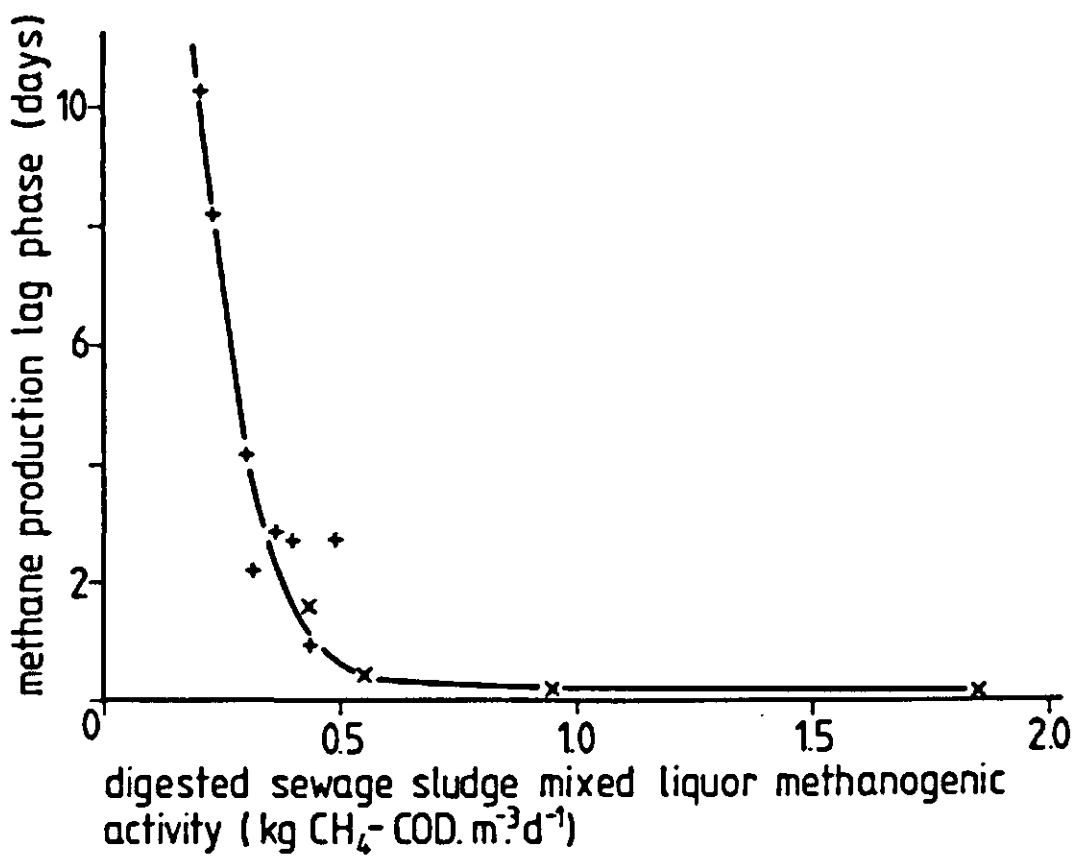


FIGURE 1
The gas production lag phase in relation to the digested sewage sludge mixed liquor methanogenic activity for samples from 9 sewage sludge digesters (+) and for one sample at 4 different sludge concentrations (x).

5.3.1.1 Addition of small amounts of well adapted UASB reactor sludge

To investigate further the influence of the active biomass concentration upon the length of the gas production lag phase, a series of standard sludge activity tests was performed, in which 1, 2 and 5 % of the VSS of the digested sewage sludge were replaced by active sludge removed from an

UASB reactor treating a mixture of acetate and propionate. The UASB reactor sludge was predominantly flocculent in appearance, and exhibited a specific activity of 0.75 $kgCH_4$-COD.$kgVSS^{-1}$.day^{-1}. The digested sewage sludge used for this series of experiments had been stored unfed for 8 months at 4 ^{o}C, and had a specific activity of only 0.06 $kgCH_4$-COD.$kgVSS^{-1}$.day^{-1}.

TABLE 1 EFFECT UPON THE METHANE PRODUCTION LAG PHASE OF THE ADDITION OF SMALL AMOUNTS OF ACTIVE UASB REACTOR SLUDGE TO DIGESTED SEWAGE SLUDGE IN BATCH FED REACTORS (1)

digested sewage sludge (% of VSS)	UASB reactor sludge (% of VSS)	lag (days)	maximum mixed liquor activity ($kgCH_4$-COD.m^{-3}.d^{-1})	
			observed	calculated (2)
100	0	14.7	0.10	-
99	1	10.1	0.10	0.11
98	2	6.5	0.13	0.13
95	5	4.3	0.15	0.16
0	100	0.2	1.35	-

(1) Total sludge concentration: 1.8 $kgVSS.m^{-3}$. Feed: 0.55 $kg.m^{-3}$ of both acetate and propionate. Experiments were performed as standard activity tests; see paragraph 3.2.1.
(2) Calculated from the activities observed in the experiments with 100 % digested sewage sludge and 100 % UASB reactor sludge.

The data in Table 1 shows that the addition of small amounts of active UASB reactor sludge to relatively inactive digested sewage sludge significantly decreased the lag phase. The addition of 1 % UASB reactor sludge, constituting a (calculated) rise in mixed liquor methanogenic activity of 13 %, resulted in a 32 % decrease of the lag phase. Keeping in mind the lag phase definition used, the extra gas production to reach the boundary level of 0.2 $kgCH_4$-COD.m^{-3} 4.6 days earlier was greater than can be explained by the gas production of the added 1 % UASB-reactor sludge alone, suggesting a positive effect of the added sludge upon the start of the gas production by the digested sewage sludge.

Exchanging 5 % of the digested sewage sludge VSS for UASB reactor sludge resulted in a 64 % (calculated) increase in the mixed liquor methanogenic activity, and a 71 % decrease of the methane production lag phase. Compared to the 100 % UASB-reactor sludge experiment the lag phase of the UASB-reactor sludge increased in the case of a 5 % addition. If no lag phase would have occurred, the boundary level of 0.2 $kgCH_4$-COD.m^{-3} should have been reached in 0.2/(0.05 x 1.8 x 0.75) = 3.0 days (neglecting any gas

production by the digested sewage sludge). In fact it took 4.3 + (0.2/0.15) = 5.6 days.

5.3.1.2 Unfed storage of sludge

Digested sewage sludge has been stored at 4 °C without feeding up to 1.5 year after sampling from different sludge digesters. At regular intervals a standard sludge activity test was applied to part of the stored sludge. The results of these measurements are summarised in Table 2.

TABLE 2 INFLUENCE OF UNFED STORAGE AT 4 °C ON THE LAG PHASE AND ON THE SLUDGE ACTIVITY OF DIGESTED SEWAGE SLUDGE (1)

sludge type (2)	storage time (days)	lag (days)	maximum sludge activity ($kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$)
a	2	1.5	0.17
	199	2.9	0.20
	294	3.3	0.12
	338	4.7	0.17
	499	5.5	0.16
	535	7.5	0.16
b	3	2.7	0.14
	77	7.5	0.12
	243	14.7	0.06

(1) Performed as standard sludge activity tests; see para. 3.2.1.
(2) Sludge types refer to the following codes (see Chapter 4, Table 1): "a"="E1" and "b"="F5".

In the case of type "a" sludge, which exerted the highest methanogenic sludge activity of the two tested, the observed methane production lag phase increased from 1.5 days in fresh digested sewage sludge to 7.5 days in 535 days old sludge. The maximum specific methanogenic activity of this particular sludge did not significantly change during the cold storage. In the case of type "b" sludge a more pronounced effect of storage upon the lag phase as well as upon the sludge activity was found.

5.3.2 EFFECT OF MECHANICAL MIXING

The effect of the mode (intermittently or continuously), as well as the intensity of stirring upon the lag phase was investigated in a number of batch experiments with digested sewage sludge samples fed with different VFA. Figure 2 shows the results of a representative series of experiments. The results are summarised in Table 3.

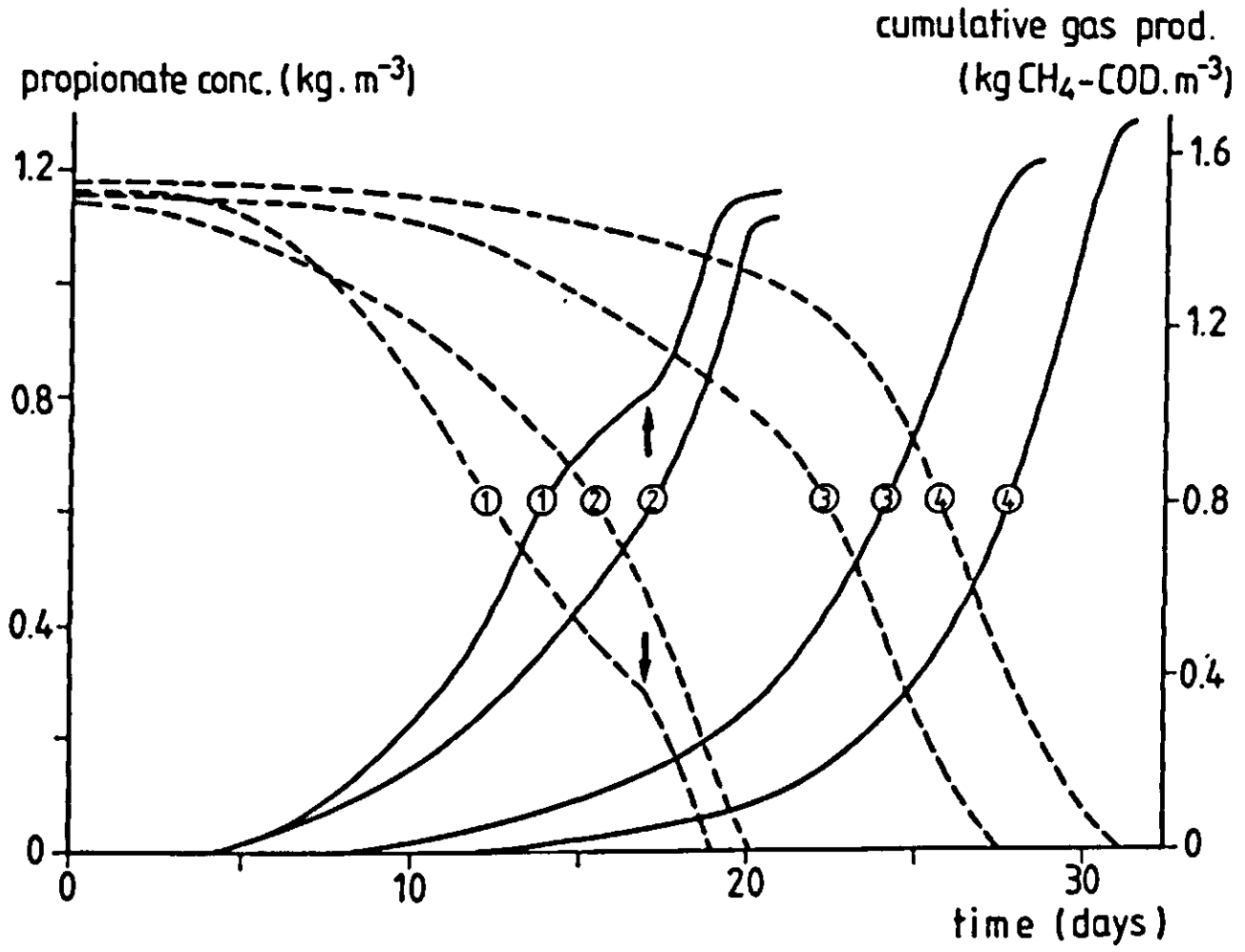


FIGURE 2 The influence of the stirring intensity upon the gas production lag phase in the batch digestion of propionate.
(——): methane production, (---): propionate concentration,
(1): no stirring, intermittent stirring started at arrow,
(2): intermittent stirring (30 sec at 140 rpm every 5 min),
(3): continuous stirring at 40 rpm,
(4): continuous stirring at 200 rpm.

TABLE 3 INFLUENCE OF THE MODE OF STIRRING UPON THE START OF THE GAS PRODUCTION FROM DIFFERENT VFA IN BATCH REACTORS (1)

stirring	gas production lag phase in the digestion of:		
	acetate (days) relat.	butyrate (days) relat.	propionate (days) relat.
no (2)	3.0 (0.9)	3.7 (0.85)	8.3 (0.85)
intermittent; 140 rpm(3)	3.4 (1.0)	4.5 (1.0)	9.8 (1.0)
continuous; 40 rpm	5.7 (1.7)	8.75 (1.95)	17.3 (1.75)
continuous; 200 rpm	8.1 (2.4)	11.8 (2.6)	22.4 (2.3)

(1) Digester volume 5.5 liter; flat blade impeller; sludge code "E1" (Chapter 4, Table 1); sludge conc. 1.8 ± 0.2 kgVSS.m^{-3}; initial pH 6.9 ± 0.1; acetate and n-butyrate conc. 1.5 kg.m^{-3}, propionate concentration 0.2 kg.m^{-3}.
(2) No mechanical stirring; manually stirred once a day before sampling.
(3) 30 sec at 140 rpm followed by a 5 min pause.

The lag phase was shortest when no stirring was applied. Intermittent stirring increased the lag phase with 10 - 15 %. The effect of continuous compared with intermittent stirring was more pronounced. Continuous

stirring resulted in a 50 to 85 % longer lag phase at an impeller speed of 40 rpm. At an impeller speed of 200 rpm the lag phase was 110 to 150 % longer, illustrating the strong effect of the intensity of stirring (Table 3).

The difference in length of the lag phase in unstirred and intermittently stirred batch reactors was significantly shorter at an initial pH (pH_o) of 7.0, than at a pH_o of 6.1 (Table 4). Apparently a suboptimal pH_o reinforces the negative effect of stirring.

TABLE 4 INFLUENCE OF THE MODE OF STIRRING AND THE INITIAL pH UPON THE GAS PRODUCTION LAG PHASE IN BATCH FED REACTORS (1)

substrate	initial pH	ratio of lag phases not stirred : intermittently stirred
propionate ($1.2\ kg.m^{-3}$)	7.0	1 : 1.16
	6.1	1 : 1.51
acetate + propionate (1.5 and $1.2\ kg.m^{-3}$)	7.0	1 : 1.08
	6.15	1 : 1.50

(1) The experiments were performed as described in Table 3. Sludge type: "E1"

Table 3 shows, that the duration of the lag phase for propionate digestion was much longer than for acetate and butyrate. For all modes of stirring investigated, the ratio of the lag phases was about 1.0 : 1.3 : 2.5 for acetate, butyrate and propionate digestion respectively at the prevailing conditions. The ratio of molar concentrations was 1.5 : 1 : 1, and the ratio of maximum specific degradation rates was 2.7 : 1 : 1 (as $moles.kgVSS^{-1}.d^{-1}$) for acetate, butyrate and propionate respectively.

5.3.3 EFFECT OF THE INITIAL SUBSTRATE CONCENTRATION

Three series of experiments have been performed to examine the influence of the initial substrate concentration upon the gas production lag phase (Table 5).

In one series of 3 experiments (Table 5; sludge type A) beetsugar campaign wastewater was used as substrate (predominantly consisting of acetate and

propionate). No influence of the substrate concentration upon the lag phase was observed at the relatively high sludge concentration used and the relatively low COD concentration range studied.

In the second series of experiments with another type of digested sewage sludge the effect of the initial substrate concentration upon the gas production lag phase was also insignificant at low substrate concentrations (Table 5, type B). The latter results were obtained with a VFA mixture (acetate, propionate and butyrate) as substrate. The volatile fatty acids were fed to the batch reactors in the acid form. For neutralization an equivalent amount of Na-bicarbonate was added simultaniously to the mixed liquor. This experimental set-up may have resulted in an initial pH (pH_o) variation (unfortunately, pH readings were omitted) with relatively low pH values at high VFA levels, thus adding a pH-effect to the effect of the substrate concentration upon the length of the gas production lag phase (see also paragraph 5.3.4). The lag phase of 23 days at a COD-concentration of 3.9 $kg.m^{-3}$ would probably have been shorter, if a pH_o of 7.0 had been maintained (Table 5).

TABLE 5 INFLUENCE OF THE INITIAL SUBSTRATE CONCENTRATION UPON THE GAS PRODUCTION LAG PHASE IN BATCH FED REACTORS (1)

digested sewage sludge		substrate		lag
type (2)	concentration ($kgVSS.m^{-3}$)	type (3)	concentration ($kgCOD.m^{-3}$)	(days)
A	5.6	beetsugar	0.2	0.35
	5.6	campaign	0.6	0.35
	5.6	wastewater	1.6	0.35
B	8.45	acetate +	0.5	4.7
	8.45	propionate +	0.9	4.2
	8.45	butyrate	1.8	6.2
	8.45		3.9	23
C	2.0	propionate	0.45	9.1
	2.0		0.9	10.7
	2.0		1.8	11.6
	2.0		2.75	12.9

(1) The experiments were performed as described in para. 3.2.1.
(2) Digested sewage sludge types A, B, and C refer to codes "H", "F1", and "E1" respectively, of chapter 4, table 1.
(3) Approximately 75 % of the COD of beetsugar campaign wastewater fed to sludge type A consisted of acetate and propionate. The VFA mixture fed to sludge type B was composed of acetate, propionate, and butyrate in a molar ratio of 1 : 0.8 : 0.7

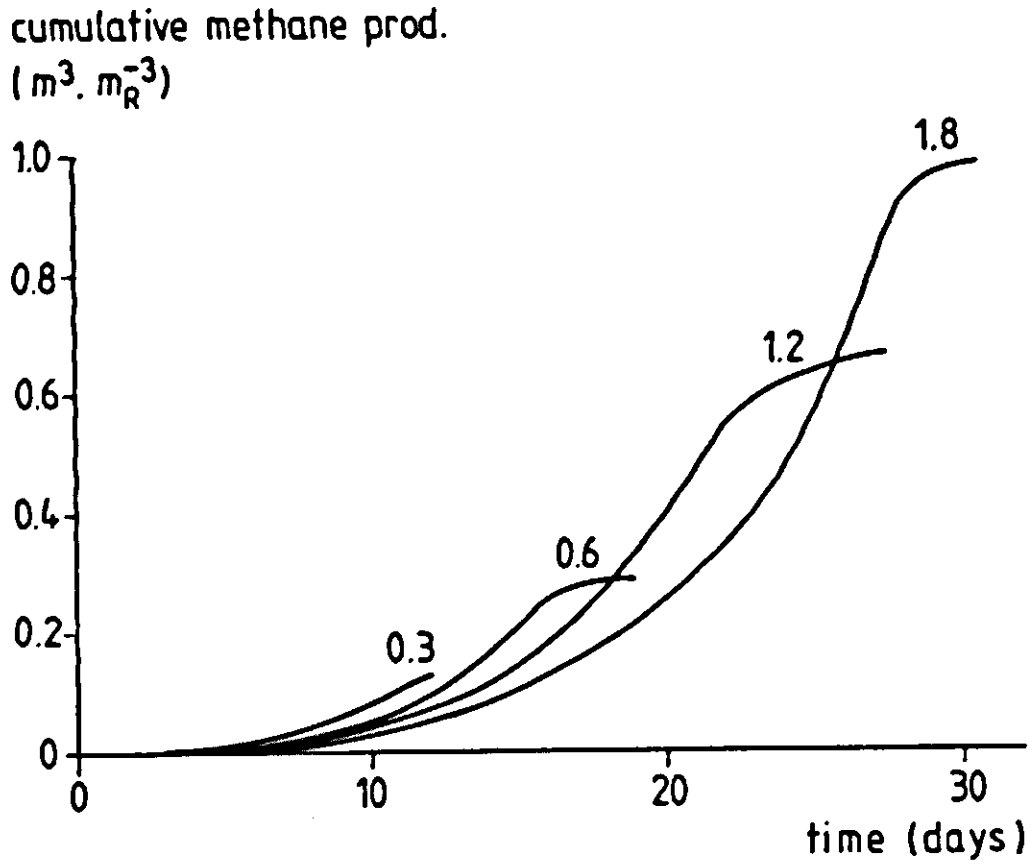


FIGURE 3
Cumulative methane production in the batch digestion of different initial propionate concentrations; 0.3, 0.6, 1.2 and 1.8 kg.m^{-3}.

In the third series yet another type of digested sewage sludge was fed different concentrations of Na-propionate. Here the lag phases showed a significant increase from 9.1 to 12.9 days, when increasing the propionate concentration from 0.45 to 2.73 kgCOD.m^{-3} (figure 3 and table 5, type C). The pH_o in this, as well as in the first series of experiments was 7.0.

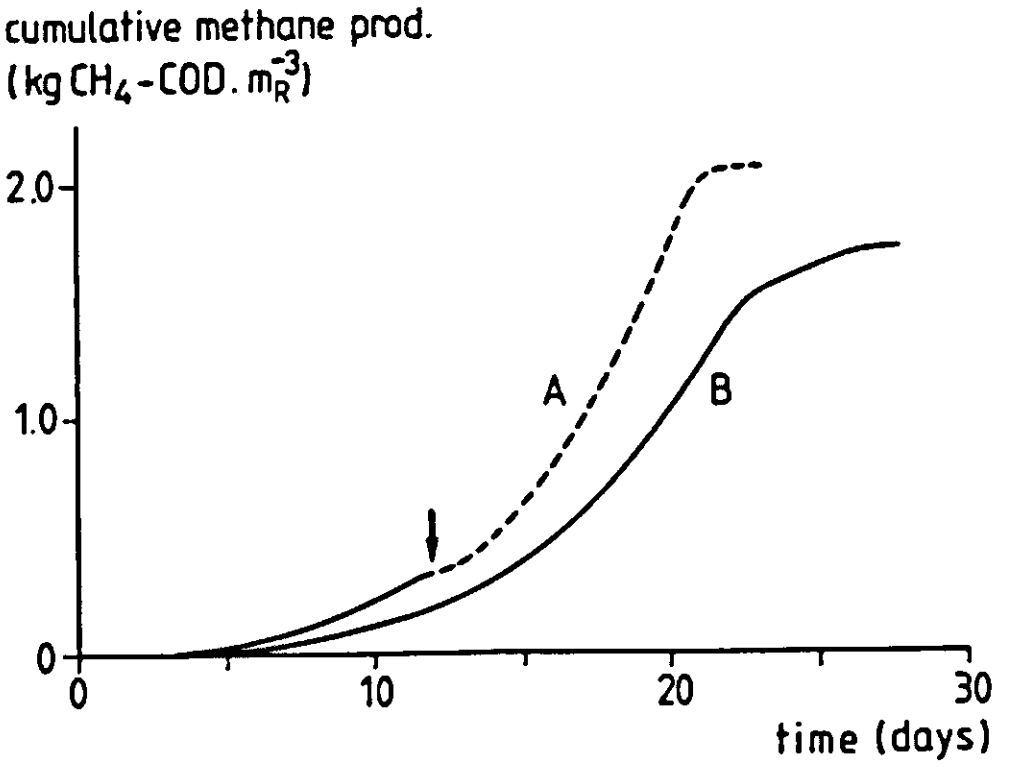


FIGURE 4
Effect upon the gas production lag phase of feeding a batch reactor with 1.2 kg.m^{-3} propionate (B) or 0.3 plus 1.2 kg.m^{-3} propionate (A). At the arrow the 0.3 kg.m^{-3} feed is fully digested and the supplementary 1.2 kg.m^{-3} feed is administered.

Once active gas production had started, an additional feed was digested virtually without any lag phase. This was shown in the batch reactor fed with 0.3 kg.m^{-3} propionate. At day 12 (figure 4) 1.2 kg.m^{-3} propionate was

added, which was digested readily within 10 days. The digestion time of propionate in the reactor fed at the start with 1.2 $kg.m^{-3}$ was about 25 days.

The virtually complete disappearance of a gas production lag phase is a common phenomenon in batch reactors, when fed a second time immediately after complete digestion of the first feed (table 6).

TABLE 6 LAG PHASES IN BATCH REACTORS PRECEDING THE DIGESTION OF THE FIRST AND THE SECOND FEED

sludge type (2)	lag phase (days) (1)	
	first feed	second feed
G	2.3	0.5
B	2.9	0.25
I	4.2	1.0
J	10.2	0.7

(1) Standard sludge activity test; see para. 3.2.1.
(2) Codes refer to table 1 of chapter 4.

5.3.4 SOME OBSERVATIONS ON THE INFLUENCE OF ACETATE AND BUTYRATE UPON THE LAG PHASE OF PROPIONATE BREAKDOWN

Apart from the methane production itself, also the breakdown of one of the constituents in the feed mixture can exhibit a lag phase. This lag phase may be caused by the absence of an adapted bacterial population or by temporarily unfavourable environmental conditions.

TABLE 7 INFLUENCE OF ACETATE AND BUTYRATE UPON THE LAG PHASE OF PROPIONATE BREAKDOWN IN BATCH REACTORS

exp. (1)	initial substrate concentration ($kg.m^{-3}$)			lag of propionate breakdown (days) (2)	
	propionate	acetate	butyrate	intermittently stirred reactor	unstirred reactor
A	1.2	0	1.5	16.5	15.2
B	1.2	1.5	0	11.0	9.9
C	1.2	0	0	6.6	5.6

(1) Sludge type "E1" was used at a concentration of 1.9 $kgVSS.m^{-3}$. Experiments were performed as described in paragraph 3.2.1. Stirring modes are defined in Table 3.
(2) Defined as the time required for the breakdown of 0.125 $kg.m^{-3}$ of propionate.

As the breakdown of propionate is often retarded during the start-up of anaerobic reactors, some additional experiments were set up to investigate the influence of acetate and butyrate upon the start of propionate digestion (A, B and C in Figure 5).

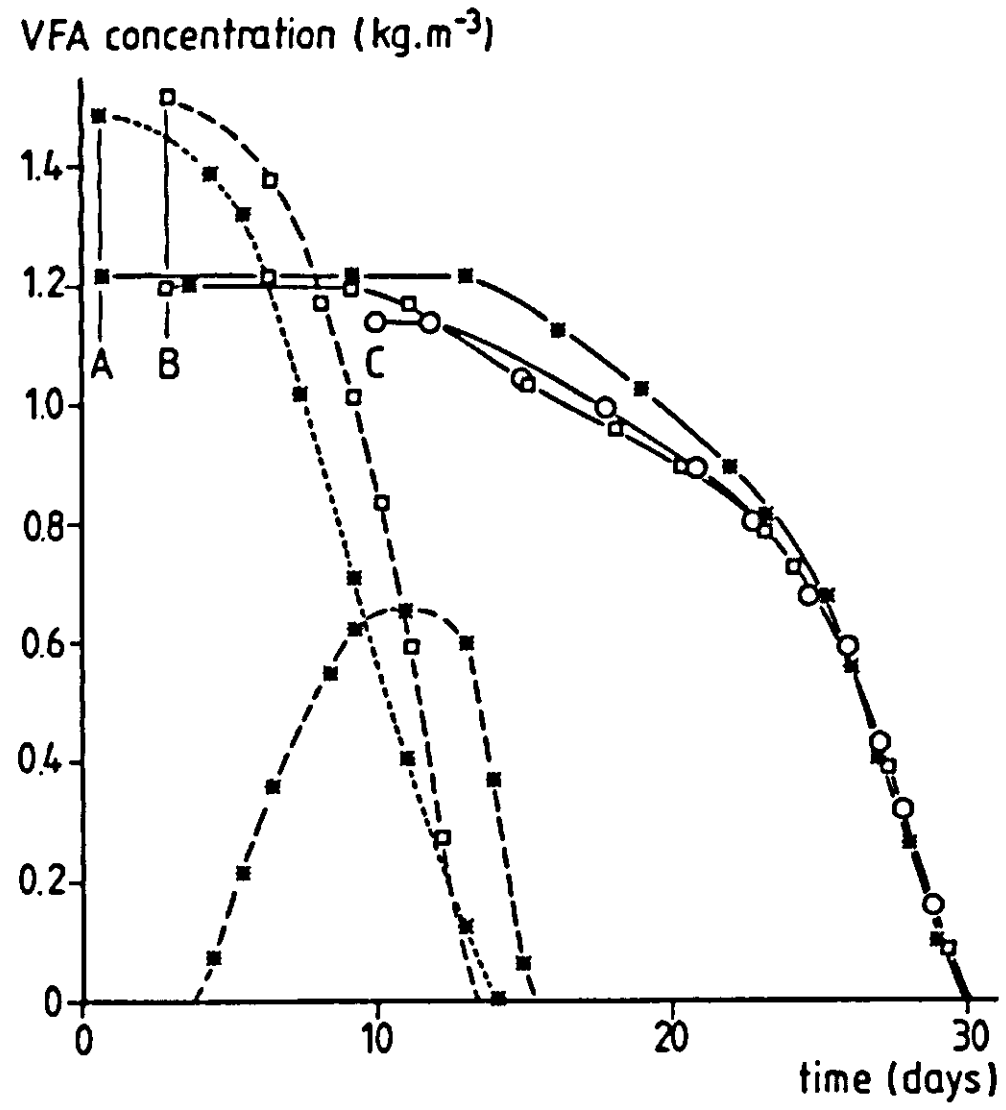


FIGURE 5 Influence of acetate and n-buryrate upon the lag phase of propionate breakdown in batch-fed reactors.
(---): acetate concentration,
(-·-·-): butyrate concentration,
(——): propionate concentration,
(*): experiment A; started on day 0.6 (butyrate added),
(□): experiment B; started on day 2.9 (acetate added), and
(o): experiment C; started on day 10.0 (propionate only).

In Figure 5 the plots of the VFA-concentration versus time have been shifted along the time axis in such a way, that the last part of the propionate concentration versus time curve coincides for these 3 experiments. Thus the differences in the length of the lag phase of propionate breakdown are clearly illustrated. Lag phase data are assembled in Table 7.

One of the reasons, that the lag phase of propionate breakdown was about 50 % longer in the presence of 17 mM (1.5 $kg.m^{-3}$) butyrate, than in the presence of 25 mM (1.5 $kg.m^{-3}$) acetate, may have been, that acetate accumulated during butyrate digestion (see Figure 5). As a result of

acetate accumulation the non-propionate VFA-concentration increased from 17 to 20 mM at day 7 (in the intermittently stirred experiments) accompanied by a pH-drop from 6.9 to 6.65. In experiment A (butyrate added) it took 4.4 days more than in experiment B (acetate added) to decrease the non-propionate VFA-concentration to 10 mM.

5.3.5 EFFECT OF OTHER ENVIRONMENTAL FACTORS

5.3.5.1 Effect of the pH

Some VFA-fed batch experiments, performed with different digested sewage sludge samples, were made to assess the influence of the pH immediately after feeding (i.e. pH_o) upon the length of the gas production lag phase (Table 8).

TABLE 8 EFFECT OF pH_o UPON THE GAS PRODUCTION LAG PHASE IN BATCH FED REACTORS

exp. (1)	feed ($kg.m^{-3}$)			sludge conc. ($kgVSS.m^{-3}$)	pH_o	lag (days)
	acetate	propionate	butyrate			
A	0.6	0.6	0.6	2.7	7.0	10.2
				5.3	6.2	15.4
				2.5	6.0	17.7
B	1.5	1.2	-	1.9	7.0	4.3
				1.8	6.15	7.5
C	-	1.2	-	1.9	7.0	9.9
				1.9	6.1	12.3

(1) The experiments were performed as described in paragraph 3.2.1. Digested sewage sludge types used in experiments A, B and C were "F3", "E1" and "E1" respectively.

Significantly longer gas production lag phases were measured at pH_o 6.0 - 6.2, than at pH_o 7.0 in all 3 experiments listed in Table 8.

In the batch reactor of experiment A with a sludge concentration of 5.3 $kgVSS.m^{-3}$ a redox potential of less than -250 mV was established in only 2.2 days. The pH_o in this experiment was 6.2. The results plotted in Figure 6 show, that an E_h low enough to permit methane production did not suffice to start the methanogenesis. This suggests an effect of the low initial pH upon the gas production lag phase (see also next paragraph).

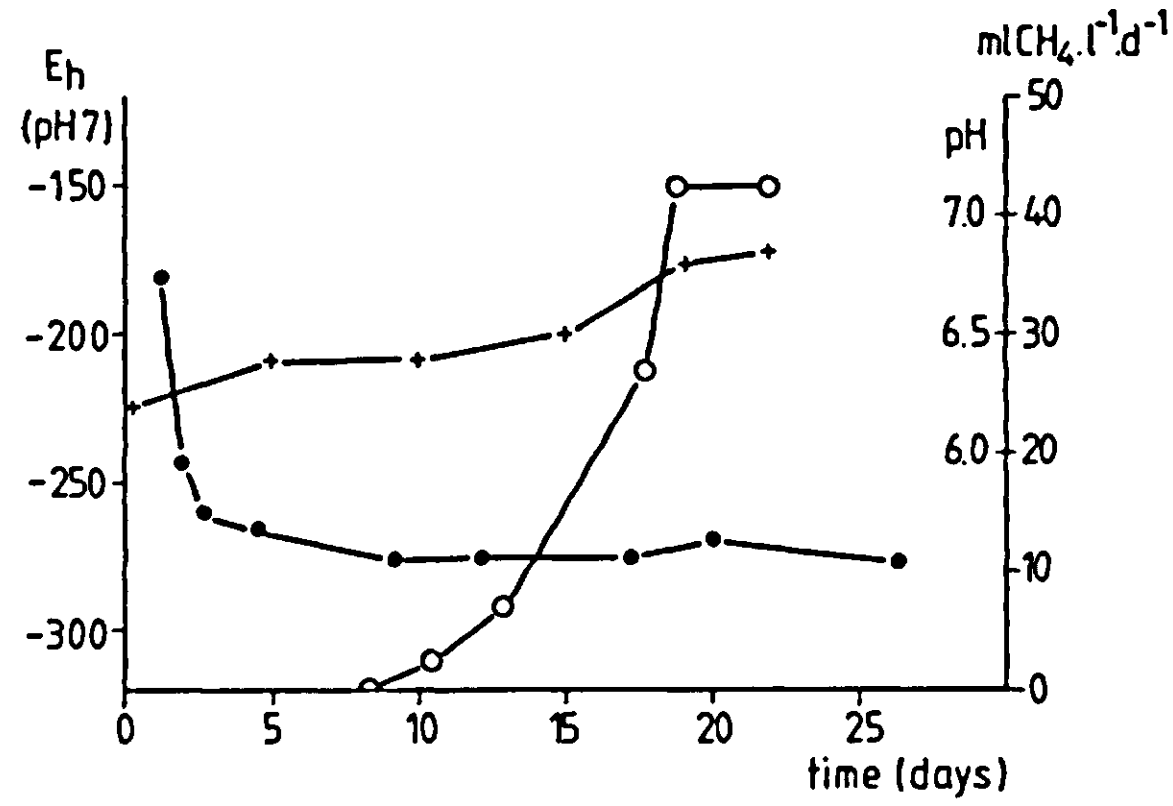


FIGURE 6 Redox potential E_h (•), pH (+) and methane production rate (o) during a standard sludge activity test with an initial pH of 6.2.

5.3.5.2 Effect of the redox potential

Redox potential measurements in a large number of batch experiments showed an E_h (pH 7.0) of -260 to -280 mV once a constant reading was obtained. This is in good agreement with literature data (Dirasian 1968, Borchardt 1971, Blanc and Molof 1973).

The time required for equilibration of the redox electrode in digested sewage sludge mixed liquors with a sludge concentration of 2 to 5 kgVSS.m^{-3} was found to be in the order of 1 to 2 days. Blanc and Molof (1973) reported similar equilibration times of 10 to 48 hrs in digesting sewage sludge. Hence, in experiments with digested sewage sludge types exhibiting a relatively short gas production lag phase no distinction can be made between the electrode equilibration time effect and a real decrease of the redox potential due to another cause. In Figure 6, with a fairly long lag period, the distinction can be made.

Another experiment in which E_h was measured and in which a relatively long lag phase occurred is shown in Figure 7. Both the redox potential E_h (pH 7.0) and the methane production rate in a standard VFA-fed batch activity test are plotted. The pH_o was 7.0. The digested sewage sludge used in this experiment had been stored unfed at 4 ^oC for about 6 months. The time required to reach an E_h (pH 7.0) of less than -250 mV was 4.5 days. The expected electrode equilibration time is only 2 days. Therefore these results do not exclude a relation between the duration of the lag phase and the relatively high redox potential during the first week.

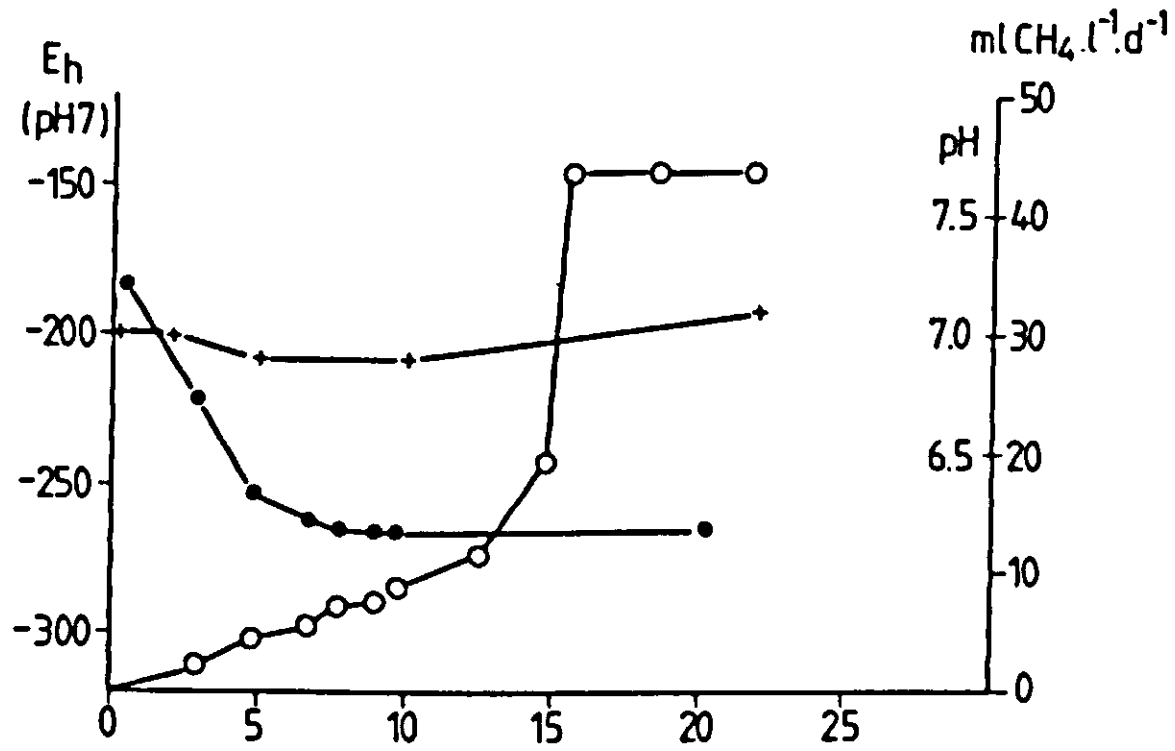


FIGURE 7 Redox potential E_h (•), pH (+) and methane production rate (o) during a standard sludge activity test (pH_o).

An artificial lowering of the E_h, e.g. by the addition of titanium(III)citrate (Zehnder and Wuhrmann 1976), has not been attempted.

Additional results relating redox potential measurements with a lag phase are reported in paragraph 5.3.5.1 and 5.3.5.3 (Figures 6 and 8).

5.3.5.3 Effect of ammonia, oxygen and sulphide

Potentially inhibitory compounds, which are most frequently encountered in anaerobic digestion, are oxygen, ammonia and sulphide.

TABLE 9 EFFECT OF AMMONIA UPON THE GAS PRODUCTION LAG PHASE IN BATCH REACTORS (1)

ammonia concentration ($kgNH_4^+-N.m^{-3}$)	lag phase (days)
0.74	1.3
1.21	3.4
2.36	26
3.52	34
4.99	48

(1) Calculated from Fig. 1 given by van Velsen (1979). Digested sewage sludge concentration: 6.8 $kgVS.m^{-3}$. Feed: VFA mixture.

Ammonia

The effect of ammonia upon the lag phase in batch experiments using digested sewage sludge has been examined by van Velsen (1979) (Table 9). According to his results a threshold concentration exists between 1.2 and 2.4 $kgNH_4^+-N.m^{-3}$, above which unadapted sludge exhibits very long lag phases. The natural background concentration in undiluted digested sewage sludge is about 0.8 $kgNH_4^+-N.m^{-3}$ (van Velsen 1979, own observation).

Oxygen

The influence of oxygen upon the start of methane production was investigated in two parallel standard VFA-fed sludge activity tests with digested sewage sludge. In batch reactor I oxygen contact was minimized by preflushing the medium in the reactor with nitrogen gas before adding the sludge. The redox potential E_h (pH 7.0) measured immediately after the addition of the sludge was lower than -100 mV (see Figure 8).

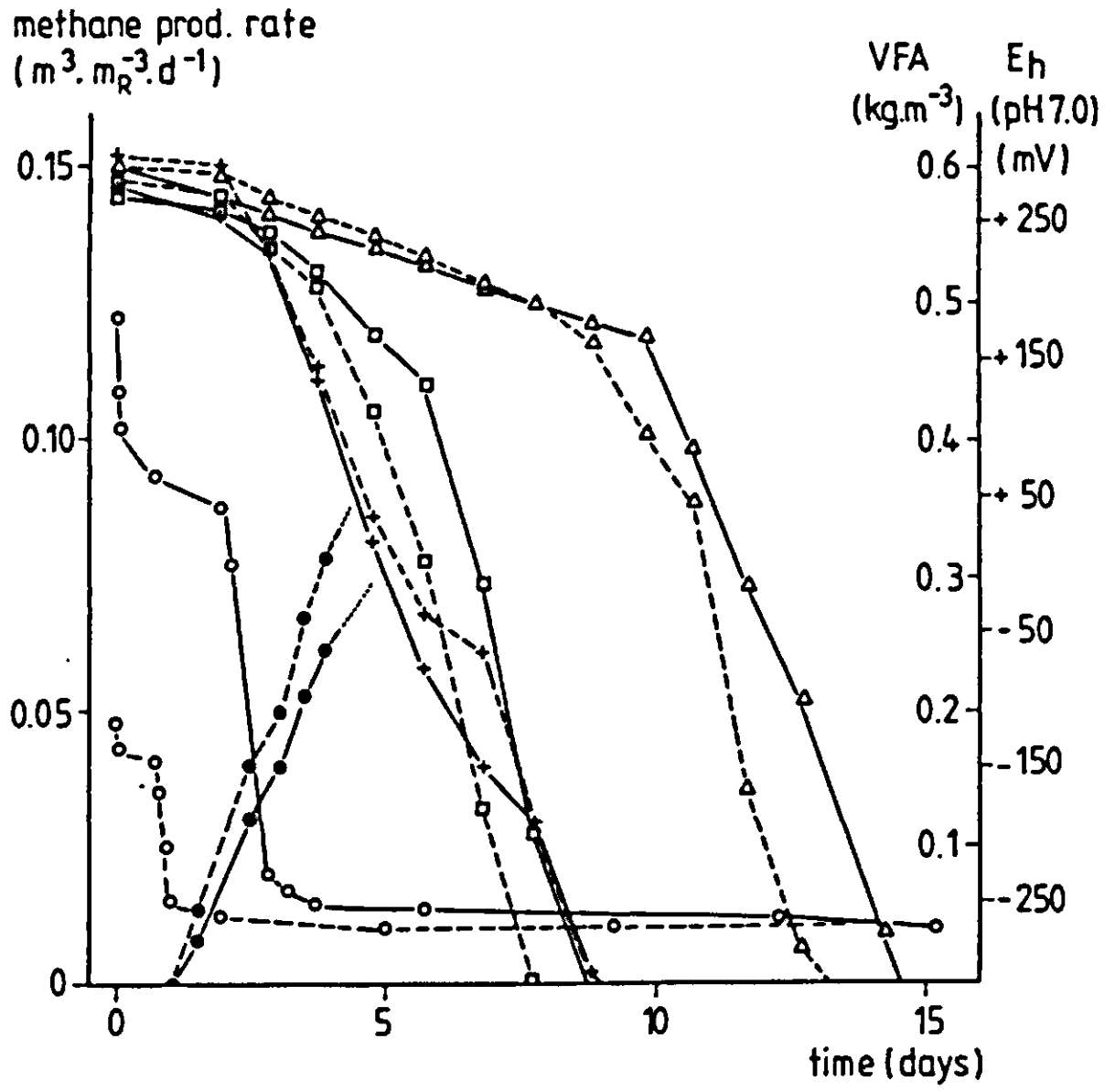


FIGURE 8 Comparison of standard batch sludge activity tests with (——) and without (---) oxygen contact during the first day.
(●): methane production rate (not shown beyond day 4),
(o): redox potential E_h, (+): acetate concentration,
(Δ): propionate concentration, (□): butyrate concentration.

In the parallel reactor II the reactor medium was saturated with air, resulting in a measured O_2-concentration of 5.1 mg/l immediately after the

consisted of nitrogen in both reactors. Results of oxygen measurements in reactor II are shown in Figure 9. The rapid decline of the oxygen concentration of the mixed liquor from 5.1 to 0.7 mg/l recorded during the first hour can be attributed to equilibration between the about 1 litre N_2 gas phase and the 5.5 litre liquid reactor volume. One to 2 % (v/v) O_2 was detected in the headspace after 24 hours. The oxygen concentration in the mixed liquor remained constant at 0.7 to 0.8 mg/l during the next 13 hours, but then dropped to zero in the period 14-24 hrs (Figure 9).

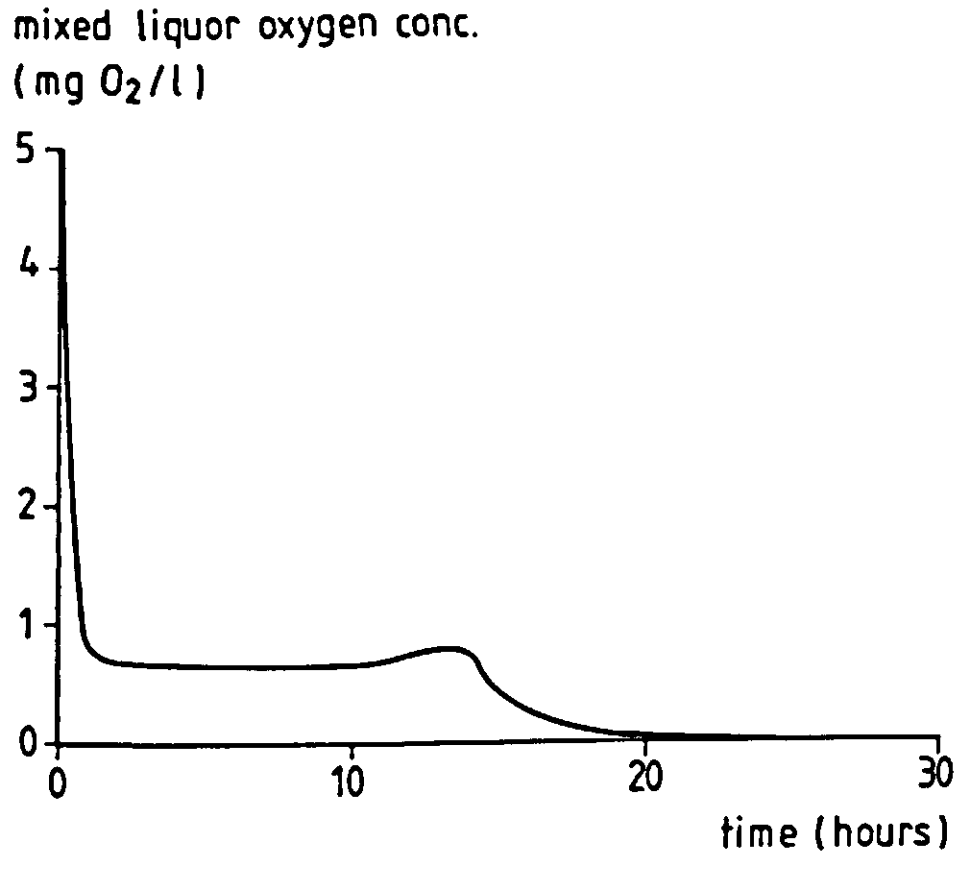


FIGURE 9
The oxygen concentration in the batch reactor of which the sludge was diluted with air saturated tap water (see Figure 8).

As shown in Figure 8 the redox potential in batch reactor II remained above zero till 52 hrs after the start of the experiment. In reactor I the E_h (pH 7.0) was about -140 mV during the first 18 hrs, and then dropped rapidly to -250 mV after 24 hrs, at which time methanogenesis started. Surprisingly the methane production in reactor II started simultaneously, despite the still prevailing high E_h. The only difference in performance between the 2 reactors was the slightly lower rate of gas production in the oxygen treated reactor. Eventually around day 8 the same maximum specific gas production rate of 0.2 $kgCH_4\text{-}COD.kgVSS^{-1}.d^{-1}$ was observed in both experiments. Shortly after the beginning of the gas production the redox potential in the oxygen contacted reactor also decreased rapidly to -250 mV (pH 7.0) (Figure 8).

Figure 10 shows the results of an additional set of experiments conducted with 2.6 $kgVSS.m^{-3}$ of digested sewage sludge (code "B"). In this experiment one batch reactor was exposed to air oxygen (sparged during 3 minutes at a rate of about 1.0 $m^3$of $air.m^{-3}.min^{-1}$). This was done during digestion of a second standard VFA feed and resulted in a measured O_2-concentration in the

O_2-concentration in the suspension dropped to zero within 3 minutes, indicating that the oxygen removal rate of the system exceeded the supply of oxygen from the headspace above the mixed liquor. As a result of the aeration methanogenesis dropped to almost zero within 6 hrs. After flushing the gas phase with nitrogen gas the methane production recovered within approximately 12 hrs.

In order to assess the influence of oxygen in the gas phase above the mixed liquor, the oxygen-free biogas was replaced by air. Once again the methane production rate dropped to almost zero, but the system recovered spontaneously within 1 day.

Taking into account all manipulations with the gas phase, the COD-balances yielded a difference between COD input and biogas-COD production, which was far too large to be explained by anaerobic sludge-growth, but which could be attributed to aerobic conversions.

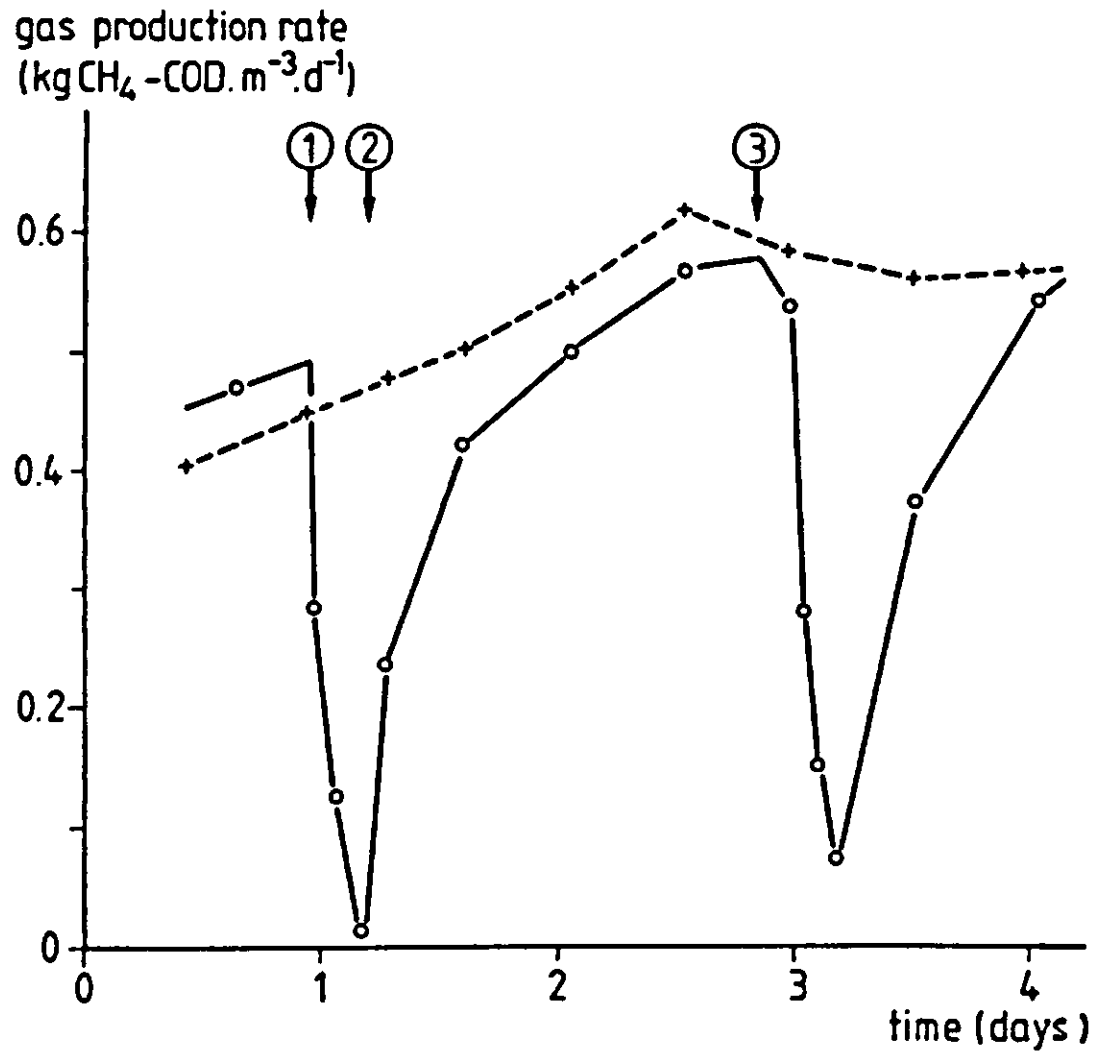


FIGURE 10 Effect of air contact upon the methane production rate in batch reactors.
(+---+): untreated reactor, (o——o): treated reactor,
(1): mixed liquor sparged with air for 3 min,
(2): headspace flushed with nitrogen gas,
(3): headspace flushed with air.

Sulphide

The influence of sulphate and sulphide upon the gas production lag phase was examined in VFA-fed batch reactors (Figure 11 and 12, and Table 10).

TABLE 10 INFLUENCE OF SULPHIDE SUPPLIED AS POTASSIUM SULPHIDE, OR SULPHIDE PRODUCED FROM THE REDUCTION OF SULPHATE UPON THE GAS PRODUCTION LAG PHASE IN BATCH REACTORS

exp. (1)	concentration (in mM) of			length of lag phase
	sulphate	sulphide (2)		
	at t=0	at t=0	at t=7 (d)	(days)
A	0	0	0	4.5
B	2.6	0	1.6	4.9
C	5.2	0	3.3	4.6
D	0	2.8	2.15	34
E	2.6	2.8	3.9	39

(1) Experiments were performed as described in para. 3.2.1. Sludge type: "F4". Feed: neutralized mixture of 0.5 kg.m^{-3} acetate and propionate. The pH_o was 7.0. During the digestion it increased to 7.4 ± 0.1.
(2) The value given for t = 0 days is the added concentration; the value at t = 7 days has been measured.

The results in Figure 11 show, that the addition of 2.6 or 5.2 mM sulphate (0.25 and 0.5 kgSO_4^{2-}.m^{-3}) hardly affected the gas production lag phase (Table 10, exp. A,B and C). Sulphate reduction and methanogenesis started simultaneously after 2 days. The results also show that propionate was used

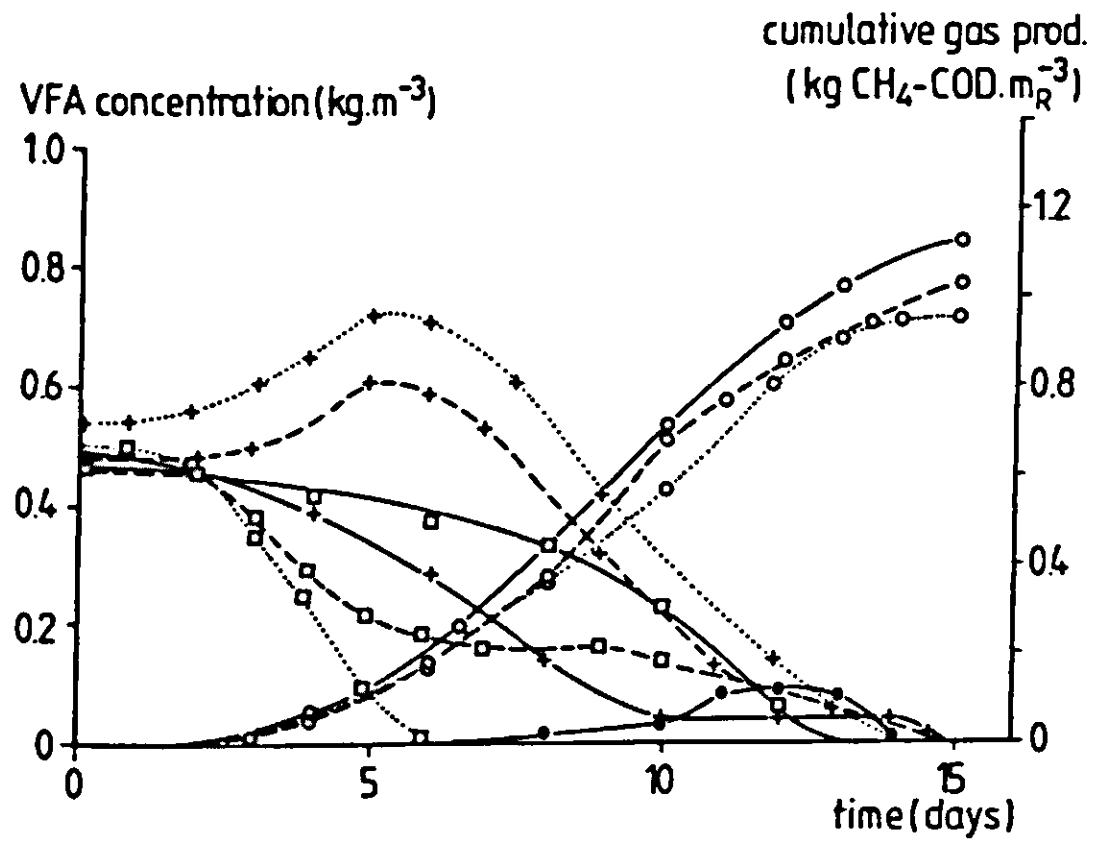


FIGURE 11 Batch digestion of acetate and propionate in the presence of sulphate.
(——): 0.0 mM, (---): 2.6 mM and (·····): 5.2 mM.
(o): methane production, (+): acetate concentration,
(□): propionate concentration, (●): butyrate concentration.

as hydrogen donor for sulphate reduction, and that some acetate accumulation took place.

Contrary to sulphate addition, the direct addition of 2.8 mM of potassium sulphide with the VFA-feed caused a considerable extension of the lag phase to 34 days (Figure 12 and Table 10, exp. D).

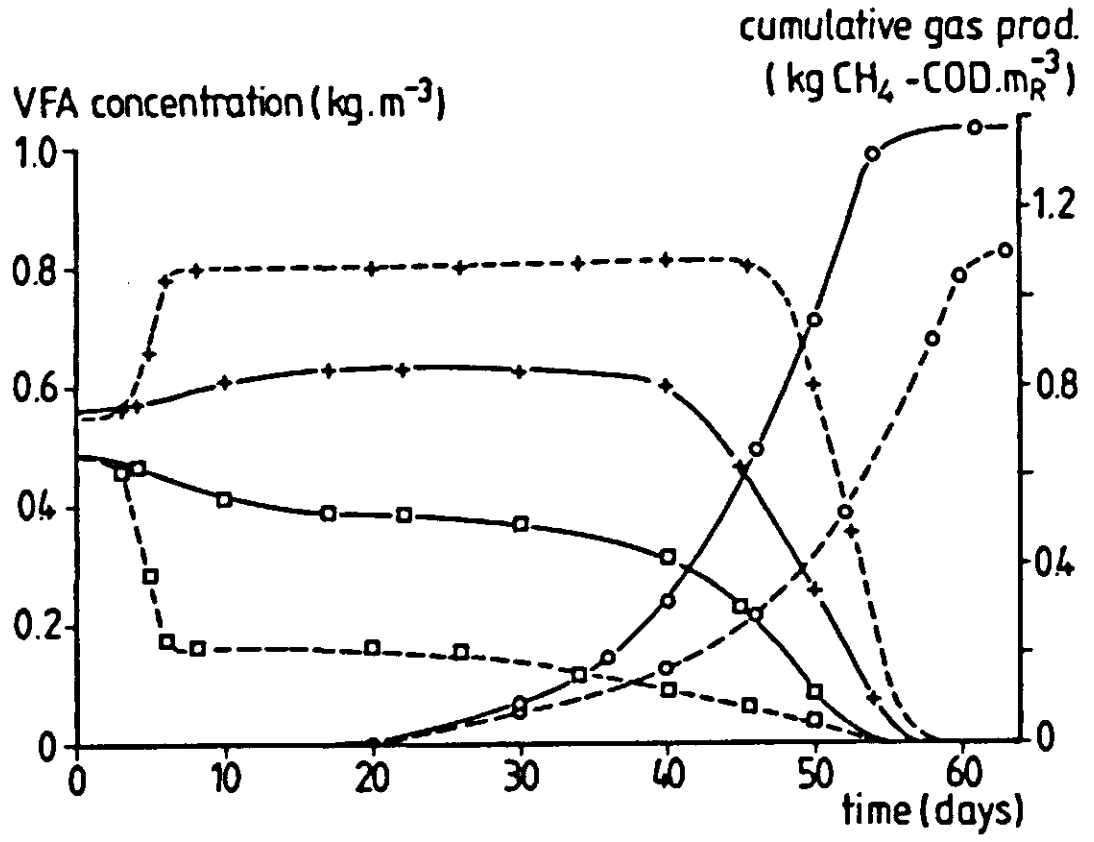


FIGURE 12 Batch digestion of acetate and propionate in the presence of 2.8 mM sulphide (——) and 2.8 mM sulphide plus 2.6 mM sulphate (---).
(o): methane production, (+): acetate concentration,
(□): propionate concentration.

The presence of an additional amount of 2.6 mM of sulphate together with 2.8 mM of sulphide even further increased the length of the lag phase to 39 days (Figure 12 and Table 10, exp. E).
In both experiment D and E a small pH-drop from 7.0 to 6.7 and 6.9 respectively was recorded at day 11. This may have aggravated the sulphide inhibition somewhat.
Once again (in experiment E) sulphate reduction started already after 2 days, as indicated by the propionate breakdown. An equivalent amount of acetate accumulated. The sulphate reduction was completed before methane production started.

5.4 DISCUSSION

Inoculum-size and the reactivation of the cellular systems

The gas production lag phase in VFA-fed batch experiments with digested sewage sludge was shown to be inoculum-size dependent (Figure 1). Above a viable biomass concentration corresponding with a mixed liquor methanogenic activity of 0.5 $kgCH_4$-COD.m^{-3}.day^{-1} (initial COD: 2.65 kg.m^{-3}) no lag phase occurs, while at lower methanogenic activities the lag phase rapidly increases (Figure 1). Accordingly an increase in the biomass concentration (or the methanogenic activity) of the system by adding small increments of a highly active VFA-cultivated anaerobic sludge to the digested sewage sludge sample, substantially shortens the gas production lag phase (Table 1). These results indicate, that the bacterial population is actively involved in providing the proper environmental conditions for methanogenesis; probably by producing (a compound with) a sufficiently low redox potential, as suggested by Borchardt (1971) and Blanc and Molof (1973). The source of low potential reducing equivalents needed to activate various catalysts of the cell, which must be reduced before they become functional, must have been the VFA used as substrate in these experiments. Stadtman (1967) reported the use of pyruvate as possible source of reducing equivalents to shorten the lag phase. However, this could not be confirmed in an additional experiment in which 1.0 mM Na-pyruvate was added to the VFA-feed.

Of the three volatile fatty acids tested, under comparable conditions, acetate shows the shortest gas production lag phase, and propionate the longest, i.e. about twice as long as butyrate (Table 3). Obviously acetate is most suited to overcome the lag phase and to start methanogenesis. Balba and Evans (1977) reported a significant decrease in the lag phase of benzoate digestion by the inclusion of acetate in the initial medium.

The assumption that part of the substrate may be required to restore the exhausted metabolic system of the cells and/or to invest in providing an environment that allows for methanogenesis, is supported by the high fraction of substrate-COD not being converted into methane during the digestion of the first batch feed (Table 11). Normal COD yield factors (Y_{COD}) reported for VFA substrates are in the order of 4 %. Such figures are found during digestion of the second batch feed. However, during

digestion of the first batch feed Y_{COD} is about 2.5 times higher (Table 11).

TABLE 11 PERCENTAGE OF SUBSTRATE-COD NOT RECOVERED AS CH_4-COD IN THE FIRST AND THE SECOND VFA BATCH FEED (1)

exp. (2)	first feed		second feed	
	gas production lag phase (days)	Y_{COD} (%)	gas production lag phase (days)	Y_{COD} (%)
A	1.7	9.8	0.1	4.4
B	1.8	8.3	0.1	3.2
C	4.8	14.0	0.3	5.4

(1) The experiments were performed as described in para. 3.2.1.
(2) A: average of 5 experiments with digested sewage sludge samples of type "E1, E2, E3, E4, E5". B and C: average of experiments with digested sewage sludge types "F5, G, I, J" and "A, B, C, E3" respectively.

Long periods of unfed storage of digested sewage sludge lengthen the gas production lag phase. As the maximum specific sludge activity remains more or less unaffected, this may also be explained in terms of time required for restoring the exhausted metabolic system of the cells .

pH_o, Initial substrate concentration, ammonia and sulphide

A suboptimal pH causes a longer lag phase (Table 8), probably because of the energy that has to be invested in restoring and maintaining an optimal interior cell pH different from the one in the bulk solution. No attempts were made to differentiate between the direct influence of a low pH and the indirect effect of a higher concentration of unionised VFA at low pH values. The total concentration of unionised VFA at pH 6.0-6.2 in the present experiments (Table 8) ranged from 0.9 to 1.8 mM, and may very well have been inhibitory to unadapted sludge (Duarte and Anderson 1982).

Longer lag phases are also caused by higher initial VFA-concentrations (Table 5), as was also reported by others (Nikitin 1968, Hobson and Shaw 1976). Again the higher concentration of unionised VFA - although less than 0.2 mM at pH 7.0 in the present experiments - may have caused the longer lag phase.

The effect of ammonia upon the length of the lag phase of unadapted sludge may be explained by the fact that NH_3 can readily enter into the cells and interfere with internal pH gradients by its equilibration with NH_4^+.

and Plahl-Wabnegg (1983) report a 45 % inhibition of the acetoclastic methanogenesis at a free H_2S concentration of 1.5 mM. At pH 7.0 this corresponds to 3 mM sulphide. In the present work the exposure of resting cells at pH 7.0 to a dissolved sulphide concentration of 3mM resulted in a considerable extension of the lag phase (Table 12). However, when the slow build-up - as a result of sulphate reduction - of the same sulphide concentration coincides with the onset of methanogenesis, the already active cells are hardly affected.

According to the literature the optimal dissolved sulphide concentration for methanogens is 0.05-0.1 mM (Zehnder and Wuhrmann 1977, Scherer and Sahm 1981).

Mixing intensity, redox potential and oxygen

Redox potential measurements in the bulk solution indicate, that low methane production rates coincide with the decrease of the redox potential to its optimal low value (Figures 7 and 8).

This was also observed by Nikitin (1968) and may be explained by the existence of pockets or micro-environments in the sludge mixed liquor (sludge flocs, bacteria attached to particles) with a local methanogenic activity higher than the average of the bulk. A low redox potential will be attained easier there and methane production will start in these pockets, thus creating the right conditions for methanogenesis in the rest of the mixed liquor.

Stirring in the reactor will interfere with this process by equalizing the environmental conditions throughout the digester. At a higher mixing intensity the size and number of these pockets will be smaller and more time is needed to attain the maximum gas production rate (Table 3).

The importance of micro-environments in the sludge may apply particularly to the breakdown of propionate, which (in the absence of sulphate) is degraded by a syntrophic consortium of acetogenic and methanogenic bacteria. This reaction is easily inhibited by high concentrations of the end products hydrogen and acetate (see Chapter 2). Stirring may upset an effective interspecies hydrogen transfer. This will then result in a higher hydrogen concentration and inhibition of the propionate breakdown. A similar inhibitory effect from stirring was observed in benzoate digestion (Ferry and Wolfe 1976).

Micro-environments in the sludge may also play a role in reducing the effect of oxygen on methanogens. Literature data on oxygen toxicity to methanogens indicate that short term exposure to air leads to a reversible loss of activity, and that the gas production rate recovers to close to its original level within several hours to two days after the removal of the oxygen (Bhuwapathanapun and Earle 1975, van den Berg et al. 1974, Zehnder and Wuhrmann 1977). Resting cells are reported to be more sensitive to oxygen inhibition than active cells (Roberton and Wolfe 1970). The present experiments confirm that the methanogenic activity of diluted digested sewage sludge is not affected significantly by short term, intensive oxygen contact and that the methane production fully recovers within about one day (Figures 8, 9 and 10). Aerobic or facultative aerobic bacteria, which are always present in small numbers in digested sewage sludge (Kotzé et al. 1968), presumably play an important role in restoring anaerobic conditions (Pirt 1975).

Implications for UASB reactor start-up

From the results presented in this chapter a number of conclusions can be derived with respect to UASB reactor start-up:

a. At an initial seed sludge concentration corresponding to a methanogenic activity of less than 0.5 $kgCH_4$-$COD.m^{-3}.d^{-1}$ a gas production lag phase can be expected.
b. Stirring the reactor contents significantly prolongs the lag phase. If stirring is necessary to ensure sufficient contact between substrate and biomass it should be done intermittently and at a low intensity.
c. A suboptimal initial pH prolongs the lag phase.
d. Above 2 $kgCOD.m^{-3}$ a negative effect of the initial VFA concentration upon the lag phase can be expected. This effect also depends on the inoculum size and the pH_o.
e. An initial sulphide concentration in excess of 2 mM causes along lag phase to resting cells.
f. The need to minimize air exposure (Clausen et al. 1981) does not exist. Oxygen contact that may occur during transport or handling of digested sewage sludge has no significant adverse effect upon the gas production lag phase or the specific sludge activity.

CHAPTER 6 SLUDGE GROWTH AND SPECIFIC SLUDGE ACTIVITY

6.1 INTRODUCTION

In the ideal case of complete biomass retention the length of the start-up period of an UASB reactor seeded with digested sewage sludge is predominantly controlled by the maximum sludge growth rate.
Well mixed batch fed digesters with a complete sludge retention can be used advantageously for the assessment of the sludge growth yield factor Y and the specific biomass methanogenic activity V for different substrates.
The growth rate μ can then be calculated as: $\mu = V.Y$

In this chapter the results are presented of batch experiments with VFA and methanol as substrate. The objective of these experiments was to determine the sludge growth yield factor under different circumstances, the specific methanogenic activity of newly formed sludge-VSS, and the sludge decay rate.

When comparing the values of the specific methanogenic activity obtained in batch fed reactors with those obtained from continuously fed (UASB) reactors, one should keep the following differences in mind:

a. A spatial substrate gradient occurs in a continuously fed upflow reactor in the lower part of the sludge bed. The steepness of the gradient depends mainly upon the mixing brought about by the gas production, the influent substrate concentration and the imposed loading rate. As a result of the mixing, sludge particles move up and down the sludge bed, although heavy particles do so to a lesser extent than lighter ones.
   The substrate concentration in a mixed batch fed reactor varies in time instead of in space. However, in both systems the biomass as a whole is alternatingly exposed to high and low substrate concentrations; albeit on different time scales. For this reason sludge growth yields as determined in a mixed reactor from a number of consecutive batch feedings are assumed to be comparable to growth yields obtained in a well functioning upflow reactor.
b. In upflow reactors an effective sludge retention is pursued through a selection process. Well settling sludge particles are retained in (the lower part of) the reactor and therefore are exposed to maximum growth

conditions, while less well settling sludge particles and dispersed bacterial matter concentrates at the top or is washed out from the system. In batch fed reactors with complete sludge retention all forms of biomass present are preserved and exposed to identical growth conditions.
Hence, different bacterial populations may proliferate in batch and upflow reactors. In the case of the digestion of VFA mixtures, it is assumed that no significant differences occur.
Microscopic observations confirm, that the most important population in the digestion of VFA, i.e. the acetate-methanogens, is identical in both systems.

6.2 MATERIALS AND METHODS

Expertimental set-up

Batch experiments were performed as described in paragraph 3.2.1, unless stated otherwise.

Sludge

Sludge type codes mentioned refer to Table 1 of Chapter 4.

Analyses

-DSS and VSS determinations were performed according to Standard Methods.
-VFA analysis was conducted as described in paragraph 3.2.1.
-Methanol was determined gaschromatographically.
-Redox potential, sulphide and sulphate were measured as described in paragraph 5.2.

6.3 RESULTS

6.3.1 SLUDGE GROWTH YIELDS ON DIFFERENT SUBSTRATES

Sludge growth yield factors were determined in batch reactors from the difference in the VSS concentration before and after the digestion of a number of batch feeds. The results obtained with different simple substrates are shown in Table 1.

TABLE 1 SLUDGE GROWTH YIELD FACTORS FOR DIFFERENT SUBSTRATES AS DETERMINED IN BATCH FED REACTORS (1)

substrate	initial conc. of batch feed ($kgCOD.m^{-3}$)	number of feeds added	pH range	average yield factor ($gVSS.gCOD_{conv.}^{-1}$)
-acetate (2)	1.6	40	6.9-7.5	0.064
-acetate (3)	2.6	-	6.7-7.5	0.020
-methanol	4.8	16	7.25	0.042
-propionate	1.8	4	7.0-7.2	0.026
-n-butyrate	2.0	10	7.0-7.1	0.021
-standard VFA mixture (4)	2.6	20	6.2-7.5	0.024

(1) Experiments performed as described in para. 3.2.1.
(2) Acetate growth experiments are reported in paragraph 6.3.1.1.
(3) Derived from results given in Figure 1 and Table 3.
(4) Mixture of acetic, propionic and butyric acid: 0.6 $kg.m^{-3}$ each.

6.3.1.1 Sludge growth yield factor with acetate as single substrate

A biomass yield factor of 0.064 $gVSS.gCOD^{-1}$ (or 4.1 $gVSS.mole^{-1}$ of substrate) was found for acetate digestion. This is surprisingly high; i.e. even significantly higher than the yield factor found for methanol of 0.042 $gVSS.gCOD^{-1}$ (or 1.0 $gVSS.mole^{-1}$ of substrate) (Table 1).

Regarding the much lower standard free energy of acetate methanogenesis (-31 kJ) compared to that of methanogenesis from methanol (-312/4 = -78 kJ) (Thauer et al. 1977), the high growth yield factor for acetate digestion probably should be attributed partly to the formation of some - still unknown - reserve material or exopolymer instead of new active cells.

In order to check this idea, the NH_4^+-N concentration was measured at the start and at the end of the acetate growth yield experiments. The data in Table 2 demonstrate, that virtually no NH_4^+-N was incorporated in the newly formed sludge-VSS. The sludge growth constituted a 45 % increase in the original total amount of sludge-VSS present.

Moreover, in the acetate fed experiments no increase in the maximum observed methanogenic activity was noted (Table 2), indicating once again that little if any new active biomass had been formed. The slight decrease in the activity in the course of the acetate experiments is not significant, as the average activity for all 40 batch feeds was 1.64 $kgCH_4$-$COD.m^{-3}.d^{-1}$ with a standard deviation of 0.13.

TABLE 2 MAXIMUM OBSERVED METHANOGENIC ACTIVITY AND AMMONIA FIXATION DURING GROWTH YIELD EXPERIMENTS WITH ACETATE ADAPTED SLUDGE (1)

substrate	maximum observed methanogenic activity ($kgCH_4$-COD.m^{-3}.d^{-1})		NH_4^+-N (2)		VSS formed	N/VSS
	at start	at end	(g) at start	(g) at end	(g)	(%)
-acetate	1.67	1.52	0.465	0.320	5.75	2.5
-acetate	1.68	1.63	1.581	1.582	4.45	0.0
-butyrate	0.33	1.95	0.358	0.131	2.15	10.6

(1) Each experiment consisted of 10 successive batch feeds of 1.5 $kg.m^{-3}$ of acetate or 1.1 $kg.m^{-3}$ of n-butyrate. Data shown for acetate digestion are averages of 2 experiments.
(2) With yeast extract 0.25 gN-Kjeldahl was added. Based on results of yeast extract digestion experiments it was assumed in the calculation that 80 % of this N either was incorporated in cell material or made available as NH_4^+-N.

In order to enable a comparison between acetate digestion and the digestion of other VFA, Table 2 also includes data of an identical experiment using n-butyrate as substrate. The experimental conditions with respect to mode of feeding, pH, nutrients (including yeast extract), and sludge type used were exactly the same as in the acetate digestion experiments. The yield factor found in case of n-butyrate as substrate was only 0.021 $gVSS.gCOD^{-1}$, and contrary to the acetate experiments a significant amount of NH_4^+-N was incorporated in the sludge-VSS and the methanogenic activity of the sludge increased during the experiment (Tabe 2).

The results mentioned above lead to the hypothesis, that at the conditions prevailing in these experiments growth of acetate digesting biomass only occurs in the presence of hydrogen that may be formed, for instance, from the breakdown of higher volatile fatty acids.

In an additional experiment, a batch reactor seeded with digested sewage sludge was given 4 successive standard VFA feeds. This resulted in an increase of the maximum observed methanogenic activity from 0.47 to 0.96 $kgCH_4$-COD.m^{-3}.d^{-1} (Figure 1). During the digestion of the next 3 standard VFA feeds (feed 5, 6 and 7 in Figure 1) an additional amount of acetic acid (up to a total of 130.4 gCOD) was added in small increments once or twice a day in order to maintain the acetate concentration in the reactor between

1.0 and 2.5 $kg.m^{-3}$ and so to enhance the growth of acetate degrading bacteria. The experiment was terminated with a normal standard VFA feed (number 8 in Figure 1).

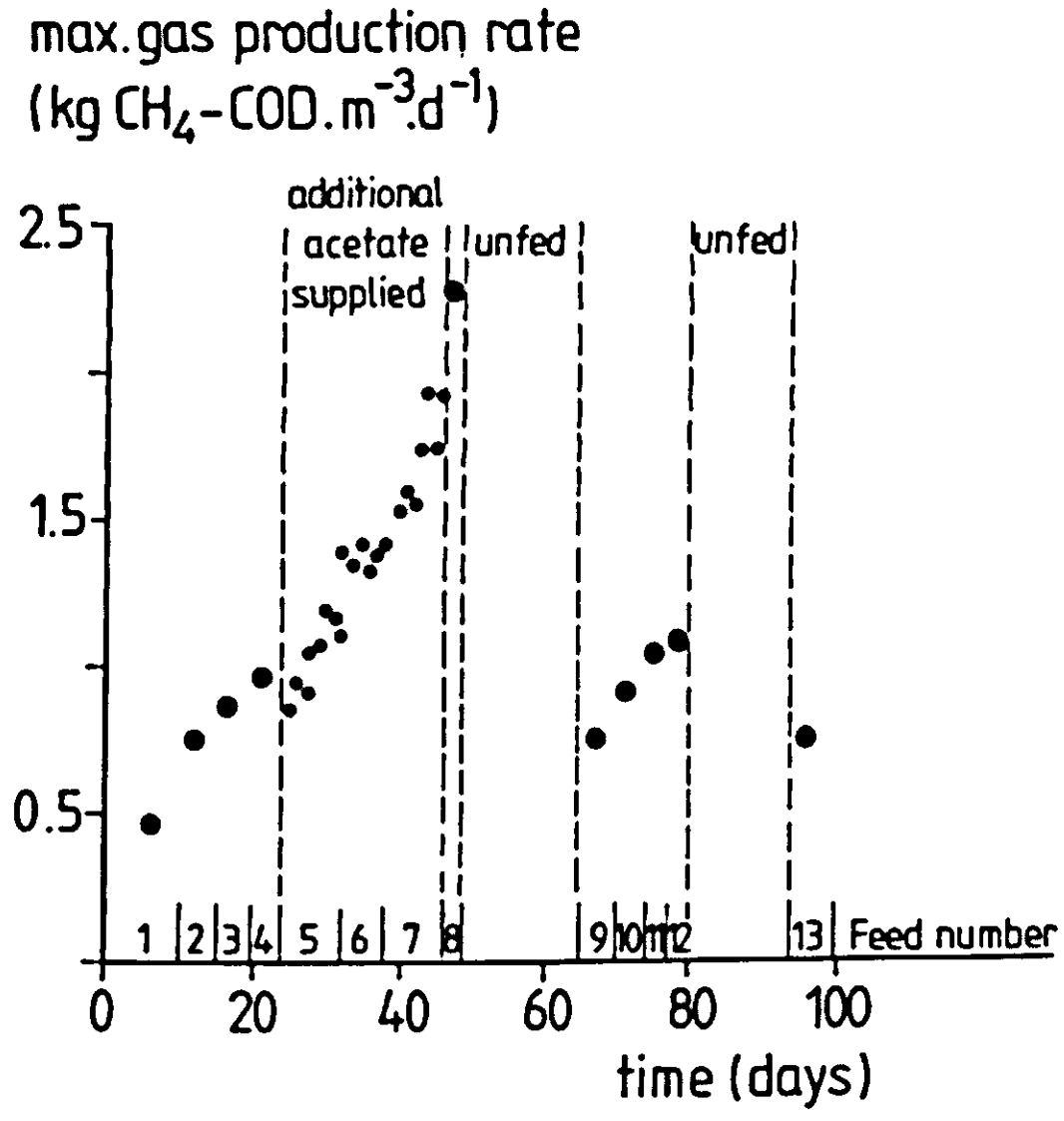


FIGURE 1 Maximum methane production rate during the digestion of successive batch feeds with the standard VFA mixture (●) in a reactor seeded with digested sewage sludge (type "E4"). During the digestion of feed number 5, 6 and 7 the acetate concentration was maintained between 2.5 and 1.0 $kg.m^{-3}$ by supplying daily (•) additional acetic acid to determine the growth yield factor. Between feed 8 and 9 and between feed 12 and 13 feeding was interrupted to determine the death and decay rate.

Figure 1 reveals a steep increase in the maximum methane production rate when feeding with extra acetate, i.e. from 0.85 to 1.92 $kgCH_4\text{-}COD.m^{-3}.d^{-1}$. In this period the sludge growth yield factor Y was 0.020 $gVSS.gCOD^{-1}$.
Of the total amount of 171 g COD converted into methane and sludge in these 3 feedings, 81.0 % was supplied in the form of acetate, 13.2 % in the form of acetate as an intermediate from propionate and butyrate breakdown, and only 5.8 % in the form of hydrogen resulting from propionate and butyrate breakdown. As a consequence practically all of the sludge growth in these feedings should be attributed to the growth of acetate methanogens. This is also clearly reflected in the observed maximum conversion rates of acetate, propionate and butyrate as shown in Table 3.
A very significant increase in the conversion rate of acetate occurred during the feeds with supplementary acetate, whereas the conversion rates

the acetate addition period. In the presence of high acetate concentrations the propionate and butyrate conversion rates dropped to about 50 % of there original level (Table 3).

TABLE 3 MAXIMUM OBSERVED CONVERSION RATES OF ACETATE, PROPIONATE AND N-BUTYRATE IN THE DIGESTION OF STANDARD VFA FEEDS WITH AND WITHOUT THE ADDITION OF (EXTRA) ACETATE

feed number	acetate conc. range ($kg.m^{-3}$)	maximum observed conversion rate of: ($kg.m^{-3}.day^{-1}$)		
		acetate	propionate	butyrate
1	0.6 - 0	0.38	0.12	0.18
2	0.6 - 0	0.65	0.16	0.29
3	0.6 - 0	0.68	0.16	0.40
4	0.6 - 0	0.75	0.20	0.51
5(2)	0.6	0.71	0.19	0.61
5	2.3 - 1.0	1.03	0.11	0.28
6	2.3 - 1.0	1.25	0.12	0.32
7	2.5 - 1.0	1.71	0.11	0.29
8	0.6 - 0	1.82	0.23	0.58

(1) Experiments performed as described in para. 3.2.1. Sludge type used "E4".
(2) Before the addition of extra acetate.

6.3.1.2 Sulphur limited sludge growth

In batch fed reactors the methane production rate increases with each new feeding, provided the environmental conditions are favourable for growth, that no sludge is removed and that starvation is avoided.

In order to establish the influence of nutrient limitation upon the growth yield and the specific methanogenic sludge activity, the addition of a sulphur source was omitted in a batch reactor fed repeatedly (41 times) with the standard VFA-mixture. The digested sewage sludge used as seed will have contained some sulphide (not analysed). Yeast extract was not supplied. Figure 2 shows the maximum methane production rate observed in the digestion of the 16^{th} to the 41^{st} feed. No further increase occurred beyond the 18^{th} feeding (day 79). The gas production rate remained in the range 1.3 to 1.5 $kgCH_4\text{-}COD.m^{-3}.d^{-1}$ during the next 17 standard VFA feedings (see Figure 2). In the absence of a sulphur deficiency a rise to at least 3 $kgCH_4\text{-}COD.m^{-3}.d^{-1}$ would have been expected from the increase in the gas

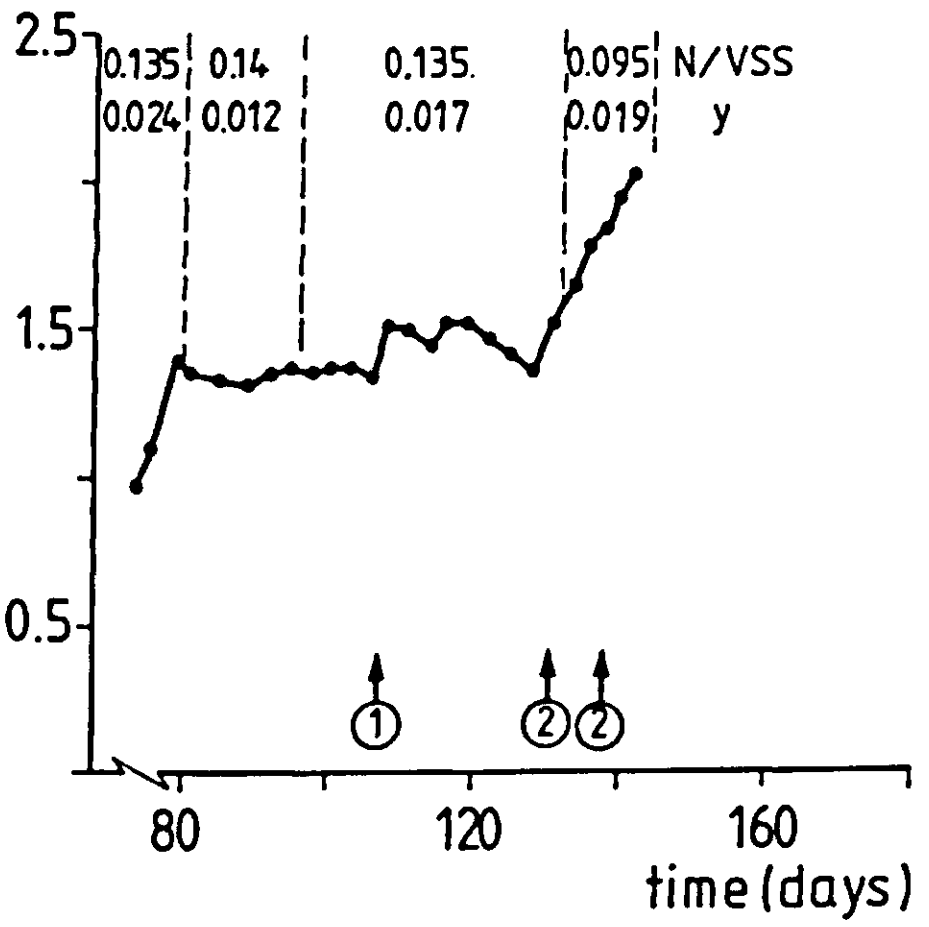


FIGURE 2 Batch reactor seeded with digested sewage sludge (type "E1"). No sulphur source was added. Adequate amounts of other nutrients were provided. The maximum observed methane production rates during the digestion of successive batch feeds with the standard VFA-mixture are shown (feed 16 till 41). No further increase occurs beyond day 79. Sulphide addition on day 129 and 137 resulted in a renewed increase in the gas production rate.
N/VSS = NH_4^+-N incorporated in sludge-VSS ($gN.gVSS^{-1}$)
Y = growth yield factor (gVSS/gCOD)
(1): Temporary increase of the pH-range during the digestion of one batch feed from pH 6.4-7.2 to 7.1-7.45. Around day 128 the pH-range was back at pH 6.5-7.3.
(2): Addition of 0.1 mM Na_2S.

In the absence of an increase in the gas production rate sludge growth still occurred, although the observed sludge growth yield factor Y of approximately 0.015 $gVSS.gCOD^{-1}$ was lower than the value found under optimal conditions of 0.024 $gVSS.gCOD^{-1}$ (Table 1).
The observed sludge growth presumably should be attributed to the growth of bacterial matter, and not to the formation of reserve material, because 0.14 $gN.gVSS^{-1}$ is incorporated during the stagnation period.
The addition of a small amount of sulphide (0.1 mM) on day 129 immediately resulted in an increase of the gas production rate (Figure 2). However, the increase was not proportional to the amount of sludge formed during the stagnation period. The slow rate of increase indicates either that the conditions were still limiting growth or that the death rate was high.

6.3.2 METHANOGENIC ACTIVITY OF NEWLY FORMED BACTERIAL MATTER

In batch fed digesters with complete sludge retention the specific methanogenic activity of newly formed sludge-VSS can be calculated by attributing the increase in the gas production rate to the sludge growth. The maximum specific methanogenic sludge activity calculated in this way was approximately 3.0 $kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$ (Table 4).

TABLE 4 CALCULATED VALUES FOR THE SPECIFIC METHANOGENIC ACTIVITY OF NEWLY FORMED SLUDGE-VSS WITH DIFFERENT SUBSTRATES

substrate	concentration range ($kgCOD.m^{-3}$)	maximum specific activity of newly formed sludge ($kgCH_4$-COD.$VSS^{-1}.d^{-1}$)
standard VFA-mixture (1)	2.65 - 0	2.85 ± 0.3
predominantly acetate(2)	2.65 - 1.1	3.0 ± 0.5
methanol	4.75 - 0	3.2 ± 0.3

(1) Consisting of 0.6 $kg.m^{-3}$ of acetic, propionic and butyric acid.
(2) 81 % acetate-COD and 19 % propionate and butyrate-COD (Figure 1).

6.3.3 EFFECT OF SHORT PERIODS WITHOUT FEEDING

In experiments with batch-fed intermittently stirred tank reactors (ISTR), indications were obtained, that interruption of the feeding caused a serious drop in the specific sludge activity. This effect was much stronger, than could be expected from results obtained in the unfed storage of sludge cultivated in UASB reactors on sugar beet wastewater (Lettinga 1978) and on sucrose (Hulshoff Pol et al. 1983b).
Results of short term starvation experiments with VFA and methanol cultivated ISTR-sludge are presented in Table 5 and Figure 1. Even a feedless period of less than 1 day resulted in a 5 to 9 % drop in the methanogenic activity of the sludge in an actively digesting ISTR.

Examination of the influence of periods of feed interruption on the conversion rates of the individual volatile fatty acids revealed that the acetate conversion rate was affected most seriously (Table 6).
Redox potential measurements (in experiment B4; Table 5 and 6) indicated, that during the entire period without substrate the E_h remained below -280 mV (pH 7.0).

TABLE 5 INFLUENCE OF SHORT PERIODS WITHOUT FEEDING UPON THE METHANOGENIC ACTIVITY IN BATCH-FED INTERMITTENTLY STIRRED TANK REACTORS AT 30 °C (1)

exp.	substrate (2)	length of unfed period (days)	original maximum methanogenic activity ($kgCH_4$-COD.m^{-3}.d^{-1})	decrease of methanogenic activity (%)
A1	standard VFA	2.1	1.02	16
2	mixture	14	1.08	31
3		15	1.86	60
4		16	2.26	67
5		50	1.87	86
B1	standard VFA	0.4	2.77	5
2	mixture	0.8	3.07	9
3		2.0	2.84	13
4		14	3.37	89
C	methanol	10	1.81	51

(1) Experiments performed as described in para. 3.2.1. Sludge used in exp. A, B and C was digested sewage sludge adapted to the respective substrates.
(2) Standard VFA mixture: 0.6 $kg.m^{-3}$ of acetic, propionic and butyric acid. Methanol: 1.6 $kg.m^{-3}$.

TABLE 6 INFLUENCE OF A 14-15 DAYS FEED INTERRUPTION UPON THE CONVERSION RATES OF INDIVIDUAL VFA IN INTERMITTENTLY STIRRED TANK REACTORS (1)

exp. (2)	original methanogenic activity ($kgCH_4$-COD.m^{-3}.d^{-1})	decrease of the conversion rate of:		
		acetate (%)	propionate (%)	n-butyrate (%)
A2	1.08	36	12	27
A3	1.86	70	38	45
B4	3.37	93	42	52

(1) Experiments performed as described in para. 3.2.1.
(2) See Table 5.

As is evident from Figure 1, the loss of methanogenic activity during periods without substrate is irreversible. The increase in the methanogenic activity after resuming the feeding, can be accounted for by normal sludge growth. Therefore a death rate constant k_d can be estimated from the loss of the methanogenic activity during feed interruptions ranging from 0.05 to 0.12 d^{-1} (Table 7).

Simultaneously with the loss of methanogenic activity during the interruption of the feeding a decrease in the sludge concentration was measured. In order to estimate the biomass decay rate the amount of active biomass originally present in the sludge mixed liquor must be known. The latter can be assessed from the measured maximum methanogenic activity (in $kgCH_4$-COD.day^{-1}) in the batch reactor preceding the starvation period and the maximum specific activity of the biomass as determined previously (Table 4). Thus a biomass decay rate constant k_D of 0.02-0.04 d^{-1} has been calculated for VFA cultivated sludge, and a k_D of 0.07 d^{-1} for methanol grown sludge.

As indicated by the standard deviations given in Table 7, the k_d and k_D values mentioned are only rough estimates.

TABLE 7 CALCULATED DEATH RATE AND DECAY RATE CONSTANTS DURING INTERRUPTION OF THE FEEDING OF VFA-CULTIVATED ISTR-SLUDGE (1)

exp. (2)	substrate	average biomass concentration ($kgVSS.m^{-3}$)	k_d (3) (d^{-1})	k_D (3) (d^{-1})
A	standard VFA	0.59 ± 0.14	0.053 ± 0.025	0.017 ± 0.013
B	mixture (1)	1.10 ± 0.12	0.119 ± 0.37	0.042 ± 0.023
C	methanol	0.57 ± 0.02	0.071	0.066 ± 0.008

(1) Experiments performed as described in para. 3.2.1. Substrate and sludge types used: see Table 6.
(2) Number of data averaged for A, B and C; n=4, n=4 and n=3 respectively (k_d of methanol is a single observation). See also Table 5 and 6.
(3) k_d = death rate constant, and k_D = biomass decay rate constant.

6.3.4 MISCELLANEOUS OBSERVATIONS

6.3.4.1 Effect of a high acetate concentration upon the propionate and butyrate removal rates

The maximum specific biomass activity of VFA-cultivated sludge (Table 4) will depend upon the concentration of the volatile fatty acids applied (assuming optimal conditions with respect to pH, nutrients, etc.).
In this study the experiments were centred around the lower VFA concentration range, i.e. below 1 $kg.m^{-3}$ of each VFA. At significantly

Shaw 1976).

High acetate concentrations may induce product inhibition in the breakdown of propionate and butyrate (see Chapter 2). This was observed in two separate experiments after increasing the acetate concentration to 1.5 (Figure 3) and 2.5 $kg.m^{-3}$ (Table 3). This increased acetate concentration caused an approximately 50 % decrease of the removal rate of propionate and in the latter case also of butyrate.

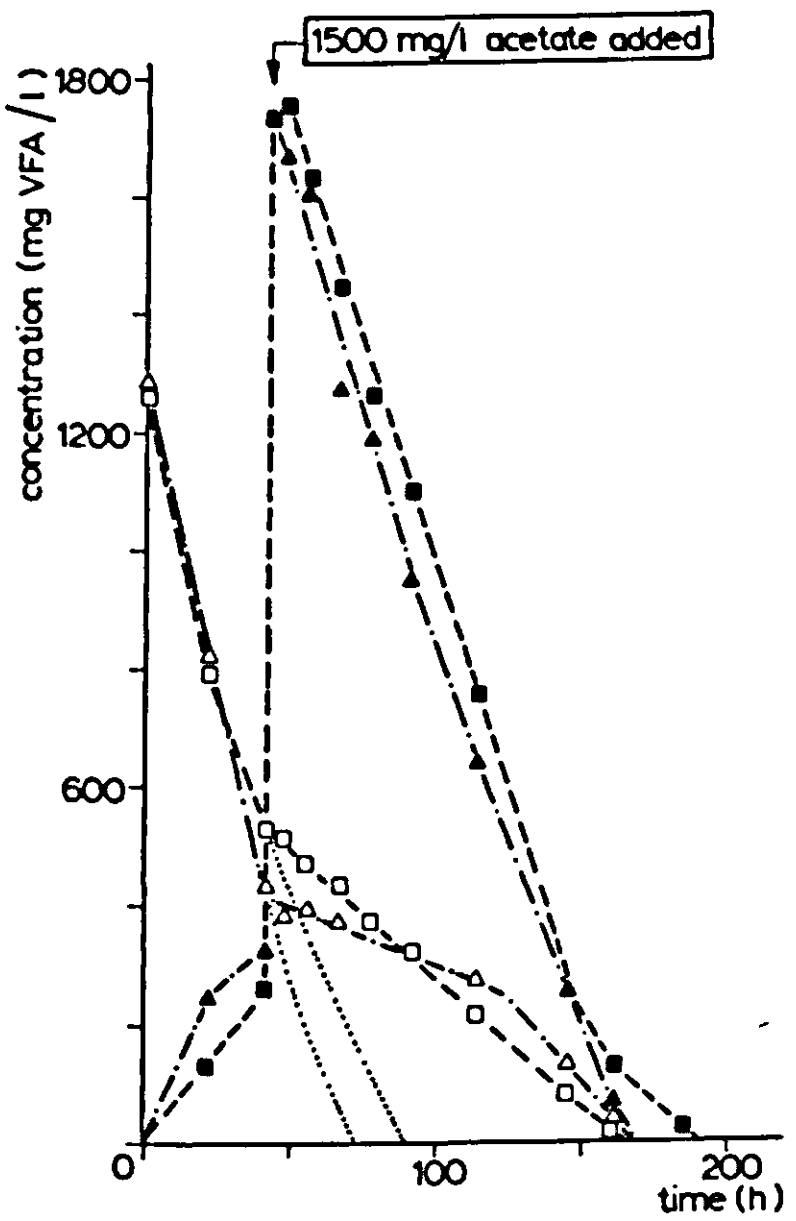


FIGURE 3
The influence upon the propionate (▵,▫) conversion rate of increasing the acetate (▲,■) concentration. Culture conditions: 5.5 liter batch fed reactors seeded with propionate-adapted digested sewage sludge (type "E1"). Continuously stirred at 40 (▫,■) and 200 rpm (▵,▲). The dotted lines indicate the expected path of the propionate depletion plot if no acetate had been added.

6.3.4.2 The COD yield factor Y_{COD} and the sludge settleability

In intermittently stirred tank reactors (ISTRs) seeded with digested sewage sludge and with nearly complete sludge retention normally the sludge settleability remains poor during the digestion of successive VFA feeds. As no selection pressure is exerted on the bacterial population, growth will not only take place in or on the sludge flocs, but dispersed growth will become increasingly important. After the stirring was stopped the more coarse sludge flocs settled out, but the supernatant solution in these ISTR-experiments remained turbid for several hours up to a few days. However, there were two remarkable exceptions.

Following the digestion of 8 successive standard VFA feeds, a digested sewage sludge seeded ISTR was given a shock load of acetic, propionic and butyric acid of 5.2 $kg.m^{-3}$ each, corresponding to 8 standard VFA feeds at once (Figure 4; day 29). About 11 days later (day 40) a remarkable improvement of the sludge settleability became apparent. The turbidity of the supernatant after settling of the flocculent sludge had almost vanished.

This improvement lasted for approximately 12 days. Then the settleability deteriorated again and the turbidity of the supernatant after settling of the flocs reappeared.

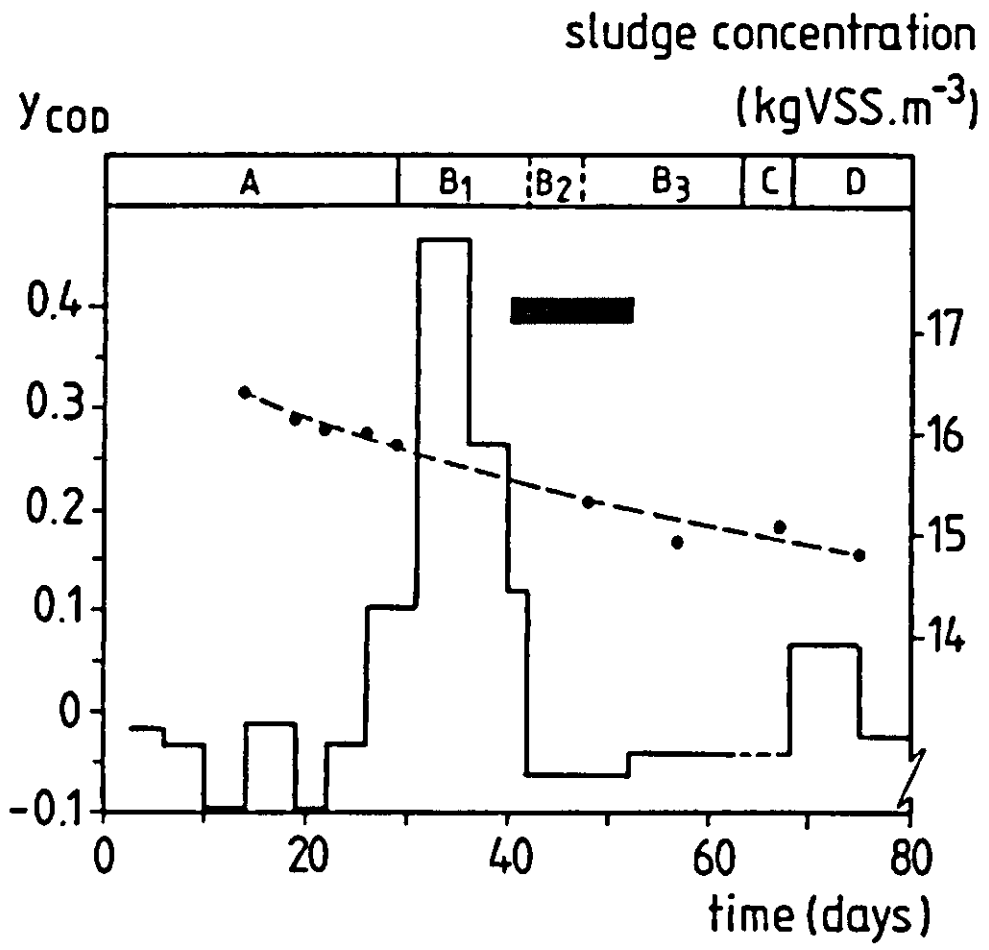


FIGURE 4 The influence of a VFA shock load upon the Y_{COD} (——) in an ISTR experiment seeded with a high concentration (-●-) of digested sewage sludge (type "E1"). Bar indicates period of improved sludge settleability.
period A: digestion of 8 successive standard VFA feeds.
period B: digestion of shock load
period C: interruption of the feeding
period D: digestion of successive standard VFA feeds

	concentration range ($kg.m^{-3}$)		
	acetate	propionate	butyrate
period A	0.8 - 0	0.6 - 0	0.6 - 0
period B1	5.1 - 2.8	5.2 - 4.1	5.2 - 0.2
period B2	2.8 - 0	4.1 - 3.6	0.2 - 0
period B3	0	3.6 - 0	0
period C	0	0	0
period D	0.6 - 0	0.6 - 0	0.6 - 0

Figure 4 shows, that the period of improved sludge settleability was preceded by a period with a remarkably high COD yield factor Y_{COD}; i.e. ($gVFA\text{-}COD_{converted}$ - $gCH_4\text{-}COD$)/$gVFA\text{-}COD_{converted}$. The Y_{COD} calculated for the periods preceding and following the shock load were mostly negative as a result of the further stabilization of the concentrated seed sludge (fresh digested sewage sludge). The VSS concentration gradually decreased. The average Y_{COD} for the digestion of the concentrated feed (period B) was 0.15 $g.g^{-1}$. If the fraction of the converted VFA-COD, that was not recovered as methane would have become sludge-COD, the VSS concentration should have increased with about 2.4 $kgVSS.m^{-3}$. However, no lasting increase was recorded, suggesting that a soluble compound had been formed. As COD analyses (next to VFA measurements) were not performed, this could not be confirmed.

A second similar observation of an improvement of the sludge settleability was made in an ISTR seeded with a different type of digested sewage sludge (type "H"; 4 $kgVSS.m^{-3}$). Following the digestion of 3 batch feeds with a mixture of acetic and propionic acid (0.4 to 0.7 $kg.m^{-3}$ of each VFA per feed) a shock load was imposed of 2.2 $kg.m^{-3}$ acetate and 1.6 $kg.m^{-3}$ propionate. At day 22 after the feeding, all propionate had been removed, while the acetate concentration still amounted to 1.6 $kg.m^{-3}$. From day 34 to day 49 an improved sludge settleability was observed. The acetate concentration during this period dropped from 0.9 to 0.0 $kg.m^{-3}$. The settling velocity of the sludge flocs increased from less than 1.0 m/h at day 36 to close to 1.6 m/h at day 41 and decreased again to 0.9 m/h at day 49. The improved settleability again was accompanied by a much clearer supernatant solution. The pH increased slowly during the digestion from 7.1 to 7.55.

Further research into this improved flocculation phenomenon seems justified.

6.3.4.3 Microscopic observations

By microscopic observation a number of morphological sludge characteristics can be described. Special attention has been given to the appearance of filamentous microorganisms resembling Methanothrix (see Huser 1981; Figure 4), and three morphologically different Methanosarcina type organisms. The latter resembled either 1) aggregates of very big clumps similar to the

young cells of Methanococcus mazei mentioned by Mah 1980 and called biotype I by Zhilina (1976), or 2) aggregates of coccoid elements presumably also belonging to Methanococcus mazei, which form spherical bodies ("cysts") (Mah 1980; Figure 3A), or 3) sarcina-type aggregates similar in form to Methanosarcina strain 227 (Mah et al. 1978; Figure 1a) and called biotype II by Zhilina (1976).

Most batch experiments described in this chapter were performed with digested sewage sludge as seed. The digesters were stirred intermittently and fed repeatedly with a VFA mixture. Except for sampling the sludge retention was complete.
Protozoic organisms were generally present in the batch digesters in varying numbers.
Sludge flocs tended to be very small and light in these intermittently stirred tank reactors (ISTRs).

Despite their relatively low numbers, the filamentous microorganisms in digested sewage sludge rapidly became abundant in the batch fed digesters. Initially they were mainly attached to the small flocs, but gradually they also occurred dispersed freely in the liquid.
Methanosarcina-type bacteria were observed regularly, but they were never present in large numbers, nor in large clumps. The same applied to the experiment in which the acetate concentration in the reactor was maintained above 20 mM for a period of 22 days (Table 3). Only very few methanosarcina-type bacteria were seen in this reactor, while the filamentous bacteria formed their characteristic bundles.

Methanol digestion

The batch experiments with methanol were performed with acetate adapted sludge cultivated from digested sewage sludge in an UASB reactor fed an acetate solution of 4.3 $kgCOD.m^{-3}$ over a period of 82 days ("f3" in Table 2 of Chapter 7). This sludge contained relatively few filamentous bacteria, and methanosarcina-type organisms prevailed. All three morphologically different Methanosarcina types were present.
The sludge was subjected to a number of batch feeds with methanol and acetate as indicated in Table 8.
As a result of the methanol digestion the number of filamentous bacteria,

TABLE 8 MICROSCOPIC OBSERVATIONS ON ACETATE CULTIVATED SLUDGE (1) FED BATCH-WISE WITH METHANOL AND ACETATE

substrate conc. (2) ($kg.m^{-3}$)	number of feeds	day no.	relative abundance of: (3)			
			filamentous bacteria A	methanosarcina type aggregates B	aggregates like young cells of M. mazei C	aggregates of coccoid elements D
meth. 1.6	6	0-38				
		9	+	++++	+	+++
		15	+	+++	+++	+++
		28(4)	++	++	+	++++
		34	+++	++	++	+++
meth. 3.2	4	38-50				
		38	+++	++	+++	+++
		50	+++	+	++++	++++
meth. 1.6 plus acet. 2.2	2	50-62				
		52	+++	++	+++	++++
		59	+++	+++	+	++++
acet. 2.2	5	62-72				
		65	+++	+++	++	++++
		72	++++	n.o.(5)	n.o.(5)	++++

(1) The sludge was taken from an UASB reactor, which had been fed with an acetate solution of 4.3 $kgCOD.m^{-3}$ for 82 days.
(2) meth.= methanol and acet.= acetic acid.
(3) The number of (+)-signs indicates the trend observed in the number of the specific morphotype. A desciption of the morphotypes A, B, C and D is given in the text.
(4) Observation at the end of a 10 day period without feeding.
(5) n.o.= no observation recorded.

As Methanothrix can grow only on acetate, these results suggest that methanol is digested at least partly via the intermediate formation of volatile fatty acids (Lettinga et al. 1981). Indeed low levels of acetate (less than 0.8 mM) and propionate (less than 0.15 mM) were detected in the mixed liquor during methanol digestion.

The methanosarcina-type aggregates originally present in clumps up to a diameter of 0.1 mm (type B in Table 8) decreased in number during the digestion of methanol and increased again when acetate was added (day 50; Table 8). Big spherical clumps frequently were empty inside.
Cell aggregates similar to clumps of young cells of Methanococcus mazei (which sometimes also showed an empty space inside) became abundant during

the digestion of methanol and tended to disappear in restarting the acetate feeding (type C in Table 8).

Spherical aggregates of very small coccoid bodies (type D in Table 8) were present in large numbers during the entire experimental period. These spherical bodies frequently showed an empty space inside.

Combinations of the three different morphological types (B, C and D in Table 8) or intermediate forms were often observed.

F420 fluorescence was very intense in type C cell aggregates, moderate in type B cells, almost absent in type D cell aggregates and completely absent in type A cells. Type D cell aggregates, therefore, presumably consisted of resting cells.

6.4 DISCUSSION

Growth yield

The biomass yield factor of 0.064 $gVSS.gCOD^{-1}$ found for acetate digestion falls well within the range of literature data reported for batch-wise or semicontinuously fed acetate digesting cultures (Table 9). Sludge growth yield factors of 0.12 to 0.4 $gVSS.gCOD^{-1}$ for acetate digestion are reported occasionally (Novak and Ramesh 1975, Ghosh and Klass 1978, Cappenberg 1975), i.e. approximately an order of magnitude higher than the average reported value. Relatively low growth yield factors are found for the digestion of propionate, butyrate and mixtures of volatile fatty acids (Table 1). These values are in accordance with literature data (Chapter 2,

TABLE 9 REPORTED GROWTH YIELDS FOR ACETATE DIGESTION IN BATCH-FED AND SEMI-CONTINUOUSLY FED DIGESTERS

Y (1) ($gVSS.gCOD^{-1}$)	culture characteristics	ref. (2)
0.015 - 0.019	Methanothrix soehngenii	*
0.025 - 0.07	Methanosarcina barkeri	*
0.06	Mixed enrichment culture	1
0.12	Mixed enrichment culture	2
0.3	Mixed enrichment culture	3
0.4	Pure culture of rod-shaped methanobacterium	4

(1) Literature data recalculated assuming 1 g bacterial mass = 0.872 gVSS (Luria 1960).
(2) *: see Table 2 of Chapter 2, 1: McCarty 1966, 2: Novak and Ramesh 1975, 3: Ghosh and Klass 1978, 4: Cappenberg 1975.

Table 4). Small variations in the growth yields for e.g. propionate digestion can be attributed to the presence in the mixed culture of either Methanothrix (Koch et al. 1983) or Methanosarcina (Lawrence and McCarty 1969), which exhibit different Y-values for acetate digestion (Table 9). In the experiments reported in this chapter Methanothrix-type bacteria prevailed and only relatively small numbers of Methanosarcina-type bacteria were observed (except for the methanol digestion experiments in which Methanosarcina-type organisms were abundant).

In Figure 5 the growth yield factors are plotted versus the composition of the feed in terms of methanogenic substrates on a common COD-basis (thus ignoring the contribution of the acetogenic bacteria to the yield factor, which was assumed to be small (Koch et al. 1983)).

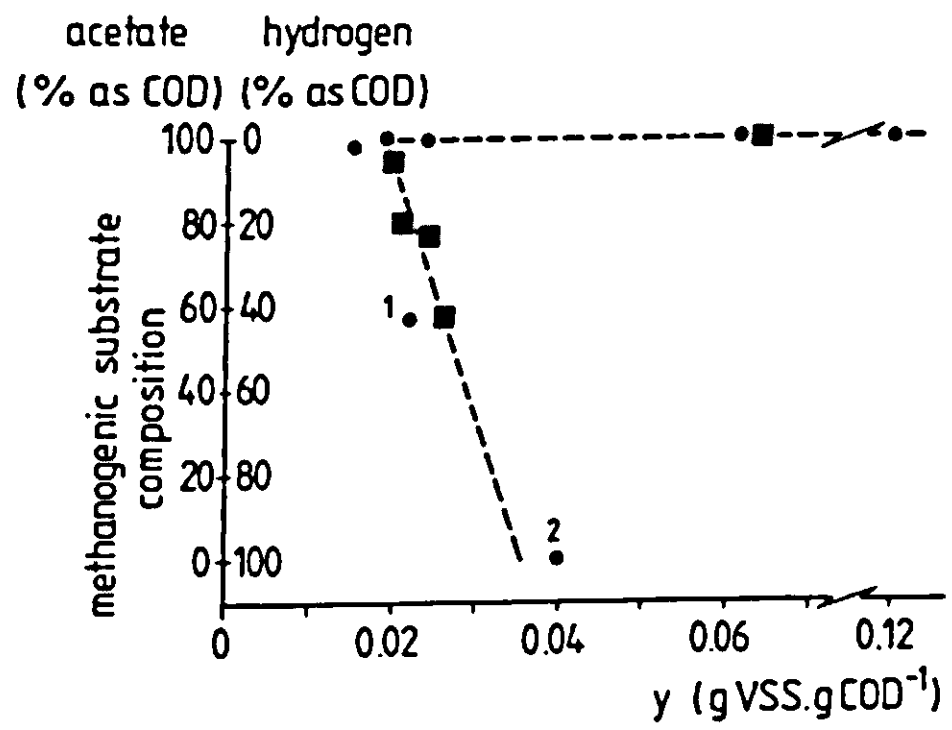


FIGURE 5 Relation between the methanogenic substrate composition as produced from the degradation of VFA (expressed as theoretical COD) and the biomass growth yield; (■): data of Table 1, (●): literature data of Table 10 and of Gujer and Zehnder (1983) (1) and Zehnder and Wuhrmann (1977) (2).

Figure 5 shows that the growth yield factors for the digestion of propionate, butyrate and mixtures of VFA lie between the lowest growth yield factors reported for pure cultures of Methanothrix and the lowest value found for a pure culture of a hydrogenotrophic methanogen (Zehnder and Wuhrmann 1977). The observation that, with acetate as sole carbon and energy source, exceptionally high growth yields may be attained without an increase in the methanogenic activity of the culture and without

NH_4^+-nitrogen fixation (Table 2), suggests the production of reserve material, rather than of viable biomass. This may be explained by some nutritional deficiency of the growth medium, which can be alleviated by excretion products of the acetogenic bacteria present in VFA-digesting mixed cultures. As suggested by Pretorius (1972), Zeikus (1977) and Mah et al. (1978), small amounts of hydrogen may be required for the generation of reducing equivalents for anabolism in a relatively poor medium. H_2 obtained from the breakdown of propionate and butyrate apparently suffices for this purpose.

The incorporation of 10.6 % NH_4^+-N in sludge-VSS for butyrate digestion and 9.5 to 13.5 % for bacterial growth on the standard VFA-mixture corresponds well with the value of 10.6 % nitrogen fixation reported by Speece and McCarty (1964) for acetate digestion.

A lower growth yield factor under conditions of sulphur-limitation (Figure 2) agrees with data of Mountfort and Asher (1979) concerning the growth of Methanosarcina barkeri on methanol in the absence and presence of a sulphur source.

Specific biomass methanogenic activity

The specific methanogenic activity of newly formed sludge-VSS, i.e. 2.85 and 3.0 $kgCH_4$-COD.$kgVSS^{-1}$.day^{-1} for the digestion of the standard VFA mixture and acetate respectively (Table 5), corresponds well with the maximum specific activity of Methanothrix soehngenii as reported in the literature, but is considerably lower than would be expected from literature data on Methanosarcina at the concentrations used (see Chapter 2, Table 2). In fact, Methanothrix-type bacteria prevailed in the experiments presented in this chapter.

The specific methanogenic activity of 3.2 $kgCH_4$-COD.$kgVSS^{-1}$.d^{-1} (Table 5) found for the digestion of methanol at concentrations up to 3 $kg.m^{-3}$ is also considerably lower than the reported maximum activity of about 10 $kgCH_4$-COD.$kgVSS^{-1}$.d^{-1} for Methanosarcina barkeri (Mountfort and Asher 1979). This supports the microscopic observations (Table 8) revealing that Methanothrix-type bacteria played an important role in the digestion of methanol in the present experiments.

As expected from the thermodynamics of the acetogenic reactions (see Table 1 of Chapter 2), elevated acetate concentrations (10 to 40 mM) significantly slow down the rate of propionate, as well as butyrate breakdown (Table 3 and Figure 3). Similar results for propionate degradation were reported by Zehnder and Koch (1983).

Sludge death and sludge decay rate

The sludge death rate constant of 0.05 to 0.1 d^{-1} as observed during feed interruptions of VFA-fed ISTR-sludge (Table 7) are higher than those reported by Lawrence and McCarty (1969), and contradict the results obtained in the unfed storage of granular UASB sludge (Lettinga 1978, Hulshoff Pol et al. 1983b). In the latter case no appreciable loss of activity was noted after a 6 weeks feed interruption at 30 °C. Only a short gas production lag phase occurred.The smaller resilience of ISTR-sludge towards starvation in comparison with granular UASB-sludge presumably can be attributed to the fact that - due to the stirring and the lack of selection pressure upon the sludge particles - ISTR-sludge consists of freely dispersed bacteria and very small sludge flocs, which will not be protected as well against adverse environmental conditions as the granular UASB-sludge bacteria embedded in capsular material.

Implications for UASB-reactor start-up

1. With acetate as the main substrate a growth rate μ of 0.06 d^{-1} can be calculated from the growth yield factor Y of 0.020 $gVSS.gCOD^{-1}$ (paragraph 6.3.1.1.) and the biomass methanogenic activity V of 3.0 $kgCH_4\text{-}COD.kgVSS^{-1}.d^{-1}$ (Table 5). Similarly a growth rate of about 0.07 d^{-1} is found for growth on the standard VFA mixture (Tables 1 and 5).
From these growth rates the theoretical minimum start-up time of an UASB reactor treating VFA and seeded with digested sewage sludge can be predicted to be in the order of 35 days (assuming complete biomass retention and an initial average activity of 1 $kgCH_4\text{-}COD.m^{-3}.d^{-1}$, and defining reaching an activity of 10 $kgCH_4\text{-}COD.m^{-3}.d^{-1}$ as the end of reactor start-up).
In reality the selection pressure upon the sludge particles brought about by sludge wash-out, represents an essential feature of the UASB process. A granular sludge type with superior settling properties will develop. UASB reactor start-up based on digested sewage sludge as seed, therefore will always take longer than the theoretical minimum time.

2. A net positive sludge growth yield does not necessarily guarantee an increase in the methanogenic activity of the sludge mixed liquor. A shortage of available sulphide in a VFA digesting system causes a stagnation in the development of the methane production rate although net sludge growth still occurs.

   When treating a waste with acetate as the sole carbon and energy source, high sludge growth yields may be observed; largely due to the production of carbonaceous matter free of nitrogen, and not to the growth of viable biomass.

CHAPTER 7 START-UP OF UASB REACTORS SEEDED WITH DIGESTED SEWAGE SLUDGE

7.1 INTRODUCTION

UASB reactor performance depends largely upon the retention of the sludge. Especially when treating dilute wastewaters at high organic loading rates, the settleability of the sludge particles has to be good. In UASB reactors this condition is met by the development of a granular type of sludge with excellent settling characteristics. Granular anaerobic sludge was first observed in a pilot scale UASB reactor seeded initially with digested sewage sludge and treating sugar-beet campaign wastewater. A sludge concentration of 45 $kgDS.m^{-3}$ was achieved in the reactor and a loading rate of 20 $kgCOD.m^{-3}.d^{-1}$ could be treated satisfactorily (Lettinga et al. 1977). In preliminary laboratory UASB-reactor experiments with this type of wastewater no sludge granules were observed. A maximum loading rate of approximately 10 $kgCOD.m^{-3}$ was reached at a sludge concentration of 15 $kgDSS.m^{-3}$ in these experiments.

Later the development of granular sludge from digested sewage sludge was also observed in treating other types of wastewater (Versprille 1978, van der Vlugt 1980, van Bellegem 1980). Similar sludge granules were reported to occur in the interstices of highly loaded anaerobic filters (Young and Dahab 1983).

The first start-up of UASB reactors using digested sewage sludge as seed may last several months (Versprille 1978, Pette et al. 1979, Frostell 1979). Because the knowledge concerning the factors influencing the duration of the start-up period was scarce, a comprehensive study was undertaken to improve the insight in this matter. Factors of importance in UASB reactor start-up are the type and concentration of the seed, the composition and the strength of the wastewater and the start-up strategy applied. Because of the complexity of the system not all factors that may influence the start-up could be studied in detail. Yet from the results obtained in the experiments presented in this chapter, the main processes controlling UASB reactor start-up can be distinguished.

7.2 MATERIALS AND METHODS

Start-up procedure

Unless otherwise stated the start-up procedure consisted of a step-wise increase of the loading rate whenever the COD removal efficiency surpassed 80 to 90 %. The COD removal efficiency never dropped below 50 %. The effluent VFA concentration was maintained generally below 1 to 1.5 kgVFA-COD.m^{-3} for the 3 to 5 kgCOD.m^{-3} substrate concentration range, and below 2.5 kgVFA-COD.m^{-3} for a substrate concentration of 11 kgCOD.m^{-3}. In the experiments with a feed concentration of 1.0 kgVFA-COD.m^{-3} the effluent concentration was kept below 0.5 kgVFA-COD.m^{-3}.

Experimental set-up

A schematic diagram of the laboratory UASB reactors used in this study is given in Figure 1. The reactor dimensions are listed in Table 1. Table 2 provides a list of all UASB experiments referred to in this chapter.

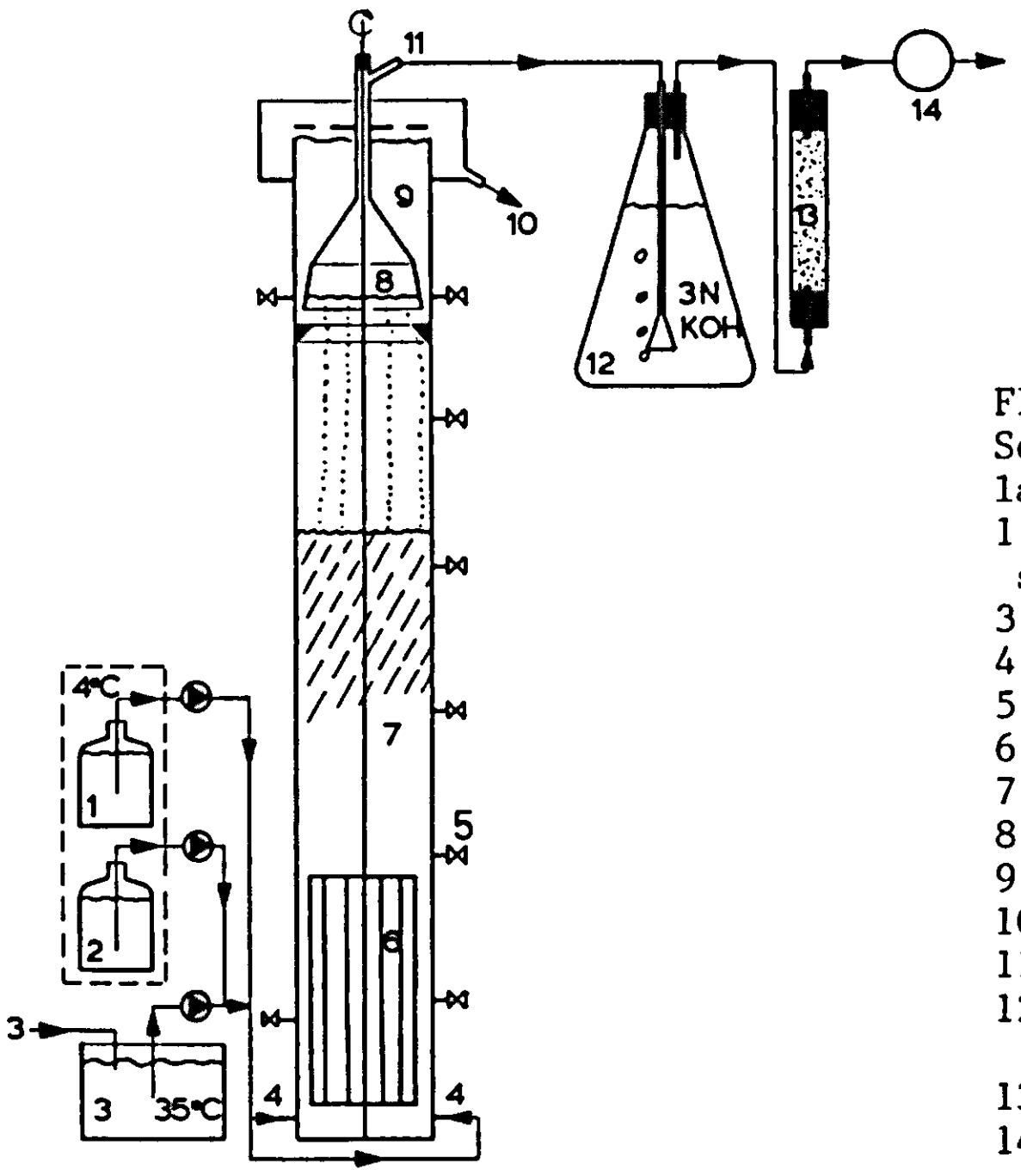


FIGURE 1
Schematic diagram of a laboratory UASB reactor.
1,2: stock solutions of substrate and nutrients,
3: dilution water,
4: influent inlet points,
5: sampling ports,
6: flat blade impeller,
7: sludge bed,
8: gas collector,
9: settler,
10: effluent discharge,
11: gas outlet,
12: CO_2 (and H_2S) stripping device,
13: granular soda lime,
14: gas meter.

The reactors were equiped with a stirring assembly, which was used only at gas production rates smaller than 1 $m^3.m^{-3}.d^{-1}$ (intermittently: 30 sec at 10-20 rpm each 10-20 min).

All experiments were conducted at 30 °C.

TABLE 1 DIMENSIONS OF LABORATORY UASB REACTORS

volume (liter)	cross sectional area (dm^2)
30	2.8
23.5	2.8
10.7	0.7
4.2	0.7
2.8	0.7

Substrate composition

The substrates used in the experiments are listed in Table 2. The volatile fatty acids were neutralized with NaOH in order to maintain a reactor pH of 7.0 - 7.5. In the case of non-VFA substrates 10 meq $NaHCO_3$ per g COD was added.

Supply of nutrients

In the experiments conducted with VFA solutions with a concentration exceeding 2.5 $kgCOD.m^{-3}$ the COD : N : P : S ratio was kept between 800:7:1:1 and 1200:7:1:1 (w/w) by addition of appropriate amounts of NH_4Cl, KH_2PO_4 and $(NH_4)_2SO_4$. In the experiments with a more dilute VFA solution and in the experiments with a carbohydrate feed a ratio of about 300:5:1:1 was adapted.

In all cases, unless otherwise stated, Difco Bacto yeast extract was supplied to the feed in a COD/YE ratio of 10-15 g/g. From the yeast extract additional amounts of approximately 0.06 gNH_4^+-N/gYE and 0.001 gPO_4^{3-}-P/gYE were released.

In all experiments 0.1 to 0.4 ml/l of a trace element solution according to Zehnder (1976) was added to the feed.

Seed sludges

The type of seed sludge used and the average reactor concentration of seed sludge supplied, are listed in Table 2.

TABLE 2 LIST OF UASB EXPERIMENTS PERFORMED

code	seed sludge		reactor volume	starting date	duration (days)	feed composition(1)		
	(2)	VSS-conc. ($kg.m^{-3}$)	(liter)			ac. (mM)	prop. (mM)	total COD ($kg.m^{-3}$)
a	E1	15	2.8	19-02-79	43	25	16	3.4
b	F3	11.5	30	05-04-79	86	20	13.5	2.8
c1	F3	12	30	21-09-79	45	35.5	24	5.0
c2	F3+ b	12+ 0.6	30	21-09-79	49	35.5	24	5.0
c3	F3	11.5	30	05-12-79	16	35.5	24	5.0
c4	F3	11.5	30	05-12-79	17	28.5	18.5	4.5
d1	F4	14.5	30	15-01-80	29	38	25.5	5.3
d2	F4	14.5	30	15-01-80	29	35.5	24	5.0
d3	F4	16	30	12-02-80	13	35.5	24	5.0
d4	F4	16	30	12-02-80	13	35.5	24	5.0
e1	E1	10	23.5	11-09-79	84	67		4.3
e2	E1	10	23.5	11-09-79	84	67		4.3
f1	E2	10	23.5	15-02-80	25	67		4.3
f2	E2	10	23.5	15-02-80	28	67		4.3
f3	E2	10	23.5	13-03-80	59	67		4.3
f4	E2	10	23.5	13-03-80	59	67		4.3
g1	E3	7.5	30	12-04-80	30	29	26	4.8
g2	E3	14.5	30	12-04-80	30	39	25	5.3
h1	E4	7.5	30	23-05-80	30	82	53	11.2
h2	E4	14.5	30	23-05-80	30	82	53	11.2
j1	E5	7.5	30	28-06-80	30	7.3	4.7	1.0
j2	E5	14.5	30	28-06-80	30	7.3	4.7	1.0
k	E5	6	30	30-07-80	30	84	55	11.5
l	F5	9	30	30-07-80	30	83	54	11.3
m	e2	12.5	4.2	08-80	50	(3)		4.8
n	E8	10	10.8	16-10-81	114	20	13.5	3.0
p1	E	11.5	30	02-82	57	38	23	5.0
p2	E	11.5	30	02-82	57	38	23	5.0
q1	E*	10	30	13-05-82	79	49	29	6.4
q2	E*	5	30	13-05-82	81	49	29	6.4
r1	E9	13	23.5	10-02-82	110	20	13(4)	3.0
r2	E9	13	23.5	10-02-82	142	1.1	.7(4)	3.0
r3	E9	13	23.5	21-06-82	46	(5)		3.1
s1	E10	10	30	25-08-82	81	23	14.5	3.0
s2	E10	10	30	25-08-82	81	23	14.5	3.0

(1) ac.= acetate; prop.= propionate

(2) Codes refer to Table 1 of Chapter 4. In experiment "c2" 0.6 $kg.m^{-3}$ of sludge cultivated in experiment "b" was added. Experiment "m" was performed with sludge cultivated in exp. "e2" Further data on the seed sludge used in exp. "p1" through "q2" are not available. Seed sludge of experiments "q1" and "q2" was incompletely digested (i.e. digesting) sewage sludge.

(3) Fed 9 days with 74 mM acetate followed by 27 days with 27 mM acetate + 27 mM propionate and 14 days with 297 mM formate.

(4) In experiments "s1" and "s2" 10 and 95 % respectively of the total COD consisted of sucrose.

Analyses

-DSS, VSS and VFA analyses were performed as described in paragraph 3.2.1.

-The specific methanogenic activity of the sludge was measured according to the standardised batch activity test described in paragraph 3.2.

-F420 analysis was performed as described in paragraph 3.3.2.

-The methane gas production was measured using a wet test gas meter after absorption of the carbon dioxide in an alkaline solution.

The effluent solution was considered to be saturated with methane, in accordance with data presented by Grin et al. (1983). The discrepancy between measured methane production and the actual methane production (i.e. including methane dissolved in the effluent) is given in Table 3. Except for experiments "j1" and "j2" the gas production rates have not been corrected for dissolved methane.

TABLE 3 CALCULATED CONTRIBUTION OF METHANE DISSOLVED IN THE EFFLUENT TO THE TOTAL METHANE PRODUCTION

exp. (1)	substrate concentration ($kgCOD.m^{-3}$)	average removal efficiency (%)	methane dissolved in effluent (% of total)
j1	1.0	54.5	15
j2	1.0	51.1	16
b	2.8	80	3.4
g	5	82	1.9
h	11.2	80	0.9

(1) Codes refer to Table 2.

7.3 RESULTS

The results obtained from the UASB reactor start-up experiments mentioned in Table 2 are presented here in such combinations as to illustrate the effect of different factors upon the course of the start-up.

Factors that were examined, include:

-the sludge wash-out in relation to the type and amount of seed sludge supplied (paragraph 7.3.2),

-the concentration and composition of the substrate (para. 7.3.3) and

-the start-up strategy (paragraph 7.3.4).

Separate paragraphs are devoted to the sludge growth yields (7.3.5) and the development of sludge granules (7.3.6).

7.3.1 GENERAL COURSE OF UASB REACTOR START-UP

The course of UASB reactor start-up is illustrated by the results of experiment 'b ' (Figures 2, 3, 4 and 5). The reactor was seeded with 11.5 $kgVSS.m^{-3}$, creating a sludge bed of 0.37 m in a 1 m high reactor. The specific activity of the seed sludge was 0.12 $kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$. An initial volumetric loading rate of 1.2 $kgCOD.m^{-3}.d^{-1}$ was employed corresponding to a sludge loading rate of 0.1 $kgCOD.kgVSS^{-1}.d^{-1}$.

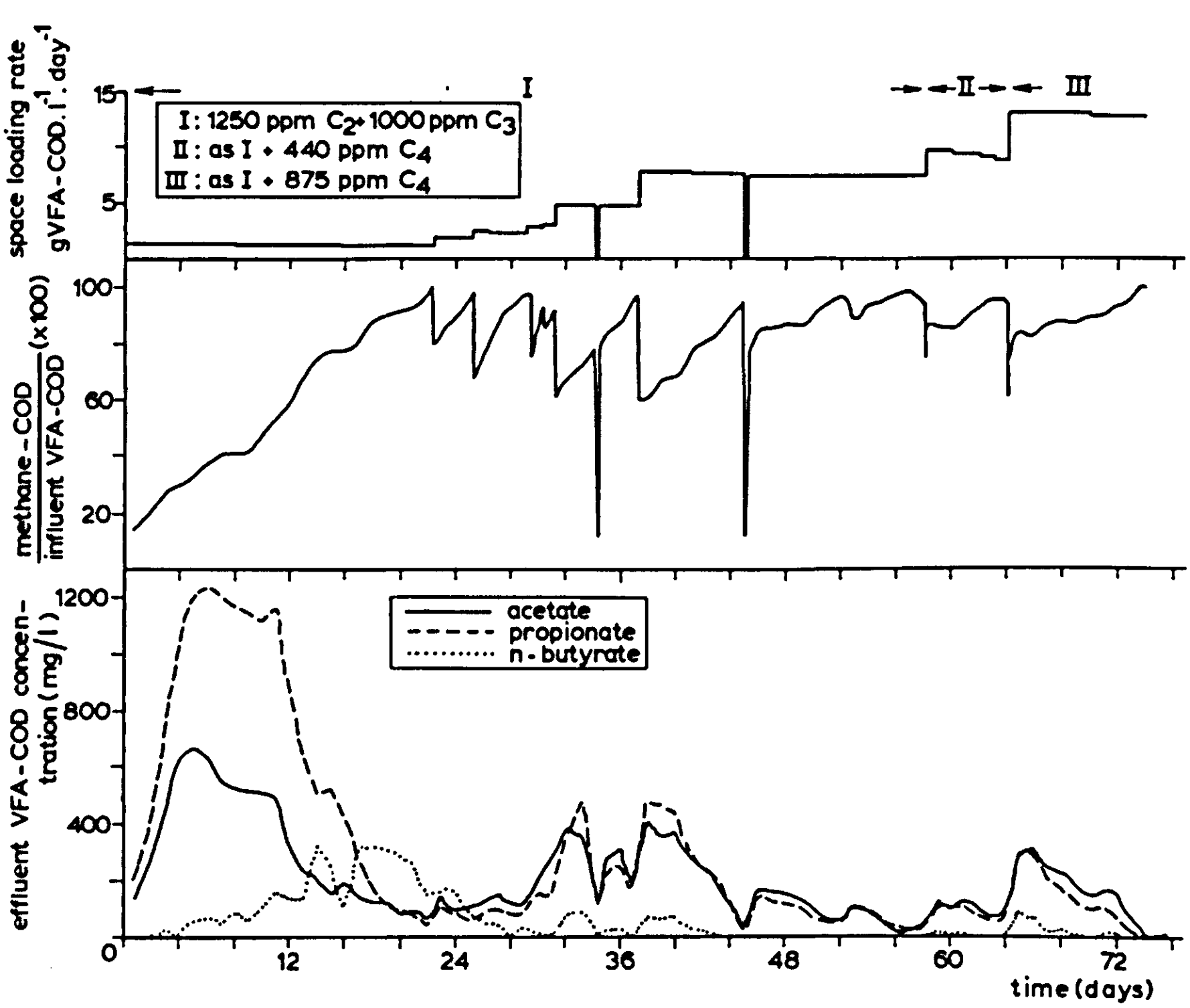


FIGURE 2 Results of an UASB-reactor start-up experiment ('b' of Table 2).
Upper part: step-wise increase of the loading rate,
Middle part: COD removal efficiency,
Lower part: Effluent VFA-COD concentrations.

This resulted in a liquid detention time of 58 hrs. As soon as the feed components, acetate and propionate, were eliminated in the process for more than 90 %, the loading rate was increased step-wise according to the adopted general start-up procedure (Figure 2).

Sludge wash-out data are shown in Figure 5 (example B).

The amount of sludge present in the reactor has been followed by measuring VSS profiles over the height of the reactor at regular time intervals (Figure 3). In combination with the sludge wash-out data a sludge balance calculation can be made; the results of which are shown in Figure 4.

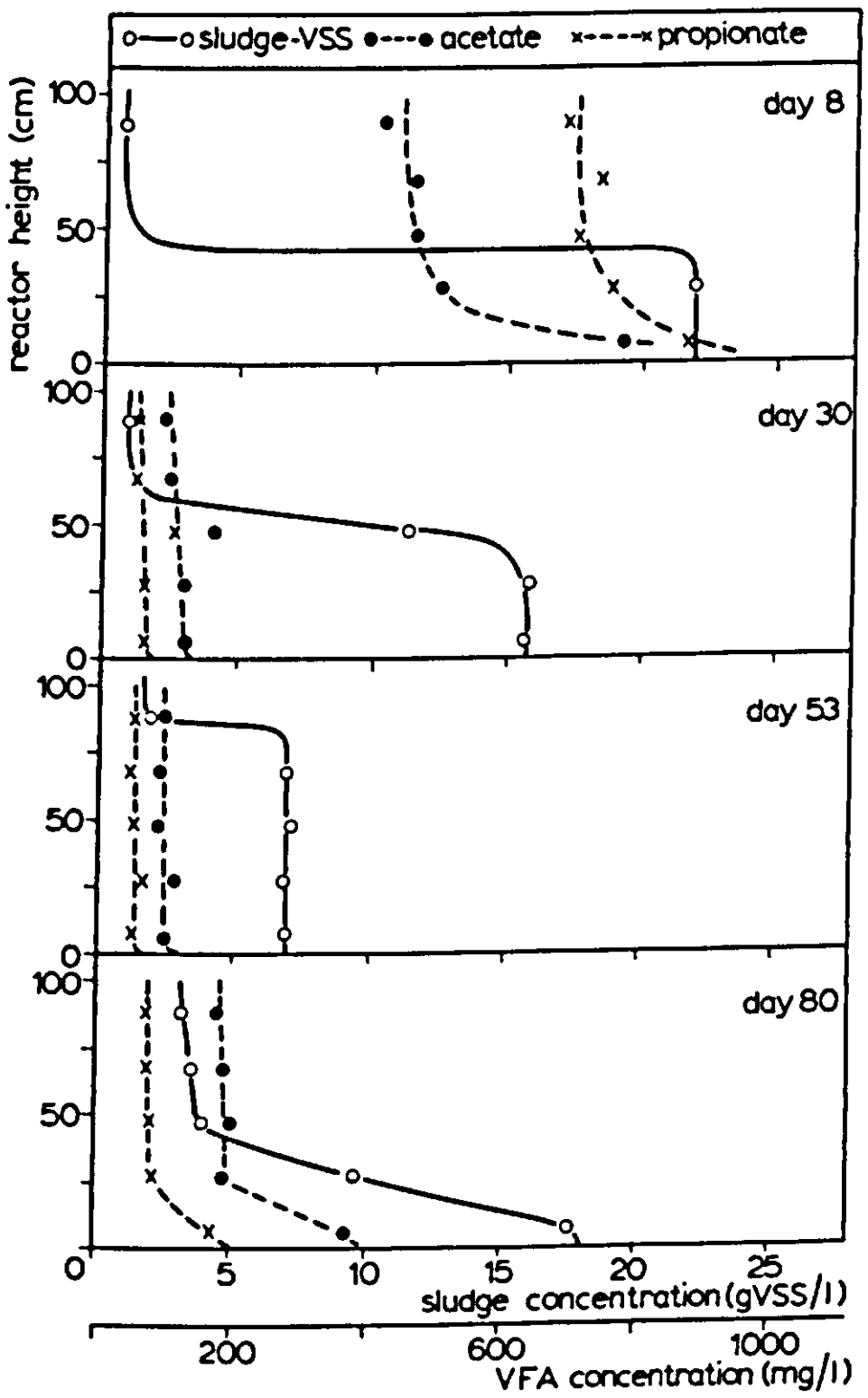


FIGURE 3 Sludge concentration and VFA concentration profiles at different moments during UASB-reactor start-up. Experiment 'b'; see also Figure 2.

The sludge bed and sludge blanket behaviour is reflected in the VSS profiles (Figure 3). At day 8 the sludge bed had only expanded from 0.37 to 0.42 m, because of the still low hydraulic loading and gas production rate. The sludge bed concentration amounted to 22 and the sludge blanket concentration to only 0.7 $kgVSS.m^{-3}$.

However, at higher loading and gas production rates the sludge bed expanded to 80 % of the reactor height, resulting in an average sludge bed concentration of 7 $kgVSS.m^{-3}$ and a sludge blanket concentration of 1.85 $kgVSS.m^{-3}$ at day 53.

Around day 40 the first sludge granules were detected. These grey-white round particles at that time were approximately 0.5 mm in size and showed a very low ash content of only 10 % of the dry matter. They were more or less uniformly dispersed over the sludge bed at day 53, but in the course of the next 4 weeks they gradually concentrated in the lower part of the reactor constituting there a 'granular sludge' bed with a sludge concentration of 17.5 $kgVSS.m^{-3}$. The sludge blanket concentration had increased to 3.5 $kgVSS.m^{-3}$ (Figure 3; day 80).

The amount of sludge retained in the reactor decreased slowly until day 50, beyond which day a slight increase could be noted (Figure 4).

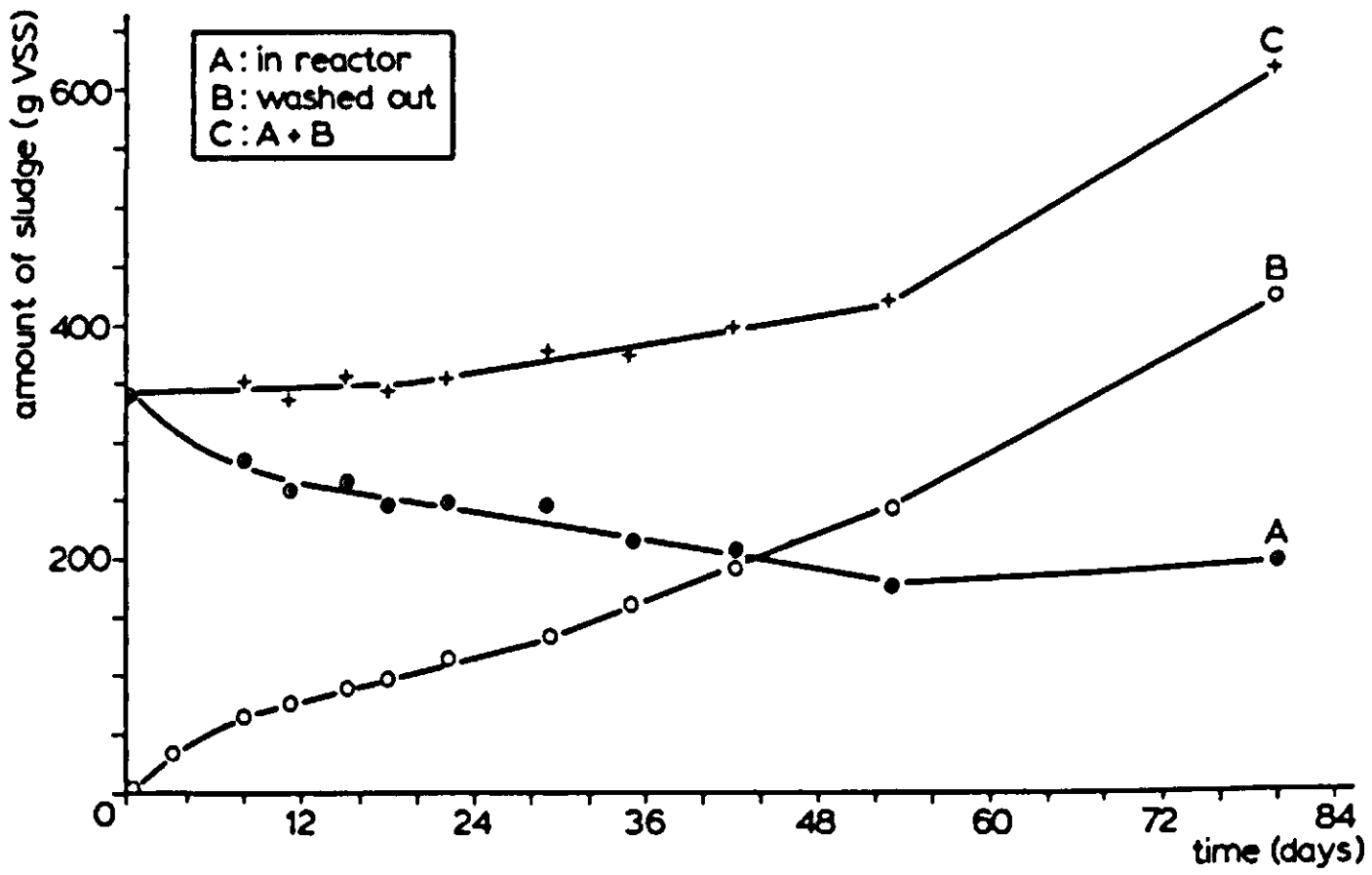


FIGURE 4 Development of the total amount of sludge in the reactor (A), the cumulative amount of washed out sludge (B) and the sum of both (C). Experiment 'b'; see also Figures 2 and 3.

The specific activity of the retained sludge increased gradually from 0.03 to 1.8 $kgCH_4\text{-}COD.kgVSS^{-1}.d^{-1}$.

As a result the gas production rate reached 11.5 $kgCH_4\text{-}COD.m^{-3}.d^{-1}$ at day 75.

7.3.2 TYPE AND QUANTITY OF SEED SLUDGE

7.3.2.1 Type of seed sludge and sludge wash-out

As reported in paragraph 4.3.2, seeding the reactor with a highly concentrated digested sewage sludge, leads to a substantial loss of sludge during the first days of the start-up due to the wash-out of 'non-settleable' solids (see Table 2 of Chapter 4 and Figure 5 of this chapter; experiment B). With that a considerable part of the methanogenic activity is lost as well (see Table 4 of Chapter 4). The remaining sludge exhibits a fairly good settleability. This is reflected in the moderate expansion of the sludge bed during the first weeks in the experiment of Figure 3.

In contrast, less concentrated types of digested sewage sludge (as used in experiment A of Figure 5) are much more homogeneous regarding their settling characteristics. Little sludge wash-out occurs during the first days of operation and little methanogenic activity is lost (see Tables 2 and 4 of Chapter 4 and Figure 5 of this chapter).

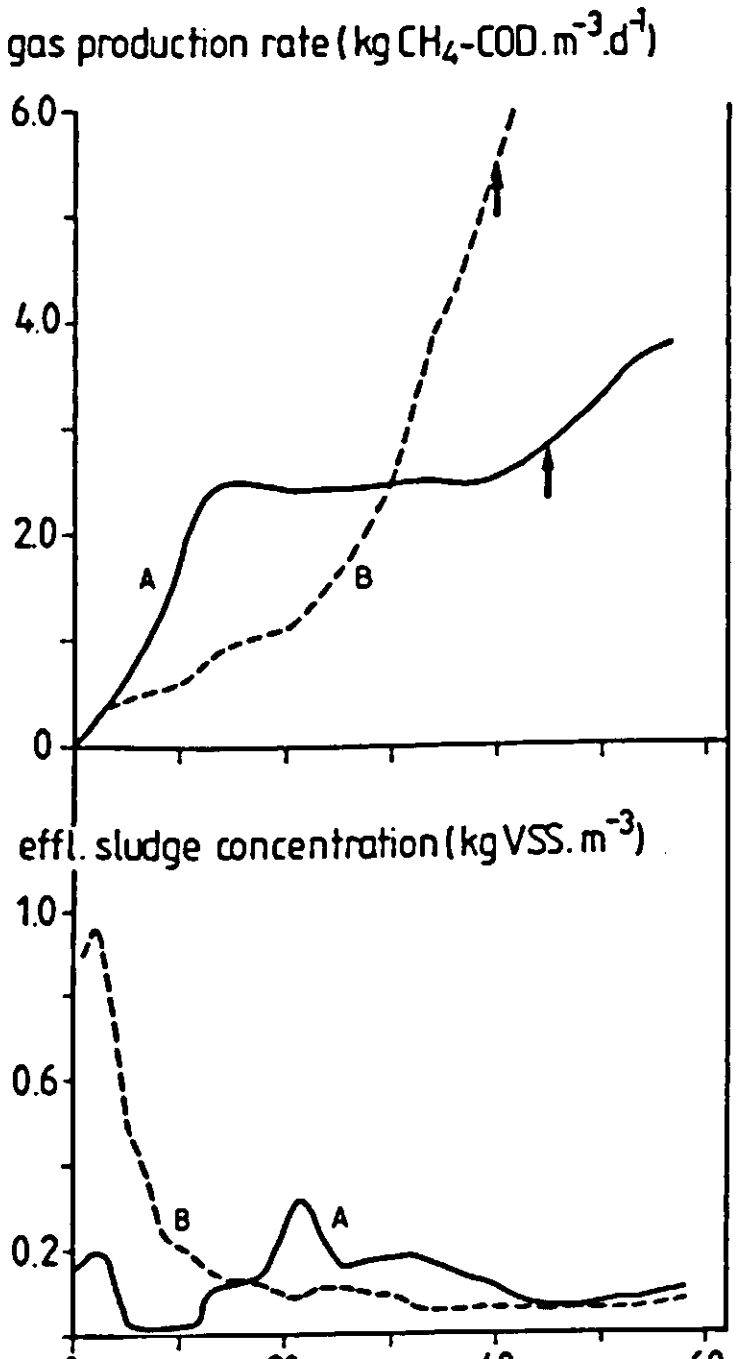


FIGURE 5
The gas production rate and the effluent sludge concentration during start-up of UASB reactors seeded with different types of digested sewage sludge.
(A): seeded with 10 kgVSS.m^{-3} of sludge type 'E10' (original conc.: 43.5 kgDSS.m^{-3}),
(B): seeded with 11.5 kgVSS.m^{-3} of sludge type 'F3' (original conc.: 79 kgDSS.m^{-3}).
Feed: mixture of acetate and propionate (3 kgVFA-COD.m^{-3}).
Arrows indicate the appearance of macroscopic granules.

The overall inferior settleability and the comparatively high methanogenic activity of the retained sludge in the latter case, cause a rapid expansion of the sludge bed into the settler, and consequently a wash-out of part of the sludge (Figure 5; experiment A from day 11 onwards).
The latter mechanism of sludge wash-out is called here 'expansion wash-out', because it is brought about by expansion of the sludge bed out of the reactor. The former type is named 'erosion wash-out', because it refers to the wash-out of sludge particles originating from the sludge bed, provided that the top of the sludge bed is still well below the settling compartment.

As reported in paragraph 4.3.2, the sludge lost during the first days of the start-up by erosion wash-out has a higher VSS content (see Table 3 of Chapter 4) and a higher specific methanogenic activity (see Table 4 of Chapter 4), than the retained sludge.
In Table 4 a comparison is made between expansion and erosion wash-out with respect to the differences in VSS content of retained and washed out sludge.

TABLE 4 THE VSS CONTENT OF SLUDGE WASHED OUT FROM THE REACTOR THROUGH EROSION AND EXPANSION WASH-OUT RESPECTIVELY AS COMPARED TO THE VSS CONTENT OF THE RETAINED SLUDGE

exp. (1)	type of wash-out	day no (2)	VSS content of sludge:	
			retained in reactor (% of DSS)	washed out from reactor (% of DSS)
b1	erosion	15	68.1	72.1
		21	66.8	72.8
		30	67.2	73.0
h2	expansion	15	64.3	64.3
		21	65.0	65.5
		30	65.7	65.9

(1) Codes refer to Table 2.
(2) Number of days elapsed since start of experiment.

Digested manure as seed sludge

Digested cow manure (7.1 % total solids, 4.8 % volatile solids) has been used as UASB-reactor seed sludge in the treatment of a VFA-solution (2.7 $kgCOD.m^{-3}$). The reactor was seeded with 15 $kgVS.m^{-3}$. A distinct sludge bed

washed out as a result of sludge bed erosion and severe wash-out was still going on. At that time 4 times the reactors' volume of wastewater had been treated. The specific activity of the retained sludge had increased from 0.01 at the start to 0.05 $kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$. The gas production rate amounted to 0.3 $kgCH_4$-COD.$m^{-3}.d^{-1}$.

From these preliminary results it appears that digested cow manure behaves in a way similar to concentrated digested sewage sludge, and that it can be used as UASB-reactor seed sludge (see also Wiegant 1983). However, the start-up with digested cow manure will take longer than with digested sewage sludge.

7.3.2.2 Quantity of seed sludge

Obviously, too little seed sludge will needlessly delay reactor start-up, whereas too much seed sludge will lead to heavy sludge wash-out from the reactor.

With the same type of digested sewage sludge (type "E"; see Table 2) a number of comparative experiments were performed to assess the effect of the initial seed concentration upon the rate of reactor start-up (Table 5 and Figure 6). The specific methanogenic activity of this sludge was 0.17 $kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$.

TABLE 5 EFFECT OF THE AMOUNT OF SEED SLUDGE UPON UASB REACTOR START-UP TREATING A VFA MIXTURE

exp. (1)	influent VFA-COD conc. ($kg.m^{-3}$)	average seed sludge conc. ($kg.m^{-3}$)	after 30 days: (2)		after 80 days: (2)	
			average sludge conc. ($kg.m^{-3}$)	specific sludge activity (3)	average sludge conc. ($kg.m^{-3}$)	specific sludge activity (3)
k	11.5	5.9	4.8	0.63		
h1	11.2	7.5	5.1	0.60		
h2	11.2	15	5.8	0.55		
g1	4.8	7.5	4.4	0.70		
g2	5.3	15	4.4	0.75		
j1	1.0	7.5	4.4	0.55		
j2	1.0	15	4.6	0.47		
q1	6.4	4.6	3.0	0.43	2.8	1.2
q2	6.4	9.2	6.4	0.29	3.7	0.6

(1) Codes refer to Table 2. Experiments "q1" and "q2" are performed with digesting instead of digested sewage sludge.

As appears from Table 5, the amount of sludge present in the reactor after a 30 days start-up period hardly depends upon the initial seed sludge concentration. An initial average sludge concentration of 15 kgVSS.m^{-3} obviously was unnecessarily high, because the major part of the sludge was lost due to sludge bed expansion. Similar or even slightly better results were obtained when starting with 6 to 7.5 kgVSS.m^{-3} of seed sludge (Figure 6).

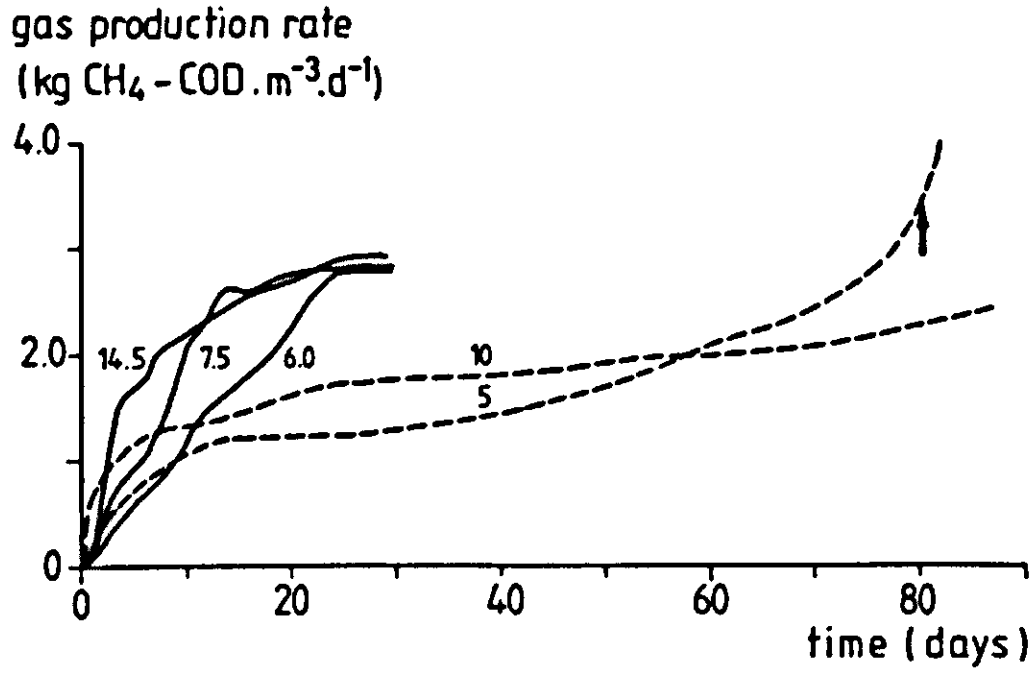


FIGURE 6 Gas production rates obtained in UASB reactor start-up experiments with different quantities of seed sludge (indicated by the numbers and expressed as kgVSS.m^{-3}).
(——): exp.'h2','h1','k'.
(---): exp. 'q1', 'q2'.
Codes refer to Table 2.
Arrow marks the time of the first appearance of macroscopic granules.

Two experiments ("q1" and "q2") were performed with actively digesting sewage sludge (instead of digested sewage sludge) obtained from the same sewage treatment plant. The specific methanogenic activity of this sludge was 0.20 kgCH_4-COD.kgVSS^{-1}.d^{-1}.
Figure 6 shows that an average seed sludge concentration of 4.6 kgVSS.m^{-3} eventually (beyond day 60) yielded better results, than a seed sludge concentration of 9.2 kgVSS.m^{-3}.
Sludge bed expansion into the settling compartment occurred from day 7 onwards in the experiment seeded with 9.2 kgVSS.m^{-3}. In the other experiment seeded with 4.6 kgVSS.m^{-3} this did not occur.

The experiments of Table 5 were all performed with rather dilute digested sewage sludge exhibiting a high specific methanogenic activity. Sludge bed expansion into the settler occurred already at initial average seed sludge concentrations as low as 6 kgVSS.m^{-3} when treating a waste with a concentration of 5 kgVFA-COD.m^{-3}.
When treating a very dilute waste of 1.0 kgCOD.m^{-3}, sludge bed expansion into the settler was just avoided in the reactor seeded with 7.5 kgVSS.m^{-3} of the same sludge (maximum bed expansion: 90 % of reactor height), but

When using a less active, more concentrated digested sewage sludge, more seed sludge can be used before expansion wash-out occurs. This was for instance observed in the experiment described in paragraph 7.3.1 ('b' in Table 2). Sludge bed expansion into the settler did not yet occur with an initial average seed sludge concentration of 11.5 $kgVSS.m^{-1}$ treating a VFA-solution with a COD concentration of only 2.8 $kg.m^{-3}$.

7.3.2.3 Effect of the addition of a small amount of active UASB reactor sludge to the seed

To enhance the rate of start-up the digested sewage sludge can be enriched with a small quantity of active UASB reactor sludge.

A start-up experiment with 12 $kgVSS.m^{-3}$ of digested sewage sludge with a low specific activity of 0.05 $kgCH_4$-$COD.kgVSS^{-1}.d^{-1}$ showed a very slow increase in the gas production rate (Figure 7; B). In a parallel experiment the same seed sludge was supplemented with 0.6 $kgVSS.m^{-3}$ of flocculent UASB-reactor cultivated sludge with a specific activity of 0.8 $kgCH_4$-$COD.kgVSS^{-1}.d^{-1}$ (Table 6).

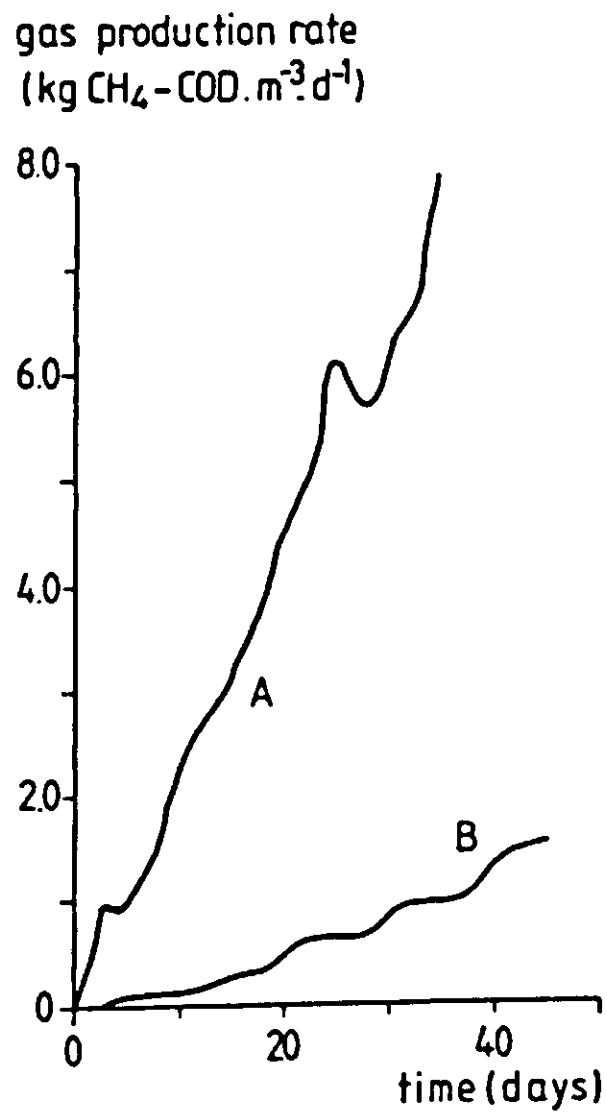


FIGURE 7
Gas production rates obtained in UASB reactor start-up experiments with (A) and without (B) the addition of a small amount of UASB reactor cultivated sludge to the digested sewage sludge seed (see Table 6).

This 5 % (VSS/VSS) addition of highly active sludge to the digested sewage sludge seed resulted in a fast reactor start-up (Figure 6; A). A gas production rate of 8 $kgCH_4$-$COD.m^{-3}.d^{-1}$ was attained within 35 days.

TABLE 6 THE EFFECT OF A 5 % ADDITION OF FLOCCULENT UASB-REACTOR CULTIVATED SLUDGE-VSS TO THE DIGESTED SEWAGE SLUDGE SEED IN UASB REACTOR START-UP (1)

exp. (2)	seed sludge concentration		situation after 35 days:	
	as digested sewage sludge ($kgVSS.m^{-3}$)	as active UASB-reactor sludge ($kgVSS.m^{-3}$)	average sludge conc. ($kgVSS.m^{-3}$)	specific sludge activity (3)
A	12.0	0.6	10	0.8
B	12.0	0	7.1	0.14

(1) The digested sewage sludge (type "F3") had been stored unfed at 4 °C for 180 days prior to use. The flocculent sludge had been washed out from the UASB reactor of experiment "b".
(2) A = exp. "c2" and B = exp. "c1"; see Table 2.
(3) Specific sludge activity expressed as $kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$.

7.3.2.4 Effect of unfed storage of seed sludge prior to use

Storage of digested sewage sludge without feeding prior to its use results in an increase of the gas production lag phase in batch experiments (see paragraph 5.3.1.2).
The effect of unfed storage of seed sludge upon UASB-reactor start-up is illustrated in Figure 8 and Table 7.

TABLE 7 EFFECT OF PROLONGED UNFED STORAGE OF DIGESTED SEWAGE SLUDGE PRIOR TO USE AS SEED SLUDGE IN UASB REACTOR START-UP EXPERIMENTS

exp. (1)	unfed storage (days)	average sludge concentration ($kgVSS.m^{-3}$)			spec. sludge activity ($kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$)	
		at start	day 28	day 40	day 28	day 40
A	2	14.5	5.0	-	0.65	-
B	280	15	15	-	0.2	-
C	14	11.5	-	7	-	0.75
D	180	12	-	11.5	-	0.12

(1) A = "g2", B = "a", C = "b" and D = "c1" (see Table 2).

With both types of digested sewage sludge tested, the reactor start-up with fresh seed sludge was considerably faster, than with sludge stored unfed for prolonged periods of time (Figure 8). It takes 4 to 6 weeks for the

stored sludge to attain a specific activity comparable with the initial specific activity of the fresh sludge. In both cases the sludge retention of the pre-stored sludge is better than that of fresh sludge at comparable gas production and hydraulic loading rates (Table 7).

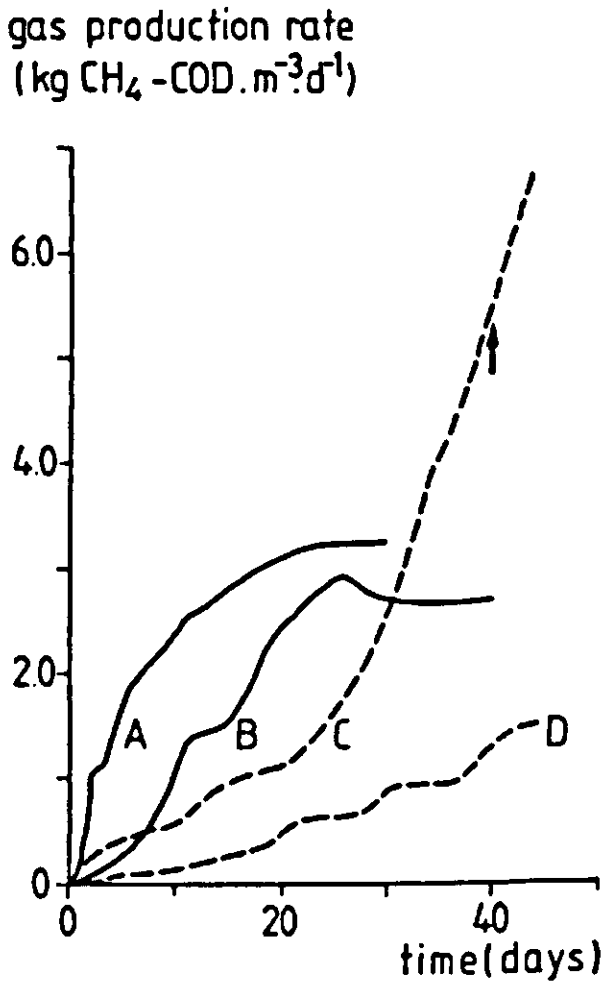


FIGURE 8
Gas production rates obtained in start-up experiments with UASB reactors seeded with fresh (A and C) and old digested sewage sludge (B and D). For details see Table 7. Arrow marks the time of the first appearance of macroscopic sludge granules.

7.3.3 EFFECT OF THE CONCENTRATION AND COMPOSITION OF THE FEED

7.3.3.1 Effect of the substrate concentration

The substrate concentration may influence the rate of reactor start-up in two ways. First a very high concentration may cause substrate inhibition. In the present work the reactor concentrations of the VFA were kept low by maintaining a high removal efficiency.
Secondly, the substrate concentration is inversely proportional to the hydraulic loading rate at a given organic loading rate. The selection pressure exerted on the sludge particles will be greater at a higher hydraulic loading rate.

As appears from the results obtained with type "E" seed sludge (Table 8), equal amounts of sludge are retained after a 30 days start-up period using substrate concentrations of 1.0 and about 5 kgVFA-COD.m^{-3} respectively. The specific activity of the retained sludge, however, is different indicating different degrees of selection. Due to the 5 times higher hydraulic loading

rate in the case of the 1.0 $kgCOD.m^{-3}$ feed, the wash-out of dispersed bacterial growth and small sludge flocs will have been greater, than in the experiment with the 5 $kgCOD.m^{-3}$ feed solution.

TABLE 8 EFFECT OF THE SUBSTRATE CONCENTRATION ON THE RATE OF UASB REACTOR START-UP

exp.	substrate VFA-COD concentr.	seed sludge		situation after 30 days:		
		type	average VSS conc.	average VSS conc.	specific sludge activity	hydraulic loading rate
(1)	($kg.m^{-3}$)	(2)	($kg.m^{-3}$)	($kg.m^{-3}$)	(3)	($m^3.m^{-3}.d^{-1}$)
j2	1.0	E	14.5	4.6	0.47	4.15
g2	5.3	E	14.5	4.4	0.75	0.86
h2	11.2	E	14.5	5.8	0.55	0.34
j1	1.0	E	7.5	4.4	0.55	4.22
g1	4.8	E	7.5	4.4	0.70	0.76
h1	11.2	E	7.5	5.1	0.60	0.36
b1	2.8	F	11.5	7.8	0.32	1.10
1	11.3	F	9	7.5	0.08	0.10

(1) Codes refer to Table 2.
(2) Codes refer to Table 1 of Chapter 4.
(3) Specific sludge activity expressed as $kgCH_4$-$COD.kgVSS^{-1}.d^{-1}$.

When using a COD concentration of 11.2 $kg.m^{-3}$, i.e. when operating the system at a lower hydraulic loading rate, a slightly higher sludge retention after 30 days of operation was achieved.

In the case of type "F" digested sewage sludge as seed material, the start-up proceeded without problems with a waste containing 2.8 $kgVFA-COD.m^{-3}$, whereas the start-up using a COD concentration of 11.3 $kgVFA-COD.m^{-3}$ showed no further increase in the gas production rate after 12 days of operation (Figure 9). The hydraulic loading rate in the latter case was still very low (Table 8). Hardly any selection through sludge wash-out can be expected at a hydraulic retention time of 10 days. In this situation dispersed bacteria can grow as fast as, or faster than they are washed out. A pseudo-steady state situation may thus be created with a retention time of the dispersed bacteria equalling the liquid retention time.

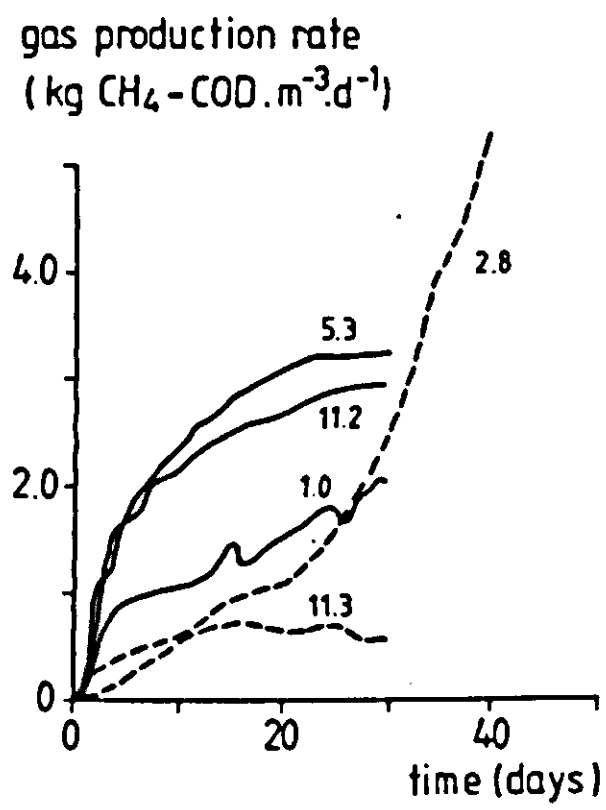


FIGURE 9
Gas production rates obtained in UASB reactor start-up experiments with different VFA-substrate concentrations (as indicated by the numbers; expressed as kgVFA-COD.m^{-3}). For details see Table 8). (——): seeded with 14.5 kgVSS.m^{-3} of sludge type 'E', (---): seeded with approx. 10 kgVSS.m^{-3} of sludge type 'F'.

7.3.3.2 Effect of the substrate composition

A mixture of acetate and propionate was used as substrate for the majority of the experiments performed in this study.A limited number of experiments was conducted using an acidifiable substrate, i.e. glucose or sucrose and skimmed milk powder.
Figure 10 shows the gas production rates obtained during reactor start-up using the same seed sludge, but different substrates.

TABLE 9 EFFECT OF SUBSTRATE COMPOSITION UPON UASB REACTOR START-UP

exp. (1)	substrate		average seed sludge-VSS conc. ($kg.m^{-3}$)	situation after 25 days:	
	composition (2)	COD conc. ($kg.m^{-3}$)		average sludge-VSS conc. ($kg.m^{-3}$)	specific sludge activity (3)
A	95% sucrose+VFA	3.0	13	11.5	0.2
B	10% sucrose+VFA	3.0	13	6	0.45
C	100% VFA	3.0	10	8	0.3
D	100% SMP	3.0	13	11	0.25

(1) A="r2", B="r1", C="n" and D="r3" (see Table 2).
(2) % sucrose = percentage of total COD supplied as sucrose.
VFA = mixture of acetate and propionate; 1.5 : 1 (mol/mol).
SMP = skimmed milk powder.
(3) Expressed as $kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$.

Adding 10 % (COD/COD) sucrose to the VFA feed seems to slightly enhance the rate of reactor start-up, although the difference may also be attributed to the difference in seed sludge concentration (compare B and C of Table 9 and Figure 10).

Reactor start-up experiments using a sucrose feed with only 5 % (COD/COD) VFA or an unacidified skimmed milk powder solution (data of the latter not shown in Figure 10) yielded similar gas production rates; without significant differences from those with 10 % or no sucrose-COD in the feed (Figure 10). However, the sludge retention in the experiments with sucrose and skimmed milk powder was considerably better, probably due to the growth of acidogenic bacteria (Table 9; A and D). Beyond day 40 (experiment A) and day 32 (experiment D) reactor start-up was interrupted by foaming problems.

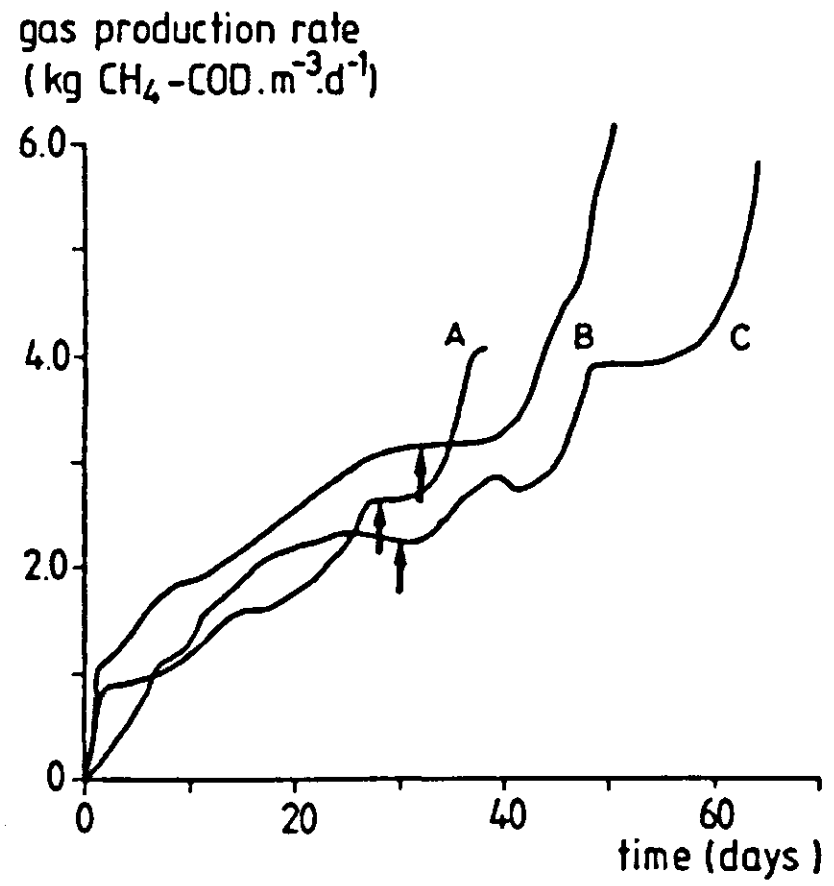


FIGURE 10 Gas production rates obtained in UASB reactor start-up experiments with a different substrate composition.
Feed concentration: 3.0 kgCOD.m^{-3}.
(A): 95% sucrose-COD + 5% VFA-COD,
(B): 10% sucrose-COD + 90% VFA-COD,
(C): 100% VFA-COD.
Arrows indicate the appearance of macroscopic granules.

7.3.3.3 Effect of sulphate and sulphide

In all UASB reactor start-up experiments presented in this chapter, sulphate was added to the feed as the sulphur source for bacterial growth, assuming that sulphide will become available through sulphate reduction.

Higher concentrations of sulphide considerably prolonged the gas production lag phase in batch experiments, whereas equivalent concentrations of sulphate did not (Chapter 5).
Results of UASB reactor experiments with increased sulphate and sulphide concentrations are shown in Figure 11 and Table 10.

The presence of 3 mM sulphate in the wastewater did not significantly influence the rate of reactor start-up. The slightly lower methane production rate in comparison with a blank can partly be accounted for by sulphate reduction (Figure 11; A and B). As appears from Table 10, sulphate reduction was not complete, although the molar ratio of propionate to sulphate of 8 would have permitted this.

TABLE 10 EFFECT OF ELEVATED LEVELS OF SULPHATE AND SULPHIDE IN THE FEED UPON UASB REACTOR START-UP

exp.	average seed sludge VSS conc.	influent		effluent		situation at end of exp.:	
		SO_4^{2-} conc.	S^{2-} conc.	SO_4^{2-} conc.	S^{2-} conc.	average sludge-VSS conc.	specific sludge activity
(1)	($kg.m^{-3}$)	(mM)	(mM)	(mM)	(mM)	($kg.m^{-3}$)	(2)
A	16	-	-	-	-	14.3	0.05
B	16	3.1	-	1.0	nd	13.7	0.06
C	14.5	3.1	-	0.8	2.0	13.5	0.17
D	14.5	-	2.8	-	2.5	14.3	0.02

(1) A="d3", B="d4", C="d2" and D="d1" (see Table 2).
(2) Specific sludge activity expressed as $kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$.

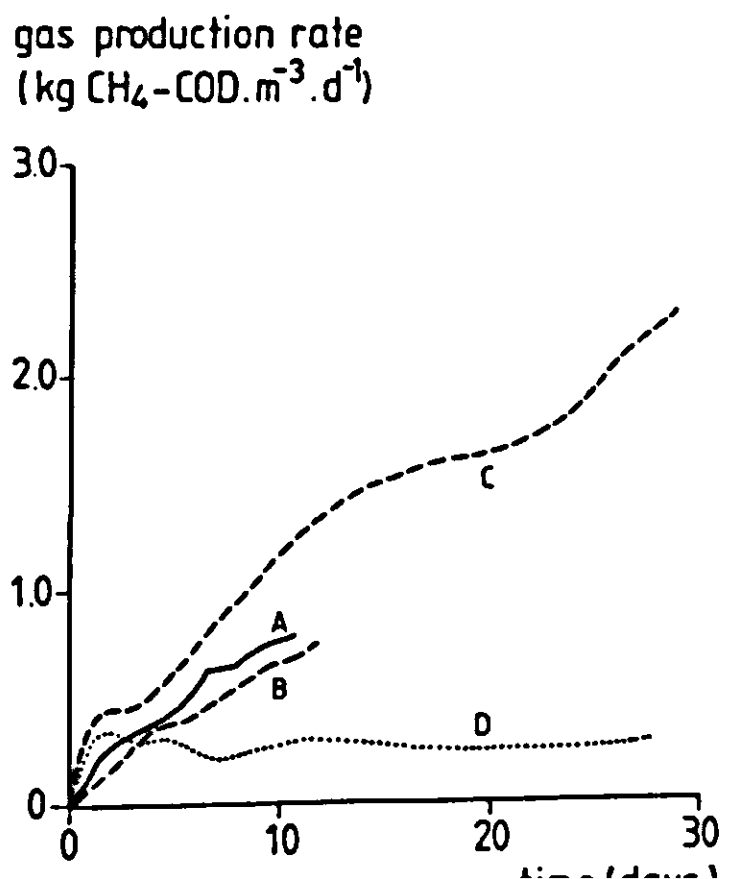


FIGURE 11
Gas production rates obtained in UASB reactor start-up experiments without (A) and with (B) 3 mM sulphate in the feed, and experiments with 3 mM sulphate (C) or 3 mM sulphide (D) in the feed. For details see Table 10.

Adding 3 mM sulphide (as K_2S) to the waste strongly inhibited the start-up (Figure 11; D). The sulphide was introduced directly into the lower part of the sludge bed independently from the substrate and nutrient solution.

7.3.4 EFFECT OF THE START-UP MODE

The UASB reactor start-up experiments presented in the previous sections were performed in such a way, that the effluent VFA concentration generally was kept below 1.0 to 1.5 $kgCOD.m^{-3}$ for the 3-5 $kgCOD.m^{-3}$ substrate concentration range, and below 2.5 $kgCOD.m^{-3}$ for a substrate concentration of 11 $kgCOD.m^{-3}$. These values correspond to a removal efficiency of 70 to 80 %. In the experiments with a dilute waste of only 1.0 $kgCOD.m^{-3}$, the effluent COD concentration was kept below 0.5 $kgVFA\text{-}COD.m^{-3}$.

In an experiment treating a mixture of acetate and propionate a comparison was made between this start-up mode with an average removal efficiency of 80 %, and a start-up procedure in which the effluent COD was maintained at a higher level, corresponding to an average removal efficiency of about 50 % (experiments C and D in Table 11 and Figure 12).

TABLE 11 UASB REACTOR START-UP MAINTAINING DIFFERENT EFFLUENT COD LEVELS

exp. (1)	seed sludge VSS conc. ($kg.m^{-3}$)	average COD concentration ($kg.m^{-3}$)		average sludge concentration ($kgVSS.m^{-3}$)		spec. sludge activity (2)	
		infl.	effl.	day 26	day 45	day 26	day 45
A	10	4.3	1.5	6.6	8.6	0.34	0.62
B	10	4.3	1.2	6.4	8.7	0.26	0.36
C	11.5	5.0	2.5	2.9	1.4	1.10	2.05
D	11.5	5.0	1.0	5.2	1.75	0.50	1.60

(1) A = "f4", B = "f3", C = "p2" and D = "p1" (Table 2).
(2) Specific sludge activity expressed as $kgCH_4\text{-}COD.kgVSS^{-1}.d^{-1}$.

Overloading turned out to give a faster reactor start-up . At day 26 an accidental loss of sludge from the overloaded reactor decreased its gas production rate (Figure 12; C). The rate of increase in the gas production rate before and after this incident, however, was significantly higher than in the reactor with 80 % removal efficiency (Figure 12; D). Significantly

the specific activity was far greater. It was not investigated whether the overloaded reactor could achieve a high removal efficiency at the same gas production rates (compare with experiments A and B). No microscopic observations were made.

A similar experiment with an acetate solution yielded similar results (Figure 12; A and B), in spite of the relatively small difference between the respective average effluent COD concentrations (Table 11; A and B). Both reactors (A and B) were equally overloaded during the initial 15 days of the start-up. The effluent concentration was 1.5 to 2.0 kgCOD.m^{-3}. After this period the start-up policy in the blank reactor (B) aimed at maintaining an effluent concentration well below 1.0 kgCOD.m^{-3}. This, however, was not achieved. The removal efficiency remained poor although the loading rate was only increased slowly. Even when maintaining a constant loading rate for some time the removal efficiency hardly improved. In the overloaded reactor (A) the loading rate was increased rapidly resulting in a concomitant increase in the gas production rate.

The difficulty in attaining a low effluent acetate concentration in the blank experiment (B) may be due to the fact that after the first 15 days of operation Methanosarcina-type bacteria with a high K_s-value for acetate conversion prevailed in both experiments.

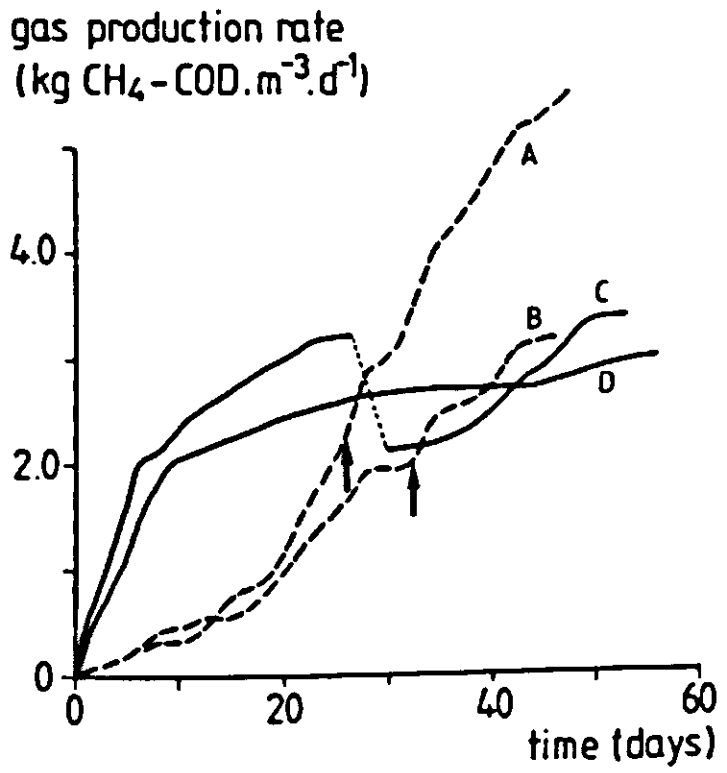


FIGURE 12
Gas production rates obtained in start-up experiments in which the reactor was overloaded deliberately (A and C) or in which a high removal efficiency was pursued (B and D). For details see Table 11.
(——): Feed: mixture of acetate and propionate (total COD of 5.0 kg.m^{-3}).
(---): Feed: acetate solution (4.3 kgCOD.m^{-3}).
Arrows indicate the appearance of macroscopic granules.

7.3.5 BIOMASS YIELD FACTORS

For a number of UASB-reactor start-up experiments the sludge balance was evaluated. The biomass yield factors calculated are listed in Table 12.

TABLE 12 BIOMASS YIELD FACTORS IN UASB-REACTORS DURING START-UP

experiments (1)	substrate		average biomass yield factor (2)
	composition (1)	COD conc. ($kg.m^{-3}$)	($kgVSS.kgCOD^{-1}$)
h1,h2,k,l	acetate+prop.	11.3	0.023 ± 0.006
g1,g2	acetate+prop.	4.8-5.3	0.018 ± 0.002
b	acetate+prop.	2.8	0.022
j1,j2	acetate+prop.	1.0	0.046 ± 0.007
e1,e2,f1-4	acetate	4.3	0.17 ± 0.09
r1	VFA+10% sucr.	3.0	0.04
r2	sucr.+5% VFA	3.0	0.13
r3	skimmed milk powder	3.0	0.15

(1) Codes refer to Table 2, which also comprises more details about the substrate composition (prop.=propionate, sucr.=sucrose).
(2) Calculated from sludge balances and the total amount of COD converted.

In spite of the big standard deviations certain meaningful observations can be made.

The biomass yield factor of approximately 0.02 $gVSS.gCOD^{-1}$ found for the digestion of a mixture of acetate and propionate is in accordance with the data from batch experiments (see paragraph 6.3.1).
The yield factor of 0.17 $gVSS.gCOD^{-1}$ found in the treatment of acetate solutions is very high, as was also the case in batch experiments.
COD-balance calculations confirmed both figures. In experiment "b" 3.1 % of the COD removed was converted into sludge-VSS, corresponding to a Y_{COD} of 0.022 $gVSS.gCOD^{-1}$. Similarly a Y_{COD} of 0.18 ± 0.08 $gVSS.gCOD^{-1}$ was found for the acetate digestion experiments. The conversion of Y_{COD} to Y_{VSS} was made on the basis of a VSS-COD of 1.42 $gCOD.gVSS^{-1}$ as had been measured for acetate digesting sludge.

It is interesting to note that the biomass yield factor of 0.046 $gVSS.gCOD^{-1}$ found for the digestion of a dilute acetate and propionate solution (1.0 $kgCOD.m^{-3}$) is distinctly higher than for more concentrated identical substrate solutions (Table 12). In fact, 85 to 88 % of the methane formed from the dilute substrate originated from acetate, because propionate conversion was rather poor in these experiments. In the

experiments with more concentrated wastes the removal efficiency of propionate was as good as that of acetate, and 77 % of the methane originated from acetate. So the sludge yield factor seems to increase when the converted substrate approaches pure acetate.

The high growth yields found for sucrose and skimmed milk powder solutions can be attributed to the growth of acidogenic bacteria.

7.3.6 SLUDGE PELLETIZATION AND RELATED MICROSCOPIC OBSERVATIONS

Four different types of sludge granules developed in UASB-reactor start-up experiments from digested sewage sludge as seed material and volatile fatty acids as substrate (Table 13). The first macroscopic granules were usually observed 30 to 45 days after the start of the experiments.
Three types (Table 13; A, C and D) consisted entirely of bacterial matter, whereas the fourth type (Table 13; B) contained an inert particle as a nucleus on which the bacteria attached.

Microscopic observation of sludge samples during the start-up experiments revealed the predominant proliferation of Methanothrix and Methanosarcina-type bacteria, as may be expected in the digestion of volatile fatty acids (Table 15).

TABLE 13 DESCRIPTION OF THE DIFFERENT TYPES OF MACROSCOPIC SLUDGE GRANULES FORMED IN UASB-REACTOR START-UP EXPERIMENTS TREATING A VFA-WASTE

A:	Compact granules mainly composed of rod-shaped bacteria resembling Methanothrix soehngenii (Hulshoff Pol et al. 1982). Good mechanical strength.
B:	Granules mainly consisting of loosely intertwined filamentous bacteria attached to an inert particle (Hulshoff Pol et al. 1982). The prevailing bacteria resemble Methanothrix soehngenii. Presumably approximately of the same mechanical strength as type A granules.
C:	Fragile granules composed of very loosely intertwined filamentous bacteria (resembling Methanothrix) containing no support material. Low resistance towards shear. Granules fall apart upon stirring or dewatering the sludge.
D:	Granules composed predominantly of Methanosarcina-type bacteria.

Only in two start-up experiments with acetate as sole carbon and energy source, in which a high reactor concentration was maintained, sludge granules developed consisting mainly of Methanosarcina-type bacteria (Table 14).

In the experiments with the non-VFA substrates large numbers of other bacterial species were also observed.

TABLE 14 FORMATION OF SLUDGE GRANULES IN UASB-REACTOR START-UP EXPERIMENTS USING DIGESTED SEWAGE SLUDGE AS SEED MATERIAL

exp. (1)	substrate (1)	situation at time of first observation of granules: time elapsed since start of experiment (days)	average sludge VSS conc. ($kg.m^{-3}$)	average sludge activity (3)	type of granules (2) A	B	C	D
b	ac.+prop.	40	7.0	0.79	x	(x)		
c2(4)	idem	33	10	0.8	x			
s2	idem	45	6.2	0.45		x		
s1	idem	45	5.9	0.49		x		
n	idem	30	5	0.45		x		
j	idem	30	4.5	0.51		x		
q2	idem	80	2.8	1.24			x	
f3	acetate	33	9.4	0.26				x
f4	idem	26	6.6	0.34	(x)			x
e1	idem	63	5.3	0.85			x	
e2	idem	41	4.3	0.82	x			
r1	VFA+10% s.	32	3.7	0.84		x		
r2	s.+5% VFA	28	11.5	0.41	x			
r3	sk.milk p.	31	7.5	0.44		x		

(1) Codes refer to Table 2. Table 2 also contains more details concerning the substrate composition (ac.=acetate, prop.= propionate, s.=sucrose, and sk.milk p.= skimmed milk powder).
(2) Granular sludge types are described in Table 13. When a second type of sludge granule is observed in the same experiment at a later date, it is indicated between brackets.
(3) Average sludge activity expressed as $kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$.
(4) The digested sewage sludge seed was supplemented with 5 % (VSS/VSS) of flocculent sludge washed out in experiment "b" (see paragraph 7.3.2.3).

The type of sludge granules formed (A, B or C) could be related to the type of seed sludge used (Table 16). Also the reactor start-up pattern depended upon the type of seed sludge used (Figure 13).

The start-up patterns shown in Figure 13 as well as the digested sewage

TABLE 15 MICROSCOPIC OBSERVATIONS DURING UASB-REACTOR START-UP

day no.	organic loading rate (1)	hydraulic loading rate ($m^3.m^{-3}.d^{-1}$)	sludge granules (2)	M.thrix-type filamentous bacteria (2)	M.sarcina-type bacteria (2)
Development of type A sludge granules (3)					
1	0.11	0.42	-	-	-
10	0.14	0.43	-	+	-
16	0.14	0.43	-	++	
31	0.41	1.33	-	+++	+
36	0.67	1.67		+++	
50	1.19	2.49	+	+++	+
58	1.23	2.53	++	+++	++
80	1.89	2.57	+++	++	+++
Development of type B sludge granules (3)					
1	0.03	0.12	-	+	-
13	0.23	0.80	-	++	-
30	0.40	1.03	-	+++	+
40	0.55	1.12	-	+++	+
49	0.70	1.40	+	+++	-
59	1.25	1.90	++	+++	+
74	2.15	2.05	+++	+++	-
Development of type D sludge granules (3)					
1	0.07	0.16	-	-	-
17	0.12	0.23	-	+	+
25	0.30	0.58	-	++	++
33	0.42	0.91	+	++	+++
59	0.55	1.23	+++	++	+++

(1) Expressed as $kgCOD.kgVSS^{-1}.d^{-1}$.
(2) -: not observed ++: many
+: some +++: very many
(3) For description of types of granules see Table 13.
Development of type A, type B and type D granules as observed in experiments "b", "s1" and "f3" respectively (see Table 2).

Figure 13 also shows the results of 2 start-up experiments using acetate as single substrate. They showed type A start-up patterns and type A granules developed (Table 15). The seed sludge used in the acetate experiments had an original sludge concentration of about 30 $kgVSS.m^{-3}$. The sludge had been stored unfed at 4 °C for 1.5 and 16 months prior to its use.

TABLE 16 MAIN CHARACTERISTICS OF THE 3 REPRESENTATIVE TYPES OF DIGESTED SEWAGE SLUDGE FROM WHICH 3 DIFFERENT TYPES OF SLUDGE GRANULES DEVELOPED USING A MIXTURE OF ACETATE AND PROPIONATE AS SUBSTRATE

original sludge conc.(1) ($kgVSS.m^{-3}$)	specific methanogenic activity (2)		type of granules (3)
	($kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$)	($kgCH_4$-COD.$m^{-3}.d^{-1}$)	
40	0.12	4.8	A
27	0.13	3.5	B
15	0.18	2.7	C

(1) As obtained from the sewage sludge digester.
(2) As determined in standard batch activity tests.
(3) As developed in UASB reactor start-up experiments. Initial seed sludge concentration approx. 10 $kgVSS.m^{-3}$.
Codes refer to Table 13.

A major difference was noted with respect to the sludge bed expansion in the start-up experiments with the 3 types of digested sewage sludge yielding sludge granules of type A, B and C, despite both the wastewater composition as well as the amount of seed sludge used (approx. 10 $kgVSS.m^{-3}$) were identical. Table 17 shows, that with sludge type B and C already during the first 2 weeks of the reactor start-up the sludge bed expanded into the settler compartment followed by increased sludge wash-out. The concomitant gas production rates were 2 and 1.2 $kgCH_4$-COD.$m^{-3}.d^{-1}$ respectively.

TABLE 17 SLUDGE BED HEIGHT (AS PERCENTAGE OF THE TOTAL REACTOR HEIGHT) DURING UASB-REACTOR START-UP EXPERIMENTS WITH DIFFERENT TYPES OF DIGESTED SEWAGE SLUDGE AS SEED SLUDGE (1)

type of sludge (1)	sludge bed height at the start of the experiment (% of total height)	maximum sludge bed height (% of total height)	concomitant gas production rate (2)
A	31	71 (at day 40)	5.2
B	39	>100 (at day 10-15)	2
C	33	>100 (at day 7)	1.2

(1) Type A, B and C seed sludge yield type A, B and C granules; see Table 16.
(2) Expressed as $kgCH_4$-COD.$m^{-3}.d^{-1}$.

In the start-up experiment with type A sludge, expansion of the sludge bed into the settler never occurred. The gas production rate at the time of maximum sludge bed expansion was 5.2 $kgCH_4$-COD.m^{-3}.d^{-1}.
In the acetate-experiments mentioned above the sludge bed did not expand into the settler compartment until around day 30 (concomitant gas production rate: 2 - 3 $kgCH_4$-COD.m^{-3}.d^{-1}).

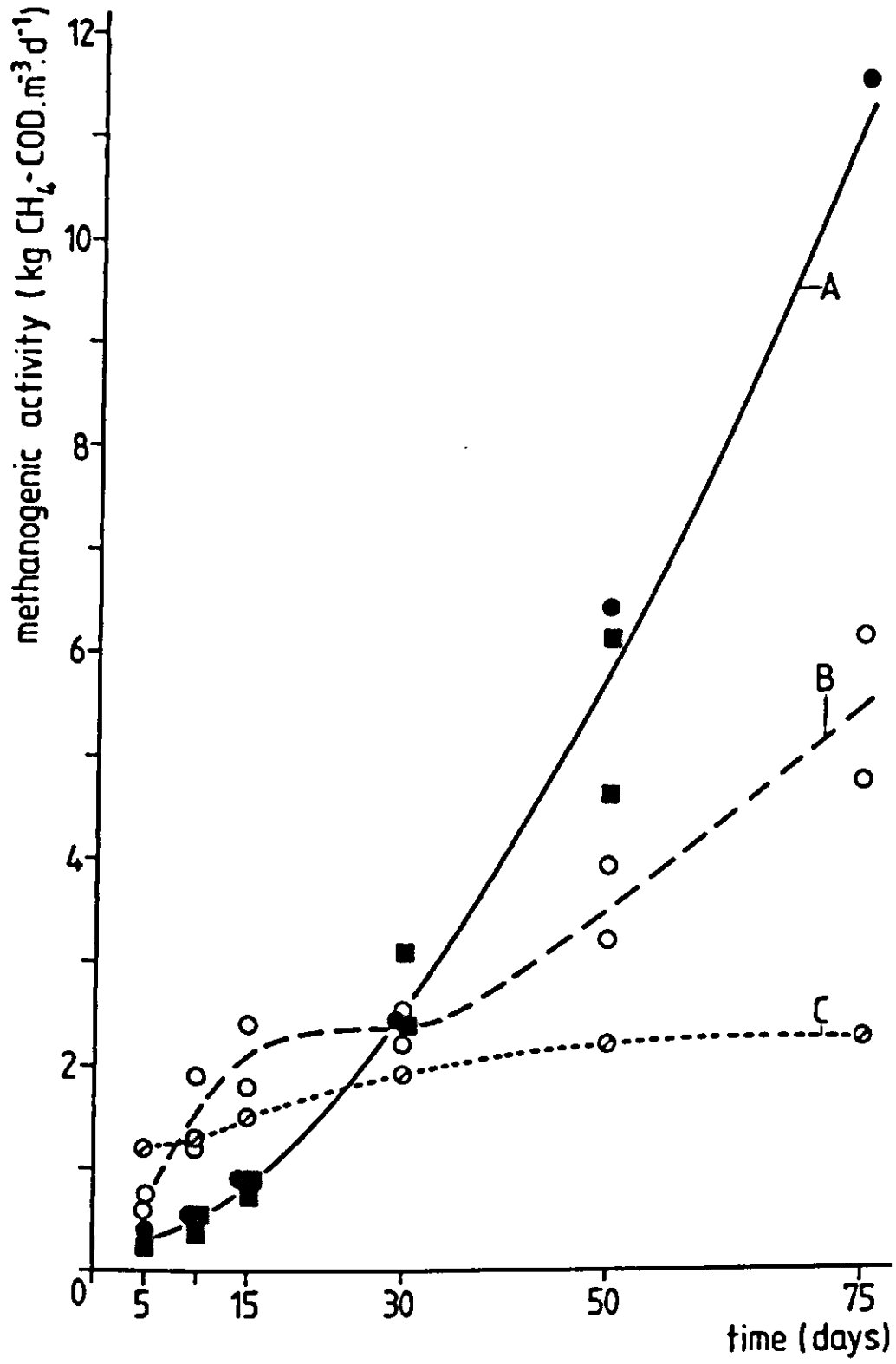


FIGURE 13 Development of the gas production rates in UASB-reactor start-up experiments using three different types of digested sewage sludge and yielding three different types of sludge granules. See Table 16.
(●,■; exp. b,e2,f4): type A seed sludge; type A granules,
(o; exp. n and s1): type B seed sludge; type B granules,
(ø; exp. q1): type C seed sludge; type C granules.
Codes of experiments refer to Table 2.
Squares denote results of start-up experiments using acetate as single substrate instead of a mixture of acetate and propionate as in the other experiments.

7.4 DISCUSSION

7.4.1 DIFFERENT STAGES IN UASB-REACTOR START-UP

All start-up experiments performed with digested sewage sludge as seed material showed basically the same pattern. This applies to changes in the average sludge concentration in the reactor, the applicable volumetric loading rate at a COD removal efficiency of about 80 %, as well as to the sludge methanogenic activity.
Roughly four stages can be distinguished.

During stage 1 the lightest, more or less 'colloidal' fraction of the digested sewage sludge is washed out from the reactor. The gas production rate increases rapidly from zero to the level corresponding to the specific activity of the residual sludge, provided a high enough organic loading rate is applied.

During stage 2 a moderate sludge wash-out continues due to sludge bed erosion exerting a mild selection pressure on the sludge particles. As a result of the combined effect of both bacterial growth and sludge wash-out, the average concentration of the retained sludge decreases, but the specific sludge activity increases.
Primarily as a result of the increasing gas production rate and also due to the increasing hydraulic loading rate, the sludge bed will expand and may eventually fill the entire reactor volume.

In some cases a stage 3 occurs, during which a further increase in the gas production rate (and to a lesser extent the hydraulic loading rate) causes the sludge bed to be pushed out of the reactor leading to a heavy wash-out of flocculent sludge (expansion wash-out). As a result of the loss of appreciable amounts of active biomass, the gas production rate may level off for some time. The specific activity of the retained sludge increases rapidly, because the loss with the effluent is a mixture of active and inactive sludge-VSS, whereas only active sludge-VSS grows in. The selection pressure on the sludge particles is much higher, than during previous stages.

reactor, thus rendering the remaining flocculent sludge into the sludge blanket.

This stage (stage 2 or stage 3) ends when no further decrease in the amount of retained sludge occurs, i.e. when the sludge growth equals the sludge wash-out.

During stage 4 the sludge growth occurs more and more on the heavier sludge particles in the sludge bed, and increasingly exceeds the wash-out of lighter sludge. As a result the total amount of sludge in the reactor begins to increase again, enabling a faster increase in the loading rate and consequently the gas production rate.
Due to the higher loading rates the selection pressure on the sludge particles increases further, and the remainder of the flocculent sludge in the sludge blanket eventually washes out. Sludge growth then exclusively takes place in the form of granular sludge. Sludge wash-out drops to a very low level.

7.4.2 FACTORS INFLUENCING THE RATE OF UASB-REACTOR START-UP

Amount of seed sludge and sludge wash-out

Two types of sludge wash-out are distinguished; sludge bed erosion wash-out and sludge bed expansion wash-out.
No lasting difference in the rate of reactor start-up was noted when applying different concentrations of seed sludge as long as it allowed for sludge bed expansion wash-out (paragraph 7.3.2.1 and 7.3.2.2).
To avoid excessive sludge wash-out, the amount of seed sludge should be small enough to maintain the sludge bed within the reactor, i.e. sludge bed expansion wash-out should be limited.

When using a concentrated digested sewage sludge type, more seed sludge can be applied, than when using a more dilute type of seed sludge (paragraph 7.3.2.2).

In the treatment of a dilute wastewater, sludge bed erosion wash-out will be greater, than in the treatment of a more concentrated waste.

Consequently more seed sludge can be applied in the former case before sludge bed expansion wash-out will occur.

Substrate concentration

If the substrate concentration is high, the hydraulic loading rate in the early stages of reactor start-up (at low organic loading rates) is low. The liquid retention time may be long enough to allow for the growth of dispersed bacteria with a growth rate equal to the dilution rate. Very little selection pressure is exerted on the sludge particles, and the start-up will stagnate or be extremely slow (paragraph 7.3.3.1).
Such a situation can be (partly) avoided by seeding with a more active seed sludge, or by recycling part of the effluent as long as the effluent suspended solids are not recycled.

A lower limit to the substrate concentration was not reached with respect to the feasibility of UASB-reactor start-up with digested sewage sludge as seed. No problems were encountered in treating a VFA solution with a COD concentration as low as 1.0 $kg.m^{-3}$.

Lag phases

No gas production lag phases were encountered in the UASB-reactor start-up experiments. In batch experiments (Chapter 5) a lag phase was found at sludge concentrations corresponding to a methanogenic activity of less than 0.5 $kgCH_4$-$COD.m^{-3}.d^{-1}$. This level was always surpassed in the UASB experiments.

Factors causing prolonged gas production lag phases in batch experiments like unfed storage of the seed sludge prior to its use, or the presence of a high sulphide concentration in the waste, slow down the rate of start-up of UASB-reactors (paragraph 7.3.2.4 and 7.3.3.3).

The addition of a small percentage of active UASB-reactor cultivated sludge to the digested sewage sludge seed decreased the gas production lag phase in batch experiments (Chapter 5) and strongly enhances the rate of UASB-reactor start-up. This is in accordance with results of Hulshoff Pol et al. (1983c).

7.4.3 SLUDGE YIELD FACTORS

The sludge yield factors found for the digestion of VFA mixtures in UASB-reactors are in accordance with those found in batch experiments (paragraph 6.3.1), and those reported in the literature (see Chapter 2, Table 2 and 4).

A very high yield factor of 0.17 $gVSS.gCOD^{-1}$ was found for the digestion of acetate as single carbon and energy source in UASB reactors. Occasionally even higher yield factors were reported in literature for the continuous digestion of acetate (Cappenberg 1975, Ghosh and Klass 1978).

In batch experiments showing a high sludge yield factor for acetate digestion (paragraph 6.3.1.1), the increase of the sludge concentration was not accompanied by an increase in the methanogenic activity of the culture. In contrast, the gas production rate (and consequently the number of bacteria) in the UASB-reactor experiments increased at a rate similar to that in experiments with a feed containing a mixture of volatile fatty acids or sucrose.

Presumably the high sludge yield factor found for the digestion of acetate should be attributed for the greater part to the formation of reserve material or exopolymers instead of to the formation of viable cells.

With respect to the mechanism underlying the development of sludge granules the presumed production of exopolymers by acetate converting methanogenic cultures deserves more research.

7.4.4 DEVELOPMENT OF GRANULAR SLUDGE

Three (A, B and C) of the four types of granules developing in UASB-reactor start-up experiments can be cultivated on the same substrate; a mixture of acetate and propionate (Table 13). The granules consisting of Methanosarcina-type bacteria (type D) were found exclusively in experiments in which the reactor concentration of acetate as single substrate was maintained above 1 $kgCOD.m^{-3}$.

The development of type A, B or C granules is apparently related to the reactor start-up pattern (Figure 13). In turn, differences in the start-up pattern are mainly dictated by the characteristics of the seed sludge used (Table 16).

The explanation for the development of particular types of sludge granules related to a particular start-up pattern can be found by comparing the average biomass retention times in the respective start-up experiments.
For this purpose, the amount of active biomass present in the reactor was calculated from the methane production rate. Based on results of VFA-fed batch experiments the maximum specific activity of pure active biomass was estimated at 2.85 $kgCH_4$-COD.$kgVSS^{-1}.d^{-1}$ (Table 5 of Chapter 6). This value is in accordance with literature data for Methanothrix (Huser 1981), which is the prevailing species in the granules, and it can be used, because during UASB-reactor start-up the substrate concentration is always well above the K_s level.
In combination with the biomass growth figures as calculated from the established yield factor of 0.024 g biomass-VSS.gCH_4-COD^{-1} (Table 1 of Chapter 6), a biomass balance calculation can be made from which the biomass retention time is estimated.

Figure 14 shows the average biomass retention time corresponding to the start-up patterns A, B and C of Figure 13. In each type a period occurs with an average biomass retention time of only 20 days. During this specific period wash-out of flocculent sludge takes place. This is due to either excessive expansion of the sludge bed, or to sludge bed erosion caused by high hydraulic and gas loading rates. This period is followed by a period of increasing biomass retention times as a result of the development of a granular sludge bed. As most experiments were terminated after the appearance of the first granules, this is only shown for one experiment (B).
Preceding the period of heavy wash-out of flocculent sludge the average biomass retention time exceeds 100 days. During this latter period the sludge bed still has room to expand in the reactor, and the loading rates are still too low to cause excessive wash-out of sludge through erosion of the sludge bed. The length of this initial period with an average biomass retention time of more than 100 days is about 40 days for start-up pattern A, compared to only 10 to 15 days for start-up pattern B. In start-up pattern C such a period could not be established, but it will have been less than 10 days.

In a well-mixed sewage sludge digester no selection pressure will be exerted on the sludge particles. Growth will take place in the form of

small sludge flocs or as freely dispersed bacteria. The latter will be washed out easily from an upflow reactor seeded with digested sewage sludge.

In UASB-reactors bacterial sludge growth can take place in 3 different modes, i.e. 1) as sludge flocs, 2) as sludge granules without carrier material, and 3) as sludge granules attached to supporting particles. These particles may originate from the seed sludge, or may be present in the wastewater as coarse suspended solids.

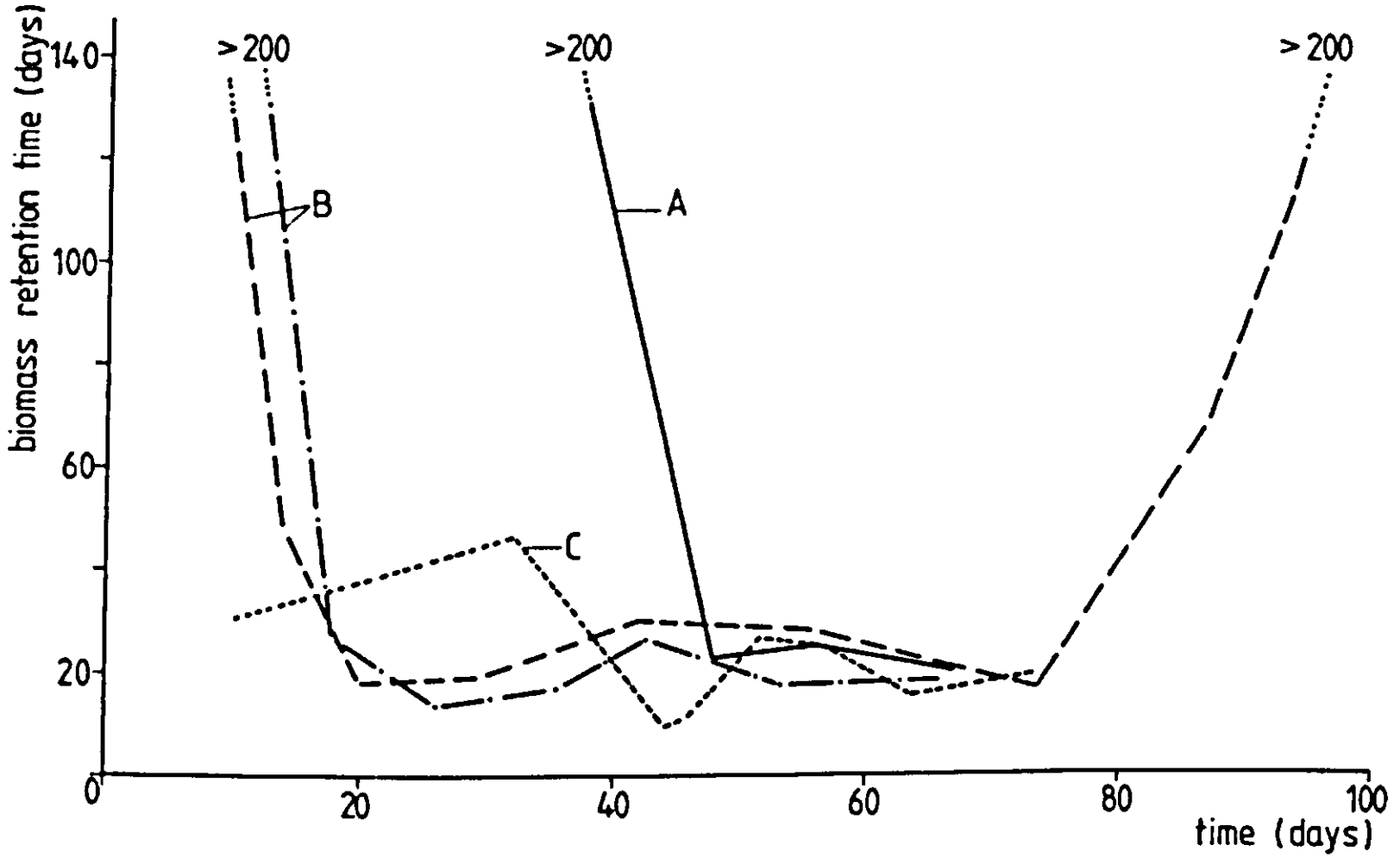


FIGURE 14 Average biomass retention time during UASB-reactor start-up with different types of seed sludge (see Table 17). For calculation see text. Seed concentration: approx. 10 $kgVSS.m^{-3}$. Feed: mixture of acetate and propionate (3-6 $kgCOD.m^{-3}$).
(A): seed sludge type A yielding type A granular sludge,
(B): seed sludge type B yielding type B granular sludge,
(C): seed sludge type C yielding type C granular sludge.

It is inherent to the low selection pressure at the modest loading rates prevailing during the initial phase of UASB-reactor start-up that the small flocs present in the seed sludge will grow rapidly and produce a so called 'bulking' anaerobic sludge. When a reactor has been seeded with much active flocculent sludge, as is the case when a relatively active digested sewage sludge type is used (start-up pattern B of Figure 13), little time is required for the sludge bed to expand out of the reactor at a gas production rate equivalent to about 2 $kgCH_4\text{-}COD.m^{-3}.d^{-1}$. Once heavy

wash-out of flocculent sludge has started (beyond day 10 to 15), the average biomass retention time becomes too short to allow for macroscopic bacterial granules to develop. Large conglomerates of bacteria can then be formed only through attachment to inert support particles, which are heavy enough to be retained longer (type B granules).

A similar explanation applies for the start-up pattern C (Figure 13). The digested sewage sludge used as seed sludge in this case has an even higher specific sludge activity at the start, than in case B. Wash-out of flocculent sludge through sludge bed expansion starts here almost from the beginning of the experiment and remains on virtually the same level. This brings on a more or less steady state of growth and wash-out of flocculent sludge. Eventually granule-like flocs devoid of inert material develop (type C; Table 13), as the sludge-VSS at last consists almost entirely of bacterial matter and some conglomerates of predominantly Methanothrix become large enough to be preferentially retained. As wall-growth occurred in the experiments in which type C granules developed, these conglomerates may have been sloughed off of the reactor wall. Type C granules have never been observed in full scale UASB-reactors, probably because they are too fragile.

Only in the case of start-up pattern A the initial period, with an average biomass retention time of more than 100 days, is evidently long enough (about 40 days) to allow for the development of firm sludge granules which consist exclusively of bacterial matter.

Sludge granules of type A, B and D first appear in a macroscopic form (diameter 0.5 mm) after 30 to 45 days of reactor start-up treating a VFA feed (Table 18).

Type D granules consist almost exclusively of bacteria resembling Methanococcus mazei. From the reported growth rate of this organism on 1 % acetate of 1.0 d^{-1} (Mah 1980), one can calculate, that at an average acetate concentration of 1.0 $kg.m^{-3}$ it will indeed take roughly one month to form a macroscopic granule when starting from a single bacterium. However the bulk of the bacteria constituting the granules of type A and B resemble Methanothrix söhngenii. The growth rate of a pure culture of Methanothrix söhngenii at 30 °C is 0.14 d^{-1} (Huser et al. 1982); being about

macroscopic granule starting from a single bacterium would then be in the order of 140 days. The fact that macroscopic granules appear after 30 to 45 days of start-up may imply either, that the growth rate of Methanothrix söhngenii in mixed cultures is much higher than in pure cultures or, that a different species is involved or, that microscopic granules (consisting of up to 500.000 bacteria) are already present in the seed sludge. The latter, however, has never been observed.

TABLE 18 RELATION BETWEEN THE DURATION OF THE INITIAL PERIOD WITH AN AVERAGE BIOMASS RETENTION TIME EXCEEDING 100 DAYS, AND THE TYPE OF MACROSCOPIC SLUDGE GRANULES FORMED

initial period with an average biomass retention time exceeding 100 days (days)	type of granules formed after 30-45 days of start-up (1)
40	A
37	A
30	D
26	D
14	B
11	B
10	B

(1) Codes refer to Table 13.

Hulshoff Pol et al. (1983c) have found that the addition of a small amount of ground type A granules to a digested sewage seed sludge, which normally yields a sludge bed of type B granules invariably leads to the development of a sludge bed of type A granules without altering the start-up pattern as far as sludge wash-out is concerned. By adding fragments of granules, which still consist of fairly large conglomerates of bacteria, the initial period with a high biomass retention time required for the development of macroscopic type A granules is obviously reduced from about 40 to 10-15 days.

Hulshoff Pol et al. (1983a) reported that no major differences could be established between type A and type B granular sludge with regard to settleability and specific methanogenic activity.
Nevertheless, a reactor start-up leading to the development of type A granules has some advantages over a start-up yielding a sludge bed with

type B granules.
In the latter case the sludge wash-out during the start-up is higher. Consequently the start-up will last longer (up to 30 % in the present experiments; see Figure 13 and Table 19). The minimum amount of sludge retained in the reactor at the time the first granules have developed is smaller. This may aggrevate problems with regard to substrate-biomass contact, because of short-circuiting of the shallow sludge bed. Also one has to depend upon the availibility of the proper support particles in the seed sludge or the waste.
These drawbacks may be overcome by the addition to the seed sludge of specific support particles, to which methanogenic bacteria attach easily (Hulshoff Pol et al., unpublished results).

The results discussed here apply primarily to the treatment of VFA substrates; i.e. completely acidified wastewaters. In the case of a one step anaerobic treatment of largely unacidified wastewaters the acid forming bacteria may also contribute to the formation of granular sludge (Hulshoff Pol et al. 1983b). In view of their far higher growth rates they may enhance the development of type A granular sludge even at low initial biomass retention times. This is illustrated by the start-up experiment with a type B digested sewage sludge as seed sludge, and a sucrose containing wastewater with a sucrose-COD/VFA-COD ratio of 95 : 5 (Table 14; experiment "r2").

7.4.5 MINIMUM START-UP TIME

From the growth rate of 0.07 d^{-1} found for growth on VFA (see Chapter 6) a theoretical minimum start-up time of a VFA-fed UASB reactor seeded with digested sewage sludge can be predicted to be in the order of 35 days, assuming complete biomass retention, an initial average reactor activity of 1 $kgCH_4$-COD.m^{-3}.d^{-1}, and defining as a goal for the first start-up a space activity of 10 $kgCH_4$-COD.m^{-3}.d^{-1}.

The actual start-up time of an UASB-reactor is longer (Table 19), because not all of the newly grown biomass is retained. Especially during the early stage of reactor start-up with a low selection pressure, a part of the growth will be in a flocculant form which will inevitably be lost at a later stage when the selection pressure is increased.

TABLE 19 THEORETICAL MINIMUM START-UP TIME AND ACTUAL START-UP TIME OF A VFA-FED (LABORATORY) UASB-REACTOR

	start-up time (1) (days)
theoretical (2)	35
granular sludge type A (3)	60
granular sludge type B (4)	80

(1) Time needed to reach a gas prod. rate of 10 $kgCH_4$-COD.m^{-3}.d^{-1}.
(2) For calculation see text.
(3) Start-up experiment "b". (4) Start-up experiment "n".

The start-up of other reactor types based on attached growth of the biomass like the fluidised bed reactor and other fixed film reactors is potentially faster, because the growth of suspended or flocculant bacterial matter is minimized from the beginning. For a pilot scale fluidised bed reactor Heijnen (1983) reported a COD conversion rate of 10 $kgCOD.m^{-3}.d^{-1}$ after 25-30 days of start-up at 37 °C. The prevailing bacterium in the biofilm resembled Methanothrix söhngenii.

The start-up of UASB reactors seeded with digested sewage sludge can be significantly accelerated by adding a few percent of the VSS in the form of UASB cultivated sludge (Hulshoff Pol et al. 1983c). Similar observations were made in the present work (paragraph 7.3.2.3).

CHAPTER 8 A DESCRIPTIVE MODEL OF UASB-REACTOR START-UP AND GUIDELINES FOR PRACTICAL APPLICATION

8.1 INTRODUCTION

Based on the results presented in the previous chapters a descriptive model of the processes controlling UASB-reactor start-up has been developed.
In this chapter the decisive microbiological processes are discussed first. As this study was directed primarily towards technological aspects, some of the microbiological claims are hypothetical, or at least require further confirmation, because they are based on only a small amount of data.
Secondly, the overall start-up process will be evaluated with respect to the wastewater characteristics and the choice of the seed sludge.
In conclusion guidelines are given for a successful start-up of UASB-reactors using digested sewage sludge as seed.

8.2 MICROBIOLOGICAL ASPECTS OF UASB REACTOR START-UP

In the treatment of VFA substrates, two bacterial genera seem of predominant importance with respect to UASB-reactor start-up and the development of granular sludge; i.e. Methanothrix and Methanosarcina. This is not surprising, because acetate is the precursor of 70 to 75 % of all methane, and both Methanothrix and Methanosarcina are acetate converting methanogens.
Of these two, Methanothrix occurs most frequently. Usually it represents the bulk of bacteria present in UASB reactor sludges.
In the treatment of more complex substrates acidogenic bacteria - many of which exhibit good floc-forming properties - may predominate.

Methanosarcina

Within the framework of this study no distinction is made between Methanosarcina and Methanococcus mazei (Mah 1980), which is assumed to be a morphotype of Methanosarcina.

Methanosarcina is well known for its ability to produce clumps of bacteria (Zhilina 1976). The tendency of Methanosarcina to cluster is independent of the selection pressure put on the system and also occurs in batch reactors

with complete sludge retention. The clumps can reach macroscopic dimensions (type D granules), and show cavities, which can be inhabited by other species (Zhilina 1976, Bochem et al. 1982).

Presumably type A granules (see Table 14 of Chapter 7) develop from Methanosarcina clumps of which the hollow centre becomes inhabited by Methanothrix bacteria. Their respective substrate affinity factors would favour such a spatial distribution. In fact large numbers of Methanosarcina bacteria were observed in the surrounding solution of young type A granules. Ultra-microtomic slices of these granules revealed the presence of clusters of embedded Methanosarcinas close to the periphery, whereas no Methanosarcinas were observed in the central part. Obviously the outer layer of Methanosarcina is easily lost once the granules attain macroscopic dimensions. In later stages of the growth and multiplication of type A granules Methanosarcina apparently is no longer involved.

Methanosarcina is not known to attach easily to alien surfaces. Therefore its retention in upflow reactors depends upon the settleability of the clumps, or - as long as Methanosarcina still occurs as clusters of only a few cells - upon the dilution rate, which should be smaller than the growth rate. The maximum growth rate is approximately 1.0 d^{-1}. However, the acetate concentration in the reactor during start-up normally will not exceed 1.0 $kg.m^{-3}$, and mostly be far lower when treating low and medium strength wastewaters at an acceptable removal efficiency. As the K_s value of Methanosarcina is high (i.e. 0.3 $kg.m^{-3}$) the actual growth rate will be much lower than 1.0 d^{-1}. In UASB-reactors during start-up the dilution rate will initially be less than 0.5 d^{-1}. When the loading rate of the reactor is increased, the dilution rate also increases and will soon become more than 1.0 d^{-1} depending on the strength of the wastewater. Only Methanosarcina clumps that have grown large enough to settle will be retained. Single cells or small clusters of Methanosarcina bacteria will then be washed out of the reactor.

In sewage sludge digesters the acetate concentration normally is very low, i.e. about 0.03 $kg.m^{-3}$ (Kaspar 1977). Consequently only few Methanosarcina bacteria are present in digested sewage sludge.

Methanothrix

Methanothrix has a high substrate affinity. Therefore UASB reactors in which a high removal efficiency is pursued, provide an excellent environment for their growth.

These bacteria form filaments.

During the initial stages of UASB-reactor start-up under conditions of a low selection pressure Methanothrix filaments grow in and on flocs and in this way are responsible for the development of a bulking anaerobic sludge.

The filaments often grow together to form thick bundles. This also occurs in batch reactors with complete sludge retention. In UASB reactors eventually from these bundles floc-like granules (type C granules) may develop under conditions of low shear forces (see Chapter 7). At high shear forces these fragile aggregates fall apart into smaller units, whereas in strongly stirred reactors Methanothrix filaments break up into small strings of only a few cells or even into single bacteria.

Methanothrix very easily attaches to all kinds of surfaces, as has been demonstrated in fixed film reactors (van den Berg and Kennedy 1981, Heynen 1983).

When a high selection pressure is exerted on the sludge particles during start-up of UASB reactors they attach to carrier particles originating from the seed sludge. In this way type B granules develop.

In contrast to type B granules, which are composed of loosely intertwined filaments, type A granules predominantly consist of Methanothrix bacteria, which are packed densely together. The latter may well be a result of the way in which type A granules develop; i.e. presumably through growth of Methanothrix in the cavities of clumps of Methanosarcina bacteria.

8.3 THE OVERALL PROCESS OF START-UP

The start-up of UASB-reactors will be discussed distinguishing three types of wastewater and two types of digested sewage seed sludge (Table 1).

As reported in Chapter 4, the two types of digested sewage sludge behave differently when used as seed in the treatment of a medium strength wastewater (Table 2).

TABLE 1 DESCRIPTION OF WASTEWATER TYPES AND SEED SLUDGE TYPES

wastewater types	
description	VFA-conc. ($kgCOD.m^{-3}$)
low strength	≤ 1
medium strength	3 - 6
high strength	≥10

digested sewage sludge types	
description	DSS-conc. ($kg.m^{-3}$)
dilute	30
concentrated	75

The start-up using a concentrated type of seed sludge will eventually b faster, because little or no expansion wash-out occurs and consequentl more sludge will be retained in the reactor, than in case an equa concentration of a dilute seed sludge is used.

TABLE 2 SLUDGE BEHAVIOUR DURING UASB-REACTOR START-UP USING DIFFERENT SEED SLUDGE TYPES

CONCENTRATED SEED SLUDGE TYPE	DILUTE SEED SLUDGE TYPE
high initial erosion wash-out	low initial erosion wash-out
↓	↓
low residual methanogenic activity	high residual methanogenic activity
↓	↓
slow initial rate of start-up	fast initial rate of start-up
↓	↓
well settleable sludge / dense sludge bed	poorly settleable sludge / thin sludge bed
↓	↓
fast growth of Methanothrix	fast growth of Methanothrix
↓	↓
limited sludge bed expansion / no expansion wash-out	excessive sludge bed expansion / expansion wash-out
↓	↓
long biomass retention time	short biomass retention time
↓	↓
many Methanosarcina bacteria	only few Methanosarcina bacteria
↓	↓
colonization of Methanosarcina clusters by Methanothrix	colonization of inert fluidised particles by Methanothrix
↓	↓
development of type A granules	development of type B granules

Expansion wash-out of sludge is an important factor in UASB-reactor start-up. A sludge bed of a dilute digested sewage sludge type exerting a relatively high initial methanogenic activity in the form of flocculent sludge will expand very rapidly leading to excessive expansion wash-out and consequently the sludge concentration in the reactor will become very low.

Expansion wash-out will, of course, occur with all types of digested sewage sludge when too much seed is used reducing the seed sludge hold-up in the reactor to its maximum value under the prevailing conditions.

Expansion wash-out primarily reduces the average sludge retention time and causes only little selection between different sludge particles. If a reactor is completely filled with sludge, as is the case when sludge bed expansion wash-out occurs, there is no settling zone left in the reactor and sludge particles which might otherwise have been retained, are also wasted. Only very coarse particles are retained selectively. The latter will be used as carrier material for the development of type B granules.
The other type of sludge wash-out, i.e. sludge bed erosion wash-out, is very selective. It only removes the lightest particles from the system in accordance with the basic concept of the UASB-reactor. A situation should be pursued in which sludge bed expansion wash-out is limited and sludge wash-out predominantly takes place in the form of sludge bed erosion wash-out. Therefore, a concentrated digested sewage sludge type is recommended as seed for the start-up of reactors treating a medium strength wastewater.

In treating a low strength instead of a medium strength wastewater, sludge bed erosion wash-out inherently plays a larger role, because at equal COD loading rates the hydraulic loading rate is greater. Therefore a seed sludge with a higher initial methanogenic activity can be used without risking the loss of large quantities of sludge through expansion wash-out.

In contrast to low and medium strength wastes, the hydraulic loading rate in the treatment of high strength wastewaters is relatively low. Both expansion wash-out and erosion wash-out will be limited simply because of the low dilution rate.
Experiments lasting long enough to yield a granular sludge have not been performed in this study with high strength wastes. From the trends observed with the other wastes it is expected that irrespective of the seed chosen the average biomass retention time will be long enough to allow for the development of type A granules. As the selection pressure exerted on the sludge particles is low, the start-up period will be long. A large fraction of the bacterial growth will take place in a dispersed or 'bulking' form,

and consequently will be washed out. Indications were obtained that start-up with a concentrated seed sludge leads to a stagnation of the production rate at a very low level (see Figure 9 of Chapter 7). It therefore recommended to use a dilute seed sludge type in the treatment a high strength wastewater.

8.4 GUIDELINES FOR THE START-UP OF UASB-REACTORS

This section gives a summary of guidelines for the start-up UASB-reactors. Two types of digested sewage sludge and three types VFA-containing wastewaters are distinguished as described in Table 1.

Seed sludge

1. As discussed in the previous section a concentrated digested sewage s sludge is recommended for the treatment of medium strength wastewater Both a dilute and a concentrated digested sewage sludge can be used seed in the treatment of low strength wastewater, but a concentrated is recommended.
   For high strength wastewater a dilute digested sewage sludge type best be used.
2. If a concentrated digested sewage sludge is not available, a dil sludge can be used after unfed storage of this sludge for a prolon period of time. Old sludge is retained better during start-up, t fresh sludge (see Table 7 of Chapter 7).
3. If no digested sewage sludge is available digested manure can be used seed sludge.
4. The rate of reactor start-up can be increased considerably by enrichi the seed sludge with a small addition of UASB-reactor sludge.
5. It is difficult to deduce the proper quantity of seed sludge to be us from laboratory scale experiments (reactor height: 1.2 m). Scale- effects with respect to the superficial gas and hydraulic loading rat due to the 4 to 8 times greater height of full scale reactors a difficult to assess.
   The quantity of seed sludge on the one hand should be small enough avoid excessive expansion wash-out, but on the other hand it should large enough to avoid unnecessary delay during start-up.
   A quantity of seed sludge corresponding to an average react concentration of 10 to 15 $kgVSS.m^{-3}$ can be recommended because

yielded satisfactory results both in laboratory scale and in full scale reactors.

Start-up conditions

1. To attain a fast reactor start-up the environmental conditions — e.g. pH, nutrients, temperature — should be optimal.
2. An influent sulphide concentration of approximately 3mM seriously hampers reactor start-up. To avoid inhibition sulphide should be removed first or its production, i.e. the reduction of sulphate, should be simultaneous with methanogenesis (one stage operation).
3. The acetate concentration in the reactor should be kept low enough to allow for the growth of both Methanothrix and Methanosarcina. Sludge granules which consist predominantly of Methanothrix bacteria will not develop if Methanosarcina becomes the dominant acetate converting methanogen. The acetate concentration should not be maintained at a high level (e.g. 1.0 $kg.m^{-3}$) for a prolonged period of time. A step-wise increase of the loading rate after reaching a low effluent concentration will ensure the right conditions for the growth of both populations (see Figure 2 of Chapter 7).
4. When treating a very concentrated wastewater, effluent recycle can be used for dilution. Washed out sludge should not be returned into the reactor, because this adversely interferes with the selection process necessary to develop a sludge with a good settleability.
5. Sludge bed erosion wash-out should not be limited, e.g. by decreasing the loading rate or by recycling the effluent suspended solids, because it represents a feature of the UASB-reactor, which is essential in the development of a granular sludge from the digested sewage seed sludge. Likewise sludge bed expansion wash-out should also be accepted; unless it leads to a decrease of the gas production rate. If the latter occurs, the biomass growth no longer compensates for the loss of active biomass and the system becomes unstable. Temporarily decreasing the loading rate will limit the sludge wash-out.

SUMMARY

The successful performance of high rate anaerobic wastewater treatr systems depends upon the retention of a high concentration of act biomass despite high superficial gas and liquid velocities. In the l decade, a number of new reactor types have been developed which achi this by way of immobilization of the bacteria. Other high rate syst depend upon the attachment of bacteria to either static or mobile carr material, e.g. sand, plastics. In UASB-reactors the bacteria attach other bacteria and form granules, which exhibit the necessary h settleability.

Presently, the Upflow Anaerobic Sludge Bed (UASB) reactor is the m widely used high rate system.

The subject of this study is the operation of UASB-reactors during initial start-up period using an unadapted/not acclimatised seed materi The reactor start-up period is defined as the period during which the fi macroscopic sludge granules develop.

Digested sewage sludge was chosen as seed material, because it is read available in industrialised countries and it contains a rich variety anaerobic bacteria. Unadapted sludge was used, because excess sludge f existing UASB-reactors is often not available.

In Chapter 2 the theoretical background is outlined with respect to microbiology and the kinetics of anaerobic digestion, and the one two-stage treatment process configuration.

In a two stage process volatile fatty acids (VFA) are the main products the first reactor, in which hydrolysis and acidogenesis take place. In second reactor the VFA are converted into methane by the combined action acetogenic and methanogenic bacteria. In this study the emphasis was pla upon the methane digestion of VFA. To simplify the system most start- experiments were performed using a mixture of acetate and propionate feed. Acetate is the methanogenic substrate responsible for 70 to 75 % all methane produced in most digesters and propionate is the acetoger substrate which thermodynamically is most difficult to convert into aceta and hydrogen.

No kinetic models that can be applied to the start-up process UASB-reactors were found in the literature. Most models of anaerob

digestion processes are based upon the Monod equation. This equation was also used in this study to evaluate certain observations in the start-up process.

When working with digested sewage sludge as seed material, the volatile suspended solids (VSS) content cannot be regarded as a measure of the viable biomass, because the larger part of the VSS consist of refractory material instead of bacterial matter. Therefore, the methanogenic activity of the sludge is used as a measure of the viable biomass present.

The methanogenic activity of digested sewage sludge is low, and frequently lag phases occur. In order to give an accurate estimate of the methanogenic activity of digested sewage sludge a standardised activity test was used, which is discussed in Chapter 3. In the same chapter the analysis of F420 - a coenzyme unique to methanogens in anaerobic digestion - is presented as an alternative way of assessing the methanogenic sludge activity. The Q_{F420}, i.e. the specific methane production rate per unit weight of F420, can be used to predict the methanogenic activity of the sludge from the measured F420 concentration. To do this, the fraction (A) of methane derived from acetate should be known. The following relationship was established for anaerobic sludges in which Methanothrix is the prevailing methanogen: $Q_{F420} = 1.3 / (1-A)$.

The characteristics of digested sewage sludge were investigated by examining samples from several sludge digesters (Chapter 4). A relationship was established between the original concentration of digested sewage sludge and factors relevant to reactor start-up, notably the VSS content, the settleability and the methanogenic activity. One extreme is concentrated digested sewage sludge (≥ 75 kgDSS.m^{-3}), which is inhomogeneous with respect to the settling characteristics. A large fraction of the VSS of concentrated digested sewage sludge will wash out from the reactor during the first week of start-up. The fraction of the sludge which is retained, exhibits a relatively good settleability and a relatively low specific methanogenic activity. The other extreme is dilute digested sewage sludge (≤ 40 kgDSS.m^{-3}). This sludge is much more homogeneous with respect to settleability and little sludge wash-out will occur during the initial stage of reactor start-up. The retained sludge exhibits a relatively low settleability and a relatively high specific methanogenic activity.

Batch-fed reactors can successfully be used to explore the influenc several factors upon the digestion process. In Chapter 5 factors affec the gas production lag phase are discussed. Conditions that tend to pro the gas production lag phase in batch-fed reactors can also be expecte have a negative influence upon the initial rate of UASB-reactor start It was found that a gas production lag phase does not occur with dige sewage sludge at biomass densities corresponding with a methanog activity of more than 0.5 $kgCH_4$-COD.m^{-3}.d^{-1}. Other factors that stro affected the duration of the gas production lag phase are the sludge (i.e. the period of storage without feeding before its use), the mi intensity in the reactor, the initial VFA concentration, the initial pH the concentration of ammonia and sulphide.

In Chapter 6 the determination of the sludge growth yield Y and the bio specific activity V in batch-fed reactors is discussed. The spec methanogenic activity of newly formed sludge-VSS was found to be in order of 3.0 $kgCH_4$-COD.$kgVSS^{-1}$.d^{-1} for the digestion of VFA. The sl growth yield for the digestion of VFA ranged from 0.020 to 0 $gVSS.gCOD^{-1}$. When using acetate as a single substrate, a much higher gr yield of 0.064 $gVSS.gCOD^{-1}$ was determined. In the latter case the sl growth was not accompanied with ammonia fixation. Therefore, i concluded that most of the growth presumably took place in the form reserve material or exopolymers. Simultaneous digestion of small quanti of propionate and butyrate decreased the acetate growth yield to expected low level of 0.020 $gVSS.gCOD^{-1}$. It is postulated acetate-converting methanogens use hydrogen obtained from the breakdow propionate and butyrate for anabolism. The growth yields determined UASB-reactor experiments with VFA mixtures are similar to those foun batch experiments. The average growth yield of 0.17 $gVSS.gCOD^{-1}$ establi in the digestion of acetate as single substrate in UASB reactors is greater than its equivalent found in batch-reactors (Chapter 7). assumed formation of exopolymers may be important with respect to development of sludge granules.

The sludge cultivated in stirred batch-fed reactors appears to be susceptible to short periods without feeding. The death rate and decay r constants are much higher than for UASB-reactor cultivated sludge.
The dominant bacterial population in the batch reactor experiments with

The effect of various factors upon UASB-reactor start-up is reported in Chapter 7. Factors causing prolonged gas production lag phases in batch experiments like unfed storage of the seed sludge prior to its use, or a high sulphide concentration in the feed, slow down the rate of start-up of UASB-reactors.

The sludge wash-out was examined in relation to the type and quantity of seed sludge supplied. Two types of sludge wash-out are distinguished; sludge bed erosion wash-out and sludge bed expansion wash-out. Sludge bed erosion wash-out represents an essential feature of the UASB concept, i.e. the selection process based on differences in the settleability of the sludge particles. Sludge bed expansion wash-out predominantly occurs when using a dilute digested sewage sludge in the treatment of medium strength wastewaters (3-6 $kgCOD.m^{-3}$). It involves little selection between sludge particles with a different settleability, and greatly reduces the average biomass retention time in the reactor. By choosing a concentrated digested sewage sludge as seed the latter type of sludge wash-out can be avoided.

The average biomass retention time during the initial stages of reactor start-up turned out to determine the type of sludge granules that develop. The first macroscopic sludge granules are normally observed after 30 - 45 days. A long biomass retention time in the reactor permits Methanosarcina bacteria to proliferate, and results in the development of granules predominantly consisting of densely packed Methanothrix bacteria; presumably by way of colonization of hollow clumps of Methanosarcina bacteria.

At short biomass retention times during the initial stage of start-up sludge granules develop through bacterial attachment to inert carrier particles originating from the seed sludge. They show loosely intertwined filaments of Methanothrix.

In experiments with a high reactor concentration of acetate as single substrate, granules develop which consist of Methanosarcina-type bacteria, and which are hollow or exhibit cavities.

To avoid excessive sludge wash-out, the amount of seed sludge should be small enough (10-15 $kgVSS.m^{-3}$) to maintain the sludge bed beneath the settler compartment during the start-up period, i.e. sludge bed expansion wash-out should be limited.

In the treatment of a high strength wastewater ($\geq$ 10 $kgVFA\text{-}COD.m^{-3}$) the dilution rate is low during the initial stages of start-up. Little

selection pressure is exerted on the sludge particles and disp
bacterial growth occurs. Consequently the start-up will be slow.
In contrast, the treatment of low strength wastewater (≤1 kgVFA-COD
is accompanied by relatively strong sludge bed erosion wash-out
consequently a high selection pressure.
A descriptive model summarizing the processes controlling UASB rea
start-up is presented in Chapter 8.
This last chapter also contains guidelines for the start-up of
reactors with respect to the type and quantity of seed sludge to be
and the start-up conditions to be maintained.

SAMENVATTING

De toepassing van hoge belastingen in anaërobe waterzuiveringsinstallati
hangt af van de retentie van een hoge concentratie van aktieve biomas
ondanks hoge oppervlaktebelastingen van gas en vloeistof. De laatste ti
jaar is een aantal nieuwe reaktortypen ontwikkeld, waarin dit wordt berei
door middel van immobilisatie. De werking van de meeste hoog belas
systemen is afhankelijk van de hechting van de bakteriën aan vaste
beweeglijke dragermaterialen (bijv. zand of kunststof). In Upflow Anaerob
Sludge Bed (UASB) reaktoren hechten de bakteriën zich aan andere bakteri
en vormen korrels, die de benodigde goede bezinkeigenschappen bezitten.
dit moment is de UASB reaktor het meest toegepaste hoog belaste systeem.
Het onderwerp van dit onderzoek is de eerste opstart van UASB-reaktoren m
behulp van niet-geadapteerd/niet-aangepast entmateriaal. De opstart perio
wordt gedefinieerd als de periode die nodig is voor de vorming van
eerste macroscopische slibkorrels. Als entmateriaal is gekozen voor slij
gistingsslib, omdat dit algemeen voorhanden is in geïndustrialiseer
landen en omdat het een grote verscheidenheid aan bakteriën bevat. Er
gekozen voor niet-geadapteerd entmateriaal, aangezien surplus-slib v
bestaande UASB reaktoren vaak nog niet beschikbaar is.

In hoofdstuk 2 wordt de theoretische achtergrond geschetst voor wat betre
de microbiologie en de kinetiek van de anaërobe gisting, en de één
twee-traps procesvoering.

In een twee-traps proces worden in de eerste trap, waarin de hydrolyse
de verzuring plaatsvinden, vluchtige vetzuren gevormd. In een twee
reaktor worden deze vetzuren in samenwerking door acetogene en methanoge
bakteriën omgezet in methaan. In dit onderzoek lag de nadruk op de methaa
vorming uit vetzuren. Teneinde het systeem te vereenvoudigen, zijn
meeste opstart-experimenten uitgevoerd met een mengsel van azijnzuur
propionzuur. Azijnzuur is het methanogene substraat waaruit 70 tot 75 % v
alle methaan ontstaat in de meeste anaerobe gistingen, terwijl propionzu
het acetogene substraat is dat thermodynamisch het moeilijkst afgebrok
wordt tot azijnzuur en waterstof.

In de literatuur zijn geen kinetische modellen aangetroffen, die kunn
worden toegepast op het opstart-proces van UASB-reaktoren. De meeste mode
len van het anaërobe gistingsproces zijn gebaseerd op de Monod-vergeli

king. Deze vergelijking is ook in dit onderzoek gebruikt bij de verklar van een aantal waarnemingen.

Bij het gebruik van slijkgistingsslib als entmateriaal kan het organis stofgehalte niet worden gebruikt als maat voor de biomassa, omdat de or nische stof grotendeels bestaat uit inert materiaal in plaats van bakter massa. Daarom wordt de methaanvormingsaktiviteit van het slib gebruikt maat voor de aanwezige aktieve biomassa. De methanogene aktiviteit slijkgistingsslib is laag en er treedt vaak een lag-fase op. Teneinde betrouwbare schatting te geven van de methanogene aktiviteit van slijkg tingsslib is een gestandaardiseerde aktiviteitstest gebruikt, die in hoo stuk 3 wordt besproken. In hetzelfde hoofdstuk wordt de bepaling van gehalte aan F420 - een coënzyme dat in de anaërobe gisting alleen voork in methaanbakteriën - behandeld als een alternatieve bepalingswijze van methaanvormende aktiviteit van het slib. De Q_{F420}, d.w.z. de specifi methaanproduktiesnelheid per eenheid F420, kan worden gebruikt om methanogene aktiviteit van het slib te voorspellen op basis van de geme F420 concentratie. Hiervoor dient men de fractie (A) van het methaangas kennen, die uit acetaat wordt gevormd. De volgende relatie werd vastgest voor anaëroob slib waarin Methanothrix de overheersende methanoge bakterie is: $Q_{F420} = 1.3 / (1-A)$.

De eigenschappen van slijkgistingsslib werden onderzocht aan de hand monsters afkomstig van verschillende slijkgistingsinstallaties (hoofdst 4). Er werd een relatie vastgesteld tussen de oorspronkelijke concentrat van het slijkgistingsslib en relevante faktoren met betrekking tot reaktor-opstart; met name het organische stofgehalte, de bezinkbaarheid de methaanvormingsaktiviteit van het slib. Eén uiterste wordt gevormd do geconcentreerd slijkgistingsslib (≥ 75 kgDSS.m^{-3}), dat niet homogeen is m betrekking tot de bezinkeigenschappen. Een aanzienlijke fractie van organische stof van geconcentreerd slijkgistingsslib zal uit de reakt spoelen tijdens de eerste week van de opstart. De slibfractie die achte blijft, vertoont een relatief goede bezinkbaarheid en een relatief la specifieke methanogene aktiviteit. Het andere uiterste is laag geconce treerd slijkgistingsslib (≤ 40 kgDSS.m^{-3}). Dit slib is veel homogener w betreft bezinkbaarheid, en tijdens de eerste fase van de reaktoropstart z slechts weinig slibuitspoeling optreden. Het achterblijvende slib vertoo een relatief lage bezinkbaarheid en een relatief hoge specifieke methan

Batch-gewijs gevoede reaktoren kunnen met succes worden gebruikt om de invloed van diverse faktoren op het anaerobe gistingsproces te onderzoeken. In hoofdstuk 5 worden faktoren behandeld die de lag-fase in de gasproduktie beïnvloeden. Van omstandigheden die de lag-fase in batch reaktoren verlengen, kan verwacht worden dat zij ook een negatieve invloed hebben op de opstartsnelheid van UASB-reaktoren. Er werd gevonden, dat met slijkgistingsslib geen lag-fase optreedt bij biomassa concentraties, die overeenkomen met een methanogene aktiviteit van meer dan 0,5 $kgCH_4$-CZV.$kgVSS^{-1}$.d^{-1}. Andere faktoren, die de lengte van de lag-fase sterk beïnvloedden, zijn de ouderdom van het slib (d.w.z. de ongevoede bewaarperiode voordat het wordt gebruikt), de roerintensiteit in de reaktor, de aanvangsvetzuurconcentratie, de aanvangs-pH en de concentratie van ammonium en sulfide.

In hoofdstuk 6 wordt de bepaling van de groei-opbrengst Y en de specifieke biomassa aktiviteit V in batch-reaktoren behandeld. Er werd een specifieke methanogene aktiviteit van nieuw gevormd organisch slib vastgesteld van ongeveer 3,0 $kgCH_4$-CZV.$kgVSS^{-1}$.d^{-1} voor de vergisting van vetzuren. De slibgroei-opbrengst voor de vergisting van vetzuren varieerde van 0,020 tot 0,026 gVSS.$gCZV^{-1}$. Wanneer azijnzuur werd gebruikt als enig substraat, trad een veel hogere groei-opbrengst op van 0,064 gVSS.$gCZV^{-1}$. In het laatste geval ging de slibgroei niet gepaard met ammoniumfixatie. Er wordt daarom gekonkludeerd, dat het merendeel van de groei plaats vond in de vorm van reserve-materiaal of exopolymeren. Gelijktijdige vergisting van kleine hoeveelheden propionzuur en boterzuur verlaagde de groei-opbrengst op azijnzuur tot het verwachte lage peil van 0,020 gVSS.$gCZV^{-1}$. Er wordt gepostuleerd, dat azijnzuur vergistende methanogenen voor hun anabolisme waterstof gebruiken afkomstig van de afbraak van propionzuur en boterzuur. De groei-opbrengsten, die in UASB-experimenten met vetzuren werden bepaald, zijn gelijk aan de waarden gevonden in batch-experimenten. De gemiddelde groei-opbrengst van 0,17 gVSS.$gCZV^{-1}$, die werd vastgesteld voor de vergisting van azijnzuur als enig substraat in UASB-reaktoren, is nog aanzienlijk groter dan de overeenkomstige waarde gevonden in batch-experimenten. De veronderstelde vorming van exopolymeren kan van belang zijn in verband met de korrelvorming.

Slib, dat in geroerde batch-reactoren was gekweekt, bleek zeer gevoelig voor korte ongevoede bewaarperioden. De afstervings- en mineralisatiesnelheidskonstantes zijn aanzienlijk groter, dan voor slib, dat in UASB-

reaktoren is gekweekt.
De dominante bakterie populatie in de experimenten met batch-reaktoren was van het Methanothrix-type.

In hoofdstuk 7 wordt verslag gedaan van het effekt van diverse faktoren op het verloop van de opstart van UASB-reaktoren. Faktoren die in batch-experimenten lange lag-fases in de gasproduktie veroorzaakten, zoals het ongevoed bewaren van het entslib voor het gebruik, of een hoge sulfide-concentratie in de voeding, vertraagden de opstartsnelheid van UASB-reaktoren.

De slibuitspoeling werd onderzocht in relatie tot het type en de hoeveelheid van het entslib. Twee soorten slibuitspoeling worden onderscheiden; nl. slibbederosie-uitspoeling en slibbedexpansie-uitspoeling. Slibbederosie-uitspoeling vertegenwoordigt een essentieel onderdeel van het UASB-koncept, namelijk het selektieproces gebaseerd op verschillen in de bezinkbaarheid van de slibdeeltjes.
Slibbedexpansie-uitspoeling treedt voornamelijk op wanneer een laag geconcentreerd slijkgistingsslib wordt gebruikt bij de zuivering van matig geconcentreerde afvalwaters (3-6 $kgCZV.m^{-3}$). Deze soort uitspoeling brengt maar weinig selektie teweeg tussen slibdeeltjes met verschillende bezinkeigenschappen, en verlaagt de gemiddelde biomassa-verblijftijd in de reaktor aanzienlijk. Wanneer geconcentreerd slijkgistingsslib als entslib wordt gebruikt, treedt veel minder snel slibbedexpansie-uitspoeling op.
De gemiddelde biomassa verblijftijd gedurende de beginperiode van de reaktor-opstart blijkt te bepalen welk type slibkorrels er wordt gevormd. De eerste macroscopische slibkorrels worden normaliter waargenomen na 30-45 dagen. Een lange biomassa-verblijftijd in de reaktor maakt het mogelijk dat Methanosarcina bakteriën zich ontwikkelen, en leidt tot de vorming van slibkorrels, die voor het overgrote deel bestaan uit dicht opelkaar gepakte Methanothrix bakteriën; waarschijnlijk door de groei van de laatsten in holle klompjes van Methanosarcina bakteriën.
Bij korte biomassa retentietijden gedurende de eerste fase van de opstart ontwikkelen zich slibkorrels door hechting van bakteriën aan inert dragermateriaal afkomstig uit het entslib. Zij vertonen een losse struktuur van Methanothrix draden.
In experimenten met een hoge reaktor concentratie van azijnzuur als enig substraat, ontstaan korrels die bestaan uit Methanosarcina bakteriën. Zij zijn hol of vertonen instulpingen.

Ter voorkoming van overmatige slibuitspoeling moet de hoeveelheid entslib zo groot zijn (10-15 kgVSS.m^{-3}), dat het slibbed tijdens de opstart beneden de bezinkerruimte blijft, d.w.z. dat slibbedexpansie-uitspoeling beperkt dient te blijven .

Bij de zuivering van hoog geconcentreerd afvalwater ($\geq$10 kgCZV.m^{-3}) is de hydraulische verblijftijd lang gedurende de eerste fase van de opstart. Er wordt weinig selektiedruk uitgeoefend op de slibdeeltjes en er treedt disperse bakteriegroei op. Als gevolg hiervan zal de opstart langzaam verlopen.

Daarentegen gaat de zuivering van laag geconcentreerd afvalwater ($\leq$1 kgCVZ.m^{-3}) gepaard met een grote slibbedexpansie-uitspoeling en dientengevolge een hoge selektiedruk.

Een beschrijvend model dat de processen samenvat, die de opstart van UASB-reaktoren beheersen, wordt in hoofdstuk 8 gepresenteerd.

Dit laatste hoofdstuk bevat ook richtlijnen voor de opstart van UASB-reaktoren met betrekking tot het aanbevolen type entslib en de te handhaven opstart omstandigheden.

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CURRICULUM VITAE

De auteur is geboren op 18 december 1950 te Ridderkerk.
In 1969 behaalde hij het diploma gymnasium β aan het Wagenings Lyceum en begon met de studie in de richting Milieuhygiëne aan de Landbouwhogeschool te Wageningen. Het doctoraalexamen, afgelegd in juni 1976, omvatte de vakken waterzuivering, kennis van de bodemverontreiniging en microbiologie.
Tot september 1977 was hij als milieuchemicus werkzaam bij het SCMB-TNO te Delft. In de periode daarna tot januari 1983 is het onderzoek voor dit proefschrift verricht bij de vakgroep waterzuivering van de Landbouwhogeschool, waaraan hij was verbonden als promotie-assistent en als gastmedewerker.
Sinds maart 1983 is de auteur als milieutechnoloog in dienst van Avebe B.A. te Veendam.

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