NN08201, 2503

Stellingen:

1. In contrast to all findings sofar the results of this dissertation demonstrate that mesophilic biomass fed under psychrophilic process conditions (10 °C) on the long term will attain a specific methanogenic activity at 30 °C comparable to that of granular sludge cultivated under thermophilic conditions (55 °C).

   Henzen, M. and Harremoes, P. 1983 Anaerobic treatment of wastewater in fixed film reactors-literature review. *Wat. Sci. Technol.*, 15: 1-102.

   Pavlostathis S.G. and Giraldo-Gomez E. 1991 Kinetics of anaerobic treatment: A critical review. *Critical Rev. in Environ. Control*, 21: 411-490

2. Contrary to the statements in Metcalf & Eddy that merely the mesophilic and thermophilic temperature range would be of practical significance for anaerobic wastewater treatment, this dissertation demonstrates that lower temperatures are not a limiting factor anymore for the application of high-rate anaerobic treatment, even not to low strength acidified wastewaters.

   Metcalf & Eddy 1991 Wastewater Engineering, Treatment, Disposal, Reuse. Third edition. McGrow-Hill, Inc.

3. The propionate oxidising bacterium (cover page), isolated from granular sludge grown on a volatile fatty acids mixture at 8 °C, is capable to oxidise propionate at high rate (1 g CODprop·$L^{-1}_{reactor}$·day^{-1}) even at 3 °C. It's classification within the bacterial domain is still a challenge for further research.

   This dissertation.

4. Strong passion and fine patience for anaerobic technology are needed to convert the storage temperature (4 °C) of anaerobic granular sludge into the operating temperature of a high-rate anaerobic EGSB reactor.

   This dissertation.

5. Use of water in the city is frequently called "consumption" of water. That is a misnomer for the use. The "consumer" does not consume in the conventional interpretation of the word. The "consumer" uses the water in order to pollute it! The function of water use in cities is to remove unwanted material from the location where the water is used. The use of water as a means of transport of waste out of cities is a disaster from the point of view of sustainability. It is not sustainable to distribute 200 litre of water per person and day, purified to drinking water standard in view of the fact that only 1 litre is use for drinking.

   Harremoës, P. 1998 Water as a transport media for waste out of towns. International WIMEK Congress, Options for closed water systems (sustainable water management), March 11-13, Wageningen, The Netherlands

6. Geduld is gelijk een boom, waarvan de wortel bitter is, maar de vruchten erg zoet zijn.
   Uit Balkan

7. Er zijn twee soorten van vrijheid: de valse, waarbij men vrij is om te doen wat men wil; en de echte, waarbij men vrij is om te doen wat men moet doen.
   Charles Kingsley

8. Inzicht hebben is meer waard dan een sterke arm.
   Euripides

9. Goed opgevoede mensen spreken anderen tegen. Wijze mensen spreken zichzelf tegen.
   Oscar Wilde

10. Het opmaken van de levensbalans bestaat voor het grootste gedeelte uit het afschrijven van illusies.
    Koos Versteeg

11. Laten we de tijd die ons gegeven is goed gebruiken, er is zoveel te doen.

Stellingen behorende bij het proefschrift "**Psychrophilic anaerobic treatment of low strength wastewaters**".

Salih Rebac
Wageningen, 16 oktober 1998

Psychrophilic Anaerobic Treatment of Low Strength Wastewaters

Salih Rebac

Promotor: Prof. Dr. Ir. G. Lettinga,
bijzonder hoogleraar in de anaërobe zuiveringstechnogie en hergebruik

Co-promotor: Dr. Ir. J.B. van Lier,
projectleider bij de leerstoelgroep Milieutechnologie

Salih Rebac

Psychrophilic Anaerobic Treatment of Low Strength Wastewaters

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus,
van de Landbouwuniversiteit Wageningen,
dr. C.M. Karssen,
in het openbaar te verdedigen
op vrijdag 16 oktober 1998
des namiddags te half twee in de Aula.

Cover page:
The propionate oxidising bacterium isolated from granular sludge grown in this research.

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Rebac, S.

Psychrophilic anaerobic treatment of low strength wastewaters / S. Rebac [S.I. : s.n.]
Thesis Wageningen Agricultural University, Wageningen, The Netherlands
- With general discussion and conclusions in Dutch.

ISBN 90-5485-943-1
Subject headings: wastewater / anaerobic treatment / psychrophilic conditions

Voor Jasmina, Nela, en Senka

Aan mijn ouders

Acknowledgements

I express my deep gratitude to Gatze Lettinga for all these years giving me the opportunity to work at this department and for his efforts in coordinating the research project that led to this dissertation.

The research presented in this dissertation was supported by Bavaria B.V., Lieshout, The Netherlands. I would like to express my acknowledgement to Koen Th. M Swinkels for funding the research, and to his employees Freddy Dekkers and Tim Tersmette who cooperated in the planing of the research. My special thanks to Ing.Soer, Herman, Rini, Martin, Jelle and Gert from Export Mouterij Netherland for their help to make part of this research possible.

Part of the research in this dissertation was the results of the efforts of several students Julia Ruskova, Sebren Gerbens, Marcel Janssen, Joost van Cappellen, Marc Vermeulen, Klaas-Jan Kramer, Friso van Bijnen, and Bed Mani Dahal, whose hard work I do acknowledge.

I would like to thank to Katja Grolle, Look Hulshoff Pol, Jane Robertson, René Wijffels, Ida Akkerman, Loudinha Florencio, Mario Kato, André Visser, Jim Field, Reyes Sierra, Ricardo Bernardes, Elias Razo Flores, Francisco Omil, Piet Lens for very pleasant friendship and unsolicited goodwill for helping me on many occasions. I express my gratitude to Dora Lettinga for her friendship and enjoyable hospitality to my family and me.

I would like to thank Jules van Lier for very good sub-optimal temperature (psychro-thermo) cooperation, which resulted that he became my co-promotor.

I would like to thank to Evert Janssen, Jan Theunissen, Andre Sanders, Eric Karruppannan, Hans De Rooy, Arthur van Munster, Mees Schimmel, Reinoud Hummelen, Peter Smit, Anne Baars, Ton Erenst and Jan Daniel Houthuizen from Biotechnion technical department for their urgent help on many occasions in my research.

It was great pleasure to work with many of researchers of the anaerobic group of this department, among them Marjo Lexmond, Anita van Langerak Renze van Houten, Sjon Kortekaas, Grietje Zeeman, Ronaldo Petruy, Robbert Kleerebezem, Adrie Veeken, Brian Donlon, Bert Hamelers, Vinnie de Wilde, Wendy Sanders, Dave van Bergen, Tarek El-metwally, Graciela Gonzalez.

It was great pleasure associate with Bordes group: Miriam van Eekert, André Visser, Jules van Lier, Albert Janssen, Goutam Gosh, Francisco Omil, Jan Weijma and Piet Lens. Annual "Bordesborrel" became nice event in the department, and should be continued.

I am also grateful to the members of the analytical staff, Johannes van der Laan and Ilse Gerrits-Bennehey for the help during the chromatographic determinations, and Jo Ackermans-Jacobs and Sjoerd Hobma for the chemical analyses and Hans Donker for PC equipment. I acknowledge Helen Vos and Liesbeth Kesaulya-Monster for their cordiality and assistance at our secretary's office. I express my appreciation to all the members of the staff at the department of Environmental Technology for having me at the department.

Last but not least, I thank my spouse Jasmina and my daughters Nela and Senka for their love, care and support in all these years.

ABSTRACT

Rebac, S. (1998) Psychrophilic Anaerobic Treatment of Low Strength Wastewaters. Ph.D. Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

The main objective of this thesis was to design a high-rate anaerobic system for the treatment low strength wastewaters under psychrophilic conditions.

Psychrophilic (3 to 20 °C) anaerobic treatment of low strength synthetic and malting wastewater was investigated using a single and two stage expanded granular sludge bed (EGSB) reactor system. The chemical oxygen demand (COD) removal efficiencies found in the experiments with synthetic wastewater exceeded 90 % in the single stage reactor at imposed organic loading rates up to 12 kg COD m^{-3} day^{-1} and a hydraulic retention time (HRT) of 1.6 h at ambient (10-12 °C) temperature using influent concentrations ranging from 500 to 800 mg COD dm^{-3}. A malting wastewater with an anaerobically biodegradable COD of about 73 %, as determined in the batch bioassays at 15 °C was also used during single stage reactor operation at 16°C. The COD removal efficiencies averaged about 56 %, at organic loading rates (OLR) ranging between 4.4 - 8.8 kg COD m^{-3} day^{-1} and a HRT of approximately 2.4 h. At 20°C, removal efficiencies were approximately 66 % and 72 %; respectively, at OLRs of 8.8 and 14.6 kg COD m^{-3} day^{-1}, corresponding to HRTs of 2.4 and 1.5 h.

Psychrophilic (3-8 °C) wastewater treatment was further optimized at the laboratory scale two stage expanded granular sludge bed (EGSB) reactor in series, fed with a VFA mixture (500-900 mg COD dm^{-3}). The COD removal efficiencies exceeded 90 % at 8 °C and 4 °C, at organic loading rates of 12 and 5 kg COD m^{-3} day^{-1}, respectively. Even at 3 °C, COD removal efficiencies averaged 80 %. High rate propionate oxidation was for the first time successfully achieved at such low temperatures. Applying this two stage EGSB system to malting wastewater in the temperature range 10-15 °C, gave removal efficiencies for soluble COD and for volatile fatty acids COD 67-78 % and 90-96 %, respectively, at an OLR between 2.8-12.3 kg COD m^{-3} day^{-1} and a HRT of 3.5 h. The second stage serves mainly as a scavenger of non-degraded volatile fatty acids (VFA) from the first stage.

The specific activities of the reactor sludge increased by a factor 3 after 300 days of reactor operation, indicating enrichment of methanogens and acetogens even at the low temperatures applied. The homoacetogenic, hydrogenotrophic and acetoclastic specific activities of the sludge at 10 °C, were 1.744, and 0.296 and 0.331 g COD g^{-1}VSS day^{-1}, respectively. At 30 °C the specific activities were 18.024, 2.732 and 2.204 g COD g^{-1}VSS day^{-1}, respectively. These high specific sludge activities can be attributed to the good and stable enrichment of methanogenic, acetogenic and homoacetogenic bacteria under psychrophilic conditions. Surprisingly, the optimal temperatures for substrate conversion of reactor sludge, after it has been exposed to long term psychrophilic conditions, were still similar to those of the original mesophilic inoculum. The results of EGSB batch reactor experiments revealed apparent half saturation constants of the acetate and propionate degraders in the range of 39-58 mg COD dm^{-3} and 7-14 mg COD dm^{-3}, respectively. For butyrate degraders, higher K_m values were found, i.e., 142-243 mg COD dm^{-3}. The observed low K_m values are in agreement with the high removal efficiencies of the EGSB reactor during anaerobic treatment of the cold, low strength, wastewater.

By adapting the process design to the expected prevailing conditions inside the reactor, the loading rates and overall stability of the anaerobic high-rate process may be distinctly improved under psychrophilic conditions. The results obtained clearly reveal the big potentials of anaerobic wastewater treatment under low ambient (10-12 °C) temperature conditions for low strength wastewaters.

CONTENTS

Chapter 1

Introduction

1. PSYCHROPHILIC ANAEROBIC TREATMENT OF LOW STRENGTH WASTEWATERS

1.1 Cold low and medium strength wastewaters

More than 50 % of the earth is exposed to moderate and cold temperature conditions. Under climate conditions many low and medium strength wastewaters are discharged at lower ambient temperatures, including domestic wastewater and a large variety of industrial wastewaters, e.g. those bottling, malting, brewery and soft drinks manufacturing. These industrial processes may produce several streams with different characteristics either in flow or in COD concentrations, thus some wastewaters may have a broad concentration range since the COD of industrial effluents depends largely on the technological process.

Low strength wastewaters can be defined as those with an organic pollution below 1500 mg COD dm^{-3}. They generally contain a variety of biodegradable compounds such as simple short-chain volatile fatty acids (VFA), alcohols, carbohydrates, but frequently also contain proteins, suspended solids of different origin, fats or long-chain fatty acid (LCFA). Moreover, they may contain dissolved oxygen concentration up to saturation level (10-12 mg O_2 dm^{-3} at 4 °C).

1.2 Anaerobic biological conversion

Fatty acids play a key role in the breakdown of complex organic matter under methanogenic conditions, as they are intermediates of polysaccharide, lipid and protein fermentation (Gottschalk, 1985). In environments with a high organic matter turn-over, e.g. anaerobic wastewater treatment bioreactors with organic loading rates up to 30 kg chemical oxygen demand (COD) per m^3 reactor per day (Lettinga, 1995), operational conditions have to be controlled to assure complete breakdown of fatty acids. Failure of the anaerobic digester performance is often accompanied by a build-up of VFAs acetate, propionate and butyrate, with propionate being the more likely VFA to accumulate initially (Lin et al., 1986; Mawson et al., 1991). Consequently, many process control strategies in anaerobic digestion are based on monitoring the effluent VFA content, either directly (Marchaim and Krause, 1993) or indirectly, via pH (Denac et al., 1988), bicarbonate (Hawkes et al., 1993) or (dissolved) hydrogen concentration (Collins and Paskins, 1987; Pauss et al., 1990).

Deterioration of the breakdown process at the level of VFAs reflects the sensitivity of their methanogenic decomposition. The latter can be attributed to the highly positive $\Delta G^{o'}$ value of acetogenic dehydrogenation reactions, e.g. +76.1 kJ/mol and +48.1 kJ/mol substrate for propionate and butyrate oxidation to acetate under standard conditions (Thauer et al., 1977). However, propionate and butyrate oxidation can become exergonic due to interspecies transfer of reducing equivalents from acetogenic to methanogenic bacteria, either as molecular hydrogen (Bryant et al., 1967; McInerney et al., 1981) or as formate (Dong et al., 1994; Thiele and Zeilus, 1988). The biochemistry (Boone, 1984; Houwen et al., 1987, 1991; Robbins, 1988; Plugge et al., 1993), kinetics (Heyes and Hall. 1983; Boone and Xun, 1987; Dong et al., 1994) and inhibition (Fukuzaki et al., 1990) of mesophilic syntrophic propionate degradation have been well documented using defined cocultures and enrichment cultures.

Propionate degradation has been studied in more complex microbial ecosystems as well, e.g. in sludges growing in different bioreactor types under mesophilic conditions, including continuously stirred tank reactors (Mawson et al., 1991; Smith and McCarty, 1989), fixed film reactors (Tholozan et al., 1988), upflow anaerobic sludge blanket reactors (Fang et al., 1995) or fluidized bed reactors (Heppner et al., 1992). These studies, mainly carried out to improve bioreactor performance, showed that besides the syntrophic oxidation of propionate to the methanogenic substrates H_2, formate and acetate, reduced end products such as alcohols (Smith and McCarty, 1989) and higher (C_4-C_7) VFAs (Lin et al, 1986; Tholozan et al., 1990; Wu et al., 1993) can be formed from propionate as well. The pathway involved in propionate formation during anaerobic ethanol fermentation in the presence of extremely low concentration or absence of sulphate is attributed to the presence of *Desulfobulbus propionicus* in the granular sludge (Stams et al., 1984; Samain et al., 1984). In general, these investigations have been done to optimise the reactor performance in the mesophilic temperature range. Little is known about the biochemistry involved and their *in situ* role in organic matter removal in bioreactors under psychrophilic conditions.

1.3 Effect of low temperature on anaerobic biological conversion

Microorganisms are classified into "temperature classes" on the basis of the optimum temperature and the temperature span where the species are able to grow and metabolise (Fig. 1.1). The overlapping growth temperature ranges in Fig. 1.1 indicate that there is no clear boundaries between these classic groups of psychrophilic, mesophilic and thermophilic

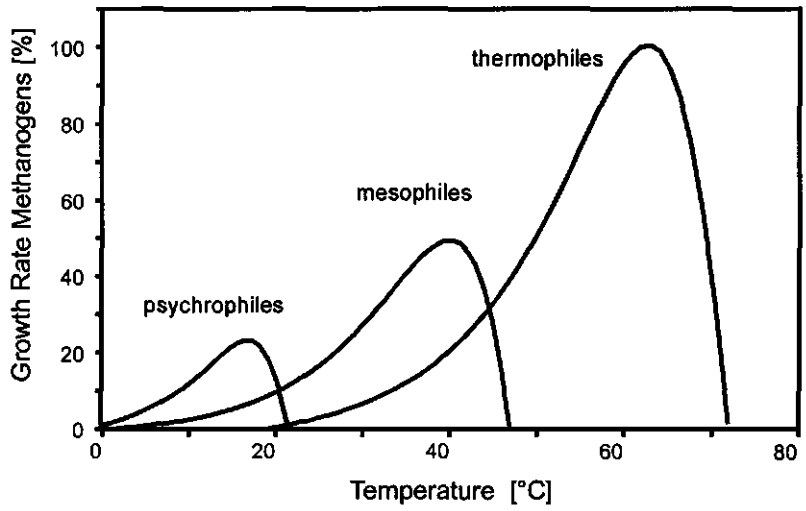

Fig. 1.1 Relative growth rates of psychrophilic, mesophilic and thermophilic methanogens, after Wiegel (1990).

microorganisms. The bacterial growth rates of methanogenic thermophiles and mesophiles from anaerobic reactors are quite well determined. However, to date, only two psychrophilic marine methanogens and a few psychrophilic (optimum below 20 °C) and psychrotrophic

acetogenic bacteria (homoacetogens) (optimum between 20-30 °C) from natural sediments have been isolated (Romesser et al., 1979; Conrad et al., 1989; Kotsyurbenko et al., 1995).
The fact that anaerobic psychrophiles have been found only in natural eco-systems illustrates the lack of information on anaerobic reactors treating wastewater under psychrophilic conditions.
At psychrophilic conditions, chemical and biological reaction rates proceed much slower at psychrophilic than at mesophilic conditions. Most reactions in the biodegradation of organic matter require more energy to proceed at low temperature then at optimum of 37 °C (Table 1.1), however some reactions i.e. hydrogenotrophic sulphate reduction, hydrogenotrophic methane production and acetate formation from hydrogen and bicarbonate require less energy (Table 1.1, reactions 9, 10 and 11, respectively).

Table 1.1 Stoichiometry and Gibbs free-energy changes[♣] of acetate, propionate, butyrate, and hydrogen anaerobic conversion in the presence and absence sulphate.

	REACTIONS	$\Delta G'$ kJ/reaction (37°C)	(10°C)
1	$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$	+ 71.8	+ 82.4
2	$CH_3CH_2COO^- + 0.75SO_4^{2-} \rightarrow CH_3COO^- + HCO_3^- + 0.75HS^- + 0.25H^+$	- 39.4	- 35.4
3	$CH_3CH_2COO^- + 1.75SO_4^{2-} \rightarrow 3HCO_3^- + 1.75HS^- + 0.25H^+$	- 88.9	- 80.7
4	$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$	+ 44.8	+ 52.7
5	$CH_3CH_2CH_2COO^- + 0.5SO_4^{2-} \rightarrow 2CH_3COO^- + 0.5HS^- + 0.5H^+$	- 29.3	- 25.9
6	$CH_3CH_2CH_2COO^- + 2.5SO_4^{2-} \rightarrow 4HCO_3^- + 2.5HS^- + 0.5H^+$	- 128.3	-116.4
7	$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$	- 49.5	- 45.3
8	$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	- 32.5	- 29.2
9	$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$	- 148.2	-157.1
10	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	- 131.3	-140.9
11	$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 4H_2O$	- 98.7	-111.8

[♣]Energy changes were calculated by using the Van 't Hoff equation, standard enthalpy values of compounds (Chang, 1977), and Gibbs free-energy changes at 25 °C (Thauer et al., 1977).

A strong temperature effect on the kinetic parameters of microorganisms has been observed by many researchers (Jewell and Morris, 1981; Lin et al., 1987; Matsushige et al., 1990; Wu et al., 1993). Lowering the operational temperature generally leads to a decrease in the maximum specific growth and substrate utilisation rates, but it also may lead to an increased biomass yield (g biomass g^{-1} substrate converted) of methanogenic population (Van den Berg, 1977; Lin et al., 1987).

1.3.1 Effect of low temperature on the physical-chemical properties of wastewater

So far, it is neither clear whether high-rate psychrophilic anaerobic wastewater treatment needs the development of psychrophilic or psychro-tolerant sub-populations, nor to what extent mesophilic sludges can become psychro-tolerant. Low temperatures change the physical-chemical properties of the wastewater, which can considerably affect design and operation of the treatment system. The solubility of gaseous compounds particularly increases with decreasing temperature below 20 °C (Fig. 1.2).

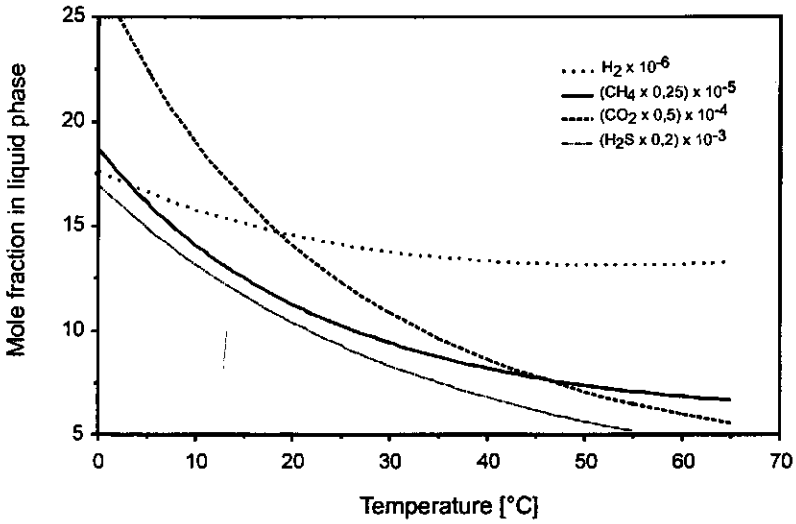

Fig. 1.2 Gas solubility in pure water at various temperatures, after Lide (1992).

This implies that the dissolved concentration of methane, hydrogen sulphide and hydrogen will be higher in the effluent of reactors operating at low temperatures. The high increase of solubility of CO_2 indicates that slightly a lower reactor pH might be expected under psychrophilic conditions.

At low temperature, the viscosity of liquids will be higher. This implies that more energy is required for mixing and that sludge bed reactors are less easily mixed particularly at low biogas production rates. In psychrophilic reactors, particles will settle slower because of a decreased liquid-solids separation at low temperatures. Related to liquid viscosity, the diffusivity of soluble compounds will be decreased by decreasing temperature as indicated in (Perry and Green, 1984):

$$D_2 = D_1 \times \left(\frac{\eta_1}{T_1}\right) \times \left(\frac{T_2}{\eta_2}\right)$$

where D = diffusion coefficient of a specific compound ($m^2\ s^{-1}$), T = temperature (K), and η=

the liquid viscosity of the solution (N s m^{-2}). The subscripts 1 and 2 refer to two different temperatures. The diffusivity of soluble compounds in the temperature range 10-40 °C, relative to the diffusivity at 30 °C is given in Table 1.2. From Table 1.2 follows that the diffusion constant of soluble compounds is about 50 % lower at 10 °C compare to the mesophilic temperature range (30-40 °C).

Table 1.2 The diffusivity of any soluble compounds at various temperature relative to the diffusivity at 30 °C[a].

Temperature [°C]	10	20	30	40	50	60
Dtemp/D30 [-]	0.57	0.77	1.00	1.26	1.55	1.88

[a] Values were calculated using equation 1 and the viscosity of pure water at the various temperatures (Lide,1992)

1.4 Anaerobic wastewater treatment technology

One of the major successes in the development of anaerobic wastewater technology was the introduction of high-rate reactors in which biomass retention and liquid retention are greatly uncoupled (McCarty, 1981; Iza et al., 1991; Lettinga, 1995). This feature comprises a crucial issue for the treatment of low(er) strength wastewaters. For those reactor systems where the sludge retention is based on the settling characteristics of sludge aggregates, like is the case for the well known Upflow Anaerobic Sludge Bed (UASB) reactors, the hydraulic load therefore will become the restrictive factor with respect to the required reactor volume when treating very low strength wastewaters.

The established sanitary wastewater engineering world so far considered anaerobic wastewater treatment of cold and very low strength wastewaters as not practically-feasible. This opinion is mainly based on prejudice and a serious lack on sound insight in the anaerobic digestion process and technology. In fact restrained scientists in the past had researched in this field (Table 1.3). Certainly, anaerobic wastewater treatment of low strength cold wastewaters indeed is not obvious, i.e. a number of bottlenecks have to be eliminated. So for instance, the low $COD_{influent}$ will result in low substrate levels (50-100 mg COD l^{-1}) inside the reactor, and in a low biogas production rate as well. In conventional anaerobic sludge bed reactors this implies a too low mixing intensity in the reactor, and consequently in a poor substrate-biomass contact. Another serious problem when treating very low strength wastewaters is that the permissible amount of sludge washout per m^3 wastewater is extremely small, which sets exceptionally high requirements on the sludge retention abilities of the reactor. Therefore the required reactor volume in case of low strength wastewaters generally will be determined by the permissible hydraulic loading rate (HLR) rather than by the organic loading rate (OLR) (Lettinga and Hulshoff Pol, 1991). Practically all full scale applications of anaerobic wastewater treatment are restricted to wastewaters with temperatures exceeding 18°C. The maximum reported organic loading rate achieved at temperatures below 15 °C are presented in Table 1.3.

Table 1.3 Literature review of anaerobic treatment of low strength wastewaters under psychrophilic conditions.

Reactor type	Influent	Concentration [g COD dm^{-3}]	OLR [kg COD m^{-3} d^{-1}]	Temperature [°C]	HRT [h]	Efficiency [%]	Reference
AAFEB[1]	Glucose	0.2 - 0.6	4 - 16	10	1 - 6	40 - 80	Switzenbaum & Jewell, 1978
AF[2]	Sewage	0.53	1.8	13-15	6	35-55	Derycke & Verstraete, 1986
UASB[3]	Vinasse	0.2 - 0.4	0.7 - 6.5	8	1.5 -14	32 - 65	De Man et al., 1988
EGSB[4]	VFA	2.6	2.0	12	32	50	De Man et al., 1988
FB[5]	Sewage	0.76	8.9	10	1.7-2.3	53-85	Sanz & Fdz-Polanco, 1990
ASF[6]	Peptone	0.2*	0.64	5 - 10	7.5	27 - 35	Matsushige et al., 1990
EGSB[4]	Sewage	0.3	4.5	9 - 11	2.1	20 - 48	Van der Last & Lettinga, 1992
UASB[3]	Beef consommé	1.4 - 7.0	2 - 10	10	16	49 - 80	Grant & Lin, 1995
ASBR[7]	Dry milk	0.6	0.6 - 2.4	5 - 10	6	65 - 85	Banik & Dague, 1996

* g BOD dm^{-3}
[1] Anaerobic attached film expanded bed reactor
[2] Anaerobic filter
[3] Upflow anaerobic sludge blanket reactor
[4] Single stage expanded granular sludge bed reactor
[5] Fluidized bed reactor
[6] Anaerobic submerged filter tank
[7] Anaerobic sequencing batch reactor

The very promising results indicated in Table 1.3, mainly from the last 20 years, were not sufficiently encouraging to implement anaerobic wastewater treatment at full scale for the treatment of cold wastewaters (t < 18°C). In fact only in the last 10 years high rate anaerobic wastewater treatment systems were accepted as 'grown-up', mainly for medium strength wastewaters under optimal mesophilic conditions.

1.5 Anaerobic treatment of low strength wastewaters at low temperature

Most studies on the effect of low temperature on anaerobic digestion show a strong negative effect on the metabolic activity of mesophilic anaerobic methanogenic bacteria as presented in Fig. 1.3. This indicates that the capacity of an anaerobic reactor seeded with mesophilic biomass will sharply drop during start-up. Temperature effects on kinetic parameters have been described mathematically by using e.g. the Arrhenius equation (Pavlostathis & Giraldo-Gomez, 1991).

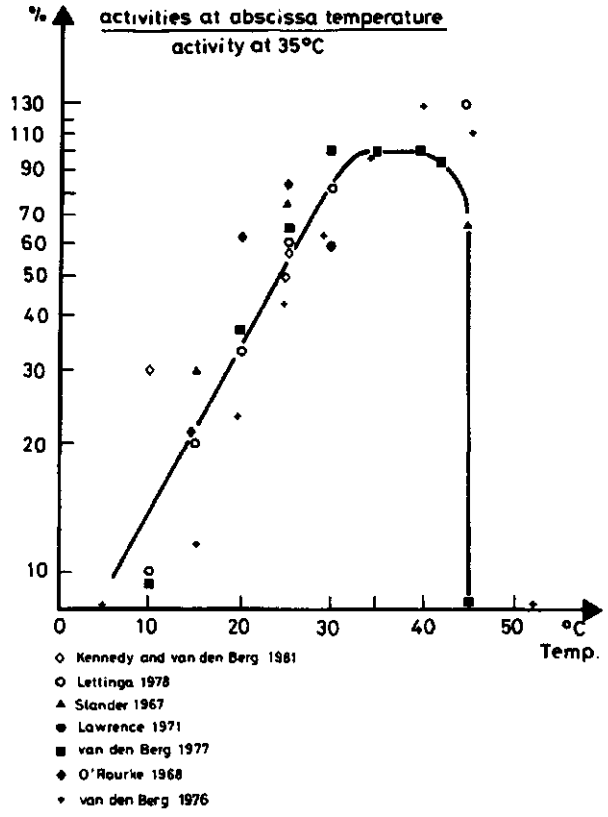

Fig. 1.3 Temperature dependency of the methane production rate of mesophilic anaerobic processes, after Henzen and Harremoes (1983).

Because temperature strongly affects the rates of the anaerobic conversion processes, some essential improvements have to be made in the conventional design of high-rate reactors in order to enable their application under 'sub-optimal' temperatures and for very low strength wastewaters. When successful, such a modified (improved) reactor system would represent a major technological break-through, because then indeed an efficient bioengineering of bacterial catalysis under sub-optimal temperatures would become possible. A successful application of psychrophilic anaerobic biocatalysis would be also of big economical importance, since

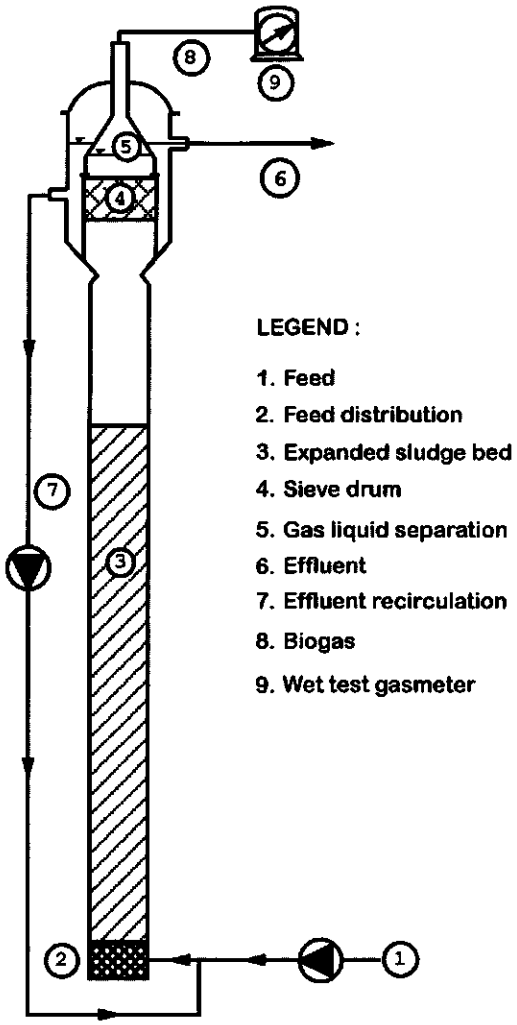

Fig. 1.4 Schematic diagram of EGSB reactor system.

generally (depending on the temperature of the wastewaters) a significant amount of energy is required for bringing the wastewater temperature in the more optimal mesophilic range (30-40°C) (e.g. Mills, 1979). This puts a heavy burden on the economy of the wastewater system. Previous experiments in optimising the sludge - wastewater contact in UASB-reactors, led to the development of an advanced reactor design, viz. the Expanded Granular Sludge Bed (EGSB) (De Man et al., 1988; Frankin et al., 1992; Rinzema et al., 1993; Kato et al., 1994). The EGSB-system (Fig. 1.4) uses exclusively granular sludge, while in anaerobic Fluidized Bed (FB) or Attached Film Expanded Bed (AFEB) reactors inert carrier materials are used for attachment of active biomass. The upflow velocities (v_{up}) which can be applied in the EGSB system are between 4 to10 m h^{-1}. These high v_{up}-values can be achieved by applying effluent recycle and/or by using tall reactors. The feasibility of high-rate anaerobic wastewater treatment systems for cold wastewaters depends primarily on:

i) the quality of the seed material used and its development under sub-mesophilic conditions.

ii) the types of the organic pollutants in the wastewater (Koster and Lettinga, 1985).

iii) the reactor configuration, especially its capacity to retain viable sludge.

1.6 Hypothesis

The high-rate anaerobic treatment of low strength wastewaters at psychrophilic conditions will become feasible if sufficient proliferation and retention of newly grown viable mesophilic biomass in an anaerobic reactor system can be achieved.

1.7 Scope of this dissertation

This thesis describes the results of research on the feasibility of the EGSB reactor system for the anaerobic treatment of low strength wastewaters under psychrophilic conditions. The performance and design of this high-rate anaerobic reactor under psychrophilic conditions for a variety of substrates including also malting wastewater was investigated. Chapter 1 presents a general introduction on the low temperature effect on anaerobic degradation of different substrates, and a review of literature on anaerobic low strength wastewater treatment at low temperatures. In Chapter 2 the design of high-rate anaerobic reactor under psychrophilic conditions is presented. The temperature dependence of the kinetic parameters of granular sludge after being exposed for a prolonged period of time under low temperature was determined. Also the effect of sulphate on the propionate and butyrate degradation kinetics under psychrophilic conditions has been investigated. The feasibility of the single stage pilot-scale EGSB reactor system for the anaerobic treatment of brewery and malting wastewater under various low temperature ranges is presented in Chapter 3. Research on process optimisation for acidified and partly acidified wastewater on realising a stable treatment process with the highest possible efficiency (> 90%) and at the lowest temperature applied (3 °C) is presented in Chapter 4. The research on optimisation of the pilot-scale EGSB process for the anaerobic treatment of malting wastewater under psychrophilic conditions is reported in Chapter 5. General discussion and conclusion of the thesis are given in Chapter 6.

1.8 REFERENCES

Banik, G.C., Dague, R.R. 1996. ASBR treatment of dilute wastewater at psychrophilic temperatures. In: Proc. of 69th Annual Water Environmental Conference, Dallas, Texas, USA, 235-246.

Boone, D.R. 1984. Propionate exchange reactions in methanogenic ecosystems. *Appl. Environ. Microbiol.* **48:** 863-864.

Boone, D.R., and Xun, L. 1987. Effect of pH, temperature and nutrients on propionate degradation by a methanogenic enrichment culture. *Appl. Environ. Microbiol.* **53:**1589-1592.

Bryant, M.P., Campbell, L.L., Reddy, C.A., and Crabill, M.R. 1967. Growth of *Desulfovibrio* in lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. *Appl. Environ. Microbiol.* **33:** 1162-1169.

Chang, R., 1977. Physical chemistry with application to biological systems. Macmillan Publishing Co, New York, USA.

Collins, L.J., and Paskins, A.R. 1987. Measurement of trace concentrations of hydrogen in biogas from anaerobic digester using an exhaled hydrogen monitor. *Water Res.* **21:**1567-1572.

Conrad, R., Bak, F., Seitz, H. J., Thebrath, B., and Mayer, H. P. 1989. Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment. *FEMS Microbiol. Ecol.* **62:** 285-294.

De Man, A.W.A., van der Last, A.R.M., Lettinga, G. 1988: The use of EGSB and UASB anaerobic systems for low strength soluble and complex wastewaters at temperatures ranging from 8 to 30°C. In: E.R. Hall and P.N. Hobson (eds.), Proceeding of the Fifth International Symposium on Anaerobic Digestion. Bologna, Italy, p. 197-209.

Denac, M., Griffin, K., Lee, P.L., and Greenfield, P.F. 1988. Selection of controlled variables for a high rate anaerobic reactor. *Environ. Technol. Letters*, **9:**1029-1040.

Derycke, D. and Verstraete, W. 1986. Anaerobic treatment of domestic wastewater in a lab and pilot scale polyurethane carrier reactor. In Proceeding Anaerobic Treatment a Grown-up Technology, Amsterdam, September 16-18, pp. 437-450.

Dong, X., Plugge, C.M., and Stams, A.J.M. 1994. Anaerobic degradation of propionate by a mesophilic acetogenic bacterium in coculture and triculture with different methanogens. *Appl. Environ. Microbiol.* **60:** 2834-2838.

Fang, H.H.P., Li, Y.Y., and Chui, H.K. 1995. Performance and sludge characteristics of UASB process treating propionate-rich wastewater. *Water Res.* **29:** 895-898.

Frankin, R.J., Koevoets, A.A., van Gils, W.M.A., van der Pas, A. 1992. Application of the BIOBED upflow fluidized bed process for anaerobic waste water treatment. *Wat. Sci. Tech.*, **25:** 373-382.

Fukuzaki, S., Nishio, N., Shobayashi, M., and Nagai, S. 1990. Inhibition of the fermentation of propionate to methane by hydrogen, acetate, and propionate. *Appl. Environ. Microbiol.* **56:** 719-723.

Gottschalk, G. 1985. Bacterial metabolism, 2nd ed. Springer-Verlag, New York.

Grant, S., Lin, K.C. 1995. Effects of temperature and organic loading on the performance of upflow anaerobic sludge blanket reactors. *Can. J. Civ. Engin.*, **22:** 143-149.

Hawkes, F.R., Guwy, A.J., Rozzi, A.G., and Hawkes, D.L. 1993. A new instrument for on-line measurement of bicarbonate alkalinity. *Water Res.* **27:**167-170.

Heitzer, A., Kohler, H.P.E., Reichert, P. and Hamer, G. 1991. Utility of phenomenological models for describing temperature dependence of bacterial growth. *Appl. Environ. Microbiol.*, **57:** 2656-2665.

Henzen, M. and Harremoes, P. 1983. Anaerobic treatment of waste water in fixed film reactors-literature review. *Wat. Sci. Technol.*, **15:** 1-102.

Heppner, B., Zellner, G., and Diekmann, H. 1992. Start-up and operation of a propionate-degrading fluidized-bed reactor. *Appl. Microbiol. Biotechnol.* **36:** 810-816.

Heyes, R.H., and Hall, R.J. 1983. Kinetics of two subgroups of propionate-using organisms in anaerobic digestion. *Appl. Environ. Microbiol.* **46:** 710-715.

Houwen, F.P., Dijkema, C., Stams, A.J.M., and Zehnder, A.J.B. 1991. Propionate metabolism in anaerobic bacteria; determination of carboxylation reactions with ^{13}C-NMR spectroscopy. *Biochim. et Biophys. Acta*, **1056:**126-132.

Iza, J., Colleran, E., Paris, J.M,. Wu, W-M. 1991, International workshop on anaerobic treatment technology for municipal and industrial wastewaters: Summary paper. *Wat. Sci. Tech.*, **24 (8):** 1-16.

Jewell, W.J., and Morris, J.W. 1981. Influence of varying temperature, flow rate an substrate concentration on the anaerobic attached film expanded bed process. In: Proceeding of the 36th Ind. waste conf., Purdue University, pp. 1 - 24.

Kato, T.M., Field, J.A., Versteeg, P., and Lettinga, G. 1994. Feasibility of Expanded Granular Sludge Bed Reactors for the anaerobic treatment of low strength soluble wastewaters. *Biotech. Bioengin.*, **44:** 469-479.

Kenedy, K. J. and Van den Berg, L. 1981. Effects of temperature and overloading on the performance of anaerobic fixed film reacttors. In Proceeding of 36th annual industrial waste conference, Purdue University, Lafayette, Indiana.

Koster, I.W., and Lettinga, G. 1985. Application of the upflow anaerobic sludge bed (UASB) process for treatment of complex wastewaters at low temperatures. *Biotechnol. and Bioengin.*, **27:** 1411-1417.

Kotsyurbenko, O. R., Simankova, M. V., Nozhevnikova, A. N., Zhilina, T. N., Bolotina, N. P., Lysenko, A. M., and Osipov, G. A. 1995. New species of psychrophilic acetogens: *Acetobacterium bakii* sp.nov., *A. paludosum* sp.nov., *A. fimetarium* sp.nov. *Arch. Microbiol.* **163:** 429-434.

Last van der, A.R.M., and Lettinga, G. 1992. Anaerobic treatment of domestic sewage under moderate climatic (Dutch) conditions using upflow reactors at increased superficial velocities. *Wat. Sci. Technol.*, **25:** 167-178.

Lettinga, G. 1995. Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek.* **67:** 3-28.

Lettinga, G, and Hulshoff Pol, L.W. 1991, UASB-process design for various types of wastewaters. *Wat. Sci. Tech.*, **24:** 87-107.

Lettinga, G. 1978. Feasibility of anaerobic digestion for purification of industrial waters. In Proceeding of 4th Symposium EAS, June, Munich, pp. 226-256.

Lide, D.R. (ed.) 1992. CRC Handbook of Chemistry and Physics, 73rd edition, 1992-1993,CRC Press, Inc., Boca Raton, USA.

Lin, C.Y., Noike ,T., Sato, K., Matsumoto, J. 1987. Temperature Characteristics of the methanogenesis process in anaerobic digestion. *Wat. Sci. Tech.*, **19:** 299-310.

Lin, C.Y., Sato, K., Noike, T., and Matsumoto, J. 1986. Methanogenic digestion using mixed substrate of acetic, propionic and butyric acids. *Water Res.* **20:**385-394.

Marchaim, U., and Krause, C. 1993. Propionic to acetic acid ratios in overload anaerobic digestion. *Bioresource Technol.* **43:**195-203.

Matsushige, K, Inamori, Y, Mizuochi, M, Hosomi, M, Sudo, R 1990. The effects of temperature on anaerobic filter treatment for low-strength organic wastewater. *Environ. Technol.*, **11:** 899-910.

Mawson, A.J., Earle, R.L., and Larsen, V.F. 1991. Degradation of acetic and propionic acids in the methane fermentation. *Water Res.* **25:**1549-1554.

McCarty, P.L. 1982. One hundred years of anaerobic treatment. In Anaerobic digestion 1981, eds Hughes, D. E., Stafford, D. A., Wheatly, B. I., Baader, W., Lettinga, G., Nyns, E. J. And Verstraete, W., Amsterdam, Elsevier, pp. 3-22.

McInerney, M.J., Bryant, M.P., Hespell, R.B., and Costerton, J.W. 1981. Syntrophomonas wolfei gen. nov. spec. nov., an anaerobic syntrophic fatty acid-oxidizing bacterium. *Appl. Environ. Microbiol.* **41:** 1029-1039.

Mills, P.J. Minimisation of energy input requirements of an anaerobic digestor. *Agricult. Wastes.*, 1979, 1: 57-66.

O' Rourke, J. T. 1968. Kinetics of anaerobic waste treatment at re-duced temperatures. Ph. D. *Thesis*, Stanford University, California, USA.

Pauss, A., Samson, R. and Guiot, S. 1990. Continuous measurement of dissolved H_2 in an anaerobic reactor using a new hydrogen/air fuel cell detector. *Biotechnol. Bioeng.* **35:** 495-501.

Pavlostathis, S.G. and Giraldo-Gomez, E. 1991. Kinetics of anaerobic treatment: a critical review. *Critical Rev. in Env. Control*, **21:** 411-490.

Perry, R.H. and Green, D.W. (eds) 1984. Perry's chemical engineers' handbook, section 14, 6th edition, McGraw-Hill Publishing Co., New York, USA.

Plugge, C.M., Dijkema, C., and Stams, A.J.M. 1993. Acetyl-CoA cleavage pathway in a syntrophic propionate oxidizing bacterium growing on fumarate in the absence of methanogens. *FEMS Microbiol. Lett.* **110:** 71-76.

Rinzema, A, van Veen, H, Lettinga, G. 1993. Anaerobic digestion of triglyceride emulsions in expanded granular sludge bed upflow reactors with modified sludge separators. *Environ. Technology*, **14:** 423-432.

Robbins, J.E. 1988. A proposed pathway for catabolism of propionate in methanogenic cocultures. *Appl. Environ. Microbiol.* **54:**1300-1301.

Romesser, J.A., Wolfe, R.S., Mayer, F., Spiess, E., and Walther-Mauruschat, A. 1979. *Methanogenium*, a new genus of marine methanogenic bacteria, and characterization

of *Methanogenium cariaci* sp. nov. *Methanogenium marisnigri* sp. nov. *Arch. Microbiol.* **121**:147-153

Samain E., Dubourguier H.C. and Albanac G. 1984. Isolation and characterization of *Desulfobulbus elongatus* sp. nov. from a mesophilic industrial digester. *Syst. Appl. Microbiol.*, **5**: 391 - 401.

Sanz, I. and Fernandez-Polanco, F. 1990. Low temperature treatment of municipal sewage in anaerobic fluidized bed reactors. *Wat. Res.*: **24**: 463-469.

Smith, D.P., and McCarty. P.L. 1989. Reduced product formation following perturbation of ethanol- and propionate-fed methanogenic CSTRs. *Biotechnol. Bioeng.* **34**: 885-895.

Stams, A.J.M., Kremer, D.R., Nicolay, K., Weenk, G.H. and Hansen , T.A. 1984. Pathway of propionate formation in *Desulfobulbus propionicus*. Arch. Microbiol., **139**: 167-173.

Switzenbaum, M.S. and Jewell, W.J. 1978. Anaerobic attached film expanded bed reactor treatment of dilute organics. In: Proceedings of the 51st Annual WPCF Conference. Anaheim, California, pp. 1-164.

Thauer, R.K., K. Decker, and K. Jungermann. 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev.* **41**:100-180.

Thiele, J.H., and Zeikus, J.G. 1988. Control of interspecies electron flow during anaerobic digestion: significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. *Appl. Environ. Microbiol.* **54**: 20-29.

Tholozan, J.L., Samain, E., Grivet, J.P., Moletta, R., Dubourguier, H.C., and Albagnac, G. 1988. Reductive carboxylation of propionate to butyrate in methanogenic ecosystems. *Appl. Environ. Microbiol.* **54**: 441-445.

Van den Berg, L. 1977. Effect of temperature on growth and activity of a methanogenic culture utilising acetate. *Can. J. Microbiol.*, **23**: 898-902.

Wiegel, J. 1990. Temerature spans for growth: hypothesis and discussion. *FEMS Microbiol. Rev.*, **75**:155-170.

Wu, W.-M., Thiele, J.H., Jain, M.K., and Zeikus, J.G. 1993. Metabolic properties and kinetics of methanogenic granules. *Appl. Microbiol. Biotechnol.* **39**:804-811.

Chapter 2

Start-up of psychrophilic EGSB systems with mesophilic granular sludge as seed material

Published as \ accepted as:

2.1 Rebac, S.; Ruskova, J.; Gerbens, S.; van Lier, J. B.; Stams, A. J. M.; Lettinga, G. (1995) High-rate anaerobic treatment of wastewater under psychrophilic conditions. *J. Ferment. Bioeng.*, **5**: 15-22.

2.2 Rebac, S.; Gerbens, S.; Lens, P.; van Lier, J. B.; Stams, A. J. M.; Lettinga, G. (1998) Kinetic of fatty acid degradation by psychrophilically cultivated anaerobic granular sludge. *Bioresource Technol.*, (in press).

2.3 Rebac, S., Visser, A., Gerbens, S., van Lier, J.B., Stams, A.J.M. & Lettinga, G., (1996) The effect of sulphate on propionate and butyrate degradation in a psycrophilic anaerobic expanded granular sludge bed (EGSB) reactor. *Environ. Technol.*, Vol 17: 997-1005.

2.1 HIGH-RATE ANAEROBIC TREATMENT OF WASTEWATER UNDER PSYCHROPHILIC CONDITIONS

ABSTRACT

The start-up and operation of an expanded granular sludge bed (EGSB) reactor under psychrophilic (10-12 °C) conditions was studied. The reactor was seeded with mesophilic methanogenic granular sludge and fed with a mixture of volatile fatty acids (VFA). Chemical oxygen demand (COD) removal efficiencies exceeded 90 % at imposed organic loading rates up to 12 g $COD \times l^{-1} \times d^{-1}$ at 10-12 °C using influent concentrations ranging from 500 to 800 mg $COD \times l^{-1}$. The applied hydraulic retention time (HRT) was between 2.5 and 1.6 h and a liquid upflow velocity of 10 $m \times h^{-1}$ was applied. The effect of temperature on the specific VFA conversion rates was assessed using batch activity assays for the seed sludge and the sludge cultivated in the reactor. The optima temperatures for substrate conversion of sludge exposed to long term psychrophilic conditions were similar to those of the original mesophilic inoculum. Both sludges exerted an optimum substrate conversion rate at 35-40 °C. The temperature dependence of acetate conversion between 10 °C and 40 °C could be described by an Arrhenius derived model. Propionate, butyrate and VFA mixtures degrading activities for the same temperature range could be described by a square root model. The specific activities of the sludge in the reactor increased in time indicating enrichment of methanogens and acetogens even at low temperature.

Key words: Expanded granular sludge bed, psychrophilic conditions, anaerobic treatment, volatile fatty acids, temperature dependence, methanogenesis.

2.1.1 INTRODUCTION

Modern 'high rate' anaerobic treatment systems are based on sludge immobilization retaining as much viable sludge as possible in the reactor. As a result of the high sludge concentration of these systems, conversion rates exceeding 40-60 kg $COD \times m^{-3} \times d^{-1}$ can be easily reached at 30-40 °C for soluble wastewaters (1). High biomass retention in principle also offers the opportunity to treat soluble wastewaters at low temperature (2,3,4,5,6,7). However, so far practically all full scale applications of anaerobic treatment are restricted to wastewaters with a temperature exceeding 18 °C. Under moderate climate conditions, many wastewaters are discharged at low ambient temperatures, e.g. those from bottling, malting and brewery. The COD concentrations of these wastewaters are generally relatively low (< 1500 mg $COD \times l^{-1}$). Anaerobic treatment of low strength wastewaters at low temperature has been limited to a few studies (4,5,8,9). The results obtained so far were not encouraging full scale application of the reactor systems at low temperature (t < 20 °C) since conditions of 'high rate' anaerobic treatment have not yet been achieved.

Anaerobic treatment of low strength wastewaters at low temperatures may give rise to a number of problems which have to be solved. The low COD concentration of the influent

results in very low substrate levels inside the reactor, and to a low biogas production rate as well. Consequently, a lower mixing intensity and a poor substrate-biomass contact can be expected. When treating low strength wastewater, the required reactor volume generally will be determined by the hydraulic retention time, rather than by the organic space load (1). This is due to the fact that treatment of very low strength wastewater at the maximum possible organic loading rate with respect to the maximum COD conversion capacity of the sludge, might cause severe hydraulic wash out of the sludge. For very low strength wastewaters, little if any wash-out of sludge can be admitted, because the amount of active biomass growing per m^3 wastewater is very low.

Previous experiments in optimizing the sludge - wastewater contact, led to the development of an advanced anaerobic reactor design, the expanded granular sludge bed reactor (EGSB) (8,10-13), which is similar to the fluidized bed (FB) systems. However, the EGSB uses granular sludge, while the FB uses inert carrier material for attachment of active biomass. The superficial liquid velocities applied in the EGSB system are between 4 to 10 $m \times h^{-1}$. The high superficial liquid velocities required can be achieved by applying effluent recycle and/or by increasing the height of the reactor. While on the one hand, a high liquid upflow velocity is used to provide expansion of the sludge bed, on the other hand this might cause high erosion of the granular aggregates in the early stages of the start up.

In the present research, we investigated the start-up and operation of a 'high rate' EGSB reactor at 10-12 °C. The reactor was seeded with mesophilic granular sludge and after seeding, the process temperature was immediately set at 10-12 °C.

2.1.2 MATERIALS AND METHODS

Experimental conditions.

Experiments were performed using a 0.05 m diameter glass EGSB reactor (Fig. 2.1.1) with total volume 4.3 l (settler included). The reactor was equipped with a screen (circle openings 1 mm) placed below the gas-liquid-solids separator device. Temperature was controlled by thermostat-cooling system which consisted of a two different cooling devices (Fryka-Kältetechnik, Germany and Rheinische Geraetebau GmbH, Switzerland) and a heat-exchanger with the pump (Iwaki magnet pump MD-15R-220N, Tokyo, Japan) connected to the double wall of the reactor. Temperature in the sludge bed was measured with a thermometer (TES 1320 type-K, Taiwan). Methane production was measured by a wet-test gas meter (Meterfariek, Dordrecht, The Netherlands) at 20 °C after the biogas had been led through a NaOH solution (10 % w/w) and a column of soda lime pellets with indicator (Merck, Darmstadt, Germany). The main flow was provided with a peristaltic pump (Watson Marlow 501 U, Falmouth, Cornwall, UK) pumping cooled tap-water of 4-6 °C, to which concentrated feed stock solution was supplied with a separate peristaltic pump (Gilson - Minipuls 2, Villiers-Le-Bel, France). Recirculation of the effluent was imposed to the system by a peristaltic pump (Watson Marlow 502 S, Falmouth,

Cornwall,UK) enabling the desired expansion of the sludge bed in the reactor. Tapwater, feed stock and recirculation flow were combined before entering the reactor.

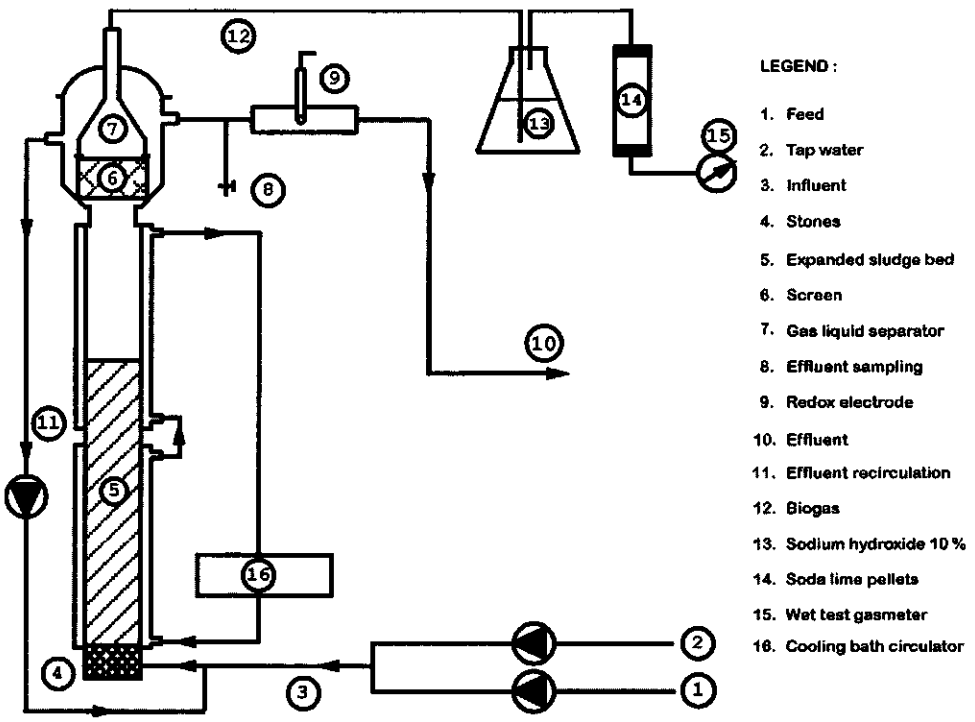

Fig. 2.1.1 Schematic diagram of 4.0 l EGSB reactor used in this study.

Biomass.

The reactor was inoculated with elutriated mesophilic granular methanogenic sludge, originating from a 760-m^3 UASB reactor (20-24 °C) of the Bavaria brewery at Lieshout, The Netherlands. The total amount of granular sludge added at the start-up was approximately 100 g volatile suspended solids (VSS).

Medium.

The reactor was fed with a concentrated stock solution of 33.36 g chemical oxygen demand (COD)×l^{-1}. The substrate consisted of a partly neutralized (pH 6.5) volatile fatty acid (VFA) mixture composed of acetate ; propionate and butyrate in the ratio 1 : 1.5 : 1.8, based on COD. After day 205 this ratio was changed to 3 : 1 : 1. The concentrations of basal nutrients in the concentrated stock solution were (g×l^{-1}): NH_4Cl, 43.5; KH_2PO_4, 7.075; $(NH_4)_2SO_4$, 7.0 (from day 30 to 161, 3.5); $MgCl_2 \times 6H_2O$, 6.25; $CaCl_2 \times 2H_2O$, 2.75; yeast extract, 0.825. After day 161

the concentration and composition of basal nutrients in the stock solution were changed to ($g \times l^{-1}$): NH_4Cl, 7.5; $MgSO_4 \times 7H_2O$, 1.5; $NaH_2PO_4 \times 2H_2O$, 27.6; K_2HPO_4, 21.2; $CaCl_2 \times 2H_2O$, 0.3; yeast extract, 0.5. To each litre of stock solution 4.5 ml of a trace element solution was added containing ($mg \times l^{-1}$): $FeCl_2 \times 4H_2O$, 2000; H_3BO_3, 50; $ZnCl_2$, 50; $CuCl_2 \times 2H_2O$, 30; $MnCl_2 \times 4H_2O$, 500; $(NH_4)_6Mo_7O_{24} \times 4H_2O$, 50; $AlCl_3 \times 6H_2O$, 90; $CoCl_2 \times 6H_2O$, 2000; $NiCl_2 \times 6H_2O$, 92; $Na_2SeO_3 \times 5H_2O$, 164; EDTA, 1000; resazurin, 200; 36% HCl, 1 $ml \times l^{-1}$. All chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany). Yeast extract was purchased from Unipath Ltd. (Basingstoke, Hampshire, UK), resazurin from Fluka (Buchs, Switzerland), and the gases from Hoekloos (Schiedam, The Netherlands).

Start-up of the reactor.

Feeding of the reactor was started immediately after inoculation with the mesophilic granular sludge, at an organic loading rate (OLR) of 7 g $COD \times l^{-1} \times d^{-1}$ and a hydraulic retention time (HRT) of 2.5 h. From the start of the experiment the temperature of the reactor was set at 11 °C. In continuous operation of the reactor, the samples of influent and effluent were taken three times per week in duplicate, except for the first week, when the samples were taken every day.

Batch experiments.

The temperature dependence of the substrate activity of the cultivated sludge and the seed material was determinated in triplicate, except for the sludge sample taken at day 235, which was determinated in duplicate. Serum bottles (120 ml) were filled with 100 ml medium and approximately 1 g volatile suspended solids $(VSS) \times l^{-1}$. Thereafter the bottles were brought to the desired temperature and were placed in a Gerhardt RO 20 rotating shaker (Bonn, Germany) at 50 (rpm). The medium consisted of approximately 3 g chemical oxygen demand $(COD) \times l^{-1}$ of either sodium acetate, sodium propionate, sodium butyrate or a neutralized VFA mixture (acetate : propionate : butyrate = 1 : 1.5 : 1.8 ; pH 6.5) as substrates. The mineral composition was ($g \times l^{-1}$): NH_4Cl, 0.28; $MgSO_4 \times 7H_2O$, 0.11; K_2HPO_4, 2.0; $NaH_2PO_4 \times 2H_2O$, 3.33; yeast extract, 0.10; and 1 $ml \times l^{-1}$ trace element solution. The Bavaria seed sludge, which before use had been stored for a period of two months at 4°C, was first activated for some days at 20°C with a VFA mixture (as above) as feed (two feedings of 3 g VFA-$COD \times l^{-1}$) before measuring the activity. Sludge samples from the psychrophilic reactor were used directly. After closing the bottles and changing the gas phase composition to N_2/CO_2 (70%/30%), Na_2S (1 $ml \times l^{-1}$ from 1 M stock solution) was added to assure completely anaerobic conditions. At various periods of time, samples were taken and analyzed for acetate, propionate and butyrate. After termination of each experiment the exact amount of VSS in the bottles was determined. The specific activity was calculated from the linear decrease of the substrate concentration which was followed until substrate concentrations dropped below 500 mg $COD \times l^{-1}$. The temperature dependence of the maximum acetate conversion rate was fitted using an Arrhenius derived equation which

describes the effect of temperature on the net microbial activity by recognizing the occurrence of a process of biosynthesis and microbial decay (14):

$$A_{max} = k_1 \bullet e^{a_1(T-x_T)} - k_2 \bullet e^{a_2(T-x_T)} \quad \text{.....1}$$

In the temperature range up to 40 °C equation (1) describes an exponential increase of the conversion rate with increasing temperature, where k_1 and a_1 are kinetic constants which can be calculated using linear regression as for temperature below the maximum temperature the second term of the eq. (1) is negligible. The kinetic constants k_2 and a_2 of the second term as well as the temperature correction factor (x_T) in both terms are calculated using a non-linear regression routine for parameter estimation (15).

The temperature dependence of the maximum butyrate, propionate and VFA mixture conversion rate was fitted using (Ratkowsy's square root empirical non-linear regression) model which describes a non-linear relationship between the net microbial activity in the entire temperature range (16):

$$\sqrt{A_{max}} = b_r \bullet (T - T_{min}) \bullet [1 - \exp[c_r \bullet (T - T_{max})]] \quad \text{.....2}$$

where b_r is a regression coefficient of the square root of microbial activity rate constant versus degrees Kelvin for temperatures below the optimal temperature. The constant c_r is an additional parameter to enable the model to fit the data for temperature above the optimal temperature. The kinetic constants b_r and c_r and T_{min} , T_{max} are calculated using the same non-linear regression routine for parameter estimation (15).

Between day 143 and 205 the EGSB reactor was operated in batch - mode for eight times in order to determine the apparent K_s value of the system for acetate, propionate and butyrate under psychrophilic conditions. Before starting the experiments, the reactor was flushed with tap water for a period of two HRTs in order to wash all substrate from the sludge bed and the reactor. In the recycle flow of the reactor then the substrate was supplied consisting of 2.6 g COD×l^{-1} sodium acetate, or 1.0 g COD×l^{-1} sodium propionate or 1.4 g COD×l^{-1} sodium butyrate with 50 ml mineral medium. The medium had the same composition as used for the batch experiments in the serum bottles. The batch experiments lasted for a period between 6 to 12 hours depending on the kind of substrate. Samples were taken every 15 to 20 minutes until substrate was completely depleted. After performing these batch experiments, the reactor immediately was operated again under continues flow conditions.

Size distribution of the sludge.

The size distribution of the sludge was determined by the image-analyzing technique. For sampling, the sludge bed was mixed by increasing the recirculation flow rate, and than settled so that a homogeneous sludge sample was obtained. A sludge sample of approximately 0.5 ml was placed in a 3.5 cm petridish. Pictures of the dishes (minimum of four plates per sample) were

digitalized and analyzed by image-analyzing software Magiscan, Genias (Version 3, 1991, Applied imaging, Gateshead, UK)

Estimation of dissolved methane in the effluent.

The solubility of the methane (CH_4) in water as a function of temperature, was derived from data in literature (17,18). The evaluated reference data were fitted to a smoothing equation 3 (18):

$$\mathrm{Ln}(X) = A_s + \frac{B_s}{\left(\frac{T}{100}\right)} + C_s \bullet \mathrm{Ln}\left(\frac{T}{100}\right) + D_s \bullet \left(\frac{T}{100}\right) \quad \ldots\ldots 3$$

where: X = litre of dissolved methane in water

T = (K)

A_s = 25.0726

B_s = -27.6867

C_s = -59.8201

D_s = 15.4911

Equation constants A_s , B_s , C_s and D_s were estimated using a non-linear regression routine for parameter estimation (15). Equation (3) is valid for the temperature range of 273.15 to 328.15 K. Dissolved methane in the effluent was corrected for the partial pressure of methane in the biogas, which was measured by gaschromatography.

Analyses

Samples for VFA analyses were centrifuged for 3 min at 10000 rpm in a Biofuge A (Heraeus Sepatech, Osterode, FRG). VFA were determined by gas chromatography. The chromatograph (Hewlett Packard 5890A, Palo Alto, USA) was equipped with a 2 m x 2mm (inner diameter) glass column, packed with Supelco port (100-120 mesh) coated with 10% Fluorad FC 431. Operating condition were: column, 130 °C; injection port, 200 °C; flame ionization detector, 280 °C. N_2 saturated with formic acid at 20 °C was used as carrier gas (30 $ml \times min^{-1}$). The biogas composition CH_4, CO_2, N_2, O_2 was determinated in 100 ml samples immediately after sampling using Fisons Instruments gas chromatography model GC 8000 series, equipped with columns connected in parallel (split 1:1) - (1.5mx2mm) teflon, packed with chromosorb 108, (60-80 mesh), and a (1.2mx2mm) stainless steel, packed with mol. sieve 5A, (60-80 mesh). Helium was used as a carrier gas (45 $ml \times min^{-1}$). The oven, detector, and injection temperature were 40 °C, 100 °C, 110 °C, respectively. Determination of the content of suspended and volatile solids in the effluent was determined after drying and incineration of the samples. Gravimetric method for the analysis of suspended and volatile solids was done according to the Netherlands norm (NEN 6621, 1988) using glass fiber filter GF 52 purchased from Schleicher

& Schuell (Dassel, Germany), determination was done in duplicate. All other analyses were determined according to standard methods (19).

2.1.3 RESULTS

Reactor performance.

The performance data of the EGSB reactor at the start up temperature of 11 °C, are shown in the Fig. 2.1.2a, b and c. During the first 40 days, the treatment efficiencies were between 40 - 80 %. Recovery of the methane production was very low compared with soluble COD removal. The observed difference between COD removal and methane yield can be attributed to sulphate reduction which was present in nutrients stock solution in concentration of 5, 2.5, and 0.6 g $SO_4^{2-} \times l^{-1}$ respectively in time and/or unforseen gas leakages. After day 50, the settler of the reactor was closed and connected to the gasmeter. This resulted in an 100 % increase of the recorded biogas production, but the gas production was still too low to account for the soluble COD removal. Another explanation might be the inaccuracy of the wet test gasmeter at low gas flow rates or a higher solubility of methane in the wastewater than that derived from data in the literature (17,18).

Despite the imposed upflow velocity of 6 $m \times h^{-1}$, sludge piston formation occurred. However, this problem was solved by increasing the upflow velocity to 10 $m \times h^{-1}$. From day 40 to 120 the efficiencies in the EGSB reactor increased gradually. After 80 days of stable continuous operation the reactor achieved a treatment efficiency above 90 %.

The apparent half saturation constant K_s was estimated in eight short term experiments of 6 -12 hours for various substrates after day 137. The apparent K_s values found in these batch-fed reactor experiments for acetate, propionate and butyrate were equal to 0.039, 0.014 and 0.142 g $COD \times l^{-1}$ respectively. The treatment efficiency of the reactor dropped considerably after resuming the continuous feed but recovered after 10 days. Propionate conversion was affected severely by the short batch operation of the reactor.

Even after 7 months operation under psychrophilic conditions, the reactor was highly susceptible to lowering the temperature by several degrees. Short temperature shocks to values as low as 5 to 7 °C, resulted in a decreased degradation rate of acetate down to 55 %, but the system recovered in the next two days to 75 % despite the very low temperature.

Degradation of individual fatty acids present in the VFA mixture showed different susceptibility to low temperature (Fig. 2.1.3). Acetate degradation was the most sensitive to low temperature, which was reflected in the high fluctuations in the removal efficiencies during the first 100 days of reactor operation. A highly stable degradation of acetate was achieved in the period from day 110 to 232. The temperature susceptibility of propionate degradation is very similar to the degradation of acetate (Fig. 2.1.3c). Even small temperature drops of 1 °C led to a substantial decrease of the propionate removal rate. Butyrate degradation was most stable towards temperature fluctuation (Fig. 2.1.3d), because the treatment efficiencies remained above 90 %.

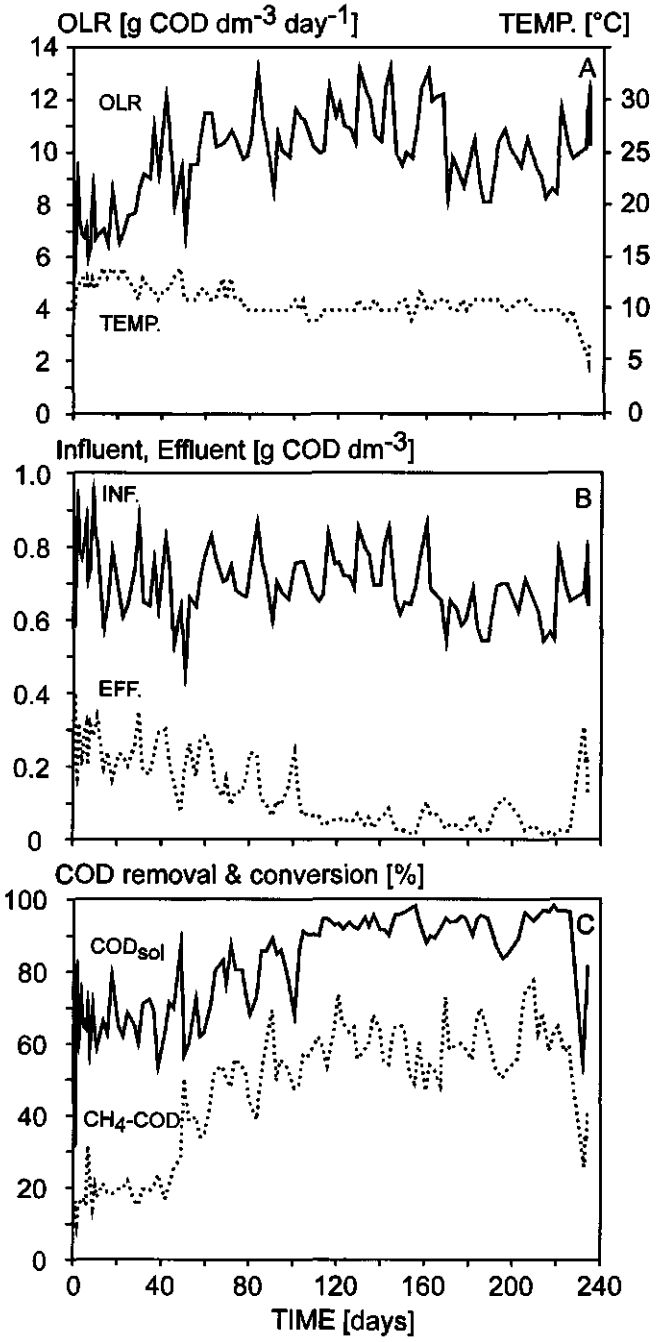

Fig. 2.1.2 Operation and efficiency of the EGSB reactor fed with low strength VFA mixtures. A. Organic loading rate (—) temperature °C (···). B. COD concentration (—) influent, (···) effluent. C. COD soluble removal (—), CH_4-COD conversion (···).

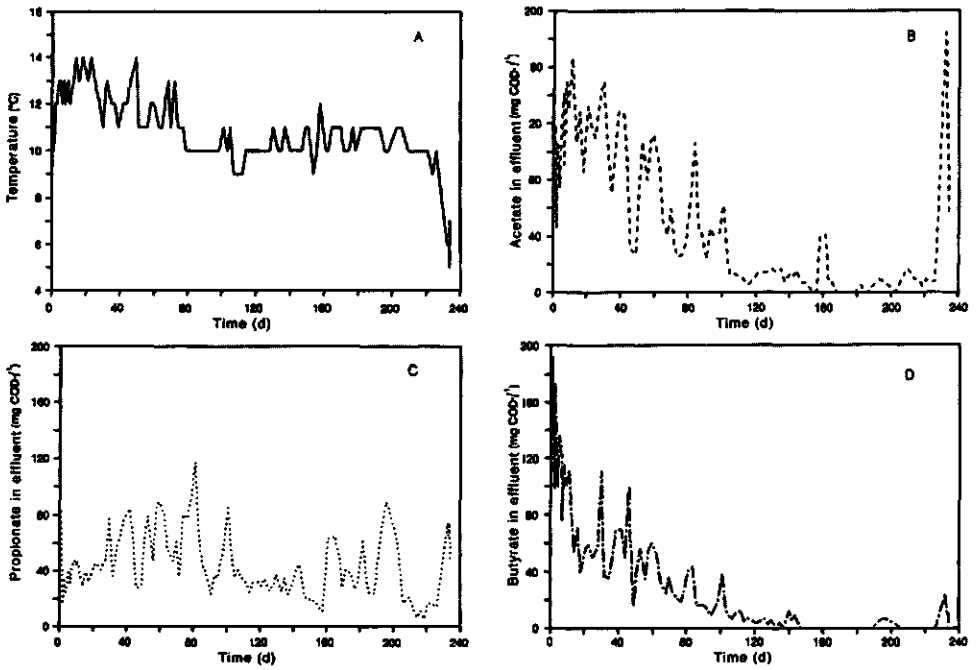

Fig. 2.1.3 Individual fatty acids degradation susceptibility to low temperatures. A. Temperature °C (—). B. Acetate in effluent (----). C. Propionate in effluent (···). D. Butyrate in effluent (– · –).

Temperature optima of the cultivated sludge.

The influence of temperature on the specific activities of acetate, butyrate, propionate and VFA mixture degradation of the sludge measured in the batch activity assays are depicted in Fig. 2.1.4a, b, c, and d. The figures clearly illustrate that the temperature curves from the sludge exposed to long term psychrophilic conditions were very similar to those of the mesophilic inoculum having optima between 30-40 °C, indicating that no specialized psychrophiles developed in the sludge or cannot be seen due to large amounts of mesophiles. The determined VSS content of the bottles incubated at 40 °C was 45 % lower compared to all other temperatures, probably resulting from cell lyses at 40 °C. Therefore, the extremely high activity found for acetate at 40°C with the sludge collected on day 235 day is in part due to high losses of VSS during these tests.

The figures also illustrate that the specific activities of the sludge in the reactor increased in time indicating enrichment of methanogens and acetogens even at the low temperatures. The increase in butyrate degrading activity was the most pronounced of all VFA.

The temperature dependence of the maximum acetate conversion rates were fitted using equation (1). The temperature dependence of the maximum butyrate, propionate and VFA

VFA mixture conversion rates were fitted using equation (2), because no reasonable fit could obtain using the first equation (20).

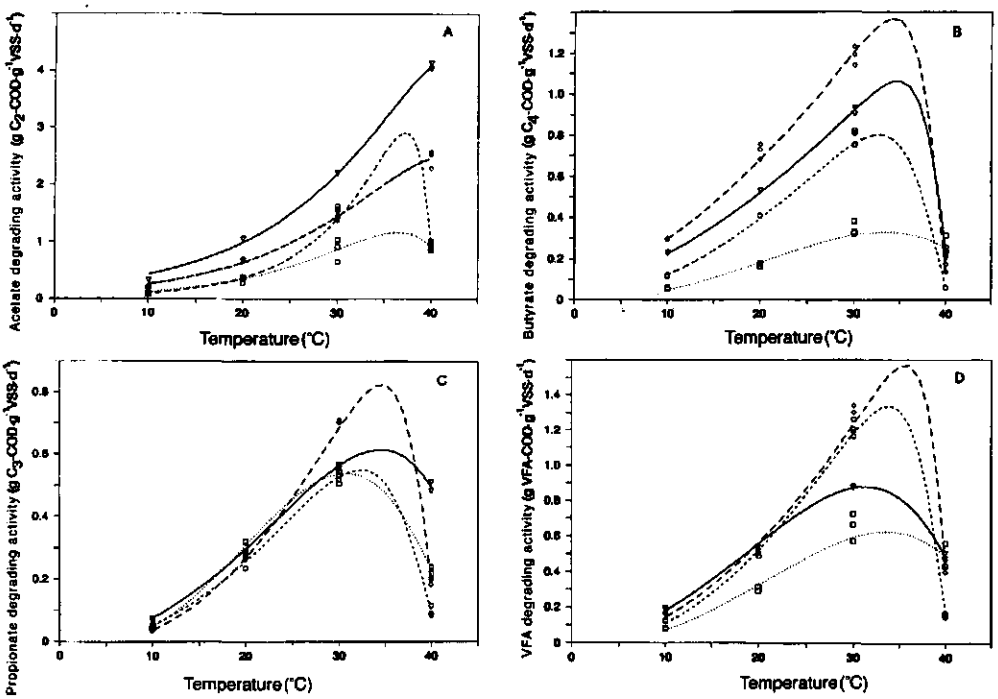

Fig. 2.1.4 Temperature characteristics of the cultivated sludge at psychrophilic conditions. A. Acetate degrading activity (g C_2-COD g^{-1}VSS d^{-1}). B. Butyrate degrading activity (g C_4-COD g^{-1}VSS d^{-1}). C. Propionate degrading activity (g C_3-COD g^{-1} VSS d^{-1}). D. VFA degrading activity (g VFA-COD g^{-1}VSS d^{-1}) at various temperatures of sludge cultivated in EGSB reactor under psychrophilic conditions at (◊) 64 days, (o) 134 days, (∇) 235 days. As a reference the activities of the mesophilic inoculum () are also depicted. The lines are computed using eq. (1) for acetate degrading activity and eq. (2) for all others degrading

Sludge washout

The EGSB reactor are characterized by intensive mixing of the bulk liquid phase which results to some extent in erosion of the sludge granules, particularly during the initial phases of the experiment. Small dispersed sludge particles were rinsed from the EGSB reactor. None the less, a satisfactory sludge hold up was achieved in the high loaded reactor. Using a theoretical cell yield of 0.03 g VSS×g^{-1} COD (7) and a 90 % COD conversion rate, the net growth of the sludge was approximately 23 mg VSS×l^{-1} . The effluent biomass concentration, measured as the fraction of suspended solids in the effluent, was low (Fig. 2.1.5a, b).

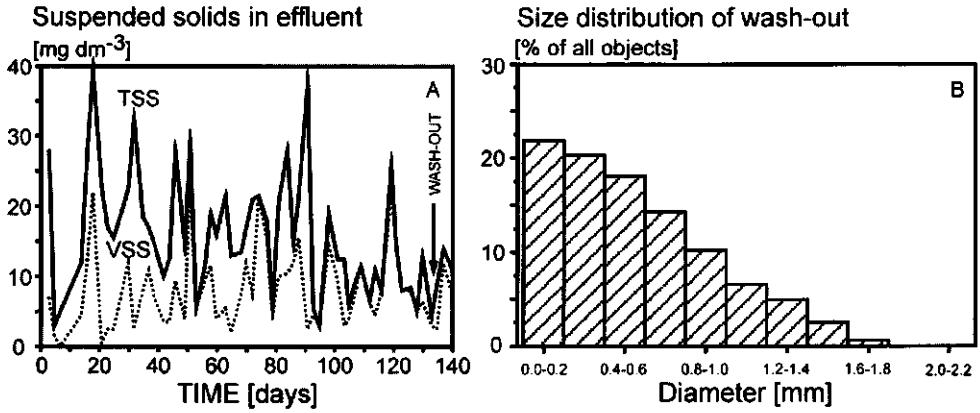

Fig. 2.1.5 Suspended solids in effluent and size distribution of wash-out. A. Total suspended solids TSS (—), volatile suspended solids VSS (···). B. Particles size distribution of wash-out.

Size distribution

The development of the granulation process in the EGSB reactor under psychrophilic conditions is depicted in Figs. 2.1.6a, b, c, and d. The results show a distinct decrease in the granules diameter at day 64, followed by a significant increase in the sludge diameter thereafter. This indicates that after an initial period of sludge erosion new biomass was becoming attached to the granules under psychrophilic conditions.

2.1.4 DISCUSSION

The results of the present study clearly reveal the potential of the EGSB reactor as a 'high rate' treatment system for low strength wastewater under psychrophilic conditions (10-12 °C). COD removal efficiencies over 90 % were achieved at organic loading rates up to 12 g $COD \times l^{-1} \times d^{-1}$ and at HRT as low as 1.6 h using a VFA-mixture as feed. These results represent a definite breakthrough with respect to the application of anaerobic treatment systems at low ambient temperatures for low strength wastewaters.

One of the main concerns of this study was to determine if psychrophilic populations of methanogens develop during long term operation at low temperature. During the course of the experiment the optimum temperature for acetoclastic activity did not decrease, instead it slightly increased indicating that the dominant populations were still mesophilic after 8 months of operation under psychrophilic conditions (Fig. 2.1.4a). Also in the case of propionate, butyrate and VFA acetoclastic and hydrogenotrophic methanogens have been isolated from sediment with a temperature optimum clearly below the mesophilic range, i.e. at 20 and 28 °C respectively (21,22,23).

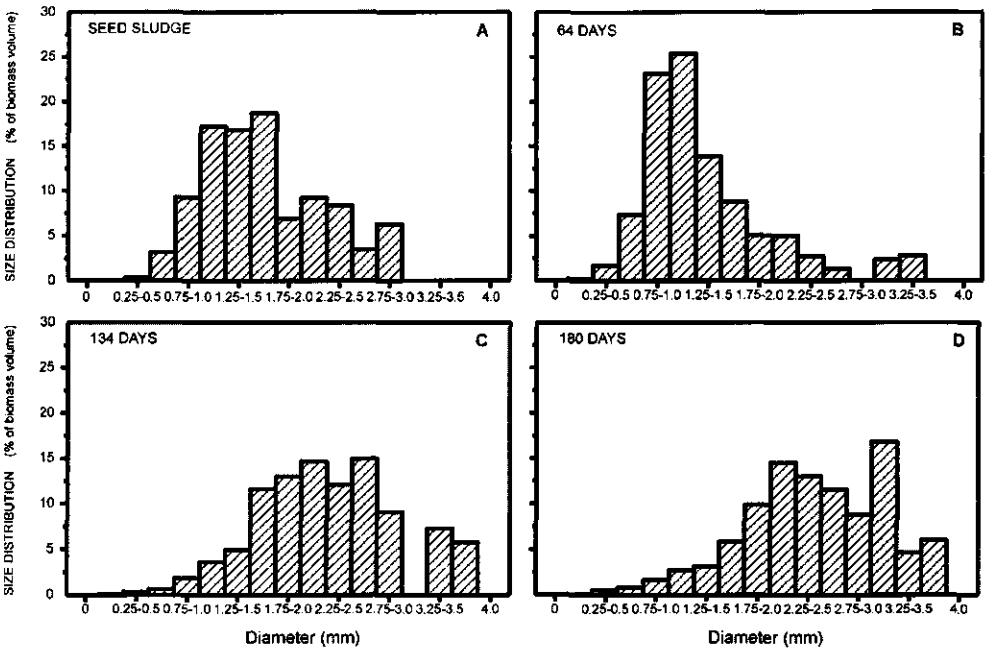

Fig. 2.1.6 Size distribution of granular sludge at the start of the experiments and at day 64, 134 and 180, expressed in percentage of the biomass volume represented by the granules.

The specific activity of the seed sludge improved significantly during the course of the experiment, which indicates that growth and enrichment of methanogens and acetogens in the reactor sludge was occurring at the low temperature used in this study.

The interesting observation that a high COD removal efficiency was still achieved at very low substrate levels can be attributed to the very low apparent K_s values of the system for different VFA substrates. This finding represents a very important step forward in the application of anaerobic treatment system. Our results demonstrate how adequate hydraulic mixing is essential for lowering the apparent K_s. Kato (13) found extremely low apparent K_s value (0.01 g COD×l^{-1}) of the EGSB system fed with ethanol at 30 °C. So far significantly higher values of apparent K_s were obtained in batch assays with 130 ml serum vials (24).

During the long period of reactor operation very little propionate degrading capacity was observed. This result suggests that propionate degraders posses a low activity under psychrophilic conditions which is in agreement with the batch activity tests.

A problem which might occur in lab-scale EGSB reactors is sludge bed piston formation which is related to the diameter of the reactor, the gas production rate and the liquid up-flow velocities. Kato (13), found that piston flotation occurs at 30 °C at low liquid velocities v_{up} 2.5 m×h^{-1}. However, in the present studies, it occurred even at 6 m×h^{-1}, despite the same internal reactor diameter of 0.05 m. Piston formation probably was enhanced by the lower biogas

production rate, the higher liquid viscosity at low temperature, and the larger sludge particles. The problem was solved by increasing the upflow velocity to 10 $m \times h^{-1}$. The higher up-flow velocity applied may enhance shear forces, as well as segregation of the granules in the sludge bed (13,25). In the present study hydraulic load of 10 $m \times h^{-1}$ resulted in higher shear forces on the granular aggregates. The granular seed sludge from the mesophilic UASB reactor might have been affected in two ways by these shear forces. 1.) The abrasion of the granules has led to an increase of dispersed sludge, which is washed out from the reactor. However the wash-out of sludge decreases with time during the experiment. The washed out particles are rather small and don't have a granular shape. The concentration of VSS in the effluent in our study was on the average 10 $mg \times l^{-1}$. The results reveal that the system can be considered as stable with respect to the biomass hold-up. Similar results were obtained for (AAFEB) anaerobic attached film expanded bed reactors (4). 2.) A second effect of shear forces on the seed granules, might be an increasing density of the granules, resulting from the new environment conditions and selection pressure for the anaerobic microorganisms. The increase density corresponds to decreasing porosity (26), which may enhance substrate diffusion limitations.

Granule size distribution was used to characterize the growth of granules in the EGSB reactor under psychrophilic conditions. The results reveal a decrease in the granule diameter after seeding probably caused by erosion. Afterwards, the sludge granules started to grow which is in congruent with their enrichment in methanogenic activity.

2.1.5 ACKNOWLEDGMENTS

We would like to thank J. van der Laan and I. Bennehey for the technical assistance with the chromatographs.

2.1.6 NOMENCLATURE

a_1 = synthetic energy constant (K^{-1})
a_2 = degradative energy constant (K^{-1})
A = specific activity ($g\ COD \times g^{-1}\ VSS \times d^{-1}$)
A_{max} = maximum specific activity ($g\ COD \times g^{-1}\ VSS \times d^{-1}$)
b_r = Ratkowsky's regression coefficient (K^{-1})
c_r = Ratkowsky's degradative constant (K^{-1})
COD = chemical oxygen demand ($g\ O_2 \times l^{-1}$; $mg\ O_2 \times l^{-1}$)
EGSB = expanded granular sludge bed
HRT = hydraulic retention time (h)
k_1 = temperature related activity constant ($g\ COD \times g^{-1}\ VSS \times d^{-1}$)
k_2 = temperature related decay constant ($g\ COD \times g^{-1}\ VSS \times d^{-1}$)
K_s = apparent half saturation constant ($g\ COD \times l^{-1}$)
OLR = organic loading rate ($g\ COD \times l^{-1} \times d^{-1}$)

t	= experimental time (d)
t	= temperature (°C)
T	= thermodynamic temperature (K)
T_{min}	= lower temperature limit (K)
T_{max}	= upper temperature limit (K)
TSS	= total suspended solids ($mg \times l^{-1}$)
UASB	= up-flow anaerobic sludge bed
v	= up-flow velocity ($m \times h^{-1}$)
VFA	= volatile fatty acids
VSS	= volatile suspended solids ($mg \times l^{-1}$)
x_T	= temperature correction factor (K)
X	= litre of methane per litre of water

2.1.7 REFERENCES

1. Lettinga, G., Hulshoff Pol, L.W.: UASB-process design for various types of wastewaters. Wat. Sci. Tech., 24, 87-107 (1991).
2. Jewell, W. J. and Morris, J. W.: Influence of varying temperature, flow rate an substrate concentration on the anaerobic attached film expanded bed process. p. 1-24. In: Proceeding of the 36th Ind. waste conf., Purdue University (1981).
3. Lettinga, G., Hulshoff Pol, L. W., Wiegant, W., de Zeeuw, W., Grin, P., Roersma, R. E., Koster, I. W. and Hobma, S. W.: High rate anaerobic waste water treatment using the UASB-reactor under a wide range of temperature conditions. Biotechnol. Genetic. Engin. Rev., 2, 253 - 284 (1984).
4. Switzenbaum, M. S. and Jewell, W. J.: Anaerobic attached film expanded bed reactor treatment of dilute organics. p. 1-164. In: Proceedings of the 51st Annual WPCF Conference. Anaheim, California (1978).
5. Kida, K., Tanemura, K. and Sonoda, Y.: Evaluation of the anaerobic treatment of sewage below 20°C by novel processes. J. Ferment. Bioeng., 76, 510-514 (1993).
6. Koster, I.W. and Lettinga, G.: Application of the upflow anaerobic sludge bed (UASB) process for treatment of complex wastewaters at low temperatures. Biotechnol. and Bioengin., 27, 1411-1417 (1985).
7. Lin, C.Y., Noike, T., Sato, K. and Matsumoto, J.: Temperature Characteristics of the methanogenesis process in anaerobic digestion. Wat. Sci. Tech., 19, 299-310 (1987).
8. de Man, A.W.A., van der Last, A.R.M. and Lettinga, G.: The use of EGSB and UASB anaerobic systems for low strength soluble and complex wastewaters at temperatures ranging from 8 to 30°C. p. 197-209. In: E.R. Hall and P.N. Hobson (eds.), Proceeding of the Fifth International Symposium on Anaerobic Digestion. Bologna, Italy (1988).

9. Matsushige, K., Inamori, Y., Mizuochi, M., Hosomi, M. and Sudo, R.: The effects of temperature on anaerobic filter treatment for low-strength organic wastewater. Environ. Technology, 11, 899-910 (1990).
10. Frankin, R.J., Koevoets, A.A., van Gils, W.M.A, van der Pas, A.: Application of the BIOBED upflow fluidized bed process for anaerobic waste water treatment. Wat. Sci. Tech., 25, 373-382 (1992).
11. Lettinga, G., Hulshoff Pol, L.W., Hobma, S.W., Grin, P., Jong, P. de, Roersma, R.E. and IJspeert, P.: The use of a floating settling granular sludge bed reactor in anaerobic treatment. p. 411-429. In: W.J. van den Brink (ed.), Proceedings of the European Symposium on Anaerobic Waste Water Treatment. Noordwijkerhout, The Netherlands (1983).
12. Rinzema, A., Veen, H. van and Lettinga, G.: Anaerobic digestion of triglyceride emulsions in expanded granular sludge bed upflow reactors with modified sludge separators. Environ. Technology, 14, 423-432 (1993).
13. Kato, T.M., Field, J.A., Versteeg, P. and Lettinga, G.: Feasibility of Expanded Granular Sludge Bed Reactors for the anaerobic treatment of low strength soluble wastewaters. Biotech. Bioengin., 44, 469-479 (1994).
14. Pavlostathis, S.G. and Giraldo-Gomez, E.: Kinetics of anaerobic treatment: a critical review. Critical Rev. in Env. Control 21, 411-490 (1991).
15. Nelder, J.A. and Mead, R.: A simplex method for function minimization. The Computer Journal, 8, 308-313 (1965).
16. Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N. and Chandler, R.E.: odel for bacterial culture growth rate throughout the entire biokinetic temperature range. J. Bacteriology, 154, 1222-1226 (1983).
17. Cosgrove, B.A. and Walkley, J.: Solubilities of gases in H_2O and ^2H_2O. Chromatography, 216, 161-167 (1981).
18. CRC Handbook of Chemistry and Physics: p. 6-3 to 6-6. In: D.R. Lide (ed.), 73rd edition, CRC Press, Inc., Boca Raton, USA (1992-1993).
19. American Public Health Association: Standard methods for the examination of water and wastewaters. 16th edition. Washington DC, USA (1985).
20. Heitzer, A., Kohler, H.P.E., Reichert, P. and Hamer, G.: Utility of phenomenological models for describing temperature dependence of bacterial growth. Appl. Environ. Microbiol., 57, 2656-2665 (1991).
21. Romesser, J.A., Wolfe, R.S., Mayer, F., Spiess, E. and Walther-Mauruschat, A.: *Methanogenium*, a new genus of marine methanogenic bacteria, and characterization of *Methanogenium cariaci* sp. nov. and *Methanogenium marisnigri* sp. nov.. Arch. Microbiol., 121, 147-153 (1979).
22. Simankova, M.V., Kotsyurbenko, O.R., Bolotina, N.P., Zhilina, T.N. and Nozhevnikova, A.N.: Psychrotrophic bacteria from several environments. p. 27-30, In:

Paper Pre prints of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa (1994).

23. Svensson, B.H.: Different temperature optima for methane formation when enrichments from acid peat are supplemented with acetate or hydrogen. Appl. Environ. Microbiology, 48, 389-394 (1984).
24. Dolfing, J.: Kinetics of methane formation by granular sludge at low substrate concentrations. The influence of mass transfer limitation. Appl. Microbiol. Biotechnol., 22, 77-81 (1985).
25. Guiot, S.R., Arcand, Y. and Chavarie, C.: Advantages of fluidization on granule size and activity development in upflow anaerobic sludge bed reactors. Wat. Sci. Tech., 26, 897-906 (1992).
26. Alphenaar, A., Pérez, C.M. and Lettinga, G.: The influence of substrate transport limitation on porosity and methanogenic activity of anaerobic sludge granules. Appl. Microbiol. Biotechnol., 39, 276-280 (1993).

2.2 KINETICS OF FATTY ACID DEGRADATION BY PSYCHROPHILICALLY CULTIVATED ANAEROBIC GRANULAR SLUDGE

ABSTRACT

The kinetic parameters of anaerobic granular sludge, grown at 10 °C in an expanded granular sludge bed (EGSB) reactor and fed with VFA mixtures, were determined in batch assays. The homoacetogenic, hydrogenotrophic and acetoclastic specific activities of the sludge at 10 °C, measured after 300 days of cultivation were 1.744, and 0.296 and 0.331 g COD g^{-1}VSS day^{-1}, respectively. At 30 °C these values were 18.024, 2.732 and 2.204 g COD g^{-1}VSS day^{-1}, respectively. These high sludge activities can be attributed to the good and stable enrichment of methanogenic, acetogenic and homoacetogenic bacteria under psychrophilic conditions. The temperature characteristics of the granular sludge and the homoacetogenic enrichment culture from cultivated sludge showed that the temperature optima are still in the mesophilic range (30-40 °C) even after long term (300 days) operation at low temperature (10-12 °C). In the overall conversion rate for acetate and propionate, evidence for the temperature compensation effect was found. In contrast, no temperature compensation effect was present for butyrate degradation. The results of EGSB batch reactor experiments revealed apparent half saturation constants of the acetate and propionate degraders in the range of 39-58 mg COD dm^{-3} and 7-14 mg COD dm^{-3}, respectively. For butyrate degraders, higher K_m values were found, i.e., 142-243 mg COD dm^{-3}. The observed low K_m values are in agreement with the high removal efficiencies of the EGSB reactor during anaerobic treatment of the cold, low strength, wastewater.

Key words: anaerobic granular sludge, EGSB reactor, Monod kinetics, Michaelis-Menten kinetics, specific substrate degrading activity.

2.2.1 INTRODUCTION

Anaerobic treatment of cold low strength wastewaters is a growing field of interest in environmental sanitation. Despite the low temperature, high-rate reactor operation for these types of wastewaters can be achieved by applying the expanded granular sludge bed (EGSB) reactor system. The advantage of EGSB systems over other high-rate anaerobic systems is the better contact between the sludge and the substrate (Zoutberg et al., 1997; Kato et al., 1994). As a consequence, higher biological conversion rates can be achieved, which is particularly the case for low strength wastewaters at lower temperatures (Rebac et al., 1995; 1997; van Lier et al., 1997).

A strong temperature effect on the kinetic parameters of microorganisms has been observed by many researchers (Lawrence & McCarty, 1969; Switzenbaum & Jewell, 1978; Lin et al., 1987; Matsushige et al., 1990; Wu et al., 1993; Rebac at al., 1995). Lowering the operational temperature generally leads to a decrease in the maximum specific growth and substrate utilisation rates, but it also may lead to an increased biomass yield (g biomass g^{-1} substrate converted) of methanogenic population (Van den Berg, 1977; Lin et al., 1987) or acidogenic sludge (van Lier et al., 1997). Temperature effects on kinetic parameters can be described

mathematically by using e.g. the Arrhenius equation (Pavlostathis & Giraldo-Gomez, 1991). However, it is hardly possible to describe with one single equation the changes in kinetic parameters induced by temperature for the various organisms present in methanogenic consortia (Heitzer et al., 1991).

An increase in the substrate affinity, characterised by a decrease in the apparent half saturation constant (K_m), can contribute to a temperature compensation effect. This effect means that at decreased temperature, the specific activity of sludge still remains high, despite the significantly lower maximum specific activity. When bacterial growth is negligible, as may be the case at low substrate concentrations and at a comparably high biomass concentration, Michaelis-Menten kinetics can be used to predict the changes in the temperature effect. The relationship between the maximum substrate degradation rate (V_{max}) and the apparent K_m, (V_{max}/K_m) has been previously used to show whether or not temperature compensation will manifest for given methanogenic consortium (Westermann et al., 1989, Lin et al., 1987). This ratio reflects the rate of substrate degradation at substrate levels much smaller than the K_m.

Mainly natural ecosystems as tundra soil, pond sediments and deep lake sediments have been investigated for methanogenesis at low temperatures (Nozhevnikova et al., 1997; Kotsyurbenko et al., 1996). To date, only two psychrophilic marine methanogens (Romesser et al., 1979) and a few psychrophilic and psychrotrophic acetogenic bacteria (homoacetogens) from natural sediments are isolated (Conrad et al., 1989; Kotsyurbenko et al., 1995). For Methanogenic granular sludge grown under psychrophilic conditions, little is known about the bacterial composition and kinetic parameters.

This paper describes the assessment of kinetic parameters for volatile fatty acids (VFA) and hydrogen conversions by methanogenic granular sludge grown in a VFA-fed EGSB reactor under psychrophilic conditions (10 °C). The temperature effect on these kinetic parameters was also determined. In order to assess the development of specialised hydrogen utilising psychrophiles or psychrotrophs in the granular sludge, a H_2 utilising enrichment culture was prepared from the sludge and the kinetic characteristics of this culture were also assessed.

2.2.2 MATERIALS AND METHODS

2.2.2.1 Source of biomass

Granular sludge was sampled from a psychrophilic (10-12 °C) EGSB reactor treating a VFA mixture of varying composition at volumetric loading rates of 10-12 g COD dm^{-3} day^{-1} for a period of 306 days. The reactor performance and physical-chemical characteristics of the sludge have been reported previously (Rebac et al. 1995).

2.2.2.2 Assessment of substrate depletion curve for the determination of A_{max}

The specific activities of the seed sludge, and the sludge cultivated in the reactor were determinated in serum bottles (0.120 dm^{-3}) which were filled with 0.1 dm^{-3} medium and approximately 1 g volatile suspended solids (VSS) dm^{-3}. At various periods of time, samples of

supernatants were taken and analysed for acetate, propionate and butyrate. At the end of the experiment the exact amount of VSS in the bottles was determined. More details of the procedure are described elsewhere (Rebac et al., 1995). Measurements were performed in triplicate, except for the sludge samples taken at day 235 and 306, which were measured in duplicate.

Activity measurements with hydrogen were performed with disintegrated granular sludge, crushed under an atmosphere of nitrogen with a mortar and pestle. Serum bottles (0.120 dm^3) contained 0.025 dm^3 medium and a gas phase of 2 atmosphere $H_2/N_2/CO_2$ (64/20/16 v/v). After addition of the biomass (approximately 0.66 g VSS dm^{-3}), 3 to 6 $\times 10^{-4}$ dm^3 gas samples were taken at various periods of time to determine hydrogen and methane in the gas phase. The homoacetogenic activity was determined in batches to which bromoethane sulphonic (Bres) acid (30 mM) was added to inhibit methanogenesis.

2.2.2.3 Assessment of substrate depletion curve for the determination of apparent K_m

In order to determine the apparent K_m value of the system for acetate, propionate and butyrate under psychrophilic conditions the EGSB reactor was operated temporarily in batch - mode. The superficial velocity in the reactor was kept at 10 m h^{-1}. Before starting the experiments, the reactor was flushed with tap water for a period of two HRT's in order to wash out all substrate still present in the reactor system. At time zero, the substrate concentration in the reactor was set at 2.6 g COD dm^{-3} acetate, or 1.0 g COD dm^{-3} propionate or 1.4 g COD dm^{-3} butyrate. The same composition of the substrate medium was used as in the batch activity tests experiments in the serum bottles. The EGSB reactor batch experiments lasted for a period between 6 to 12 hours, depending on the kind of substrate. Samples were taken every 15 to 20 minutes until the substrate was completely depleted.

The temperature dependence of the apparent K_m of the sludge was determined in duplicate using serum bottles for sludge removed at day 295 from the EGSB reactor. Serum bottles (0.120 dm^3) were filled with 0.100 dm^3 medium and approximately 5 g VSS dm^{-3}. Thereafter, the bottles were brought to the desired temperature and incubated on a Gerhardt RO 20 rotating shaker (Bonn, Germany) at 50 rpm. The same medium and substrate concentrations were used in the assessment of the substrate depletion curve for K_m determination in the reactor. At various periods of time, samples of the reactor liquor were taken and analysed for acetate, propionate and butyrate.

2.2.2.4 Calculations

The kinetics of substrate degradation by a mixed bacterial culture can be described adequately using the Monod equation (Robinson & Tiedje, 1983). When the effect of the mass transfer rate can be neglected, the Monod model (1949) yields a sigmoidal S - shaped substrate depletion curve (Fig. 1).

The maximum specific activity was calculated from the steepest linear decline in the substrate concentration, which represented at the minimum 50 % of initial substrate concentrations.

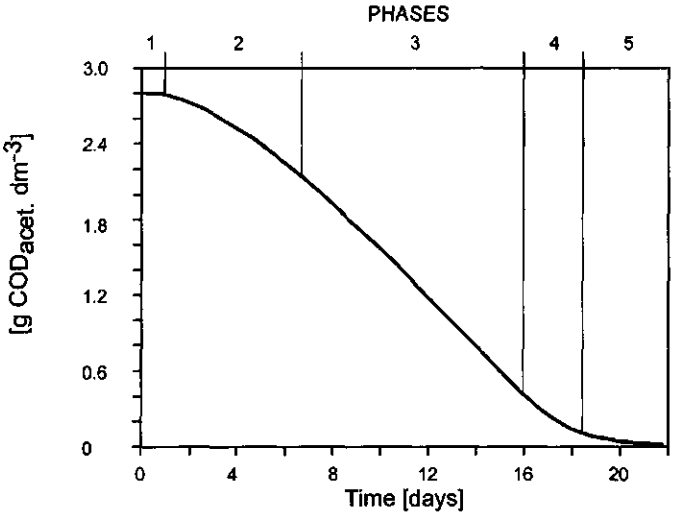

Fig. 2.2.1 Sigmoidal degradation of acetate at 10 °C. Phase 1, a lag region - no substrate degradation; Phase 2, mixed order region at S >> K_m,; Phase 3, zero-order region; Phase 4, mixed order region where S = K_m; Phase 5, first order region where S << K_m.

The apparent half saturation constants of acetate, propionate and butyrate degraders were calculated from the batch experiments described above by fitting sigmoidal substrate depletion data to the integrated Michaelis-Menten equation, using non-linear least-squares analysis as described by Visser et al. (1995).

The temperature dependence of the assessed maximum conversion rate of the homoacetogenic enrichment culture was fitted using an Arrhenius derived equation and a Ratkowsky's square root empirical non-linear regression model, using non-linear least-squares analysis as described previously (Rebac et al., 1995).

2.2.2.5 Microbial characteristics

The enrichment of microorganisms was carried out in a basal bicarbonate buffered medium described by Stams et al. (1993). Routinely, cultivation was done in 0.120 dm^3 serum vials containing 0.050 dm^3 medium and closed with butylrubber stoppers and aluminium crimp seals. For growth of hydrogenotrophic methanogens or homoacetogens, the gas phase consisted of 1.6 atmosphere of H_2/CO_2 and then organic substrates were absent in the medium. Microorganisms using hydrogen were enriched further. For this purpose, grown cultures were transferred to a fresh medium using an inoculum size of 10 %. To avoid growth of homoacetogens, 0.05 mg vancomycin was added per liter medium for enriching hydrogenotrophic methanogens.

For enumeration of microorganisms using H_2, the (n=3) most probable number (MPN) technique was used (Collins and Lyne, 1976). Dilution series of crushed granular sludge were made in medium (Stams et al., 1993) without substrate, and 5 ml of the different dilutions were added to 0.045 dm^3 medium in 0.120 dm^3 serum bottles. Bottles were incubated at 20°C in the dark, and after 93 days the gas phase and the liquid phase were analysed. In addition, the morphology of the microorganisms was analysed by microscopy.

2.2.2.6 Analyses

Volatile fatty acids and H_2 were analysed by gas chromatography as described elsewhere (Rebac et al., 1995).

2.2.3 RESULTS AND DISCUSSION

2.2.3.1 Maximum substrate degrading activities.

The long term (300 days) cultivation of granular sludge at 10 °C in the EGSB reactor resulted in very high substrate degrading activities (SDA) at 10 °C (Table 2.2.1). When assessed at 20 °C, the specific substrate degradation rates for hydrogen (homoacetogenic activity), acetate, and propionate increased by a factor 5, 3, and 5, respectively. The specific activities doubled relative to the values found at 20 °C when the activity tests were performed at 30 °C (Table 2.2.1). The SDA for hydrogen (hydrogenotrophic activity) and butyrate tripled and doubled, respectively, when temperature increased from 10 to 20 and from 20 to 30 °C. The activities in Table 1 reveal that the temperature effect is substrate dependent, which implies that a single mathematical model cannot describe accurately the temperature effect of the multiple species present in methanogenic consortia (Rebac et al., 1995; Heitzer et al., 1991).

Table 2.2.1 Temperature dependence of the maximum specific substrate degrading activities of granular sludge cultivated at 10 °C for 300 days with various methanogenic and acetogenic substrates.

Temperature	MAXIMUM SPECIFIC ACTIVITY [g COD g^{-1} VSS day^{-1}]				
(°C)	Hydrogen♠	Hydrogen♣	Acetate	Propionate	Butyrate
10	1.744 (0.374)	0.296 (0.009)	0.331 (0.003)	0.070 (0.002)	0.228 (0.002)
20	8.064 (0.624)	1.020 (0.379)	1.057 (0.004)	0.328 (0.010)	0.530 (0.002)
30	18.024 (1.170)	2.732 (0.076)	2.204 (0.011)	0.663 (0.002)	0.915 (0.025)

♠Homoacetogenic activity
♣Hydrogenotrophic activity

Multiple species substrate utilisation is also evidenced from the H_2 fed test vials. MPN counts showed that less homoacetogens, 2.62×10^4 bacteria g^{-1} VSS with 95 % confident interval [5.59×10^3 - 1.22×10^5] were present compared to hydrogenotrophic methanogens in the psychrophilic granular sludge, i.e., 9.12×10^5 bacteria g^{-1} VSS with 95 % confident interval [1.94×10^5 - 4.27×10^6]. The high homoacetogenic activity, relative to the hydrogenotrophic methanogenic activity at 10 °C, originates from a significant growth of the homoacetogenic bacteria during the experiment, since it is known that these H_2- utilisers grow relatively fast.
The substrate degrading activities of the EGSB reactor sludge at 30 °C were very high (Table 2.2.1) and even exceeded those of typical mesophilic granular sludges (e.g. Alphenaar 1994, Kato, 1994). In fact, they even approximated those of sludge from thermophilic anaerobic reactors at 55-65 °C (van Lier et al., 1996). Banik et al. (1997) reported much lower acetate degrading activities, at 30 °C, i.e., 1.62 and 1.74 g COD g^{-1} VSS day^{-1} for granular sludge cultivated for three years at 5 and 10 °C, respectively on a substrate consisting of non-fat dry milk. Likely, the good and stable enrichment can be attributed to the higher net biomass yields of these bacteria under psychrophilic condition compared to mesophilic conditions (Van den Berg, 1977; Lin et al., 1987). The higher net biomass yield can be attributed to substantial lower decay rates, K_d, under psychrophilic conditions (Van Lier et al., 1997).

2.2.3.2 Half saturation constant K_m at 10 °C

The development of the apparent half saturation constant K_m for the various substrates at 10 °C of the sludge along with the operational period is summarised in Table 2.2.2. It is clear that the apparent K_m values for the different VFA substrates imposed to the reactor indeed are quite low. This can be attributed to the prevailing adequate hydraulic mixing in the EGSB reactors (Kato et al., 1994). The lowest K_m applies for the propionate degraders, i.e., 7 mg COD dm^{-3} and the highest value, i.e., 243 mg COD dm^{-3} for butyrate degraders.

Table 2.2.2 Apparent half saturation constant K_m for acetate, propionate and butyrate degraders in the batch-fed reactor system with 30 VSS dm^{-3} at 10°C.

	Apparent half saturation constant K_m [g COD dm^{-3}]		
	period	period	period
Substrate	days 145 - 155	days 194 - 205	day 210
Acetate	0.162	0.039	0.058
Propionate	0.007	0.014	ND
Butyrate	0.243	0.142	ND
Mean diameter [mm]	2.2	2.7	2.7

ND = not determined

The K_m values found for the sludge in our EGSB experiments are comparable with those reported for other types of granular sludge. The K_m values assessed for the acetate degraders from our experiment (Table 2.2.2) are close to those reported by Tramper et al. (1984), Dolfing (1985) and Morvai et al. (1992) for mesophiliccally grown granular sludges at a temperature of 30 °C. The K_m values of the propionate degraders (Table 2.2.2) correspond to the values of Wu et al. (1993), to the lowest value reported by Tramper et al. (1984), and also to the lower values found for the mixed culture reported by Heyes & Hall (1983). The latter authors attributed the low K_m value to the presence of *Syntrophobacter wolinii* in their sludge, because it is known that these organisms have a very low intrinsic K_m value. According to Van Lier et al. (1996), Dolfing (1985) and Tramper et al. (1984), a strong correlation prevails between the K_m for the acetate degraders and the diameter of the granules at low substrate concentration. Such a clear correlation between K_m and the granules diameter was not found for propionate degraders (Dolfing, 1985; Van Lier, 1996).

The values for V_{max}/K_m ratio calculated for the same temperature, are presented Table 2.2.3 column "A". It appears that they increase 4, 7 and 7 fold, for acetate, propionate and butyrate, respectively, when the temperature is raised from 10 to 20 °C. However this factor (column "A") drops for acetate and propionate, but not for butyrate, when the temperature is raised further from 20 to 30 °C. The values for the V_{max}/K_m ratio presented in column "B" of Table 2.2.3, in which K_m values assessed at 30 °C were used are significantly lower for acetate and propionate compared to the values presented in column "A" at the same tested temperatures.

Table 2.2.3 Effect of temperature on the ratio maximum substrate degrading activity, V_{max} and apparent half saturation constant, K_m, in granular sludge for acetate, propionate and butyrate. Sludge was sampled after 295 days of reactor operation.

	Ratio V_{max}/K_m					
Temperature	Acetate		Propionate		Butyrate	
[°C]	A	B	A	B	A	B
10	2.35	0.68	0.34	0.09	0.70	2.28
20	9.30	2.71	2.30	0.51	4.80	5.28
30	5.65	5.65	1.00	1.00	9.05	9.05

A, calculated by using K_m values obtained at the same temperature;
B, calculated by using K_m values obtained at 30 °C.

These lower values are clear evidence of the prevalence of a temperature compensation effect in the overall conversion for acetate and propionate at lower temperature. However, for butyrate apparently any temperature compensation is absent when elevating the temperature from 10 to 30 °C, because the values for the V_{max}/K_m ratio in column "A" and "B" don't differ significantly. For a pure culture of *Methanosarcina barkeri* 227 Westermann et al. (1989) found a

temperature compensation effect based on V_{max}/K_m ratio for hydrogen when decreasing the temperature from 30 to 12 °C and for acetate when the temperature was decreased from 37 to 20 °C. The absence of a temperature compensation effect for butyrate (Table 2.2.3) can be due our observation that the K_m value decreased when temperature was elevated from 10 to 30 °C. This agrees with Lin et al. (1987) who found a decrease from 600 mg COD dm^{-3} to 150 mg COD dm^{-3} for the apparent K_m

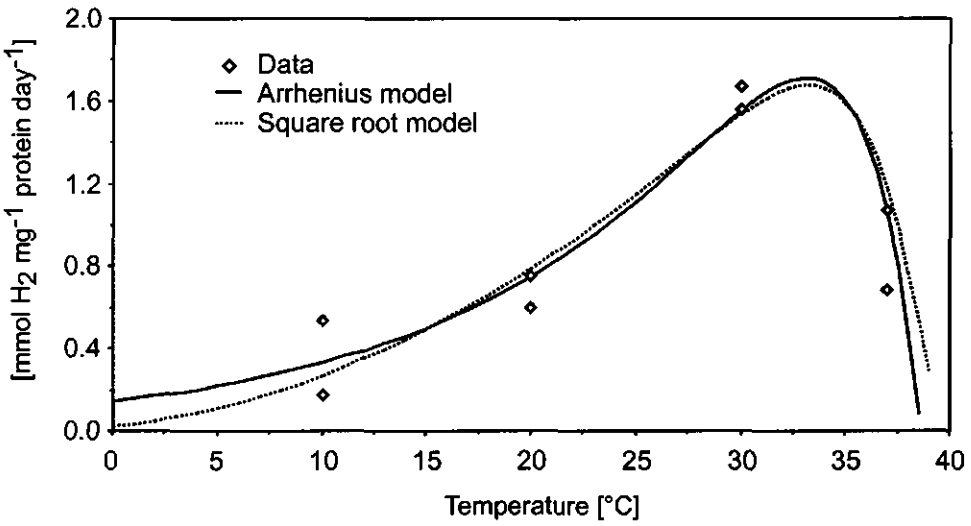

Fig. 2.2.2 Temperature characteristics of the homoacetogenic enrichment culture. Measured data (◊); Arrhenius derived model (-); Ratkowsky empirical square root model (· · ·).

for a VFA mixture (acetate: propionate: butyrate = 2:1:1, based on COD) when increasing the temperature from 15 to 35 °C.

2.2.3.3 Enrichment culture

Figure 2.2.2 shows the relationship found between temperature and the specific activity of the homoacetogenic enrichment culture prepared from the psychrophilically cultivated EGSB sludge. One of the aims of this study was to find out whether or not any significant enrichment of psychrophilic and/or psychrotrophic homoacetogenic bacteria had occurred during the long term operation of the reactor at low temperature. The very high homoacetogenic activity of the granular sludge that found at 10 °C might suggest this (Table 2.2.1). The assessed homoacetogenic specific activity of the granular sludge and of the homoacetogenic enrichment culture show an exponential increase with the temperature in the range 10-30 °C. Moreover, Table 4 shows the effect of the acetate concentrations on the lag phase of the hydrogenotrophic activity of the homoacetogenic enrichment culture (at 10 °C). Table 4 shows that lag phase significantly increases at elevated initial acetate concentrations. This seems not to be the case

for their maximum activity (Table 2.2.4). The calculated Gibbs free energy (ΔG kJ mol^{-1}) of the homoacetogenic reaction at 10 °C changes from −111.73 to −104.98 when the acetate concentration increases from 0 to 60 mM. This suggests that an increase in the lag phase is not caused by product inhibition, because the Gibbs free energy of the reaction is decreased only for 6.75 kJ mol^{-1}. However, under thermophilic conditions Van Lier et al. (1993) found that the formed products could inhibit growth of acetogens already at concentration exceeding 5 mM. The lag phase very likely can be attributed to H_2 diffusion from the gas phase to liquid phase, because this in fact is the rate-limiting step and according to van Houten (1996) the solubility of H_2 decreases with increasing acetate concentrations.

Table 2.2.4 Effect of initial acetate concentration on maximum hydrogenotrophic activity of the homoacetogenic enrichment culture at 10 °C.

Acetate	[mMol]	0	15	30	45	60
Maximum hydrogen activity*	[%]	100	74.8	97.6	81.5	104.7
Lag phase	[days]	10	13	16	22	26

*Relative to maximum hydrogen activity with acetate concentration of 0 [mMol].

Both the Arrhenius model and Ratkowsky square root model were used to fit the experimental data. In both models a clear temperature optimum is found between 30-35 °C, indicating that little if any specialized psychrophilic and/or psychrotrophic homoacetogens had developed in the sludge, although it is obvious that they hardly can manifest due to a large predominance of mesophilic species. As the seed initial sludge was mainly mesophilic, and since these organisms are still sufficiently active at low temperature, they simply will overgrow the psychrotrophs. The application of Arrhenius model is likely inappropriate for temperatures < 5 °C, because of the high specific activity prediction. So the conclusion is that the existence of psychrophilic homologues in sludge from anaerobic reactors remains unclear. Nonetheless, H_2 turnover by psychrotrophic homoacetogens (*Acetobacterium carbinolicum* strain HP4 and *Acetobacterium spp*) in low temperature lake sediments has been reported (Conrad et al., 1989; Kotsyurbenko et al., 1995).

2.2.4 CONCLUSIONS

1. The assessed very high substrate degrading activities both at 10 and 30 °C, of psychrophilically (10 °C) grown mesophilic granular sludge suggest a good enrichment of methanogens, syntrophs and homoacetogens at this low temperature. Although, low temperatures limit the anaerobic degradation rates, this study shows that low temperatures are not hampering the development of methanogenic consortia. When these sludges are exposed to mesophilic conditions, their specific activity is higher than of sludges cultivated under mesophilic conditions and fed with the same substrate.

2. The granular sludge cultivated under psychrophilic conditions still shows optimum growth rates in the mesophilic temperature range (above 30 °C).

3. The low apparent K_m of the EGSB system for the various VFAs can be maintained when operating the system for prolonged periods under psychrophilic conditions. This finding is of eminent importance because low K_m values are essential for achieving a good treatment efficiency in anaerobic treatment of low strength wastewaters.

4. The temperature characteristics of psychrophilically grown mesophilic seed sludge and a homoacetogenic enrichment culture are very similar to mesophilic sludges, even after a long-term operation (± 300 days) at 10 °C. This indicates that mesophilic sludge is quite well capable to grow under low temperature conditions and consequently anaerobic reactors can be considered feasible under psychrophilic conditions. Moreover there is no need psychrophilic populations for a high-rate anaerobic reactors to be operated under low temperature.

2.2.5 ACKNOWLEDGMENTS

We would like to thank J. van der Laan and I. Bennehey for the technical assistance with the chromatographs.

2.2.6 NOMENCLATURE

A	= specific activity (g COD g^{-1} VSS day^{-1})
A_{max}	= maximum specific activity (g COD g^{-1} VSS day^{-1})
COD	= chemical oxygen demand (g O_2 dm^{-3})
EGSB	= expanded granular sludge bed
K_m	= apparent half saturation constant (g COD dm^{-3})
OLR	= organic loading rate (g COD dm^{-3} day^{-1})
T	= temperature (°C)
VFA	= volatile fatty acids
V_0	= initial substrate degradation rate (g COD dm^{-3} day^{-1})
V_{max}	= maximum substrate degradation rate (g COD dm^{-3} day^{-1})
VSS	= volatile suspended solids
X	= biomass concentration (g VSS dm^{-3})
Y	= biomass yield (g VSS g^{-1} $COD_{converted}$)

2.2.7 REFERENCES

Alphenaar, A. (1994) Anaerobic granular sludge: characterization, and factors affecting its functioning, Ph.D. thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Banik, G. C., Ellis, T. G. & Dague, R. R. (1997) Structure and methanogenic activity of granules from an ASBR treating dilute wastewater at low temperatures. *Proc. of 8th International Conference on Anaerobic Digestion*, Sendai, Japan, Vol. 2, pp 548-556.

Berg van der, L. (1977) Effect of temperature on growth and activity of a methanogenic culture utilising acetate. *Can. J. Microbiol.*, **23**, 898-902.

Collins, C. H. & Lyne, P. M. (1976) Microbiological Methods. 4th ed. London, Butterworths.

Conrad, R., Bak, F., Seitz, H. J., Thebrath, B. & Mayer, H. P. (1989) Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment. FEMS *Microbiol. Ecol.* **62**, 285-294.

Dolfing, J. (1985) Kinetics of methane formation by granular sludge at low substrate concentrations. The influence of mass transfer limitation. *Appl. Microbiol. Biotechnol.* **22**, 77-81.

Heitzer, A., Kohler, H.P.E., Reichert, P. & Hamer, G. (1991) Utility of phenomenological models for describing temperature dependence of bacterial growth. *Appl. Environ. Microbiol.*, **57**, 2656-2665.

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

Houten, R.T. van (1996) Biological sulphate reduction with synthesis gas, Ph.D. thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Kato, T. M., Field, J. A., Versteeg, P. and Lettinga, G. (1994) Feasibility of expanded granular sludge bed reactors for the anaerobic treatment of low strength soluble wastewaters. *Biotechnol. Bioeng.*, **44**: 469-479.

Kato, T. M. (1994) The anaerobic treatment of low strength soluble wastewaters, Ph.D. thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Kotsyurbenko, O. R., Nozhevnikova, A. N., Soloviova, T. I. and Zavarzin, G.A. 1996. Methanogenesis at low temperature by microflora of tundra wet land soil. *Antonie van Leeuwenhoek*, **69**: 75-86.

Kotsyurbenko, O. R., Simankova, M. V., Nozhevnikova, A. N., Zhilina, T. N., Bolotina, N. P., Lysenko, A. M. and Osipov, G. A. (1995) New species of psychrophilic acetogens: *Acetobacterium bakii* sp.nov., *A. paludosumsp*.nov., *A. fimetarium* sp.nov. *Arch. Microbiol.* **163**: 429-434.

Lawrence, A.W. & McCarty P.L. (1969) Kinetics of methane fermentation in anaerobic treatment. *J. Water Pollut. Control Fed.*, **41**, R1-R17.

Lin, C.Y., Noike, T., Sato, K. & Matsumoto, J. (1987) Temperature Characteristics of the methanogenesis process in anaerobic digestion. *Wat. Sci. Tech.*, **19**, 299-310.

Matsushige, K., Inamori, Y., Mizuochi, M., Hosomi, M. and Sudo, R. (1990) The effects of temperature on anaerobic filter treatment for low-strength organic wastewater. *Environ. Technol.*, **11**, 899-910.

Monod, J. (1949) The growth of bacterial cultures. *Ann. Rev. Microbiol.*, **3**, 371-394.

Morvai, L., Mihàltz, P. and Hollò, J. (1992) Comparison of the kinetics of acetate biomethanation by raw and granular sludges. *Appl. Microbiol. Biotechnol.*, **36**, 561-567.

Nozhevnikova, A. N., Holliger, C., Ammann, A. and Zehnder, A. J. B. 1997. Psychrophilic methanogenesis in sediments of deep lakes. *Wat. Sci. Technol.*, **36**: 57-64.

Pavlostathis, S.G. & Giraldo-Gomez, E. (1991) Kinetics of anaerobic treatment: a critical review. *Critical Rev. in Env. Control*, **21 (5,6)**, 411-490.

Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N. & Chandler, R.E. (1983) Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *J. Bacteriology*, **154**, 1222-1226.

Rebac, S., Ruskova, J., Gerbens, S., van Lier, J.B., Stams, A.J.M. & Lettinga, G. (1995) High-rate anaerobic treatment of wastewater under psychrophilic conditions. *J. Fermen. Bioengin.*, **80-5**, 15-22.

Robinson, J.A. & Tiedje, J.M. (1983) Nonlinear estimation of Monod groth kinetic parameters from a single substrate depletion curve. *Appl. Environ. Microbiol.*, **45**, 1453-1458.

Stams, A.J.M, van Dijk, J.B, Dijkema, C. & Plugge, C.M. (1993) Growth of syntrophic propionate-oxidizing bacteria with fumarate in the absence of methanogenic bacteria. *Appl. Environ. Microbiol.*, **59**, 1114-1119

Switzenbaum, M. S. & Jewell, W. J. (1978) Anaerobic attached film expanded bed reactor treatment of dilute organics. In: *Proc. of the 51st Annual WPCF Conference.* Anaheim, California, pp. 1-164.

Tramper, J., van Groenestijn, J.W., Luyben, K.Ch.A.M. & Hulshoff Pol, L.W. (1984) Some physical and kinetic properties of granular anaerobic sludge. p. 145-155. In: E.H. Houwink and R.R. van der Meer (eds.) Elsevier, Amsterdam, The Netherlands.

Van Lier, J. B., Rebac, S., Lens, P., van Bijnen, F., Oude Elferink, S. J. W., Stams, J. M. & Lettinga, G. (1997) Anaerobic treatment of partly acidified wastewater in a two-stage expanded granular sludge bed (EGSB) system at 8 °C. *Wat. Sci. Tech.*, **36-6/7**: 317-324.

Van Lier, J. B., Debets, M. M. W. H. and Lettinga, G. (1996) Development of thermophilic methanogenic sludge in compartmentalized upflow reactors. Biotechnol. and Bioeng., **50**: 115-124.

Lier, J.B. van, Sanz Martin, J.L. and Lettinga, G. (1996). Effect of temperature on the anaerobic thermophilic conversion of volatile fatty acids by dispersed and granular sludge. Wat. Res., **30**-1: 199-207.

Van Lier, J.B., K.C.F. Grolle, C.T.M.J. Frijters, A.J.M. Stams and G. Lettinga (1993). Effect of acetate, propionate and butyrate on the thermophilic anaerobic degradation of propionate in methanogenic sludge and defined cultures. Appl. and Environ. Microbiol., **57**: 1003-1011.

Zoutberg, G. R., Heynekamp, K. & Versprille, A. I. (1997) Anaerobic treatment of chemical waste water in Biobed® EGSB reactors., In *Proc. of 8th International Conference on Anaerobic Digestion*, Sendai, Japan., Vol. 2, pp. 167-174,

Westermann, P., Ahring, B.K. & Mah, R.A. (1989) Temperature compensation in Methanosarcina barkeri by modulation of hydrogen and acetate affinity. *Appl. Environ. Microbiol.*, **55**, 1262-1266.

Wu, W., Thiele, J. H., Jain, M.K., Pankratz, H. S., Hickey, R. F. & Zeikus J. G. (1993) Comparison of rod-versus filament-type methanogenic granules: microbial population and reactor performance. *Appl. Microbiol. Biotechnol.*, **39**, 795-803.

Wu, W., Thiele, J. H., Jain, M. K. & Zeikus J. G. (1993) Metabolic properties and kinetics of methanogenic granules. *Appl. Microbiol. Biotechnol.*, **39**, 804-811.

2.3 THE EFFECT OF SULPHATE ON PROPIONATE AND BUTYRATE DEGRADATION IN A PSYCROPHILIC ANAEROBIC EXPANDED GRANULAR SLUDGE BED (EGSB) REACTOR

ABSTRACT

The effect of sulphate on the anaerobic psychrophilic degradation of propionate and butyrate was studied using an expanded granular sludge bed (EGSB) reactor. The EGSB reactor was operated at organic loading rates 7 - 12 kg $COD \cdot m^{-3} \cdot day^{-1}$, at hydraulic retention times of 2.5 - 1.6 h and at three different sulphate concentrations. The results of the continuous flow experiments and of batch experiments with sludge from the EGSB reactor reveal that the psychrophilic degradation of propionate is strongly affected by the presence of sulphate. Addition of 0.266 g SO_4^{2-}-S·dm-3 to the medium resulted in a 35 % increase in the propionate conversion rate. In contrast, the butyrate degradation rate decreased by 32 % in the presence of sulphate. Syntrophic acetogenic butyrate-oxidizing consortia seem to compete effectively with sulphate reducing bacteria.

Key words: Sulphate, psychrophilic conditions, anaerobic treatment, volatile fatty acids, expanded granular sludge bed.

2.3.1 INTRODUCTION

Expanded granular sludge bed (EGSB) reactors are characterized by a high up flow velocity which is brought about by a high recirculation rate. This type of reactor configuration is beneficial for the anaerobic treatment of low strength waste waters [1-4]. The advantage of EGSB systems over other anaerobic reactors is the improved sludge-substrate contact. Consequently, higher volumetric loading rates can be applied, even at low temperatures [5].

In the anaerobic digestion process fermentation products such as propionate and butyrate are oxidized by acetogenic bacteria (AB) to acetate and H_2, which are then converted to methane by methane producing bacteria (MPB). Based on thermodynamic considerations, reactions carried out by the acetogens require a low H_2 partial pressure. Therefore, acetogenic bacteria are only able to grow in syntrophy with hydrogen consuming bacteria, e.g. hydrogenotrophic methanogens or homoacetogens [6-9].

In the presence of sulphate the oxidation of propionate and butyrate to acetate can be accomplished either by syntrophic associations of acetogenic bacteria with hydrogenotrophic sulphate-reducing bacteria (SRB) or SRB directly [10-12]. If sulphate is present in the wastewater, SRB will compete with AB for propionate and butyrate and with MPB for acetate and H_2 [11]. Competition studies done so far show that in anaerobic digesters fed with high sulphate concentrations, the direct oxidation of propionate by SRB becomes the most significant route for propionate utilization [12-16]. Interestingly, acetogenic butyrate oxidizers were found to compete well with sulphate reducers for the available butyrate, even at high sulphate concentrations [14].

Generally, hydrogen produced in the anaerobic conversion of organic matter is used by SRB when sulphate is present [17,18]. This has been explained by the fact that kinetic considerations favour SRB over MPB [11,19-21]. With respect to acetate the situation is less clear. The complete conversion of acetate by MPB, at high sulphate concentrations, as well as the predominance of SRB growing on acetate have both been reported [18,22,23]. These aspects have been reviewed recently [11]. The present article deals with the role of sulphate in the degradation of propionate and butyrate in a continuous flow anaerobic EGSB reactor under psychrophilic conditions (10° - 12°C).

2.3.2 MATERIALS AND METHODS

Experimental conditions

The experiments were performed using a 0.05 m diameter glass EGSB reactor (Fig. 2.1.1, Chapter 2.1) with a total volume of 4.3 dm^3 (internal settler included), as described in Chapter 2.1.

Biomass

The reactor was inoculated with elutriated mesophilic granular methanogenic sludge, originating from a 760 m^3 UASB reactor (20 - 24°C) at the Bavaria brewery at Lieshout, The Netherlands. The total amount of granular sludge added to the reactor was approximately 100 g volatile suspended solids (VSS).

Medium

The reactor was fed with a concentrated stock solution of 33 g chemical oxygen demand (COD)·dm^{-3}. The substrate consisted of a partly neutralized (pH 6.5) volatile fatty acid (VFA) mixture composed of acetate, propionate and butyrate with ratio 1 : 1.5 : 1.8, based on COD. After day 205, this ratio was changed to 3 : 1 : 1. The concentration of basal nutrients in the concentrated stock solution were (g·dm^{-3}): NH_4Cl, 43.5; KH_2PO_4, 7.08; $(NH_4)_2SO_4$, 7.0 (from day 30 to 161, 3.5); $MgCl_2 \cdot 6H_2O$, 6.25; $CaCl_2 \cdot 2H_2O$, 2.75; yeast extract, 0.83. After day 161 the concentration and composition of the basal nutrients in the stock solution were changed to (g·dm^{-3}): NH_4Cl, 7.5; $MgSO_4 \cdot 7H_2O$, 1.5; $NaH_2PO_4 \cdot 2H_2O$, 27.6; K_2HPO_4, 21.2; $CaCl_2 \cdot 2H_2O$, 0.3; yeast extract, 0.5. To each dm^3 of stock solution 0.0045 dm^3 of a trace element solution was added containing (mg·dm^{-3}): $FeCl_2 \cdot 4H_2O$, 2000; H_3BO_3, 50; $ZnCl_2$, 50; $CuCl_2 \cdot 2H_2O$, 30; $MnCl_2 \cdot 4H_2O$, 500; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 50; $AlCl_3 \cdot 6H_2O$, 90; $CoCl_2 \cdot 6H_2O$, 2000; $NiCl_2 \cdot 6H_2O$, 92; $Na_2SeO_3 \cdot 5H_2O$, 164; EDTA, 1000; resazurin, 200; 36% Hcl, 0.001 $dm^3 \cdot dm^{-3}$. The average SO_4^{2-}/COD ratios were: 0.15 (day 1 - 30), 0.08 (day 31 - 160), 0.02 (day 161 -235). All chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany). Yeast extract was purchased from Unipath Ltd. (Basingstoke, England). Resazurin was purchased from Fluka (Buchs, Switzerland). The gases were from Hoekloos (Schiedam, The Netherlands).

Start-up of the reactor

Feeding of the reactor was started immediately after inoculation with the elutriated mesophilic granular sludge. The reactor was started-up at an organic loading rate (OLR) of 7 kg COD·$m^{-3}$$day^{-1}$ and a hydraulic retention time (HRT) of 2.5 h. At the start of the experiment the temperature of the reactor was set at 11°C.

Batch experiments

The activity of the sludge from the reactor was measured in triplicate for the control bottles, and in duplicate for high sulphate and high sulphide concentrations. The measurements were performed in 0.120 dm^3 serum bottles filled with 0.100 dm^3 medium and approximately 1 g volatile suspended solids (VSS)·dm^{-3}. Sludge samples from the psychrophilic EGSB reactor were used directly after sampling. The carbon source consisted of approximately 3 g COD·dm^{-3} of either sodium propionate or sodium butyrate. The nutrient concentrations were (g·dm^{-3}): NH_4Cl, 0.28; $MgSO_4 \cdot 7H_2O$, 0.11; K_2HPO_4, 2.0; $NaH_2PO_4 \cdot 2H_2O$, 3.33; yeast extract, 0.10; and 0.001 $dm^3 \cdot dm^{-3}$ of the trace element solution. Sulphate concentrations up to 0.3 (g SO_4^{2-}-S·dm^{-3}) were obtained by adding Na_2SO_4. This resulted in a SO_4^{2-}/COD ratio of approximately 0.3. After closing the bottles and changing the gas phase composition to N_2/CO_2 (70%/30%), Na_2S (0.001 $dm^3 \cdot dm^{-3}$ from a 1 Mol stock solution) was added to obtain complete anaerobic conditions. High sulphide concentrations were obtained by adding 0.005 and 0.01 $dm^3 \cdot dm^{-3}$ of the same solution to the medium. The serum bottles were incubated at 10°C on a Gerhardt RO 20 rotating shaker (Bonn, Germany) at 50 "rpm". Periodically, samples were taken for analyzing propionate, butyrate and sulphate concentrations. After each experiment the exact amount of VSS per bottle was measured [24]. The specific activity was calculated from the linear decrease of the substrate concentrations which was followed until the substrate levels dropped below 500 mg COD·dm^{-3}.

Analyses

Samples for VFA analyses were centrifuged for 3 min at 10000 rpm in a Biofuge A (Heraeus Sepatech, Osterode, Germany). VFA were determined by gas chromatography. The chromatograph (Hewlett Packard 5890A, Palo Alto, USA) was equipped with a 2 m (6mm x mm) glass column, packed with Supelco port (100-120 mesh) coated with 10 % Fluorad FC 431. Operating conditions were: column, 130 °C; injection port, 200 °C; flame ionization detector, 280 °C. N_2-gas saturated with formic acid at 20 °C was used as carrier gas (0.030 $dm^3 \cdot min^{-1}$).Sulphate was measured by ion chromatography (Packing Chrompack Ionosphere, differential refractometer, eluent potassium biphatlate 0.04 M, pH 4.2).The biogas composition CH_4, CO_2, N_2, O_2 was determined in 10^{-4} dm^3 samples immediately after sampling by gas chromatography, using a GC 8000 chromatograph (Fisons Instruments, Milano, Italy). The gas chromatograph was equipped with columns connected in parallel (split 1:1) - (1.5mx2mm) teflon, packed with chromosorb 108, (60-80 mesh), and a (1.2m x 2mm) stainless steel, packed with mol. sieve 5A, (60-80 mesh). Helium was used as a carrier

gas (0.045 $dm^3 \cdot min^{-1}$). The oven, detector, and injection temperature were 40 °C, 100 °C and 110 °C, respectively. All other analyses were carried out according to standard methods [24].

The substrate used in this study consisted of acetate, propionate, or butyrate. Table 1.1 (Chapter 1) shows possible anaerobic conversion reactions of these compounds with or without the presence of sulphate as the electron acceptor. Data for free energy change under standard conditions at pH 7 ($\Delta G°'$) and standard enthalpy at pH 7 ($\Delta H°'$) were taken from handbooks [25,26]. The free energy changes at 10° and 37°C were calculated using the Van't Hoff equation [25]. Four different scenarios for SRB being involved in propionate and butyrate degradation are possible: (i) complete oxidation of the VFA to CO_2 and simultaneous reduction of sulphate to sulphide by sulphate reducers; (ii) incomplete oxidation to acetate by sulphate reducers, followed by acetate conversion by aceticlastic methanogens; (iii) syntrophic degradation by acetogens coupled to H_2 utilization by SRB and (iv) syntrophic degradation by SRB coupled to H_2 and acetate-consuming methanogens.

Calculation of the fraction of organic substrate used by MPB and SRB.

For mineralization of 1 mol of propionate, which is equivalent to 112 g COD, the amount of acetate and hydrogen produced according to reaction 1, Table 1.1 (Chapter 1) can be calculated as $COD_{acet.} = 0.57 * COD_{prop.}$ and $COD_{hydr.} = 0.43 * COD_{prop.}$.

For mineralisation of 1 mol of butyrate, which is equivalent to 160 g COD, the amount of acetate and hydrogen produced according to reaction 4, Table 1.1 can be calculated as $COD_{acet.} = 0.8 * COD_{butyr.}$ and $COD_{hydr.} = 0.2 * COD_{butyr.}$.

The amount of organic COD used for sulphate reduction according to reaction 7, Table 1.1 is expressed as $SO_4^{2-}\text{-COD} = 2 * SO_4^{2-}\text{-S}$.

2.3.3 RESULTS

The overall performance of the EGSB reactor has been presented elsewhere [5]. Overall COD removal efficiencies exceeding 90 % at organic loading rates up to 12 kg $COD \cdot m^{-3} \cdot day^{-1}$ and at HRT as low as 1.6 h were achieved. During the first 30 days of operation, when the reactor was operated at a SO_4^{2-}/COD ratio of 0.15, a high propionate removal efficiency of 80-90 % was obtained (Fig. 2.3.2b). At days 29 and 159 the sulphate concentrations in the influent were reduced so that SO_4^{2-}/COD ratios became 0.08 and 0.02, respectively (Fig. 2.3.2a, 2b arrows 1, 3).

The first measurement after the first decrease in sulphate concentration apparently did not significantly affect the propionate removal efficiencies in the EGSB reactor, because the SO_4^{2-}/COD ratio remained high (Fig. 2.3.2b arrow 2). Changes in the propionate degradation rate in the period day 0 - 70 could also be attributed to the prevalence of temperature fluctuations of 2-4 °C occurring at that time (Fig. 2.3.2a). After the second decrease in the

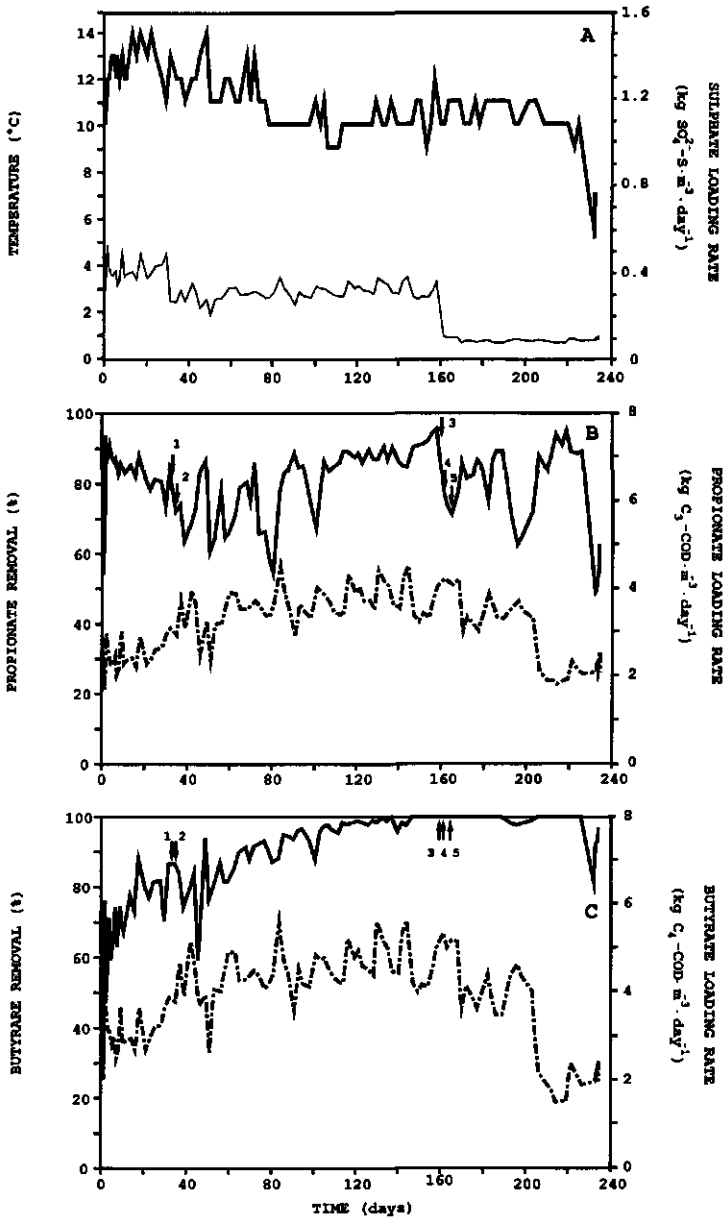

Fig. 2.3.2 Treatment of low strength VFA mixtures in EGSB reactor. A. Sulphate loading rate (kg SO_4^{2-}-S·m^{-3}·day^{-1}) (—), Temperature °C (—). B. Propionate loading rate (kg C_3-COD·m^{-3}·day^{-1}) (–·–), Percentage of propionate removal (—). C. Butyrate loading rate (kg C_4-COD·m^{-3}·day^{-1}) (–·–), Percentage of butyrate removal (—). Arrows 1 and 3 in Figures 2b and 2c indicate days on which influent sulphate concentrations were lowered. Arrows 2, 4 and 5 indicate days on which measurements were next made after reductions in influent sulphate concentration.

sulphate concentration, at measurement on day 161 and 163 (Fig. 2.3.2b arrows 4, 5) the propionate removal efficiencies were strongly affected. A decrease in the sulphate concentration had little if any effect on butyrate removal (Fig. 2.3.2c arrows 2, 4, 5).

Batch activity assays conducted at low and high sulphate concentration and using propionate and butyrate as substrate were performed with sludge removed from the reactor at day 134. The results of these experiments are depicted in Fig. 2.3.3a and 3b and summarized in Tables 2.3.2 and 2.3.3.

Table 2.3.2 Specific propionate and butyrate degrading activity of psychrophilic granular sludge with and without sulphate at 10 °C, taken from the EGSB reactor on day 134.

Sulphate concentration in	Degrading activity (g COD·g^{-1} VSS·day^{-1})	
Bottles A: 0.3 (g SO_4^{2-}-S·dm^{-3})	Propionate	Butyrate
Bottles A: before depletion of SO_4^{2-}	0.085 ± 0.011	0.079 ± 0.006
Bottles A: after depletion of SO_4^{2-}	0.016 ± 0.001	0.045 ± 0.003
Bottles B*: Control	0.055 ± 0.009	0.116 ± 0.003

*When no sulphate was added, the level of sulphate in batch media was 0.01.

In the presence of sulphate (0.266 g SO_4^{2-}-S·dm^{-3}), the propionate degrading activity was 35 % higher than in the control bottles. The specific sulphate reducing activity of the granular sludge in this experiment amounted to 0.010 ±0.001 (g SO_4^{2-}-S·g^{-1} VSS·day^{-1}). However, after depletion of sulphate, the degradation of propionate proceeded at a distinctly slower rate. Under the latter conditions the activity was even lower than that measured in the control bottles (Fig. 2.3.3a). In contrast, the butyrate degrading activity was lower in the presence of sulphate (0.308 g SO_4^{2-}-S·dm^{-3}), than in the control bottles (Fig. 2.3.3b). The specific sulphate reducing activity of the granular sludge for butyrate was 0.004 ±0.0008 (g SO_4^{2-}-S·g^{-1} VSS·day^{-1}) which was 60 % lower than for propionate (Fig. 2.3.3b).

The assessed COD mass balance in Table 2.3.3 was based on the values measured at start and the end. Calculation to determine COD equivalents for the intemediats were based on equations presented in Table 1.1 (Chapter 1).

The effect of various sulphide concentrations on the propionate and butyrate degradation rates of sludge samples removed from the reactor at day 270 are presented in Table 2.3.4. These results reveal that the presence of sulphide had a greater inhibitory effect on propionate degradation than on butyrate degradation.

2.3.4 DISCUSSION

The results obtained in the continuous experiments show that under psychrophilic conditions sulphate enhances the degradation of propionate. Decreasing the influent SO_4^{2-}/COD ratio from 0.08 to 0.03 resulted in a decrease in the propionate removal efficiency from 92 to 72

% (Fig. 2.3.2b, arrows 4 and 5). These results are in agreement with the results from the batch

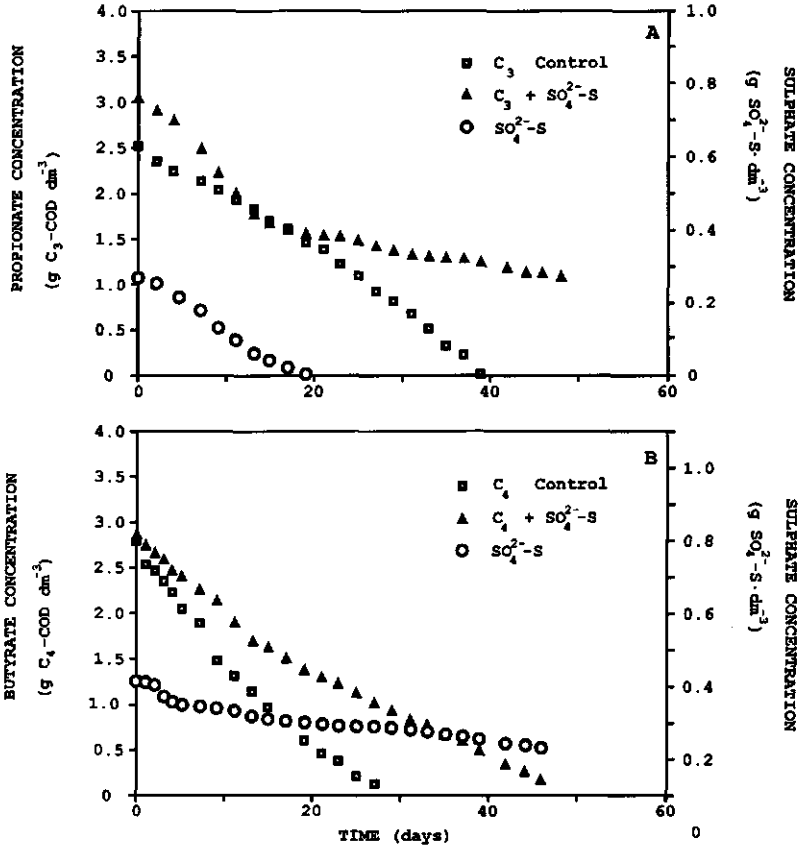

Fig. 2.3.3 Propionate and butyrate concentrations versus time at 10°C in the presence of sulphate (Bottles A) and absence (Bottles B) in batch experiments. A. Propionate concentration in in bottles A (Δ) and control bottles B (□), as well as SO_4^{2-}-S concentration in bottles A(O). B. Butyrate concentration in in bottles A (Δ) and control bottles B (□), as well as SO_4^{2-}-S concentration in bottles A(O).

experiments, which reveal that in the presence of sulphate the degradation rate of propionate was approximately 35 % higher than in the control bottles (Fig 2.3.3a and Table 2.3.2).

Under conditions of excess sulphate, different groups of SRB can be involved in degradation of propionate. Direct oxidation of propionate by SRB e.g. by *Desulfobulbus*-like bacteria, has been observed in the granular sludge from a brewery UASB reactor. These bacteria are able to couple propionate oxidation with sulphate reduction if sulphate is available [13]. Organisms like *Desulfobulbus propionicus* and *Desulfobulbus elongatus* oxidize propionate incompletely to acetate and CO_2 (Table 1: reaction 2) [27,28]. An

increased degradation of propionate under mesophilic conditions in the presence of sulphate has been reported by various other researchers [12,14,16,29]. Our results suggest that sulphate also has a strong effect on propionate degradation at low temperature. According to Visser *et al.* [14], degradation of propionate by hydrogen producing acetogens is more important at low sulphate concentrations. It has also been found that SRB can act as acetogens, degrading propionate in syntrophy with other SRB or MPB in the absence of sulphate [13]. The degradation rate of propionate dropped significantly after the sulphate had been depleted (Fig. 2.3.3 and Table 2.3.2). This may be attributed to inhibition by the produced sulphide up to 0.26 (g S^{2-}-S·dm^{-3}), or to the sudden deficiency of sulphate as the electron acceptor [12,23,30,31]. The results of the experiments conducted at various sulphide concentrations reveal a strong inhibition of the propionate degradation rate (Table 2.3.4). An inhibition of the propionate degradation in granular sludge of approximately 50 % was found at a sulphide concentration of 0.16 (g S^{2-}-S·dm^{-3}) under mesophilic conditions by Rinzema [23].

Table 2.3.3 COD balance for propionate and butyrate degradation in batch experiments at 10°C at initial sulphate concentrations of 0.266 and 0.308 (g SO_4^{2-}-S·dm^{-3}), respectively. Sludge was sampled on day 134.

Propionate		Butyrate	
COD converted	(g COD·dm^{-3})	COD converted	(g COD·dm^{-3})
$COD_{prop.}$	1.95 ±0.07	$COD_{butyr.}$	2.66 ±0.11
SO_4^{2-}-COD	0.53 ±0.00	SO_4^{2-}-COD	0.33 ±0.01
$COD_{hydr.}$	0.82 ±0.03	$COD_{hydr.}$	0.52 ±0.02
$COD_{acet.}$	1.13 ±0.04	$COD_{acet.}$	2.13 ±0.09
COD by SRB* (%)	27.19 ±1.0	COD by SRB* (%)	11.75 ±0.5

* ratio of SO_4^{2-}-COD over $COD_{prop.}$ or $COD_{butyr.}$

In contrast to propionate, the degradation of butyrate remained almost unaffected by the presence of sulphate in the continuous flow experiments (Fig. 2.3.2c arrows 2, 4, 5). In batch experiments the butyrate degrading activity at high sulphate concentrations became even lower (Fig. 2.3.3b and Table 2.3.2). The calculated sulphate reducing activity in the batch experiments appeared to be about 60 % lower with butyrate than with propionate. This indicates that under psychrophilic conditions the acetogenic butyrate oxidizers can compete well with the SRB. Similar observations were made previously under mesophilic conditions [14,15]. The butyrate degraders seems to be less sensitive to high sulphide concentrations than the propionate degraders (Table 2.3.4).

The oxidation of H_2 can proceed via various pathways (Table 1.1, Chapter 1). During the oxidation of propionate and butyrate, reducing equivalents can be disposed of as hydrogen or

may be used directly by SRB for the reduction of sulphate to sulphide. Using mass balances only, a distinction between oxidation of molecular hydrogen by SRB or MPB and the direct oxidation of fatty acids by SRB cannot be made. Table 3 shows that in the oxidation of both propionate and butyrate about 64 % of the reducing equivalents were used by SRB, whereas about 36 % were used by MPB.

Table 2.3.4 Propionate and butyrate degrading activity at various sulphide concentrations at 10 °C. Sludge was sampled on day 270.

Sulphide concentration	Degrading activity (g COD·g^{-1} VSS·day^{-1})	
(g S^{2-}-S·dm^{-3})	Propionate	Butyrate
0.03	0.061 ± 0.003	0.190 ± 0.006
0.16	0.035 ± 0.000	0.171 ± 0.001
0.32	0.025 ± 0.001	0.133 ± 0.000

Both for thermodynamic and kinetic reasons, the H_2 - oxidising SRB should have a competitive advantage over methanogens under the conditions prevailing in digesters [32]. Based on the assessed mass balance (Table 2.3.3), it can be concluded that acetate was hardly, if at all used by SRB. Instead, it was converted into methane. This shows once again that methanogens compete efficiently with acetate degrading SRB in a reactor fed with low sulphate concentration [11, 21].

2.3.5 ACKNOWLEDGMENTS

We would like to thank J. van der Laan and I. Bennehey for the technical assistance with the chromatographs.

2.3.6 NOTATION

A = specific degrading activity (g COD·g^{-1} VSS·day^{-1})
A_{max} = maximum specific degrading activity (g COD·g^{-1} VSS·day^{-1})
AB = acetogenic bacteria
COD = chemical oxygen demand (g O_2·dm^{-3})
C_2 = acetate
C_3 = propionate
C_4 = butyrate
$\Delta G°'$ = free energy change at pH 7
$\Delta H°'$ = standard enthalpy at pH 7
EGSB = expanded granular sludge bed

HRT = hydraulic retention time (hours)
MPB = methane producing bacteria
OLR = organic loading rate (kg COD·m^{-3}·day^{-1})
SO_4^{2-}-COD = organic substrate required for sulphate reduction
SRB = sulphate reducing bacteria
UASB = upflow anaerobic sludge bed
VFA = volatile fatty acids
VSS = volatile suspended solids (g·dm^{-3})

2.3.7 REFERENCES

1. Lettinga G., Hulshoff Pol L.W., Hobma S.W., Grin P., Jong P. de, Roersma R. and IJspeert P., The use of a floating settling granular sludge bed reactor in anaerobic treatment. In: W.J. van den Brink (ed.), *Proceedings of the European Symposium on Anaerobic Waste Water Treatment.* Noordwijkerhout, The Netherlands, pp. 411-429 (1983).
2. Lettinga G., de Man A.W.A., van der Last A.R.M., Kato T.M., Wang K., Rebac S., Possible role of anaerobic digestion in sewage treatment under moderate climatic conditions. *Paper presented at the 2nd Japan/Dutch Workshop on Water Quality Management.* Ube, Japan (1993).
3. Man A.W.A. de, van der Last A.R.M. and Lettinga G., The use of EGSB and UASB anaerobic systems for low strength soluble and complex wastewaters at temperatures ranging from 8 to 30°C. In: E.R. Hall and P.N. Hobson (eds.), *Proceeding of the Fifth International Symposium on Anaerobic Digestion.* Bologna, Italy, pp. 197-209 (1988).
4. Frankin R.J., Koevoets A.A., Gils W.M.A. van and Pas A. van der, Application of the BIOBED upflow fluidized bed process for anaerobic waste water treatment. *Wat. Sci. Tech.*, **25**, 373-382 (1992).
5. Rebac S., Ruskova J., Gerbens S., van Lier J.B., Stams A.J.M. and Lettinga G., High-rate anaerobic treatment of wastewater under psychrophilic conditions. *J. Ferment. Bioeng.*, **80**, 499-506 (1995).
6. Dolfing J., Acetogenesis. In: Zehnder A.J.B. (ed) *Biology of anaerobic microorganisms.* Wiley & Sons, New York, pp 417-468 (1988).
7. Conrad R. and Wetter B., Influence of temperature on energetics of hydrogen metabolism in homoacetogenic, methanogenic, and other anaerobic bacteria. *Arch. Microbiol.*, **155**, 94-98 (1990).
8. Schink B., Syntrophism among prokaryotes. In: Balows A., Trüper H.G., Dworkin M., Harder W., Scheifer K.H. (eds) *The prokaryotes.* Springer, Berlin, Heidelberg, New York, pp 276- 299 (1992).
9. Stams A.J.M., Metabolic interactions between anaerobic bacteria in methanogenic environments. *Antonie van Leeuwenhoek*, **66**, 271-294 (1994).

10. Widdel F., Microbiology and ecology of sulphate- and sulphur-reducing bacteria. In: Zehnder A.J.B. (ed) *Biology of anaerobic microorganisms*, Wiley & Sons, New York, pp. 469-586 (1988).
11. Oude Elferink S.J.W.H., Visser A., Hulshoff Pol L.W. and Stams A.J.M., Sulfate reduction in methanogenic bioreactors. *FEMS Microbiol. Reviews*, **15**, 119-136 (1994).
12. Colleran E., Finnegan S. and Lens P., Anaerobic treatment of sulphate - containing waste streams. *Antonie van Leeuwenhoek*, **67**, 29-46 (1995).
13. Wu W-M., Hickey R.F. and Zeikus J.G., Characterization of metabolic performance of methanogenic granules treating brewery wastewater: Role of sulfate-reducing bacteria. *Appl. Environ. Microbiol.*, **57**, 3438-3449 (1991).
14. Visser A., Beeksma I., van der Zee F., Stams A.J.M. and Lettinga G., Anaerobic degradation of volatile fatty acids at different sulfate concentrations. *Appl. Microbiol. Biotechnol.*, **40**, 549-556 (1993).
15. Visser A., Alphenaar A., Gao Y., van Rossum G. and Lettinga G., Granulation and immobilisation of methanogenic and sulfate- reducing bacteria in high-rate anaerobic reactors. *Appl. Microbiol. Biotechnol.*, **40**, 575-581 (1993).
16. Harada H., Uemura S. and Momonoi K., Interaction between sulphate-reducing bacteria and methane-producing bacteria in UASB reactors fed with low stength wastes containing different levels of sulfate. *Wat. Res.*, **28**, 355-367 (1994).
17. Rinzema A., Paardekooper A.H., de Vegt A.L. and Lettinga G., Anaerobic treatment of edible oil refinery waste water in granular sludge UASB reactors. *Proceeding of the NVA/EWPCA Conference Anaerobic treatment a Grown-Up Technology*, 15 - 19 September 1986, RAI-Halls, Amsterdam, The Netherlands, Industrial presentations (Europe) B.V., Schiedam, The Netherlands, pp. 205-217 (1986).
18. Alphenaar A., Anaerobic granular sludge: characterization, and factors affecting its functioning, PhD thesis, Wageningen Agricultural University. Wageningen, The Netherlands (1994) .
19. Kristjansson J.K., Schönheit P. and Thauer R.K., Different K_S values for hydrogen of methanogenic bacteria and sulfate-reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulfate. *Arch. Microbiol.*, **131**, 278-282 (1982).
20. Robinson J.A. and Tiedje J.M., Competition between sulfate- reducing and methanogenic bacteria for H_2 under resting and growing conditions. *Arch. Microbiol.*, **137**, 26-32 (1984).
21. Rinzema A. and Lettinga G., Anaerobic treatment of sulphate containing wastewaters. In: Wise D.L. (ed) *Bioenvironmental systems*, Vol 1, CRC Press, Boca Raton, Fl, pp 65-109 (1988).
22. Mulder A., The effects of high sulfate concentrations on the methane fermentation of waste water. *Prog. Environ. Microbiol.*, **20**, 133-134 (1984).

23. Rinzema A., Anaerobic treatment of waste water with high concentrations of lipids or sulphate. PhD thesis, Wageningen Agricultural University. Wageningen, The Netherlands (1988).
24. American Public Health Association, Standard methods for the examination of water and wastewaters. 16th edition. Washington DC, USA (1985).
25. Chang R., Physical chemistry with application to biological systems. Macmillan Publishing Co, New York, USA (1977).
26. Macmillan's Chemical and Physical Data, James A.M. & Lord M.P. (eds), The Macmillan press Ltd., London, Great Britain, pp 449-501 (1992).
27. Widdel F. and Pfennig N., Studies on dissimilatory sulfate- reducing bacteria that decompose fatty acids II. Incomplete oxidation of propionate by *Desulfobulbus propionicus* gen. nov., sp. nov. *Arch. Microbiol.*, **131**, 360-365 (1982).
28. Samain E., Dubourguier H.C. and Albanac G., Isolation and characterization of *Desulfobulbus elongatus* sp. nov. from a mesophilic industrial digester. *Syst. Appl. Microbiol.*, **5**, 391 - 401 (1984).
29. Qatibi A.I., Bories A., Garcia J.L., Effects of sulfate on lactate and C_2, C_3 volatile fatty acid anaerobic degradation by a mixed microbial culture. *Antonie van Leeuwenhoek*, **58**, 241-248 (1990).
30. Boone D.R. and Bryant M.P., Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystems. *Appl. Environ. Microbiol.*, **40**, 626-632 (1980).
31. Tursman J.F. and Cork D.J., Influence of sulphate and sulphate-reducing bacteria on anaerobic digestion technology. *Biol. Processes*, **12**, 273-285 (1989).
32. Zinder S.H., Physiological ecology of methanogens. In: Feery J.G. (ed) *Methanogens: Ecology, Physiology, Biochemistry and Genetics*. Chapman & Hall, London/New York, pp 128-206 (1993).

Chapter 3

Anaerobic treatment of low strength industrial wastewaters at low temperatures

Published as \ accepted as:

3.1 Kato, M.T.; Rebac, S.; Lettinga, G. (1998) The anaerobic treatment of low-strength brewery wastewater in expanded granular sludge bed. *Appl. Biochemist. Biotechnol.*, (in press).

3.2 Rebac, S.; van Lier, J. B.; Janssen, M.G.J.; Dekkers, F.; Swinkels, K.T.M.; Lettinga, G. (1997) High-rate anaerobic treatment of malting waste water in a pilot-scale EGSB system under psychrophilic conditions. *J. Chem. Tech. Biotechnol.*, **68**: 135-146.

3.1 THE ANAEROBIC TREATMENT OF LOW-STRENGTH BREWERY WASTEWATER IN EXPANDED GRANULAR SLUDGE BED REACTOR

ABSTRACT

The anaerobic treatment of low-strength brewery wastewater, with influent total COD (COD_{in}) concentrations ranging from 550 to 825 mg/L, in a pilot-scale 225.5 L expanded granular sludge bed (EGSB) reactor was investigated. In an experiment where the temperature was lowered step-wise from 30 °C to 12 °C, the chemical oxygen demand (COD) removal efficiency decreased from 73 % to 35 %, at organic loading rates (OLR) of 11 to 16.5 g COD/L.d. The applied hydraulic retention time (HRT) and liquid upflow velocity (V_{up}) were 1.2 h and of 5.8 m/h, respectively. Under these conditions, the acidified fraction of the COD_{in} varied from 45 % up to 90 %. Besides the expected drop in reactor performance, problems with sludge retention were also observed. In a subsequent experiment set at 20 °C, COD removal efficiencies exceeding 80 % were obtained, at an OLR up to 12.6 g COD/L.d, with COD_{in} between 630 and 715 mg/L. The values of HRT and V_{up} applied were 2.1 to 1.2 h, and 4.4 to 7.2 m/h, respectively. The acidified fraction of the COD_{in} was above 90 % whereas, sludge washout was not significant. These results indicate that the EGSB potentials can be further explored for the anaerobic treatment of low-strength brewery wastewater, even at lower temperatures.

Keywords: anaerobic treatment, expanded granular sludge bed reactor, brewery wastewater, acidified low-strength wastewater, low temperature.

3.1.1 INTRODUCTION

Low-strength effluents can be considered as those containing chemical oxygen demand (COD) concentrations below 2000, though many even contain concentrations of less than 800 mg/L (12). Dilute wastewater streams may be discharged at a number of industrial processes (2,6,11,27). Important examples are effluents from alcoholic and soft drink bottling industries, paper recycle and papermaking mills, fruit and vegetable canneries, and malting and brewing processes (4,11,17,22). Some wastewaters may have a broad concentration range since the COD of industrial effluents depends largely on the technological process, depending on the water used and recycled as well as the internal sources of wastewaters. Typical examples are brewery industry wastewaters, which can have COD concentrations as low as 0.6-0.9 g/L or as high as 160 g/L, since these effluents can consist of a mixture of process streams from the malting and brewing processes, spent grains and hops pressing liquor wastes. Moreover, many breweries include also a soft drink bottling section, which also discharges dilute wastewaters (3,4,5,8,9,14,21). Regardless the concentration, brewery-like wastewaters are attractive for anaerobic treatment processes since they mostly contain simple substrates (16,18). The anaerobic treatment processes are well-established methods for the elimination of easily

biodegradable organic matter from wastewater. Since its earlier development, the upflow anaerobic sludge bed (UASB)-like systems have been more widely applied in practice than other anaerobic systems (18,20). However, in order to improve the applicability of the UASB reactor, some modifications have been proposed. High hydraulic mixing intensity is important in the treatment of dilute wastewater since the gas production is lower compared with that generated from higher strength wastewaters.

A modification of the UASB reactor is the expanded granular sludge bed (EGSB) reactor. In this reactor type, the granular sludge bed is expanded and the hydraulic mixing is intensified in order to improve the wastewater-biomass contact (15,23). A higher superficial liquid velocity is achieved by applying effluent recirculation or by using taller reactors. In the UASB reactor, the liquid upflow velocity (V_{up}) is usually in the range of 0.5 to 1.5 m/h, whereas the EGSB utilizes V_{up} exceeding 5 to 6 m/h (23).

Earlier experiments with EGSB reactors showed the importance of high V_{up} and that such reactors can potentially be applied for the treatment of several wastewaters. Experiments with dilute vinasse at 8 °C showed the importance of a high V_{up} of 5 m/h compared to when 0.5 m/h was applied for influents with a low COD (23). Other investigations were carried out with complex wastewaters as lipid containing effluents and pre-settled domestic sewage (19). A higher treatment performance was obtained in the EGSB compared with the UASB reactor for the degradation of sodium caprate and sodium laurate solutions at 30 °C (26). The experiments with pre-settled domestic sewage were conducted at temperatures ranging from 8 °C to 20 °C (19). The total influent COD (COD_{in}) concentration ranged from 100 to 650 mg/L but with a soluble COD fraction (membrane filtered) of around 50 %. The results showed that the removal efficiency of the soluble COD fraction was between 62 % and 95 % at hydraulic loading rate (HRT) of 1.0 to 3.5 h, when the temperature was above 13 °C and the COD was above 350 mg/L. The feasibility of EGSB reactors treating ethanol containing wastewaters was demonstrated at 30 °C. COD removal efficiency above 80 %, at organic loading rate (OLR) up to 12 g COD/L.d, was achieved with COD concentrations as low as 100 to 200 mg/L (13). A common aspect showed by those results is that the high performance of EGSB reactor treating dilute wastewaters can be attributed to the very high mixing intensity and efficient contact between the biomass and the substrate.

An additional important aspect is that low temperatures in anaerobic treatment have always been associated with low methanogenic sludge activity. However, this does not necessarily mean that psychrophilic wastewater treatment is unfeasible (15,23). Further investigation with the treatment of dilute acidified wastewater in an EGSB reactor at 10-13 °C fed with COD ranging from 600 to 900 mg/L, COD removal efficiency of approximately 100 % was observed after 100 days of operation (19). The HRT applied was less than 2 h and the OLR was in the range of 10 to 13 g COD/L.d.

Since an intense mixing is very important for the treatment of low-strength and cold wastewaters, the EGSB does fulfill that requirement. The objective of this study was to evaluate the application of the EGSB reactor for the treatment of low-strength brewery-type wastewater. The experiments were conducted in a 225.5 L pilot-scale reactor at a temperature range of 30 ° to 12 °C.

3.1.2 MATERIALS AND METHODS

Experiments in EGSB reactor. EGSB experiments were conducted with brewery wastewater in a pilot-scale 225.5-L poly-acrylate reactor, with a height of 7.5 m and 20 cm inner diameter. The experiments were conducted with the temperature varying from 30 °C to 12 °C and later set at 20 °C. FIGURE 3.1.1 shows the schematic diagram of the used EGSB reactor system. The total amount of biomass inoculated, including 6 kg of fines (granules with diameter below 0.8 mm),

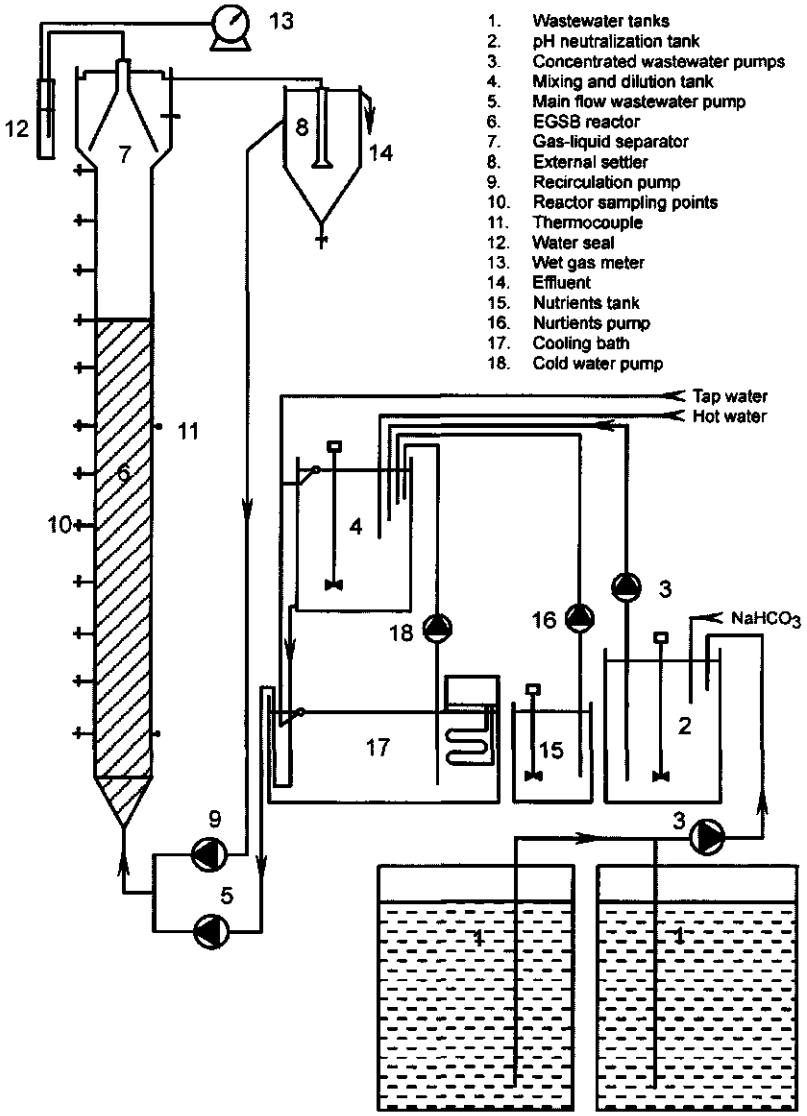

Fig. 3.1.1 Schematic diagram of the 225.5 dm^3 pilot scale EGSB system used in this study.

was 74 kg of wet granular sludge, which corresponded to 24.6 g VSS/L reactor.. The wastewater, originating from Bavaria Brewery, Lieshout, The Netherlands, was stored in two 3-m^3 tanks at ambient temperature. Its total COD was 60 g/L, of which ethanol and VFA concentrations were 71% and 12%, respectively. During the course of the experiments, concentrations and composition of the original wastewater fed to the EGSB reactor changed, due to sedimentation of suspended solids and acidification in the storage tanks. When the system was started-up, ethanol and VFA concentrations of the original wastewater had already changed to 30% and 45% of the total COD, respectively.

Inoculum. All the experiments were conducted using anaerobic granular sludge obtained from a full-scale UASB reactor treating alcohol distillery wastewater at 35 °C (Nedalco, Bergen op Zoom, The Netherlands). The composition of the distillery wastewater on a COD basis was: butane-diol 50 %; higher alcohols 20 %; acetone 4 %; acetic acid 20 %; and propionic acid 2 %. The sludge was stored at 4 °C during 4 months before starting the experiments. The volatile suspended solids (VSS) content was 7.5 % of the weight of wet sludge, respectively. Wet sludge refers to the solids before drying overnight in an oven at 100-103 °C. The mean density, settling velocity, and granule diameter were 1019 g/L sludge, 35 m/h, and 1.2 mm. The granule size distribution test revealed that 28 % of the sludge weight was in the diameter range between 0.7 and 1.0 mm, 42 % between 1.0 and 1.5 mm, and 30 % between 1.5 and 1.9 mm.

Experimental design. The reactor was run under two different operational conditions of temperature. In the first experiment, the reactor temperature was decreased step-by-step from 30 °C to 12 °C. In the second experiment, the temperature was maintained at 20 °C. Monitoring consisted of daily reactor influent and effluent sampling. COD removal efficiency refers COD_{in} to effluent COD of the internal settler supernatant.

Batch methanogenic activity assay. The specific methanogenic activity of the sludges was determined using 0.6-L glass serum flasks sealed with a rubber septum and a screw cap. The sludge was added to the flasks and then the liquid volume was completed to 0.5 L with the basal mineral medium solution. The final substrate and sludge concentration were 4 g COD/L and 1.5 g VSS/L, respectively. The used acetate and VFA solutions were neutralized. The composition of the VFA-mixture solution was 24 (C_2) : 34 (C_3) : 41 (C_4) on a COD basis. After flushing the medium with nitrogen gas, the flasks were sealed and incubated in a temperature-controlled room at 20 and 30 ± 2 °C. The flasks were provided with a second feeding when more than 80 % of the substrate COD supplied in the first feeding was converted to methane. Monitoring consisted of periodic measurements of methane production by modified Mariotte flasks. The flasks contained a 3 % (w/v) NaOH solution to remove the carbon dioxide from the biogas. The maximum specific methanogenic activity was calculated from the slope of the methane production versus time curve. The assays were conducted in duplicate under static conditions.

Granule size distribution. A sedimentation assay was performed in duplicate or triplicate to determine the particle size distribution and the mean granule diameter of the sludge, as described

by Hulshoff Pol et al. (10). The method is based on relating sedimentation velocities to the size and density of the granules.

Basal media. For the batch methanogenic activity assay, a concentrated stock solution of essential inorganic macro- and micronutrients was prepared. After a fivefold dilution, the basal medium solution contained (in mg/L): NH_4Cl: 280, $K_2HPO_4 \cdot 3H_2O$: 327.4, $MgSO_4 \cdot 7H_2O$:100, $CaCl_2 \cdot 2H_2O$: 10, yeast extract: 100, H_3BO_3: 0.05, $FeCl_2 \cdot 4H_20$: 2, $ZnCl_2$: 0.05, $MnCl_2 \cdot 4H_2O$: 0.05, $(NH_4)6Mo_7O_{24} \cdot 4H_2O$: 0.05, $AlCl_3 \cdot 6H_2O$: 0.09, $CoCl_2 \cdot 6H_2O$: 2, $NiCl_2 \cdot 6H_2O$: 0.05, $CuCl_2 \cdot 2H_2O$: 0.03, $NaSeO_3 \cdot 5H_2O$: 0.1, EDTA: 1, resazurin: 0.2, and 36% HCl 0.001 mL/L. Alkalinity was provided as sodium bicarbonate in an amount depending on the type and concentration of the substrate utilized. The bicarbonate concentration in mg $NaHCO_3$/L was 2100 using ethanol, and 400 for acetate and volatile fatty acids (VFA)-mixture solutions.
For the reactor experiments, the following nutrients were added to the substrate (brewery wastewater) (in mg/L influent): NH_4Cl: 45, K_2HPO_4: 16, $(NH_4)SO_4$: 10, $CaCl_2 \cdot 2H_2O$: 4, $MgCl_2 \cdot 6H_2O$: 9. Alkalinity was also provided as sodium bicarbonate, in an amount (336 to 924 mg $NaHCO_3$/L influent) depending on the influent COD concentration.

Analyses and chemicals. Ethanol and VFA were determined with a Hewlett Packard 5890 gas chromatograph (Palo Alto, CA). Before use, the chromatograph was calibrated with standard solutions of ethanol or VFA. For the biogas composition of the reactor, a 200-mL glass sampler was used for collection prior to the analyses. The CH_4, CO_2, and H_2S were analyzed with the same sample of 100 µL injected into a Packard Becker 433 chromatograph (Delft, The Netherlands). All gas sample analyses were conducted after calibration with standards of known amounts of the respective gases. The characteristics and operation conditions of each chromatograph were described elsewhere (12,13). The concentrations of ethanol and VFA as well as the methane production are referred to in COD units. Conversion factors utilized were 2.087 g COD/g ethanol, 1.067 g COD/g acetate (C_2), 1.515 g COD/g propionate (C_3), and 1.820 g COD/g butyrate (C_4). For methane, a factor of 2.577 g COD/L CH_4 at 30°C was utilized. This factor was corrected for other temperatures. The soluble COD, ethanol, and VFA of reactor effluents are referred to samples centrifuged at 13,000 *rpm* for 3 min.
Measurements of pH were conducted immediately after sampling with a Knick 510 pH/mV-meter (Berlin, Germany) and a Schott Nederland N61 double electrode (Tiel, The Netherlands). The COD, solids and other analyses were determined according to the Standard Methods (1).
All chemicals were of analytical grade and purchased from Merck (Darmstad, Germany). Exceptions were the yeast extract from Gist-Brocades (Delft, The Netherlands); resazurin from Fluka (Buchs, Switzerland); the gases from Hoekloos (Schiedam, The Netherlands); and the sodium bicarbonate (99.5 %) added to the influent of the reactor, from Boom (Meppel, The Netherlands).

3.1.3 RESULTS

EGSB reactor treatment efficiency. The operational conditions and performance of the pilot-scale EGSB reactor treating brewery wastewater are shown in Figures 3.1.2, 3.1.3 and 3.1.4. The average values of the applied COD_{in}, HRT, OLR and SLR, and the treatment efficiencies during the various periods of the two experiments are also summarized in Table 3.1.1.

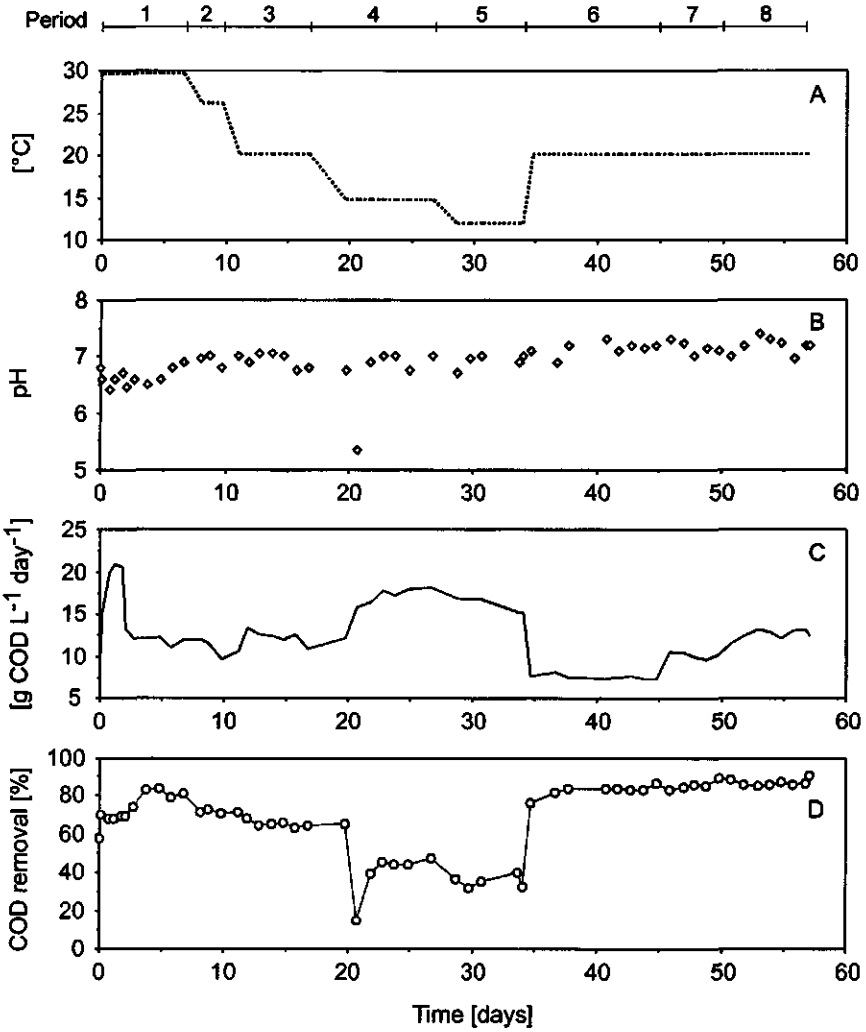

Fig. 3.1.2 Operational conditions and performance of the EGSB system. A. Temperature. B. pH in the reactor. C. Organic loading rate. D. Removal efficiency, based on COD_{tot}.

During the first experiment, each period corresponded to one range of temperature. The reactor was fed with a COD_{in} concentration ranging from 550 to 825 mg/L and the applied HRT was maintained at 1.2 h, resulting in a V_{up} value of 5.8 m/h in all the 5 periods (Table 3.1.1). No recirculation was applied. Decreasing the temperature from 30 °C to 20 °C

Table 3.1.1 Average values of the operational conditions and reactor performance of the pilot-scale EGSB reactor treating brewery wastewater.

Parameter	Experiment I					Experiment II		
Period	1	2	3	4	5	6	7	8
Days	0-7	8-10	11-17	20-27	29-34	35-45	46-50	51-57
Temperature (°C)	30	26	20	15	12	20	20	20
HRT (h)	1.2	1.2	1.2	1.2	1.2	2.1	1.7	1.2
V_{up} (m/h)	5.8	5.8	5.8	5.8	5.8	4.4	6.2	7.2
COD_{inf} (mg/L)	720	550	600	825	805	656	715	630
OLR (g COD/L.d)	14.4	11.0	12.0	16.5	16.1	7.5	10.1	12.6
SLR (g COD/g VSS.d)	0.62	0.53	0.60	0.94	1.07	0.56	0.77	1.01
COD removal (%)	73	71	66	43	35	81	85	86

SLR was corrected for the sludge washed out and values refer to the end of each experimental period.

corresponded to a gradual decrease in the COD removal efficiency from 73 % to 66 % (periods 1 to 3, Table 3.1.1), when operating at an OLR and SLR of 11.0 to 14.4 g COD/L.d and 0.53 to 0.62 g COD/g VSS.d, respectively. It should be noted that in addition to the influence of low temperatures in those periods, the OLR values also significantly increased to 16.1-16.5 g COD/L.d due to the increase in COD_{in} concentrations which resulted in overloading of the reactor in the periods 4 and 5. A significant sludge washed out occured in these periods. Since the sludge was not returned to the reactor, the applied SLR (0.94-1.07 g COD/g VSS.d) was 40-60 % higher compared to the initial 3 periods. At day 21 (period 4), an unintended pH drop (Fig. 3.1.2b) due to the bicarbonate pump failure resulted in a very sharp decrease in COD removal efficiency (Fig. 3.1.2d). This might have contributed to the poor performance in that period, despite the apparent recovery of the reactor system after 24 h to the level of 43 % of COD removal, which remained until the end.

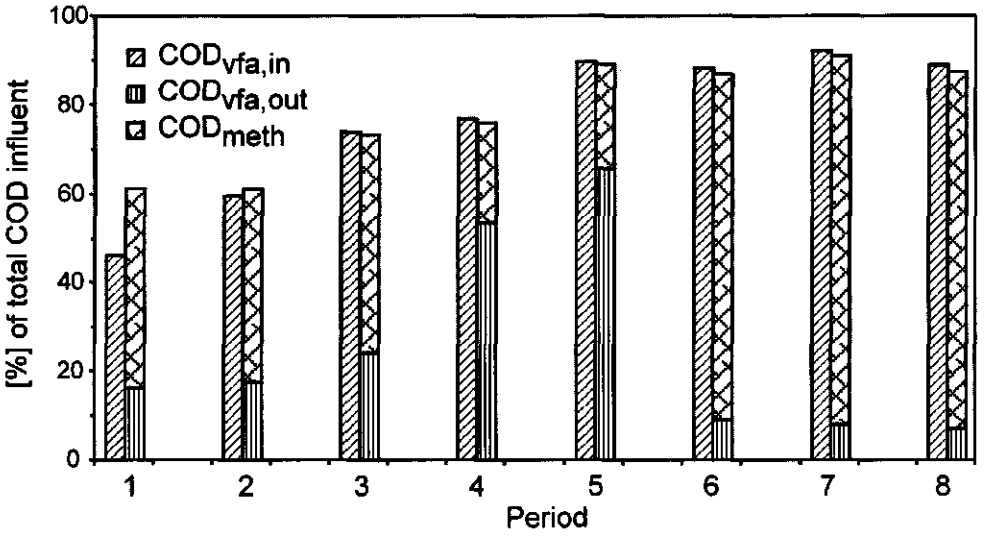

Fig. 3.1.3 Fate of COD_{sol} in the EGSB system. First bar 'COD_{vfa}' (///) of the influent, second bar 'effluent COD_{vfa} (|||) + COD_{meth} (×××)' expressed as percentage of average influent total COD in the experimental periods.

The degradation of acetate on the COD basis was almost complete in the periods 1 to 3, but decreased to about 50 % in the periods 4 to 5 (Fig. 3.1.4), when operating at 15 °C (Fig. 3.1.2a). In the case of propionate, a slight degradation occurred in the periods 1 and 2; however in the periods 3 to 5, the degradation was completely retarded (Fig. 3.1.4 a, b). It was also observed that the influent VFA-COD increased from 45 % of the total COD in the beginning of period 1 to 90 % of the total COD at the end of period 5 (Fig. 3.1.3). In this experiment, decreasing the temperature from 30 °C to 12 °C resulted in a decrease in the COD_{in} converted into methane, from 45 to 16 % (Fig. 3.1.3). This corresponded to a reduction in the biogas production from 833 L/d to 42 L/d.

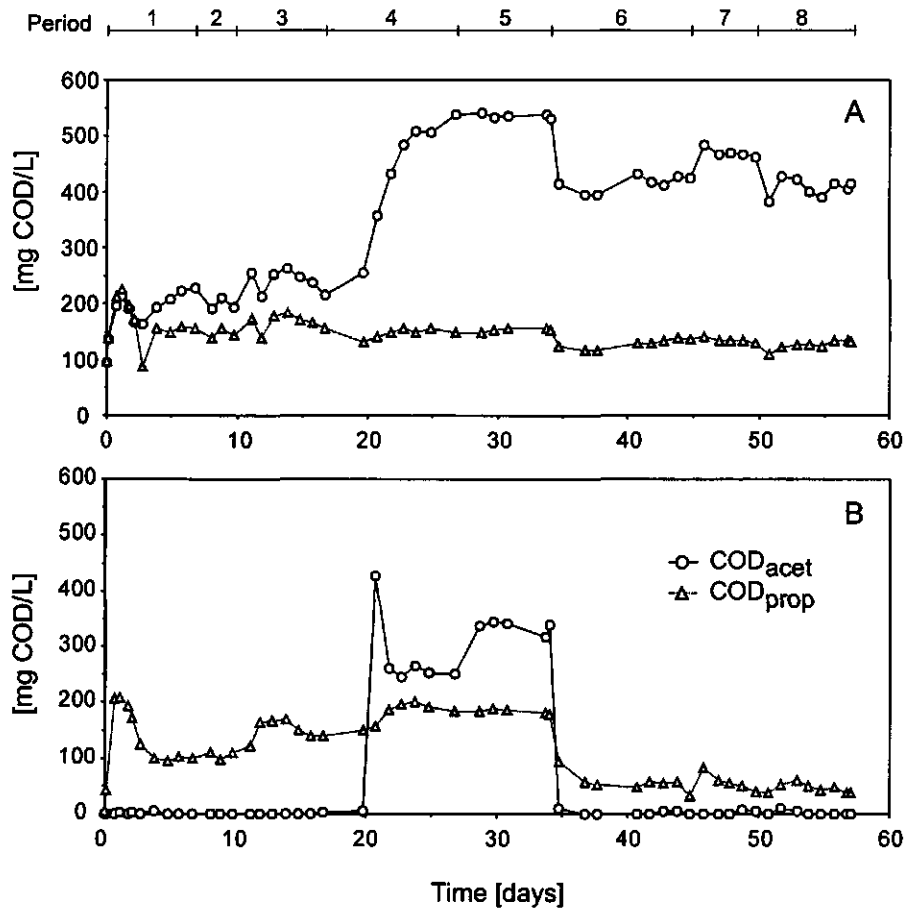

Fig. 3.1.4 Evolution of the VFA concentration in the influent (A) and the effluent (B) as a function of time: (O) $COD_{acet.}$, (Δ) $COD_{prop.}$.

During the second experiment, the reactor temperature was set at 20 °C and different HRT values ranging from 2.1 h to 1.2 h were applied in periods 6 to 8 (Table 3.1.1). Differences in the applied liquid upflow velocities were obtained by using effluent recirculation in a ratio up to 0.8. Compared to experiment I the reactor was fed with COD_{in} levels of 630 to 715 mg/L, corresponding to OLRs up to 12.6 g COD/L.d. A good treatment performance with a COD removal efficiency exceeding 80% was obtained at an OLR of 7.5 g COD/L.d when the was reactor operated at a V_{up} of 4.4 m/h (period 6). Even when the reactor was operated up to 12.6 g COD/L.d, the efficiency exceeded 85 %. In this case, the reactor was operated at a V_{up} of 7.2 m/h (period 8). Much less sludge washout occurred in this experiment compared to the previous periods. Nevertheless, due to the accumulated sludge washed out until the beginning of period 8 which was not returned to reactor, resulting in an applied SLR of about 1.01 g COD/g VSS.d. In this experiment, the wastewater fed into

the reactor can be considered as completely acidified since the COD_{in} was almost composed of VFA, above 90 % (Fig. 3.1.3). The degradation of acetate was almost complete in these last 3 periods; in the case of propionate, the degradation increased from 0 % in the beginning of period 6 up to 60-70 % at the end of period 8 (Fig. 3.1.4 a, b).

Sludge washout. After the starting and throughout the reactor experiments, an operational difficulty observed was related to sludge washout, especially during specific days of experiment 1. The total amount of sludge washed out collected in an external settler in the periods 1-5 was 546, 314, 378, 935 and 359 g VSS, respectively. This signifies that at the end of experiment 1 about 45 % of the seed sludge was washed out. Significant sludge washout mostly occurred during the days when reactor temperature changed from one experimental period to another. The occurrence of high washout at the upward liquid velocity of 5.8 m/h applied in the whole experiment I, was due to the excessive expansion of the sludge bed. Moreover, during the days of maximal washout, sludge flotation because of gas bubbles attached to granules was also observed. The flotation resulted in buoyancy forces driving part of the sludge bed upward from the bottom of the reactor. When this occurred, sludge washout occurred in a very short period of time. In the second experiment, despite even higher V_{up} values were applied, less expansion of sludge bed occurred since the initial amount of sludge was already reduced to almost half. The sludge washout in experiment II was decreased to only 10 % of the values measured in periods 3 to 5, corresponding to a average effluent concentration of only 0.006 g COD/L.

Sludge characteristic changes. During the course of the experiments, changes in reactor sludge characteristics were observed (Table 3.1.2-3.1.3). The changes were mainly related to the methanogenic activity and the granule size of reactor sludge. The maximum specific methanogenic activities of reactor sludge samples with ethanol as the substrate at 20°C are shown in Table 3.1.2. The assays were conducted with the seed sludge, the bottom and the top sludges from the EGSB reactor at day 34 and day 57. The sludge activity with ethanol decreased from day 0 to day 34 by approximately 15 %, either for the bottom or top reactor sludge. At day 57 the decrease was 30 % for the bottom sludge, but in contrast activity of the top sludge increased by 40 % compared with that of day 0. The decreases of sludge activity on ethanol as substrate might mainly be due to changes of the characteristic of the brewery wastewater fed into reactor during the experimental periods. The increased activity of the top sludge sample at the end of experiment II might be due to the higher granule segregation that occurred in the sludge bed in the last days of the experiments, compared with that of day 0 and day 34. Apparently, the smaller granules in the top of reactor were characterized by a higher sludge activity compared to that of the bottom sludge of higher diameter. Interestingly, the batch activity tests also showed that the ethanol conversion pathway changed during the experimental periods (Fig. 3.1.5). Mostly acetate accumulated as intermediate during ethanol conversion by the seed sludge. However, when sludge exposed to the brewery wastewater during 34 days and 57 days with the bottom reactor sludge, a significant shift occurred in the intermediate formation, since then propionate accumulated up to 25 % and 38 % of the initial

Table 3.1.2 Maximum specific methanogenic activity of the seed and the EGSB sludge with ethanol as the substrate. Standard deviation is given between parentheses.

Time	Temperature	Maximum specific methanogenic activity (g CH_4-COD/g VSS.d)	
		Ethanol	
	[°C]	1st feeding[d]	2nd feeding
Day 0[a]	30	1.780 (0.086)	1.760 (0.367)
	20	0.629 (0.021)	0.666 (0.022)
Bottom sludge			
Day 34[b]	20	0.533 (0.047)	0.585 (0.062)
Day 57[c]	20	0.434 (0.003)	0.351 (0.038)
Top sludge			
Day 34	20	0.532 (0.113)	0.549 (0.002)
Day 57	20	0.863 (0.006)	0.950 (0.038)

[a] Nedalco seed sludge after 4 months of storage at 4 °C.
[b] End of experiment 1.
[c] End of experiment 2.
[d] Product spectra of ethanol biodegradation by these corresponding sludges are given in Fig. 3.1.5.

ethanol COD, respectively. Similarly, propionate though less, accumulated up to 21 % and 19 %, respectively with the top reactor sludge at the same days of sampling.

The maximum specific methanogenic activity of the seed sludge and reactor sludge sampled on day 57 was assessed with the VFA mixture and the results obtained are presented in Table 3.1.3. The results reveal that methanogenic activity of the seed sludge at 20 °C was very low, even in the second feeding. However, the activity was increased by a factor 2.5 in a short period of time (34 days), taking into consideration that the sludge was overloaded (periods 4-5) with a new type of wastewater at the low temperatures applied (12-15 °C).

Table 3.1.3 Maximum specific methanogenic activity of the seed and the EGSB sludge with VFA mixture as the substrate. Standard deviation is given between parentheses.

Time	Temperature	Maximum specific methanogenic activity (g CH_4-COD/g VSS.d)	
		VFA-mixture	
	[°C]	1st feeding	2nd feeding
Day 0[a]	30	0.334 (0.013)	0.875 (0.020)
	20	0.142 (0.018)	0.160 (0.005)
Day 57[b]	20	0.346 (0.005)	0.403 (0.019)

[a] Nedalco seed sludge after 4 months of storage at 4 °C.
[b] End of experiment 2 with mixed sludge from the EGSB reactor.

The granular size distribution at the end of the experiment II (day 57) was compared with that of the start. Fig. 3.1.6 shows that the seed sludge was formed by granules of 0.7 to 1.9 mm with mean diameter of 1.2 mm, while the final sludge was formed by granules of up to 2.6 mm with mean diameter of 1.6 mm. Additionally, the size distribution was more uniform for the final sludge compared with that of the seed sludge, since the fractions of granules between 1.3 and 2.6 mm were approximately the same. Moreover, while in the seed sludge the granules with diameter below 1.2 mm were 40 % of the total weight, in the final sludge the granules in that diameter range was only 20%. Aside the increase in sludge diameter, the fines were washed out with the effluent during the course of the experimental periods, especially in the first experiment. Fig. 3.1.7 shows the result of the size distribution assay from the sludge washed out during periods 1, 3 and 5. The fraction of smaller granules with diameter below 1.2 mm was 31 %, 75 % and 55 % of the total sludge weight, respectively.

3.1.4 DISCUSSION AND CONCLUSIONS

This study showed that EGSB reactor system is feasible for the direct treatment of low-strength partially to almost completely acidified brewery wastewater (<1000 mg COD/L influent) at lower temperatures (12-20 °C). Decreasing the temperature from 30 °C to 20 °C, resulted in a drop of the COD removal efficiency of about 10 % (Table 3.1.1). A more serious drop in COD removal efficiency (43 %) when the operating the system temperature was lowered down to the range of 15-12 °C. The decreasing efficiencies when lowering the temperature can firstly be attributed to overloading. The very high OLR applied (16.5 g COD/L.d) was above the maximum biological capacity of the reactor in almost any time, resulting in decreasing values of specific methanogenic activity of the sludge (Table 3.1.2, 3.1.3). Secondly, the reactor capacity was also influenced by severe sludge washout. Thirdly, the lower activities can be due to the gradual change in the wastewater composition because of the increasing degree of acidification (Fig. 3.1.3). Partially acidified wastewater (300-400 mg VFA-COD/L) is important for obtaining high treatment efficiency at low temperatures, as showed in further experiments conducted in the same EGSB system with malting wastewater (24). In the present experiments, the influent contained VFA concentrations above that range. However, the reactor did not remove propionate (Fig. 3.1.4b), which corroborates with low activity tests (Table 3.1.3, 20 °C). Thus, the shift in ethanol fermentation product from acetate to propionate (Fig. 3.1.5) also contributed to the lower COD removal capacity of the EGSB reactor (Fig. 3.1.2d). These results indicate that lower organic loads should be applied to achieve a higher treatment performance of low-strength acidified brewery-type wastewater at temperatures lower than 20 °C in EGSB reactor system. Application of higher values of OLR (> 8 g COD/L.d) requires long term operation which allows the development of a specific bacterial population in the granular sludge, as demonstrated with an EGSB system treating low-strength malting wastewater at low temperatures (20 to 13 °C), when very good and stable reactor performance was obtained after 60 days of operation (24).

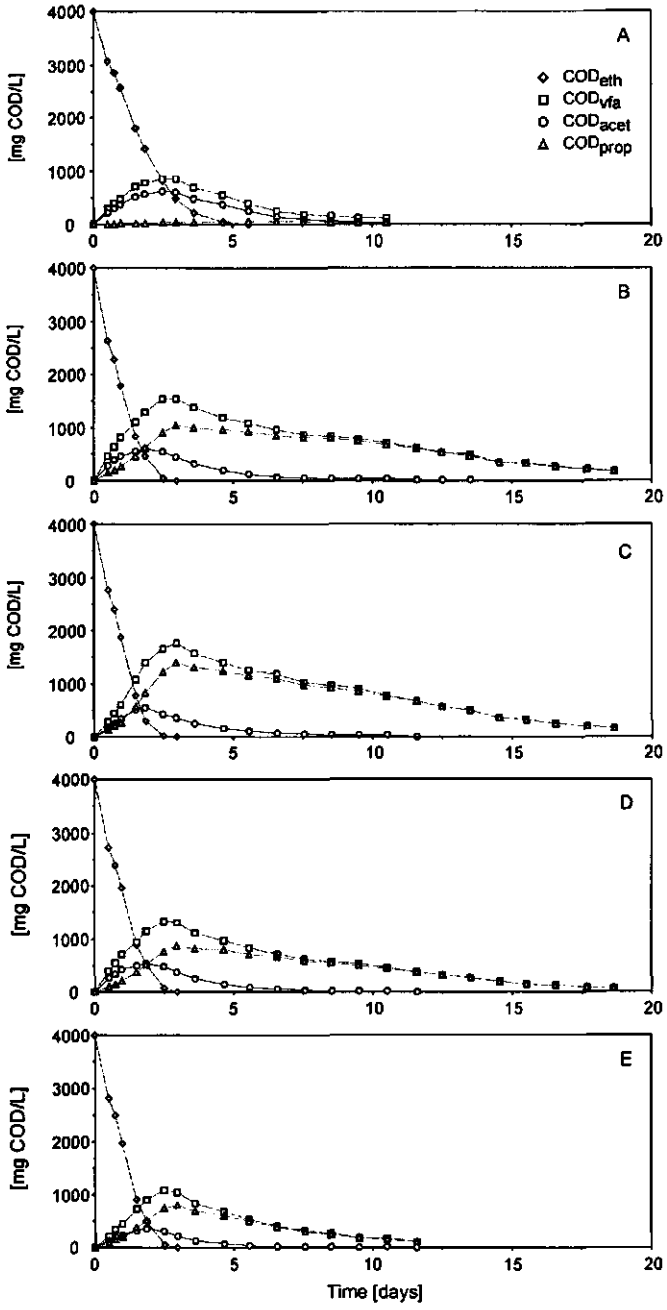

Fig. 3.1.5 Degradation of ethanol in batch experiments. A. seed sludge. B. Bottom sludge at day 34. C. Bottom sludge at day 57. D. Top sludge at day 34. E. Top sludge at day 57. (◊) COD_{eth}, (□) $COD_{vfa.}$, (O) $COD_{acet.}$, (Δ) $COD_{prop.}$.

In this study, when treating low-strength acidified brewery wastewater at 20 °C, the EGSB reactor system showed good COD removal efficiency exceeding 80 % at organic loading rates up to 12.6 g COD/L.d. Such high organic loads could be accommodated at an HRT as low as 1.2 h at liquid upflow velocity applied up to 7.2 m/h. These results reveal that the potential of anaerobic treatment for various types of low-strength wastewaters at lower temperatures can be further explored using the EGSB reactor system, as was already shown in experiments with malting and acidified wastewaters (24,25). One common observation in such experiments was that adequate hydraulic mixing is essential for achieving high COD removal efficiencies. Previous research with EGSB reactors also showed the importance of meeting that condition (13). Since at low temperatures gas production is low and high hydraulic loads are to be applied to improve mixing intensity. The present results indicate that a higher COD removal efficiency was achieved when lower HRT combined with higher V_{up} using effluent recirculation were applied, despite the increased organic loads (Fig. 3.1.2 d). The primary condition of good expansion of the sludge bed was also met concerning the

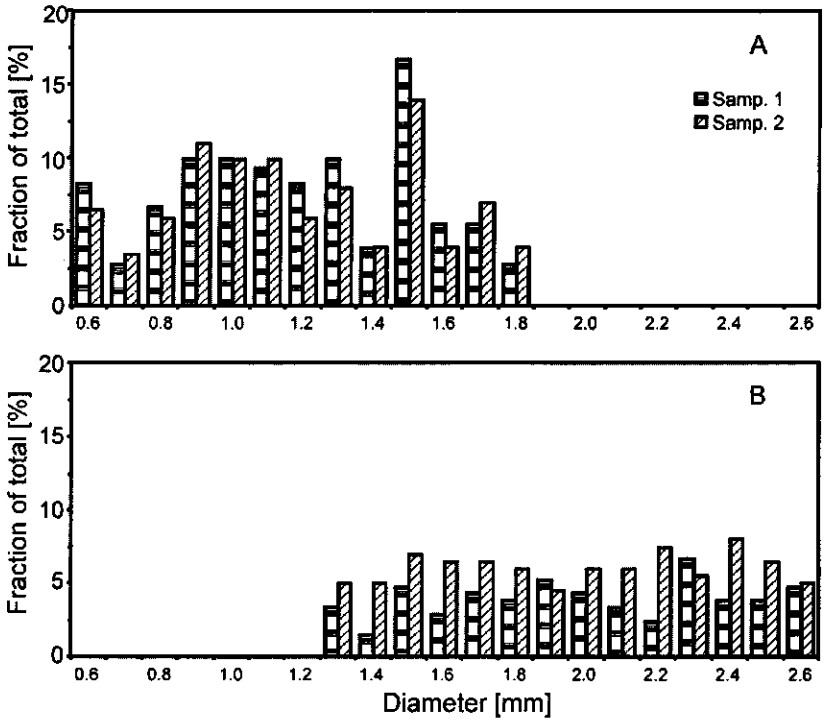

Fig. 3.1.6 Size distribution of mixed granular sludge at the start of the experiment (A) and at day 57 (B), expressed in percentage of the biomass weight represented by the granules. Duplicate samples are presented by bars.

sludge hold-up, since substantial less washout occurred compared to the previous experiment of this study. The high reactor performance can also be explained by the increased reactor sludge activity. The specific methanogenic activity of the sludge at the end of this experiment

with acetate or the VFA-mixture as substrates in batch tests at 20 °C (Table 3.1.3) increased about threefold compared with that of the seed sludge. This can be an indication that some growth and enrichment of methanogens and acetogens occurred, probably mainly after the reactor was set at 20 °C in experiment II. This agrees with the increase in granule diameter (Fig. 3.1.6). Additionally, almost no acetate was detected in the effluent and more than 60 % of the influent propionate was removed at the end of the experiments (Fig. 3.1.4).

The changes occurred in the specific methanogenic activity of the reactor sludge during the experiments can be attributed to the changes in temperature and wastewater characteristics, which influenced the formation of intermediate products and consequently, the microbial populations. The considerable change in the ethanol degrading pathway in the batch activity tests, especially concerning the formation of propionate as intermediate and its further degradation (Fig. 3.1.5), might be due to the presence of sulfate at very low sulfate concentration in the influent of reactor system. The latter might support the growth of *Desulfobulbus propionicus* in the granular sludge, a bacterium known to form propionate during the anaerobic degradation of ethanol. Similar low influent sulfate concentrations and shifts in ethanol degrading pathway have also been observed during the treatment of malting wastewater (24).

The increased mean diameter of reactor sludge at the end of the experiments confirms the effect of high liquid upflow velocities applied in the EGSB, as shown in other studies (7,13,24). High upflow velocity enhances the shear forces on granules that may result in erosion, and some granule segregation in the sludge bed. The fact that a significant amount of sludge washed out was of small particles can be attributed to erosion of the seed granules resulting in fines, and the particles of worst settleability (Fig. 3.1.7). A large amount of fines could visually be well observed in the collected sludge washed out. Very fine suspended anaerobic solids with powder-like appearance floated in the surface of the external settler. The apparently more polished and uniform size distribution of reactor final sludge also indicates the effect of higher shear forces (Fig. 3.1.6).

The granule segregation in the expanded sludge bed, due to the liquid upflow velocities applied, also was observed in EGSB reactor experiments (13,24). Firstly, despite the high values of V_{up} in the experiments resulting in an expansion of the bed over the whole reactor height, the down part remained less expanded compared to the top part. This was especially the case when the reactor was started-up and until the end of experiment I because the inlet system was probably not optimal. The poor expansion of the down bed may have caused dead zones, channeling and gas pockets. Much of the sludge washout was hydraulically assisted since the expansion was already excessive. However, the strong washout observed was very likely due to the gas accumulated in the dense unexpended lower sludge bed, since it occurred only on certain days. Gas pockets exploded and sludge bed moved upward due to the buoyancy forces dragging up the particles. Examples of such occurrence were observed in periods 1 and 2, when higher gas production up to 833 L/d resulted in pocket volumes up to 35 L every hour. This volume represented about 33 % of the volume occupied by the sludge in the reactor. Later in the experiment II, since the sludge washed out was not returned to reactor resulting in a smaller sludge bed and since

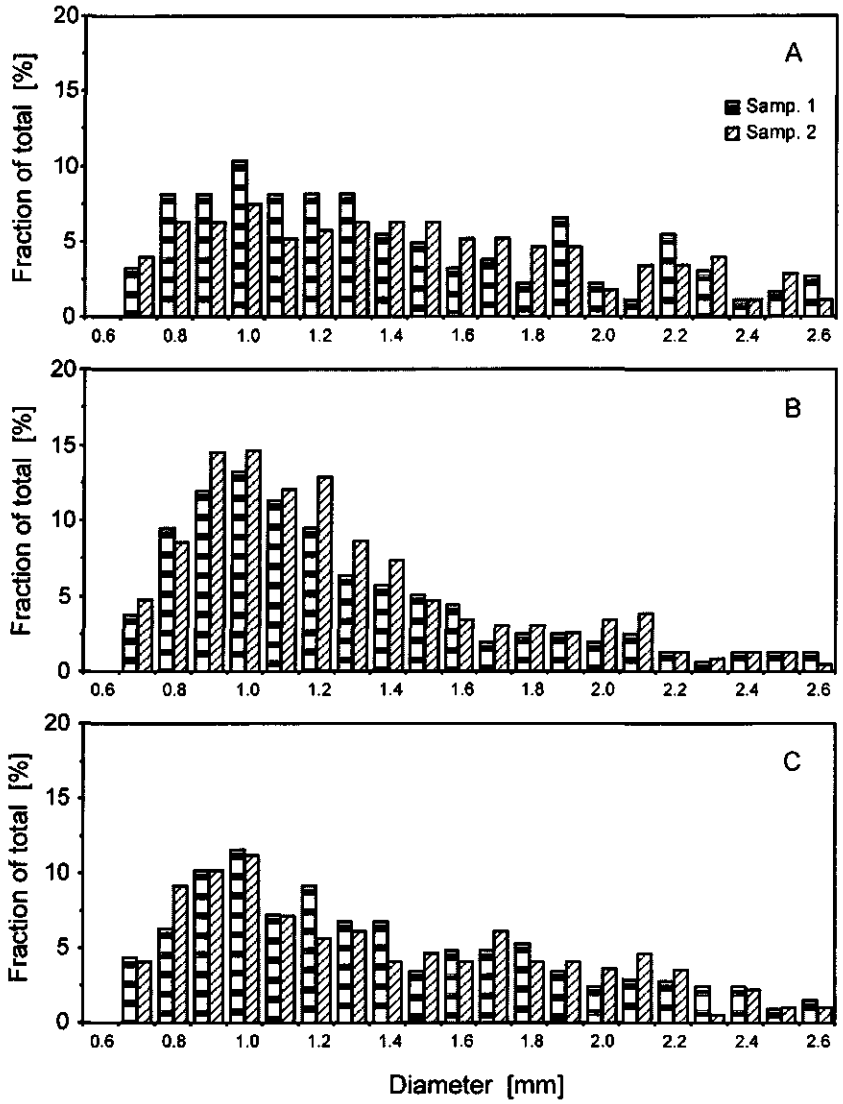

Fig. 3.1.7 Size distribution of washout of granular sludge during experiments (A) Period 1, days 0-7, (B) Period 3, days 11-17, (C) Period 5, days 29-34, expressed in percentage of the biomass weight represented by the granules. Duplicate samples are presented by bars.

recirculation started to be applied increasing the V_{up}, the expansion bed was more homogeneous causing significant less problem of sludge hold-up. Secondly, the size and settleability of granules can indicate segregation by gravitational forces. Based on the sludge size, a clear segregation between the top and down part of reactor bed was previously demonstrated (13,24), as well in this experiment. Higher granule diameters were found for the sludge at the bottom compared with those of the top. Based on the results of this study, a

higher treatment efficiencies can be expected using EGSB reactor system in the case of low-strength acidified brewery-type wastewater at low temperatures. The results with this and other EGSB experiments confirm the requirement of high hydraulic loads to ultimately enhance the wastewater-biomass contact. In addition to set the proper operational conditions needed to meet that requirement, the EGSB reactor also needs an improved device for solid-liquid-gas separation. The development of a good system for sludge hold-up will minimize the limitation for the EGSB application in practice.

3.1.5 ACKNOWLEDGEMENTS

The financial aid of the Ministry of Education/CAPES, Brazil to M. T. Kato and the Wageningen Agricultural University to S. Rebac is gratefully acknowledged. We thank J. van der Laan and Ilse Bennehey for the technical assistance with the chromatographs. We also thank Nedalco and Bavaria B.V., The Netherlands for the cooperation.

3.1.6 REFERENCES

1. American Public Health Association. (1985), *Standard Methods for the Examination of Water and Wastewater*, 16th ed., Washington, D.C.
2. Bhaskaran, T. R. (1973), *Guidelines for the Control of Industrial Wastes. 8. Brewery Wastes.* World Health Organization (WHO/WD/73.15), Geneva, Switzerland.
3. Cheng, S. S., Lay, J. J., Wei, Y. T., Wu, M. H., Roam, G. D. and Chang, T. C. (1989), in *Proc. Int. Symp. Waste Management Problems in Agro-industries*, Istanbul, Turkey, pp. 213-220.
4. Craveiro, A. M., Soares, H. M. and Schmidell, W. (1986), *Wat. Sci. Technol.* **18,** 123-134.
5. Fang, H. H. P., Guohua, L., Jinfu, Z., Bute, C. and Guowei, G. (1990), *J. Environ. Eng.* **116,** 454-460.
6. García, P. A., Rico, J. L. and Fernandez-Polanco, F. (1991), *Environ. Technol.* **12,** 355-362.
7. Guiot, S. R., Arcand, Y. and Chavarie, C. (1992), *Wat. Sci. Technol.* **25,** 897-906.
8. Hanaoka, T., Nakahata, S., Shouji, T., Sasaki, M., Maaskant, W. and Wildschut, L. (1994), in *Proc. Seventh Int. Symp. Anaerobic Digestion*, Cape Town, South Africa, pp. 518-527.
9. Hulshoff Pol, L. W., Dekkers, F. and Tersmette, T. (1991), in *Proc. Sixth Int. Symp. Anaerobic Digestion*, São Paulo, Brazil (poster).
10. Hulshoff Pol, L. W., Worp, J. J. M. van de, Lettinga, G. and Beverloo, W. A. (1986), in *Proc. Water Treatment Conf. AQUATECH '86,* Amsterdam, The Netherlands, pp. 89-101
11. Isaac, P. C. G. and McFiggans, A. (1981), in *Food Industry wastes: Disposal and Recovery*, Herzka, A. and Booth, R. G., eds., Applied Science, London, pp. 144-160.
12. Kato, M. T. (1994.), PhD thesis, Landbouwuniversiteit Wageningen, The Netherlands.
13. Kato, M. T., Field, J. A., Versteeg, P. and Lettinga, G. (1994), *Biotechnol. Bioeng.* **44,** 469-479.
14. Keenan, J. D. and Kormi, I. (1981), *J. Wat. Poll. Contr. Fed.* **53,** 66-77.

15. Last, A. R. M. van der and Lettinga, G. (1991), in *Proc. Sixth Int. Symp. Anaerobic Digestion*, São Paulo, Brazil, pp. 153-154.
16. Lettinga, G. (1982), in *Anaerobic Reactor Technology*, Agricultural University, Wageningen, The Netherlands, pp. 88-95.
17. Lettinga, G. and Hulshoff Pol, L. (1986), *Wat. Sci. Technol.* **18,** 99-108.
18. Lettinga, G. and Hulshoff Pol, L. W. (1991), *Wat. Sci. Technol.* **24,** 87-107.
19. Lettinga, G., Man, A. de, Last, A. van der, Kato, M., Wang, K. and Rebac, S. (1993), in *The 2nd Japan/Dutch Workshop on Water Quality Management*, Ube, Japan.
20. Lettinga, G., Rinzema, A. and Hulshoff Pol, L. W. (1992), in *Proc. Third Workshop Working Group on Biogas Production Technologies. CNREE Network on Biogas Production and Conversion for Energy,* Baader, W., ed., Braunschweig, Germany, pp. 73-90.
21. Lovan, C. R. and Foree, E. G. (1971), in *Proc. 26th Industrial Wastes Conf.*, Purdue University, West Lafayette, U.S.A, pp. 1074-1086.
22. Maaskant, W., Zeevalkink, J. A. (1983), in *Proc. European Symp. Anaerobic Waste Water Treatment* , van den Brink, W. J., ed., Noordwijkerhout, The Netherlands, pp. 430-439.
23. Man, A. W. A. de, Last, A. R. M. van der and Lettinga, G. (1988), in *Proc. Fifth Int. Symp. Anaerobic Digestion,* Bologna, Italy, Hall, E.R. and Hobson, P. N., eds., Pergamon, Oxford, England, pp. 197-209.
24. Rebac, S., Lier, J. B. van, Janssen, M. G. J., Dekkers, F., Swinkels, K. Th. M. and Lettinga, G. (1997), *J. Chem. Tech. Biotechnol.* **68,** 135-146.
25. Rebac. S., Ruskova, J., Gerbens, S., Lier, J. B. van, Stams, A. J. M. and Lettinga, G. (1995), *J. Ferm. Bioeng.* **80** (5), 499-506.
26. Rinzema, A. (1988), PhD thesis, Landbouwuniversiteit, Wageningen, The Netherlands.
27. Vereyken, T. L. F. M., Swinkels, K. Th. M. and Hack, P. J. F. M. (1986), in *Proc. Water Treatment Conf. Anaerobic Treatment a Grown-up Technology, AQUATECH '86.* Amsterdam, The Netherlands, pp. 283-296.

3.2 HIGH-RATE ANAEROBIC TREATMENT OF MALTING WASTE WATER IN A PILOT-SCALE EGSB SYSTEM UNDER PSYCHROPHILIC CONDITIONS

ABSTRACT

The feasibility of the expanded granular sludge bed (EGSB) system for the treatment of malting waste water under psychrophilic conditions was investigated by operating a pilot-scale 225.5 dm^3 EGSB-reactor system in the temperature range from 13° to 20°C. The concentration of chemical oxygen demand (COD) in the malting waste water was between 282 - 1436 mg dm^{-3}. The anaerobically biodegradable COD of the wastewater was about 73 %, as determined in the batch bioassays. During reactor operation at 16°C, the COD removal efficiencies averaged about 56 %, at organic loading rates (OLR) ranging between 4.4 - 8.8 kg COD m^{-3} day^{-1} and a hydraulic retention time (HRT) of approximately 2.4 h. At 20°C, removal efficiencies were approximately 66% and 72 %; respectively, at OLRs of 8.8 and 14.6 kg COD m^{-3} day^{-1}, corresponding to HRTs of 2.4 and 1.5h. The specific methanogenic activity with the sludge from the reactor, assessed on acetate and VFA mixture as substrates, significantly increased (80 %) in time, indicating an enrichment of methanogens and acetogens even at the low temperatures applied. These findings are of considerable practical importance because they indicate that anaerobic treatment of low strength waste waters at low temperature might become a feasible option.

Key words: Malting wastewater, expanded granular sludge bed, psychrophilic conditions, volatile fatty acids, methanogenesis.

3.2.1 INTRODUCTION

Anaerobic treatment of industrial waste waters is a well established technology which has proven to be successful in a wide range of applications.[1-3] One of the major succeses in the development of anaerobic waste water treatment systems was the introduction of high-rate reactors in which biomass retention and liquid retention were uncoupled.[1,4] So far, anaerobic treatment technology is mostly applied at mesophilic temperatures between 20° and 40°C. However, under moderate climate conditions, many highly soluble waste waters are discharged at low ambient temperatures, e.g. those from bottling, malting and brewery industry. Because temperature strongly affects the rates of the anaerobic conversion processes, changes to the conventional design are required when applying high-rate reactor systems at 'sub-optimal' operation temperatures. Previous research has demonstrated that very efficient anaerobic treatment can be achieved at temperatures as low as 10°C, provided the waste water with the methanogenic sludge is well mixed.[5-10] Continuous flow experiments were performed by using expanded granular sludge bed (EGSB) reactors which are characterised by a high upflow velocity (4 -10 m h^{-1}), brought about by a high effluent recirculation rate. The results show that psychrophilic high-rate anaerobic treatment is

feasible despite the slow growth rate and activity of the methanogenic bacteria at low temperatures.[5] Generally at low temperatures, the amount of energy required for maintaining the reactor temperature in the mesophilic microbial optimum range (30-40°C), accounts for the largest proportion of the total energy requirement of a reactor treating cold waste waters.[11] Therefore, operation of the process at low ambient temperatures offers a significant reduction in operational costs. The feasibility of high-rate anaerobic treatment of waste water at low ambient temperature depends on various factors such as: i) the quality of the seed material used; ii) the characteristics of the waste water and, in particular, the complexity of the organic pollutants;[12] and iii) as mentioned above, a proper reactor design. This paper describes the results of a pilot-scale novel high-rate reactor system for the treatment of malting waste water under psychrophilic conditions.

3.2.2 MATERIALS AND METHODS

Pilot-scale EGSB reactor

A schematic representation of the pilot-scale EGSB reactor and the experimental set-up is presented in Fig. 3.2.1. The dimensions of the poly-acrylate reactor were an internal diameter of 0.2 m and a height of 7.5 m. The total volume was 225.5 dm^3 (internal settler included). The reactor was equipped with a commonly used gas-liquid-solid (GLS) separator in the upper part.[13] Biogas production was measured with a wet test gasmeter connected to the gas-liquid-solids separator via a water seal. The reactor was equipped with three thermocouples to measure the temperature in the reactor at a height of 0.5 m, 3.5 m and 7.0 m. The system was equipped with an external settler to collect the sludge rinsed from the reactor. The main flow was provided with a monopump (Seepex, max. flow 350 $dm^3\ h^{-1}$, Germany) pumping cooled malting wastewater (10 - 14°C). Recirculation of the effluent from the external settler was imposed to the system by another monopump (Stober, max. flow 150 $dm^3\ h^{-1}$, Germany). The main flow and the recirculation flow were combined before entering the reactor, giving a total superficial upflow velocity of about 6 m h^{-1}. Influent samples were taken after the mainflow pump, but before the connection with the recirculation flow. Effluent samples were taken from the reactor outlet. The system had two influent storage tanks (3 m^3 each) which were refilled daily. One of the tanks was connected to a household cooling system to cool the malting wastewater to 10-14 °C. The malting wastewater was continuously homogenised by a centrifugal pump (Pedrollo, max. flow 2700 $dm^3\ h^{-1}$, Italy). The basal nutrients were supplied with a peristaltic pump (Gilson - Minipuls 2, Villiers-Le-Bel, France). The buffer solution was supplied to the influent by a peristaltic pump (Watson Marlow 502 S, Falmouth, Cornwall, UK).

Biomass

The reactor was inoculated with mesophilic methanogenic granular sludge, originating from a 760 m^3 full scale UASB reactor (20 - 24°C) of the Bavaria brewery at Lieshout, The Nether-

lands. The seed sludge had been stored unfed at 4°C for about three months prior to the reactor start-up. The total amount of granular sludge inoculated was approximately 60 kg of wet sludge, or 30 g volatile suspended solids (VSS) dm^{-3} of reactor.

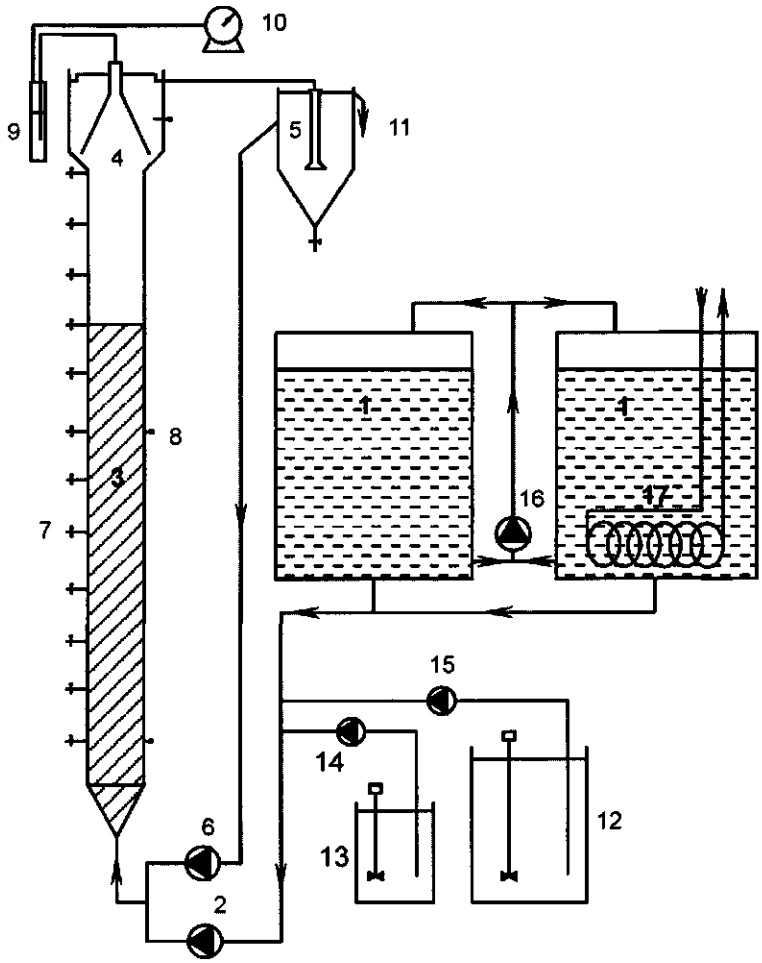

Fig. 3.2.1 Schematic diagram of the 225.5 dm^3 pilot scale EGSB system used in this study. 1, wastewater tanks ; 2, main flow pump; 3, EGSB reactor; 4, gas-liquid separator; 5, external settler; 6, recirculation pump; 7, reactor sampling points; 8, thermocouple; 9, water seal; 10, wet gas meter; 11, effluent; 12, sodium bicarbonate solution tank; 13, nutrient tank; 14, nutrients pump; 15, bicarbonate pump; 16, equalisation pump; 17, cooling device.

Malting waste water

The malting waste water originated from the batch steep process of the Bavaria B.V. malting factory, Wageningen, The Netherlands. Fresh waste water samples of 4 m^3 were brought

every second day or daily, depending on the applied hydraulic retention time of the EGSB reactor, i.e., 2.5 and 1.5 h, respectively. The waste water contained both anaerobically completely biodegradable compounds, such as different kinds of sugars, lactic acid, glycerol, ethanol and volatile fatty acids (VFA), as well as compounds which are only partially or slowly biodegraded at low temperature, such as fats, proteins, tannin, cellulose and barley grains suspended solids. The COD of the settleable and the colloidal suspended solids in the malting waste water were ranged (mg dm^{-3}) 20-231; 0-176, respectively. The waste water also contained Cl^-, Nkj, NH_4^+-N, PO_4^{3-}-P, SO_4^{2-}-S in concentration ranges of (mg dm^{-3}) 80-100; 30-50; 3-17; 6-18; 13-17, respectively.

Basal nutrients and buffer chemicals

The malting waste water was suplemented with inorganic macro- and micro-nutrients. The concentrations of basal nutrients in the concentrated stock solution were (g dm^{-3} tap water): NH_4Cl, 64.35; KH_2PO_4, 23.45; $(NH_4)_2SO_4$, 14.4; $MgCl_2 \cdot 6H_2O$, 13.03; $CaCl_2 \cdot 2H_2O$, 5.08. To each dm^3 of stock solution 0.068 dm^3 of a trace element solution was added, of which the composition is reported elsewhere.[5] From day 0 to 171, approximately 0.001 dm^3 of nutrients were supplied per dm^{-3} of waste water.

The sodium bicarbonate solution was supplied to the waste water in the range of 0.502 - 0.672 g dm^{-3} to ensure a reactor pH in the range 6.5 to 7.5.

Start-up of the reactor

Feeding of the reactor was started immediately after inoculation with the mesophilic granular sludge, at an organic loading rate (OLR) of 13 kg COD m^{-3} day^{-1} and HRT of 2.4 h. From the start of the experiment the temperature of the reactor was set at 15°C. During the continuous operation of the reactor, the influent and effluent samples were taken three times per week.

Activity assays

The specific methanogenic activity of the sludge was determined in duplicate using 0.6 dm^3 glass serum bottles sealed with a rubber septum and a screw cap under static conditions. Serum bottles were filled with 0.5 dm^3 medium and approximately 1.5 g VSS dm^{-3}. The medium consisted of approximately 4 g COD dm^{-3} of either ethanol, sodium acetate or a neutralised VFA mixture (acetate : propionate : butyrate = 1 : 1.5 : 1.8, based on COD; pH 6.5) as the substrates. The mineral composition was (g dm^{-3}): NH_4Cl, 0.28; KH_2PO_4, 0.327; $MgSO_4 \cdot 7H_2O$, 0.11; $CaCl_2 \cdot 2H_2O$, 0.010; yeast extract, 0.10; and $10^{-3} dm^3$ dm^{-3} trace element solution. The sodium bicarbonate concentration was 0.4 g dm^{-3} in the tests with acetate and VFA, and 4.0 g dm^{-3} in the test with ethanol. The mesophilic seed sludge had been stored for a period of five months at 4 °C, before it was used in the batch experiments. Sludge samples from the psychrophilic reactor were used directly. After flushing the medium with N_2 gas, serum bottles were sealed and incubated in a temperature controlled room at 20 ± 2°C. The assays were initiated with a second feeding after more than 90% of the substrate from the first

feeding was converted into methane. The CH_4 production was periodically measured by using Mariotte bottles, which were filled with a 3% NaOH solution to remove CO_2 from the biogas. The maximum specific methanogenic activity was calculated from the cumulative methane production versus time.

The specific substrate degrading activity of the sludges were performed at 20 and 15 ±2°C in duplicate using 0.3 dm^3 glass serum bottles sealed with a rubber septum and a screw cap under static conditions. Serum bottles were filled with 0.25 dm^3 medium and approximately 1.5 g volatile suspended solids (VSS) dm^{-3}. The medium and methods were the same as described above. The assays were initiated with a second feeding when more than 90% of substrate supplied in the first feeding was converted. At various periods of time, samples were taken from the bottles and analysed for acetate, VFA and ethanol. The specific substrate degrading activity was calculated from the linear decrease of the substrate concentration, which was followed until the substrate concentration dropped below 500 mg COD dm^{-3}.

Biodegradability assays

The anaerobic biodegradability of the malting waste water was assessed in 6 dm^3 batch pots. Batch pots were filled with 5 dm^3 malting waste water, 0.2 dm^3 of mineral medium and 6 g VSS dm^{-3} granular seed sludge and 1 g dm^{-3} sodium bicarbonate. The pots were stirred each 20 minutes during 5 seconds and incubated at 15 ±2°C. The CH_4 production was measured by using a gas bag which was connected to the batch pot via a column of soda lime pellets for stripping CO_2. The volume of methane in the gasbag was determined by a vacuum pump and wet gas meter. The experiment included a blank without substrate. At various time intervals, methane production was measured and samples were taken from the batch pots and analysed for total COD, soluble COD, and VFA. The results reported are corrected for the results of the blank.

Between day 114 and 117 the EGSB reactor was operated in batch-mode for 2 days in order to determine the biodegradability of the malting waste water under the reactor conditions. The temperature in the reactor was set in the range 14 - 16°C. The experiment was started by interrupting the influent flow and increasing the recirculation flow in order to maintain the upflow velocity in the reactor at 6 m h^{-1}. Before starting the experiment, a representative influent sample was taken from malting waste water entering the reactor. At various time intervals, methane production was measured and samples were taken from the settler of the reactor and analysed for total COD, soluble COD and VFA.

Size distribution of the sludge

The size distribution and settling properties of the sludge was determined with a modified sedimentation balance as described by Hulshoff Pol.[14]

Analyses

Samples for COD were analysed colorimetrically using the micromethod as described by Jirka and Carter.[15] Samples for soluble COD were obtained after filtration through a 0.45 μm pore size membrane filter "Micronsep" (MSI, Westboro, MA, USA).

VFAs were measured by gas chromatography as described elsewhere.[5] Ethanol was measured in the same gas chromatograph, which was used for VFA determination. Operating conditions were the same except that the oven temperature was 70°C.

Sulphate was measured by ion chromatography (Packing Chrompack Ionospere, USA) equipped with differential refractometer, using 0.04 mol dm^{-3} potassium biphatlate, (pH 4.2) as eluent. Biogas samples were collected in a 0.2 dm^3 glass sampler and analysed by gas chromatography as described elsewhere.[5] Hydrogen was determined in 0.001 dm^3 samples by gas chromatography with a Hewlett Packard 5890 gas chromatograph equipped with a thermal conductivity detector and molecular sieve 25H (60-80 mesh). The column size was 1.5 m x 6.4 mm. Argon was used as carrier gas at a flow rate of 0.025 dm^3 min^{-1}. Temperatures were column, 40°, injection port, 110°, and detector, 125°C.

All other analyses were determined according to standard methods.[16]

3.2.3 RESULTS AND DISCUSSION

Anaerobic biodegradability of malting waste water.

Fig. 3.2.2a shows the decrease in COD concentration and concomitant increase in methane production during the first 4 days of the biodegradability assays which were followed for a period of 18 days. The biodegradability of the malting waste water under the fed-batch EGSB reactor conditions is presented in Fig. 3.2.2b. Before the experiment started the EGSB reactor had been operated continuously for 114 days. The total duration of the fed-batch EGSB experiments was about 18 hours, however, the degradation of the malting waste water was apparently finished after 4 hours (Fig. 3.2.2b). The total COD of the malting waste water was slightly higher at 7.5 h, presumably due to a higher concentration of suspended solids in the sample. The increase in methane production after 4 hours was probably related to digestion of the biomass and/or acumulated substrate in the biomass. Adapting the sludge in the EGSB system for 114 days, significantly enhanced the biodegradation rate. However, the ultimate biodegradable fraction remained unaffected (71-73 % of the soluble COD). The biodegradability of the malting waste water varies slightly over time, depending on the composition of the malting waste water, i.e., the type of barley used, and its growth conditions prior to harvesting. VFAs were readily metabolized upon starting the tests. Also, fermentable compounds were degraded, as indicated by the steady decrease in COD_{sol}. However, the particulate COD (COD_{tot}-COD_{sol}) did not decrease in the experimental period, showing that the malting waste water is only partly anaerobically biodegradable under psychrophilic conditions.

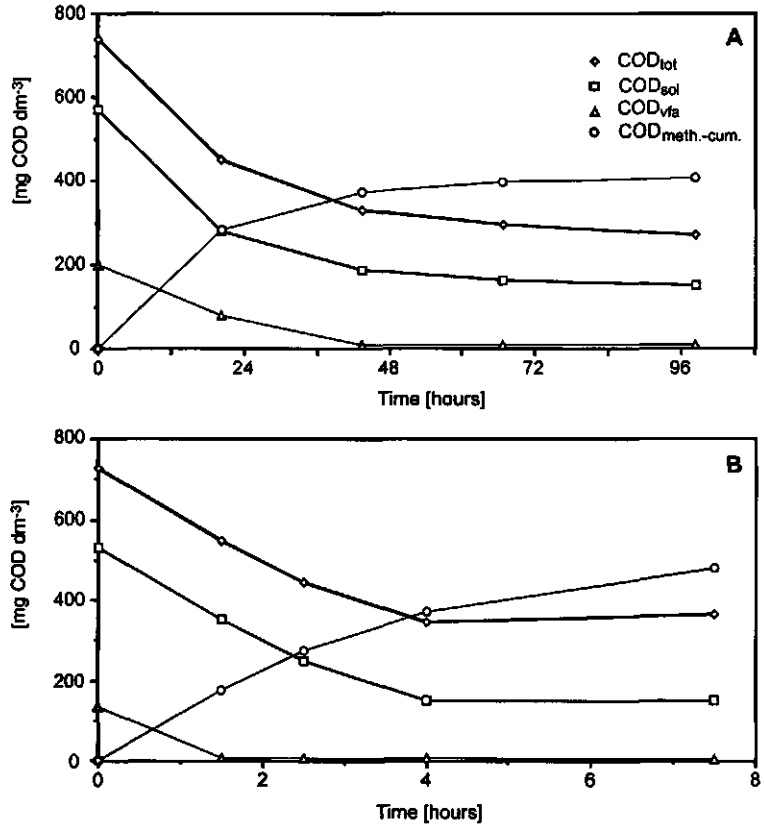

Fig. 3.2.2 Biodegradability of malting waste water. A. Batch experiments with 6 g VSS dm^{-3} of seed sludge. B. EGSB reactor in batch-mode with 30 g VSS dm^{-3} at day 115, (◊) COD_{tot}, (□) COD_{sol} (Δ) COD_{vfa}, (O) $COD_{meth-cum.}$

Reactor performance

The performance data of the EGSB system operating under psychrophilic conditions (13 to 20°C) are shown in Fig. 3.2.3 and summarized in Table 3.2.1. The first 20 days are considered as the start-up period, during which the biomass adapted to a new type of wastewater. This start-up period was characterised by strong variations in removal efficiencies (Fig. 3.2.3d), which can partly be attributed to system overloadings. In accordance to earlier recommendations, an OLR above 3 kg COD m^{-3} day^{-1} was found to be too high for a psychrophilic start-up (Fig. 3.2.3b).[13]

After this start-up period, the efficiency of the system at a HRT of 2.4 h (period II) gradually increased reaching values approaching the biodegradability of the waste water COD obtained in the batch test (Fig. 3.2.2a). The EGSB system displayed a remarkably stable performance despite the strong variations in OLR, ranging from 4.4 to 8.8 kg COD m^{-3} day^{-1}. However, operating the EGSB system at 13°C and a HRT of 1.5 h led to a drop in the treatment efficiencies in period III. It should be noted that prior to period III, the system was unfed for

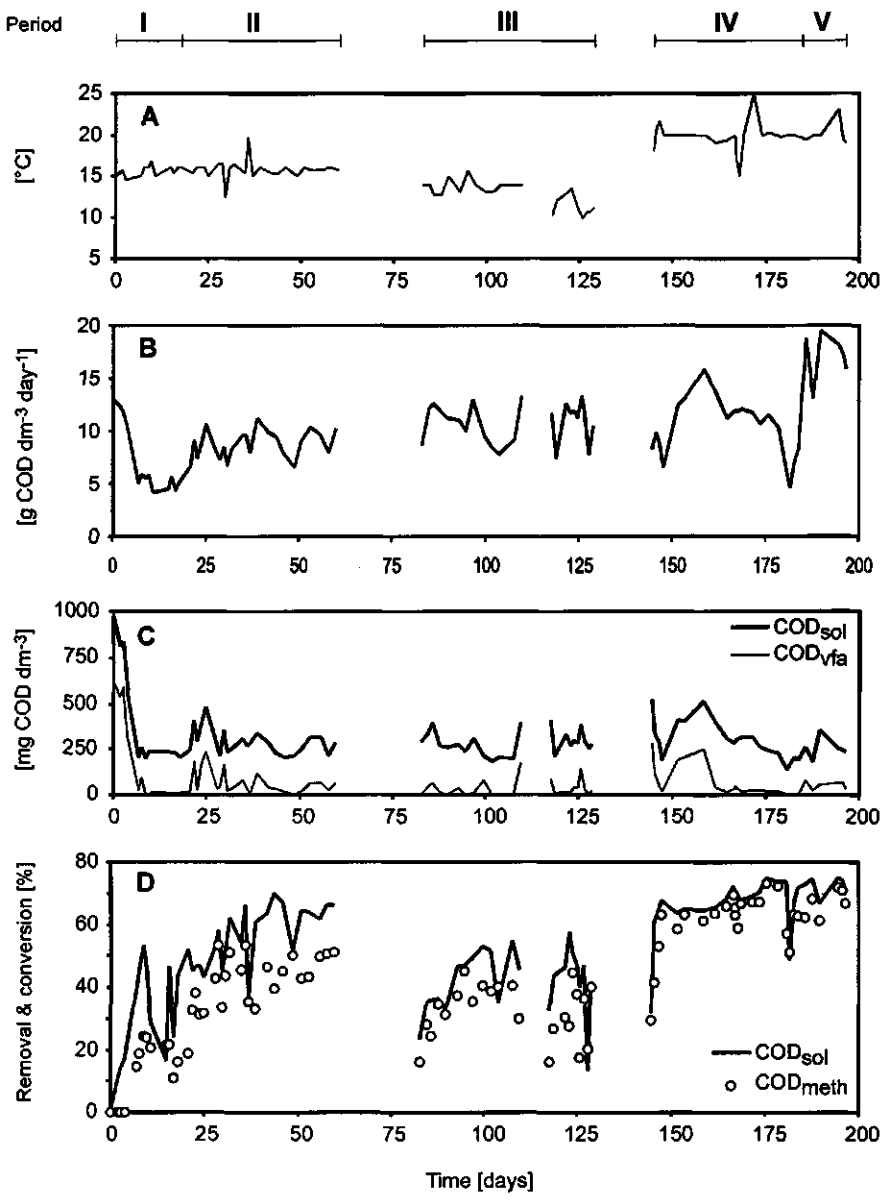

Fig. 3.2.3 Operational conditions and performance of the EGSB system. A. Temperature. B. Organic loading rate based on total COD. C. Effluent COD concentrations, (—) COD_{sol}, (—) COD_{vfa}. D. Removal efficiency, (—) COD_{sol} , Conversion to (O) COD_{meth}.

three weeks. Also, the VFA level in the waste water was extremely low (2 - 287 mg COD dm^{-3}) in period III, depriving the acetogenic and methanogenic subpopulations of substrate. In addition to the influence of changes in waste water characteristics, the fraction of slowly

Table 3.2.1 Performance data of the pilot-scale EGSB reactor treating maltery waste water. Average values are presented in parentheses.

Period		OLR_{sol}	HRT	Temp.	$COD_{sol,in}$	$COD_{sol,out}$	VFA_{in}	VFA_{out}	COD removal	VFA removal
[day]		[kg COD m^{-3} day^{-1}]	[h]	[°C]	[mg COD dm^{-3}]	[mg COD dm^{-3}]	[mg COD dm^{-3}]	[mg COD dm^{-3}]	[%]	[%]
I	0-20	3.0-10.6 (5.8)	2.4	(16)	282- 986 (555)	205-980 (433)	8-643 (255)	3-619 (202)	0-53 (28)	-26-99 (42)
II	21-60	4.4-8.8 (6.8)	2.4	(16)	436- 875 (672)	205-480 (288)	123-411 (298)	0-234 (64)	36-70 (56)	48-100 (80)
III	85-129	5.1-11.7 (8.2)	1.5	(13)	314- 729 (509)	183-411 (286)	2-287 (177)	0-173 (37)	33-55 (43)	38-100 (83)
IV	145-184	3.2-14.1 (8.8)	2.4	(20)	588-1436 (895)	133-514 (300)	212-574 (430)	0-282 (63)	49-75 (66)	62-100 (85)
V	186-197	11.2-16.8 (14.6)	1.5	(20)	715-1064 (915)	181-352 (254)	348-579 (465)	0- 79 (53)	67-75 (72)	84- 93 (89)

biodegradable organic matter probably accumulated at a higher rate at the operation temperature of 13°C when the reactor was loaded with about 8.2 kg COD m^{-3} day^{-1}. Indeed, increasing the temperature from 13° to 20°C had a significant positive effect on the achievable treatment capacity of the system (period IV and V, Fig. 3d) despite an unfed period from day 130 to day 144. The pilot-scale EGSB system maintained COD removal efficiencies of about 70%, accommodating organic loading rates up to 16.8 kg COD m^{-3} day^{-1} and a HRT of 2.4 - 1.5 h. Apparently, an expanded granular sludge bed provides good conditions for a very efficient treatment of low strength complex waste water at ambient temperatures.

Stability of the psychrophilic reactor

The sensitivity of the system to sudden changes in temperature was investigated at days 29, 35, 170 and 172. A decrease in temperature from 15° to 12°C at day 29, resulted in a drop in the COD removal efficiency by 15%. In contrast, methanogenesis was not hampered when the temperature was decreased from 20° to 15°C at day 170. The system showed a slight increase in efficiency when the temperature was increased from 15° to 20° and from 20° to 25°C at day 35 and 172, respectively.

Throughout the continuous flow experiment, a sharp drop in treatment efficiency was observed when fresh malting waste water was utilised which was characterised by an extremely low VFA content (< 150 mg COD dm^{-3}). On the basis of Monod kinetics, low treatment efficiencies can be expected if the concentration of readily biodegradable compounds (including VFA) are low in the malting waste water. This hypothesis is supported by the observation that a 2 times dilution of malting waste water on day 181 and 182, resulted in a sharp drop in the COD removal efficiencies (Fig. 3.2.3d). Obviously, the COD removal efficiencies drop when the system operates below its K_s value.[5,10] In contrast, the system was characterized by high treatment efficiencies if VFA concentrations were higher than 300 - 400 mg COD dm^{-3}.

The results presented in Fig. 3.2.4 show that in the expanded sludge bed a more-or-less sequenced degradation of the partially acidified waste water occurs. The VFA concentration profile over the reactor height at day 60 (Fig. 3.2.4), indicates that acidification of the fermentable content in malting waste water takes place predominantly in the lower region of the expanded sludge bed (<1m). Nonetheless, the hydraulic mixing conditions in the reactor are adequate, and substrate is available over the entire expanded sludge bed. At day 60 a recirculation ratio of 1:1 was applied.

Hydrogen concentrations in biogas in periods I to V were ranging between (ppm) 302-626; 105-652; 102-750; 85-220; 153-355, respectively. A lower COD removal efficiency was concomitant with high H_2 concentrations in the biogas. An imbalance between the acidification rate and methanogenesis in the reactor might have led to higher H_2 concentrations, which subsequently could have influenced the anaerobic conversion of VFA.[18]

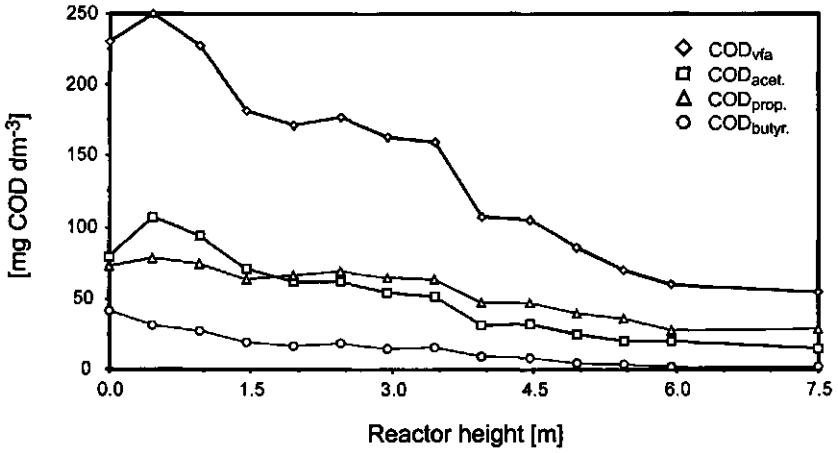

Fig. 3.2.4 Volatile fatty acids (VFA) profile over the reactor height at day 60, (◊)COD_{vfa} , (□) $COD_{acet.}$, (Δ) $COD_{prop.}$, (O) $COD_{butyr.}$.

To assess the necessity of adding nutrients, the supply of nutrients and trace elements to the system was stopped on day 173 till the end of the experiments. No effects on the methane production content of biogas and/or the treatment efficiencies were observed. Analysis of nutrients in the malting wastewater showed that sufficient nitrogen, phosphorus and sulphur were present in the waste water and thus, their addition was not necessary.

The hold-up of any type of granular sludge is controlled by the liquid upflow velocity.[10] The fraction which is rinsed first from the system contains the particles with the worst settling properties. During the experiments, the active biomass which washed out from the EGSB reactor was collected in an external settler. The total amount of granular sludge and fines washed out from the reactor were 795, 286 and 644 g VSS or 6, 2 and 5 mg VSS dm^{-3} of effluent in period, day 0-60, 85-129 and 145-197, respectively. Because the net biomass yield in the reactor was much higher than the lost fraction, the system performance remained stable. Despite the satisfactory biomass hold-up we had some concern regarding the loss of granular sludge. The 225 dm^3 EGSB reactor was equipped with the commonly used 'reversed funnel' design. However, recent experiments with a more advanced GLS design equipped with a drum screen show significant improvements regarding the retention of active granular sludge (S. Rebac, unpublished data).

Metabolic characteristics of the sludge

The maximum specific degrading activities at 15° and 20°C, with ethanol, acetate, and the VFA mixture as the substrate, are presented in Table 3.2.2 and Table 3.2.3, respectively. Tests were performed with the seed sludge, the bottom sludge and the top sludge from the expanded sludge bed. The maximum specific methanogenic activities of the same sludge samples at 20°C are presented in Table 3.2.4. The specific acetate and VFA degrading activities at 20°C, measured on day 60 and day 129, were about 85-95% and 65-85% higher,

Table 3.2.2 Maximum specific substrate degrading activities at 15°C of the inoculum, and the EGSB sludge. Standard deviation is presented in parentheses.

MAXIMUM SPECIFIC SUBSTRATE DEGRADING ACTIVITY [g COD g^{-1} VSS day^{-1}]						
Time	Ethanol♣		Acetate		VFA - mixture	
	1st feeding	2nd feeding	1st feeding	2nd feeding	1st feeding	2nd feeding
day 0*	0.397 (0.013)	0.537 (0.011)	0.064 (0.010)	0.051 (0.004)	0.108 (0.003)	0.163 (0.002)
Bottom sludge						
day 60	0.504 (0.032)	0.819 (0.030)	0.140 (0.007)	0.150 (0.000)	0.168 (0.007)	0.208 (0.005)
day 129	0.803 (0.019)	0.859 (0.005)	0.148 (0.001)	0.146 (0.001)	0.188 (0.005)	0.250 (0.002)
Top sludge						
day 60	0.435 (0.066)	0.744 (0.033)	0.172 (0.014)	0.158 (0.009)	0.227 (0.001)	0.282 (0.000)
day 129	0.395 (0.011)	0.642 (0.012)	0.219 (0.001)	0.163 (0.008)	0.258 (0.004)	0.310 (0.002)

♣ Ethanol acidifying activity

* Seed sludge after 5.5 months of storage

Table 3.2.3 Maximum specific substrate degrading activities at 20°C of the inoculum, and the EGSB sludge. Standard deviation is presented in parentheses.

MAXIMUM SPECIFIC SUBSTRATE DEGRADING ACTIVITY [g COD g^{-1} VSS day^{-1}]						
Time	Ethanol[♣]		Acetate		VFA - mixture	
	1st feeding	2nd feeding	1st feeding	2nd feeding	1st feeding	2nd feeding
day 0[*]	0.592 (0.011)	1.201 (0.006)	0.129 (0.012)	0.117 (0.004)	0.210 (0.005)	0.265 (0.005)
Bottom sludge						
day 60	1.009 (0.000)	1.473 (0.096)	0.268 (0.001)	0.263 (0.001)	0.276 (0.003)	0.351 (0.009)
day 129	1.294 (0.085)	1.693 (0.127)	0.287 (0.000)	0.248 (0.006)	0.348 (0.006)	0.437 (0.008)
day 197	1.022 (0.033)	1.321 (0.032)	0.374 (0.000)	0.488 (0.007)	0.449 (0.008)	0.585 (0.029)
Top sludge						
day 60	0.863 (0.010)	1.259 (0.016)	0.299 (0.020)	0.268 (0.022)	0.337 (0.003)	0.453 (0.023)
day 129	0.877 (0.003)	1.200 (0.028)	0.410 (0.021)	0.355 (0.004)	0.449 (0.009)	0.526 (0.036)
day 197	1.064 (0.003)	1.321 (0.003)	0.429 (0.005)	0.550 (0.003)	0.508 (0.007)	0.628 (0.027)

[♣] Ethanol acidifying activity
[*] Seed sludge after 5.5 months of storage

Table 3.2.4 Maximum specific methanogenic activities at 20°C of the inoculum, and the EGSB sludge. Standard deviation is presented in parentheses.

MAXIMUM SPECIFIC METHANOGENIC ACTIVITY [g CH_4 -COD g^{-1} VSS day^{-1}]						
Time	Ethanol		Acetate		VFA - mixture	
	1st feeding	2nd feeding	1st feeding	2nd feeding	1st feeding	2nd feeding
day 0*	0.288 (0.038)	0.526 (0.028)	0.142 (0.008)	0.138 (0.004)	0.170 (0.012)	0.246 (0.002)
Bottom sludge						
day 60	0.346 (0.014)	0.526 (0.008)	0.256 (0.010)	0.228 (0.008)	0.278 (0.010)	0.344 (0.006)
day 129	0.388 (0.022)	0.438 (0.006)	0.266 (0.040)	0.310 (0.024)	0.316 (0.000)	0.390 (0.026)
day 197	0.446 (0.136)	0.452 (0.086)	0.316 (0.034)	0.346 (0.034)	0.354 (0.002)	0.564 (0.030)
Top sludge						
day 60	0.654 (0.006)	0.852 (0.010)	0.300 (0.016)	0.290 (0.010)	0.334 (0.004)	0.458 (0.000)
day 129	0.772 (0.024)	0.872 (0.020)	0.388 (0.002)	0.362 (0.000)	0.446 (0.000)	0.542 (0.002)
day 197	0.482 (0.014)	0.700 (0.046)	0.456 (0.018)	0.480 (0.024)	0.474 (0.008)	0.620 (0.016)

* Seed sludge after 5.5 months of storage

respectively, compared to the activities measured at 15 °C. In some cases the specific acetate conversion rates and methanogenic activities, measured during the second feeding, are lower compared to the first feeding. This might be attributed to an unintended increase in pH value to 8 during the second feeding. A slight decline in the ethanol acidifying activity of the sludge at the bottom of reactor on day 197, compared to the activity at day 129, might be due to higher degree of preacidification of the malting waste water before entering the reactor in periods IV and V.

Tables 3.2.2-3.2.4 clearly illustrate that the specific substrate degrading activities and methanogenic activities of the sludge samples from the reactor had increased in time, indicating an enrichment of methanogens and acetogens, even at the low temperatures applied in this study. Specific substrate degrading and methanogenic activities of the sludge from the reactor at the end of experiment, had almost doubled compared to the seed sludge. The higher activities found during the second feeding with VFA mixture (Table 3.2.3 and 3.2.4) indicate that the maximum activities may even be higher. The increase in activity during the second feeding should be attributed to sludge adaptation to batch conditions rather than to biomass growth during the first feeding. The amount of newly grown biomass was calculated to be less than 5 % of the initial amount, assuming a sludge yield of 0.02 (g VSS g^{-1} removed COD).

The microbial populations and intermediate product formation in the granular sludge is influenced by the organic and inorganic pollutants present in the waste water.[17,18] Batch activity tests showed that the conversion pathway of ethanol changed considerably during reactor operation. In the seed sludge the conversion of ethanol mainly yielded an accumulation of acetate as an intermediate (Fig. 3.2.5a). In contrast, propionate accumulated up to 27 % and 38 % of the initial ethanol COD value, using the sludge from the bottom at day 60 (Fig. 3.2.5b) and 197 (Fig. 3.2.5c), respectively. The same shift in intermediate product formation was observed in the top sludge, however, here, propionate formation was retarded until the experimental period, day 145 - 197 (results not shown). Methanogenic activity with sludge from the top of the reactor fed with ethanol was up to 80 % higher in comparison with the sludge from the bottom, except on day 197 when the methanogenic activity of the sludge from the top was significantly lower (Table 3.2.4). The change in the ethanol conversion pathway might be attributed to the continuous presence of a small amount of sulphate in the waste water (50 mg SO_4^{2-} dm^{-3}) in the malting waste water, and 10 - 12 mg SO_4^{2-} dm^{-3} through the macronutrients supply). This sulphate concentration in the low strength malting waste water (500 - 900 mg COD dm^{-3}), yielded a SO_4^{2-}/ COD ratio of 0.07 to 0.11. Although the sulphate/ COD ratio was too low to significantly alter the methane production rate, it may have supported the development of sulphate reducers[19,20,21] in the granular sludge, stimulating the degradation of propionate under psychrophilic conditions.[22] The pathway involved in propionate formation during anaerobic ethanol degradation in the presence of extremly low sulphate concentrations, might be attributed to the presence of *Desulfobulbus propionicus*[23,24] in the granular sludge.

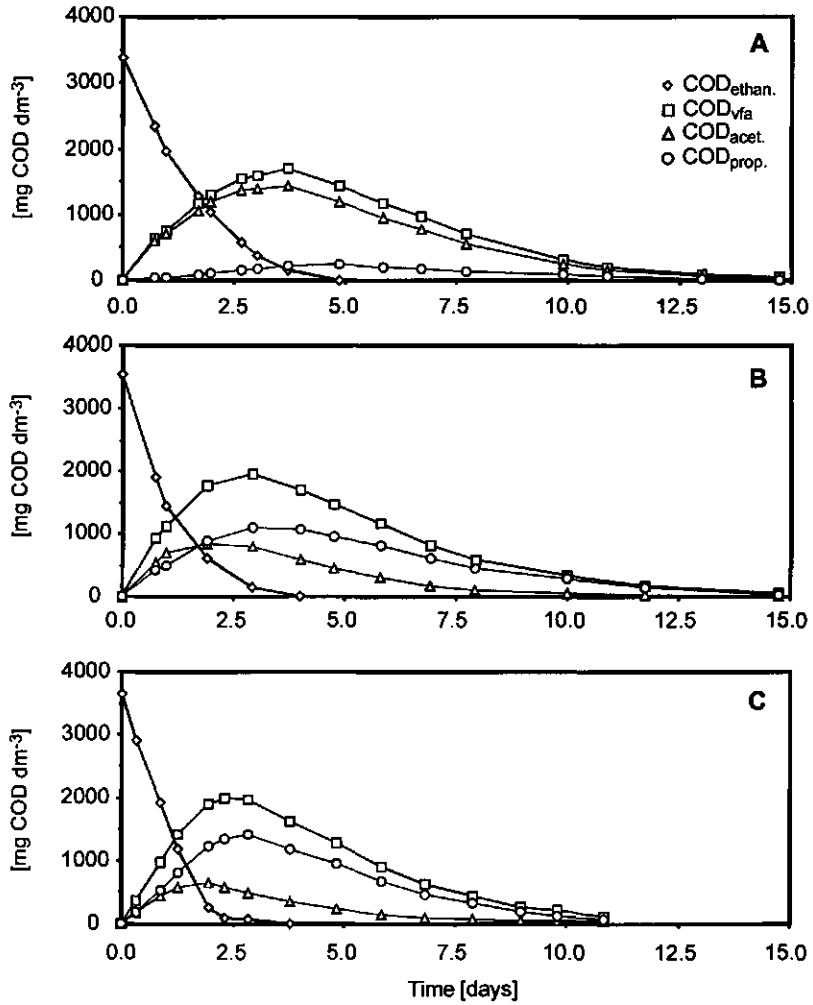

Fig. 3.2.5 Degradation of ethanol in batch experiments. A. The seed sludge. B. The sludge from the bottom at day 60. C. The sludge from the bottom at day 197. (◊) COD_{eth}, (□) $COD_{vfa.}$, (Δ) $COD_{acet..}$, (O) $COD_{prop.}$.

Physico-chemical characteristics of the sludge

The development of the granular sludge in the EGSB reactor under psychrophilic conditions is depicted in Fig. 3.2.6. During the first 129 days of operation, the diameter of the granules increased slightly. Fig. 3.2.6b shows that at day 129, the fractions of granules with a diameter between 0.9 and 2.7 mm is about equal. This probably can be attributed to erosion of the seed granules and wash out of the small particles. The sludge diameter had increased significantly at day 197 (Fig. 3.2.6c). The growth of granules is congruent with the enrichment of

methanogenic, acetogenic and acidogenic populations in the granules, and confirms lab-scale EGSB reactor studies.[5]

The up-flow velocity applied in the EGSB reactor enhances segregation by gravitational forces based on the size and the settling properties of granules.[5,25] The size distribution of the sludge from the top and the bottom of the expanded granular sludge bed is presented in Fig. 3.2.7.

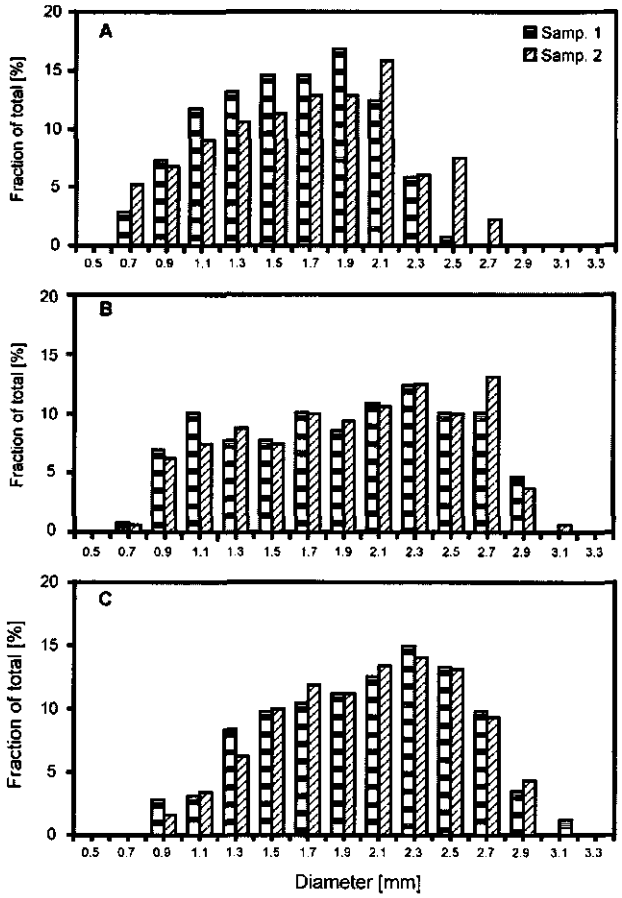

Fig. 3.2.6 Size distribution of mixed granular sludge at the start of the experiment (A) and at day 129 (B) and 197 (C), expressed in percentage of the biomass weight represented by the granules. Duplicate samples are presented by bars.

The results clearly confirm segregation of granules in the sludge bed based on the diameter size. The specific substrate degrading and methanogenic activities of the small sized top sludge, were about 11 - 40 % and 20 - 45 % higher, for acetate and VFA mixture, respectively

(Tables 3.2.2, 3.2.3, 3.2.4). Apparently, the fraction of methanogens and acetogens were much higher in the small sized granules than in the large granules present at the bottom of the

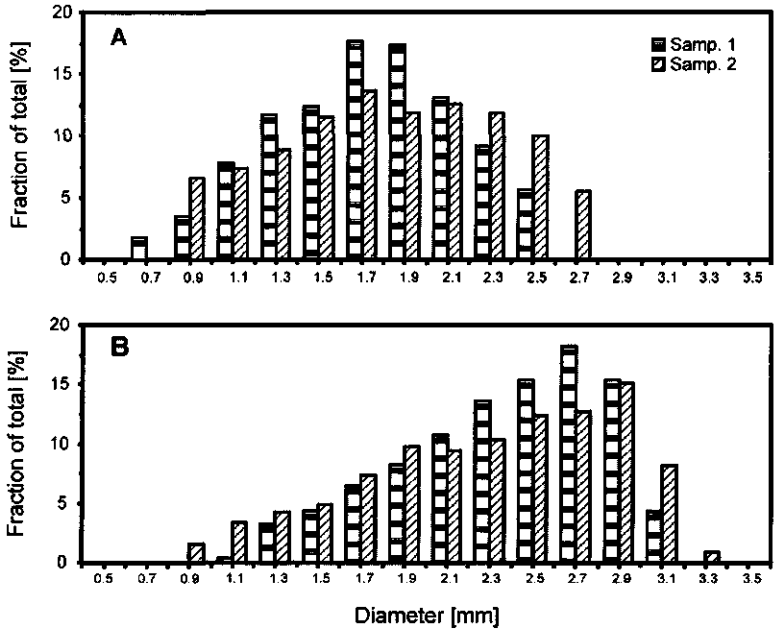

Fig. 3.2.7 Size distribution of granular sludge from the top (A) and the bottom (B) of the expanded granular sludge bed at day 197, expressed in percentage of the biomass weight represented by the granules. Duplicate samples are presented by bars.

reactor. In addition, the prevailing environmental conditions and selection pressure in the EGSB reactor, may have resulted in an increased density of the sludge granules, probably corresponding to a decreased porosity.[26] Consequently the bigger granules that segregated to the bottom, were characterised by a lower activity due to the possible occurrence of substrate diffusion limitations.[27,28,29]

3.2.4 CONCLUSIONS

The maximum conversion rate of the methanogenic sludge increased significantly in time, despite the fact that the reactor was operated under psychrophilic conditions (15 °C). The newly grown viable methanogenic biomass is apparently sufficiently well retained in the EGSB-pilot reactor investigated. The results also show that the sludge-water contact accomplished in the EGSB system was quite satisfactory. By meeting these two conditions, anaerobic treatment of low strength cold waste waters in principle looks a quite feasible option. Moreover, the investigated EGSB system was characterized by a remarkable long term stable high-rate performance. Our results are particularly encouraging for full scale application of anaerobic treatment of low strength waste water under psychrophilic

conditions. By applying the EGSB systems, low temperatures are not any longer a limitation for 'high-rate' anaerobic waste water treatment. Further improvement in the performance very likely can be achieved by staging the EGSB reactor and by developing sophisticated GLS separator which retain practically all viable sludge. This will be a point for future research.

3.2.5 ACKNOWLEDGEMENTS

We would like to thank J. van der Laan and I. Bennehey for their technical assistance with the chromatographs and Jim Field for his critical remarks on the first draft of the paper.

3.2.6 NOTATION

A_{max} = maximum specific activity [g COD g^{-1} VSS day^{-1}]
COD = chemical oxygen demand [g O_2 dm^{-3}]
COD_{tot} = total chemical oxygen demand [g O_2 dm^{-3}]
COD_{sol} = soluble chemical oxygen demand [g O_2 dm^{-3}]
COD_{vfa} = VFA chemical oxygen demand [g O_2 dm^{-3}]
COD_{meth} = methane chemical oxygen demand [g O_2 dm^{-3}]
$COD_{meth\text{-}cum}$ = cumulative methane chemical oxygen demand [g O_2 dm^{-3}]
COD_{acet} = acetate chemical oxygen demand [g O_2 dm^{-3}]
COD_{prop} = propionate chemical oxygen demand [g O_2 dm^{-3}]
COD_{butyr} = butyrate chemical oxygen demand [g O_2 dm^{-3}]
EGSB = expanded granular sludge bed
HRT = hydraulic retention time (hours)
GLSS = gas liquid solid separator
OLR = organic loading rate [kg COD m^{-3} day^{-1}]
t = experimental time [days]
UASB = upflow anaerobic sludge bed
v = upflow velocity [m h^{-1}]
VFA = volatile fatty acids
VSS = volatile suspended solids [g dm^{-3}]

3.2.7 REFERENCES

1. Lettinga, G., Anaerobic digestion and wastewater treatment systems. Antonie van Leeuwenhoek. **67** (1995) 3-28.
2. Nyns, E. J., A guide to successful industrial implementation of biomethanisation technologies in the European Union, Successes and Guidelines. Report prepared for

the European Commission, Directorated general for energy, Thermie programme, DG XVII. (1994).

3. Wheatley, A., Anaerobic digestion: a waste treatment technology. Elsevier Applied Science, London, UK. (1990).
4. Iza, J., Colleran, E., Paris, J. M. & Wu, W.-M., International workshop on anaerobic treatment technology for municipal and industrial wastewaters: Summary paper. Wat. Sci. Tech., Vol **24**, No. **8**, (1991) 1-16.
5. Rebac, S., Ruskova, J., Gerbens, S., van Lier, J.B., Stams, A.J.M. & Lettinga,G., High-rate anaerobic treatment of wastewater under psychrophilic conditions. *J. Ferment. Bioeng.*, Vol. 80, **5**, (1995) 499-506.
6. Lettinga, G., Hulshoff Pol, L. W., Hobma S. W., Grin, P., Jong, P. de, Roersma, R. & IJspeert, P., The use of a floating settling granular sludge bed reactor in anaerobic treatment. In *Proceedings of the European Symposium on Anaerobic Waste Water Treatment*, ed. W.J. van den Brink, Noordwijkerhout, The Netherlands, 1983, pp. 411-429.
7. Man, A. W. A. de, van der Last, A. R. M. & Lettinga, G., The use of EGSB and UASB anaerobic systems for low strength soluble and complex wastewaters at temperatures ranging from 8 to 30°C. In *Proceeding of the Fifth International Symposium on Anaerobic Digestion.* ed. E.R. Hall and P.N. Hobson, Bologna, Italy, 1988, pp. 197-209.
8. Frankin, R. J., Koevoets, A. A., Gils, W. M. A. van & Pas, A. van der, Application of the BIOBED upflow fluidized bed process for anaerobic wastewater treatment. *Wat. Sci. Tech.*, **25**, (1992) 373-382.
9. Rinzema, A., Veen, H. van & Lettinga, G.: Anaerobic digestion of triglyceride emulsions in expanded granular sludge bed upflow reactors with modified sludge separators. *Environ. Technology*, **14**, (1993) 423-432.
10. Kato, T. M., Field, J. A., Versteeg, P. & Lettinga, G., Feasibility of Expanded Granular Sludge Bed Reactors for the anaerobic treatment of low strength soluble wastewaters. *Biotech. Bioengin.*, **44**, (1994) 469-479.
11. Mills, P. J., Minimisation of energy input requirements of an anaerobic digestor. *Agricult. Wastes.*, **1**, (1979) 57-66.
12. Koster, I.W. & Lettinga, G.: Application of the upflow anaerobic sludge bed (UASB) process for treatment of complex wastewaters at low temperatures. *Biotechnol. Bioengin.*, **27**, (1985) 1411-1417.
13. Lettinga, G. & Hulshoff Pol, L. W., UASB-process design for various types of wastewaters. *Wat. Sci. Tech.*, **24**, (1991) 87-107.
14. Hulshoff Pol, L. W., van de Worp, J. J. M., Lettinga, G. & Beverloo, W. A., Physical characterization of anaerobic granular sludge. In *Proceeding of International Conference Anaerobic Treatment A Grown-up Technology*, Amsterdam, The Netherlands, 1986, pp 89-101.
15. Jirka, A. M. & Carter, M. J., Micro semi-automated analysis of surface and waste waters for chemical oxygen demand. Analytical Chemistry, Vol. 47, **8**,(1975) 1397-1402.

16. American Public Health Association: Standard methods for the examination of water and wastewaters. 16th edition. Washington DC, USA, (1985).
17. Stams, A. J. M., Metabolic interactions between anaerobic bacteria in methanogenic environments. Antonie van Leeuwenhoek. **66** (1994) 271-294.
18. Gujer, W. & Zehnder, A. J. B., Conversion processes in anaerobic digestion. Wat. Sci. Tech., Vol. **15**, (1983) 127-167.
19. Visser A., Beeksma I., van der Zee F., Stams A.J.M. & Lettinga G., Anaerobic degradation of volatile fatty acids at different sulfate concentrations. *Appl. Microbiol. Biotechnol.*, **40**, (1993) 549-556.
20. Harada H., Uemura S. and Momonoi K., Interaction between sulphate-reducing bacteria and methane-producing bacteria in UASB reactors fed with low stength wastes containing different levels of sulfate. *Wat. Res.*, **28**, (1994) 355-367.
21. Oude Elferink S.J.W.H., Visser A., Hulshoff Pol L.W. & Stams A.J.M., Sulfate reduction in methanogenic bioreactors. *FEMS Microbiol. Reviews*, **15**, (1994) 119-136.
22. Rebac, S., Visser, A., Gerbens, S., van Lier, J.B., Stams, A.J.M. & Lettinga, G., The effect of sulphate on propionate and butyrate degradation in a psycrophilic anaerobic expanded granular sludge bed (EGSB) reactor. Environ. Technol., Vol **17**, (1996) 997-1005.
23. Stams, A.J.M., Kremer, D.R., Nicolay, K., Weenk, G.H. & Hansen , T.A., Pathway of propionate formation in *Desulfobulbus propionicus*. Arch. Microbiol., **139**, (1984) 167-173.
24. Wu, W-M., Hickey, R. F. & Zeikus, J.G., Characterization of metabolic performance of methanogenic graniles treating brewery wastewater: Role of sulfate-reducing bacteria. Appl. Environ. Microbiol., **57**, (1991) 3438-3449.
25. Guiot, S. R., Arcand, Y. & Chavarie, C., Advantages of fluidization on granule size and activity development in upflow anaerobic sludge bed reactors. *Wat. Sci. Tech.*, **26**, (1992) 897-906.
26. Alphenaar, A., Pérez, C. M. & Lettinga, G., The influence of substrate transport limitation on porosity and methanogenic activity of anaerobic sludge granules. *Appl. Microbiol. Biotechnol.*, **39**, (1993) 276-280.
27. Tramper, J., van Groenestijn, J.W., Luyben, K.Ch.A.M. & Hulshoff Pol, L.W, Some physical and kinetic properties of granular anaerobic sludge.. In: E.H. Houwink and R. R. van der Meer (eds.) Elsevier, Amsterdam, The Netherlands, 1984, pp. 145-155
28. Dolfing, J., Kinetics of methane formation by granular sludge at low substrate concentrations. The influence of mass transfer limitation. Appl. Microbiol. Biotechnol., **22**, (1985) 77-81.
29. Wijffels, R. H., Englund, G., Hunik, J. H., Leenen, E. J. T. M., Bakketun, Á., Obón de Castro, J. M. & Tramper, J., Effects of limitation on immobilized nitrifying microorganisms at low temperatures. Biotechnol. Bioeng., **45**: (1995) 1-9.

Chapter 4

Optimization of psychrophilic anaerobic wastewater treatment systems

Published as \ submited as:

4.1 Lettinga, G., Rebac, S., Parshina, S., Nozevnikova, A., Van Lier, J. B., Stams, A. J. M. (1998) Staged high-rate anaerobic treatment of organic wastewater at psychrophilic conditions. *Appl. Environ. Microbiol.*, (submitted).

4.2 Van Lier, J. B., Rebac, S., Lens, P., Van Bijnen, F., Oude Elferink, S. J. W., Stams, A. J. M. and Lettinga, G. (1997) Anaerobic treatment of partly acidified wastewater in a two-stage expanded granular sludge bed (EGSB) system at 8 °C. *Wat. Sci. Tech.* **36**:317-324.

4.1 STAGED HIGH RATE ANAEROBIC TREATMENT AT PSYCHROPHILIC CONDITION

ABSTRACT

High-rate anaerobic treatment of a volatile fatty acid mixture (VFA) was investigated under psychrophilic (3-8 °C) conditions at the laboratory scale using two expanded granular sludge bed (EGSB) reactor modules in series. The reactor system was seeded with mesophilic methanogenic granular sludge and fed with a mixture of VFA having a chemical oxygen demand (COD) of 0.5-0.9 g l^{-1}. The COD removal efficiencies exceeded 90 % at 8 °C and 4 °C, at organic loading rates of 12 and 5 g COD l^{-1} d^{-1}, respectively. Even at 3 °C, COD removal efficiencies averaged 80 %. High rate propionate oxidation was for the first time successfully achieved at such low temperatures. The temperature optima (30-40 °C) of biomass cultivated for 1.5 years under psychrophilic conditions indicated that the dominant populations were still mesophilic. The specific VFA degrading activities of the sludge present in each module doubled during system operation for 150 days indicating a good enrichment of methanogens and acetogenic bacteria at low temperatures. The most abundant methanogenic populations observed by electronic microscopy in the psychrophilically cultivated biomass were acetate consuming *Methanosaeta* sp. and the hydrogenotrophic *Methanospirillum* sp. organisms. These findings represent a completely new insight of the high capability of anaerobic bioreactors for direct, high-rate wastewater treatment at extremely low temperatures.

Key words: Expanded granular sludge bed reactor system, two stage, psychrophilic, volatile fatty acids, methanogenesis, *Methanosaeta* sp., *Methanospirillum* sp., propionate oxidizing bacteria.

4.1.1 INTRODUCTION

Anaerobic treatment of industrial wastewaters is presently considered as a well-established technology, which has been proven for in a wide range of applications (23). However, so far, practically all full-scale applications of anaerobic treatment are restricted to wastewaters with a temperature exceeding 18°C. Under moderate climate conditions, many low strength wastewaters, including domestic and industrial wastewaters, are discharged at low ambient temperatures. Besides low concentrations of organic matter, typically 0.3–1.0 g chemical oxygen demand (COD) per l, these wastewaters usually contain a high dissolved oxygen concentration, sometimes even up to 10 mg O_2 l^{-1}. Results obtained so far have not been encouraging for anaerobic treatment for low strength wastewaters under psychrophilic conditions (6, 11, 12, 19). Because temperature strongly affects the rates of the anaerobic conversion processes, some essential improvements have to be made in the design of the conventional high-rate reactors in order to enable their application under 'sub-optimal'

temperatures and for very low strength wastewaters. A successful application of psychrophilic anaerobic treatment undoubtedly would be of big economical importance, because it presents the use of a significant amount of energy to increase the temperature of cold wastewater to the more optimal mesophilic range (30-40 °C). The feasibility of high-rate anaerobic reactor systems for cold wastewaters depends primarily on: i) the quality of the seed sludge in the reactors used and its development under psychrophilic conditions, ii) the nature of the organic pollutants in the wastewater, and iii) the reactor configuration, especially its capacity to retain viable sludge. Single or multi-compartment (staged) granular sludge reactors can be applied for psychrophilic anaerobic wastewater treatment. For accomplishing the highest possible overall treatment efficiency, especially in case of multi-component wastewaters, staged reactors offer significantly better potentials than single compartment reactors. In these reactor types, the sludge developing and retained in the separate compartments with specializes for substrates and intermediates produced in the different stages of the degradation process.

To date, only two psychrophilic marine methanogens and a few psychrophilic and psychrotrophic acetogenic bacteria (homoacetogens) from natural sediments have been isolated (4, 10, 17). The prevalence and interactions of methanogens and/or acetogens in anaerobic wastewater treatment reactor systems under psychrophilic conditions have not yet been reported. Mainly natural ecosystems as tundra soil, pond sediments (9, 14) and sediments of deep lakes (13) have been investigated for methanogenesis at extremely low temperatures. One of the key interactions in anaerobic digestion operations is the degradation of propionate, because it only proceeds in well-balanced anaerobic microbial systems. The proper syntrophic associations between propionate oxidizing bacteria and hydrogenotrophic methanogens are required (18). These bacterial partners operate in an overall reaction process which is endergonic but becomes exergonic for the first partner only though maintenance of a low interspecies hydrogen partial pressure by the second partner.

So far, it is not clear if a high-rate psychrophilic anaerobic wastewater treatment system really would require the development of psychrophilic or psychro-tolerant sub-populations, or to what extent mesophilic sludges are or can become sufficiently psychro-tolerant.

The present paper describes a novel approach for the start-up and the operation of an anaerobic high-rate staged expanded granular sludge bed (EGSB) system at 3-8 °C, treating low strength soluble wastewaters (0.5- 0.9 g COD l^{-1}), containing 12 mg O_2 l^{-1}. The paper also presents results of the temperature dependence, dynamics and metabolic routes of propionate and hydrogen degradation at 5 °C.

4.1.2 MATERIALS AND METHODS

Experimental conditions. Experiments were performed with two stage EGSB system, consisting of two 0.05 m diameter glass EGSB reactors operated in series (Fig. 4.1.1) with a total volume of 8.6 l (internal settlers included). The same reactor system as described by Rebac et al. (16) was used, in the present experiments the temperature in the sludge bed was

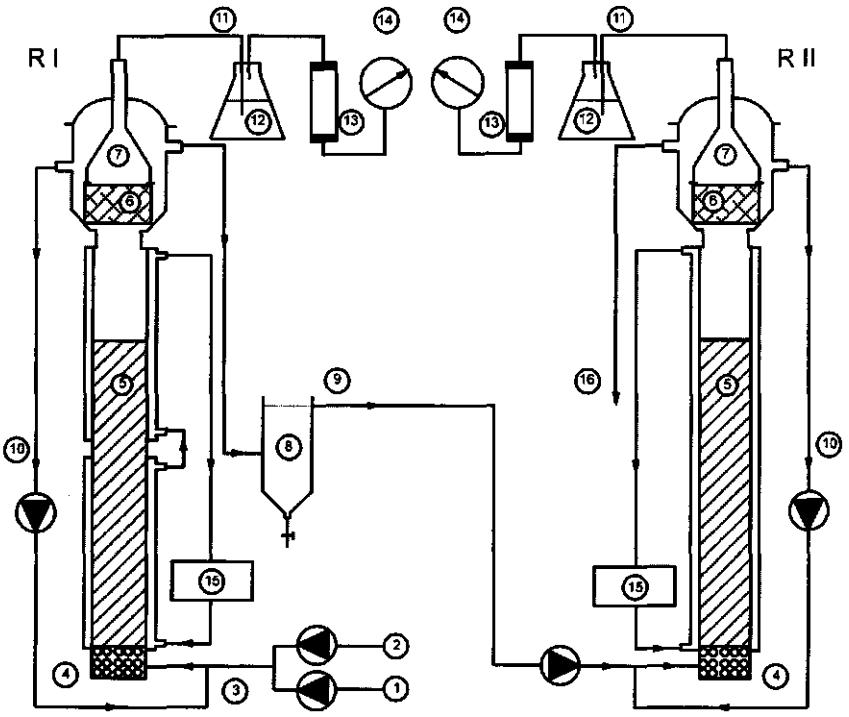

Fig. 4.1.1 Schematic diagram of the 8.6 l two stage EGSB reactor system used in this study. 1, Feed; 2, tap water; 3, influent; 4, stones; 5, expanded sludge bed; 6, screen; 7, gas-liquid-solid separator; 8, external settler; 9, effluent from first stage = influent for second stage; 10, effluent recirculation; 11, biogas; 12, sodium hydroxide (10 %); 13, soda lime pellets; 14, wet test gasmeter; 15, cooling bath circulator; 16, effluent from system.

measured with thermocouples (Shimaden, type SD 10, Tokyo, Japan) and controlled by cooling devices, connected to the house cooling system.

Biomass. The reactor system was inoculated with 260 g volatile suspended solids (VSS) methanogenic granular sludge, cultivated in a 225.5 l pilot-scale EGSB reactor treating malting wastewater (15) at temperatures between 12° to 20°C.

Medium. The reactor was fed with a concentrated stock solution of 33.36 g chemical oxygen demand (COD)•l^{-1}, consisting of a partly neutralized (pH 6.5) volatile fatty acid (VFA) mixture composed of acetate; propionate and butyrate in the ratio 1 : 1.5 : 1.8, based on COD. The concentrations of basal nutrients in the concentrated stock solution were (g l^{-1}): NH_4Cl, 7.5; $MgSO_4•7H_2O$, 1.5; $NaH_2PO_4•2H_2O$, 27.6; K_2HPO_4, 21.2; $CaCl_2•2H_2O$, 0.3; yeast extract, 0.5. To each litre of stock solution 4.5 ml of a trace element solution was added containing (mg l^{-1}): $FeCl_2•4H_2O$, 2000; H_3BO_3, 50; $ZnCl_2$, 50; $CuCl_2•2H_2O$, 30; $MnCl_2•4H_2O$, 500; $(NH_4)_6Mo_7O_{24}•4H_2O$, 50; $AlCl_3•6H_2O$, 90; $CoCl_2•6H_2O$, 2000; $NiCl_2•6H_2O$, 92; $Na_2SeO_3•5H_2O$,

164; EDTA, 1000; resazurin, 200; 36% HCl, 1 ml l^{-1}. All chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany).

Start-up of the system. The operation of reactor system was started immediately after inoculation with granular sludge, by feeding the synthetic wastewater at an OLR of 3 g COD l^{-1} d^{-1} and a HRT of 5.3 hours. From the start of the experiment, the temperature of the system was set at 9 °C.

Batch experiments. Specific substrate degrading activities were performed as described previously (16). In order to determine the apparent K_m value of the sludge from the second module for propionate under reactor conditions, the sludge was sampled at day 152 and put in two small 80-ml EGSB reactors with 30 g VSS l^{-1} sludge operated at 10 °C at upflow velocity of 6 m h^{-1}. At time zero, the substrate concentration in the reactor was set at 0.3 g COD_{prop} l^{-1}. The EGSB batch experiment lasted for a 14 hours period. Samples were taken every 20 minutes until the substrate was completely depleted. The K_m value was calculated by fitting substrate depletion data to the integrated Michaelis-Menten equation, using non-linear least-squares analysis as described previously (16).
Isotope experiments were performed at 10 °C with granular sludge from second stage. One month before the experiment, sludge was fed with sodium propionate (final concentration 0.06 g COD l^{-1} day^{-1}). One day before the experiment 12.5 ml portions of sludge were placed into 25-ml serum bottles, flushed with nitrogen and pre-incubated 24 hours with 0.11 g COD l^{-1} sodium propionate per liter at 10 °C. At the day of the experiment, one hour before the isotope solutions of labeled ^{14}C-acetate and ^{14}C-bicarbonate were added, sodium propionate was added at a final concentration of about 0.12 g COD l^{-1}. For isotope experiments VFA were analyzed with ion-exchange chromatography (1). Radioactive methane and carbon dioxide were measured by a modified method of Zehnder et al. (25).

Analyses. The pH and redox potential was determined in situ at the effluent line with a Microprocessor WTW 196 pH/mV-meter (Weilheim, Germany). Measurement of pH were conducted with Schott Nederland N61 double electrode (Tiel, The Netherlands). Redox potential was measured with combined platinum indicating and silver chloride reference electrodes (Schott Nederland PT 6180). Samples of influent and the effluent of both stages were taken three times per week in duplicate, except for the last 10 days when the samples were taken daily. Analyses of VFA and the biogas compositions (CH_4 and H_2) in the reactor and batch experiments were performed as described previously (16).

Microbiological experiments with diluted biomass from the second module. Granular sludge from the second stage was sampled at day 182 and stored in a refrigerator at 4 °C before the microbiological experiments were started. For long-term batch experiments, granular sludge of the EGSB-reactor was crushed in a glass mortar under nitrogen flow. The

modified Pfennig medium with 0.12 or 1.2 g COD l^{-1} propionate as the substrate was used for dilution of the cell suspension (9). The experiments were performed in 32 ml serum bottles with 20 ml of cultural liquid flushed with a nitrogen/carbon dioxide (70/30 %) gas mixture. The initial biomass concentrations were 5%, 0.5% and 0.05% (v/v). The experiments were performed at 5, 10, 15, 20, 25 and 30 °C in duplicates. The maximum rates were calculated for the first 1-2 days of the process. The temperature dependence of the maximum methane formation and propionate oxidation rate were fitted using, an Arrhenius derived model and a Ratkowsky's square root empirical model, respectively (16). Bromo-ethanesulfonic acid (BES) was added in a final concentration of 35 mM in order to inhibit methanogenesis. Gases and VFA were analyzed by gas chromatography (9). Microscopic observations were performed with a light microscope MBI-3 (Russia).

4.1.3 RESULTS

Performance of the system. The performance data of the two stage EGSB reactor system, are shown in the Fig. 4.1.2 The organic loading rate imposed to the system was gradually

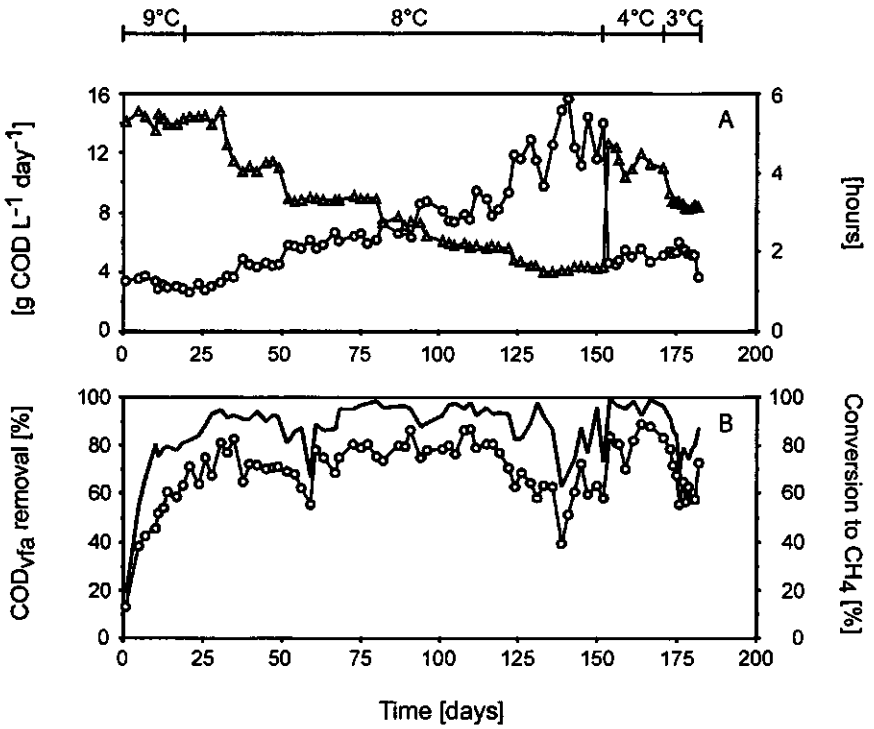

Fig. 4.1.2 Operation parameters and efficiency of the two stage EGSB reactor system fed with VFA mixture. A. Organic loading rate (O), hydraulic retention time (Δ); B. COD_{vfa} removal (—), Conversion to CH_4 (O).

increased from 3.5 to 15.5 g COD l^{-1} d^{-1} by decreasing the hydraulic retention time (HRT) from 5.3 to 1.5 hours (Fig. 4.1.2a) at an average temperature of 8 °C. Increments in loading rate were only imposed once the COD removal efficiency reached 90 %. The results in Fig. 4.1.2 reveal that the system maintained a remarkable stability and high efficiency over the period ranging from days 33 to 133, where the HRT was decreased from 5 to 2 h, and consequently the organic loading rate (OLR) was raised accordingly up to 12.5 g COD l^{-1} d^{-1}. At an imposed HRT of 1.5-1.6 h during the period between days 133 and 152, the system received peak loads 12-15.5 g COD l^{-1} d^{-1}. This resulted in stronger variations in the COD removal efficiency (63 - 92 %). The influent contained 12 mg O_2 l^{-1}, which gave maximum the dissolved oxygen loads of 0.2 g O_2 l^{-1} d^{-1}, consequently values relatively low compared to OLR. The redox potential of the reactor effluent always remained at -350 to -380 mV indicating that satisfactory anaerobic conditions prevailed in both modules of the system (data not shown). In period between days 154 and 171, the system was operated at a temperature of 4 °C and at an HRT of 4-5 h, still corresponding to an OLR of 4-5 g COD l^{-1} d^{-1}. Even under these extreme conditions the removal efficiency exceeded 90 %. The temperature of the system was further lowered to 3 °C in the period days 173-181 and at a HRT of 3 h, corresponding to an OLR of about 5.5 g COD l^{-1} d^{-1}, the treatment efficiency still could be maintained at about 80 %.

The acetate removal efficiency ranged between 90-100 % throughout the whole experiment, and the system could accommodate acetate loading rates of 10 g $COD_{acet.}$ l^{-1} d^{-1} (Table 4.1.1). The butyrate removal efficiency gradually increased to 100 %, and remained stable over the experimental period (Table 4.1.1). The system clearly has slightly some more problems with

Table 4.1.1 Average removal efficiency of acetate and butyrate in each stage and total system as percentage of the influent COD of particular volatile fatty acid in each stage and total system.

	AVERAGE REMOVAL EFFICIENCY [%]					
Temperature	ACETATE			BUTYRATE		
[°C]	1ST module	2ND module	Total system	1ST module	2ND module	Total system
8	84	94	97	59	86	90
4	80	99	100	94	100	100
3	57	86	86	83	97	99

propionic acid, as can be deduced from the results depicted in Fig. 4.1.3, which shows the degradation of propionate in each module of the system as a function of propionate OLR. The propionate degradation was far from complete in the first stage (Fig. 4.1.3a), but the total system was capable to accommodate propionate-loading rates up to 4 g $COD_{prop.}$ l^{-1} d^{-1} with

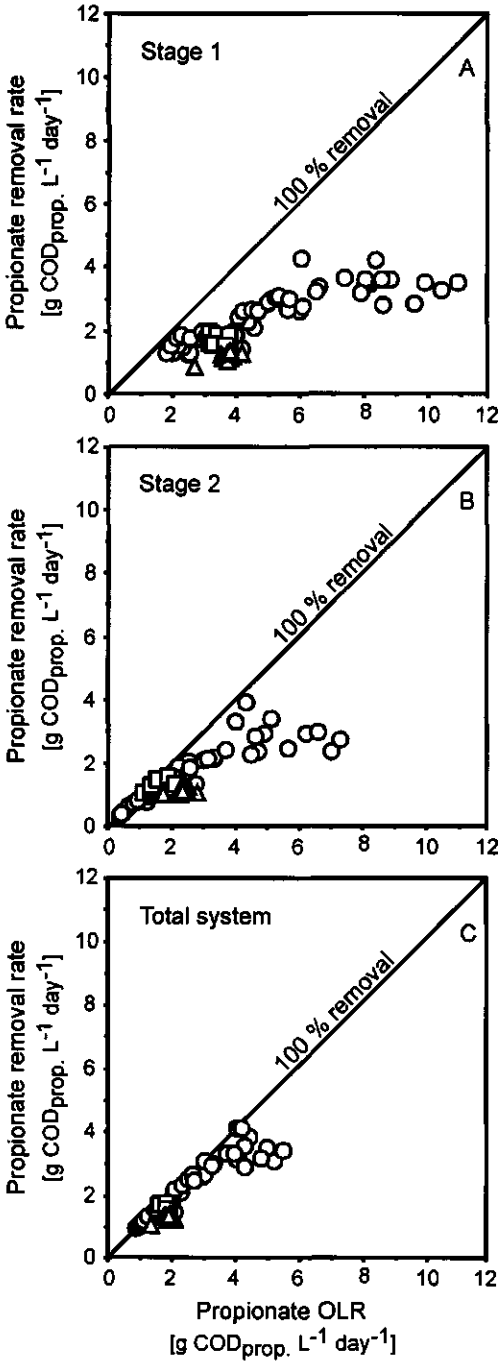

Fig. 4.1.3 Propionate removal rate versus propionate organic loading rate in each stage and the total system. A. Propionate removal rate at 8 °C (O); B. Propionate removal rate at 4 °C (); C. Propionate removal rate at 3 °C (Δ).

90-95 % degradation at an average temperature of 8 °C. At 4 °C, removal efficiencies over 80 % were achieved at propionate loading rates of 2 g $COD_{prop.}$ l^{-1} d^{-1}.
Extremely low hydrogen concentration in the biogas was measured in both stage of the EGSB system during continuous experiments (Table 4.1.2).

Table 4.1.2 The hydrogen concentration measured in the biogas of each stage of the system during continuous experiments.

T	[°C]	9	8	8	8	8	8	4	3
HRT	[h]	5	4	3	2.5	2	1.5	4	3
OLR*	[g COD $l^{-1}d^{-1}$]	3	4.8	6.4	7.7	9.6	12.8	4.8	6.4
$^1H_2$	[mg l^{-1}]	0.009	0.011	0.015	0.013	0.013	0.015	0.008	0.007
$^2H_2$	[mg l^{-1}]	0.010	0.006	0.010	0.012	0.011	0.016	0.007	0.006

*Average loading rate in the system
[1]Average hydrogen concentration in the biogas in the first stage
[2]Average hydrogen concentration in biogas in the second stage

Metabolic characteristics of the granule sludge. Table 4.1.3 presents the maximum specific degrading activities (A_{max}) at 10 °C of the inoculum and of sludge samples removed from each of the stages for propionate, butyrate and for a VFA mixture composed of acetate : propionate : butyrate in a ratio of 1 : 1.5 : 1.8 based on COD. These results clearly reveal that the specific substrate degrading activities of the sludge had increased significantly in time, indicating a very satisfactory enrichment of methanogens and acetogens at the low temperature conditions despite the very short liquid detention times applied. The specific

Table 4.1.3 Maximum specific substrate degrading activities at 10 °C of inoculum, and the EGSB sludge. Standard deviation is presented between parentheses.

Maximum specific substrate degrading activity [g COD g^{-1} VSS day^{-1}]			
Time	Propionate	Butyrate	VFA-mixture
day 0	0.097 (0.007)	0.053 (0.001)	0.106 (0.000)
First stage			
day 48	0.082 (0.000)	0.056 (0.006)	0.140 (0.005)
day 152	0.093 (0.017)	0.139 (0.001)	0.214 (0.002)
Second stage			
day 48	0.111 (0.008)	0.068 (0.002)	0.143 (0.002)
day 152	0.110 (0.000)	0.112 (0.009)	0.205 (0.002)

activity of the sludge for VFA mixture had doubled and its specific butyrate degrading activity even more than doubled after 152 days of operation. Most of the increase in butyrate degrading activity occurred in the period between days 48 and 152, which corresponds with the high butyrate removal efficiency in this period, even already achieved in the first stage. The specific propionate degrading activity of the sludge on the other hand did not improve substantially.

In order to measure the methane formation rates from acetate and from bicarbonate, during propionate degradation, experiments with addition of traces of ^{14}C-acetate and ^{14}C-bicarbonate and non-labeled propionate were performed. A linear methane formation rate in presence of the isotope traces was observed during the first 10 hours of the experiment. The rate of methanogenesis from ^{14}C-acetate was 3-5 times lower than that from ^{14}C-bicarbonate: 0.015-0.020 and 0.058-0.072 g COD_{meth} g^{-1}VSS.day^{-1} of sludge, respectively.

The apparent half saturation constant (K_m) for propionate of the sludge present in the second module was estimated in batch experiment after day 152 days of operation and was found to amount to 3.75 ± 0.56 mg COD_{prop} l^{-1}.

Metabolic characteristics of diluted biomass. The dynamics of the methane formation from propionate was investigated in batch experiments at 5-30 °C with 5% (v/v) of biomass (Fig. 4.1.4). The maximum methane production rate from 1.25 g COD l^{-1} of propionate was reached

within one day at 30 °C and after two days of incubation no propionate could be detected anymore in the medium. The rate of propionate degradation and methane formation decreased sharply at lower temperatures, but nevertheless, still a relatively high rate (0.15 g COD_{meth} l^{-1} d^{-1}) of methanogenesis was measured at 5 °C. The maximum concentration of acetate detected during the propionate degradation at 25 °C was 38 mg $COD_{acet.}$ l^{-1}, and at 5 °C, a value of 178 mg $COD_{acet.}$ l^{-1} was found. These values for acetate reflect the difference in the rate of acetate formation and degradation. The results in Fig. 4.1.4 clearly illustrate that the temperature optima of methane formation and propionate degradation anyhow than 30 °C, despite the fact that the mesophilic biomass was exposed to growth temperature of 3-8 °C for 180 days.

Light microscopic observations of the sludge samples from the second module at the end of the continuous experiments, showed that in spite of granular disruption at the start of the enrichment, practically all microbial cells were re-aggregated. *Methanosaeta-* and *Methanospirillum-* cells together with oval cells of propionate oxidizing bacteria were the most abundant microorganisms in these micro aggregates (Fig. 4.1.5b).

Fig. 4.1.6 illustrates conversion of propionate at 5 °C with very low inoculum concentration (0.5 % v/v) in batch assay. It took more than 160 days to degrade 1.25 g $COD_{prop.}$ l^{-1} propionate. In this incubation, acetate accumulation proceeded much faster than methane formation during the first two months. Acetate depletion started after about two months, and coincided with the increase in methane production. Once again, the formation of

microaggregates from disrupted sludge, containing *Methanosaeta*, *Methanospirillum*- cells, as well as separate large oval cells of propionate oxidizing bacteria were observed under the microscope at the end of cultivation.

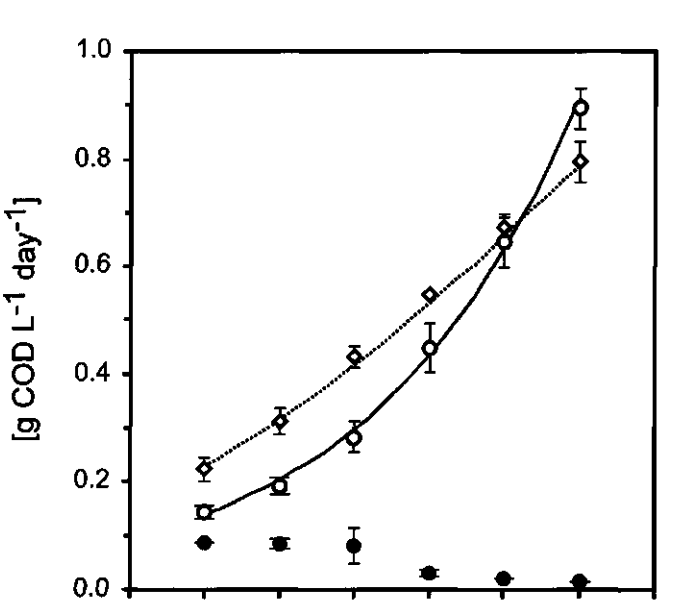

Fig. 4.1.4 Temperature characteristics of mesophilic biomass (20 times diluted) exposed for prolonged period of time to psychrophilic conditions. (O) Methane production rate (g COD l^{-1} d^{-1}); (◊) Propionate oxidition rate (g COD l^{-1} d^{-1}); (•) Acetate accumulation rate (g COD l^{-1} d^{-1}). The lines are computed using the Arrhenius model for the methane production rate (—) and the Square root model for the propionate degradation rate (-----).

In an experiment with 0.05% (v/v) biomass, only half of the initial propionate concentration (1.25 g $COD_{prop.}$ l^{-1}) was degraded after 300 days of incubation (data not shown). Contrary to the experiment with 0.5 % (v/v) biomass the cells remained mainly dispersed. Only a few microaggregates were formed and these aggregates contained *Methanosaeta*- cells and *Methanospirillum*- cells as well as oval cells of propionate oxidizing bacteria after one year of cultivation (Fig. 4.1.5c).

The methane formation rate from hydrogen conversion at 5 °C (Fig. 4.1.7) amounted to 0.012 g COD_{meth} l^{-1} d^{-1} using 0.5 % (v/v) biomass as inoculum compared to 0.004 g COD_{meth} l^{-1} d^{-1} from propionate at the same dilution of biomass (Fig. 4.1.6). A *Methanospirillum*-like bacterium was enriched at 10 °C with H_2/CO_2 as substrate from the granular sludge from the EGSB-reactor. This culture is also able to grow on formate.

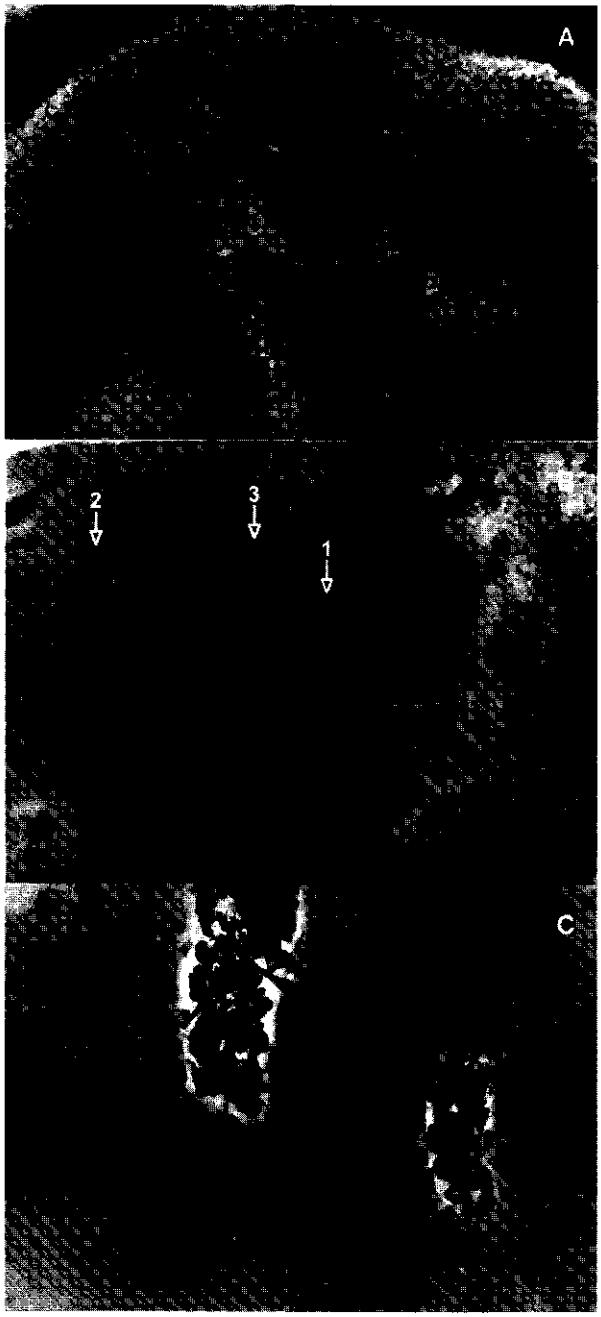

Fig. 4.1.5 Anaerobic cells aggregate and microbial cells. A. Anaerobic cell aggregate at the end of cultivation at 10 °C with 0.5 % inoculum size, magnification 100×3.2×2. B. Anaerobic cells of *Methanosaeta* (1), *Methanospirillum* (2), propionate oxidizing bacteria cells (3), at the end of cultivation at 10 °C with 0.5 % inoculum size, magnification 100×3.2×2. C. Anaerobic cell aggregates at the end of cultivation at 5 °C with 0.05 % inoculum size, magnification 100×3.2×2.

Hydrogen did not accumulate as an intermediate in any experiments during propionate degradation. In the presence of the specific methanogenic inhibitor BES, 0.020 g COD l^{-1} formate accumulated in one day from 0.11 g COD l^{-1} of propionate.

4.1.4 DISCUSSION

The results of the present study clearly reveal that high-rate anaerobic treatment in a two stage EGSB system is quite well feasible under relatively very low temperature conditions, i.e. down to 3°C. For a VFA-mixture as substrate, COD removal efficiencies exceeding 90 % can be achieved at 8°C and 4°C at organic loading rates of 12 and 5 g COD l^{-1} d^{-1}, respectively. The two stages EGSB concept was capable to accommodate 5-10 times higher OLRs at a 90 % COD_{vfa} removal efficiency than reported so far for psychrophilic anaerobic wastewater treatment (2, 7). The results obtained clearly demonstrate the feasibility of high-rate anaerobic wastewater treatment systems at temperature down to approximately 4 °C for VFA-substrate, even at very low substrate levels, which corresponds with the exceptionally low K_m value found for propionate as substrate. These low apparent K_m values probably can be attributed to excellent mixing conditions prevailing in EGSB reactor systems (8, 16).

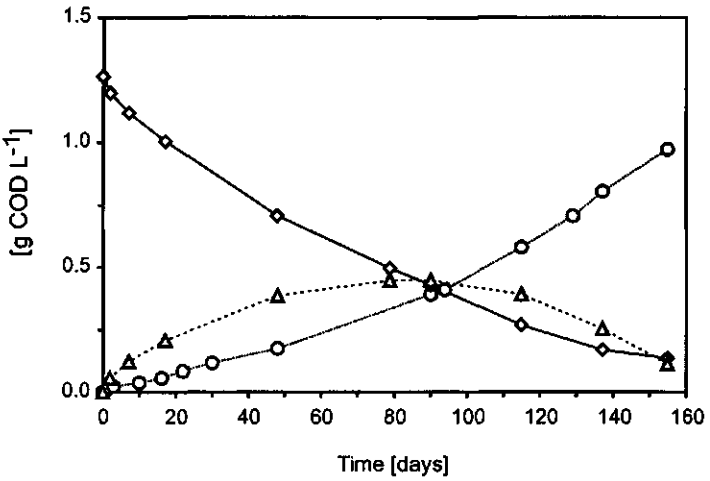

Fig. 4.1.6 Degradation of propionate at 5 °C with 200 times diluted biomass. (◊) propionate; (O) methane; (Δ) acetate.

The low hydrogen concentration in the biogas can be attributed to high activity of hydrogenotrophic methanogens (Fig. 4.1.4) and also to an increased solubility of hydrogen at such low temperatures (Table 4.1.2).

Compared to a single stage reactor system (16), the degradation of propionate improved significantly in a two stage EGSB system (Fig. 4.1.3). The good degradation of fatty acids like acetate and butyrate in the first module clearly improved the overall propionate degradation. Similar observations were previously made at 'sub-optimal' thermophilic range (55-65 °C) (20, 24). This distinct enhancement of the biodegradation of propionate in a properly designed and operated staged reactor system can be attributed to i) the development of

a balanced micro- ecosystem in the sludge in the separate reactor modules and ii) the improvement of environmental conditions, such as the lower extent of product inhibition in the

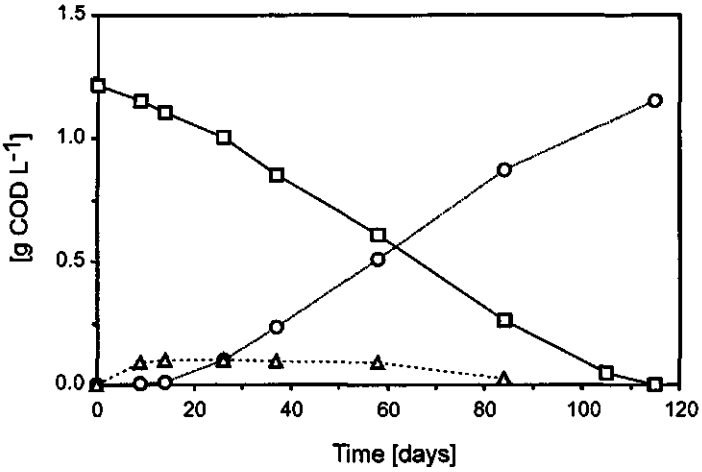

Fig. 4.1.7 Conversion of hydrogen at 5 °C with 200 times diluted biomass. (□) hydrogen; (O) methane; (Δ) acetate.

conversion of propionate.

Particularly in the second stage, because acetate can be maintained at relatively low level here, and consequently the conditions for propionate degradation then are much more optimal (3). As a consequence of moduling, and when newly ingrown bacterial matter is well retained a sludge with an exceptionally high specific acetogenic and methanogenic activity will develop in the second module, and this will lead to a substantial increase in the organic loading potentials of the system.

The observed sharp increase in specific activities of the granular sludge in time (Table 4.1.3) indicates that growth and enrichment of methanogens and butyrate oxidizers indeed proceeded quite well at the low temperatures applied. For the sludge of both stages the specific activity at 10°C, i.e. for propionate and for the VFA-mixture (Table 4.1.3) were found to be higher than grown in a single stage system after 235 days of continuous operation on a VFA substrate at 10 °C (16). On the other hand the results in Table 4.1.3 and Fig. 4.1.6 surprisingly don't show any clear evidence of growth-in of propionate oxidizers, because remain at roughly of original value. The results in Fig. 4.1.4 and Fig. 4.1.6 show a significant accumulation of acetate during the propionate degradation. As propionate oxidizers are known to be quite sensitive micro-organisms (3), their growth might have been inhibited by acetate (21) due to decreased activity of acetoclastic methanogens under these extremely low temperatures. Contrary to propionate oxidizers, the butyrate oxidizing organisms grew-in quite well, particularly regarding very low butyrate activity of the seed sludge, even in the first module. This low butyrate activity of the seed sludge may be attributed to the fact that

the seed sludge was cultivated on malting wastewater, which hardly contained butyrate (15) < 0.030 g COD_{but} l^{-1}.

At higher incubating temperature a significant higher propionate oxidation and methane formation is found for the crushed sludge (Fig. 4.1.4). Apparently even after 1.5 year period of feeding an originally 'mesophilic' sludge at temperatures below 20 °C, predominant population of specialized psychrophiles did not develop. Moreover, the results also indicate that a mesophilic sludge is quite well capable when conditions are provided to improve significantly in quality under extreme low temperature conditions. In addition to the excellent entrapment of newly grown organisms in the immobilized biomass, the good and stable enrichment of methanogens and butyrate oxidizers can be attributed to the prevailing very low decay rates, K_d, under psychrophilic conditions (22). These features facilitate practical implementation of high-rate anaerobic reactors for application under low temperature, as there is no need to develop specialized psychrophilic populations.

At temperatures below 15 °C (Figs. 4.1.4, 4.1.6), the observed accumulation of acetate could be due to the lower activity of acetoclastic methanogens, because the hydrogen concentration was much lower (less than 0.01 mg l^{-1}), than can be accomplished by homoacetogenic bacteria (about 0.6 mg l^{-1}) (5). Accumulation of formate in the presence of BES indeed could be demonstrated. Moreover, formate- and hydrogen-utilising *Methanospirillum* sp. as the predominant methanogens were found in the enrichment. Thus, at all temperatures investigated, the reducing equivalents (hydrogen or formate), formed during metabolism of propionate, were mainly utilized by methanogenic bacteria, not by homoacetogenic bacteria. This is further supported by the results of the experiments with hydrogen in Fig. 4.1.7, which show a four times higher methane production rate compared to acetate production rate and from the fact that that ^{14}C labeled methane production rates from labeled bicarbonate were 3 to 5 fold higher than from labeled acetate. Indeed, autotrophic methanogenesis has been reported to play an important role at extremely low temperature in sediment (14).

The rate of acetoclastic methanogenesis in the reactor sludge was much lower than the rate of autotrophic methanogenesis, as measured with labeled substrates. The acetoclastic methanogenesis is more strongly affected by a decreasing temperature (9, 13). This also corresponds with the observed accumulation of acetate during propionate degradation at low temperatures (Fig. 4.1.6). However, a complete propionate degradation requires a sufficiently high concentration of acetoclastic methanogens. Thus, a high density of acetoclastic methanogens as prevailing in the sludge of second stage supports the high efficiency of propionate degradation in that module (Table 4.1.1, Fig. 4.1.3b). We can conclude from our experiments that a methanogenic community degrading volatile fatty acids completely to CH_4 and CO_2 including propionate at low temperature was achieved in the EGSB-reactor system.

4.1.5 NOTATION

A_m = maximum specific degrading activity [g COD g^{-1} VSS day^{-1}]

COD = chemical oxygen demand [g O_2 l^{-1}]
COD_{vfa} = VFA chemical oxygen demand [g O_2 l^{-1}]
COD_{acet} = acetate chemical oxygen demand [g O_2 l^{-1}]
COD_{prop} = propionate chemical oxygen demand [g O_2 l^{-1}]
COD_{butyr} = butyrate chemical oxygen demand [g O_2 l^{-1}]
COD_{meth} = methane chemical oxygen demand [g O_2 l^{-1}]
EGSB = expanded granular sludge bed
HRT = hydraulic retention time [hours]
K_m = substrate half saturation constant [g COD l^{-1}]
K_d = decay rate [d^{-1}]
OLR = organic loading rate [g COD l^{-1} d^{-1}]
t = experimental time [days]
v = upflow velocity [m h^{-1}]
VFA = volatile fatty acids
VSS = volatile suspended solids [g l^{-1}]

4.1.6 ACKNOWLEDGEMENT

We are grateful to Klaas-Jan Kramer for his excellent assistance in performing reactor experiments.

4.1.7 REFERENCES

1. **Ammann, A. A., and T. B. Ruttimenn.** 1995. Simultaneous determination of small organic and inorganic anions in environmental water samples by ion-exchnge chromatography. J. Chromatography **706:** 259-269.
2. **Banik, G. C., and R. R. Dague.** 1996. ASBR treatment of dilute wastewater at psychrophilic temperatures. p 235-246 in: Proceeding of 69th Annual Water Environmental Conference, Dallas, Texas, USA.
3. **Boone, D. R., and M. P. Bryant.** 1980. Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystem. Appl. Environ. Microbiol. **40:**626-632.
4. **Conrad, R., F. Bak, H. J. Seitz, B. Thebrath, and H. P. Mayer.** 1989. Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment. FEMS Microbiol. Ecol. **62:**285-294.
5. **Cord-Ruwisch, R., H. J. Seitz, and R. Conrad.** 1988. The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on redox potential of terminal electron acceptor. Arch. Microbiol. **149:**350-357.

6. **De Man, A. W. A., A. R. M. Van der Last, and G. Lettinga.** 1988. The use of EGSB and UASB anaerobic systems for low strength soluble and complex wastewaters at temperatures ranging from 8 to 30°C. p. 197-209 in: Proceeding of the Fifth International Symposium on Anaerobic Digestion, Bologna, Italy, Hall, E. R. and Hobson, P. N. (ed.).
7. **Grant, S, and K. C. Lin.** 1995. Effects of temperature and organic loading on the performance of upflow anaerobic sludge blanket reactors. Can. J. Civ. Eng.. **22:**143-149.
8. **Kato, T. M., J. A. Field, P. Versteeg, and G. Lettinga.** 1994. Feasibility of expanded granular sludge bed reactors for the anaerobic treatment of low strength soluble wastewaters. Biotechnol. Bioeng. **44:**469-479.
9. **Kotsyurbenko, O. R., A. N. Nozhevnikova, T. I. Soloviova, and G.A. Zavarzin.** 1996. Methanogenesis at low temperature by microflora of tundra wet land soil. Antonie van Leeuwenhoek **69:**75-86.
10. **Kotsyurbenko, O. R., M. V. Simankova, A. N. Nozhevnikova, T. N. Zhilina, N. P. Bolotina, A. M. Lysenko, and G. A. Osipov.** 1995. New species of psychrophilic acetogens: *Acetobacterium bakii* sp.nov., *A. paludosum* sp.nov., *A. fimetarium* sp.nov. Arch. Microbiol. **163:**429-434.
11. **Last van der, A. R. M., and G. Lettinga.** 1992. Anaerobic treatment of domestic sewage under moderate climatic (Dutch) conditions using upflow reactors at increased superficial velocities. Wat. Sci. Tech.. **25:**167-178.
12. **Matsushige, K., Y. Inamori, M. Mizuochi, M. Hosomi, and R. Sudo.** 1990. The effects of temperature on anaerobic filter treatment for low-strength organic wastewater. Environ. Technol. **11:**899-910.
13. **Nozhevnikova, A. N., C. Holliger, A. Ammann, and A. J. B. Zehnder.** 1997. Psychrophilic methanogenesis in sediments of deep lakes. Wat. Sci. Tech. **36:**57-64.
14. **Parshina, S. N., A. N. Nozhevnikova, and S. V. Kalyuzhnyi.** 1993. Decomposition of protein substrates at low temperature by the microflora of pig manure. Microbiology **62:**121-129.
15. **Rebac, S., J. B. Van Lier, M. G. J. Janssen, F. Dekkers, K. Th. M. Swinkels, and G. Lettinga.** 1997. High-rate anaerobic treatment of malting waste water in a pilot-scale EGSB system under psychrophilic conditions. J. Chem. Tech. Biotechnol. **68:**135-146.
16. **Rebac, S., J. Ruskova, S. Gerbens, J. B. Van Lier, A. J. M. Stams, and G. Lettinga.** 1995. High-rate anaerobic treatment of wastwater under psychrophilic conditions. J. Ferment. Bioeng. **80:**499-506.
17. **Romesser, J. A., R. S. Wolfe, F. Mayer, E. Spiess, and A. Walther-Mauruschat.** 1979. *Methanogenium*, a new genus of marine methanogenic bacteria, and characterization of *Methanogenium cariaci* sp. nov. *Methanogenium marisnigri* sp. nov. Arch. Microbiol. **121:**147-153

18. **Schink, B.** 1997. Energetics of syntrophic cooperation in methanogenic degradation. Microbiol. Mol. Biol. Rev. **61**:262-280.
19. **Switzenbaum, M.S., and W. J. Jewell.** 1978. Anaerobic attached film expanded bed reactor treatment of dilute organics. p. 1-164 in: Proceedings of the 51st Annual WPCF Conference, Anaheim, California, USA.
20. **Van Lier, J. B., F. Boersma, M. M. W. H. Debets, and G. Lettinga.** 1994. High-rate thermophilic anaerobic wastewater treatment in compartmentalized upflow reactors. Wat. Sci. Tech. **30**:251-261.
21. **Van Lier, J. B., K. C. F. Grolle, C. T. M. J. Frijters, A. J. M. Stams, and G. Lettinga.** 1993. Effect of acetate, propionate and butyrate on the thermophilic anaerobic degradation of propionate in methanogenic sludge and defined cultures. Appl. Environ. Microbiol. **57**:1003-1011.
22. **Van Lier, J. B., S. Rebac, P. Lens, F. Van Bijnen, S. J. W. Oude Elferink, A. J. M. Stams, and G. Lettinga.** 1997. Anaerobic treatment of partly acidified wastewater in a two-stage expanded granular sludge bed (EGSB) system at 8 °C. Wat. Sci. Tech. **36**:317-324.
23. **Wheatley, A.** (ed.) 1990. Anaerobic digestion: a waste treatment technology. Elsevier Applied Science, London, UK.
24. **Wiegant, W. M., M. Hennink, and G. Lettinga.** 1986. Separation of the propionate degradation to improve the efficiency of thermophilic anaerobic treatment of acidified wastewaters. Wat. Res. **20**:517-524.
25. **Zehnder, A. J. B., B. Huser, and T. D. Brock.** 1979. Measuring radioactive methane with the liquid scintillation caunter. Appl. Environ. Microbiol. **37**:897-899.

4.2 ANAEROBIC TREATMENT OF PARTLY ACIDIFIED WASTEWATER IN A TWO- STAGE EXPANDED GRANULAR SLUDGE BED (EGSB) SYSTEM AT 8 °C

ABSTRACT

Psychrophilic (8 °C) anaerobic treatment of partly acidified wastewater was investigated using a two stage EGSB system with a total volume of 8.6 dm^3. The reactor system was operated at an up-flow velocity of 10 $m \cdot h^{-1}$ and was fed with a sucrose-VFA mixture of 550-1100 mg COD dm^{-3}. The average COD_{sol} and VFA-COD removal efficiencies were 97 and 90 %, respectively, at total organic loading rates (OLR) ranging between 5.1 - 6.7 g COD $dm^{-3} \cdot day^{-1}$, sucrose loading rates up to 1 g COD dm^{-3} day^{-1} and a hydraulic retention time (HRT) of 4 h. An increase in the sucrose loading rates resulted in a significant wash-out of biomass from the first stage. The second stage satisfactory served as a scavenger of non-degraded VFA from the first stage.

Specific activity assays showed an increase of 15 % in the specific methanogenic activity of the sludge present in the second stage and a decrease of 9 % in the first stage. Apparently, an enrichment of methanogens and acetogens in the anaerobic sludge in the second stage took place at temperatures as low as 8°C. The acidogenic population became much more dominant in the first stage, resulting in a higher acidifying activity and a decreased methanogenic activity. 16S rRNA probe-techniques (dot blot hybridization) showed that the acetate consuming *Methanosaeta* (formerly *Methanothrix*) and the hydrogenotrophic *Methanobrevibacter* species (or relatives) were the most abundant methanogens present in the psychrophilic sludge. The ratio between bacterial and methanobacterial hybridization signal of the first stage was 3 times higher than that of the second stage. By using NMR techniques, a higher effective diffusion coefficient was found for the smaller sized granules in both reactors, which is in congruent with the higher maximum specific acetate degrading activity of the smaller granules.

Key words: Expanded granular sludge bed reactor, psychrophilic, sucrose, volatile fatty acids, methanogenesis, two stage.

4.2.1 INTRODUCTION

Generally, high-rate anaerobic reactor systems are applied in the temperature range between 25-40 °C. Successful application in the lower temperature range, i.e., 5-20 °C, requires various adaptations of the conventional high-rate reactor design. Most important is the degree of mixing between the methanogenic biomass and the wastewater. Since the specific biogas production rate is relatively low under psychrophilic conditions, efficient mixing may be achieved by increased liquid upflow velocities (Rebac et al. 1995; 1997). The latter concept was recently studied by using expanded granular sludge bed (EGSB) reactor systems,

operated at superficial upflow velocities up to 10 $m \cdot h^{-1}$. Results indeed show a high treatment capacity of the system despite the low temperatures applied, i.e., < 15 °C (Rebac et al. 1995). The performance and stability of psychrophilic treatment systems obviously depends on the influent composition. This paper describes the use of a two stage EGSB reactor for the psychrophilic (8 °C) treatment of partly acidified waste water. The performance of the system at various specific sucrose loading rates is presented. The effects of the operational conditions on the biological and physico-chemical sludge characteristics are also described.

4.2.2 MATERIALS AND METHODS

Experimental conditions

The two stage EGSB system, consisted of two 0.05 m inner diameter glass EGSB reactors (2 x 4.3 dm^3) operated in series as described by Rebac et al. (1995). The imposed liquid up-flow velocity in both stages was 10 m h^{-1}. Each reactor was equipped with an external water circuit in which cooled water (4 ±1 °C) was pumped through the double wall of the reactor. The reactor system was started at 8 °C immediately after inoculation, using a synthetic wastewater at an OLR of 4-5 g COD m^{-3} day^{-1} and at a HRT of 4 hours. Duplicate influent and effluent samples of both stages were taken three times per week. The oxygen concentration in the dilution water was decreased from day 139 to the end of experiment from 12 to 2 mg O_2 dm^{-3} by stripping O_2 from the liquid using N_2-gas.

Biomass

The first and the second EGSB stage were inoculated with 137 and 132 g, respectively, of volatile suspended solids (VSS) methanogenic granular sludge, cultivated during 6 months in the same EGSB reactor system treating a VFA mixture at temperatures between 2 to 9 °C (manuscript in preparation).The sludge was stored for 2 months at 4 °C prior to use.

Medium

During the first 2 weeks the synthetic wastewater consisted of acetate, propionate and butyrate in a COD ratio 1 : 1.5 : 1.8 and macro and micro nutrients as described previously (Rebac et al., 1995). From day 14 onwards, also sucrose was present in the feed by supplying a sucrose stock solution of 2 g COD dm^{-3} and 2 g $NaHCO_3$ dm^{-3}. Different ratios VFA : sucrose were studied.

Biological properties of psychrophilic granular sludge

The apparent half saturation constant (K_m) of each stage of the system for acetate at 8 °C was determined at day 136 by using the substrate depletion method as described elsewhere (Rebac et al., 1995). Specific substrate degradation assays were performed at 10 °C in 0.120 dm^{-3} penicillin bottles (triplicates) containing 0.1 dm^{-3} of basal medium supplemented with 1 and 5 g VSS dm^{-3} for tests with, respectively, acetate (initial concentration 1.5 g COD dm^{-3}) and

sucrose (initial concentration 3 g COD dm^{-3}) as described elsewhere (Rebac et al., 1995). Acetoclastic methanogenic activity was measured at 10 °C using the pressure head space method of Colleran & Pistilli (1994), and with penicillin bottles containing 1 g VSS dm^{-3} and 0.05 dm^{-3} of basal medium supplemented with acetate (initially 1.5 g COD dm^{-3}). The decay rate (K_d) of acidifiers was calculated from the semi logarithmic plot of the decrease in sucrose acidifying activity of sludge from the first stage versus time. The corresponding sucrose acidifying activity of the sludge was measured in triplicates at 10 °C after 0, 48, 82 and 174 days of incubation without substrate. The methanogenic community present in the reactor sludge on day 116 was analysed using 16S rRNA dot blot hybridization with group-specific 16S rRNA oligonucleotide probes as described by Raskin et al. (1994). The following specific probes were used: *Methanococcales* (MC 1109), *Methanobacteriales* (MB 1174), *Methanogenium* (MG 1200), *Methanosarcina* (MS 821) and *Methanosaeta* (MX 825), Archaea (ARC915) and Bacteria (EUB338).

Physico-chemical characterisation of the granular sludge

The size distribution of the sludge was determined using image-analyzing techniques as described elsewhere (Rebac et al., 1995). Granule strength was determined as the resistance against axial compression forces (Alphenaar1994). Apparent effective diffusion coefficients (D_{eff}) were determined at 21 (± 1) °C by pulsed-field gradient nuclear magnetic resonance (PFG-NMR) spectroscopy (observation time Δ of 13.1 ms) as described by Lens et al. (1997).

Analyses. VFA analyses and the biogas compositions for CH_4, CO_2, O_2, N_2 and H_2 were performed as described elsewhere (Rebac et al., 1995; 1997). Sucrose was determined by HPLC, equipped with an Ion 300 Organic Acids (30 cm) column (20 °C) a Refractive Index ERC 7510 detector, and a Spectra Physics 8810 precision isocratic pump. The mobile phase was 1.25 mmol H_2SO_4 at flow rate of 0.5 ml min^{-1}.

4.2.3 RESULTS

Performance of the system

The performance of the entire two stage EGSB system at 8 °C, is depicted in Fig. 4.2.1 and summarized in Table 4.2.1. The separate stages are shown in Figs. 4.2.2 and 4.2.3. The first two weeks (period I) were used for re-activation of the unfed stored sludge (2 months at 4 °C). Immediately after the start very high removal efficiencies were obtained (90-98 %) at OLRs between 4.1-6.2 g COD dm^{-3} day^{-1} and at HRT = 4 h. In period II (days 15-57), the same OLR of VFA as in period I was applied, but in addition the feed contained 50 mg sucrose-CODdm^{-3}. As shown in Fig. 4.2.1c and Table 4.2.1 this resulted in a slight increase of the COD removal efficiency. In period III (days 58-87), the sucrose concentration was increased up to 150 mg COD dm^{-3}, resulting in an OLR of sucrose of 0.9 g COD_{suc} dm^{-3} day^{-1}. The overall COD removal efficiency remained unaffected, but the COD_{vfa} removal decreased slightly. However, the height of the granular sludge bed in the first stage increased markedly

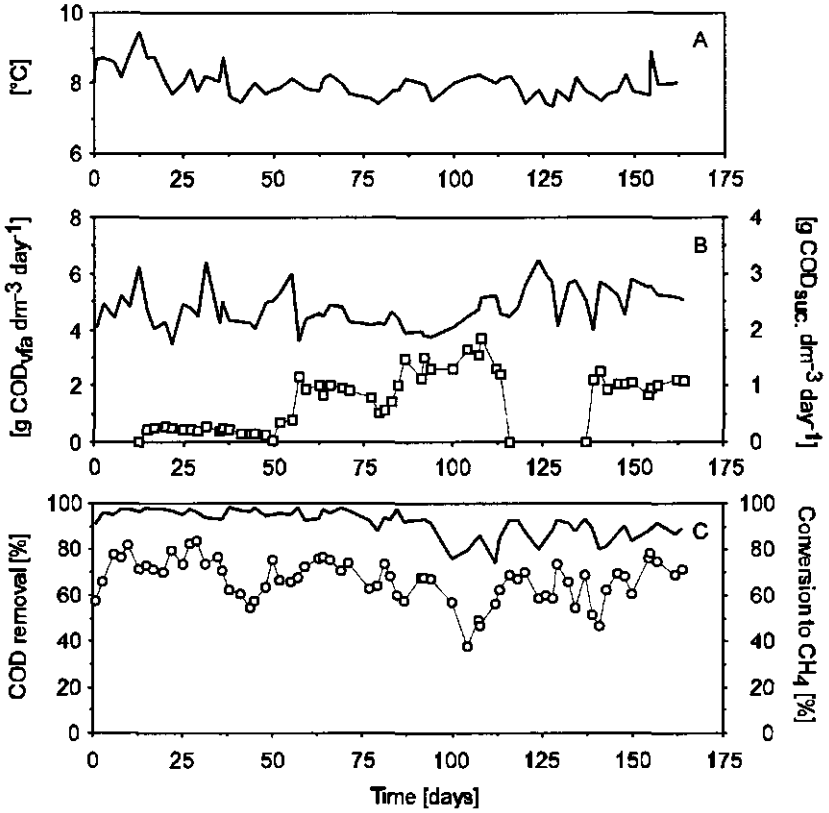

Fig. 4.2.1 Operation parameters and efficiency of the total system of the two stage EGSB reactor system fed with partly acidified waste water. A. Temperature (—); B. VFA organic loading rate (—), Sucrose organic loading rate (); C. COD removal (—) efficiency, CH_4 conversion relative to influent COD(O).

and wash-out of newly grown, filamentous biomass occurred in this period. A further increase of the sucrose loading from 0.9 to 1.8 g COD_{suc} dm^{-3} day^{-1} (Period IV, days 88-116), led to a severe wash-out of methanogenic granules entrapped in attached voluminous, filamentous, acidogenic biomass. In period IV, excess biomass had to be withdrawn from the first stage on a daily basis. This however, resulted in a significant drop in the sludge residence time (SRT) of the first stage and, concomitantly, in the overall COD removal efficiency (Fig. 4.2.1c, Table 4.2.1). The drop in COD removal efficiency was most severe in the first stage, i.e. from 50-60 % to 30 % (Fig. 4.2.2B1). The declining efficiencies of the first stage led to an increase of the OLR_{vfa} in the second stage, i.e. from 4 to 8 g COD_{vfa} dm^{-3} day^{-1}, resulting in a drop in the COD removal efficiency from 80 to 65 % (Fig. 4.2.2A2, 4.2.B2).

In order to allow a system recovery, the sucrose feeding was stopped in period V (days 117-138). The COD removal efficiency of the second stage immediately recovered (Fig. 4.2.2B2),

Table 4.2.1 Performance data of the two stage EGSB system. Average values are given in parentheses.

Period	Days	OLR_{VFA} [g COD $dm^{-3}day^{-1}$]	OLR_{suc} [g COD $dm^{-3}day^{-1}$]	T [°C]	HRT [h]	COD_{rem} [%]	VFA_{rem} [%]	COD_{meth} [%]
I	0-14	4.1-6.2 (4.9)	0	(8)	4.0	90- 98 (95)	90-98 (95)	57-82 (72)
II	15-57	3.7-6.3 (4.9)	0.1-0.4 (0.2)	(8)	4.1	95-100 (98)	93-98 (96)	55-84 (70)
III	58-87	4.6-5.7 (5.1)	0.5-1.0 (0.9)	(8)	4.0	94-100 (97)	84-93 (90)	60-77 (70)
IV	88-116	4.9-6.8 (5.6)	1.2-1.8 (1.4)	(8)	3.0	75- 95 (87)	69-85 (78)	38-68 (57)
V	117-138	4.1-6.5 (5.4)	0	(8)	3.1	79- 94 (89)	78-94 (89)	55-74 (65)
VI	139-164	4.9-6.8 (6.1)	0.8-1.3 (1.0)	(8)	2.9	83- 92 (89)	75-85 (83)	47-78 (66)

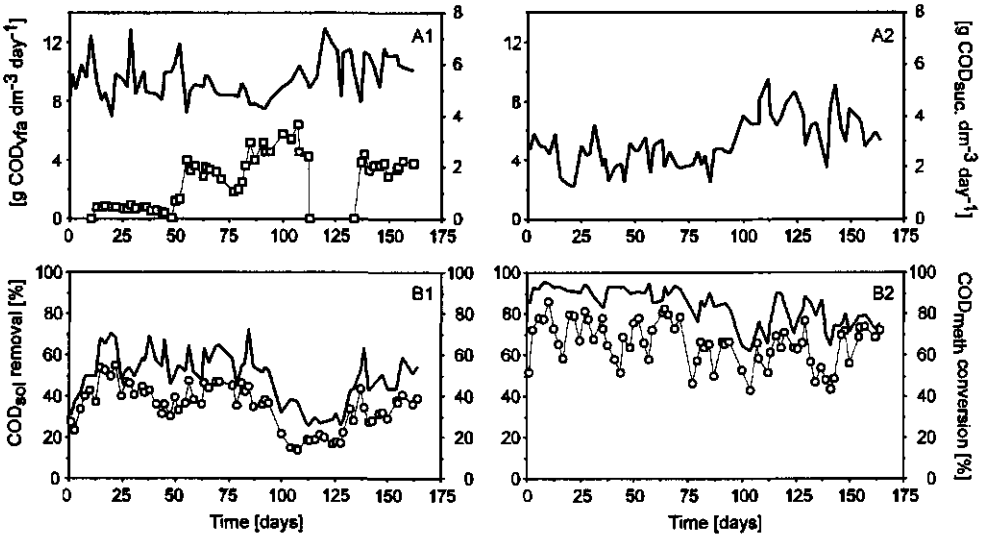

Fig. 4.2.2 Organic loading rate and efficiency of each stage of the two stage EGSB reactor system. A. VFA organic loading rate (—), Sucrose organic loading rate () in the first stage; B. COD removal (—), CH_4 conversion (O). The numbers following the abbreviation refer to the stage of the system.

obviously due to the lower imposed organic loading rate. However, no recovery was observed in the first stage during period days 117-127. After the excess sludge collected from the first stage in period IV was returned to the reactor on day 128, the COD removal efficiency of the first stage improved from 30 % to about 50 %. In period VI (days 139-164), the system was fed once again with sucrose at loading rates ranging from 0.8 to 1.3 g COD_{suc} dm^{-3} day^{-1} (Fig. 4.2.1c). Since the dilution water was pre-flushed with N_2-gas in this period, the oxygen concentration of the influent was reduced from 12 to about 2 mg O_2 dm^{-3}. The overall COD and COD_{vfa} removal efficiencies ranged between 83-92 % and 75-85 %, respectively. These removal efficiencies were only 8-10 % lower compared to period II when the reactor was operated at significantly lower sucrose loading rates (Table 4.2.1). Moreover, sludge growth was rather satisfactory in period VI and the sludge withdrawal from the first stage on a daily basis was not needed.

Staged substrate conversion

In periods II to IV, the first stage also acted as an acidogenic reactor where all sucrose was fermented (Fig. 4.2.3a, b). This resulted in an excessive growth of acidogenic biomass compared to the methanogenic sludge granules present. Flotation of granular sludge manifested at sucrose sludge loading rates (SLR) ranging from 0.021 to 0.027 g COD g^{-1} VSS day^{-1} (based on the initial VSS content) and an up-flow velocity of 10 m h^{-1} (Period III and IV). The fermentation of sucrose in the first stage led to elevated hydrogen concentrations in the biogas

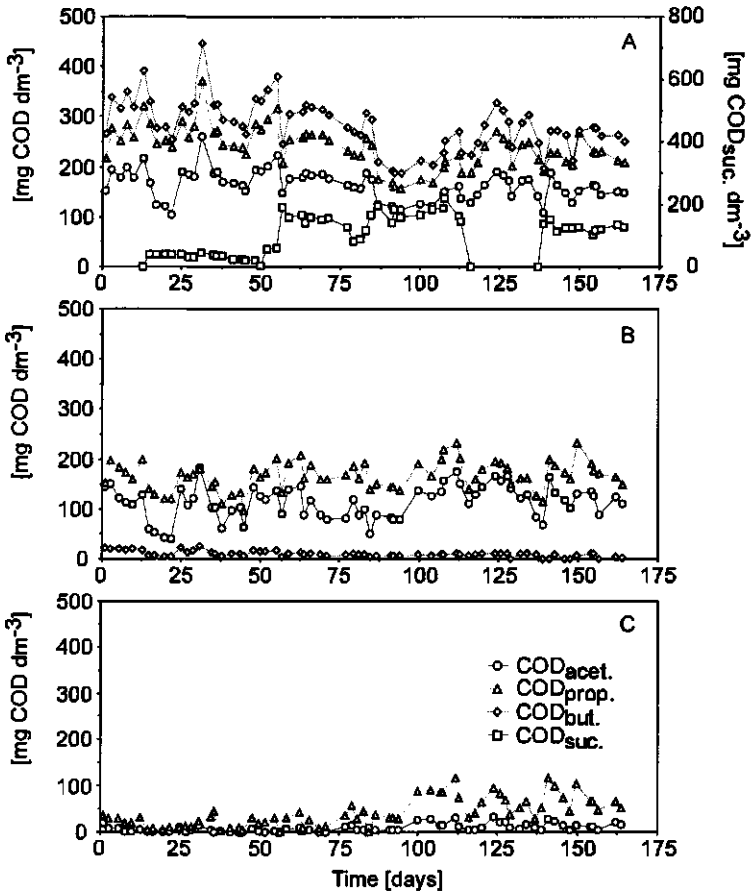

Fig. 4.2.3 Concentrations of volatile fatty acids and sucrose in influent and effluent of each stage. A. Influent, B. Effluent first stage, C. Effluent second stage. Acetate (O), Propionate (Δ), Butyrate (◊), Sucrose ().

of the first stage. During periods I to VI, the hydrogen concentrations ranged between (ppm): 7-10, 65-75, 250-301, 200-214, 9-11 and 90-101, respectively. In contrast, H_2 concentrations remained low in the second stage viz. (ppm): 10-12, 18-20, 20-25, 20-22, 14-16 and 18-23, respectively in periods I to VI.

Metabolic and microbial sludge characteristics

Table 4.2.2 provides the data concerning the evolution of the maximum specific sucrose acidifying activity (SAA), the methanogenic activities (MA), and the acetate degrading activities (ADA) at 10 °C of the sludge, sampled after various periods of time. The imbalance between MA and ADA, which is most pronounced for the first stage, could indicate the presence of alternative acetate removal pathways in the sludge. The maximum ADAs of the top and the bottom sludge sampled at day 161 from both stages, are presented in Table 4.2.3.

The MA of the sludge from the first stage, sampled at day 79, was lower compared to the results found at day 14. However, likely due to the omission of sucrose from the influent in period V, the MA had again increased in the next sampling at day 161. In contrast, MAs and ADAs of the sludge in the second stage gradually increased in time. SAAs of the sludge sampled from both reactors at day 79 (period III) were very high.

Table 4.2.2. Maximum specific sucrose, and acetate degrading activity as well as methanogenic activity at 10°C of the EGSB sludge. Standard deviation is given between parentheses.

Day	Maximum specific activity [g COD g^{-1} VSS day^{-1}]					
	FIRST STAGE			SECOND STAGE		
	SAA*	ADA♣	MA♥	SAA*	ADA♣	MA♥
14	ND	0.198 (0.003)	0.173 (0.003)	ND	0.196 (0.019)	0.175 (0.001)
79	0.810 (0.047)	0.192 (0.009)	0.158 (0.007)	0.686 (0.003)	0.205 (0.014)	0.190 (0.004)
161	0.688 (0.032)	0.208 (0.009)	0.163 (0.003)	0.584 (0.028)	0.227 (0.030)	0.201 (0.003)

ND - not determined ♣Acetate degrading activity
*Sucrose acidifying activity ♥Methanogenic activity

The decay rate, K_d, of acidifiers in the sludge sampled from the first stage at day 79 amounted $(1.57 \pm 0.2) \times 10^{-5}$ h^{-1} at 10 °C. The apparent half saturation constant K_m, for acetate were 0.035 and 0.067 g COD dm^{-3} at 8 °C for the first and the second stage, respectively, at day 136.

A good hybridization signal, corresponding to about 50 % of the total methanogenic 16S rRNA, was obtained with the MB 1174 probe, indicating that *Methanobrevibacter* or relatives are the dominant methanogenic hydrogen consumers in the sludge from both stages. This type of bacterium, which is characterized by a high H_2 affinity at low H_2 concentrations, was previously found to be the most important H_2-scavenger in propionate grown granular sludge (Grotenhuis et al., 1991). *Methanosaeta* sp. probably was the main acetate degrading methanogen in the both sludges (probe MX 825), since approximately 49 % of the total methanogenic 16S rRNA originated from this group of methanogens. The predominance of *Methanosaeta* species, which are characterized by a very low K_m, agrees with the in-reactor K_m values of 35 and 67 mg COD dm^{-3} for the first and the second stage, respectively. In contrast, a very low hybridization signal was obtained with the MS 821 probe (less than 1 % of the total methanogenic 16S rRNA), suggesting that *Methanosarcina* sp. did not play an important role in methanogenic acetate removal. No hybridization signal with the MC 1109 and MG 1200 probes were found, indicating that *Methanococcales* and *Methanomicrobiales* were present below the detection limit in both sludges. The ratio between methanogenic and bacterial 16S rRNA hybridization signal was three time lower in the sludge of the first stage than in the second stage. However, this ratio could not be extrapolated to bacterial numbers.

Physico-chemical characteristics of the sludge

The up-flow velocity applied in the EGSB reactor (10 $m \cdot h^{-1}$) enhances segregation in the sludge bed by gravitational forces based on size and settling properties of granules (Fig. 4.2.4). Similar observations were made previously (Rebac et al., 1995, 1997). The specific

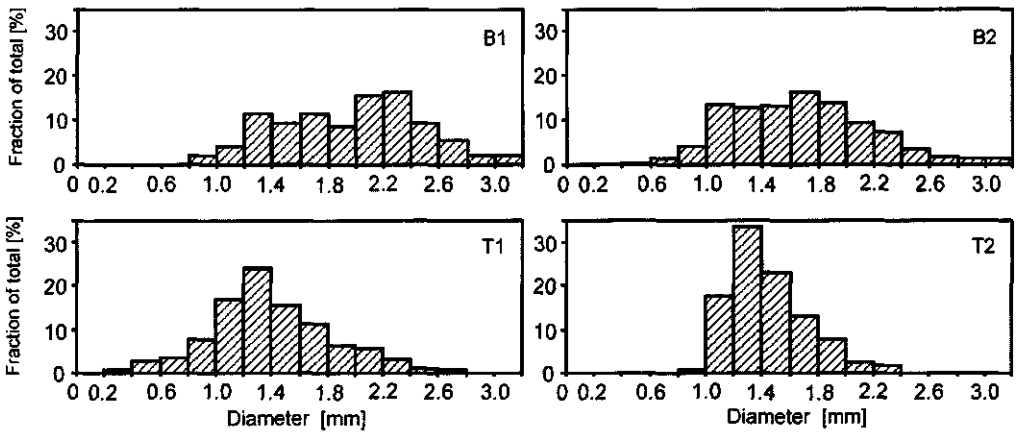

Fig. 4.2.4 Size distribution of granular sludge from the bottom and the top of each stage of EGSB system at day 161, expressed in percentage of the biomass volume represented by the granules. B = bottom; T = top; the figures following the abbreviation refer to the stage of the system.

acetate degrading activities of the small sized top sludge, were about 18 % and 48 % higher for sludge present in the first and second stage, respectively (Table 4.2.3). Apparently, the fractions of active methanogens were much higher in the small sized granules than in the large granules present at the bottom of the reactor. Also, a higher effective diffusion coefficient was found with the small sized granules, which agrees with the higher maximum

Table 4.2.3. Maximum acetate degrading activity at 10 °C, apparent diffusion coefficient, granule strength and mean diameter of the EGSB sludge from the top and the bottom at day 161. Standard deviation is given in parentheses.

		FIRST STAGE		SECOND STAGE	
		Top	Bottom	Top	Bottom
ADA*	[g COD g^{-1} VSS day^{-1}]	0.211 (0.012)	0.178 (0.007)	0.304 (0.014)	0.205 (0.031)
D_{eff} coefficient	[10^{-9} m^2 s^{-1}]	1.42 (0.01)	1.11 (0.01)	1.25 (0.01)	1.12 (0.01)
Strength	[kN m^{-2}]	94.3 (3.3)	76.1 (6.5)	90.6 (2.7)	116.0 (4.8)
Mean diameter	[mm]	1.25	1.95	1.30	1.55

*Acetate degrading activity

specific activity of the same sludge (Table 4.2.3). In addition, the prevailing environmental conditions and selection pressure at the bottom of the EGSB reactors may have resulted in an increased density of the sludge granules, probably corresponding to a decreased porosity (Alphenaar et al., 1994). Consequently, the bigger granules that segregated to the bottom were characterized by a lower activity, possibly due to the occurrence of substrate diffusion limitations. No clear trend between D_{eff} and the granule strength was observed (Table 4.2.3).

4.2.4 DISCUSSION

Reactor performance

The two stage EGSB reactor system showed a highly stable and efficient performance when treating merely pre-acidified waste water (Period I, II and V) at low temperatures (8 °C) and OLRs ranging from 4.1 - 6.5 g COD dm^{-3} day^{-1} with HRTs of 4.0 and 3.1 h. During these periods, both EGSB stages were operated as two methane reactors in series. The start-up period proceeded rapidly and a 95 % COD removal efficiency was obtained within two weeks (Fig. 4.2.1 and Table 4.2.1). Apparently, an unfed storage period of 2 months does not affect the methanogenic capacity of the sludge, which is previously grown under psychrophilic conditions. The butyrate removal efficiency in the first stage was 90 %, and remained stable throughout the experimental period (Fig. 4.2.3) Also propionate was satisfactory removed in the system (Fig. 4.2.3). Treating merely pre-acidified waste water (Periods I, II and V), the two stage EGSB system is capable to accommodate a three times higher OLR at a 90 % COD_{vfa} removal efficiency than reported sofar (Banik and Dague, 1996; Matsushige et al., 1990). Even when the system was fed with partly acidified wastewater, the overall performance was very satisfactory (Fig. 4.2.1c, period III, VI and to a lesser extent IV). However, under such conditions an effective retention of acidifying biomass in the first stage is a pre-requisite for stable reactor operation as explained in more detail below.

Reactor stability

The retention of granular sludge in the EGSB reactor system depends on the design of the sludge separator, on the hydraulic and biogas loading rates (Kato et al., 1994). When also acidogenesis occurs, the sludge retention becomes difficult, due to formation of an attached layer of acidifiers around the methanogenic granular sludge. This may lead to gas entrapment and subsequent granule flotation (Alphenaar, 1994). Flotation of mesophilic (30 °C) granular sludge was found at a sucrose loading of 0.325 g COD g^{-1} VSS day^{-1} and a liquid up-flow velocity of 0.5 m h^{-1} (Alphenaar, 1994). The present study shows that sludge deterioration appears at significantly lower sucrose sludge loading rates under psychrophilic conditions. Possibly, high up-flow velocities (10 m h^{-1}) enhance sludge flotation, especially when granules with a fluffy, acidifying, outer layer are formed. From the observed five times higher SAA compared to MA of the first-stage-sludge sampled at day 79 (Table 4.2.2), it is clear that a considerable acidifying population was already present in period III. Remarkably, a 3-4 times higher SAA compared to MA was also found for the second stage. Apparantly, a high number

of acidifiers was rinsed from the first to the second stage. The subsequent decrease in SAA of the sludge from both stages sampled at day 161, compared to day 79 might be due to sluicing of active acidifying sludge in periods III and IV and the absence of growth of acidifiers in period V.

Compared to mesophilic conditions, under psychrophilic conditions, the acidifiers have a very high biomass yield (0.22 g VSS-COD g^{-1} $COD_{removed}$, Rebac et al., unpublished data), meanwhile the starvation rate is very low (1.57×10^{-5} h^{-1}). This might explain the extremely low SRT prevailing in the first stage during periods III and IV. Additionally, in periods I-V, due to the 12 mg O_2 dm^{-3} present in the waste water, also fast growing facultative aerobic sludge might have developed. The presence of oxygen probably results in the coexistence of anaerobic (acetogenic and methanogenic) bacteria and facultative bacteria consuming oxygen (Shen and Guiot, 1996; Gerritse and Gottschal, 1992).

The system accommodated well the presence of non acidified substrate up to 10 % of the influent COD (Period II). However, at high sucrose loads (period III and IV), acidogenic biomass grew in dispersed form in the reactor. Since this sludge poorly settles, the wash-out of dipersed type of biomass is inevitable at up-flow velocities generally applied in EGSB reactors. The concomitant severe loss of acetogenic and methanogenic biomass in period IV from the first stage, strongly affected the VFA removal efficiencies in the first stage. Nonetheless, also in this period the second stage effectively served as a scavenger of non-degraded VFA, resulting in high 'overall' removal efficiencies (Fig. 4.2.1c).

Our results show that on a long term, the acidogenic and/or facultative population would 'overgrow' the methanogenic and acetogenic population in a psychrophilic reactor fed with partly acidified waste water. Flotation of granular sludge, due to excessive growth of the acidogenic population, determines the need for the development of a proper first stage reactor for the application of anaerobic treatment under psychrophilic conditions. On the other hand, a low O_2 influent concentration resulted in a more or less stable reactor performance even at relative high sucrose loading rates in the first stage (period VI). The significant decrease in the acidifying biomass yield in period VI, might be explained by the fact that facultative and/or aerobic bacteria decrease their growth-yield by a factor 10 under anaerobic conditions (Shen and Guiot, 1996; Gerritse and Gottschal, 1993). In addition, in period VI, a larger fraction of acidifiers might have been strictly anaerobic, characterized by a lower biomass yield. Apparently, when the O_2 concentrations of the discharged waste water is low (< 2 mg O_2 dm^{-3}) and measures are taken to prevent sludge carry-over from the first stage to the second stage, the proposed two stage system, likely provides a feasible process design for psychrophilic anaerobic waste water treatment.

4.2.5 CONCLUSIONS

EGSB reactor systems are very efficient for the anaerobic treatment of low-temperature (8°C) wastewaters. Particularly for the more complex type of waste waters,i.e. non- or partly-

acidified, a two-stage systems is characterized by a stable long term operation, provided the second stage can be operated at a sufficiently high sludge residence time. Production of dispersed growing acidifying sludge can be mimimized by operating the system under strictly anaerobic conditions.

4.2.6 REFERENCES

Alphenaar, A. (1994) Anaerobic granular sludge: characterization, and factor affecting its functioning. PhD. *Thesis*, Agricultural University Wageningen, The Netherlands, pp 93-112.

Banik, G. C and Dague, R. R (1996) ASBR treatment of dilute wastewater at psychrophilic temperatures. *Proceeding of 69th Annual Water Environmental Conference*, Dallas, Texas, USA, pp 235-246.

Colleran, E and Pistili, A (1994). Activity test system for determining the toxicity of xenobiotic chemicals to the methanogenic process. *Ann. Microbiol. Enzimol.*, **44**, 1-20.

Gerritse, J. and Gottschal, J. C (1993) Oxic and anoxic growth of a new Citrobacter species on amino acids. Arch. Microbiol., **160**, 51-61.

Grotenhuis, J. T. C., Smit, M., Plugge, C. M., X.u, Y., van Lammeren, A. A. M., Stams, A. J. M and Zehnder, A. J. B (1991) Bacteriological composition and structure of granular sludge adapted to different substrates. Appl. Environ. Microbiol., **57**, 1942-1949.

Kato, T. M., Field, J. A., Versteeg, P and Lettinga, G (1994) Feasibility of Expanded Granular Sludge Bed Reactors for the anaerobic treatment of low strength soluble wastewaters. *Biotech. Bioeng.*, **44**, 469-479.

Lens, P., Hulshoff Pol, L., Lettinga, G. and van As, H (1997) ^{1}H NMR study of water transport processes in sulfidogenic granular sludge. Wat. Sci. Tech., in press.

Matsushige, K., Inamori, Y., Mizuochi, M., Hosomi M and Sudo, R (1990) The effects of temperature on anaerobic filter treatment for low-strength organic wastewater. *Environ. Technol.*, **11**, 899-910.

Raskin, L., Poulsen, L. K., Noguera, D. R., Rittmann, B. E and Stahl, D. A (1994) Quantification of methanogenic groups in anaerobic biological reactors by oligonucleotide probe hybridization. *Appl. Environ. Microbiol.*, **60**, 1241-1248.

Rebac, S., van Lier, J. B., Janssen, M. G. J., Dekkers, F., Swinkels, K. T. M and Lettinga, G (1997) High-rate anaerobic treatment of malting waste water in a pilot-scale EGSB system under psychrophilic conditions. *J. Chem. Tech. Biotechnol.*, **68**, 135-146.

Rebac S., Ruskova J., Gerbens S., van Lier J. B., Stams A. J. M., Lettinga G. (1995) High-rate anaerobic treatment of wastewater under psychrophilic conditions. *J. Ferment. Bioeng.*, **5**, 15-22.

Shen, C. F. and Guiot, S. R (1996) Long-term impact of dissolved O_2 on the activity of anaerobic granules. *Biotechnol. Bioeng.*, **49**, 611-620.

Chapter 5

Application of an advanced system for psychrophilic treatment of industrial wastewater

Published as \ accepted as:

5.1 Rebac, S., van Lier, J.B., Lens, P., van Cappellen, J., Vermeulen, M., Stams, A.J.M., Dekkers, F., Swinkels, K.Th.M. and Lettinga, G. (1998) Psychrophilic (6-15 °C) high-rate anaerobic treatment of malting waste water in a two module EGSB system. *Biotechnol. Progress*, (in press).

5.1 PSYCHROPHILIC (6 - 15 °C) HIGH-RATE ANAEROBIC TREATMENT OF MALTING WASTE WATER IN A TWO MODULE EGSB SYSTEM

ABSTRACT

Psychrophilic (6 to 15 °C) anaerobic treatment of malting wastewater was investigated using a two module expanded granular sludge bed (EGSB) reactor system with a total volume of 140 dm^3. The reactor system was fed with malting wastewater with a soluble and total chemical oxygen demand (COD) between 233-1778 mg dm^{-3} and 317-4422 mg dm^{-3}, respectively. The anaerobic biodegradability of the malting wastewater was estimated a 73 % at 15 °C. Operation of this system at 6 °C gave removal efficiencies of 47 and 71 % of the average soluble and volatile fatty acids COD, respectively, at organic loading rates (OLR) ranging between 3.3-5.8 kg COD m^{-3} day^{-1} and a hydraulic retention time (HRT) of 4.9 h. In the temperature range 10-15 °C, removal efficiencies for soluble COD and volatile fatty acids COD were 67-78 and 90-96 %, respectively, at an OLR between 2.8 - 12.3 kg COD m^{-3} day^{-1} and a HRT of 3.5 h. The specific methanogenic activity of the sludge present in each module increased 2- to 3-fold during system operation for 400 days. The relatively high concentration of suspended solids in the influent (25 % of the total COD) caused a deterioration of the sludge bed in the first reactor module. This was aggravated by excessive growth of acidifying biomass, which persisted in the first-module sludge bed and resulted in granular sludge flotation. The latter significantly decreased the solid residence time, which caused a drop in the methanogenic capacity of the first module. However, the second module could accomodate the increased OLR, thus providing a very high effluent quality (soluble COD < 200 mg dm^{-3}) of the total system. When module I was fed with highly acidified wastewater, organic matter was well eliminated and converted into methane. The stability of module I concerning suspended solids could be restored by pre-settling the wastewater.

Key words: Expanded granular sludge bed reactor, malting waste water, psychrophilic, volatile fatty acids, methanogenesis, two stage.

5.1.1 INTRODUCTION

Many highly soluble low strength wastewaters with a chemical oxygen demand (COD) of less than 1500 mg dm^{-3} are relatively low in temperature (5-25 °C), e.g. wastewaters from bottling, malting, soft drink, and brewery manufacturing. Anaerobic treatment of these dilute wastewaters can be accompanied with problems due to the presence 5-10 mg dm^{-3} dissolved oxygen concentrations and suboptimal operation temperatures. The direct anaerobic treatment of wastewaters at low temperatures would offer a significant reduction in operational costs, because the amount of energy required for maintaining the reactor temperature in the mesophilic range (30-40°C), accounts for the largest proportion (35 %) of the total energy requirement of a reactor treating cold wastewaters (Mills, 1979).

Since temperature strongly affects the rates of anaerobic bioconversions, adaptation of the conventional process design is likely required in order to apply high volumetric organic loading rates at sub-optimal operation temperatures (Banik & Dague, 1996; Van Lier et al., 1997). The feasibility of high-rate anaerobic wastewater treatment at low ambient temperatures depends on various factors such as: i) the quality of the seed material used and its development under sub-mesophilic conditions; ii) the types of the organic pollutants in the wastewater (Koster & Lettinga, 1985) and iii) the reactor configuration especially, the retention of viable sludge. Despite the low growth rate and the inferior specific activity of the methanogenic consortia at low temperatures, efficient anaerobic treatment can be achieved at temperatures ranging between 10-20 °C at reasonably high volumetric organic loading rates, by using expanded granular sludge bed (EGSB) reactors (Rebac et al., 1995; 1997). The EGSB reactor design in particular is advantageous for anaerobic treatment of dilute wastewaters, because the applied high upflow velocities (4-10 m h^{-1}) guarantees a good contact of the wastewater and the methanogenic sludge (De Man et al., 1988; Frankin et al., 1992; Rinzema et al., 1992; Kato et al., 1994).

Moduling the anaerobic conversion process is another way to distinctly improve the overall treatment efficiency. This particularly is applied for wastewaters that contain a large quantity of soluble and partially soluble, non-acidified compounds (Weber et al., 1984; Cohen et al., 1985; Dinopoulou & Lester, 1989; Komatsu et al., 1991) or to produce an effluent with low VFA concentrations (Wiegant et al., 1986; Van Lier et al., 1994, 1997).

This paper describes the use of a two module EGSB reactor system for the psychrophilic treatment of malting wastewater. The rationale for the module separated process is to create niches where biomass can adapt to the prevailing conditions in each module with respect to substrates and intermediates. The performance of the system as a function of the COD and suspended solids (SS) load is presented. The effects of the operational conditions on the biological and physical-chemical sludge characteristics are also described.

5.1.2 MATERIALS AND METHODS

Experimental set-up

Two stainless steel reactors (internal diameter 0.15 m; height 3.0 m) were used in the two module pilot-scale EGSB reactor system, with a total volume of 140 dm^3, internal reactor settlers included (Fig. 5.1.1). Each module was equipped with a screen type gas-liquid-solid (GLS) separator at the top part (Hong Yu Cai et al., 1988). Biogas production was measured with a wet test gasmeter (Meterfabriek, Dordrecht, The Netherlands) connected to the GLS separator via a water seal. The temperature in the module was measured at a height of 1.5 m by using a Pt-100 electrode and a West 6700 controller (West Instruments Ltd, Brighton, England). Each module was equipped with an external water circuit in which water of the desired temperature was pumped through the jacket of the reactor. Both, wastewater flow and

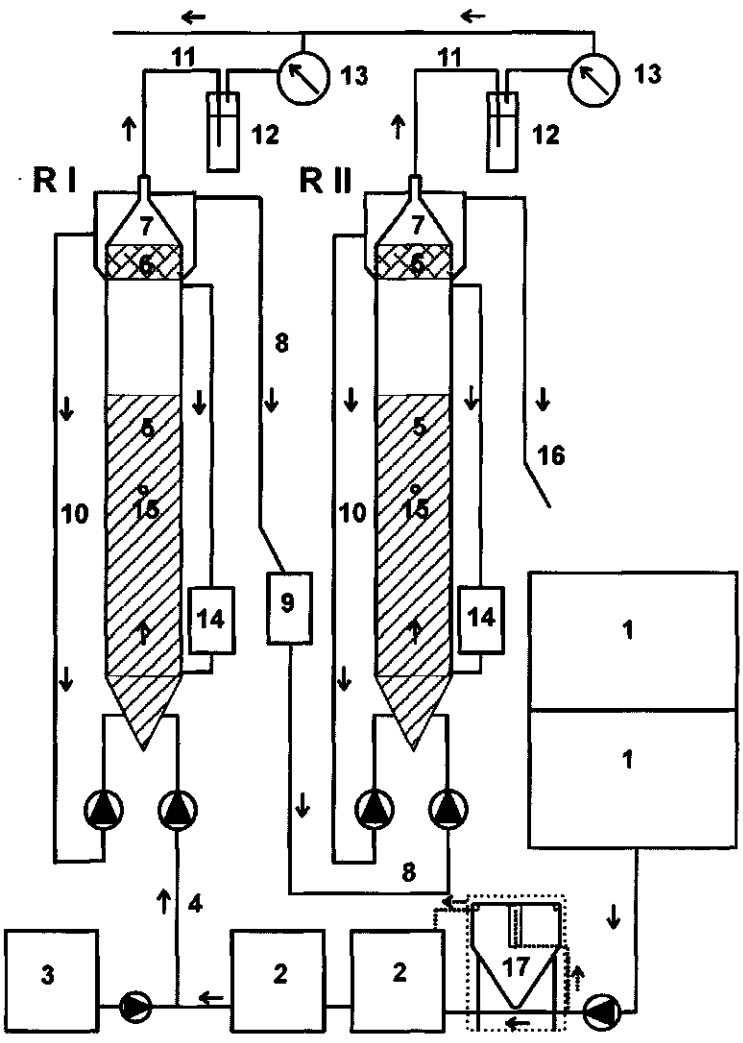

Fig. 5.1.1 Schematic diagram (not in scale) of the 140 dm^3 two module pilot-scale EGSB system used in this study. 1, waste water tanks (2 x 600 dm^3); 2, waste water tanks 2 x 200 dm^3; 3, sodium bicarbonate solution tank; 4, influent first stage; 5, EGSB reactors; 6, segmented drum screens; 7, gas-liquid separators; 8, effluent module I = influent module II; 9, intermediate tank (20 dm^3); 10, recirculation flow; 11, biogas; 12, water seals; 13, wet gas meters; 14, cooling circulator; 15, Pt 100 electrode; 16, effluent second stage; 17, settler (240 dm^3).

effluent recirculation flow, were provided by monopumps (Seepex, Germany) with a maximum flow of 200 and 220 $dm^3\ h^{-1}$, respectively. The wastewater flow and the recirculation flow were mixed at the bottom of each module, resulting in a total superficial upflow velocity between 4 - 6 $m\ h^{-1}$ inside the module. Influent samples were taken from the

wastewater flow prior to mixing. Effluent samples from each module were taken from the module outlets.

Inoculum

The first module was inoculated with fresh mesophilic (20-24 °C) methanogenic granular sludge, originating from a 760 m^3 full scale UASB reactor of the Bavaria brewery (Lieshout, The Netherlands). The second module was inoculated with methanogenic granular sludge cultivated in a 225,5 dm^3 pilot-scale EGSB reactor treating malting waste water at 12-20 °C (Rebac et al., 1997). The latter sludge had been stored unfed at 4°C for about 9 months prior to use. The total amount of granular sludge inoculated on day 0 in module 1 and 2 were approximately 30 and 33 g volatile suspended solids (VSS) dm^{-3}, respectively. For reasons described below, the first module of the system was re-inoculated with 14 and 27 g VSS dm^{-3} of new mesophilic sludge at days 234 and 337, respectively.

Wastewater

The malting wastewater (Table 5.1.1) originated from the batch steep process of a malting factory (Bavaria B.V. Wageningen, The Netherlands). Previously, the malting wastewater was found to be 73 % anaerobically biodegradable at 15 °C (Rebac et al., 1997). Sodium bicarbonate (0.502 - 0.672 g dm^{-3}) was supplied to the wastewater to ensure a reactor pH in the range of 6.5 to 7.5. Considering the wastewater composition, no nutrients were added (Rebac et al., 1997). The composition of the malting wastewater depended on the type of barley used in the steeping process, and its growth conditions prior to harvesting. Fresh wastewater samples were collected daily after 3 hours from the wet steeping process and stored in two 0.2 m^3 tanks each during the first 30 days of operation (Fig. 5.1.1, pos. 2). Thereafter, two extra 0.6 m^3 tanks were used (Fig. 5.1.1, pos. 1).

System operation

Feeding of the system was started immediately after inoculation (day 0) at 13 °C, and at an organic loading rate (OLR) of 8 kg COD m^{-3} day^{-1} and a hydraulic retention time (HRT) of 4.9 hours. In periods I, III and V, the malting wastewater contained a relatively high concentration of suspended solids (SS). In period VI (days 330-390), pre-settled malting wastewater was used as feed (Fig. 5.1.1, pos. 17). The volume of the settler was 240 dm^3. During continuous operation of the system, duplicate samples of the influent and the effluent of both modules were taken three times per week, except for periods days 275-340 and days 345-390, when the samples were taken only once and twice per week, respectively.

Activity tests

Assessment of the specific substrate degradation rates at 10 °C were performed with the seed sludge and reactor sludge samples with acetate, a VFA mixture (acetate : propionate : butyrate = 1 : 1.5 : 1.8, based on COD), ethanol or sucrose as the substrates as described by Rebac et al.

Table 5.1.1 Characteristics of COD load of the malting waste water in the different periods of the investigation. Average values are given between parentheses.

Period		COD_{tot}	COD_{ss}	COD_{sol}	COD^{*}	COD_{vfa}
[days]		[mg COD dm^{-3}]	[mg COD dm^{-3}]	[mg COD dm^{-3}]	[mg COD dm^{-3}]	[mg COD dm^{-3}]
I	0 - 33	1115-4422 (2106)	176-2988 (1053)	485-1751 (1053)	70-708 (222)	241-830 (438)
II	34 - 153	317-2089 (1157)	34-410 (234)	233-1778 (923)	31-196 (94)	70-796 (416)
III	182 - 217	418-1593 (940)	92-386 (227)	276-1258 (713)	14-477 (83)	68-344 (229)
IV	234 - 265	822-1387 (1076)	59-682 (196)	679-1176 (880)	30-265 (107)	197-505 (308)
V	266 - 330	898-1336 (1118)	101-535 (303)	443-1235 (815)	7-206 (40)	125-425 (282)
VI	337 - 393	621-1626 (1042)	109-237 (163)	494-1437 (879)	33-202 (82)	159-913 (410)

*$COD_{\text{identified compounds, without VFA}}$: COD of sugars, ethanol, glycerol, lactate, succinate, citric acid.

(1997). The maximum specific substrate degradation rates (A_{max}) with initial concentration of acetate of 1.5 g COD dm^{-3} and the apparent half saturation constants (K_m) were determined at 10 (±1) °C in 2.5 dm^3 batch reactors intermittently stirred for 10 seconds at 60 rpm, every 6 min. and contained 1.5 g VSS dm^{-3} sludge. Depletion of the acetate concentration was followed until the substrate concentration was below the detection limit (< 0.1 mg COD dm^{-3}).

The apparent K_m values were estimated from substrate depletion curves, by using a Michaelis-Menten derived equation. The substrate conversion rate depends on the biomass concentration and the specific activity of the biomass according to

$$\frac{dS}{dt} = -AX \qquad (1)$$

$$A = A_{max}\frac{S}{K_m + S} \qquad (2)$$

with:	X	biomass concentration	[g VSS dm^{-3}]
	A	substrate degradation rate	[g COD_{acet} g^{-1}VSS day^{-1}]
	S	substrate concentration	[g COD_{acet} dm^{-3}]

Integration of the combined equations (1) and (2) then yields:

$$K_m \ln\left(\frac{S_t}{S_0}\right) + S_t - S_0 = -A_{max}Xt \qquad (3)$$

with :	S_t	substrate concentration at time t	[g COD_{acet} dm^{-3}]
	S_o	initial substrate concentration	[g COD_{acet} dm^{-3}]

A similar equation was successfully used by Wu et al. (1993) and Van Lier et al. (1995) for estimating the apparent K_m of mesophilic and thermophilic sludge. The constants were estimated by using a non-linear regression routine for parameter estimation as described by Rebac et al. (1995). Changes in A_{max} and K_m of the granular sludge, which was present at the bottom (0.2 m) as well as at the top (1.73 m) of each module of the EGSB system, were determined in the period between days 230-390.

Physical-chemical characterisation of the granular sludge

The size distribution and settling properties of the sludge were determined with a modified sedimentation balance, by recording the increase of the weight of the settled sludge fraction as a function of the sedimentation time, as described by Hulshoff Pol et al. (1986). Granule strength was determined as the resistance against axial compression forces according to Hulshoff Pol et al. (1986). Diffusion coefficients were determined at 21 (± 2)°C by using pulsed-field gradient nuclear magnetic resonance (NMR) and subsequent non-linear least square (NLLS) mono-exponential analysis of the data as described by Lens et al. (1997).

Analysis

Total and soluble COD, VFA, ethanol, sugars, glycerol, succinate, lactate, citric acid and the biogas composition (CH_4, H_2) were determined as described by Rebac et al. (1997). Total suspended solids (TSS), VSS and the granule density were analysed according to standard methods (APHA, 1985).

5.1.3 RESULTS

System performance treating cold malting wastewater

The performance of the two module pilot scale EGSB system under psychrophilic conditions (6 to 15 °C) is shown in Figs. 5.1.2, 5.1.4, 5.1.5 and is summarized in Table 5.1.2. The COD conversion to methane (g CH_4-COD g^{-1} $COD_{sol,inf}$) varied in all periods of the investigation. These variations can be attributed to the daily fluctuations in the strength and composition of the malting wastewater (Fig. 5.1.2a and 5.1.2b).

Period I (the first 33 days), can be considered as the start-up period of the system, when the mesophilic methanogenic granular sludge supplied in the first stage was allowed to acclimatize to the new wastewater and the low temperature (11-15°C). The system achieved a COD removal efficiency of about 70 % within one week at an imposed OLR during this period ranging from 2.4 to 8.6 kg COD m^{-3} day^{-1} (Fig. 5.1.2b) and an HRT of 4.9 h. It should be noted that during this period the suspended solids (SS) content of the wastewater was high (COD_{ss}, Table 5.1.1). The SS accumulated in the reactor settler and/or were partly entrapped in the expanded sludge bed of the first stage (data not shown).

In period II, the COD_{sol} removal efficiencies exceeded 70 % at imposed OLRs ranging from 2.8 - 12.3 kg COD m^{-3} day^{-1} at an HRT of 3.5 h (particularly during days 70-153). About 45 % of the malting wastewater was pre-acidified (Fig. 5.1.3). As shown in Fig. 5.1.3, a considerable conversion of COD to methane (up to 40 % of COD_{sol}) already occurred in the module I.

In period III (days 182-217), the system was operated at a shorter HRT of 2.4 h. However, since the influent COD_{sol} concentrations in period III were lower, the imposed OLR remained almost unchanged (Fig. 5.1.2a, b; Table 5.1.1). Nonetheless, the COD_{sol} removal efficiencies of the system dropped to 54 % (Fig. 5.1.2c), when only 26 % of the wastewater was pre-acidified (Table 5.1.1). An additional 20-25 % of the COD_{sol} was acidified in the module I of the system (Fig. 5.1.3). The overall system still provided a high (86 %) COD_{vfa} removal efficiency (Table 5.1.2), despite the extreme loading of the module II (8-16 kg COD m^{-3} day^{-1}, Fig 5.1.4 a2). During this period, the system was again subjected to an increasing levels of the suspended solids, due to changes in the schedule of the barley steeping which led to an accumulation of SS in the wastewater. This caused a severe wash-out of the methanogenic granular sludge from module I during period III. After the end of period III (day 234), module I was re-seeded with 14 g VSS dm^{-3} fresh inoculum.

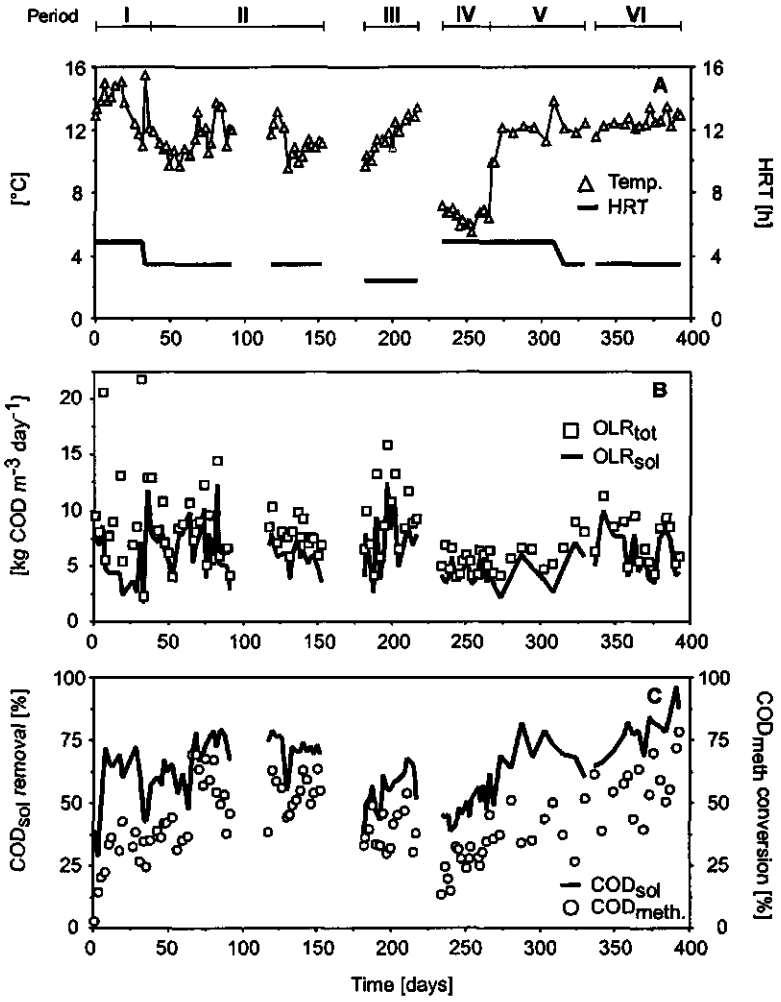

Fig. 5.1.2 Overall operational conditions and overall performance of the two module EGSB system. A. Temperature (Δ) and HRT (—). B. Total organic loading rate based on COD_{tot} (□) and COD_{sol} (—). C. Removal efficiency based on COD_{sol} (—) and Conversion of COD_{sol} to $COD_{meth.}$ (*O*).

In period IV (days 234-265), the EGSB system was exposed to a temperature of 6 °C and the imposed OLR remained at 4.3 kg COD m^{-3} day^{-1} with an HRT of 4.9 h. The removal efficiency of the total system based on COD_{sol} and COD_{vfa} dropped to 47 and 71 %, respectively. During this period, methanogenesis in the module I remained very low (Fig. 5.1.3), which likely, can be attributed to overloading of the fresh mesophilic biomass which had probably a low methanogenic capacity at 6 °C.

In period V (days 266-330), the temperature was increased from 6 to 12 °C (Fig. 5.1.2a), while the HRT was the same as in period IV. The system responded immediately with higher COD_{sol} and COD_{vfa} removal efficiencies. They increased by 46 and 35 %, respectively (Table

Table 5.1.2 Performance data of the two module psychrophilic pilot-scale EGSB system treating malting waste water. Average values are given between parentheses.

Period		OLR_{tot}	OLR_{sol}	HRT	Temp.	$COD_{sol,out}$	VFA_{out}	COD_{sol} removal	VFA removal
	[days]	[kg COD m^{-3} day^{-1}]	[kg COD m^{-3} day^{-1}]	[h]	[°C]	[mg COD dm^{-3}]	[mg COD dm^{-3}]	[%]	[%]
I	0 - 33	7.7 - 21.8 (10.5)	2.4 - 8.6 (5.2)	4.9	11 - 15 (13)	149 - 995 (464)	0 - 613 (178)	29 - 72 (59)	-43 - 100 (67)
II	34 - 153	2.2 - 14.5 (8.0)	2.8 - 12.3 (6.4)	3.5	10 - 15 (11)	133 - 989 (308)	0 - 558 (59)	42 - 79 (67)	54 - 100 (90)
III	182 - 217	4.1 - 15.8 (9.3)	2.6 - 12.5 (7.1)	2.4	10 - 13 (12)	116 - 559 (319)	7 - 198 (64)	36 - 67 (54)	70 - 97 (86)
IV	234 - 265	4.0 - 6.8 (5.3)	3.3 - 5.8 (4.3)	4.9	6 - 7 (6)	332 - 721 (463)	30 - 322 (140)	39 - 57 (47)	46 - 87 (71)
V	266 - 330	4.1 - 9.0 (6.1)	2.2 - 7.1 (4.5)	3.5 - 4.9	10 - 14 (12)	140 - 367 (260)	0 - 66 (22)	49 - 82 (68)	91 - 100 (96)
VI	337 - 393	4.3 - 11.3 (7.2)	3.8 - 9.9 (6.1)	3.5	12 - 14 (13)	22 - 487 (202)	7 - 114 (29)	64 - 96 (78)	93 - 98 (95)

5.1.2). A decrease in the HRT (on day 310) from 4.9 to 3.5 h hardly affected the COD_{sol} and COD_{vfa} removal efficiencies. During this period, the SS content of the wastewater again was high ($COD_{ss} \cong 0.59$ g dm^{-3}, Table 5.1.1). Once again a high continuous wash-out of methanogenic granular sludge occurred from module I.

At the start of period VI (day 337), module I was re-seeded for the second time with 27 g VSS dm^{-3} fresh mesophilic granular sludge. The feed from day 337 to the end of the experiment consisted of pre-settled malting wastewater (Fig. 5.1.1, pos.17) in order to eliminate the performance disturbances caused by high SS concentrations. The measurement of COD_{tot} and COD_{sol} of the malting wastewater before and after the settler showed a reduction in COD_{tot} and COD_{ss} concentrations of 30-40 %, and 80-90 %, respectively, whereas the COD_{sol} remained almost unchanged (data not shown). The COD_{sol} and COD_{vfa} removal efficiencies achieved during period VI, amounting to 78 and 95 %, respectively, were the highest over the whole experimental period.

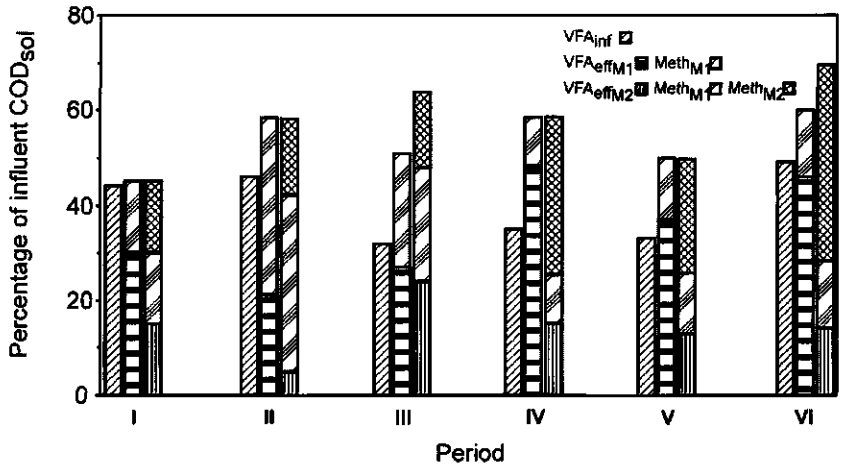

Fig. 5.1.3 Fate of COD_{sol} in the two module EGSB system. First bar 'COD_{vfa}' of the influent, second bar '$COD_{vfa\text{-}M1}$ + $COD_{meth\text{-}M1}$' of the first module, third bar '$COD_{vfa\text{-}M2}$ + $COD_{meth\text{-}M1}$ + $COD_{meth\text{-}M2}$' of the second module and COD_{meth} of the first module expressed as percentage of average influent COD_{sol} in the experimental periods. Note that only 73 % of the COD_{sol} is biodegradable.

Performance of module I versus module II

The performance of each module of the pilot scale EGSB system in the temperature range from 6 to 15 °C is shown in Fig. 5.1.4. Module I was exposed to extremely high variations in OLR from 4 to up to 24 kg COD m^{-3} day^{-1} over the experimental periods (Fig. 5.1.4a1). This affected the COD_{sol} removal efficiency of this module (Fig. 5.1.4b1). In periods III to VI, a relatively high acidification (an additional 10-25 % of influent COD_{sol}) occurred in the module I (Fig. 5.1.3 and 5.1.5 a, b), which resulted in an extensive growth of the acidogenic populations on the methanogenic sludge granules.

It was observed that flotation of granular sludge occurred at sugar sludge loading rates (SLR) ranging from 0.023 to 0.036 g COD g^{-1} VSS day^{-1} (based on the start-up value of the VSS content) and at up-flow velocities of 4-6 m h^{-1} (Periods III, IV and V). The fermentation of non-acidified compounds in module I led to higher hydrogen concentrations in the biogas. In periods I to VI hydrogen concentrations in the biogas of module I ranged between (ppm) 18-54, 65-460, 344-468, 97-368, 29-367 and 101-450, respectively. The higher hydrogen concentrations, were concomitant with a retarded conversion of propionate in module I (Fig. 5.1.5a, b). In module II, the H_2 concentrations ranged between (ppm) 5-11, 10-38, 60-116, 10-119, 20-634 and 30-83, respectively in periods I to VI. In module II, elevated H_2 concentrations were only formed at the end of periods III and V. The latter can be due to the fact that a considerable acidification also took place in module II, as a result of the significant biomass losses in module I.

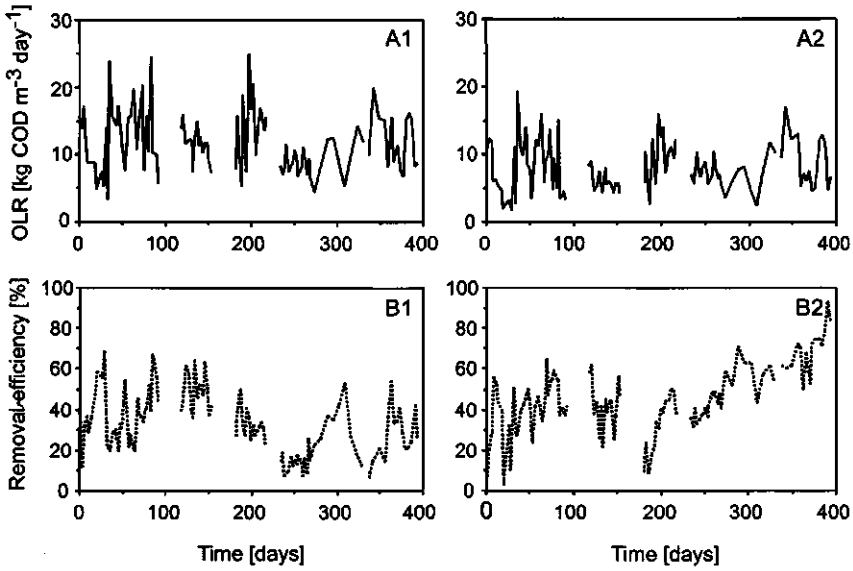

Fig. 5.1.4 Organic loading rate and efficiency of each module of the moduled EGSB system. A. COD_{sol} organic loading rate (—), B. COD_{sol} removal efficiency (----). The numbers following the abbreviation refer to the module of the system.

Metabolic characteristics of the sludge

The measured maximum specific substrate degrading activities (A_{max}) at 10 °C of the inoculum and the sludge sampled at day 140 (Period II) and 217 (end of Period III), with acetate, the VFA mixture, ethanol and sucrose as the substrates, are presented in Table 5.1.3. The maximum specific acetate degrading activities and apparent K_m values of the inoculum and the sludge samples from the bottom and the top of both modules at day 295 (Period IV), 350 (Period V) and 393 (Period VI), are presented in Table 5.1.4. It appears from Table 5.1.3 that the specific acetate and VFA degrading activities at 10 °C of the mesophilic module I

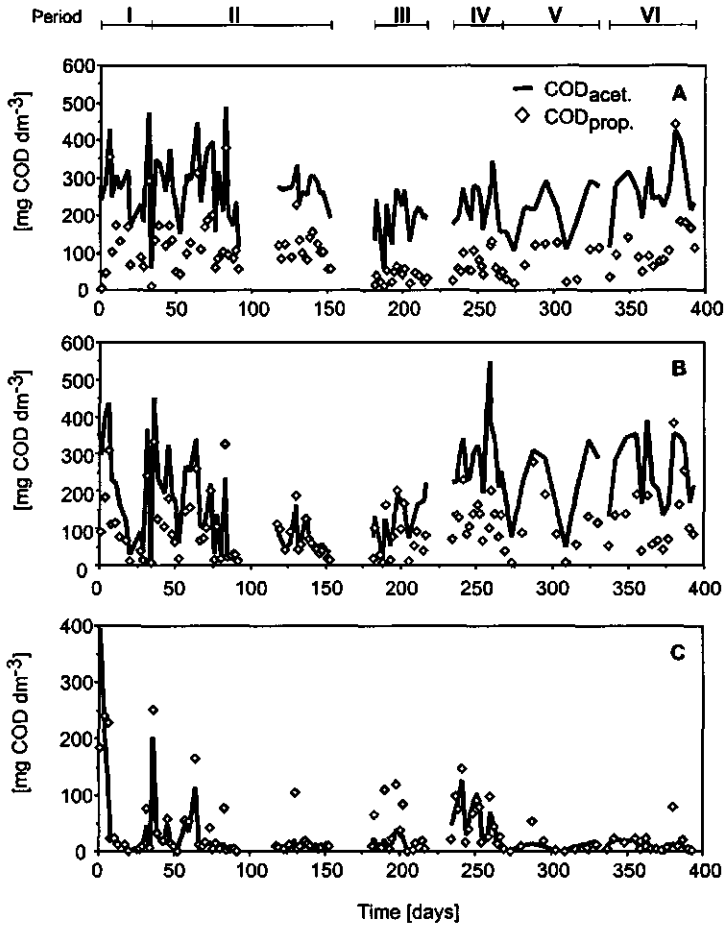

Fig. 5.1.5 VFA concentration in the influent and effluent of each module: A. Influent module I B. Effluent module I C. Effluent module II. $COD_{acet.}$ (—), $COD_{prop.}$ (◊).

inoculum increased by a factor 15 and 20, respectively, after 140 days of continuous operation under psychrophilic conditions (10-13 °C). The activity with acetate and the VFA mixture assessed at the end of period III (day 217) was slightly lower than at day 140. Apparently the sludge was detrimentally affected by sludge washout and sludge bed deterioration that occurred in module I during period III. The high ethanol and sucrose acidifying activity of the sludge from module I at day 217 reflects the high degree of anaerobic acidification in the module I during period III. The conversion pathway of ethanol with the sludge from module I changed considerably during reactor operation. With the seed sludge, acetate was the main intermediate in the conversion of ethanol, while propionate accumulated up to 33 % and 51 % of the initial ethanol COD value with the collected sludge

Table 5.1.3 Maximum specific substrate degrading activities at 10°C of the inoculum and the EGSB sludge. Standard deviation is given between parentheses.

MAXIMUM SPECIFIC SUBSTRATE DEGRADING ACTIVITY [g COD g^{-1} VSS day^{-1}]								
Time	MODULE I				MODULE II			
	Acetate	VFA - mixture	Ethanol♣	Sucrose♣	Acetate	VFA - mixture	Ethanol♣	Sucrose♣
day 0*	0.009 (0.002)	0.005(0.001)	0.127 (0.025)	ND	0.099 (0.009)	0.113 (0.011)	0.241 (0.107)	ND
day 140	0.143 (0.007)	0.091 (0.007)	0.472 (0.036)	ND	0.169 (0.003)	0.103 (0.001)	0.188 (0.019)	ND
day 217	0.104 (0.013)	0.082 (0.000)	0.594 (0.003)	0.874 (0.033)	0.171 (0.009)	0.090 (0.000)	0.354 (0.071)	0.373 (0.061)

ND = Not determined

♣ Ethanol and sucrose acidifying activity.

* Seed sludge after 5 (1st module) and 14 (2nd module) months of storage at 4 °C.

Table 5.1.4 Maximum specific acetate degrading activities (A_{max}) and apparent half saturation constant (K_m) at 10°C of the inoculum and the EGSB sludge. Standard deviation is given between parentheses.

MAXIMUM SPECIFIC ACETATE DEGRADING ACTIVITY [g COD g^{-1} VSS day^{-1}] AND APPARENT K_m [g COD dm^{-3}]				
Time	MODULE I		MODULE II	
	A_{max}	K_m	A_{max}	K_m
day 0	0.009 (0.002)	ND	0.099 (0.009)	ND
day 234*	0.021 (0.001)	0.012 (0.004)	0.093 (0.002)	0.018 (0.012)
Bottom sludge				
day 295	0.043 (0.001)	0.020 (0.009)	0.229 (0.003)	0.142 (0.064)
day 350	0.035♠ (0.001)	0.018♠ (0.001)	0.241 (0.003)	0.169 (0.025)
day 393	0.069 (0.009)	0.048 (0.009)	0.260 (0.020)	0.123 (0.026)
Top sludge				
day 295	0.057 (0.000)	0.026 (0.003)	0.241 (0.002)	0.092 (0.011)
day 350	0.042♠ (0.003)	0.016♠ (0.003)	0.247 (0.002)	0.130 (0.009)
day 393	0.074 (0.007)	0.059 (0.017)	0.274 (0.005)	0.116 (0.009)

*Module I: First re-inoculation with new mesophilic sludge at day 234; Module II: Seed sludge after 20 months of storage at 4°C

♠15 days after second re-inoculation with new mesophilic sludge at day 337

ND = Not determined

samples at day 140 and 217, respectively, (results not shown).

The specific acetate degrading activity of the sludge from the module II after 140 days of operation increased by 58 % compared to the seed sludge. However, its specific activity with the VFA-mixture as the substrate did not increase. The latter observation corroborates with the very low butyrate concentrations (< 30 mg COD_{but} dm^{-3}) in the effluent of the first stage. The specific VFA degrading activity of the sludge sampled from module II on day 217 was lower than that of sludge sampled on day 140 (Table 5.1.3). This very likely, can be attributed to the sludge wash-out from the first stage and subsequent accumulation of these solids in the sludge bed of module II. Indicatively, the ethanol acidifying activity at day 217 of the sludge from module II had increased by 95 %, compared to day 140.

The development of the maximum specific acetate degrading activities and the apparent half saturation constant (K_m) of both, the sludge sampled at the bottom and top from module I during period days 234 - 393 (Table 5.1.4), was temporary prevented by the severe sludge wash-out occurring during period V (days 266-330). The relatively low maximum specific acetate degrading activities of this sludge, between days 234 and 295, can be attributed to the introduction of new sludge in the module I on day 234 (Table 5.1.4). Also the measurement conducted between days 350 and 393, was affected by the re-inoculatation of module I on day 337. Despite the fact that the fresh mesophilic sludge was exposed instantaneously to psychrophilic conditions (6-7 °C), its acetate degrading activity measured at 10 °C doubled in a period of about 60 days (days 234-295). The specific acetate degrading activities of the sludge from the top of module I were higher (7-32 %) than that of the sludge from the bottom of that module. The K_m values for acetate of the sludge sampled from module I on day 393 were higher than those of the sludge samples on day 350.

The maximum acetate degrading activities of the sludge sampled from both the bottom and the top of module II were similar and they steadily increased in time between days 295-393. The apparent K_m values of the sludge in the module II were identical over the height of the reactor and remained unchanged during 159 days of reactor operation. Interestingly, the specific activity of the seed sludge did not change when comparing its activity on day 0 and measured again on day 234 (after 6 months storage at 4 °C). It remained stable at 0.09 g COD g^{-1} VSS day^{-1} (Table 5.1.4).

Physical-chemical characteristics of the sludge

The development of the size distribution of the sludge granules in each module over a period of 217 days is depicted in Fig. 5.1.6. During the first 140 days of operation, the mean diameter of the granules in module I increased significantly (Fig. 5.1.6 A1 and B1). This can be partly attributed to the extensive growth of an acidogenic population as illustrated by its distinct increase in acidifying activity (Table 5.1.3). In contrast, the diameter of the granules in module II hardly increased, probably due to fact that the feed of module II consisted merely of VFA (Fig. 5.1.6 A2 and B2). The strong effect of an acidifying population on the

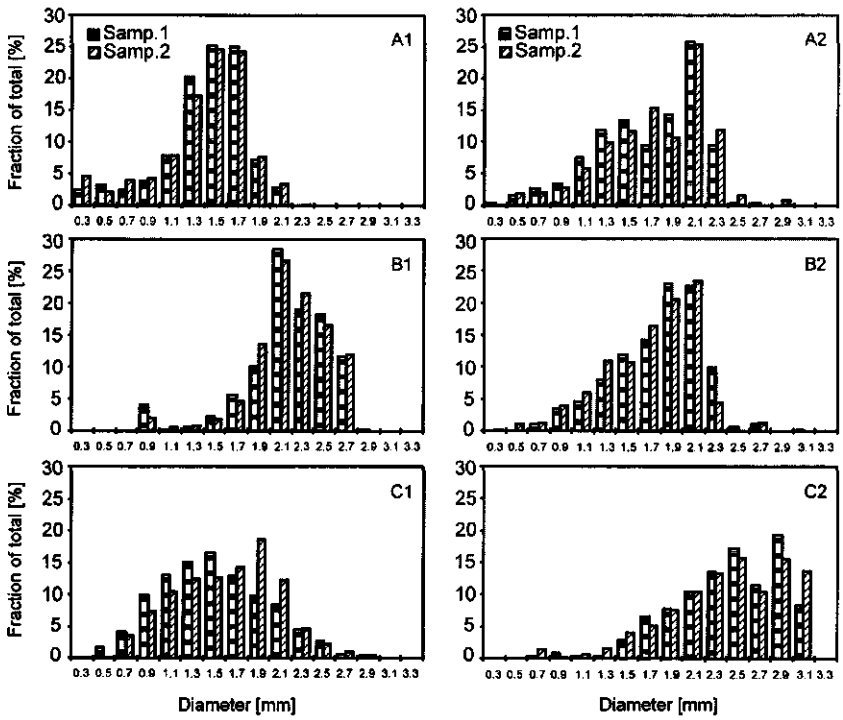

Fig. 5.1.6 Development of the size distribution of mixed granular sludge from each module expressed as percentage of the biomass weight of the granules. (A) Start of the experiment, (B) at day 140 and (C2) day 217. (C1) re-inoculation of the first stage at day 234. Duplicate samples are presented by the bars. The figures following the abbreviation refer to the module of the system.

diameter of sludge granules is also confirmed by results from the sludge sample taken from module II on day 217. In period III, due to a loss of biomass from the module I, the acidogenic organisms grew in the second module and their attachment to the granules resulted in larger granules in module II (Fig. 5.1.6 C2). The size distribution of the seed sludge, used for inoculation of module I on day 234 is depicted in Fig. 5.1.6 C1. Size distribution measurements could not be made on day 217 due to the small amount of sludge that remained in module I.

Table 5.1.5 compares the density, apparent diffusion coefficient, strength and mean diameter of the seed sludge from the bottom and the top of both modules, sampled on days 295, 350 and 393. The difference in the mean diameter between the sludge samples from the bottom and top of the module I (Table 5.1.5), reveals the occurrence of sludge granule segregation over the height of the sludge bed. The sludge samples from module I on day 350 and 393 (Table 5.1.4 and 5.1.5), originate from the second seed sludge used for the re-inoculation of the module I on day 337. The apparent diffusion coefficients of the sludge sampled at the top and the bottom of the module II were very similar during the period

Table 5.1.5 Physical characteristics of the inoculum and the psychrophilic EGSB sludge. Standard deviation is given between parentheses.

DENSITY, APPARENT DIFFUSION COEFFICIENT, STRENGTH AND MEAN DIAMETER OF GRANULAR SLUDGE								
Time	MODULE I				MODULE II			
	Density [kg m^{-3}]	Diff. coefficient [10^{-9} m^2 s^{-1}]	Strength [kN m^{-2}]	Mean diameter [mm]	Density [kg m^{-3}]	Diff. coefficient [10^{-9} m^2 s^{-1}]	Strength [kN m^{-2}]	Mean diameter [mm]
day 234*	1042 (5.6)	1.82 (0.03)	95 (25.4)	1.47 (0.17)	1027 (1.1)	1.33 (0.03)	88 (3.5)	1.78 (0.09)
Bottom sludge								
day 295	1044 (0.1)	1.19 (0.01)	113 (9.6)	2.03 (0.05)	1029 (7.6)	1.47 (0.03)	77 (1.7)	2.40 (0.10)
day 350	1049♠ (4.3)	1.49♠ (0.14)	143♠ (1.7)	2.03 (0.15)	1030 (1.5)	1.31 (0.02)	72 (7.0)	2.40 (0.04)
day 393	1042 (9.7)	0.99 (0.23)	108 (0.0)	2.09 (0.02)	1030 (2.4)	1.28 (0.06)	70 (3.5)	2.43 (0.16)
Top sludge								
day 295	1029 (28.6)	1.18 (0.00)	97 (0.8)	1.78 (0.20)	1023 (0.8)	1.35 (0.01)	73 (4.4)	2.02 (0.13)
day 350	1046♠ (4.9)	2.05♠ (0.03)	121♠ (7.4)	1.26 (0.01)	1022 (0.8)	1.38 (0.02)	71 (1.8)	2.19 (0.08)
day 393	1041 (2.5)	1.44 (0.10)	104 (7.0)	1.54 (0.03)	1034 (0.2)	1.28 (0.08)	68 (0.0)	2.15 (0.05)

*Module I: First re-inoculation with new mesophilic sludge at day 234; Module II: Seed sludge after 20 months of storage at 4 °C
♠15 days after second re-inoculation with new mesophilic sludge at day 337

between days 295-393 (Table 5.1.5). In contrast, the sludge samples from the bottom of the module I, had an apparent diffusion coefficient that was markedly lower between days 350 and 393 (Table 5.1.5). The density of the sludge from module I and II ranged between 1029-1049 and 1022-1034 kg m^{-3}, respectively. The strength of the granules present in module I had increased compared to the inoculum, but it decreased during the course of the experiment. The strength of the sludge samples from the module II decreased to a small extent during the course of the experiment.

5.1.4 DISCUSSION

Reactor performance with highly acidified wastewater (Periods II and VI)

The results obtained in this study show the feasibility of direct anaerobic treatment of malting wastewater under psychrophilic conditions at relatively high volumetric organic loading rates. When treating presettled highly acidified wastewater, the two module EGSB system achieved higher and more constant COD_{sol} removal efficiencies at 11 °C (Table 5.1.2, period VI) compared to 66 % obtained in a single module EGSB system operating at 20°C, treating the same type of malting wastewater (Rebac et al., 1997). It should, however, be noted that the HRT in the two module system was 3.5 h and in the single module system 2.5 h.

When treating highly acidified wastewater, module I achieved a high methanogenic capacity (methanation of about 40 % of influent soluble COD), whereas module II served as a scavenger of non-degraded VFA from module I (Fig. 5.1.3). Comparison of Fig. 5.1.5b with Fig. 5.1.5c shows that when the VFA concentrations entering the module II are below 200 mg COD dm^{-3} (Fig. 5.1.5b, c), the treatment efficiency of that module remain still very satisfactory (period II). This might be attributed to the very low apparent K_m (18 mg COD dm^{-3}) of the sludge (Table 5.1.4). These low K_m values once again support the advantages of using EGSB reactors in anaerobic treatment of low strength wastewaters, as previously found by Kato et al. (1994) and Rebac et al. (1995). Propionate, known as the most difficult VFA intermediate during methanogenic anaerobic degradation (Gujer and Zehnder, 1983; Lettinga, 1995), was efficiently removed by the moduled psychrophilic reactor system (Fig. 5.1.5b, c). The second module offered excellent conditions for propionate removal, as inhibitory effects of oxygen, hydrogen, and other VFAs were minimal in this module. This confirms the good propionate removal efficiency of moduled psychrophilic (8 °C) laboratory scale reactors treating a VFA-sucrose mixture (Van Lier et al., 1997).

When treating low strength highly acidified wastewater, the biomass retention in both modules was excellent and newly grown methanogenic biomass was well retained as granular sludge (Table 5.1.5, Fig. 5.1.6). This allowed the two module EGSB system to accommodate, with a 90 % COD_{vfa} removal efficiency, three to five times higher OLRs of acidified wastewater, compared to those reported so far for other psychrophilic anaerobic wastewater treatment systems (Banik and Dague, 1996; Grant and Lin, 1995; Matsushige et al., 1990; de Man et al., 1988; Switzenbaum and Jewell, 1978). Moreover, the COD removal efficiencies

were very high and comparable to the maximum efficiency of 85 % found during the mesophilic anaerobic treatment of brewery wastewaters at 30 °C at an OLR of 20 kg COD m^{-3} day^{-1} and a HRT of 2.1 h (Pereboom, 1994). Similar results were even found at 37 °C where an efficiency of 80 % was obtained in a three stage system at an OLR of 25 kg COD m^{-3} day^{-1} and a HRT of 6 h (Stadlbauer et al., 1994). The removal efficiencies achieved in this study are even comparable to the results of Perez et al. (1997) who reported an 82 % COD removal by a thermophilic (55 °C) anaerobic fluidized bed reactor operated at an OLR of 32 kg COD m^{-3} day^{-1} and an HRT of 11 h.

Reactor performance with highly unacidified wastewater (periods III, IV and V)

When treating highly unacidified wastewaters, obviously Module I receives the highest OLR and the highest wastewater complexity (Table 1, periods I, III and V), This leads more rapidly to a reactor imbalance, as evidenced by the sludge wash-out, the low COD removal capacity (Fig. 5.1.4a1 versus 5.1.4b1) and the elevated H_2 concentrations in the biogas. In contrast, module II performed stable and remained highly efficient as the feed of this module consisted of a wastewater with a much more constant composition, substantially in highly acidified COD (Fig. 5.1.5b).

The imbalance of acidification and methanogenesis in the module I (Fig. 5.1.3, periods III, IV and V) resulted in elevated H_2 concentrations. The latter probably negatively affected the anaerobic conversion of propionate, as evidenced from the absence of propionate degradation in module I in these periods (Fig. 5.1.5a, b). This phenomena is well known in mesophilic anaerobic waste processing technology (Lettinga, 1995). This study shows that the phenomenon is also valid for psychrophilic conditions (Rebac et al., 1997).

The observed sludge flotation may results from negative effect of the wastewater composition. The presence of even small amounts (< 100 mg dm^{-3}) of surface active compounds (Verstraete et al., 1996) or long chain fatty acids (Rinzema et al., 1989) in the malting wastewater might have contributed to the observed flotation of granular sludge. However, the high content of non-acidified matter was the principal cause of flotation. Acidification of nonacidified organic matter results in the formation of a layer of acidifying sludge around granules. This fluffy coating reduces the settling velocity and can lead to gas entrapment inside the granule. Subsequently, flotation of the granules occurs under the high hydraulic regime, i..e., ten times higher up-flow velocities (4-6 m h^{-1}) compared to UASB reactors (Alphenaar, 1994; Yoda and Nishimure, 1997). It was reported, that flotation of mesophilic granular sludge occurred at a sugar SLR of 0.325 g COD g^{-1} VSS day^{-1} and at a liquid up-flow velocity of 0.5 m h^{-1} (Alphenaar, 1994). Flotation of granular sludge under psychrophilic conditions is enhanced by the very high biomass yield (0.22 g VSS-COD g^{-1} $COD_{removed}$ of glucose at 10 °C, Van Lier et al., 1997), and the extremely low starvation rate (1.57×10^{-5} h^{-1} at 10 °C, Van Lier et al., 1997) of the acidifying bacteria. The presence of oxygen (up to 10 mg dm^{-3}) in the malting wastewater (steeping process-aeration process), enables fast growing facultative anaerobic bacteria, which have 10 times higher growth yields, to develop in the sludge (Shen and Guiot, 1996; Kato et al.,

1993; Gerritse and Gottschal, 1992). They also may form a fluffy coating of sludge. Flotation of granules with a fluffy acidifying outer layer in module I is a persistent problem which requires further research on the growth and granulation rate of acidifiers under psychrophilic conditions.

Also the presence of SS, originating from the barley (period I, III and V, Table 5.1.1), is known to induce a severe wash-out of granular sludge. In this case, it led to a piston formation in the sludge bed, a phenomena, which probably can be due to the relatively narrow reactor diameter at pilot-scale. The degradation of SS in module I can hardly be expected because the hydrolysis rate of SS drops sharply at low temperatures and even approximates zero for various types of solids (Zeeman et al, 1996; Van der Last & Lettinga, 1992; De Man et al., 1986). An effective and plain solution for the problems of these SS was pre-settling of the solids (Period VI, Table 5.1.1).

Biomass characteristics

The maximum specific conversion rate (A_{max}) at 10 °C of the methanogenic sludge in module II increased by a factor 2.5 compared to that of the seed sludge, when operating the system at 6 - 15 °C during a period of 393 days (Table 5.1.4). This indicates an efficient growth and, consequently, enrichment of methanogens in the module II - sludge. Interestingly, the enrichment proceeded even at the very low VFA concentrations supplied (Fig. 5.1.5b). In the period between days 295-393, (see Table 5.1.4) A_{max} values were in the range of those found at 10 °C, for sludge cultivated in a one-step EGSB reactor fed with VFA for 12 months (Rebac et al., 1995). Obviously of considerable practical importance is the observation that the methanogenic sludge preserved its achieved methanogenic activity at 10 °C, even after 6 months storage at 4 °C (see Table 5.1.4, second module, day 0 versus day 234). This indicates that the starvation rate at 4 °C of methanogens grown at these low temperatures is extremely low. Apparently, the cultivation of sludge on low strength wastewater under psychrophilic conditions enables slow, but stable enrichment of a methanogenic consortium.

It should be noted that when psychrophilic sludges are tested under mesophilic conditions, the extremely high (8-fold increase) maximum specific conversion rates were obtained (Rebac et al., 1995). Cultivation of granular sludge under psychrophilic conditions can be a method to produce sludges with an extremely high methanogenic potential when applied in mesophilic reactor systems. Futhermore, these sludge types can be used to reduce the start-up time of both psychrophilic and mesophilic reactors, substantially.

The acetate degrading activity (Table 5.1.3 and 5.1.4) of the sludge present in module I, slowly developed at 10 °C, probably due to high inflow of non-acidified COD and suspended solids. Table 5.1.4 shows that, at low operating temperatures (< 10 °), longer periods are required to achieve an acetate degrading activity comparable to values obtained at 15 °C (Rebac et al., 1997). This is supported by results of continuous flow experiments, where a temperature increase from 6 to 12 °C (period V), significantly increased the COD removal rate in module I (Fig. 5.1.4 b1). Hence, the methanogenic activity of the mesophilic

seed sludge is of utmost importance for the start-up of high-rate psychrophilic anaerobic reactor (< 15 °C), when considering that at 10 °C, fresh active mesophilic sludge expresses only 10-12 % of its methanogenic activity at 30 °C (Lettinga, 1978; Henzen and Harremoes, 1983; Rebac et al., 1995). This indicates that temperature determines the maximum applicable OLR of a psychrophilic EGSB system. In view of the OLR fluctuations when treating real wastewater under psychrophilic conditions, this may have a strong influence on the performance of the system, particularly when the mesophilic inoculum does not have yet sufficient methanogenic capacity.

The intermediate product formation in the granular sludge is influenced by both the organic and inorganic compounds present in the wastewater (Stams, 1994). The conversion pathway of ethanol degradation changed considerably during operation of module I (period 0-217 days). At the end of this period propionate accumulated as the major intermediate while acetate accumulated using the seed sludge. This might be explained by the continuous presence of a small amount of sulphate (50 mg SO_4^{2-} dm^{-3}) in the malting wastewater (Rebac et al., 1997). The formation of propionate during anaerobic ethanol degradation can be attributed to the presence of *Desulfobulbus propionicus* population in the granular sludge, a sulphate reducing bacteria known to convert ethanol into propionate when growing in the absence of sulphate (Stams et al., 1984; Wu et al., 1991).

The increase of the apparent K_m of the sludge in module I at days 234 and 393 compared to days 295 and 350 (Table 5.1.4, 5.1.5), might be attributed to the formation of extracellular polymers (Vanderhaegen et al., 1992; Bhatti et al., 1995), or to extensive growth of acidifying bacteria on the surface of methanogenic granules (Alphenaar, 1994). Interestingly, the observed increase in K_m was accompanied by a severe drop in the apparent diffusion coefficient (Table 5.1.5). The larger granules formed that segregated to the bottom (Table 5.1.5, mean diameter) and had a lower activity (Table 5.1.4), possibly due to substrate diffusion limitation (Wijffels et al., 1995; Dolfing, 1985). In contrast, small changes in apparent K_m and apparent D_{eff} coefficient of the sludge present in module II over a long period of time (days 295-393, Table 5.1.4 and 5.1.5) as results of the slow growth of methanogens at low temperature. The small differences in the mean diameter of the sludges sampled on days 295, 350 and 393 of the bottom and the top (Table 5.1.5, mean diameter) are probably due to the mixing of granules over the sludge bed on the long term (295 days). So, for instance overloading of the module II in period IV (average OLR for second stage was 7.9 kg COD m^{-3} day^{-1} at 6 °C), and resulted in a slight flotation of granules.

The strength of the granules in the module I initially increased (Table 5.1.5), possibly as a result of the fast additional formation of extracellular polymers inside the granules (Vanderhaegen et al., 1992). However, later the strength of the granules dropped, probably due to the formation of an attached fluffy acidifying layer (Hulshoff Pol et al., 1986). Compared to the seed material, also the granules from the module II decreased in strength, which confirms previous findings with mesophilic granules fed with ethanol (Kato et al., 1994) and acidified wastewaters (Pereboom, 1994).

5.1.5 CONCLUSIONS

1. Cold (8-12 °C), low strength (< 1000 mg COD dm^{-3}), acidified (60-70 %) wastewaters can be efficiently treated in a 'high-rate' anaerobic EGSB reactor configuration. The results of the present investigations clearly reveal the prospects of full scale application of anaerobic treatment for highly acidified low strength wastewater under psychrophilic conditions.
2. When applying a two module EGSB system, anaerobic treatment of acidified malting wastewater in the temperature range 8-12 °C can be accomplished at an OLR up to 12 kg COD m^{-3} day^{-1} and at an HRT of 3.5 h. Moduling the EGSB system markedly improves the performance and stability of the system compared to a one-step process. When operates properly a moduled system provides a remarkable long term stable performance at low temperatures even when imposing the system with strong variations in OLR between 3 to 12 kg COD m^{-3} day^{-1}.
3. The maximum specific conversion rate of the methanogenic seed sludge in the second module increased by a factor 2.5 within 393 days of operation at 6 - 15 °C. This study clearly indicates that mesophiles grow and methabolize at suboptimal temperatures. Newly grown viable methanogenic biomass is sufficiently well retained in the second module. The methanogenic sludge preserved its methanogenic activity achieved under psychrophilic conditions, even after prolonged unfed storage period (6 months at 4 °C).
4. Granular sludge flotation comprises problem in the first module for insufficiently acidified wastewaters. This can be attributed to excessive growth of acidogenic and facultative anaerobic populations on the methanogenic granules. It is, therefore, needed to apply a pre-acidification step of which the design criteria should be assessed.
5. High suspended solids concentrations in the influent negatively affect the sludge and sludge bed characteristics of the EGSB reactor. At least a part of these suspended solids present in the original wastewater should be removed, prior to feeding the wastewater in the EGSB system.

5.1.6 ACKNOWLEDGEMENTS

The financial support of Bavaria BV, Lieshout, The Netherlands is gratefully acknowledged. We would like to thank Jim Field for his critical remarks on the manuscript, J. van der Laan and I. Bennehey for their technical assistance with the chromatographs and S. Hobma for his assistance in performing some experiments.

5.1.7 NOTATION

A_{max} = specific substrate degrading activity [g COD g^{-1} VSS day^{-1}]
COD = chemical oxygen demand [g O_2 dm^{-3}]
COD_{tot} = total chemical oxygen demand [g O_2 dm^{-3}]
COD_{sol} = soluble chemical oxygen demand [g O_2 dm^{-3}]

COD_{ss} = chemical oxygen demand of suspended solids [g O_2 dm^{-3}]
COD_{vfa} = VFA chemical oxygen demand [g O_2 dm^{-3}]
COD_{meth} = chemical oxygen demand of methane[g O_2 dm^{-3}]
COD_{acet} = chemical oxygen demand of acetate[g O_2 dm^{-3}]
COD_{prop} = chemical oxygen demand of propionate[g O_2 dm^{-3}]
COD_{butyr} = chemical oxygen demand of butyrate[g O_2 dm^{-3}]
EGSB = expanded granular sludge bed
HRT = hydraulic retention time [hours]
K_m = apparent half saturation constant [g COD dm^{-3}]
OLR = organic loading rate [g COD dm^{-3} day^{-1}]
SLR = sludge loading rate [g COD g^{-1} VSS day^{-1}]
SS = suspended solids
t = time [days]
UASB = upflow anaerobic sludge bed
v = up-flow velocity [m h^{-1}]
VFA = volatile fatty acids [g dm^{-3}]
VSS = volatile suspended solids [g dm^{-3}]

5.1.8 LITERATURE CITED

Alphenaar, A. Anaerobic granular sludge: characterization, and factor affecting its functioning. PhD. Thesis, Agricultural University Wageningen, The Netherlands, pp 93-112, 1994.

American Public Health Association Standard methods for the examination of water and wastewaters. 16th edition. Washington DC, USA, 1985.

Banik, G. C.; Dague, R. R. ASBR treatment of dilute wastewater at psychrophilic temperatures. In *Proc. of 69th Annual Water Environmental Conference*, Dallas, Texas, USA, 1996; pp 235-246.

Bhatti, Z.I.; Furukawa, K.; Fujita, M. Comparative composition and characteristics of methanogenic granular sludges treating industrial wastes under different conditions. *J. Ferment. Bioeng.*, **1995**, 3, 273-280.

Cohen, A.; Breure, A. M.; Van Andel, J. G.; Van Deursen, A. Significance of partial pre-acidification of glucose for methanogenesis in an anaerobic digestion process. *Appl. Microbiol. Biotechnol.*, **1985**, 21, 404-408.

Dinopoulou, G.; Lester, J. N. Optimization of a two-phase anaerobic digestion system treating a complex wastewater. *Environ. Tech. Letters*, **1989**, 10, 799-814.

Dolfing, J. Kinetics of methane formation by granular sludge at low substrate concentrations. The influence of mass transfer limitation. *Appl. Microbiol. Biotechnol.*, **1985**, 22, 77-81.

Dusschoten, van D.; de Jager, P. A.; van As, H. Extracting diffusion constants from echo-time-dependent PFG NMR data using relation-time information. *J. Magn. Reson.*, **1995**, 116, 22-28.

Frankin, R. J.; Koevoets, A. A.; van Gils, W. M. A.; van der Pas, A. Application of the BIOBED upflow fluidized bed process for anaerobic waste water treatment. *Wat. Sci. Tech.*, **1992**, 25, 373-382.

Gerritse, J.; Gottschal, J. C Oxic and anoxic growth of a new Citrobacter species on amino acids. *Arch. Microbiol.*, **1993**, 160, 51-61.

Grant, S.; Lin, K. C. Effects of temperature and organic loading on the performance of upflow anaerobic sludge blanket reactors. *Can. J. Civ. Engin.*, **1995**, 22, 143-149.

Guiot, S. R.; Arcand, Y.; Chavarie, C. Advantages of fluidization on granule size and activity development in upflow anaerobic sludge bed reactors. *Wat. Sci. Technol.*, **1992**, 26, 897-906.

Gujer, W.; Zehnder, A. J. B. Conversion processes in anaerobic digestion. *Wat. Sci. Technol.*, **1983**, 15, 127-167.

Henzen, M.; Harremoes, P. Anaerobic treatment of waste water in fixed film reactors-literature review. *Wat. Sci. Technol.*, **1983**, 15, 1-102.

Hong, Yu Cai; Lettinga, G., Willers, H. Use of the modified UASB for the anaerobic treatment of vinasse from beet sugar molasses. In: *Proc. of the Fifth International Symposium on Anaerobic Digestion.* ed. ER Hall and PN Hobson, Bologna, Italy, 1988; pp 533-539.

Hulshoff Pol, L. W.; van de Worp, J. J. M.; Lettinga, G.; Beverloo, W. A. Physical characterization of anaerobic granular sludge. In: *Proc. of International Conference Anaerobic Treatment A Grown-up Technology*, Amsterdam, The Netherlands, 1986; pp 89-101.

Kato, M. T.; Field, J. A.; Lettinga, G. Methanogenesis in granular sludge exposed to oxygen. FEMS *Microbiol. Lett.*, **1993**, 11, 317-324.

Kato, T. M.; Field, J. A.; Versteeg, P.; Lettinga, G. Feasibility of Expanded Granular Sludge Bed Reactors for the anaerobic treatment of low strength soluble wastewaters. *Biotech. Bioeng.*, **1994**, 44, 469-479.

Komatsu, T.; Hanaki, K.; Matsuo, T. Prevention of lipid inhibition in anaerobic processes by introducing a two-phase system. *Wat. Sci. Technol.*, **1991**, 23, 1189-1200.

Koster, I. W.; Lettinga, G. Application of the upflow anaerobic sludge bed (UASB) process for treatment of complex wastewaters at low temperatures. *Biotechnol. Bioengin.*, **1985**, 27, 1411-1417.

Last van der, A. R. M.; Lettinga, G. Anaerobic treatment of domestic sewage under moderate climatic (Dutch) conditions using upflow reactors at increased superficial velocities. *Wat. Sci. Technol.*, **1992**, 25, 167-178.

Lens, P.; Hulshoff Pol, L.; Lettinga, G.; Van As, H. ^{1}H NMR study of water transport processes in sulfidogenic granular sludge. *Wat. Sci. Technol.*, **1997**, in press.

Lettinga, G. Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek*, **1995**, 67, 3-28.

Lettinga, G.; Hulshoff Pol, L. W. UASB-process design for various types of wastewaters. *Wat. Sci. Technol.*, **1991**, 24, 87-107.

Lettinga, G.; Hulshoff Pol, L. W.; Wiegant, W.; de Zeeuw, W. J.; Klapwijk, A. Use of the Upflow Anaerobic Sludge Blanket (UASB) reactor. *Biotechnol. Bioeng.*, **1980**, 22, 699-734.

Lettinga, G. Feasibility of anaerobic digestion for the purification of industrial waste waters. In: *Proceeding 4th Symposium EAS*, München, Germany, 1978; pp 226-256.

Lier van, J. B.; Boersma, F.; Debets, M. M. W. H.; Lettinga, G. High-rate thermophilic anaerobic wastewater treatment in compartmentalized upflow reactors. *Wat. Sci. Technol.*, **1994**, 30, 251-261.

Lier van, J. B.; Sanz Martin, J. L.; Lettinga, G. Effect of temperature on the anaerobic thermophilic conversion of volatile fatty acids by dispersed and granular sludge. Wat. Res., **1996**, 30, 199-207

Lier van, J. B.; Rebac, S.; Lens, P.; Bijnen van, F.; Oude Elferink, S. J. W. H.; Stams, A. J. M.; Lettinga, G. Anaerobic treatment of partly acidified wastewater in a two-stage expanded granular sludge bed (EGSB) system at 8 °C. *Wat. Sci. Technol.*, **1997**, 36, 317-324.

Man de, A. W. A.; van der Last, A. R. M.; Lettinga, G. The use of EGSB and UASB anaerobic systems for low strength soluble and complex wastewaters at temperatures ranging from 8 to 30°C. In: *Proc. of the Fifth International Symposium on Anaerobic Digestion*. ed. ER Hall and PN Hobson, Bologna, Italy, 1988; pp 197-209.

Man de, A. W. A.; Grin, P. C.; Roersma, R. E.; Grolle, K. C. F.; Lettinga, G. Anaerobic treatment of municipal wastewater at low temperatures. In: *Proc. Conference Anaerobic Treatment a grown up Technology*, Amsterdam, The Netherlands, 1986; pp 453-466.

Matsushige, K.; Inamori, Y.; Mizuochi, M.; Hosomi, M.; Sudo, R. The effects of temperature on anaerobic filter treatment for low-strength organic wastewater. *Environ. Technol.*, **1990**, 11, 899-910.

Mills, P.J. Minimisation of energy input requirements of an anaerobic digestor. *Agricult. Wastes*, 1979, 1, 57-66.

Nyns, E.J. A guide to successful industrial implementation of biomethanisation technologies in the European Union, Successes and Guidelines. Report \prepared for the European Commission, Directorated general for energy, Thermie programme, DG XVII, 1994.

Pereboom, J. H. F. Methanogenic granule development in full scale internal circulation reactors. *Wat. Sci. Technol.*, **1994**, 30, 211-221.

Péres, M.; Romero, L. I.; Sales, D. Thermophilic anaerobic degradation of distillery wastewater in continuous-flow fluidized bed bioreactors. *Biotechnol. Prog.*, **1997**, 13, 33-38

Rebac, S.; van Lier, J. B.; Janssen, M. G. J.; Dekkers, F.; Swinkels, K. T. M.; Lettinga, G. High-rate anaerobic treatment of malting waste water in a pilot-scale EGSB system under psychrophilic conditions. *J. Chem. Tech. Biotechnol.*, **1997**, 68, 135-146.

Rebac, S.; Ruskova, J.; Gerbens, S.; van Lier, J. B.; Stams, A. J. M.; Lettinga, G. High-rate anaerobic treatment of wastewater under psychrophilic conditions. *J. Ferment. Bioeng.*, **1995**, 5, 15-22.

Rinzema, A.; van Veen, H.; Lettinga, G. Anaerobic digestion of triglyceride emulsions in expanded granular sludge bed upflow reactors with modified sludge separators. *Environ. Technol.*, **1993**, 14, 423-432

Rinzema, A.; Alphenaar, A.; Lettinga, G. The effect of lauric acid shock loads on the biological and physical performance of granular sludge in UASB reactors digesting acetate. *J. Chem. Tech. Biotechnol.*, **1989**, 46, 257-266.

Stadlbauer, E. A.; Oey, L. N.; Weber, B.; Jansen, K.; Weidle, R.; Löhr, H.; Ohme, W.; Döll, G. Anaerobic purification of brevery wastewater in biofilm reactors with and without a methanation cascade. *Wat. Sci. Technol.*, **1994**, 30, 395-404

Shen, C. F.; Guiot, S. R. Long-term impact of dissolved O2 on the activity of anaerobic granules. *Biotechnol. Bioeng.*, **1996**, 49, 611-620.

Stams, A. J. M. Metabolic interactions between anaerobic bacteria in methanogenic enviroments. *Antonie van Leeuwenhoek*, **1994**, 66, 271-294.

Stams, A. J. M.; Kremer, D. R.; Nicolay, K.; Weenk, G. H.; Hansen, T. A. Pathway of propionate formation in *Desulfobulbus propionicus*. *Arch. Microbiol.*, **1984**, 139, 167-173.

Switzenbaum, M. S.; Jewell, W. J. Anaerobic attached film expanded bed reactor treatment of dilute organics. In: *Proceedings of the 51st Annual WPCF Conference*, Anaheim, California, USA, 1978, pp 1-164.

Vanderhaegen, B.; Ysebaert, E.; Favere, K.; Van Wanbeke, M.; Peeters, T.; Panic, V.; Vandenlangenbergh, V.; Verstraete, W. Acidogenesis in relation to in-reactor granule yield. *Wat. Sci. Tech.*, **1992**, 25, no. 7, 21-30

Verstraete, W.; de Beer, D.; Pena, M., Lettinga, G.; Lens, P. Anaerobic bioprocessing of waste. *World J. of Microbiol. Biotechnol.*, **1996**, 12, 221-238.

Wijffels, R. H.; Englund, G.; Hunik, J. H.; Leenen, E. J. T. M.; Bakketun, Á.; Obón de Castro, J. M., Tramper, J. Effects of limitation on immobilized nitrifying microorganisms at low temperatures. *Biotechnol. Bioeng.*, **1995**, 45, 1-9.

Wheatley, A. Anaerobic digestion: a waste treatment technology. Elsevier Applied Science, London, UK, 1990.

Wiegant, W. M.; Hennik ,M.; Lettinga, G. Separation of the propionate degradation to improve the efficiecy of thermophilic anaerobic treatment of acidified wastewaters. *Wat. Res.*, **1986**, 20, 517-524.

Wu, W-M.; Thiele, J.H.; Mahendra, K J.; Zeikus, J. G. Methabolic properties and kinetics of methanogenic granules. *Appl. Microbiol. Biotechnol.*, **1993**, 39, 804-811.

Wu, W-M.; Hickey, R. F.; Zeikus, J.G. Characterization of metabolic performance of methanogenic granules treating brewery wastewater: Role of sulfate-reducing bacteria. *Appl. Environ. Microbiol.*, **1991**, 57, 3438-3449.

Yoda, M.; Nishimure, S. Controling granular sludge flotation in UASB reactors. *Wat. Sci. Technol.*, **1997**, 36, 579-586.

Zeeman, G.; Sanders, W. T. M.; Wang, K. Y.; Lettinga, G. Anaerobic treatment of complex wastewater and waste activated sludge. In *Proc. Conference Advanced Wastewater Treatment*, Amsterdam, The Netherlands, 1996, pp 225-232.

Chapter 6

Psychrophilic anaerobic treatment of low strength wastewaters General discussion and conclusions

Published as \ accepted as:

6.1 Rebac, S., van Lier, J.B., Lens, P., Stams, A.J.M., Dekkers, F., Swinkels, K.Th.M. and Lettinga, G. (1998) Psychrophilic anaerobic treatment of low strength wastewaters, *Wat. Sci. Tech.*, (accepted for publication).

6.1 PSYCHROPHILIC ANAEROBIC TREATMENT OF LOW STRENGTH WASTEWATERS
General Discussion and Conclusions

6.1.1 INTRODUCTION

Under moderate climate conditions, the ambient temperatures of many wastewaters are considerably lower than the optima of the biological wastewater treatment processes, i.e. nitrification, denitrification and mesophilic methanogenisis. These "cool" wastewaters comprise leachates of landfills or sulphidic areas, urban drain-off water and domestic sewage as well as several industrial wastewaters, such as particularly those from the food (canning) and drink (bottling, malting and brewery) industry. The COD concentrations of these wastewaters are generally relatively low (< 1500 mg COD dm^{-3}). One of the major concerns in anaerobic low-temperature reactors is the very low biogas production rate, which may result in a low mixing intensity and a poor substrate-biomass contact. Another major point of concern, particularly for low strength wastewaters is accomplish an extremely well biomass retention. Because temperature strongly affects the anaerobic conversion process, changes to the conventional design are likely required if high-rate reactor systems are going to be applied at 'sub-optimal' temperatures. The reactor design has to guarantee efficient bioconversion under these extreme conditions. A attractive and feasible possible reactor system under these conditions comprises the expanded granular sludge bed (EGSB) reactor (De Man et al., 1988; Van der Last and Lettinga, 1992; Kato, 1994).

This thesis describes the results of the research on concerning the feasibility of the EGSB reactor system for the anaerobic treatment of the low strength wastewaters under psychrophilic conditions. The performance and design of this high-rate anaerobic reactor under psychrophilic conditions, for variety of substrates including malting wastewater was investigated, and in connection with that the temperature dependence of the kinetic parameters of the granular sludge after it has been exposed for prolonged period of time under low temperature were determined. The feasibility of a single module EGSB reactor system for the anaerobic treatment of malting wastewater at low temperatures was assessed. Furthermore the research was focused on process optimization with respect to application to partly acidified wastewater in order to realize a stable treatment process with the highest possible efficiency and at the lowest temperature.

6.1.2 START-UP OF EGSB REACTOR UNDER PSYCHROPHILIC CONDITIONS

The EGSB reactor was inoculated with mesophilic granular sludge from a full scale UASB reactor treating brewery wastewater. The use of granular sludge aggregates allows application of up-flow velocities up to 10 m h^{-1}, which provides sufficient expansion of the sludge bed in the reactor. A successful start-up of EGSB reactors under temperatures below 15 °C requires

an higher initial biomass concentration of 30 g VSS dm^{-3} to alleviate strong negative effect of low temperature on capacity of mesophilic sludge (Chapters 2.1; 3.2, 5).

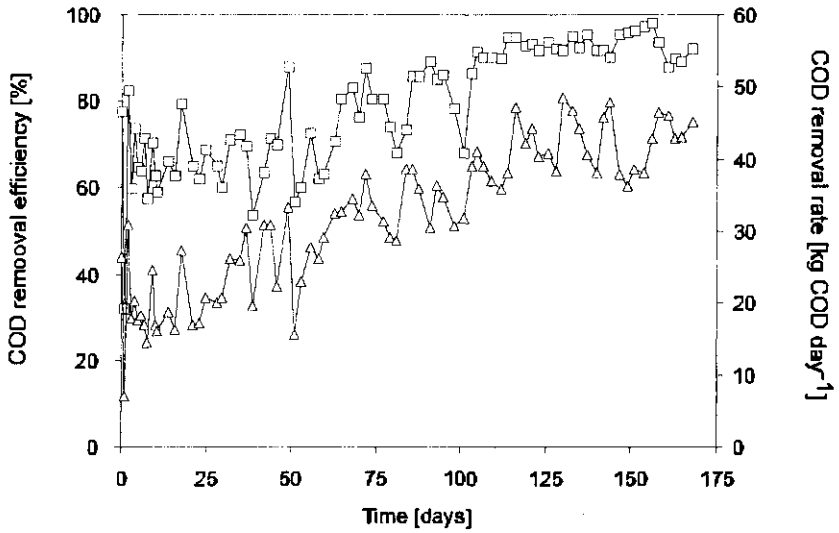

Fig. 6.1.1 Start-up of a 4 l EGSB reactor fed with low strength VFA mixtures at 10-12 °C. (□) Removal efficiency, (Δ) removal rate (Chapter 2.1)

In experiments of Kato (1994) in fact also higher quantities of sludge should have been applied in order to enable the application of high (or very high) organic loading rates. When during the start-up, the temperature is set immediately in the low temperature range (10-15 °C), removal efficiencies for low strength acidified wastewater are expected not to exceed 40-60 % at imposed space loading rates of 8 kg COD m^{-3} day^{-1}. However, as shown in Fig. 6.1.1, a full and stable conversion capacity of psychrophilic EGSB systems already was achieved within 3 months. The system was capable to accommodate even an organic loading rate as high as 12 kg COD m^{-3} day^{-1} (Chapter 2.1). When using malting wastewater (anaerobically biodegradable of 73 % at 15 °C), two months were needed for a pilot-scale EGSB reactor to achieve 70 % COD reduction at 16 °C at its full capacity of 8-10 kg COD m^{-3} day^{-1}, at a HRT of 2.4 h (Chapter 3.2). The reasons for the relatively long start-up period lays in the fact that the methanogenic capacity of the mesophilic granular sludge is strongly affected by lowering the temperature (Lettinga, 1978; Henze and Harremoes, 1983). Hence, assessment of the development in the methanogenic activity of a mesophilic seed sludge when exposed to low temperature is of utmost importance with respect to the start-up of high-rate psychrophilic anaerobic reactors (Chapters 2.2, 4.1 and 5.1).

6.1.3 GRANULAR SLUDGE CHARACTERISTICS

6.1.3.1 Metabolic characteristics

The long-term operation performance of the lab-scale and pilot-scale reactor systems reveals a quite satisfactory development of the methanogenic activity of the mesophilic sludge, under low temperature conditions. However, so far, the presence of substantial fraction of psychrophilic bacteria in the sludge remains unclear. The assessed temperature response curves of the psychrophilically (3-12 °C) grown sludge still reveal a clear optimum temperature in the mesophilic range (30-40 °C) (Table 6.1.1, 6.1.2). But this obviously not means those psychrophilic organisms completely would be absent (Chapter 2.1, 2.2 and 4.1).

Table 6.1.1 Temperature dependence of the maximum specific degrading activities of mesophilic seed granular sludge from full scale UASB reactor with various substrates. Standard deviation is given between parentheses.

Temperature	MAXIMUM SPECIFIC ACTIVITY [g COD g^{-1} VSS day^{-1}]		
(°C)	Acetate	Propionate	Butyrate
10	0.090 (0.000)	0.050 (0.002)	0.050 (0.010)
20	0.380 (0.010)	0.301 (0.020)	0.172 (0.013)
30	0.980 (0.120)	0.551 (0.031)	0.331 (0.042)

In case these psychrophilic homologues would have developed in the sludge, they likely cannot sufficiently manifest due to presence of the large amounts of mesophiles. Since, the specific methanogenic activity of the seed sludge improved significantly during the course of the experiment, low temperatures apparently do not hamper the growth and enrichment of methanogens and acetogens (Table 6.1.1 and 6.1.2). The substrate degrading activities of the sludge at 30 °C (Table 6.1.2) are very high, viz. exceeding the values found previously for a typical mesophilic granular sludge by Alphenaar, (1994) and Kato (1994) and even approaching those found for a sludge from thermophilic 55-65 °C anaerobic reactors (van Lier, 1996).

The high propionate degrading activities measured at 5 °C indicate that the existing propionate oxidizing population maintained its activity (Chapter 4.1) but they surprisingly don't show clear evidence of growth-in of propionate oxidizers. The results in Chapter 4.1 reveal a significant accumulation of the acetate concentration during propionate oxidation at 5 °C.

As propionate oxidizers are known as sensitive micro-organisms (Boone and Bryant, 1980), the growth of this micro-organisms might have become inhibited by acetate (Van Lier et al., 1993). Hydrogen in these experiments very likely didn't prevail, thus could not contribute to inhibition of propionate (Chapter 4.1). A higher hydrogen concentration in biogas indicates an imbalance between the acidification rate and methanogenisis which inhibit conversion of

VFA (Gujer and Zehnder, 1983), but particularly conversion of propionate (Chapter 3.2, 4.2, 5.1).

Table 6.1.2 Temperature dependence of the maximum specific degrading activities of granular sludge cultivated at 10 °C for 300 days with various substrates. Standard deviation is given between parentheses.

Temperature	MAXIMUM SPECIFIC ACTIVITY [g COD g^{-1} VSS day^{-1}]				
(°C)	Hydrogen♠	Hydrogen♣	Acetate	Propionate	Butyrate
10	1.744 (0.374)	0.296 (0.009)	0.331 (0.003)	0.070 (0.002)	0.228 (0.002)
20	8.064 (0.624)	1.020 (0.379)	1.057 (0.004)	0.328 (0.010)	0.530 (0.002)
30	18.024 (1.170)	2.732 (0.076)	2.204 (0.011)	0.663 (0.002)	0.915 (0.025)

♠Homoacetogenic activity
♣Hydrogenotrophic activity

In contrast, the butyrate degrading activity significantly increased within 100 days, indicating high enrichment of these bacteria, also in the first module (Chapter 4.1). Moreover, we also found, and this is considerable practical importance that the sludge preserved its achieved higher methanogenic activity (Chapter 5.1) even after 6 months storage period at 4 °C, indicating that the starvation rate of methanogens grown in the granular sludge under the low temperature indeed is extremely low. From these observation it can be concluded that apparently slow but stable enrichment of a methanogenic population can be achieved in sludge on a low strength VFA wastewaters under psychrophilic conditions (Chapter 2.2), thus a mesophilic seed sludge even might attain a higher methanogenic activity than it would develop under optimal mesophilic growth conditions. Consequently, psychrophilically grown sludge will enable a good and fast start-up of new psychrophilic reactor systems.

The apparent K_m values of the sludge (module II, Chapter 5.1) were identical over the height of the reactor and remained unchanged during 159 days of reactor operation. This observation, which infect implies that apparent K_m and apparent diffusion coefficient in the sludge remained unchanged over such long period, is very important (Chapter 5.1). It means that a high VFA removal efficiency can be maintained with feeds with extremely low VFA concentrations (< 400 mg COD dm^{-3}). These low K_m values prevailing in EGSB reactors once again support the advantages of this reactor concept (Chapters 2.1, 2.2, 4.1) for the anaerobic treatment of low strength wastewaters, as proposed previously Kato (1994).

6.1.3.2 Granular sludge morphology

The most abundant methanogens present in the psychrophilically cultivated sludge were acetate consuming *Methanosaeta*, the hydrogenotrophic *Methanobrevibacter* species (or relatives) and formate- and hydrogen-utilising *Methanospirillum* species (Chapter 4.1 and

4.2). *Methanosacina* sp. was found to represent less than 1 % of the total methanogenic 16S rRNA, suggesting that these bacteria did not play an important role in methanogenic acetate removal (Chapter 4.2). The low level of *Methanosacina* spp. in the psychrophilic granular sludge might be related to the low acetate concentrations in the reactor system over a long period of time. The fact that K_m values on acetate for *Methanosaeta* spp. are 5-10 times lower than for *Methanosacina* spp. (Jetten et al., 1992), likely implies that latter organisms have been out competed for acetate in the EGSB reactors.

6.1.3.3 Effect of sulphate on ethanol and propionate degradation

The intermediate product formation in the granular sludge is influenced both by the organic and inorganic compounds present in the wastewater (Stams, 1994). The conversion pathway of the ethanol degradation in the sludge changed considerably during reactor operation compared to the seed sludge. From acetate as major intermediate product formation in the seed sludge has been changed to propionate in the reactor sludge (Chapters 3.1, 3.2 and 5.1). This change in the intermediate accumulation might be explained by the continuous presence of a small amount of sulphate (50 mg SO_4^{2-} dm^{-3}) in the malting wastewater and 10-12 mg SO_4^{2-} dm^{-3} through the macronutrients supply (Chapters 2.3, 3.2). As a result the sulphate concentration in the low strength malting wastewater (500 - 1000 mg COD dm^{-3}), amounted to a SO_4^{2-}/ COD ratio of 0.07 to 0.11. Although such a sulphate/COD ratio was too low to alter significantly the methane production rate, it certainly supported the development of sulphate reducers (Visser, 1995; Wu et al., 1991) in the granular sludge.

The results in Chapter 2.3 with a VFA mixture, propionate oxidation was stimulated in continuous EGSB experiments at SO_4^{2-}/ COD ratio of 0.15, but butyrate oxidation remained almost unaffected by this ratio. This effect of sulphate on propionate and butyrate oxidation was confirmed in batch experiments (Chapter 2.3). The formation of propionate during anaerobic ethanol degradation with reactor sludge (Chapter 3.1, 3.2 and 5.1) and propionate oxidation (Chapter 2.3) likely can be attributed to the presence of *Desulfobulbus propionicus* population in the granular sludge, a sulphate reducing bacteria known to convert ethanol into propionate when growing in the absence of sulphate (Stams et al., 1984; Wu et al., 1991).

6.1.4 PSYCHROPHILIC PROCESS TECHNOLOGY

6.1.4.1 Treatment of acidified wastewaters

The results of the EGSB reactor studies described in this thesis (Table 6.1.3) clearly reveal the big potentials of the EGSB system for the treatment of low strength wastewater under psychrophilic conditions (4-20 °C). COD removal efficiencies over 90 % can be achieved at organic loading rates up to 12 kg COD m^{-3} day^{-1} at HRT's as low as 1.6 h using a VFA-mixture as feed. In applying a single stage reactor system, propionate appeared to be the limiting compound in anaerobic treatment of pre-acidified wastewater (Chapters 2.1, 3.2). Propionate indeed is known to comprise the most difficult VFA intermediate in methanogenic anaerobic

Table 6.1.3 Results of the psychrophilic EGSB reactor studies in this thesis

Substrate	Volume [dm^3]	Concentration [g COD dm^{-3}]	OLR [kg COD m^{-3} d^{-1}]	Temp. [°C]	HRT [h]	COD removal [%]	Thesis
VFA	1 x 4	0.5 - 0.8	10-12	10-12	1.6 - 2.5	90	Chapter 2.1
VFA	2 x 4	0.5 - 0.9	5 - 12	4 - 8	2 - 4	90	Chapter 4.1
VFA	2 x 4	0.5 - 0.9	5	3	4	80	Chapter 4.1
Sucrose + VFA	2 x 4	0.5 - 1.1	5 - 7	8	4	90	Chapter 4.2
Beer	1 x 225	0.5 - 0.8	12	20	1.5	80 - 85	Chapter 3.1
Malting	1 x 225	0.3 -1.4	4 - 8	16	2.4	56	Chapter 3.2
Malting	1 x 225	0.3 -1.4	9 - 15	20	1.5 - 2.4	66 - 72	Chapter 3.2
Malting	2 x 70	0.2 - 1.8	3 - 6	6	4.9	47	Chapter 5.1
Malting	2 x 70	0.2 - 1.8	3 - 12	10-15	3.5	67 - 78	Chapter 5.1

degradation (Gujer and Zehnder, 1983; Lettinga, 1995). Apparently, also under psychrophilic conditions, it hardly develops, probably mainly due to the same thermodynamic considerations as prevailing for mesophilic conditions (Stams, 1994).

Introducing a two stage EGSB system (in series) can optimize the psychrophilic anaerobic treatment process. Experimental results with such a two stage EGSB set-up clearly reveal that high-rate anaerobic treatment is very well feasible at extremely low temperatures, i.e. down to 3 °C (Chapter 4.1). While treating a VFA-mixture, COD removal efficiencies exceeding 90 % were achieved at 8 °C and 4 °C at organic loading rates of 12 and 5 kg COD m^{-3} day^{-1} and at HRTs of 2.0 and 4.0 hours, respectively. The two stage EGSB concept is capable to accommodate a three to five times higher OLR at a 90 % COD_{vfa} removal efficiency than reported so far for psychrophilic anaerobic waste water treatment systems (Banik and Dague, 1996; Grant and Lin, 1995; Matsushige et al., 1990; de Man et al., 1988; Switzenbaum and Jewell, 1978).

The enhancement of the psychrophilic conversion processes in a staged system should be attributed to the presence and maintenance of a more balanced micro-ecosystem in the separate reactors, particularly in the sludge bed of the second stage. The most important aspect in this respect is the significant decrease of product inhibition during conversion of propionate in the second stage. For thermodynamic reasons, the degradation of propionate doesn't easily proceed under conditions with high H_2 and acetate concentrations. However, when this reaction can be delegates to the second stage the conversion of propionate becomes a self-regulating process. Similar observations were made in the 'sub-optimal' thermophilic range (van Lier et al., 1994, Wiegant et al., 1986). As a result of staging the number of bacteria involved in the acetogenic conversions will increase in the second stage, leading to an even higher increase in the organic loading capacity compared to single stage. The extraordinary potentials of the staged system become particularly apparent from the high COD removal efficiencies achieved, even at the very low influent concentrations applied. This extremely important feature can be attributed to the prevailing very low values for the substrate affinity constants K_m, at 10 °C. For acetate, propionate and butyrate values as low as 0.04, 0.01 and 0.14 g COD dm^{-3}, respectively, were found (Chapter 2.2, 4.1). These findings represent a major step forward in the application of the anaerobic treatment system. In addition, the results demonstrate the importance of adequate hydraulic mixing in anaerobic system for lowering the apparent K_m (Chapters, 2-5; Kato, 1994).

6.1.4.2 Treatment of partly acidified wastewaters

The concentration of soluble and total COD in the malting wastewater used in our investigations fluctuated between 230-1800 mg dm^{-3} and 320-4450 mg dm^{-3}, respectively (Chapters 3.2, 5). The strength of the malting wastewater can vary strongly, depending on the type of barley used, and its growth conditions prior to harvesting. The anaerobically biodegradable COD of the waste water amounted to about 73 %, as determined in the batch bioassays at 15 °C. The wastewater contained both anaerobically completely biodegradable

compounds, such as different kinds of sugars, lactic acid, glycerol, ethanol and volatile fatty acids (VFA), as well as compounds which are only partially or slowly biodegradable at low temperature, such as fats, proteins, tannin, cellulose and barley grains suspended solids. The COD of the settleable and the colloidal suspended solids in the malting wastewater were in the range (mg dm^{-3}) 20-231; 0-176, respectively (Chapters 3.2, 5.1).

In anaerobic treatment of malting wastewater in a single stage EGSB reactor at 16°C, the COD removal efficiencies averaged about 56 %, at imposed organic loading rates (OLR) ranging between 4.4 - 8.8 kg COD m^{-3} day^{-1} and a HRT of approximately 2.4 h. At 20°C, the removal efficiencies were approximately 66% and 72 %; respectively, at imposed OLRs of 8.8 and 14.6 kg COD m^{-3} day^{-1}, corresponding to HRTs of 2.4 and 1.5h (Chapter 3.2). A lower COD removal efficiency was obtained at high H_2 concentrations in the biogas. An imbalance between the acidification rate and methanogenesis in the reactor may lead to higher H_2 concentrations, which then subsequently will detrimentally affect the anaerobic conversion of VFA (Stams, 1994). With regard to the removal of non-soluble COD fraction, a psychrophilic single EGSB reactor system is not effective, due to the high superficial velocity in the reactor (Van der Last and Lettinga, 1992).

When applying a two stage pilot-scale EGSB system, satisfactory anaerobic treatment of partly acidified malting wastewater in the temperature range of 8-12 °C can be accomplished at an OLR up to 12 kg COD m^{-3} day^{-1} and at an HRT of 3.5 h. When operated properly, such a staged system provides a remarkable long term (6 months) stable performance at low temperatures even when imposing strong variations in OLR between 3 to 12 kg COD m^{-3} day^{-1} (Chapter 5.1). Staging the EGSB system markedly improves the performance and stability of the system compared to an one-step process. Moreover, the COD removal efficiencies were very high and comparable to the maximum efficiency of 85 % found in the mesophilic anaerobic treatment of brewery wastewaters at 30 °C (Pereboom, 1994). Similar results were found at 37 °C in a three stage system (Stadlbauer et al., 1994), an efficiency of 80 % at an imposed load of 25 kg COD m^{-3} day^{-1} and at an HRT of 6 h. The removal efficiencies achieved in this study are comparable to those found by Perez et al. (1997) who reported, a 82 % COD removal using a thermophilic (55 °C) anaerobic fluidized bed reactor. However, in applying the system to partly acidified malting wastewaters under psychrophilic conditions problem will manifest with respect to the formation of a layer of acidifying sludge around the granules present in the first module. This may lead to gas entrapment inside the granule, initiating flotation of these granules (Chapter 5.1). Compared to mesophilic conditions (Alphenaar, 1994), the acidifiers have a very high biomass yield (0.22 g VSS-COD g^{-1} $COD_{removed}$; Chapter 4.2) under psychrophilic conditions, but they also show an extremely low starvation rate (1.57×10^{-5} h^{-1} at 10 °C; Chapter 4.2). Additionally, the presence of oxygen in the wastewater, can support the growth of facultative anaerobic and/or aerobic organisms, which have an even higher growth-yield (Shen and Guiot, 1996; Kato et al., 1993; Gerritse and Gottschal, 1992). The two stage EGSB system was found capable to accommodate the presence of non acidified substrate

up to values of 10 % of the influent COD at temperatures as low as 8 °C, if the oxygen level in the wastewater is remained below 2 mg O_2 dm^{-3} (Chapters 4.2, 5.1).
Also the suspended solids (SS) concentrations (20-30 % of total COD) in the influent negatively affect the sludge and sludge bed characteristics of the EGSB reactor (Chapter 5.1). At least part of these suspended solids present in the wastewater should be removed, prior to feeding the wastewater to the EGSB system. A significant SS degradation in psychrophilic reactors can be hardly expected, because at low temperatures the hydrolysis rate of SS drops sharply and even may approach zero for various types of solids (Zeeman et al, 1996; Van der Last & Lettinga, 1992; De Man et al., 1986).

6.1.5 CONCLUSIONS

1. Cold (8-12 °C), low strength (< 1000 mg COD dm^{-3}) and well acidified wastewaters can be efficiently (>90 %) treated in a single module 'high-rate' anaerobic EGSB reactor configuration up to OLR of 12 kg COD m^{-3} day^{-1}.
2. When applying a two stage EGSB system, to partly acidified malting wastewater in the temperature range 8-12 °C, OLR up to 12 kg COD m^{-3} day^{-1} can be applied at an HRT of 3.5 h. Staging the EGSB system markedly improves the performance and stability of the system compared to a one-step process. When operated properly, a stageded system provides a remarkable long term stable performance at low temperatures even when imposing the system with strong variations in OLR in the range 3 to 12 kg COD m^{-3} day^{-1}.
3. The maximum specific conversion rate of the methanogenic seed sludge increased by a factor 3 within 300 days of operation at 10-12 °C. The results of this study clearly reveal that mesophiles grow and metabolize at suboptimal temperatures. Newly grown-in viable methanogenic biomass is sufficiently well retained in the second module. The methanogenic sludge preserved its methanogenic activity attained under psychrophilic conditions, even after prolonged periods of unfed storage (6 months at 4 °C).
4. Flotation of granular sludge may comprise problems in the first stage in case of insufficiently acidified wastewaters. This can be attributed to excessive growth of acidogenic and facultative anaerobic populations on the methanogenic granules. In order to prevent these problems, it therefore, is needed to apply a pre-acidification step. The design criteria of a psychrophilic high rate pre-acidification step still have to be assessed.
5. The suspended solids concentrations (> 10-15 % of total COD) in the influent may negatively affect the sludge and sludge bed characteristics of the EGSB reactor. At least part of these suspended solids present in the original wastewater should be removed, prior to feeding the wastewater in the EGSB system.
6. These results represent a definite breakthrough with respect to the application of anaerobic treatment systems at low ambient temperatures for low strength wastewaters. Our results

are particularly encouraging for full scale application of anaerobic treatment of low strength waste water under psychrophilic conditions.

Consequently it can be concluded that anaerobic wastewater treatment now represents a feasible and very attractive alternative for the treatment of low strength acidified wastewaters particularly regarding its principle advantages over conventional aerobic treatment systems.

6.1.6 REFERENCES

Alphenaar, A. Anaerobic granular sludge: characterization, and factor affecting its functioning. PhD. Thesis, Agricultural University Wageningen, The Netherlands, pp 93-112, 1994.

Banik, G. C.; Dague, R. R. ASBR treatment of dilute wastewater at psychrophilic temperatures. In *Proc. of 69th Annual Water Environmental Conference*, Dallas, Texas, USA, 1996; pp 235-246.

Boone, D. R., Bryant, M. P. Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystem. *Appl. Environ. Microbiol.*, **1980**, 40, 626-632.

Grant, S.; Lin, K. C. Effects of temperature and organic loading on the performance of upflow anaerobic sludge blanket reactors. *Can. J. Civ. Engin.*, **1995**, 22, 143-149.

Gujer, W.; Zehnder, A. J. B. Conversion processes in anaerobic digestion. *Wat. Sci. Technol.*, **1983**, 15, 127-167.

Henzen, M.; Harremoes, P. Anaerobic treatment of waste water in fixed film reactors-literature review. *Wat. Sci. Technol.*, **1983**, 15, 1-102.

Jetten, M.S.M., Stams, A.J.M. and Zehnder, A.J.B. 1992. Methanogenesis from acetate: A comparison of the metabolism in *Methanothrox soehngenii* and *Methanosarcina* spp. FEMS *Microbiol. Rev.* **88**: 181-198.

Kato, M. T. The anaerobic treatment of low strength soluble wastewaters. Ph.D. Thesis, Agricultural University Wageningen, The Netherlands, pp 101-128, 1994.

Last van der, A. R. M.; Lettinga, G. Anaerobic treatment of domestic sewage under moderate climatic (Dutch) conditions using upflow reactors at increased superficial velocities. *Wat. Sci. Technol.*, **1992**, 25, 167-178.

Lettinga, G. Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek*, **1995**, 67, 3-28.

Lettinga, G. Feasibility of anaerobic digestion for the purification of industrial waste waters. In: *Proceeding 4th Symposium EAS*, München, Germany, 1978; pp 226-256.

Lier van, J.B. Thermophilic anaerobic wastewater treatment; temperature aspects and process stability. Ph.D. Thesis, Agricultural University Wageningen, The Netherlands, pp 124-144, 1995.

Van Lier, J.B., Grolle, K.C.F., Frijters, C.T.M.J., Stams, A.J.M. and Lettinga, G. Effect of acetate, propionate and butyrate on the thermophilic anaerobic degradation of ropionate in

methanogenic sludge and defined cultures. *Appl. and Environ. Microbiol.*, **1993**, 57: 1003-1011.

Man de, A. W. A.; van der Last, A. R. M.; Lettinga, G. The use of EGSB and UASB anaerobic systems for low strength soluble and complex wastewaters at temperatures ranging from 8 to 30°C. In: *Proc. of the Fifth International Symposium on Anaerobic Digestion*. ed. ER Hall and PN Hobson, Bologna, Italy, 1988; pp 197-209.

Man de, A. W. A.; Grin, P. C.; Roersma, R. E.; Grolle, K. C. F.; Lettinga, G. Anaerobic treatment of municipal wastewater at low temperatures. In: *Proc. Conference Anaerobic Treatment a grown up Technology*, Amsterdam, The Netherlands, 1986; pp 453-466.

Matsushige, K.; Inamori, Y.; Mizuochi, M.; Hosomi, M.; Sudo, R. The effects of temperature on anaerobic filter treatment for low-strength organic wastewater. *Environ. Technol.*, **1990**, 11, 899-910.

Pereboom, J. H. F. Methanogenic granule development in full scale internal circulation reactors. *Wat. Sci. Technol.*, **1994**, 30, 211-221.

Péres, M.; Romero, L. I.; Sales, D. Thermophilic anaerobic degradation of distillery wastewater in continuous-flow fluidized bed bioreactors. *Biotechnol. Prog.*, **1997**, 13, 33-38.

Stadlbauer, E. A.; Oey, L. N.; Weber, B.; Jansen, K.; Weidle, R.; Löhr, H.; Ohme, W.; Döll, G. Anaerobic purification of brevery wastewater in biofilm reactors with and without a methanation cascade. *Wat. Sci. Technol.*, **1994**, 30, 395-404

Stams, A. J. M. Metabolic interactions between anaerobic bacteria in methanogenic enviroments. *Antonie van Leeuwenhoek*, **1994**, 66, 271-294.

Stams, A. J. M.; Kremer, D. R.; Nicolay, K.; Weenk, G. H.; Hansen, T. A. Pathway of propionate formation in *Desulfobulbus propionicus*. *Arch. Microbiol.*, **1984**, 139, 167-173.

Switzenbaum, M. S.; Jewell, W. J. Anaerobic attached film expanded bed reactor treatment of dilute organics. In: *Proceedings of the 51st Annual WPCF Conference*, Anaheim, California, USA, 1978, pp 1-164.

Visser, A. The anaerobic treatment of sulphate containing wastewater. Ph.D. Thesis, Agricultural University Wageningen, The Netherlands, pp 109-120, 1995.

Zeeman, G.; Sanders, W. T. M.; Wang, K. Y.; Lettinga, G. Anaerobic treatment of complex wastewater and waste activated sludge. In *Proc. Conference Advanced Wastewater Treatment*, Amsterdam, The Netherlands, 1996, pp 225-232.

Wiegant, W. M. Thermophilic anaerobic digestion for waste and wastewater treatment. Ph.D. Thesis, Agricultural University Wageningen, The Netherlands, pp 95-111, 1986.

Wu, W-M.; Hickey, R. F.; Zeikus, J.G. Characterization of metabolic performance of methanogenic graniles treating brewery wastewater: Role of sulfate-reducing bacteria. *Appl. Environ. Microbiol.*, **1991**, 57, 3438-3449.

6.1 PSYCHROFIELE ANAEROBE BEHANDELING VAN VERDUNDE AFVALWATERSTROMEN
Algemene Discussie en Conclusies

6.1.1 INLEIDING

Onder gematigde klimaatcondities is de temperatuur van vele afvalwaters aanzienlijk lager dan de optima van de temperatuur biologische afvalwaterzuiveringsprocessen, zoals nitrificatie, denitrificatie en mesofiele methanogenese. Voorbeelden van dergelijke koude afvalwaters zijn lekwater van stortplaatsen of zwavelrijke ertsgebieden, stedelijk drain-off water en huishoudelijk afvalwater, als ook verschillende industriële afvalwaters, zoals die van de voedings- (inblikking) en drank- (bottelerij, mouterij en brouwerij) industrie. De CZV concentratie van deze afvalwaters is meestal vrij laag (< 1500 mg CZV dm^{-3}). Eén van de belangrijkste problemen van anaërobe reactoren, die bij een lage temperatuur worden bedreven is de zeer lage biogas productiesnelheid. Dit kan leiden tot een slechte menging en beperkt substraat-biomassa contact. Een ander belangrijk punt, vooral voor laag geconcentreerde afvalwaters, is het bewerkstelligen van een goede biomassa retentie. Omdat de temperatuur sterk de snelheid van anaërobe omzettingsprocessen bepaalt, zullen hoogst waarschijnlijk veranderingen in de gangbare ontwerpen noodzakelijk zijn, als hoogbelaste reactorsystemen zullen worden toegepast bij sub-optimale temperatuur. Het reactorontwerp moet een efficiënte bioconversie onder deze condities garanderen. Een aantrekkelijk en mogelijk toepasbaar reactor systeem voor deze condities is EGSB (Expanded granular sludge bed) reactor (De Man et al., 1988; Van der Last and Lettinga, 1992; Kato, 1994).

Deze thesis beschrijft de resultaten van het onderzoek betreffende de toepasbaarheid van het EGSB reactorsysteem voor de anaërobe behandeling van laag geconcentreerde afvalwaters onder psychrofiele condities. De resultaten met en het ontwerp van deze hoogbelaste anaërobe reactoren onder psychrofiele condities werd onderzocht voor een aantal substraten, waaronder mouterij afvalwater, en in verband hiermee werd de temperatuur afhankelijkheid van de kinetische parameters van het korrelslib bepaald nadat het gedurende een lange periode was blootgesteld aan lage temperaturen. De toepasbaarheid van één enkele EGSB reactor module voor de anaërobe behandeling van mouterij afvalwater bij lage temperaturen werd bepaald. Verder werd het onderzoek gericht op de procesoptimalisatie van de behandeling van gedeeltelijk verzuurd afvalwater om tot een stabiel proces met de hoogst mogelijke efficiëntie bij de laagst mogelijke temperatuur te kunnen komen.

6.1.2 OPSTART VAN EGSB REACTOR ONDER PSYCHROFIELE CONDITIES

Een EGSB reactor werd geinoculeerd met mesofiel korrelslibslib van een praktijkschaal UASB reactor waarmee die brouwerijafvalwater wordt behandeld. Het gebruik van korrelslib laat bij opstroomsnelheden tot 10 $m \cdot h^{-1}$, een goede expansie van het slibbed in de reactor toe. Een succesvolle opstart van de EGSB reactor bij temperaturen lager dan 15 °C vereist een

initiële biomassa concentratie hoger dan 30 g VSS·dm^{-3} om het sterk negatief effect van de lage temperatuur op de capaciteit van mesofiel slib af te zwakken (Hoofdstukken 2.1; 3.2 en 5).

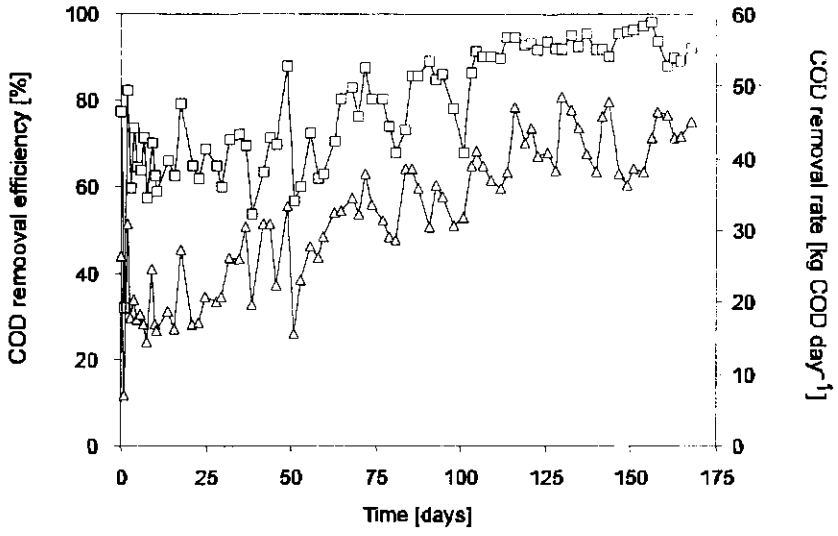

Fig. 6.1.1 Opstart van een 4 l EGSB reactor gevoed met een laag geconcentreerd VVZ mengsel bij 10-12 °C. (□) CZV-Verwijderingsrendement, (Δ) CZV-Verwijderingssnelheid (Hoofdstuk 2.1)

Ook in de experimenten van Kato (1994) moesten grote hoeveelheden slib gebruikt moeten worden om de toepassing van hoge (of zeer hoge) organische belastingen mogelijk te maken. Als gedurende de opstart de temperatuur onmiddellijk in de lage temperaturen range (10-15 °C) wordt gebracht, zullen de verwijderingsefficienties voor laag geconcentreerde, verzuurde afvalwaters 40-60 % niet overschreiden bij belastingen van 8 kg CZV m^{-3} dag^{-1}. Echter, zoals geïllustreerd in Fig. 6.1.1, kan een volledige en stabiele conversiecapaciteit van psychrofiel EGSB systemen reeds binnen 3 maand bereikt worden. Het systeem kon zelfs een organische belasting van 12 kg CZV m^{-3} dag^{-1} behandelen (Hoofdstuk 2.1). Met mouterijafvalwater (anaërobe biodegradeerbaarheid van 73 % bij 15 °C), was twee maanden nodig om met een pilot-schaal EGSB reactor 70 % COD reductie bij 16 °C te bereiken bij een volumebelasting van 8-10 kg COD m^{-3} dag^{-1} en een hydraulische verblijftijd (HVT) van 2.4 h (Hoofdstuk 3.2). De redenen voor deze relatief lange opstart periode ligt aan het feit dat de methanogene capaciteit van mesofiel korrelslib sterk door temperatuurverlagingen wordt bepaald (Lettinga, 1978; Henze en Harremoes, 1983). Dus, bepaling van de ontwikkeling van de methanogene activiteit van een mesofiel inoculum slib bij blootstelling aan lage temperaturen is van groot belang voor de opstart van hoogbelast psychrofiele anaërobe reactoren (Hoofdstukken 2.2, 4.1 en 5).

6.1.3 KORRELSLIB KARAKTERISTIEKEN

6.1.3.1 Metabole karakteristieken

De lange-termijn resultaten met laboratorium- en pilot-schaal reactorsystemen bedreven onder lage temperatuur condities vertoonden een vrij bevredigende ontwikkeling van de methanogene activiteit van het mesofiel ent slib. Het blijft echter onduidelijk of er ook een aanzienlijke, de aanwezigheid van een aanzielijk aandeel populatie aan psychrofiele bacteriën in het slib aanwezig is. De temperatuur responsiecurven van psychrofiel (3-12 °C) gegroeid slib vertoonde nog steeds een duidelijk optimum in het mesofiele temperatuursgebied (30-40 °C) (Tabel 6.1.1, 6.1.2). Maar dit beduidt daarom niet

Tabel 6.1.1 Temperatuurafhankelijkheid van de maximale specifieke afbraak activiteiten van mesofiel korrelslib van een paktijkschaal UASB reactor met diverse substraten. Standaard afwijking is tussen haakjes gegeven.

Temperatuur	MAXIMALE SPECIFIEKE ACTIVITEIT [g CZV g^{-1} OS dag^{-1}]		
(°C)	Acetaat	Propionaat	Butyraat
10	0.090 (0.000)	0.050 (0.002)	0.050 (0.010)
20	0.380 (0.010)	0.301 (0.020)	0.172 (0.013)
30	0.980 (0.120)	0.551 (0.031)	0.331 (0.042)

dat psychrofiele micro-organismen helemaal afwezig zouden zijn (Hoofdstukken 2.1, 2.2 en 4.1). In het geval deze psychrofiele homologen zich in het slib ontwikkeld zouden hebben, kan hun activiteit ongemerkt blijven door de aanwezigheid van de grote hoeveelheid mesofiele organismen. Aangezien de specifieke methanogene activiteit van het inoculum slib aanzienlijk verhoogde gedurende het experiment, verhinderen de lage temperaturen blijkbaar de groei en aanrijking van methanogenen en acetogenen niet (Tabellen 6.1.1 en 6.1.2). De maximale specifieke afbraak activiteiten van het slib bij 30 °C (Tabel 6.1.2) zijn erg hoog, en overtreffen de vroeger gevonden waarden voor een typisch mesofiel korrelslib door Alphenaar (1994) en Kato (1994) en evenaren zelfs de activiteiten gevonden voor slib uit thermofiele (55-65 °C) anaërobe reactoren (van Lier, 1996).

De hoge propionaat afbraakactiviteiten gemeten bij 5 °C tonen aan dat de bestaande propionaat oxiderende populatie zijn activiteit behield (Hoofdstuk 4.1), maar verrassend genoeg werden geen duidelijke aanwijzingen gevonden voor de ingroei van propionaat oxideerders. De resultaten in Hoofdstuk 4.1 tonen een significante accumulatie van acetaat gedurende propionaat oxidatie bij 5 °C. Gezien het feit dat propionaat oxideerders bekend staan als gevoelige micro-organismen (Boone en Bryant, 1980), kan de groei van deze micro-organismen geinhibeerd worden door acetaat (Van Lier et al., 1993). Waterstof accumuleerde in deze experimenten niet, en kan dus niet bijdragen aan de inhibitie van de propionaat oxidatie (Hoofdstuk 4.1). Een hogere waterstofconcentratie in het biogas wijst op een

Tabel 6.1.2 Temperatuurafhankelijkheid van de maximale specifieke afbraak activiteit van korrelslib gegroeid bij 10 °C gedurende 300 dagen op diverse substraten. Standaard afwijking is tussen haakjes gegeven.

Temperatuur	MAXIMALE SPECIFIEKE ACTIVITEIT [g CZV g^{-1} OS dag^{-1}]				
(°C)	Waterstof♠	Waterstof♣	Acetaat	Propionaat	Butyraat
10	1.744 (0.374)	0.296 (0.009)	0.331 (0.003)	0.070 (0.002)	0.228 (0.002)
20	8.064 (0.624)	1.020 (0.379)	1.057 (0.004)	0.328 (0.010)	0.530 (0.002)
30	18.024 (1.170)	2.732 (0.076)	2.204 (0.011)	0.663 (0.002)	0.915 (0.025)

♠Homoacetogene activiteit
♣Hydrogenotrofe activiteit

onbalans tussen de acidificatie- en de methaanvormingssnelheid en inhibeert de conversie van vluchtige vetzuren (Gujer en Zehnder, 1983), in het bijzonder de propionaatconversie (Hoofdstukken 3.2, 4.2 en 5.1). De butyraatafbraak activiteit nam daarentegen significant toe binnen 100 dagen, wat wijst op een aanrijking van deze bacteriën, ook in de eerste trap (Hoofdstuk 4.1). Bovendien werd gevonden, en dit is van aanzienlijk belang voor de praktijk, dat het slib zijn hogere methanogene activiteit bewaarde (Hoofdstuk 5.1), zelfs na een opslagperiode van 6 maand bij 4 °C. Dit wijst erop dat de afstervingssnelheid van de methanogenen in het korrelslib bij lage temperatuur extreem laag is. Uit deze waarnemingen kan geconcludeerd worden dat er een schijnbaar trage, maar stabiele aanrijking van de methanogene populatie kan worden, bereikt in het slib bij de behandeling van laag geconcentreerde vluchtige vetzuur (VVZ) afvalwaters onder psychrofiele condities (Hoofdstuk 2.2), zodat het mesofiele inoculum slib zelfs een hogere methanogene activiteit kan bereiken, ten opzichte van slib ontwikkeld onder optimale mesofiele groeicondities. Bijgevolg, psychrofiel gekweekt slib laat een goede en snelle opstart van nieuwe psychrofiele reactorsystemen toe.

De schijnbare K_m-waarden van het slib (trap II, Hoofdstuk 5.1) waren laag en identiek op alle bemonsterde hoogtes in de reactor en bleven onveranderd gedurende de 159 dagen van reactor bedrijfsvoering. Deze waarneming, die eigenlijk betekent dat de schijnbare K_m en de schijnbare diffusie coëfficiënt van het slib onveranderd bleven over een lange tijdsperiode, is zeer belangrijk (Hoofdstuk 5.1). Het betekent dat een hoge VVZ verwijderingsefficiëntie kan worden behouden bij voedingen met extreem lage VVZ concentraties (< 400 mg COD dm^{-3}). Deze lage K_m waarden van EGSB reactoren tonen opnieuw het voordeel van dit reactor concept aan (Hoofdstukken 2.1, 2.2 en 4.1) voor de anaërobe behandeling van laag geconcentreerde afvalwaters, zoals reeds gepostuleerd door Kato (1994).

6.1.3.2 Korrelslib morfologie

De meest voorkomende methanogenen aanwezig in het psychrofiele gegroeide slib waren de acetaat verbruikende *Methanosaeta*, de hydrogenotrofe *Methanobrevibacter* species (of verwanten) en formiaat- en waterstofverbruikende *Methanospirillum* species (Hoofdstukken 4.1 en 4.2). *Methanosarcina* sp. vertegenwoordigde minder dan 1 % van de totale methanogene 16S rRNA hoeveelheid, wat suggereert dat deze bacteriën geen belangrijke rol in de methanogene acetaatverwijdering speelden (Hoofdstuk 4.2). Het lage gehalte *Methanosacina* spp. in het psychrofiel granulair slib kan gerelateerd zijn aan de lage acetaat concentraties in het reactorsysteem gedurende een lange tijdsperiode. Het feit dat de K_m waarde voor acetaat van *Methanosaeta* spp. 5-10 maal lager is dan voor *Methanosarcina* spp. (Jetten et al., 1992), kan bijgedragen hebben dat deze laatste organismen werden weggeconcurreerd in de EGSB reactoren.

6.1.3.3 Effect van sulfaat op ethanol en propionaat afbraak

De vorming van intermediaren in korrelslib wordt beïnvloed door zowel de organische als de anorganische bestanddelen van het afvalwater (Stams, 1994). De afbraakroute gevolgd gedurende de ethanolafbraak in het slib kan aanzienlijk veranderen gedurende het bedrijven van EGSB reactoren. Het belangrijkste dat gevormd werd in een EGSB intermediair veranderde van acetaat in het ent slib naar propionaat in het reactorslib (Hoofdstukken 3.1, 3.2 en 5.1). Deze verandering kan mogelijk verklaard worden door de continue aanwezigheid van een kleine hoeveelheid sulfaat (50 mg SO_4^{2-} dm^{-3}) in het mouterij afvalwater en 10-12 mg SO_4^{2-} dm^{-3} door de toevoeging van macronutriënten (Hoofdstukken 2.3 en 3.2). Ten gevolge van de sulfaatconcentratie in het laag geconcentreerde mouterijafvalwater (500 - 1000 mg CZV dm^{-3}), bedroeg de SO_4^{2-}/CZV verhouding 0.07 tot 0.11. Alhoewel een dergelijke sulfaat/CZV verhouding te laag was om de methaanproductiesnelheid significant te veranderen, kan het wel de ontwikkeling van sulfaatreduceerders (Visser, 1995; Wu et al., 1991) in het korrelslib ondersteunen.

De resultaten in Hoofdstuk 2.3 met een VVZ-mengsel toonden aan dat propionaat oxidatie gestimuleerd werd in een continu EGSB experiment door een SO_4^{2-}/CZV verhouding van 0.15, maar butyraat oxidatie bleef vrijwel onbeïnvloed door deze verhouding. Dit effect van sulfaat op propionaat- en butyraatoxidatie werd bevestigd in batch experimenten (Hoofdstuk 2.3). De vorming van propionaat gedurende anaërobe ethanol afbraak met reactorslib (Hoofdstukken 3.1, 3.2 en 5.1) alsook de propionaatoxidatie (Hoofdstuk 2.3) kunnen mogelijks door de aanwezigheid van een *Desulfobulbus propionicus* populatie in het korrelslib worden verklaard. Deze sulfaatreducerende bacterie kan ethanol in propionaat omzetten gedurende groei in afwezigheid van sulfaat (Stams et al., 1984; Wu et al., 1991).

6.1.4 PSYCHROFIELE PROCESTECHNOLOGIE

6.1.4.1 Behandeling van verzuurde afvalwaters

De in deze thesis beschreven resultaten over de EGSB reactor studies (Tabel 6.1.3) toonde duidelijk de grote mogelijkheden van het EGSB systeem voor de behandeling van laag geconcentreerde afvalwaters onder psychrofiele condities (4-20 °C) aan. CZV verwijderingsefficienties hoger dan 90 % kunnen worden bereikt bij een organische belasting van 12 kg CZV m^{-3} dag^{-1} bij een HVT van 1.6 h bij de behandeling van een VVZ-mengsel als substraat.

Bij het gebruik van een ééntrap reactor systeem, bleek propionaat de beperkende faktor voor de anaërobe behandeling van voorverzuurd afvalwater (Hoofdstukken 2.1 en 3.2). Propionaat staat inderdaad bekend als het moeilijkst afbreekbare VVZ intermediair gedurende methanogene anaërobe afbraak (Gujer en Zehnder, 1983; Lettinga, 1995). Blijkbaar, ook onder psychrofiele condities, ontwikkelt propionaat afbrekende capaciteit van het slib zich langzaam, waarschijnlijk hoofdzakelijk ten gevolge van dezelfde thermodynamische overwegingen als die spelen onder mesofiele omstandigheden (Stams, 1994).

Het gebruik van een tweetraps EGSB systeem (in serie) kan het psychrofiele anaërobe zuiveringsproces verbeteren. Experimentele resultaten met zo'n tweetraps EGSB set-up toonden duidelijk aan dat een hoogbelaste anaërobe zuivering goed mogelijk is bij extreem lage temperaturen, zelfs tot 3 °C (Hoofdstuk 4.1). Bij de behandeling van een VVZ-mengsel werden CZV verwijderingsrendementen die de 90 % overtroffen behaald bij 8 °C en 4 °C bij organische belastingen van 12 en 5 kg CZV m^{-3} dag^{-1} en bij HVT van 2.0 en 4.0 uur, respectievelijk. Het tweetraps EGSB concept kan een drie tot vijf maal hogere organische volume belasting (OVB) aan (met een 90 % CZV_{VVZ} verwijderingsrendement) dan tot dusverre vermeld voor psychrofiele anaërobe afvalwater zuiveringssystemen (Banik en Dague, 1996; Grant en Lin, 1995; Matsushige et al., 1990; de Man et al., 1988; Switzenbaum en Jewell, 1978).

De versnelling van de psychrofiele conversieprocessen in een gefaseerd systeem is waarschijnlijk te danken aan de aanwezigheid en het behoud van meer gebalanceerd micro-ecosystemen in de afzonderlijke reactoren, vooral in het slibbed van de tweede trap. Het meest belangrijke aspect in deze context is de significante afname van productinhibitie gedurende de conversie van propionaat in de tweede trap. Om thermodynamische redenen, verloopt de afbraak van propionaat niet gemakkelijk onder omstandigheden met hoge H_2 en acetaat concentraties. Echter, als deze reactie kan worden verplaatst naar de tweede trap, wordt de conversie van propionaat een zelfregulerend proces. Gelijkaardige waarnemingen werden gedaan onder 'sub-optimale' thermofiele omstandigheden (van Lier et al., 1994, Wiegant et al., 1986). Ten gevolge van faseren neemt het aantal bacteriën betrokken bij de acetogene omzettingen toe in de tweede trap, leidend tot een toename van de toepasbare organische belasting in vergelijking tot het ééntraps proces. De uitzonderlijke mogelijkheden van de gefaseerde systemen wordt vooral zichtbaar door de hoge bereikte CZV verwijderingsefficiëncies, zelfs bij de zeer lage influent concentraties die werden toegepast. Dit

Tabel 6.1.3 Overzicht van de operationele condities en resultaten van de in dit onderzoek bedreven psychrofiele EGSB reactoren.

Substraat	Volume [dm^3]	Concentratie [g CZV dm^{-3}]	OVB [kg CZV m^{-3} d^{-1}]	Temp. [°C]	HVT [h]	CZV verwijdering [%]	Proefschrift
VVZ	1 x 4	0.5 - 0.8	10-12	10-12	1.6 - 2.5	90	Hoofdstuk 2.1
VVZ	2 x 4	0.5 - 0.9	5 - 12	4 - 8	2 - 4	90	Hoofdstuk 4.1
VVZ	2 x 4	0.5 - 0.9	5	3	4	80	Hoofdstuk 4.1
Sucrose + VVZ	2 x 4	0.5 - 1.1	5 - 7	8	4	90	Hoofdstuk 4.2
Bier	1 x 225	0.5 - 0.8	12	20	1.5	80 - 85	Hoofdstuk 3.1
Mout	1 x 225	0.3 -1.4	4 - 8	16	2.4	56	Hoofdstuk 3.2
Mout	1 x 225	0.3 -1.4	9 - 15	20	1.5 - 2.4	66 - 72	Hoofdstuk 3.2
Mout	2 x 70	0.2 - 1.8	3 - 6	6	4.9	47	Hoofdstuk 5.1
Mout	2 x 70	0.2 - 1.8	3 - 12	10-15	3.5	67 - 78	Hoofdstuk 5.1

kan toegeschreven worden aan de heersende lage waarden voor de substraataffiniteitsconstante K_m, bij 10 °C. Er werden voor acetaat, propionaat en butyraat waarden van respectievelijk, 0.04, 0.01 en 0.14 g CZV dm^{-3}, gevonden (Hoofdstukken 2.2 en 4.1). Deze bevindingen zijn een grote stap voorwaarts bij de verbreding van het toepassingsgebied van anaërobe behandelingssystemen. Bovendien, de resultaten illustreren het belang van een adequate hydraulische menging in anaërobe systemen ter verlaging van de apparent K_m (Hoofdstukken 2 -5; Kato, 1994).

6.1.4.2 Behandeling van gedeeltelijk verzuurde afvalwaters

De concentratie aan opgeloste en totale CZV in het mouterijafvalwater gebruikt voor dit onderzoek varieerde tussen 230-1800 mg dm^{-3} en 320-4450 mg dm^{-3}, respectievelijk (Hoofdstukken 3.2 en 5.1). De sterkte van het mouterijafvalwater kan erg variëren, afhankelijk van het gebruikte type gerst, en de groeiomstandigheden voor de oogst. De anaërobe biodegradeerbare CZV van het afvalwater bedroeg ongeveer 73 %, zoals bleek uit batch proeven bij 15 °C. Het afvalwater bevatte zowel anaëroob volledig biodegradeerbare stoffen, zoals verschillende soorten suikers, melkzuur, glycerol, ethanol en VVZ, alsook stoffen die slechts gedeeltelijk of langzaam biodegradeerbaar zijn bij lage temperatuur, zoals vetten, eiwitten, tannines, cellulose en gesuspendeerde gerstdeeltjes. De CZV van de bezinkbare en de colloïdale gesuspendeerde stoffen in het mouterij afvalwater bedroeg tussen de (mg dm^{-3}) 20-231 en 0-176, respectievelijk (Hoofdstukken 3.2 en 5.1).

In de anaërobe behandeling van mouterij afvalwater in een ééntraps EGSB reactor bij 16 °C, waren de CZV verwijderingsefficienties gemiddeld 56 %, bij een toegepaste organische belasting variërend tussen 4.4 - 8.8 kg CZV m^{-3} dag^{-1} en een HVT van ongeveer 2.4 h. Bij 20 °C, bedroegen de verwijderingsefficienties ongeveer 66 % en 72 %; respectievelijk, bij toegepaste OVBs van 8.8 en 14.6 kg CZV m^{-3} dag^{-1}, overeenkomend met een HVT tussen de 2.4 en 1.5h (Hoofdstuk 3.2). Een lagere CZV verwijderingsefficiëntie werd verkregen bij hoge H_2 concentraties in het biogas. Een verstoord evenwicht tussen de acidificatiesnelheid en methanogenese in de reactor kan keiden tot hogere H_2 concentraties, die dan vervolgens de verdere anaërobe omzetting van de VVZ negatief zullen beïnvloeden (Stams, 1994). Met betrekking tot de verwijdering van de niet oplosbare CZV fractie, is een psychrofiel één-traps EGSB reactor systeem niet effectief, ten gevolge van de hoge superficiële snelheid in de reactor (Van der Last en Lettinga, 1992).

Bij de toepassing van een twee-traps piloot-schaal EGSB systeem kan de anaërobe behandeling van gedeeltelijk verzuurd mouterijafvalwater in de temperatuursrange van 8-12 °C succesvol verlopen bij een OVB tot 12 kg CZV m^{-3} dag^{-1} en bij een HVT van 3.5 h. Wanneer goed onderhouden, bieden dergelijke meer-traps systemen een merkwaardig stabiele performantie gedurende een lange termijn (6 maand) bij lage temperaturen, zelfs wanneer sterke variaties in de OVB, tussen 3 en 12 kg CZV m^{-3} dag^{-1}, worden toegepast (Hoofdstuk 5.1). Het gebruik van meedere stappen van het EGSB systeem verhoogd merkbaar de resultaten en de stabiliteit van het systeem in vergelijking met het ééntraps proces.

Bovendien, de CZV verwijderingsefficienties waren zeer hoog en vergelijkbaar met een maximum efficiëntie van 85 % gevonden voor de mesofiele anaërobe behandeling van brouwerij afvalwaters bij 30 °C (Pereboom, 1994). Gelijkaardige resultaten zijn gevonden bij 37 °C in een drie traps systeem (Stadlbauer et al., 1994): een efficiëntie van 80 % bij een toegepaste belasting van 25 kg CZV m^{-3} dag^{-1} en een HVT van 6 h. De in dit onderzoek verkregen verwijderingsefficiënties zijn vergelijkbaar met die gevonden door Perez et al. (1997) die 82 % CZV verwijdering vonden bij een thermofiele (55 °C) anaërobe wervelbed reactor. Echter, wanneer het systeem wordt gebruikt voor partieel verzuurd mouterijafvalwater onder psychrofiele omstandigheden, zullen problemen ontstaan met betrekking tot de vorming van een laag verzurend slib rond de granules aanwezig in de eerste module. Dit kan leiden tot gasinvang binnenin de korrel, wat tot flotatie van deze korrels kan einden (Hoofdstuk 5.1). In vergelijking met mesofiele condities (Alphenaar, 1994), hebben de verzuurders een zeer hoge biomassaopbrengst (0.22 g OS-CZV g^{-1} $CZV_{verwijderd}$; Hoofdstuk 4.2) onder psychrofiele omstandigheden, maar ze hebben ook een extreem lage afstervingssnelheid (1.57 10^{-5} h^{-1} bij 10 °C; Hoofdstuk 4.2). Bovendien, de aanwezigheid van zuurstof in het afvalwater kan leiden tot groei van facultatief anaërobe en/of aërobe organismen, die een nog hogere groei-opbrengst hebben (Shen en Guiot, 1996; Kato et al., 1993; Gerritse en Gottschal, 1992). Het twee-traps EGSB systeem kan de aanwezigheid van niet verzuurd substraat tot 10 % van de influent CZV verwerken bij een temperatuur van 8 °C, als de zuurstofconcentratie in het afvalwater lager dan 2 mg O_2 dm^{-3} blijft (Hoofdstukken 4.2 en 5.1).

Ook de concentratie aan gesuspendeerde stoffen (20-30 % van de totale CZV) in het influent beïnvloedt het slib en de slibbedkarakteristieken van de EGSB reactor negatief (Hoofdstuk 5.1). Minstens een deel van de in het afvalwater aanwezige gesuspendeerde stoffen moet worden verwijderd, alvorens het afvalwater in het EGSB systeem te brengen. Een significante afbraak van gesuspendeerde stoffen kan in psychrofiele reactoren nauwelijks worden verwacht, omdat bij lage temperaturen de hydrolysesnelheid van gesuspendeerd materiaal scherp afneemt en zelfs tot nul nadert voor verschillende soorten particulair materiaal (Zeeman et al, 1996; Van der Last en Lettinga, 1992; De Man et al., 1986).

6.1.5 CONCLUSIES

1. Koude (8-12 °C), laag geconcentreerde (< 1000 mg CZV dm^{-3}) en vergaand verzuurde afvalwaters kunnen efficiënt (>90 %) behandeld worden in een ééntraps 'hoogbelast' anaërobe EGSB reactor bij een OVB van 12 kg CZV m^{-3} dag^{-1}.
2. Bij het toepassen van een twee traps EGSB systeem voor partieel verzuurd mouterij afvalwater in de temperatuursrange 8-12 °C, kan een OVB tot 12 kg CZV m^{-3} dag^{-1} worden toegepast bij een HVT van 3.5 h. Het moduleren van het EGSB systeem verhoogd sterk de resultaten en stabiliteit van het systeem in vergelijking met het éénstaps proces. Een tweetraps systeem gaf een stabiele bedrijfsvoering gedurende lange termijn bij lage

temperaturen, zelfs als sterke fluctuaties in de OVB tussen de 3 tot 12 kg CZV m^{-3} dag^{-1} optraden.

3. De maximum specifieke omzettingssnelheid van het methanogene inoculum slib verhoogde met een factor 3 na een bedrijfsvoering van 300 dagen bij een temperatuur van 10-12 °C. De resultaten van dit onderzoek toonden duidelijk aan dat mesofiele micro-organismen groeien en metaboliseren bij sub-optimale temperaturen. Nieuw gevormde methanogene biomassa wordt voldoende in de tweede module vastgehouden. Het methanogene slib behield de methanogene activiteit verworven onder psychrofiele condities, zelfs na een langdurige, ongevoede opslagperiode (6 maand bij 4 °C).
4. Flotatie van korrelslib kan problemen veroorzaken in de eerste trap, in geval van onvoldoende verzuurd afvalwaters. Dit kan te wijten zijn aan de overmatige groei van acidogene en facultatief anaërobe populaties rondom de methanogene granules. Om deze problemen te voorkomen, is een voorverzuringsstap noodzakelijk. De ontwerpcriteria van dergelijke psychrofiele hoogbelast voorverzuringsstap moeten nog worden bepaald.
5. De gesuspendeerde stof concentraties (> 10-15 % van de totale CZV) in het influent kunnen een negatief effect op het slib en de slibbedkarakteristieken van de EGSB reactoren hebben. Ten minste een deel van de in het influent aanwezige gesuspendeerde stoffen moeten worden verwijderd alvorens het afvalwater aan het EGSB systeem te voeden.
6. De verkregen resultaten zijn een definitieve doorbraak voor de toepassing van anaërobe behandelingssystemen bij lage omgevingstemperaturen voor laag geconcentreerde afvalwaters. Onze resultaten zijn vooral aanmoedigend voor de praktijkschaal toepassing van anaërobe behandeling van laag geconcentreerde afvalwaters onder psychrofiele condities.

Bijgevolg kan worden besloten dat anaërobe afvalwaterzuivering nu een haalbaar en zeer aantrekkelijk alternatief vormt voor de behandeling van laag geconcentreerde verzuurde afvalwaters, vooral gezien de principiële voordelen ten opzichte van conventionele aërobe zuiveringssystemen.

6.1.6 REFERENTIES

Alphenaar, A. Anaerobic granular sludge: characterization, and factor affecting its functioning. PhD. Thesis, Agricultural University Wageningen, The Netherlands, pp 93-112, 1994.

Banik, G. C.; Dague, R. R. ASBR treatment of dilute wastewater at psychrophilic temperatures. In *Proc. of 69th Annual Water Environmental Conference*, Dallas, Texas, USA, 1996; pp 235-246.

Boone, D. R., Bryant, M. P. Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystem. *Appl. Environ. Microbiol.*, **1980**, 40, 626-632.

Grant, S.; Lin, K. C. Effects of temperature and organic loading on the performance of upflow anaerobic sludge blanket reactors. *Can. J. Civ. Engin.*, **1995**, 22, 143-149.

Gujer, W.; Zehnder, A. J. B. Conversion processes in anaerobic digestion. *Wat. Sci. Technol.*, **1983**, 15, 127-167.

Henzen, M.; Harremoes, P. Anaerobic treatment of waste water in fixed film reactors-literature review. *Wat. Sci. Technol.*, **1983**, 15, 1-102.

Jetten, M.S.M., Stams, A.J.M. and Zehnder, A.J.B. 1992. Methanogenesis from acetate: A comparison of the metabolism in *Methanothrox soehngenii* and *Methanosarcina* spp. FEMS *Microbiol. Rev.* **88**: 181-198.

Kato, M. T. The anaerobic treatment of low strength soluble wastewaters. Ph.D. Thesis, Agricultural University Wageningen, The Netherlands, pp 101-128, 1994.

Last van der, A. R. M.; Lettinga, G. Anaerobic treatment of domestic sewage under moderate climatic (Dutch) conditions using upflow reactors at increased superficial velocities. *Wat. Sci. Technol.*, **1992**, 25, 167-178.

Lettinga, G. Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek*, **1995**, 67, 3-28.

Lettinga, G. Feasibility of anaerobic digestion for the purification of industrial waste waters. In: *Proceeding 4th Symposium EAS,* München, Germany, 1978; pp 226-256.

Lier van, J.B. Thermophilic anaerobic wastewater treatment; temperature aspects and process stability. Ph.D. Thesis, Agricultural University Wageningen, The Netherlands, pp 124-144, 1995.

Van Lier, J.B., Grolle, K.C.F., Frijters, C.T.M.J., Stams, A.J.M. and Lettinga, G. Effect of acetate, propionate and butyrate on the thermophilic anaerobic degradation of ropionate in methanogenic sludge and defined cultures. *Appl. and Environ. Microbiol.*, **1993**, 57: 1003-1011.

Man de, A. W. A.; van der Last, A. R. M.; Lettinga, G. The use of EGSB and UASB anaerobic systems for low strength soluble and complex wastewaters at temperatures ranging from 8 to 30°C. In: *Proc. of the Fifth International Symposium on Anaerobic Digestion*. ed. ER Hall and PN Hobson, Bologna, Italy, 1988; pp 197-209.

Man de, A. W. A.; Grin, P. C.; Roersma, R. E.; Grolle, K. C. F.; Lettinga, G. Anaerobic treatment of municipal wastewater at low temperatures. In: *Proc. Conference Anaerobic Treatment a grown up Technology*, Amsterdam, The Netherlands, 1986; pp 453-466.

Matsushige, K.; Inamori, Y.; Mizuochi, M.; Hosomi, M.; Sudo, R. The effects of temperature on anaerobic filter treatment for low-strength organic wastewater. *Environ. Technol.*, **1990**, 11, 899-910.

Pereboom, J. H. F. Methanogenic granule development in full scale internal circulation reactors. *Wat. Sci. Technol.*, **1994**, 30, 211-221.

Péres, M.; Romero, L. I.; Sales, D. Thermophilic anaerobic degradation of distillery wastewater in continuous-flow fluidized bed bioreactors. *Biotechnol. Prog.*, **1997**, 13, 33-38

Stadlbauer, E. A.; Oey, L. N.; Weber, B.; Jansen, K.; Weidle, R.; Löhr, H.; Ohme, W.; Döll, G. Anaerobic purification of brevery wastewater in biofilm reactors with and without a methanation cascade. *Wat. Sci. Technol.*, **1994**, 30, 395-404

Stams, A. J. M. Metabolic interactions between anaerobic bacteria in methanogenic enviroments. *Antonie van Leeuwenhoek*, **1994**, 66, 271-294.

Stams, A. J. M.; Kremer, D. R.; Nicolay, K.; Weenk, G. H.; Hansen, T. A. Pathway of propionate formation in *Desulfobulbus propionicus*. *Arch. Microbiol.*, **1984**, 139, 167-173.

Switzenbaum, M. S.; Jewell, W. J. Anaerobic attached film expanded bed reactor treatment of dilute organics. In: *Proceedings of the 51st Annual WPCF Conference*, Anaheim, California, USA, 1978, pp 1-164.

Visser, A. The anaerobic treatment of sulphate containing wastewater. Ph.D. Thesis, Agricultural University Wageningen, The Netherlands, pp 109-120, 1995.

Zeeman, G.; Sanders, W. T. M.; Wang, K. Y.; Lettinga, G. Anaerobic treatment of complex wastewater and waste activated sludge. In *Proc. Conference Advanced Wastewater Treatment*, Amsterdam, The Netherlands, 1996, pp 225-232.

Wiegant, W. M. Thermophilic anaerobic digestion for waste and wastewater treatment. Ph.D. Thesis, Agricultural University Wageningen, The Netherlands, pp 95-111, 1986.

Wu, W-M.; Hickey, R. F.; Zeikus, J.G. Characterization of metabolic performance of methanogenic granules treating brewery wastewater: Role of sulfate-reducing bacteria. *Appl. Environ. Microbiol.*, **1991**, 57, 3438-3449.

BIBLIOGRAPHY

Rebac, S. (1991) Phosphate removal from domestic wastewater. Journal "Nase Vode" Sarajevo, Yugoslavia.

Lettinga, G., De Man, A., van der Last, A., Kato, M. Wang, K., Rebac, S. (1993) Possible role of anaerobic digestion in sewage treatment under moderate climatic conditions. Paper presented at the 2nd Japan/Dutch Workshop on Water Quality Manegement. Ube, Japan.

Van Lier J.B., S. Rebac, and G. Lettinga (1995). Advanced process design for anaerobic wastewater treatment under low and high temperature conditions. *In*: M. Dohányos et al. (ed), Proc. of Sludge and Solid Waste Conference - Czech Technical Society, Brno, Czech Republic, October 17-19, 1995, pp: 263-280.

Rebac, S., J. Ruskova, S. Gerbens, J.B. van Lier, A.J.M. Stams and G. Lettinga (1995). High-rate anaerobic treatment of wastewater under psychrophilic conditions. J. Fermen. Bioengin., 80: 499-506.

Van Lier J.B., S. Rebac, and G. Lettinga (1996). High-rate anaerobic wastewater treatment under psychrophilic and thermophilic conditions. *In*: Proc. of the IAWQ-NVA conference on Advanced Wastewater Treatment, September 23-25,1996, Amsterdam, The Netherlands.

Rebac, S., A. Visser, S. Gerbens, J.B. van Lier, A.J.M. Stams and G. Lettinga (1996). The effect of sulphate on propionate and butyrate degradation in a psychrophilic anaerobic expanded granular sludge bed (EGSB) reactor. Environ. Technol., 17: 997-1005.

Kato, M.T., Rebac, S., Field, J.A. and Lettinga, G. (1996) Tratamento anaeróbio de águas residuárias em reatores relge. In: Memorias IV Seminario-Taller Latinoamericano sobre Tratamiento Anaerobio de Águas Residuales. Bogota, Colombia, October 1996, pp 175-185.

Rebac, S., J.B. van Lier, M.G.J. Janssen, F. Dekkers, K.T.M. Swinkels, and G. Lettinga (1997). High-rate anaerobic treatment of malting waste water in a pilot-scale EGSB system under psychrophilic conditions. J. Chem. Tech. Biotechnol., 68: 135-146.

Van Lier, J.B., S. Rebac, P. Lens, F. van Bijnen, S.J.W.H. Oude Elferink, A.J.M. Stams and G. Lettinga (1997). Anaerobic treatment of partly acidified wastewater in a two- stage expanded granular sludge bed (EGSB) system at 8 °C. *In:* Proceedings of 8th Intl. Conf. on Anaerobic Digestion. 25-29 May, 1997, Sendai, Japan.

Van Lier J.B., S. Rebac, and G. Lettinga (1997). High-rate anaerobic wastewater treatment under psychrophilic and thermophilic conditions. Wat. Sci. Technol., 35-10: 199-206.

Rebac, S., F. Omil, J.B. van Lier and G. Lettinga (1997). Tratamiento anaerobio de aguas residuales de baja carga en condiciones psicrofilas (10-12 °C). Tecnologia del Agua, 17 nr. 167: 41-48.

Van Lier, J.B., S. Rebac, P. Lens, F. van Bijnen, S.J.W.H. Oude Elferink, A.J.M. Stams and G. Lettinga (1997). Anaerobic treatment of partly acidified wastewater in a two- stage expanded granular sludge bed (EGSB) system at 8 °C. Wat. Sci. Technol., 36-6/7: 317-324.

Rebac, S., van Lier, J.B., Lens, P., Stams, A.J.M., Dekkers, F., Swinkels, K.Th.M. and Lettinga, G. (1998) Psychrophilic anaerobic treatment of low strength wastewaters. In: Proceeding of International WIMEK Congress Options for Closed Water Systems, Wageningen, The Netherlands, March 11-13.

Lettinga, G., Rebac, S., van Lier, J.B., Zeeman, G. (1998) The potentials of sub-mesophilic and/or psychrophilic anaerobic tretment of low strength wastewaters. In: R. Margesin and F. Schinner (eds.), Cold-Adapted Organisms – Fundamentals and Applications, Environmental biotechnology, Landes Bioscience, Georgetown, Texas, USA, (in press).

Kato, M.T.; Rebac, S.; Lettinga, G. (1998) The anaerobic treatment of low-strength brewery wastewater in expanded granular sludge bed. *Appl. Biochemist. Biotechnol.* (in press).

Rebac, S., van Lier, J.B., Lens, P., van Cappellen, J., Vermeulen, M., Stams, A.J.M., Dekkers, F., Swinkels, K.Th.M. and Lettinga, G. (1998) Psychrophilic (6 - 15 °C) high-rate anaerobic treatment of malting waste water in a two module EGSB system. *Biotechnol. Progress* (in press).

Rebac, S.; Gerbens, S.; Lens, P.; van Lier, J. B.; Stams, A. J. M.; Lettinga, G. (1998) Kinetic of fatty acid degradation by psychrophilically cultivated anaerobic granular sludge. *Bioresource Technol.*, (in press).

Submitted:

Rebac, S., van Lier, J.B., Lens, P., Stams, A.J.M., Dekkers, F., Swinkels, K.Th.M. and Lettinga, G. (1998) Psychrophilic anaerobic treatment of low strength wastewaters, *Wat. Sci. Techol.*, (modified version accepted for publication).

Lettinga, G., Rebac, S., Parshina, S., Nozevnikova, A., Van Lier, J. B., Stams, A. J. M. (1998) Staged high-rate anaerobic treatment of organic wastewater at psychrophilic conditions. *Appl. Environ. Microbiol.*, submitted.

Curriculum vitae

The author of this dissertation was born in Mostar, Ex-Yugoslavia. He received his B.Sc. degree in Processing Technique Environmental Protection from Mechanical Engineering Faculty, University of Sarajevo, Yugoslavia. His Master of Science degree was granted from International Institute for Hydraulic and Sanitary Engineering Environmental Engineering, Delft The Netherlands in 1986 and Civil Engineering Faculty, University of Zagreb, Yugoslavia in 1990. The topic of master thesis was on "Phosphate removal from domestic wastewater". He worked as sanitary engineer in consulting company developing design and plants in the field of domestic and industrial wastewater treatment systems. From June 1992 till January 1994 he worked as guest researcher at Department of Environmental Technology at Wageningen Agricultural University. In January 1994 he started his Ph.D. studies at Department of Environmental Technology at Wageningen Agricultural University. In December 1997 he became citizen of The Netherlands. Since January 1997 he has been working on several research projects for anaerobic treatment of domestic and industrial wastewaters at Department of Environmental Technology at Wageningen Agricultural University.

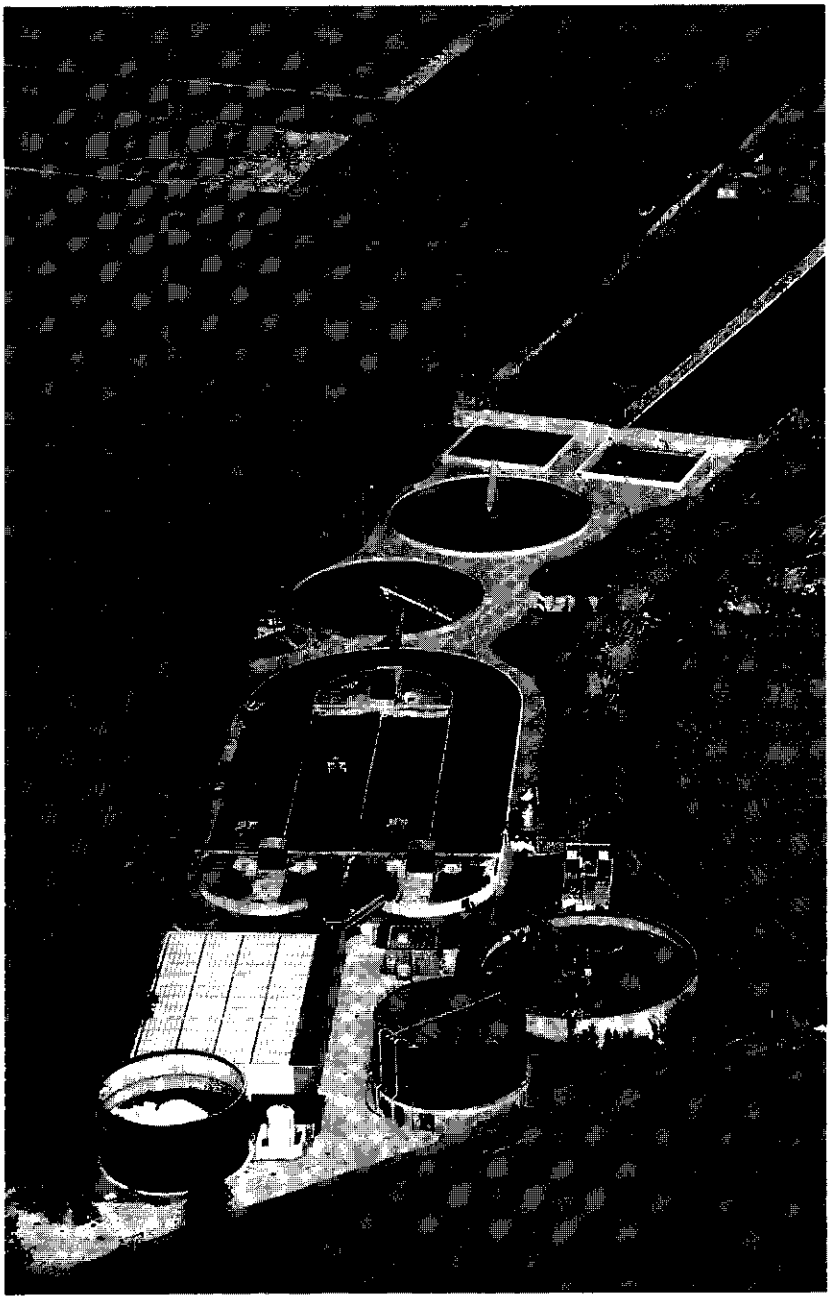

Photo: The seed granular sludge used in this research originate from the UASB reactor of the wastewater treatment plant BAVARIA B.V., Lieshout, The Netherlands.