ANAEROBIC TREATMENT OF PROTEIN, LIPID AND CARBOHYDRATE CONTAINING WASTEWATERS USING THE EGSB TECHNOLOGY

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Aos meus pais

e à Mariza

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RONALDO PETRUY

PROPOSITIONS

1-The relatively high values of rate constants mentioned in literature for the hydrolysis of lipids are rather exception than rule and therefore provide a far too favorable picture of what actually can be achieved in modern high rate anaerobic treatment systems in practice.

Gujer, W. & Zehnder, A. J. B. (1983). Conversion processes in anaerobic digestion. Wat. Sci. Tech., 15, 127-167.

2-A sieve drum gas liquid solids separator represents an attractive option for retaining buoying granular sludge in EGSB-reactors and therefore offers big potentials for treating wastewaters containing lipids and higher fatty acids as pollutants.

This thesis

3-In anaerobic treatment of wastewaters containing proteins and sugars as major pollutants it should be taken into account that sugars are the preferred substrates for anaerobic organisms.

Breure, A. M., Mooijman, K. A. & van Andel, J.G. (1986). Protein degradation in anaerobic digestion: influence of volatile fatty acids and carbohydrates on hydrolysis and acidogenic fermentation of gelatin. Appl. Microbiol. Biotechnol., 24, 426-431.

4-To reach a tenable development in third word countries environmental protection should constitute an integral part of the development process.

United Nations-Rio de Janeiro-Brazil-June 3-4,1992, Principle 4.

5-For the treatment of wastwaters containing soluble proteins and carbohydrates in high rate anaerobic treatment systems like the EGSB-system, the application of a separate acidogenic reactor may constitute an additional essential process step.

This thesis

6-As sulfate reducers can contribute to an effective elimination of long chain fatty acids from wastewaters, the practical application of the process of sulfate reduction in the treatment of lipid containing wastewaters deserves serious consideration.

Yamaguchi, T., Harada, H. & Tseng, I-C. (1997). Competitive exclusion of methane-producing bacteria by sulfate-reducing bacteria in anaerobic degradation of long-chain fatty acids. In: Proceedings of The 8th International Conference on Anaerobic Digestion, May 25-29, Sendai, Japan, 2, 362-370.

7-Conventional 'high tech' aerobic wastewater treatment systems should not be implemented in developing countries and countries suffering from a poor economy.

8-Domestic waste and wastewater problems need be attacked much more multidisciplinary than the presently is the case.

9-The competence level of sanitary engineerings is reflected in excessive size of conventional sewage treatment plants projected by them.

10-The police implemented in Curitiba to accomplish a socially more friendly and environmentally more sustainable society deserves worldwide imitation.

Propositions belonging to the thesis **"Anaerobic treatment of protein, lipid and carbohydrate containing wastewaters using the EGSB technology"**.
Ronaldo Petruy
Wageningen, March 31, 1999.

ABSTRACT

Petruy, R. (1999) Anaerobic Treatment of Protein, Lipid and Carbohydrate Containing Wastewaters using the EGSB technology.
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Industries such as margarine, meat packing, dairy, slaughterhouse, edible oil (palm and olive oil) generate large amount of effluents. Strict environment laws in numerous countries has forced these agro-industries to apply suitable wastewater treatment in order to reduce the organic pollution load before discharging the effluents to receiving waters. Anaerobic treatment comprises a very attractive and suitable method for these industries, where the effluents frequently are composed of mixtures of proteins, lipids and carbohydrates. The research described in this thesis deals with the feasibility of anaerobic treatment for complex types of wastewaters, e.g. containing mixtures of lipids, proteins, and carbohydrates. In the research particular emphasis was afforded to the application of Expanded Granular Sludge Bed (EGSB) reactors, because results obtained in earlier research indicated that the EGSB reactor-system might represent special promise for lipid containing wastewaters.

Chapter 1 provides a brief literature survey of relevant reports dealing with the production of complex industrial wastewaters and with information about the feasibility of anaerobic treatment systems like anaerobic sludge bed reactors, viz. the well known UASB-system and the more recently introduced EGSB-reactor.

Chapter 2 deals with investigations concerning the degradation of a milk-fat emulsion using a closed circuit with an EGSB reactor as treatment system. The results of the experiment show that 70 % of the lipids were adsorbed on the granular sludge. This adsorbed fraction remained greatly non-degraded; as a matter of fact only biodegradation was found for the colloidal fraction, although the process proceeded very slowly, which mainly could be due to the very slow rate of hydrolysis, viz. a value of the hydrolysis rate k_h of 0.01 d^{-1} was assessed. The main mechanism prevailing in the lipid removal in an EGSB-system comprises a sorption process.

Chapter 3 deals with investigations conducted with an expanded granular sludge bed (EGSB) reactor equipped with a sieve drum as gas-liquid-solid separator device (GLS) to prevent the wash-out of buouying sludge. Two sieve drum designs were evaluated in experiments conducted with complex synthetic wastewaters composed of mixtures of carbohydrate, protein and lipids. One of these devices was capable to retain floating granular sludge efficiently and without damaging the granular sludge structure. The results of this experiment also revealed that 85 % of effluent leaving the reactor consisted of 'soluble' type of pollutants. The hydrolysis and acidification of this fraction was found to proceed very slowly. Moreover a peculiar phenomenon manifested, i.e. drops in the concentration of non-acidified soluble COD (NAS) in the effluent coincided with an increase in the amount of colloidal COD and vice versa. Results presented of batch biodegradability assays with different types of proteins, e.g. originating from potato, corn, milk, egg, gelatin and bovine. The results revealed that the deamination generally proceeds well, but the conversion of the proteins into methane-COD looks rather unsatisfactory.

The investigations in Chapter 4 were addressed to the application of the EGSB technology to complex synthetic wastewater composed of carbohydrates, beer, proteins like gelatin and a milk-fat lipid emulsion. The results show that the organic pollutants originating from or present in beer and gelatin were well removed at a high COD removal efficiency (90-95 %), and at a satisfactory COD-conversion efficiency to methane, i.e. for 85 %, at imposed OLR's up to 12 g COD/l d. The deamination of gelatin amounted to 86-89 %. Lipids, applied in a concentration of 0.260 g COD/l, did not detrimentally affect the reactor performance. However, on the other hand the degradation of lipids did not proceed satisfactorily.

Chapter 5 presents results of research dealing with the applicability of the EGSB reactor to wastewaters composed of mixtures of gelatin and sucrose using non-adapted granular sludge and in presence/absence of macro-nutrients. The results show that the degradation (i.e. the deamination) of gelatin reaches 70-90 % at space loads up to 7 g COD/l.d , but once again the acidification remained poor and the conversion to methane amounted only to 65 %. Nevertheless a high COD removal efficiency was found with a figure in the range 85-90 %, indicating that a certain fraction of the gelatin or its intermediates have become sorbed to the sludge surface or precipitated in the reactor. Addition of sucrose to the experimental feed solution resulted in a slight improvement of its deamination, but also
in further decline in the fermentation process. After addition of nutrients to the feed solution, viz. simultaneously with gelatin and sucrose in the feed storage vessel, the pre-acidification of both these substrates improved . But when gelatin and sucrose were introduced via separate lines in the reactor, the fermentation of

gelatin dropped sharply. From these observations it can be concluded that apparently sucrose seriously depresses the fermentation of gelatin.

Chapter 6 summarizes the results of the experiments, and the scientific relevant aspects are highlighted and discussed. Moreover, based on the insights obtained, some recommendations for the operation of an EGSB-system have been provided.

CONTENTS

Chapter 1

ANAEROBIC TREATMENT OF PROTEIN, LIPID AND CARBOHYDRATE CONTAINING WASTEWATERS USING THE EGSB TECHNOLOGY

General Introduction

Many industries such as margarine, meat packing, dairy, slaughterhouse, edible oil (palm oil and olive oil) industries produce large amounts of effluent that pose a high threat to the environment of urban regions if these effluents do not receive any kind of treatment. The extend of the discharge of these industries is considerable. So in Malaysia in 1980 the amount of wastewater discharged from palm oil industries to receiving waters was up to 330 tons/day of BOD (Ng *et al.*, 1985). In the Mediterranean area up to 1.4-1.8 million tons of olive oil is processed yearly, which results in a high pollution because 45-55 kg BOD_5 per ton of olives is produced in olive oil production. The pollution load of the olive oil mills therefore amounts up to 2800-3600 tons per day over a season of 100 days (Boari *et al.*, 1984). In the USA, the annual whey production amounts to approximately 16.8×10^9 kg (Palmer & Marguardt, 1978 in Switzenbaum, 1982). In the UK dairy processing plants generate approximately 700 m^3/day of wastewater (Kasapgil *et al.*, 1994), while in the State of Paraná, (Brazil) the amount of wastewater from dairy industries in 1977 was 6486 kg BOD/day, equivalent to a population of 120 thousand people (Bório & Pawlowsky, 1977). In Western Australia 546 dairy farms (Master, 1993) in 1989 produced 1.66×10^9 kg of liquid whey, in Canada the production of sweet whey in 1981 amounted to 1.69×10^9 kg (Schmidtke & Bisset, 1985) and in the European Union up to 39.52 million of tons liquid whey (Gurguis *et al.*, 1993). Dairy plants in Turkey produce 1500 m^3 of wastewater per day (Orhon *et al.*, 1993). Meat packing plants in the USA produce 15140 m^3/day of wastewater (Kane & Osantowiski, 1980). From these numbers it is clear that these countries need to invest in wastewater treatment. The principal components in the effluents from these industries are proteins, lipids and carbohydrates. The pollution power of these components can be expressed in terms of COD, the amount of oxygen required for the complete chemical oxidation. The higher the biodegradable COD-content of an industrial effluent, the higher is the pollution load they cause when discharge to receiving waters, because it will result in a serious deficit of oxygen to oxygen-dependent aquatic organisms. This clearly can lead to a serious deterioration of aquatic life. Strict environment laws established in numerous countries has forced agro-industries to apply proper wastewater treatment methods to reduce the organic pollution load of the effluents prior to their discharged into receiving waters. For this reason there is an increased interest at these industries in the development of low cost, efficient and easy to operate treatment systems. Anaerobic treatment comprises for many of the agro-industries an extremely attractive wastewater treatment option, because the method in principle is effective in removing proteins, lipids and carbohydrates, which in various ratios are the main constituents of the effluents of these industries. Under anaerobic conditions these compounds are converted into methane, carbon dioxide and water (Pavlostathis & Giraldo-Gomes, 1990; Petruy, 1989). The

advantages of anaerobic treatment over conventional treatment systems are well known, viz. they comprise for instance the small area of land required because frequently compact reactor systems can be applied (i.e. high organic and hydraulic space loads), very low energy consumption, small amount of excess of sludge generated, the production of biogas which represents an attractive energy source. Since also adapted anaerobic sludge can be preserved for long period of time under unfed conditions, the anaerobic treatment options therefore is attractive for agro-industries.

The anaerobic digestion process proceeds via a number of separate steps as has described by Breure (1984) and Gujer and Zhender (1983) and many others: for complex insoluble organic pollutants the first step is hydrolysis and/or liquefaction of organic polymers, which leads to the formation of smaller soluble 'monomers'. These monomers can be taken up by bacteria. So proteins are degraded to a variety of amino acids, polysaccharides to sugar monomers and fats/oils to glycerol and long-chain fatty acids (LCFA). In he second step, known as the acidogenesis step, the intermediates of the hydrolysis step are fermented to volatile fatty acids, alcohols, lactic acid, ammonia and also carbon dioxide and hydrogen gas will produced. These fermentation processes comprise the source of energy for the acidogenic population. The organic fermentation products constitute the substrate for the organisms prevailing in the third step, the acetogenic bacteria. These organisms oxidize higher fatty acids to acetic acid, carbon dioxide and hydrogen which are the main substrates for the methanogens. It is only in the last (forth) step that major part of the biodegradable COD is eliminated from the solution. According to Hanaki *et al.* (1981), Breure *et al.* (1986*a*, 1986*b*), Briant (1979), Fox and Polhand (1994) methanogenesis only proceeds completely when the partial pressure of the hydrogen is kept at a very low level, consequently when hydrogen eliminated from the system, e.g. by methanogenes or sulfate reducing organisms. In methanogenic step, the predominant reactions proceeding are the reduction of carbon dioxide by hydrogen, and the conversion of acetate to methane and carbon dioxide. In addition to these compounds, also methanol is a substrate for a specific group of methanogens.

The four steps described above are involved in the anaerobic wastewater treatment of complex types of wastewaters, e.g. those containing high molecular compounds. Every change in the conditions imposed to the system, either physical, chemical or biological, will need an adaptation process of the system, which ultimately will lead to the required associated microbiological communities. As consequence changes in bacterial metabolism, through alterations at the enzymatic level, or growth or decay of certain metabolically active members of the population can occur. Therefore process conditions such as pH, temperature and retention time are very important for maintaining certain kinds of bacteria best suited for the habitat. McCarty and Mosey (1991) (in Nachaiyasit & Stuckey, 1995) suggested that the relative ratios of reduced end products produced by the catabolism of carbohydrates are controlled more by populations dynamics than by kinetical or thermodynamics factors: a competition between propionic and butyric acid producing bacteria would be important.

A specific model of anaerobic digestion, know as the "Rain Barrel" model, has been proposed by Schink in 1988 (in Nachaiyasit & Stuckey, 1995) (Fig.1). This model involves a

community of fermentative bacteria in which the complex substrate flowing in at A is transformed to the products flowing out at B, C or D. At B low-energy products of fermentation process are released, such as acetate, hydrogen, and carbon dioxide. At position C the more reduced intermediates such as propionate, butyrate and others are released. Under normal conditions (water level E), nearly all substrate carbon passes exclusively through outlet B. Outlet C is used only if the capacity of B (as a function of methanogenic activity) is not sufficient to balance the inflow or otherwise at normal substrate inflow, if outlet B is partially or completely blocked as a consequence of inhibition of methanogenic bacteria. However, a complete blocking of this outlet could lead to higher accumulation of diverse fatty acids (level E), which would lower the pH and as further consequence would even cause ethanol and lactate to be formed (outlet D). The flux of fatty acids never is zero if a complex substrate mixture is degraded. This model represents graphically the anaerobic process and its regulation.

According to the above described steps in anaerobic treatment, proteins defined as linear chains of amino acids with peptide bound (Lehninger, 1975), are hydrolyzed to amino acids in the first step (Jeris & McCarty, 1965; McInerney, 1988). The various amino acids are deaminated via Strickland reaction (Nielsen, 1954) to e.g. higher fatty acids, which next are fermented to acetic acid and finally converted to methane plus CO_2. Lipids, which for the most part are tryglycerides composed of glycerol esterified with long-chain fatty acids (Mulder & Walstra, 1974; Larson, 1994; Lehninger, 1975) are hydrolyzed to long-chain fatty acids under anaerobic conditions. The long-chain fatty acids are degraded further by β-oxidation into mainly acetic acid, which is converted to methane and CO_2 (Jeris & McCarty, 1965; McInerney, 1988). The rate limiting step in the degradation of lipids could comprise the diffusion of lipids and/or degradation of LCFA (Heukelekian & Muller, 1958) or the liquefaction of lipids, particularly when lipids are present in adsorbed form in/on granular sludge or present in agglomerates (Sayed, 1987). The product of the hydrolysis step, long-chain fatty acids were found to represent serious inhibitors of acetogenic and methanogenic organisms (Rinzema, 1988; Jeris & McCarty, 1965; Hanaki *et al.*, 1981; Novak & Carlson, 1970). Carbohydrates, polymers made of glucose sub-units (Lehninger, 1975), are converted to volatile fatty acids as main intermediates and next via acetic acid and hydrogen to methane and CO_2 (Jeris & McCarty, 1965; Zoetemeyer *et* al., 1982; Cohen *et al.*, 1979; Breure, 1986).

Anaerobic wastewater treatment is applied in reactors systems, where optimal operational conditions can be maintained, particularly with respect to the retention of the sludge and the amount of contact between sludge and wastewater. The feed generally is supplied in a continuous mode. In the early 1970's, big advances were made in anaerobic wastewater treatment technology with the development of the Upflow Anaerobic Sludge

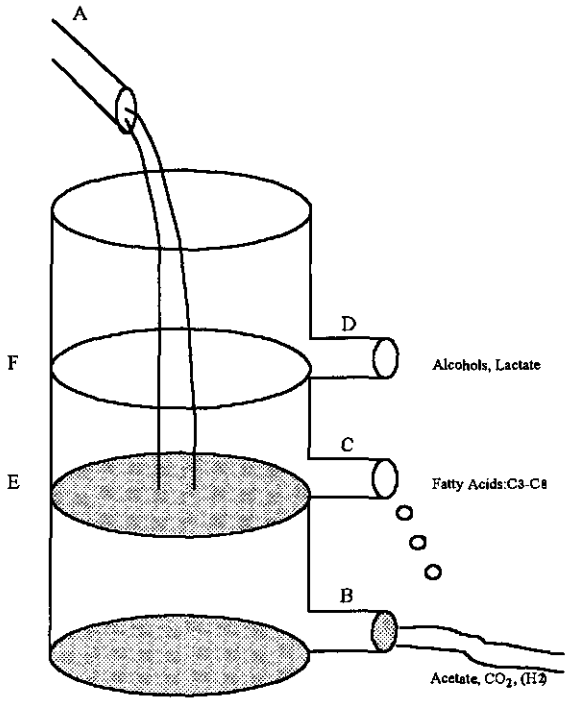


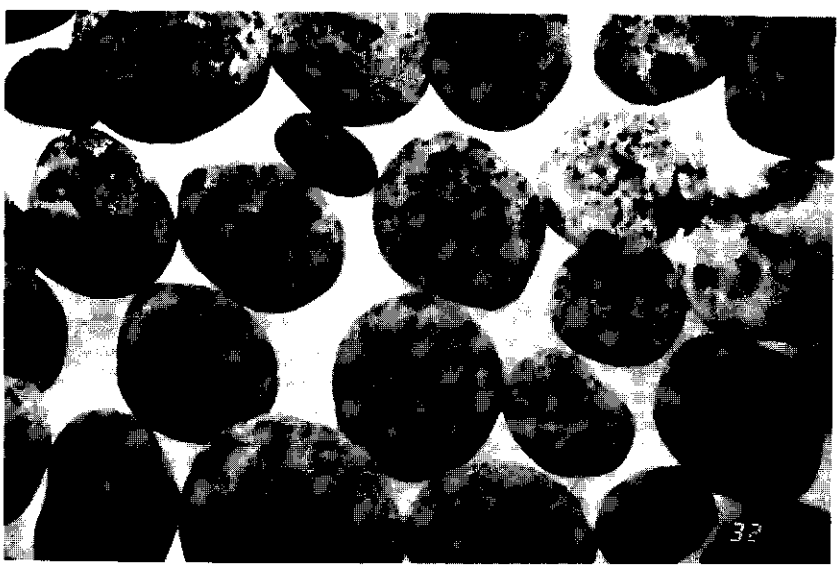
Fig. 1 *Rain barrel model of carbon and electron flow in methanogenic degradation.* (A)inflow. (B) low outlet, representing low-energy products. (C) medium outlet, representing fatty acids. (D) high outlet, representing high-energy products. (E) normal water level. (F) high water level. Hydrogen at lower outlet is writing in brackets to indicate that its partial pressure has to be kept very low (from Schink, 1988, in Nachaiyasit & Stuckey, 1995).

Bed (UASB) reactor system (Lettinga *et al.*, 1980). The basic idea underlying the UASB reactor concept is that wastewater is introduced at bottom of the reactor and passes contacts a dense layer of viable well settleable sludge, and then passes an internal baffle system for separation of gas liquid and solids (GLS-device). In this settler part of GLS-device dispersed sludge particles, which frequently are composed of self-aggregating bacterial communities, are allowed to settle out and to return back to the digestion compartment. The systems ensures an adequate sludge retention, resulting in the required high retention of anaerobic sludge in the reactor (Pette *et* al., 1980).

The first 6 m^3 pilot plant was built in 1972 with at Centrale Suiker Maatschappij (CSM), The Netherlands (Pette *et al.*, 1980), treating sugar beet wastewater. Based on the very promising results obtained in this pilot plant a 30 m^3 and later a 200 m^3 demonstration UASB-reactor was constructed in the period 1974 and 1976. Encouraged with the excellent results obtained in the 200 m^3 UASB-plant the first full-scale UASB-treatment plant with volume capacity of 1000 m^3 was put in operation in 1997 for treating the entire effluent flow of 240 m^3/h at CSM sugar beet factory in Halfweg, near Amsterdam.

An interesting and quite important phenomenon proceeding in treating sugar beet wastewater in the UASB pilot plants was the granulation of the anaerobic sludge. Granules of 0.25-2.5 mm developed in the reactor; the bacteria present in these aggregates mainly consisted of rod shaped organisms. The mechanism underlying the granulation process is based on the selection pressure prevailing in the system which results in a wash-out of finely dispersed mater, and on strong auto-immobilization characteristic of the organisms involved in the granulation process. The granules formed posses a high settleability velocity, and as a result they are well retained (Forster & Quarmby, 1995). The microorganisms in the granules are usually present in densely packed balanced communities; little space is lost for inert support materials. The spherical granules provide a maximal microorganism-to-space ratio. Although buoyant densities of granules are equal to densities for discrete bacterial cell, granules show excellent settling properties because of their large size (Stoke's law) (Guiot *et al*, 1992; Hulshoff Pol *et al.*,1986). Due to the long period of viable storage which can be applied, the granular sludge of a UASB reactor can be used to seed another reactor. In this way the period required for first start up of anaerobic treatment systems can be very substantially reduced (Picture 1).

For illustration, the model for structure of granular sludge as aggregates as proposed by Guiot *et al.* (1992) is shown in Figure 2.



Picture 1. Granular Sludge from full scale UASB reactor, Industriewater, Eerbeek, The Netherlands.

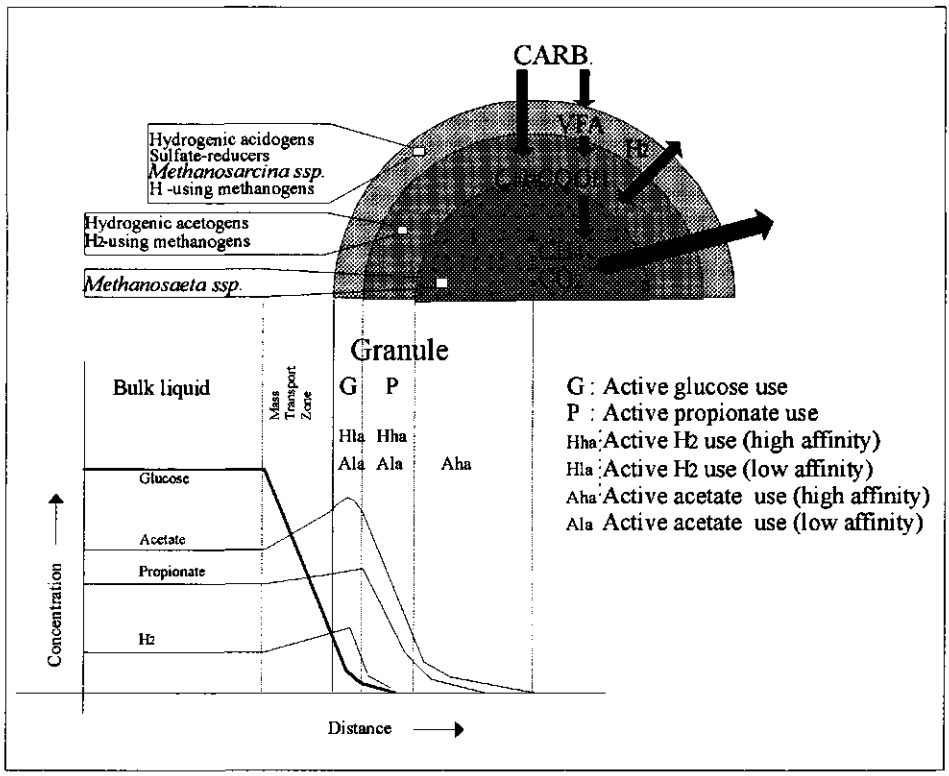


Fig. 2 Proposed structure of the granule population arrangement, related to a substrate and product diffusion model for glucose-fed granules, from Guiot *et al.*(1992).

With the development of the UASB reactor concept, anaerobic wastewater treatment received increasingly more credit for the treatment of a large variety of liquid organic effluents of different types of agro-industries and recently even of wastewaters of chemical industries. The achieved efficiencies were equal or sometimes even superior over other wastewater treatment systems, while the operational cost were distinctly lower. At data a large number of the reactors have been put in operation worldwide, in 1996 the registered number of the reactors amounted to about 914 (see in Table 1).

Problems in anaerobic treatment using complex wastewater

Anaerobic treatment, e.g. when using UASB reactors, for complex wastewaters such as those composed of proteins and lipids may be accompanied with serious operational problems. Problems as foaming and flotation of granular sludge in UASB reactors have been reported with wastewaters containing proteins as major pollutant. This for instance was the case for potato processing wastewaters. As a result of these problems, heavy wash-out of granular sludge can occur. Moreover as a high concentration of ammonia is

Table 1. Full-scale UASB installations*

Factory	Country	Type of wastewater	Year of contract	Design capacity kg COD/d	Design Volume (m^3)
Kufbo, Waspik	Netherlands	potato processing	1981	1920	330
Residentia Slachthuis, The Haage	Netherlands	slaughterhouse	1983	10000	1000
Douwe Egberts, Joure	Netherlands	coffee	1986	-	435
Antarctica Niger, Ribeirão Preto, SP	Brazil	brewery	1986	6400	800
Kaiser S/A, Jacaraí , SP	Brazil	brewery	1987	20000	2000
Antarctica Polar, Estrêla, RS	Brazil	brewery	1987	6144	900
Brahma, RJ	Brazil	brewery	1988	21600	2000
Nechar Alimentos, Rio das Pedras, SP	Brazil	candy	1988	1800	250
Coca Cola, Vitória, ES	Brazil	soft drinks	1988	824	250
Sucocitrico Cutrale, S/A, Araraquara, SP	Brazil	orange juice	1989	48000	2 x 2000
CCPL, RJ	Brazil	dairy	1990	2117	300
Caninha 51, Pirassununga, SP	Brazil	alcoholic drinks	1990	3000	300
Sousa Cruz, RJ	Brazil	cigarette factory	1992	600	100
Fleischmann-Royal, Escada	Brazil	yeast production	1990	11500	1250
Anaheim Citrus Products, Los Angeles	USA	Lemon & orange peal wash.	1988	33 000	1900

* Look Hulshoff Pol (personal communication)

produced in the degradation of proteins, also inhibition of the process could occur (Hulshoff Pol & Lettinga, 1986; Lettinga & Hulshoff Pol, 1986; Fang *et al.*, 1994).

In laboratory scale experiments Breure *et al.* (1986*b*) found that the degradation of gelatin (protein) becomes retarded at increase dilution rates, while their degradation also may became inhibited by higher concentration of carbohydrates, such as glucose and lactose. In continuos experiments conducted by Breure *et al.*(1985) it was observed that turned quite slimy after exposing the granular sludge to gelatin, and as a consequence it became more susceptible for flotation and adherence to the wall of the reactor.

The anaerobic treatment of wastewater containing lipids in UASB reactors particularly can suffer from seriously problems, once again due to heavy flotation of the granules, and consequently wash-out of sludge. Another detrimental effect of the presence of lipids concerns the formation of scum layers resulting from flotation; this can cause clogging of gas lines and pipes, and due to that a poorer treatment efficiency may be found together with other troubles (Sayed, 1987; Andersen & Schmid, 1985).

Lipids also tend to precipitate in the reactor (Rinzema, 1988), which may results in operational problems of the reactor. So the precipitated lipids can directly detrimentally affect methanogenesis due to substrate transport limitation problems, i.e. especially when they adsorbed in dense films around the grains, and due to serious inhibition (Perle *et al.*, 1995) particularly due to hydrolysis products, viz. long-chain fatty acids (Jeris & McCarty, 1965; Hanaki *et al.*, 1981; Koster, 1987; Koster & Cramer, 1987; Rinzema, 1988).

The problem with lipids is not especially a poor COD removal efficiency but the comparably low conversion into methane which mainly can be attributed to their easy adsorption to the sludge surface and/or precipitation in the sludge. As a result the contact between bacteria and substrate becomes extremely poor (Rinzema, 1988). The precipitation of lipids in the UASB reactors also may lead to an increase of the dead volume in the system, causing a decrease of the amount of active biomass, which obviously also negatively will affect the reactor degradation capacity, consequently the performance of the system.

The main objective of this thesis is to improve the understanding of the mechanisms of the degradation of complex components like proteins, lipids and carbohydrates, particularly when present in mixture, and the causes of operational problems as mentioned above. An improved understanding may result in a better and wider application of anaerobic treatment.

The EGSB concept

In experiments conducting with raw sewage at lower ambient temperatures using 0.12 m^3 pilot scale UASB reactor with granular sludge it was found that a significant accumulation of suspended solids may occur due to the very slow hydrolysis of the entrapped solids (van der Last & Lettinga, 1992). The entrapped solids consist for a major part of lipids! Results obtained in 6 and 20 m^3 pilot UASB-reactors with granular sludge even gave distinctly lower efficiencies as compared to 0.12 m^3 reactor, which was attributed to the poor sludge water contact and the poorer removal of suspended solids. In order to improve the sludge water contact and to reduce the dead space, some improvement can be made in the design and/or

operation of granular sludge bed reactors, i.e. the use of an increased in number of feed inlet points and application of higher superficial velocities. For that reason the expanded granular sludge bed (EGSB) reactor was developed (van der Last & Lettinga, 1992; de Man *et al.*, 1988). In this EGSB-reactor the applied higher liquid superficial velocities, viz. 4-8 m/h, cause the granular sludge bed to expand and partially also fluidize, which results in a better sludge-wastewater contact and therefore use of the degradation capacity present in the system, and in a decreased accumulation of flocculent sludge between the granular sludge. The required high liquid superficial velocities can be achieved by applying a higher height/diameter ratio for the reactor and/or by applying recirculation of effluent. In conventional UASB reactor the granular sludge behaves more as a static bed.

The performance characteristics of the EGSB-reactor-concept were found to be superior over the conventional UASB-system for wastewaters containing higher fatty acids like lauric acid (Rinzema, 1988; Lettinga *et al.*, 1993). Based on these promising findings the EGSB reactor was chosen in this Ph.D-research. The objective was therefore to assess whether or not this reactor system represents a feasible option for the treatment of complex wastewaters; e.g. consisting of a mixture of lipids, carbohydrates and proteins. Special attention has been afforded to development of a special sieve-drum device at the top of reactor for retaining buoying granular sludge, because this one of the dominant problems manifesting with lipid containing wastewaters, and also wastewater containing higher fatty acids.

The EGSB system so far has been applied at full scale mainly for soluble industrial wastewaters with easily biodegradable compounds. Table 2 provides some examples of the application of the EGSB concept.

Scope of this thesis

Chapter 2 presents a survey of experiments conducted concerning the degradation of a milk-fat emulsion in a laboratory scale EGSB recycle system. Special attention was given to the mechanism underlying the lipid removal.

Chapter 3 describes investigations concerning the application of a pilot scale EGSB-system with a complex substrate composed of protein, carbohydrate and lipid. In these experiments the also feasibility of a modified sieve-drum design as GLS-device was tested for the retention of buoying granular sludge. Special attention was given to the degradation of complex pollutants, and the interactions between these components with respect to their degradation.

Chapter 4 reports results of investigations dealing with the application of an EGSB reactor for a mixture of beer, gelatin and a milk-fat emulsion. The EGSB reactor was seeded with a granular sludge from a full scale UASB reactor treating recycle paper wastewater. Different concentrations of milk-fat lipids were investigated.

The results in Chapter 5 deal with investigations of a substrate mixture of gelatin and sucrose. The effect of the presence and absence of nutrients was investigated. Attention was afforded in these investigations to the effect of pre-acidification on the performance of a lab scale EGSB reactor.

Table 2. Full-scale EGSB installations*

Factory	Country	Type of wastewater	Year of contract	Design capacity kg COD/d	Design Volume (m^3)
Gist-brocades, Delft	Netherlands	backers yeast/antibiotics	1984	20000	2 X 380
Gist-brocades, Prouvy	France	backers yeast	1984	7000	2 x 125
Gist-brocades, Delft	Netherlands	backers yeast/antibiotics	1985	20000	2 x 380
Uniferm, Monhein	Germany	backers yeast	1985	3500	125
Caldic Europort, Rozenburg	Netherlands	chemical	1992	2800	275
Heineken, Zoeterwoude	Netherlands	brewery	1992	15000	780
Midwest Grain products, Pekin. ill	USA	starch	1993	27200	1750

* Look Hulshoff Pol (personal communication)

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Chapter 2

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ANAEROBIC BIODEGRADATION OF A MILK-FAT EMULSION IN AN EXPANDED GRANULAR SLUDGE BED REACTOR

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Abstract

In the literature relatively little information is available dealing with research concerning the anaerobic digestibility of lipids. In this study the anaerobic biodegradability of lipids in the form of a milk-fat emulsion was evaluated using a closed circuit with an Expanded Granular Sludge Bed (EGSB) reactor as treatment system. The results obtained reveal that 70 % of the lipids were adsorbed on the granular sludge within approximately 1 day and thereafter the remaining lipids were slowly converted to methane gas. After 26 days only 22 % of the milk-fat COD was converted to methane. The biodegradation of lipids mainly resulted from colloidal COD-fraction. The adsorbed COD remained greatly unaffected. The poor biodegradation of milk-fat could be attributed to the very slow rate of liquefaction, as evidenced by the assessed very low hydrolysis constant k_h, viz. being only 0.01 d^{-1}.

In order to prepare the milk-fat emulsion an anionic surfactant, sodium dodecylbenzenesulfonate, was used. As anionic surfactants are reported as toxic compounds to methanogenic bacteria, a toxicity test was carried out to choose a subtoxic surfactant concentration in order to prepare an emulsion that after dilution would not present inhibition.

Key words: milk-fat; lipids; EGSB reactor; hydrolysis; biodegradability; adsorption.

INTRODUCTION

Lipids are natural neutral fats formed by esterification of long-chain fatty acids with glycerol (Mulder & Walstra, 1974; Larsson, 1994). Many industries produce effluents with appreciable amounts of lipids; such as dairy; slaughterhouse; edible oil and fat refining; rendering industry; margarine; palm oil processing; wool scouring and meat packing industries (Koster, 1987; Rinzema, 1988; Brown & Fico, 1979; Andersen & Schmid, 1985).

Many problems are encountered in the anaerobic treatment of lipid-containing wastewaters. Both in industrial-and laboratory-scale, application of anaerobic reactors to treat wastewaters rich in lipids a heavy granular sludge flotation and wash-out has been frequently observed (Samson *et al.*, 1985; Rinzema, 1988; Rinzema *et al.*, 1993). In a pilot plant using complex slaughterhouse wastewater, accumulated lipids formed a floating scum-layer at top of the reactor which caused clogging in the gas and effluent lines (Andersen & Schmid, 1985). Moreover lipids, and long chain fatty acids resulting from lipid hydrolysis, cause inhibition of methanogenic activity and also decrease the concentration of adenosine triphosphate (ATP) (Perle *et al.*, 1995; Hanaki *et al.*, 1981; Novak & Carlson, 1970; Jeris &

McCarty, 1965; Koster & Cramer, 1987). Problems are encountered with adsorption of lipids on granular sludge causing disintegration of the sludge aggregates by adsorption of long-chain fatty acids (Sam-Soon *et al.*, 1991; Boari *et al.*, 1984). Furthermore, lipids prevent granulation of flocculent anaerobic seed sludge in Upflow Anaerobic Sludge Bed (UASB) reactors. Lipids frequently are poorly biodegradable due to the fact that the are poorly available for organisms. Sayed (1987) studied the treatability of paper filtered slaughterhouse wastewater and he found that only 58 % and 46 % of the COD applied was converted into methane at 30 and at 20 °C; respectively, indicating a relatively poor biodegradability. These results are in accordance with the maximum extent of conversion into methane, i.e. varying between 40 and 49 %, obtained by Neave and Bruswell (1928) in their study on digestibility of the fatty fraction in sewage at temperatures of 25 and 35° C.

In the literature, few studies are available dealing with the anaerobic digestion of semipurified lipids, i.e. lipids composed only of triglycerides; most of the results reported were been obtained with synthetic substrates or real wastewater containing other products beside lipids such as carbohydrates and proteins. While such studies provide useful information about the feasibility of anaerobic treatment for different kinds of lipid-containing industrial wastewaters, they do not allow the assessment of limiting conditions or reliable insight into the fate of lipids during anaerobic wastewater-treatment.

In order to prevent problems resulting from the poor bioavaliability of lipids, Rinzema (1988) and Rinzema *et al.* (1993) used feed solutions consisting of emulsified lipids in a lab-scale EGSB-reactor which was equipped with a sieve-drum at the top of the reactor to separate liquid and solid phases and to prevent floating sludge from wash-out. The main problem encountered in the experiments was sludge flotation due to the poor conversion of accumulated lipids into methane; consequently Rinzema *et al* (1988) recommended to conduct more research in this specific field. Rinzema demonstrated that the EGSB for anaerobic treatment of complex wastewaters like sodium salts of lauric acid and capric acid is superior in efficiency to the conventional UASB reactor concepts because organic loading rates up to 30 kg $COD.m^3.d^{-1}$ could be accommodated at hydraulic retention times as low as 2 hours at 30 °C. Based on these very promising results we decided to use the EGSB-reactor system in this study.

In our study semi-purified lipids as milk-fat or butter-oil in the form of an emulsion was chosen as a lipid substrate; so interference in the interpretation of data due to conversion of possible co-substrates (e.g. proteins or carbohydrates) was be avoided.

The compositions of common fatty acids of triglycerides from milk-fat are shown in Table 1. Palmitic, oleic, myristic and stearic acids are the major components in milk-fat.

Milk-fat was applied in the form of a stable and homogeneous emulsion, because lipids particles then have more surface area due to their small particle size (approximately 1μm) and; therefore they are more easily accessible for enzymes. For the preparation of the emulsion, the surfactant sodium dodecylbenzenesulfonate was used (Prins & Walstra personal communication).

The anaerobic biodegradability of emulsified milk-fat was assessed using an EGSB reactor under conditions of low sludge-loading rates. An additional objective of the

investigation was to estimate the toxicity of the surfactant used for the preparation of the emulsion in order to assess a subtoxic concentration to be used in the experiment. An additional issue of the study was to observe the behavior of the sludge bed when lipids became adsorbed on the granular sludge.

Table 1. The Fatty acids composition of Triglycerides of Milk Fat

DESCRIPTION	NOTATION	% OF TOTAL ACIDS
Butyric	4:0	8.5
Caproic	6:0	4.0
Caprylic	8:0	1.8
Capric	10:0	3.0
Lauric	12:0	3.6
Myristic	14:0	10.5
Palmitic	16:0	23.5
Stearic	18:0	10.0
Oleic	18:1	21.0
Linoleic	18:2	1.8
Linolenic	18:3	0.4

From Mulder and Walstra (1974)

METHODS

Preparation of emulsion and substrate

The emulsion used in the experiment consisted of 200 g of butter oil or milk-fat added to 800 g of tap water together with 1.6 g of surfactant sodium dodecylbenzenesulfonate. Under stirring it was then transferred to a Rannie homogenizator (APV Rannie AS- Albertslund, Danmark) to emulsify the mixture and this resulted in a milk-fat micelle particle size of approximately 1 μm in diameter after 20 minutes mixing under 30 Kg/cm2 of pressure and at a temperature of 60 °C. Afterwards, the emulsion was cooled down slowly to room temperature and put into the refrigerator as a stock solution. In this way, it was possible to obtain a stable and homogenous mixture of milk-fat in water for application as feed to the EGSB reactor system.

Surfactants are reported as toxic compounds to microorganisms; therefore, we conducted a toxicity test on methanogenesis in granular sludge. The toxicity test was performed in accordance with a method described by Donlon *et al.* (1995). The concentrations of the surfactant, sodium dodecylbenzenesulfonate, tested were 1,10, 50, 75, 100, 125 and 150 mg/l.

The substrate used in this study was prepared from a mixture of 59.3g of milk-fat emulsion (stock solution) plus 2 liter water containing a basal nutrient solution, plus 0.5 g of yeast extract and it was then completed to 10 liters with tap water; the final concentration amounted to 2.7 g COD/l, corresponding to 9.5 mg of surfactant per liter.

The milk-fat or butter oil contained 99.8 % of triglycerides, viz. only 0.2 % of water. It was kindly supplied by the Dept. of Food Science, section Dairy and Food Physics at the Wageningen Agricultural University, Wageningen, The Netherlands.

Analysis

The gas production and pH were measured daily. The pH was determined immediately after sampling in order to avoid a pH increase due to the loss of carbon dioxide from the liquid. Samples were taken from the effluent of the EGSB reactor for assessing the Volatile Fatty Acids content (VFA) in a membrane-filtered (0.45 μm) sample. In addition the COD of paper filtered samples was assessed (COD_{PF}) and soluble COD (COD_{SOL}) of the membrane-filtered samples. The COD_{IN} was determined before starting the experiment and other samples of the reactor contents were determined during the experiment. VFA were determined with gas chromatography using a GC HP 5890 II (Hewlett Packard, Palo Alto, USA) equipped with a 2 m*6 mm*2 mm glass column packed with 10 % Fluorad 431 on Supelco-port 100-120 mesh. The temperatures of the column, the injection port and the flame ionization detector (FID) were 130, 280, 200 °C, respectively. Nitrogen, saturated with formic acid, was used as gas carrier at a flow rate of 40 ml/min. The automatic injection volume was 1 μl. COD (colorimetric micro-method), total volatile solids (TSS) and volatile suspended solids (VSS) were determined according to Standard Methods (18^{th} ed. 1992) and Dutch Standard Methods (NEN 3235-4.1 and NEN 3235-5.3).

The amount of lipids in the emulsion preparation was determined with the Gerber test (gravimetric), using sulfuric acid and isoamyl alcohol (Standard Methods for the Examination of Dairy Products, 1978).

Biomass

The active biomass used in the study consisted of a two-year-old unfed granular sludge from a full-scale UASB reactor treating wheat-starch (Latenstein, in the The Netherlands). Before use the seed sludge was rinsed in order to remove the fines and it then was stored at 4 °C until used. The specific methanogenic activity of the sludge tested with a VFA-mixture amounted to 0.6 g COD/g VSS.d. at 30 °C.

The specific methanogenic activity test was performed using a 0.6 l glass serum flask with a rubber septum and a screw cap. Granular sludge (1.5 g VSS/l) was transferred to the serum bottle containing 0.1 l of tl.e basal nutrient and VFA mixture from a stock solution of 100:100:100 g per kg acetate; propionate and butyrate (24:31:41 COD basis) neutralized and diluted to obtain a final substrate concentration of 4 g COD/l. Distilled water was added to complete the medium volume to 500 ml. The liquid was flushed with nitrogen gas and the flasks were sealed with a rubber septum and a screw cap and incubated in a temperature-controlled room at 30±2 °C. Methane production was monitored periodically during the assays with modified Marriotte flasks. These flasks were filled with a 3% NaOH solution, which served to remove the CO2, contained in the biogas.

The specific methanogenic activity, which is expressed as the amount of CH4 (as COD) produced by 1 g of sludge VSS per day (g CH4-COD/gVSS.d), was calculated from the slope of the methane production versus time curve and divided by the quantity of VSS.

The seed sludge contained 15.72 % total suspended solids (TSS) and 14.2 % VSS. For the methanogenic-toxicity test, a granular sludge was used which originated from a full-scale UASB treating chemical industry wastewater of Shell Nederland Chemie at Moerdijk, the Netherlands. The specific acetoclastic activity amounted to 0.744 g COD/gVSS.d, viz. assessed with neutralized acetate.

Basal nutrients

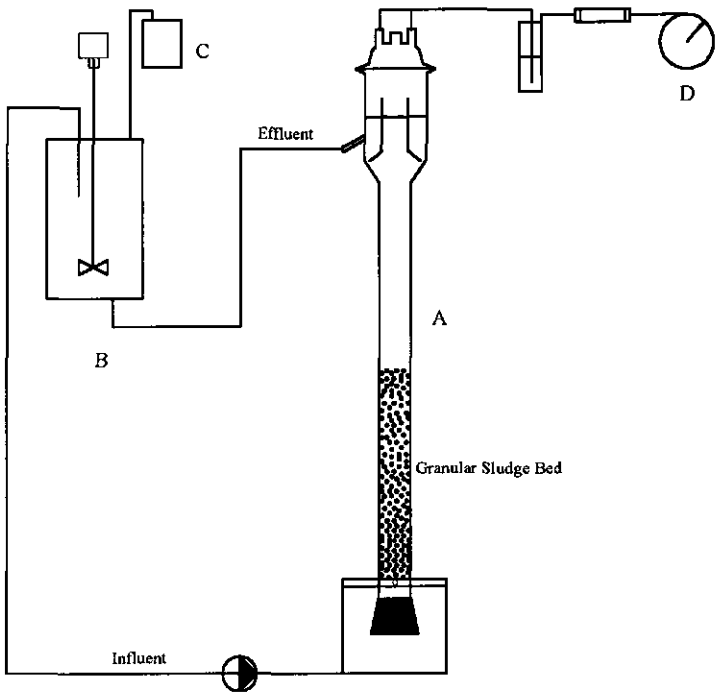
The basal nutrient solution used in the EGSB experiment contained:
NH_4Cl 56 mg/l; K_2HPO_4 20 mg/l; $MgSO_4.7.H_2O$ 66 mg/l; $CaCl_2$ 1.5 mg/l; $NaHCO_3$ 80 mg/l.
Micro elements: $FeCl_2.4H_2O$ 4 mg/l; H_3BO_3 0.1 mg/l; $ZnCl_2$ 0.1 mg/l; $CuCl_2.2H_2O$ 0.08 mg/l; $MnCl_2.4H_2O$ 1 mg/l; $(NH_4)_6Mo_7O_{24}.4H_2O$ 0.1 mg/l; $AlCl_3.6H_2O$ 0.2 mg/l; $CoCl_2.6H_2O$ 4 mg/l; $NiCl_2.6H_2O$ 0.2 mg/l; $Na_2SeO_3.5H_2O$ 0.3 mg/l; EDTA $C_{10}H_{16}N_2O_8$ 2 mg/l; Resazurine 0.4 mg/l; HCl 36 % 0.002 ml. The basal nutrients used in the specific methanogenic activity and toxicity assays had the same composition as outlined above, but a five-fold more concentrated solution was used. All chemicals were of analytical grade (Merck, Darmstad, FRG). The yeast extract was supplied by Gist-Brocades (Delft, The Netherlands).

Reactor

A glass EGSB reactor (2.5 l), supplied with granular sludge (6.35 g/l VSS), was connected with a polyvinyl chloride (5 l) store the substrate vessel (Figure 1). The substrate was pumped from this vessel to the bottom of the reactor at a flow rate ranging from 0.33 to 0.5 l/min. Effluent was recirculated from top of the column to the bottom of the vessel in a closed circuit. Gas was collected from the reactor and was monitored with a wet test gas meter and the gas produced in the vessel was monitored with a Mariotte flask. The reactor was equipped with a simple liquid separator (GLS)-device at its top, viz. a device consisting of an upside-down funnel. Table 2 summarizes the operational conditions imposed to the system during the experiment. The upflow velocity applied in the reactor ranged from 9.0 to 13.65 m/h. The hydraulic regime imitated that of a granular sludge bed in a full-scale EGSB reactor, and allowed good contact between bacteria and substrate.

Calculations

The calculations were based on the COD-balance, for which purpose the methane-COD is calculated using a conversion factor of 2.485 g COD/l liter of moist CH4 at 30 °C and 720 mm Hg. Other data of COD were obtained by analysis. In order to describe the performance of reactor the following parameters were used:
COD_{IN}= total COD of the unfiltered feed solution at the start of the experiment (t=0) (mg/l).
COD_{PF}= COD of paper filtered sample from the reactor contents (mg/l).



A EGSB-REACTOR
B STORAGE VESSEL
C MARRIOTTE FLASK
D WET TEST GAS METER

Fig. 1. Schematic presentation of EGSB reactor system.

COD_{MF}= COD of a membrane filtered sample of the reactor contents (soluble COD) (mg/l).
COD_{COLL}= colloidal COD (COD_{PF} - COD_{MF}) (mg/l).
COD_{VFA}= COD corresponding to VFA concentration (mg/l).
NAS= COD of non-acidified soluble fraction (=COD_{MF}-COD_{VFA}) (mg/l).
M= COD corresponding to methane produced (mg/l liquid contents of reactor).

These basic data enable the calculation of the various COD-fractions defined below.
Fraction of COD eliminated (COD_{FEL}) defined as percentage of COD removed during the experiment,

$$COD_{FEL} = \left(\frac{COD_{IN} - COD_{PF}}{COD_{IN}}\right) x100$$

Table 2. Summarize of Operational Conditions of EGSB reactor system

DESCRIPTION	DATA
REACTOR-EGSB(Volume Working) (l)	2.35
HEIGHT (m)	1.07
FLOW (l/min)	* 0.5; 0.36; 0.33
SUPERFICIAL VELOCITY (m/h)	* * 13.65; 9.8; 9.0
CONCENTRATION OF LIPIDS (g COD/l)	2.7
AMOUNT OF INFLUENT(SUBSTRATE) (l)	7.35
LOAD SLUDGE (g COD/g VSS)	1.32
VOLATILE SUSPENDED SOLIDS (g/l)(EGSB)	6.35
INFLUENT VESSEL (l)	5
TEMPERATURE (°C)	30

* range of flow

* * range of superficial velocities

Fraction of COD adsorbed (COD_{FAD}) defined as percentage of COD adsorbed during the experiment in the reactor. In this calculation, it is assumed that COD used for cell production is negligible compared to COD adsorbed,

$$COD_{FAD} = \left(\frac{COD_{IN} - (COD_{PF} + M)}{COD_{IN}}\right)x100$$

Fraction of COD colloidal (COD_{COLL}) defined as percentage of COD colloidal during the experiment,

$$COD_{FCOLL} = \left(\frac{COD_{PF} - COD_{MF}}{COD_{IN}}\right)x100$$

Fraction of liquefied COD:

$$COD_{FL} = \left(\frac{COD_{MF} + M}{COD_{IN}}\right)x100$$

Fraction of acidified COD:

$$COD_{FA} = \left(\frac{M + VFA}{COD_{IN}}\right)x100$$

Fraction of COD converted to methane:

$$COD_{FM} = \left(\frac{M}{COD_{IN}}\right)x100$$

Fraction of COD remaining:

$$COD_{FREM} = COD_{FCOLL} + COD_{FAD}$$

RESULTS

Toxicity

Since anionic surfactants like alkylbenzenesulfonate are known to be toxic to microorganisms we carried out a toxicity test for methanogenic bacteria in granular sludge. The inhibitory effects of various concentrations of the surfactant sodium dodecylbenzenesulfonate on the methanogenic activity of granular anaerobic sludge therefore should be assessed.

The results in Figure 2A shows the cumulative methane productions as a function of time in the test conducted at different concentrations of surfactant. A severe decrease in the methane production occurred at higher concentrations. The decrease in methane production rate per unit concentration of surfactant was more pronounced at low than at high concentrations. The results in Figure 2B show the percentage of activity left at each concentration of surfactant in relation with control (without surfactant, assuming 100 % of the methanogenic activity) as a function the surfactant concentration. The 50 % inhibitory concentration (IC) obtained was 22 mg/l and the 80 % IC was 55 mg.

In the EGSB-experiments described in this thesis, a surfactant concentration was chosen in the stock solution of the emulsion which that after dilution will give only a subtoxic effect, viz. 9.5 mg/l of the substrate solution. According to Figure 2B this will cause 38 % inhibition of the methanogenic activity.

Biodegradability

A laboratory-scale EGSB reactor operating in closed circuit was used to assess the anaerobic biodegradation of the milk-fat emulsion. The results in Figure 3A shows an elimination of COD_{IN} after a contact period of one day, reaching a value of 70 %. However this elimination mainly can be attributed to adsorption of lipids on the granular sludge.

COD converted to methane

The calculated COD-fractions liquefied, acidified and converted COD to methane reached only 10 %, 5 % and 2 %, respectively (Figure 3B), illustrating that only very poor biodegradation had occurred at this time. Therefore the elimination of COD obviously proceeds via a sorption mechanism, and the rate biodegradation is very low.

Following the first day after the start of the assay, it can be observed that the adsorbed mount of COD remained roughly unchanged, while the COD_{COLL} decreased slowly reaching low values of this fraction by the termination of the assay (Figure 3A). At day 27 the fractions of COD liquefied, acidified and converted to methane reached values of 30, 22.5, and 22 % respectively (Figure 3B), and coincided with the loss of COD_{COLL} (Figure 3A). The accumulated amount of VFA reached a peak value of 120 mg COD/l at day 5 and then it started to decline to low concentrations around 20 mg COD/l (Figure 4). During the first days of the experiment, the pH dropped from 7.6 to 6.8; thereafter, the pH remained constant at a value of approximately 6.8 (Figure 4).

From Figure 3B it can also be observed that the NAS fraction roughly is 10 % of the total COD. Since this fraction remained constant throughout the entire experiment, it was most likely not biodegradable.

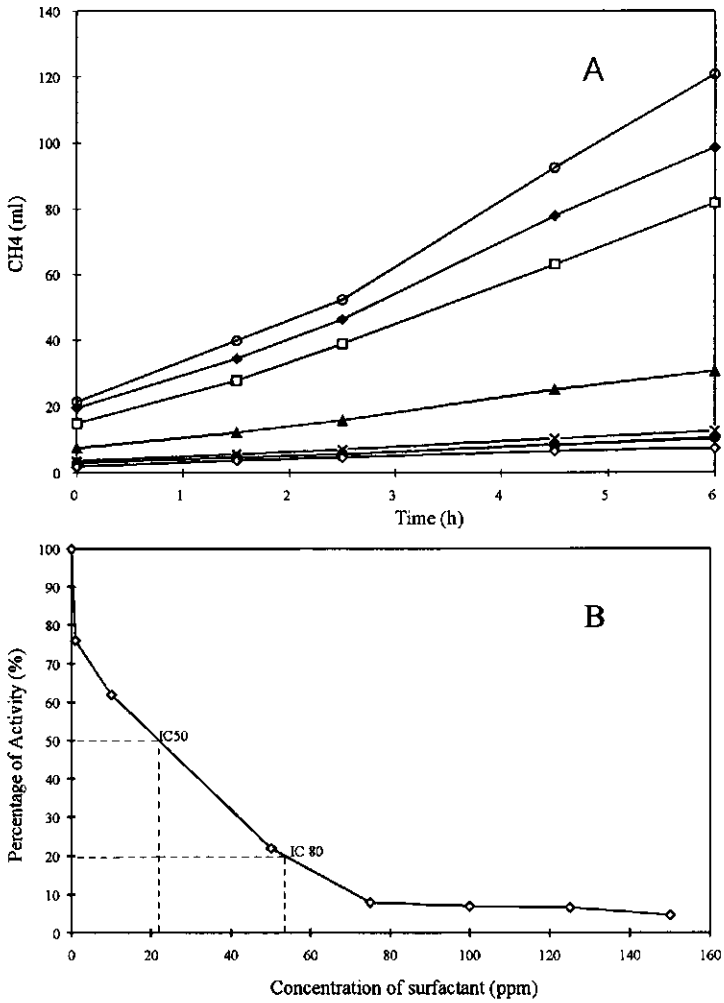
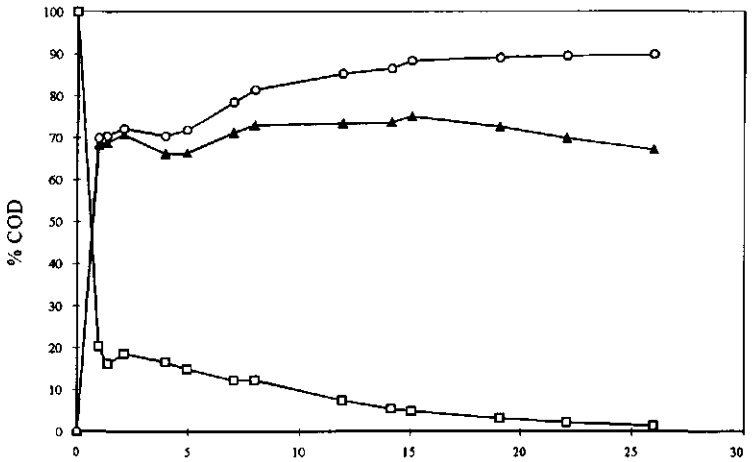


Fig. 2 Toxicity test of the surfactant, sodium dodecylbenzenesulfonate.
A- Methane accumulation at different concentrations of surfactant.
o - No surfactant ▲ - 50 ppm ◇ - 150 ppm
◆- 1 ppm × - 75 ppm
□- 10 ppm ● - 100 and 125 ppm
B- The percentage of left Methanogenic Activity at different concentrations of surfactant relative to the activity assessed in the inhibited assay.

The results in Figure 3B demonstrate that the liquefaction is the limiting rate step in lipid biodegradation. With the exception of the non biodegradable NAS fraction mentioned



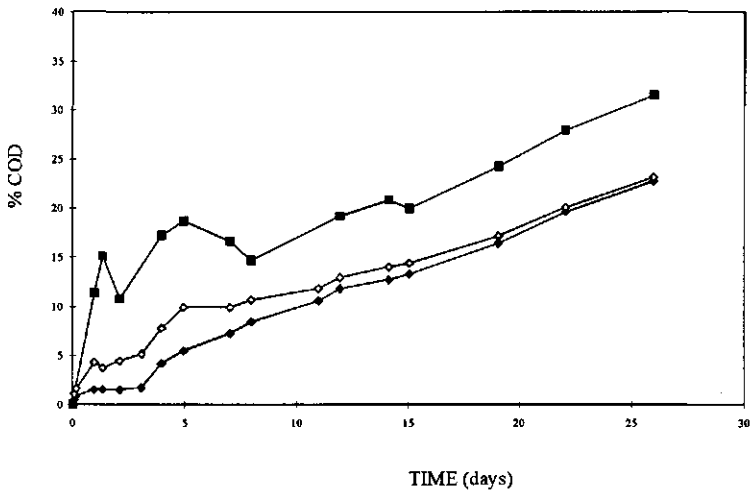


Fig. 3. A- The course of some COD-fractions in relation to time.
- o Fraction of COD eliminated
- ▲ Fraction of COD absorbed
- □ Fraction of COD colloidal

B- Kinetics of fractions of COD.
- ■ COD liquefied
- ◇ COD acidified
- ◆ COD converted to methane

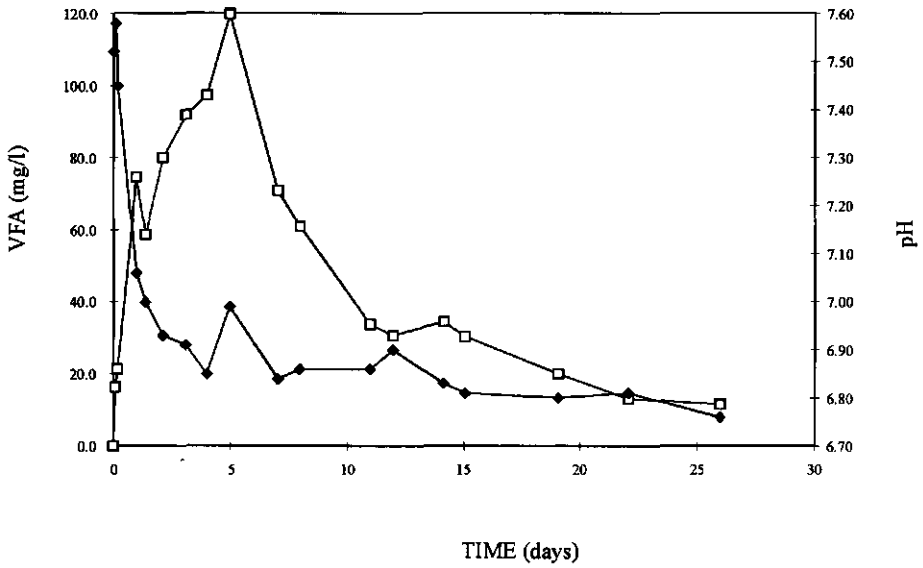


Fig. 4. Assessed VFA (Volatile Fatty Acids) concentrations, and pH.
□ VFA
◆ pH

above, the rest of the liquefied substrate was readily acidified and converted to methane. The acidification and methanogenisis increased in parallel with the liquefaction of the milk-fat.

In order to demonstrate that the liquefaction was the rate-limiting step, the hydrolysis constant was calculated by applying the first order equation of Eastman & Ferguson (1981) to this study, as follows:

$$\frac{\partial F}{\partial t} = -k_h . F \quad (1)$$

After integration:

$$\ln \frac{F0}{F} = k_h . t \quad (2)$$

The equation describes the rate of hydrolysis of an initial concentration of lipids with time, where:

F= fraction of lipids remaining = COD_{FCOLL} + COD_{FAD}

F_0= initial concentration of lipids

k_h= constant of hydrolysis

t= time of duration of experiment

In Figure 5, $\ln F_0/F$ is plotted against time (t). From the linear regression the hydrolysis constant k_h was extracted. The value found amounted to 0.01 d^{-1},which indeed indicates that

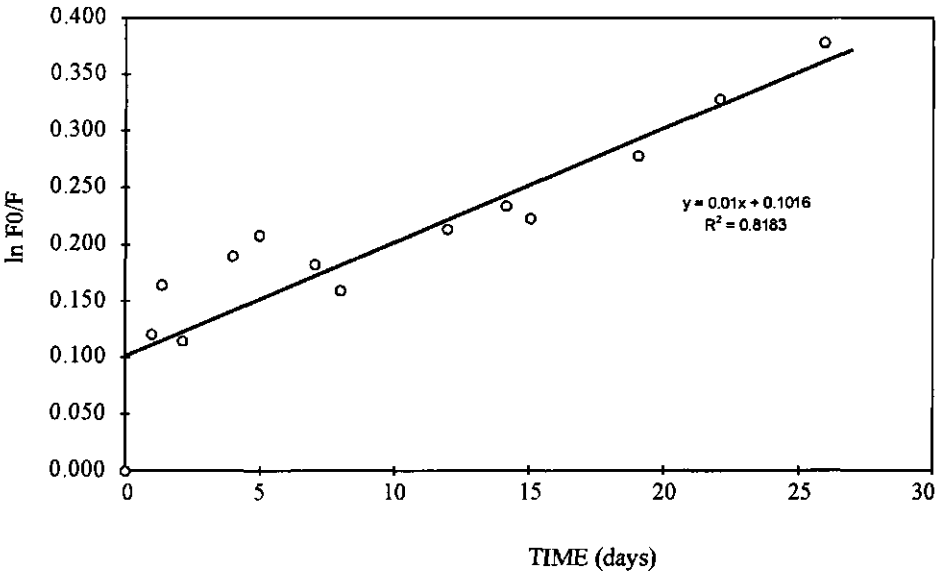


Fig.5. Plot of ln F_0/F against time to obtain the Hydrolysis Constant (k_h).

milk-fat biodegradation in the EGSB reactor proceeds very slowly.

The milk-fat emulsion added to the EGSB reactor clearly rapidly adsorbed to the sludge. This factor can be considered to very big importance, because adsorbed lipids can strongly affect the behavior of the sludge bed, i.e. causing flotation or other perturbations. However, adsorbed milk-fat on the Latenstein granular sludge used in this study did not cause sludge flotation. The adsorbed lipids could be seen by difference of color of the granules (the milk-fat emulsion was white, Picture 1). Adsorption of lipids in the form of films on the surface also will result in a steep drop of the degradation rate.

DISCUSSION

Anionic surfactants are reported to be toxic to methanogenic bacteria and to be nonbiodegradable by anaerobic sludge (Wagner & Schink, 1987). The results show that sodium dodecylbenzenesulfonate used in this study to prepare the milk-fat emulsion caused a 50 % inhibition of the maximum specific methanogenic activity at a concentration of 22 mg/l and an 80 % inhibition at a concentration of 55 mg/l. Wagner and Schink (1987) found a 50 % inhibition at a concentration of 62.5 mg/l and an 80 % inhibition at 100 mg/l concentration of sodium docecylbenzenesulfonate, indicating that our results were in agreement. The concentrations used in the preparation of the feed emulsion, viz. 9.5 mg/l, can be considered as subtoxic. In the reactor, the impact of the subtoxic concentration of surfactant may be less than the estimated value, because of adsorption on the granular sludge during the experiment.

Picture 1. Granules with adsorbed milk-fat.

On the other hand it could also be argued that the surfactant increases the toxicity of lipids due to dispersion and increased availability. However, in the present we did not investigate the effect of the surfactant on the toxicity of the lipids in more detail.

Previous applications of anaerobic treatment to lipid-containing wastewaters, such as those from the dairy industry, indeed demonstrated a poor biodegradability of lipids (Rinzema, 1988). In this study, the biodegradability of a milk-fat emulsion found in a laboratory-scale EGSB reactor was very poor, viz. only 30 % of liquefaction, 22 % of acidification and methanogenesis, after 27 days of the experiment. The experiment showed that a large part of the COD is eliminated rapidly by adsorption onto granular sludge. Clear evidence was obtained that adsorption was the main mechanism of COD removal.

Sayed (1987) studied the degradation of a paper-filtered fraction separated from slaughterhouse wastewater. The COD removal observed in his experiment amounted to 86 % and 82 % at 30 and 20 °C, while the conversions to methane were only 58 and 46 %; respectively. The poor conversion to methane clearly indicated adsorption of COD. As lipids constitute 67 % of the colloidal fraction of slaughterhouse wastewater, they may have had an important role in the adsorption. The results of our experiment with respect to the COD removal of 70 %, was similar to that obtained by Rinzema (1988) and Rinzema *et al.* (1993)

with 80-85 % COD removal being emulsified lipids. They also found a low conversion to methane of only 40 %.

The biological elimination of COD was very slow in our study. The methanogenisis reached only 22 % of COD_{IN}. The rate limiting step is the poor liquefaction of the milk-fat emulsion, as evidenced by the very low value of the calculated hydrolysis constant of k_h= 0.01 d^{-1}.

In the literature, the values for hydrolysis constants of lipids are dubious. There are various data available dealing with sewage to calculate the hydrolysis constant of lipid, yet the methods, and definition of lipids, are vague (Pavlostatis & Giraldo-Gomes, 1991). Most researchers do not mention the method they used in the calculation and for the lipids analysis; moreover lipids were frequently the minor component in the substrate (Gujer & Zehender, 1983 using data from Heukelekian & Muller, 1958 and Woods & Malina, 1965).

In the present experiments, the biodegradation that occurred (30 %) was due to loss of non-adsorbed colloidal lipids, as follows from the results in Figure 3A. The adsorbed lipids were not significantly converted until the end of the experiment, indicating that it was this fraction which was responsible for the poor hydrolysis of lipids. Presumably this poor degradation can be attributed to the fact that the seed sludge used in our experiments can be considered as greatly non-adapted to lipid degradation, and to the imposed relatively high sludge loads.

The sludge flotation problems experienced by other researchers (Samson *et al.*, 1985; Rinzema, 1988; Rinzema *et al.*, 1993) did not occur in this study, which likely can be due to the prevailing very low gas production and the fact that the total amount of lipids (relative to the amount of sludge) was still too low. In other studies, conducted elsewhere the amount of biodegradable co-substrate (e.g. protein) generally present, led to substantially higher gas production rates, and consequently sludge flotation then would occur easier in view of difficulties in the release of the entrapped gas from the granules due to lipid films covering the sludge.

CONCLUSIONS

Milk-fat lipid emulsions are very poorly biodegradable in the EGSB-reactors containing a non-adapted granular sludge. However, a large fraction of the lipids will become eliminated from the liquid phase by adsorption. They will be present here as a film on the granular surface, and in this state their biodegradability is extremely poor. On the other hand the colloidal lipid fraction that remains in the liquid phase will be slowly hydrolyzed. However only a part of the liquefied lipids will be converted to methane, another part, representing 10 % of the COD, apparently can be converted into non-biodegradable matter, at least with overloaded non-adapted granular sludge.

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Chapter 3

Submitted

PILOT SCALE EXPANDED GRANULAR SLUDGE BED REACTOR WITH A NEW SIEVE-DRUM DEVICE FOR THE ANAEROBIC TREATMENT OF COMPLEX WASTEWATER CONTAINING PROTEINS, CARBOHYDRATES AND LIPIDS.

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Abstract

Anaerobic treatment of wastewaters that contain high concentrations of proteins and lipids are problematic since they cause sludge flotation and sludge wash-out in upflow anaerobic reactors using granular sludge. In this study, expanded granular sludge bed (EGSB) reactors were used with a sieve drum, gas-liquid-solid separator (GLS) device, at the top of reactor to prevent wash-out of floating granular sludge. Two sieve drum designs were evaluated in EGSB reactor treating complex synthetic wastewaters composed of carbohydrates, protein and lipids. The second design tested could successfully retain floating sludge without damaging granular structure. After 42 days of operation with complex wastewater, 86 % of sludge bed was retained. The COD elimination during reactor operation averaged 54 % with 40 % conversion of wastewater COD to methane. The biodegradation of the protein, gelatin, was evaluated in the EGSB reactor. Gelatin-N was mineralized up 90 % during reactor start-up as a sole substrate in the influent. However after addition of sucrose to the influent, N-mineralization decreased to 69 % due in part to the extra N-uptake of the cells growing on the sugar. Batch experiments also indicated an extensive degradation of various proteins by anaerobic granular sludge. The average mineralization of protein N was >95 % and the average conversion of protein COD to methane was 70 % after 2 weeks of incubation, and in the specific case of gelatin, the mineralization was 95 % and the conversion to methane was 61 %. Lipids (0.1 g/l) in the form of a milk-fat emulsion were added to the reactor and. had no toxic effect on anaerobic treatability of the other wastewater components, sucrose and gelatin. The presented elimination of 62 %. and the principal mechanism for lipid removal presumably was adsorption. In the experiment, the major part of effluent COD consisted of soluble fraction, approximately. 85 %. The acidification of this fraction proceeded very slowly. In specific periods, decreases in non-acidified soluble COD (NAS) were found to be accompanied by increases in colloidal COD (COD_{COLL}) and *vice versa, therefore, deterioration of system should be resulted from poor degradation of soluble COD fraction. The results of this study indicate that EGSB reactors could be operated successfully with a complex wastewater without major loss of granular sludge.*

Key words: EGSB reactors, sieve drum, proteins, carbohydrates, beer, gelatin, sucrose, lipid, milk-fat, emulsion.

INTRODUCTION

The application of anaerobic treatment processes for the treatment of industrial wastewaters was introduced in the 1960's. The acceptance of the anaerobic treatment systems increased after introduction of innovative reactor designs with a high biomass retention were introduced. Biomass in these systems either retained on a fixed support material such as the anaerobic filter (Young & McCarty, 1969; Andersen & Schmid, 1985) or in dense sludge granules such as the Anaerobic Upflow Sludge Bed (UASB) reactor (operating with upflow velocities up to 2 m/h, Lettinga *et al.*, 1980; Lettinga & Hulshoff Pol, 1986) or the Expanded Granular Sludge Bed (EGSB) reactor (operating at upflow velocities exceeding 5 m/h in order to expand the sludge bed, van der Last, 1991; van der Last *et al.*, 1992; de Man *et al.*, 1988; Rinzema, 1988). A key feature in the design of the UASB and EGSB reactors is the gas-liquid-solid separator (GLS). Traditionally the GLS consists of a tapered gas cap compartment with baffles to deflect gas under the cap and a settler compartment above the gas cap to settle out the sludge particles, e.g. flocs and/or granules from the out-flowing effluent. Most full-scale anaerobic reactors installed so far are applied for wastewaters containing relatively simple substrates like organic acids and carbohydrates.

Wastewaters containing a high concentration of proteins or fats may suffer from problems associated with foaming and scum formation, which induce sludge flotation and subsequently -as a result- in a serious wash-out of viable sludge (Öztürk, *et al.*, 1993; Lettinga & Hulshoff Pol, 1991; Lettinga & Hulshoff Pol, 1986). This is a potentially very serious problem for application of UASB or EGSB processes to these types of complex wastewater, such as those from dairy and meat processing industries (Fang *et al.*, 1994).

In order to deal with sludge wash-out problems when treating these types of complex wastewaters, a new type of GLS was introduced for EGSB and UASB reactors based on the use of a sieve drum placed at the top of the reactor (Rinzema, 1988; Yucai *et al.*, 1988). However, even with these proposed sieve drum GLS devices, granular sludge wash-out remained still too high in case of treating lipid-containing wastewaters (Rinzema, 1988).

As a matter of fact many of the complex substrates responsible for sludge flotation are potentially anaerobically biodegradable, although the reports in the literature concerning the degradation for instance of proteins in continuous anaerobic reactors are rather conflicting. High removal efficiencies of casein and gelatin were observed in reactors acclimatised to these proteins (or their hydrolysates) as sole substrates (Shulze *et al.*, 1988; Thaveesri *et al.*, 1994; Fang *et al.*, 1994; Alphenaar, 1994). On the other hand, a poor protein degradation was reported in the case the biomass used in the experiment, had been previously adapted to carbohydrate substrates (Breure *et al.*, 1986; Perle *et al.*, 1995). Moreover, one also should account for inhibition from ammonia, released from high concentrations of proteins in wastewaters. It can lead to a serious inhibition of methanogens, consequently to reactor upset (e.g. Morgan *et al.*, 1990).

Also in the anaerobic treatment of long chain fatty acids (LCFA) and lipids, serious toxicity problems may occur (Koster, 1987; Perle *et al.*, 1995). Moreover, these compounds were found to form precipitates and stimulate the formation of scum layers inside the anaerobic reactors (Rinzema, 1988). In specific cases, such as with substrates like lauric and capric acids precipitate formation could be avoided by improving the mixing inside the reactor using effluent recirculation

such as can be applied in the EGSB reactor (Rinzema, 1993). In the EGSB reactors, these LCFA were found to be well degraded and almost completely converted into methane (Rinzema, 1993). In the other hand it was also found that emulsions of lipids were relatively very poorly degraded in EGSB-systems (Rinzema, 1988)! As explained in chapter 2 a large fraction of the lipid COD easily and rapidly adsorbs onto the sludge and once absorbed, its liquefaction proceeds very slowly (Petruy & Lettinga, 1997)!

The experiments described in this paper concern the use of a modified design of the sieve drum gas-liquid-solid separator for granular sludge bed reactors, in order to improve the granular sludge retention of the reactor, especially for the treatment of complex wastewaters composed of proteins, carbohydrates and/or lipids. The modified sieve drum was tested in order to establish its feasibility of granular sludge bed reactors for treating these types of wastewaters. A second important objective of the investigation was to improve the insight in the fate of the proteins and lipids when exposed to anaerobic treatment granular sludge bed reactors. In connection the anaerobic biodegradability of various types of proteins was assessed in a number of batch assays.

METHODS

Analysis

The gas production, pH and flow rate were measured daily. The pH was determined immediately after effluent sampling. Effluent samples for chemical oxygen demand (COD) measurements were made unfiltered (COD_{EFF}), paper filtered (COD_{PF}) with a Schleicher & Schuell n° $595^{1/2}$ filter (porosity 4-7 µm, Dassel, Germany) or membrane filtered (COD_{SOL}) with a Schleicher & Schuell ME filter (0.45µm Ø50 mm, Dassel, Germany). The COD of the samples influent (COD_{IN}) was measured unfiltered on unfiltered samples. Samples for volatile fatty acids (VFA) analyses were always membrane filtered.

The VFA composition was determined by gas chromatography according the methods described in Chapter 2.

Measurements of effluent COD and VFA concentration were conducted three times a week. COD (colorimetric micro-method), total volatile solids (TSS) and volatile suspended solids (VSS) were determined according to Standard Methods (18^{th} ed., 1992, American Public Health Association).

The Gerber test (gravimetric) was used to determine the amount of lipids in the milk-fat emulsion preparation, using sulfuric acid and isoamyl alcohol as reagents (Standard Methods for the Examination of Dairy products, 1978). In this test the milk-fats separate from other compounds such as carbohydrate and proteins by precipitation.

Lipids in the reactor effluent were determined gravimetrically after extraction with n-hexane plus terc-butyl methyl ether (80 % and 20 % respectively) according to Standard Methods (18^{th} edn., 1992, American Public Health Association).

Ammonium concentration in the influent, determined periodically, was analyzed by a photometric method using an autoanalyzer according to Dutch Standard Methods (NEN 6646 and NEN 6652, 1992).

The EGSB Reactor

The experiments were carried out in an EGSB reactor (see Fig.1). The diameter of the reactor (constructed from PVC) was 29 cm and its height was 154 cm, corresponding to a total volume of 101.7 l and a working liquid volume of 96 l. The reactor was equipped with one of either two types of a sieve drum GLS-device. In experiment 1, the sieve drum was designed according to system described previously by Yucai *et al.* (1988) in Figure 2A. In experiment 2, a modified sieve drum design was used as shown in Fig.2B. The concentric sieve drums were equipped with 3 brushers elaborated with plastic material (each brush was 20 cm long, 2 cm wide and 1 cm thick), that were rotated against the internal face of the sieve drum to prevent clogging of the sieve. (Fig. 2C). The brushers were operated continuously at 120 revolution per minute [rpm]) in experiment 1 and intermittently at 120 rpm, 6 seconds of operation each 20 minutes in experiment 2. The sieve drum was installed in the central upper part of the reactor. The main objective of using the sieve drum was to prevent granular sludge from washing-out. The treated wastewater (including the recycle water) leaves the system via this sieve (Picture 1, 2 and 3).

The biogas left the reactor via a 15 cm height water seal, Figure 1 D1. The amount of biogas produced was measured using a wet test gas meter (type 1, Schlumberger, Dordrecht, The Netherlands). The effluent overflowed into a small external settler with 4 l of volume. (Figure 1 E).

The reactor system was continuously fed from the bottom with stock solutions containing the influent substrates and hot dilution water (40 °C) via four separate lines using three peristaltic pumps, a model 503 U (Fig.1 H1) and an 101 U (Fig.1 H2) Watson-Marlow pump Wilmington, Massachusetts, USA) and one Heidolph (Kelheim, Germany) (Fig.1 H3).

Effluent was recycled (Fig.1 G) at a ratio depending on the selected superficial upflow velocity imposed to the systems using a high flow pump (Seepex mono pump, Fritz Seeberger KG, Bottrop, Germany, Fig.1 H4), which was connected to an inverted tube leading to the inner compartment of the sieve drum.

An influent distribution system was located at the bottom of reactor (Fig.1 I), and consisted of 4 tubes for the influent and/or bypass and one for effluent recycle. The reactors were placed in a temperature controlled room at 40 °C.

In experiment 1, the sieve drum used consisted of a column with a surface area of 754 cm^2 (12 cm of diameter x 20 cm height) with slots of 0.4 x 4.0 mm corresponding to 21 % of open area on the sieve face. The upper rim of the sieve drum was placed 9.8 cm from the top of reactor. The distance between the liquid-gas interface and the upper rim of the sieve drum was 4.8 cm, corresponding to a quiescent zone above the sieve drum with a volume of 2.6 l (Figure 2A).

The feed inlet distribution system (one tube) at the bottom of the reactor used 8 apertures (holes of 1.6 mm of diameter).

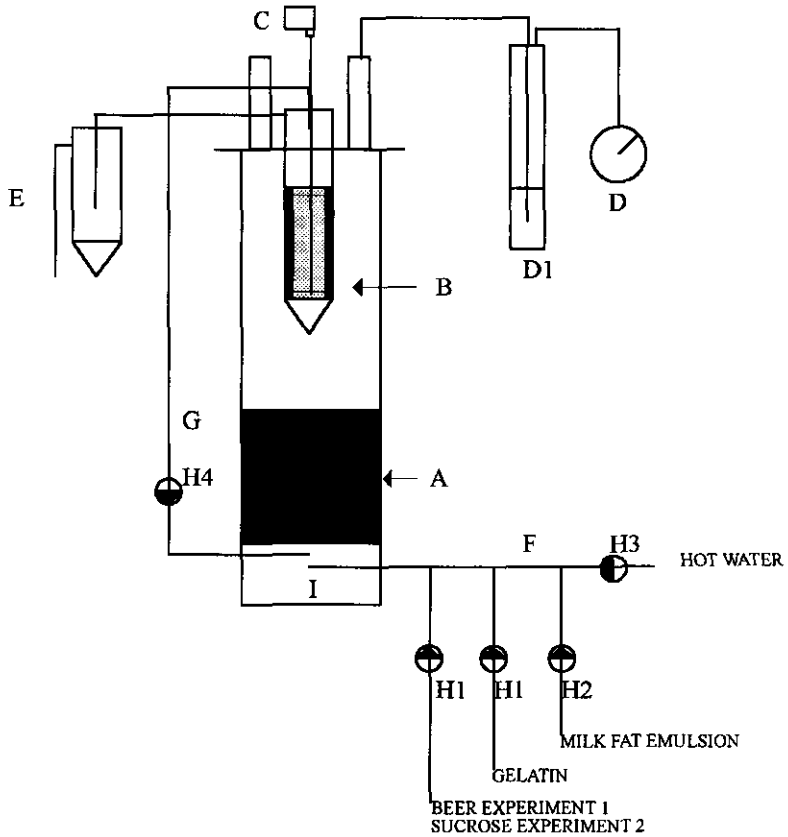


Fig. 1 Schematic presentation of the pilot scale EGSB reactor used in this study. (A) EGSB reactor. (B) Sieve drum GLS device. (C) Motor operating rotation of brush to clean GLS.(D) Wet test gas meter. (D1) Water seal. (E) Effluent overflow and sludge settler. (F) Peristaltic pumps going the influent. (G) Recirculation line. (H) Pumps.

Initially, in fact only in the "first" start-up, the reactor was operated at an upflow velocity of 6 m/h resulting in a flux of 26 $m^3/m^2.h$ through the apertures of the sieve drum. The imposed hydraulic retention time (HTR) was 6 hours and the organic loading rate (OLR) amounted to 8.4 g COD/l.d at an influent COD 2.19 g/l. The recirculation rate was 24:1. A second start-up was carried out with a new batch of fresh granular sludge and several changes in the operational conditions were made. The substrate concentration was decreased to 1.13 g COD/l, the OLR to 4.4 g COD/l.d and the upflow velocity was reduced from 6 m/h to 0.84 m/h, corresponding to a flux through the sieve drum apertures of 4 $m^3/m^2.h$. The recirculation rate in the new start-up was 4:1. The experiment was terminated after 1 month of operation.

In experiment 2, different sieve drum was tested, viz. with holes of 2 mm in diameter, placed at 3.5 mm distance from one another (Fig.2B) providing an aperture area of 30 % of the

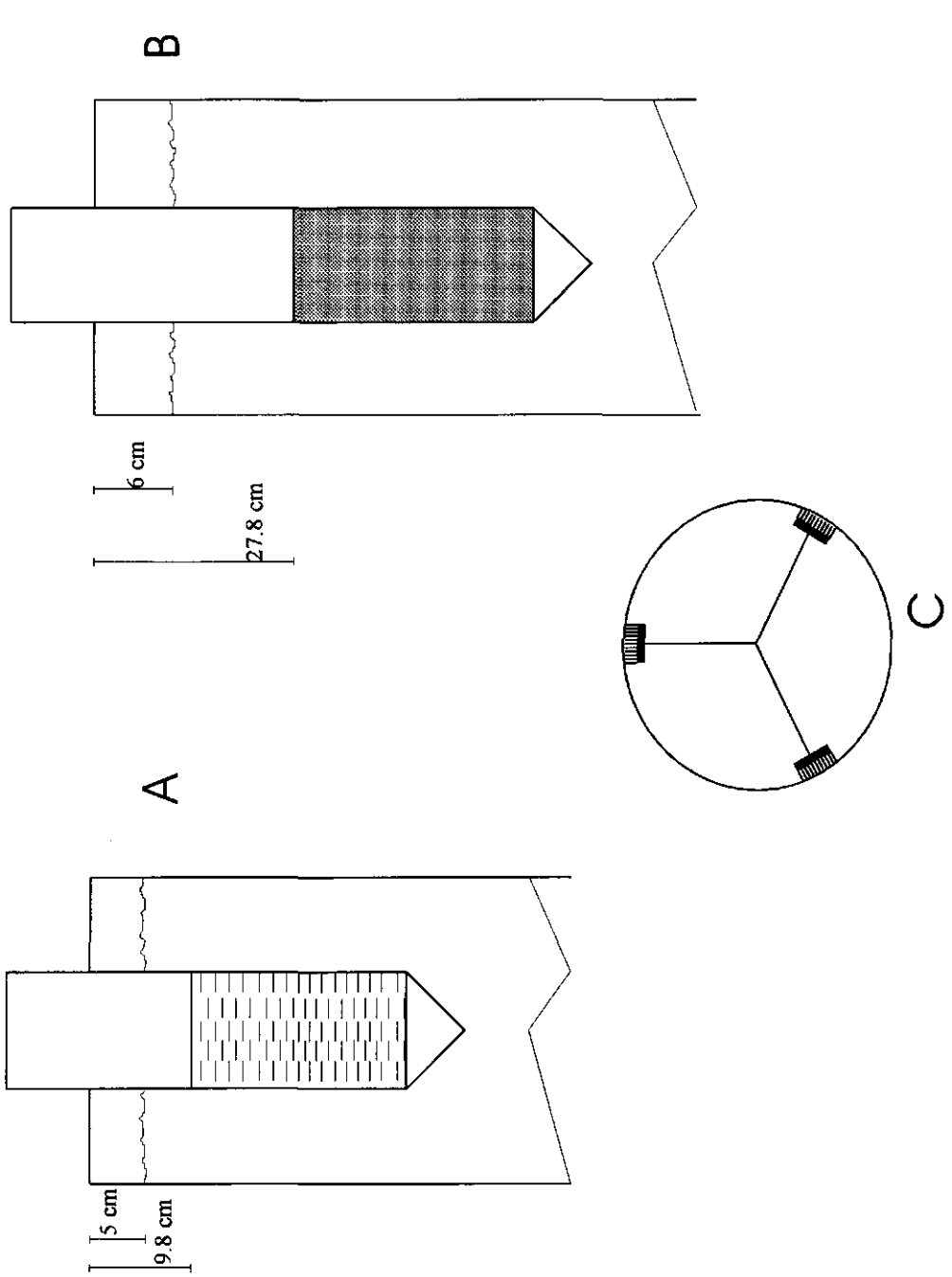
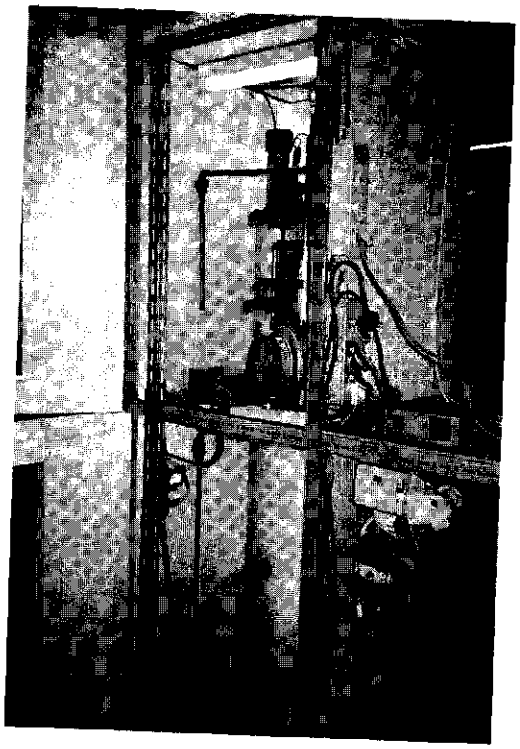
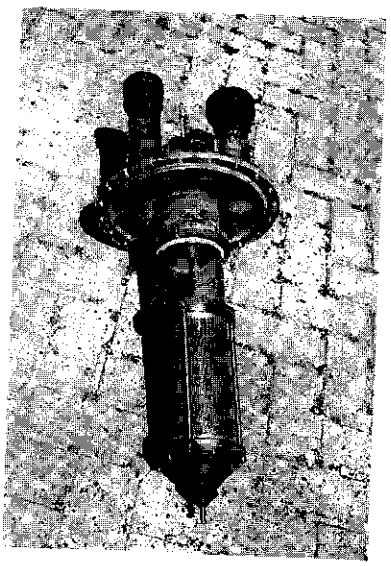


Fig. 2. Design of sieve drum used by Yucai *et al.* (1988) (**A**) used in experiment 1 and the new design (**B**) used in experiment 2. In (**C**), schematic representation of brusher inside the sieve drum.



Picture 1. EGSB reactor plant.

Picture 2. Sieve used in experiment 2.



Picture 3. Sieve drum used in experiment 2.

sieve drum surface area. The sieve surface area was 754 cm^2 (12 cm of diameter x 20 cm of height). The sieve used was punctured. The flux through the aperture was 18 m^3/m^2 at an operational upflow velocity imposed of 6 m/h in the reactor. The recirculation rate in the experiment 2 was 25:1. The sieve drum was placed 17 cm below that in experiment 1; consequently, 21.8 cm below the liquid-gas interface resulting in a quiescence zone above the sieve drum of 12 l.

The feed inlet distribution system in experiment 2 consisted of 12 distribution points (holes of 2 mm of diameter), directed to the bottom of reactor. A 15 cm thick layer of gravel, glass balls (size 1-2 cm) and of ceramic rings (size 0.5 x 1.0 cm) was placed above the inlet pipes in order to distribute the influent better and to reduce shear forces of high incoming liquid flow velocities on the overlying granular sludge.

Preparation of the wastewater

The wastewater used in experiment 1 was composed of beer, gelatin and a milk-fat lipid emulsion in a COD ratio of 1.0; 1.0; 0.13 g/l, giving a COD concentration of 2.13 g/l. For the second start-up, the concentrations of gelatin and beer were decreased to 0.5 and 0.5 g/l COD, respectively, while the lipid was maintained at the same concentration as in the first start-up.

The beer used was a commercially available low alcohol beer (Brouw Meester 1.5 % vol. alcohol, Brouwerij B.V. West, The Netherlands) with a COD of 80 g/l.

The gelatin used was technical grade obtained from Boom B.V., Meppel, The Netherlands, with a COD-content of 1.15 g COD/g of gelatin. The amount of gelatin-nitrogen (gelatin-N) was 0.157 g N/g gelatin (0.137 g N/g COD).

The lipid emulsion was prepared according to method described in Chapter II (Petruy and Lettinga, 1997) with pure milk-fat (99.8 %) which was kindly provided by the Dept. of Food Technology, Wageningen Agricultural University, Wageningen, The Netherlands. The milk-fat lipid contained 2.61 g COD/g of lipid.

The wastewater used in experiment 2 was composed of sucrose (Suiker Unie, Breda, The Netherlands) gelatin and lipid emulsion. The relative proportions of each substrate were varied. Initially, only gelatin was used and the next period a mixture of gelatin and sucrose, and finally a mixture of gelatin, sucrose and lipids according to the feeding schedule shown in Table 1. The COD concentration of the influent as well as the OLR applied are also shown in Table 1.

Each substrate component was prepared in concentrated stock solutions with the tap water and were diluted with hot water before being added as feed to the reactor.

Sodium bicarbonate, commercial grade was added to the influent as a buffer in experiment 1 and 2 at a rate of 1 g of bicarbonate for each g influent COD applied. Nutrients were not added in any of the experiments.

Biomass

The sludge used in experiment 1 was obtained from a full scale UASB treating potato processing

wastewater (Aviko, Steenderen, The Netherlands). The sludge was elutriated to remove the fines and then stored for 4 weeks at ambient temperature until it was used. The maximum specific methanogenic activity of the sludge amounted to 0.5 g COD/gVSS.d at 30 °C using 4 g COD/l

Table 1. Feeding schedule and feed composition during the operation of EGSB reactor in experiment 2

DESCRIPTION		DATA		
Scheme of Feeding		Influent COD	OLR**	SLR***
days	feed composition*	(g COD/l)	(g COD/l.d)	(g COD/gVSS.d)
0-1	(G)	0.23	0.9	0.03
1-2	(G)	0.42	1.7	0.05
2-6	(G)	0.74	3.6	0.1
6-8	(G+S)	0.74+0.37	5.4	0.16
8-13	(G+S)	0.74+0.72	6.5-7.3	0.2
13-14	(G+S)	1.16+1.18	11.2	0.3
14-42	(G+S+L)	1.23+1.23+0.26	11.4-14.1	0.4

* G=gelatin

S=sucrose

L=lipid (milk-fat) emulsion

**OLR=organic loading rate

***SLR=sludge loading rate

of a VFA-mixture (24:31:41 acetate; propionate; butyrate on a COD basis) as substrate. The sludge sedimentation velocity was 13 m/h. Approximately 24.5 kg of wet sludge was applied to the reactor in the first start-up experiment, providing an initial sludge concentration of 21.5 g VSS/l inside the reactor. In the second start-up also 24.5 kg of new sludge was used.

The anaerobic granular sludge used in experiment 2 was obtained from a full-scale UASB reactor treating paper manufacturing wastewater (Industriewater, Eerbeek, The Netherlands). The sludge was elutriated to remove the fines and than stored for five months at ambient temperature until it was used. This sludge was more dense and its sedimentation velocity amounted to 84.4 m/h. The maximum specific methanogenic activities for the Eerbeek sludge at 40 °C (simulating the temperature conditions in the continuous reactor) using either 4 g COD/l of the VFA-mixture

or acetate as substrates, were 0.495 and 0.627 g COD/g VSS.d; respectively. Approximately 20 kg wet sludge was supplied to the reactor, which corresponded to a sludge concentration of 32.75 g VSS/l inside the reactor at start-up.

Batch tests for assessment of biodegradability and specific methanogenic activity

The anaerobic biodegradability of various types of proteins to methane was evaluated in static batch assays of 0.5 l at 30 °C. The Aviko granular sludge was used at a concentration of 2 g VSS/l. In each batch, 0.1 l of basal nutrients (Brons *et al.*, 1985) was added. The duration of experiment was 2 weeks. The initial pH was set between 7.0 to 7.4. The proteins used originated from potato (Aviko), corn (zein, Sigma, St. Louis, MO, USA), milk (casein, Sigma), gelatin (Sigma), egg (Dep. of Food Technology, Wageningen Agricultural University, The Netherlands) and bovine serum albumin (Sigma) and they were tested at a concentration of 3.6 to 5.8 g COD/l. In the experiment, sludge blanks were used to correct for background methane and NH_4^+-N production.

The maximum specific activities of the sludge were assessed according Chapter 2.

Calculations

The performance of experiment 2 was evaluated by using parameters based on the following calculations.

COD removal efficiency (E) = $100*(COD_{IN}-COD_{PF})/COD_{IN}$

Methanogenisis (M) = $100*(l_{CH}/d$ from the reactor)$*2.494/(V_R*OLR)$; where, 2.494 is factor of conversion CH_4 (l) to COD (g) at 40 °C

Acidogenesis (A) = $M+100*(VFA/COD_{IN})$

Colloidal COD (COD_{COLL}) = $COD_{PF} - COD_{MF}$

Coarse suspended solids COD (COD_{COARSE}) = $COD_{EFF} - COD_{PF}$

Non-Acidified Soluble COD (NAS) = COD_{MF} -VFA

% of mineralization of protein = NH_4^+-N_{EFF}/N_{IN} x 100

N_{IN} = concentration of gelatin (g/l) * 15.7 % (concentration of nitrogen in gelatin)

Where:

VFA = volatile fatty acids effluent COD (mg/l)

COD_{IN} = unfiltered influent COD (g/l)

COD_{EFF} = unfiltered effluent COD (g/l)

COD_{PF} = paper filtered effluent COD (g/l)

COD_{MF} = membrane filtered effluent COD (g/l) = COD_{SOL} = soluble COD (g/l)

V_R = volume of reactor (l)

OLR = organic loading rate (g COD/l.d)

N_{IN} = concentration of gelatin-N (g N /l)

NH_4^+-N_{EFF} = concentration of ammonium nitrogen measured in the effluent

RESULTS

Gas-liquid-solid separator device performance.

Two experiments were conducted with the EGSB reactor using a wastewater composed of carbohydrates, proteins and lipids as feed. These experiments were conducted to compare the two sieve drum designs for use as GLS device.

In experiment 1, the sieve drum design of Yucai (1988) was used (Figure 2A) and in experiment 2, a improved design for the sieve drum was utilized (Figure 2B).

The EGSB reactor in experiment 1 was started with a solution containing 2.13 g COD/l and at an OLR of 8.4 g COD/l.d at a HRT= 6 hrs. The sludge loading rate (SLR) applied amounted to 0.4 g COD/gVSS.d. The upflow velocity used was of 6 m/h and a recirculation factor of 24:1 was applied.

In this experiment, already after 17 hours of operation, the granular sludge bed floated heavily due to adsorption or poor release of biogas produced. While floating, the granular sludge was sucked onto the sieve drum surface due to the flux of effluent across the sieve drum as is depicted in the illustration in Figure 3A. Consequently, the granular sludge was crushed by being pressed on the slots of the sieve drum. The fine particles formed rinsed out from the reactor and consequently they also were recycled for major part due to the high recycle factor applied. The continuous operation of the brusher (Fig.2C) also contributed to the crushing of granular sludge. The situation soon aggravated by the continuous recirculation of washed out sludge particles with the effluent. As a consequence, the sieve drum became severely clogged and the experiment therefore had to be terminated already after 17 hours of operation. At that time, the liquid phase present in the reactor appeared to completely black due to the presence of suspended crushed fine sludge particles. A part of these fines formed a scum layer of the liquid interface at the top of the reactor. Moreover the granular sludge used was almost completely lost.

In order to find a proper solution for these problems, a second start-up was carried out 4 days later with a new batch of sludge, but under different operational conditions, such as decreased OLR to 4 g COD/l.d, and at a reduced up-flow velocity from 6 m/h to 0.84 m/h. Nonetheless, sludge flotation still occurred, once again due to a poor release of biogas from the granules. However, the floating granules now mostly reached the quiescent zone present in the top of reactor, because the suction flux across the sieve-drum was lower than before. In the quiescent zone part of the granules stayed sufficiently long to release the adsorbed gas. However as depicted in Figure 3B, the volume of the quiescent zone in the reactor was still very small, only 2.6 l and therefore, the slowly accumulating layer of floated granular sludge also in this reactor soon came in contact with the sieve drum where it was exposed to the crushing action of the brusher. As result of these detrimental conditions more than half of the seed sludge was lost already after one month of operation. At that time the amount of sludge present in the reactor amounted to 10.75 g VSS/l.

In order to improve the sludge retention, a new GLS device (Figure 2B) was used in experiment 2. The main feature of this new design is the larger quiescent zone; for this purpose the upper rim of the sieve drum was now placed 21.8 cm below the gas-liquid interface. As a

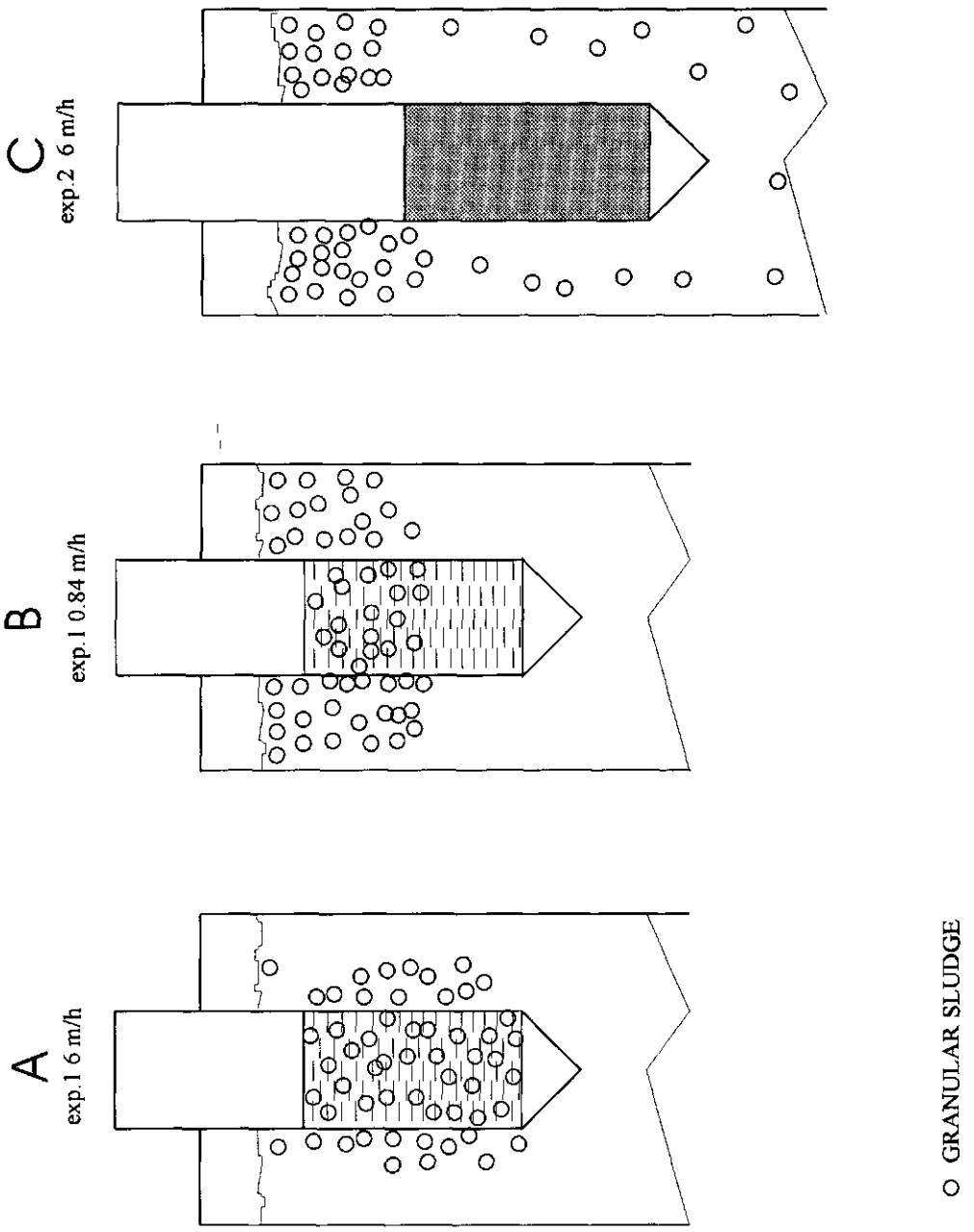


Fig. 3. Illustrations depicting the behavior of floating sludge in the sieve drum device, i.e.(**A**) and (**B**) the system used in experiment 1and (**C**) in experiment 2.

result of the larger "quiescent" zone, the floating sludge was enabled to release the adsorbed gas sufficiently rapidly, so that the layer of buoying granular sludge remained small and the sludge was not exposed to crushing by the brusher (Figure 3C). Moreover, because the aperture area of new design was approximately 50 % larger, the flux through the sieve drum also was lower. Also a different type of granular sludge was used in the experiment with a superior settleability (84.4 m/h). A new distribution system was installed at the bottom of reactor to provide a better distribution of flow and less erosion of the granules by shear forces of the incoming influent flow. As a result of all these modifications little if any crushing of the sludge and therefore sludge wash-out occurred, and consequently the sludge retention of the reactor was substantially better, as is evident from the low average value of coarse suspended solids in the effluent (COD_{COARSE}=54 mg/l). At the end of experiment, after 42 days of operation, an amount of 28 g VSS/l as granular sludge was measured in the reactor which is approximately 86 % of the initial sludge concentration.

Treatment performance of the continuously operated EGSB in experiment 2

The performance of the reactor in experiment 2 also can provide useful in information about the processes that occur in the system, especially with respect to the major polluting components of the feed used in the experiment. A thorough evaluation of the data therefore is worthwhile. The reactor was fed for the first 6 days (period 1) with gelatin as the mere substrate. Despite the low OLR imposed to the system, a poor conversion of COD to $_{CH}$ (approx. 20 %) was found (Fig.4). In the subsequent period, from day 6 to 14, the feed consisted of a mixture of sucrose and gelatin. Although a higher OLR was applied, the conversion efficiency increased, viz. up to 50 % for methanogenesis at up to 60 % for acidogenesis about 70 % of the COD elimination (based on COD_{PF}). These values obviously are still far from satisfactory! Based on the performance found during this phase we can conclude that apparently sucrose was a better substrate than gelatin, but the system (sludge) still was overloaded.

The biodegradation of gelatin can be evaluated on the basis of mineralization of organic nitrogen contained in gelatin, consequently the formation of NH_4^+-N. For this purpose, we therefore measured the effluent ammonium concentration. During period 2, from day 6 up to day 14, the mineralization of the gelatin-N ammounted to approximately 90 %. However, after increasing the OLR at day 13 from approximately.. 7 to 11, the gelatin-N mineralization dropped to 69 % (Table 2) and the treatment efficiency dropped from approximately 75 to 50 %. Obviously the imposed OLR is far too high!

Starting from day 14, 0.26 g COD/l of lipids were added to the influent of the reactor for the rest of the experiment, but the OLR remained unchanged at approximately 13. The results in Table 3 show the recovery of lipids in the reactor effluent. The elimination of lipids averaged to 62 %. The principal mechanism for lipid removal presumably was adsorption.

The results in Fig. 4 reveal that during period 14-42 days, both the COD removal efficiency and the conversion into $_{CH}$ gradually dropped further. At day 42, only 35 % of the COD influent was converted to methane, which is 15 % less, compared to day 14 (Figure 4).

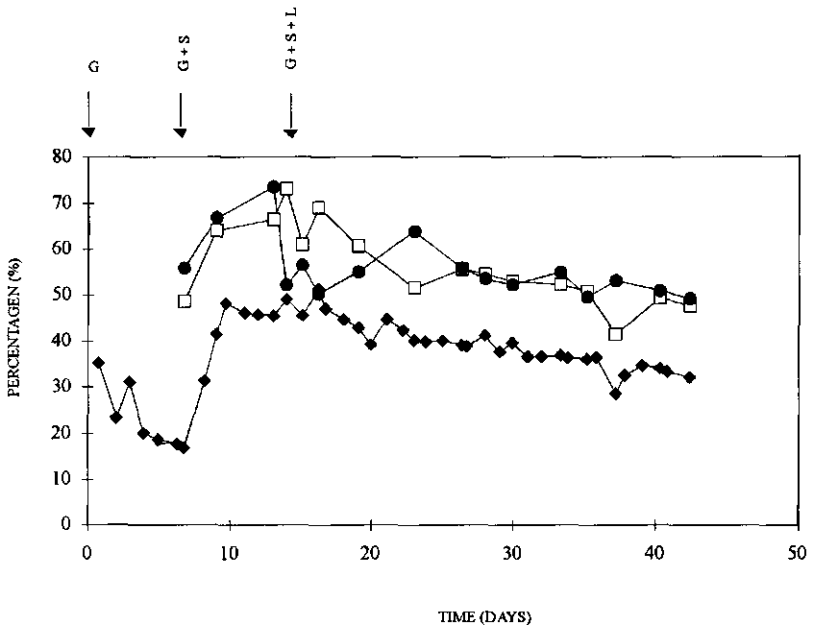


Fig. 4. Course of treatment efficiency obtained in EGSB reactor experiment 2; based on COD_{PF} elimination (● E%), conversion to VFA and methane, acidogenesis (□A%) and conversion to methane, methanogenesis (◆M%).

The results of the COD analysis made on the effluent, i.e. total, paper filtered and membrane filtered COD are summarized in Table 4 together with the calculated values for COD_{COARSE} and COD_{COLL}. Moreover Table 4 summarizes also the assessed values for the VFA and the calculated values of non-acidified soluble COD. The colloidal solids in the effluent ranged from 8 to 280 mg/l of COD, approximately 0.6 to 23 % of the unfiltered effluent COD. The largest fraction of COD in the effluent consists of soluble matter viz. it accounted for approximately 85 % of the unfiltered effluent COD. The effluent VFA concentrations varied in the range 300 to 500 mg/l and appreciable fluctuations were found in the NAS, viz. they ranged from approximately 400 to 850 mg/l of COD. Apparently the acidification of part of soluble COD proceeded slowly if at all. From the results shown in Fig. 5 it appears that fluctuations in NAS might be associated with changes in the COD_{COLL}. Decreases in NAS were found to be accompanied by increases in COD_{COLL} and vice versa. The reactor pH in experiment 2 was stable between 6.5 to 7.0 (Fig.6).

Table 2. The extent of nitrogen mineralization during the operation of EGSB reactor in experiment 2

DAY	N_{IN} (mg/l)	$NH4\text{-}N_{EFF}$ (measured) (mg/l)	Unrecovered* Nitrogen (mg/l)	% Mineralization** of protein-N
7	103	118	-	115
9	94	83	11	89
13	104	85	19	82
14	159	141	18	89
15	182	123	59	68
16	159	137	22	86
19	175	135	40	77
23	170	93	77	54
26	173	131	42	75
28	166	99	67	60
30	177	99	78	56
33	175	123	52	70
35	173	116	57	67
37	140	876	64	54
40	164	117	47	71
42	153	98	55	64
49	-	287	-	-
51	-	290	-	-

*N_{IN} - $NH_4\text{-}N_{EFF}$

N_{IN} = influent N concentration based on N contained in gelatin.

$NH_4\text{-}N_{EFF}$ = effluent ammonium -N concentration.

** calculus of % mineralization N.

Table 3. Lipid concentration in influent and effluent sample during the operation of the EGSB reactor in experiment 2

DAY	COD_{IN} (g/l)	COD_{EFF} (g/l)	% REMOVAL
20	0.32	0.091	72
23	0.22	0.071	68
26	0.21	0.096	54
30	0.21	0.120	43
33	0.25	0.104	58
36	0.25	0.093	63
40	0.22	0.084	66
42	0.21	0.052	75

1 g of milk fat lipid of the emulsion = 2.61 g COD

Table 4. The COD concentrations in influent and effluent samples during the operation of the EGSB reactor in experiment 2

DAY	COD_{IN} (mg/l)	COD_{EFF} (mg/l)	COD_{PF} (mg/l)	COD_{SOL} (mg/l)	COD_{COARSE} (mg/l)	COD_{COLL} (mg/l)	VFA (mg COD/l)	NAS* (mg/l)	COD_{COLL} + NAS*
7	1128	507	498	498	9	0	358	140	140
9	1379	517	457	407	60	50	312	95	145
13	1514	401	401	320	0	81	318	2	83
14	2341	1229	1118	1002	111	116	562	440	556
15	2623	1198	1139	996	59	143	416	580	723
16	2271	1222	1129	1005	93	124	421	584	708
19	2661	1224	1194	1085	30	109	473	612	721
23	2830	1013	964	695	49	269	306	389	658
26	2702	1228	1219	1176	9	43	458	718	761
28	2644	1237	1228	1194	9	34	356	838	872
30	2606	1311	1243	1209	68	34	348	861	895
33	2737	1243	1232	1224	11	8	418	806	814
35	2839	1466	1428	1148	38	280	418	730	1010
37	2202	1094	1031	766	63	265	286	480	745
40	2585	1355	1269	1084	86	185	395	689	874
42	2431	1304	1235	1226	69	9	379	847	856

*non-acididfied soluble COD

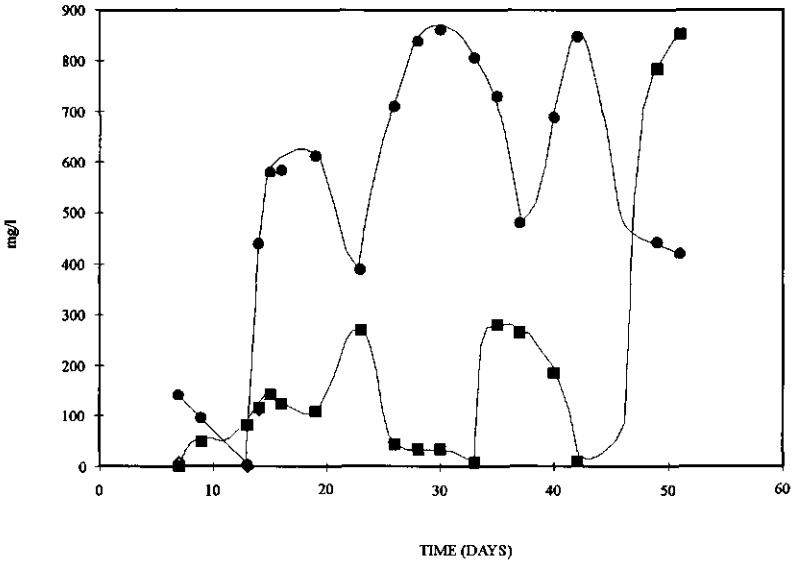


Fig. 5. The concentration of effluent colloidal COD (■) and non-acidified soluble COD (●) during the pilot-scale EGSB reactor operation.

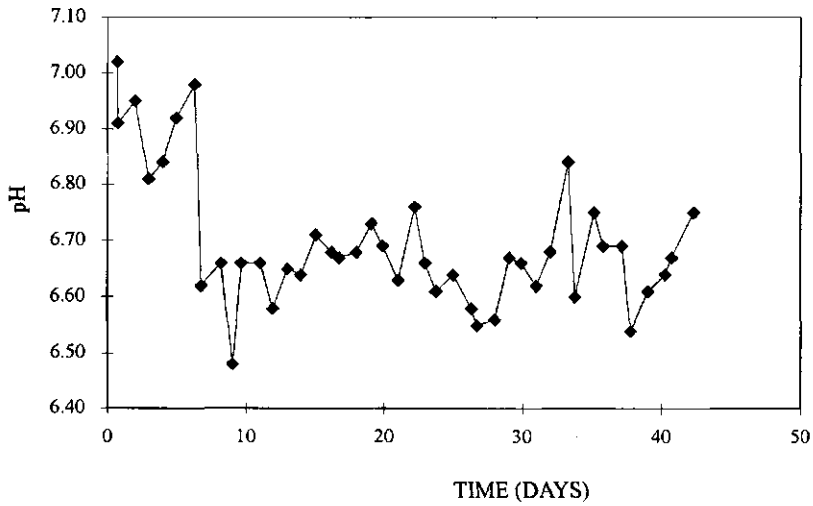


Fig. 6. The variations in effluent pH during the pilot-scale EGSB reactor operation.

Batch test

In order to assess the anaerobic biodegradability of proteins a batch test was carried out. The results of batch biodegradability assay with different types of proteins originating from potato, corn (zein), milk (casein), gelatin, egg and bovine serum albumin (>95 %) are summarized in Table 5. It appears that all proteins are deaminated (mineralized) quite well, but their conversion into CH_4 is much lower, i.e. it ranged from 55 to 86 % after 2 weeks at 30 °C. In the specific case of gelatin, the mineralization of protein-N was 95 %, while its conversion into methane amounted to 61.4 %.

Table 5. The anaerobic degradation of various proteins to CH_4 and NH_4^+-N after 2 weeks at 30 °C, in a batch assay

Protein type	COD:TS	Concentration COD g/l	TKN	Methaniz. * % COD	Mineral. % TKN
potato	1.22	5.84	0.675	72.9	96.0
corn	1.11	3.62	0.443	85.9	96.5
milk	1.32	3.62	0.364	77.8	103.8
gelatin	1.12	5.81	0.777	61.4	95.0
egg	1.34	3.62	0.484	66.0	103.9
BSA**	1.21	5.81	0.605	54.7	104.1

*Methaniz.= percent conversion COD to CH_4
Mineral. = percent conversion TKN of protein to NH_4^+-N
**bovine serum albumin
TS=total solid protein (g)
TKN= total kjeldahl nitrogen

DISCUSSION

The results obtained clearly revealed that serious problems may manifest in the anaerobic treatment of solutions containing higher concentrations of proteins and lipids, such as heavy granular sludge flotation and wash-out. Consequently, a granular sludge bed reactor like the EGSB system needs devices to prevent sludge wash-out when treating wastewaters with proteins and lipids. The sieve drum GLS device, which uses a screen at the top of reactor to prevent granular sludge wash-out represents a potential solution for these problems in EGSB reactors.

The results of the investigations conducted clearly demonstrated that the sieve drum design proposed by Yucai (1988) does not represent the proper system for granular sludge retention. This is especially true for big fluxes through the aperture area of the sieve (26 $m^3/m^2.h$ at upflow velocity of 6 m/h) but the problems still prevail after the up-flow velocity was reduced to 0.84 m/h, corresponding to a flux through the sieve drum face of only $4m^3/m^2.h$. At this low flux sludge wash-out continued to occur due to the fact that the floating granules did not release the gas sufficiently rapidly or that they stayed to long at the gas-liquid-solid interface. As a matter of the volume of the quiescent zone above the upper rim of the sieve drum should be increased,

in order to prevent the granules to be exposed to the crushing forces of the brusher. In such as enlarged sludge scum layer volume, sufficient time is allowed for the granules to release their accumulated gas, so that they settle back into the sludge bed in time.

The modified design of the sieve drum used in experiment 2 gave an improved sludge retention mainly because of the lower flux as a result of greater aperture area (from 21 to 30 %) and the lower position of the sieve drum in the reactor and consequently the greater volume of the quiescent zone so that floating sludge granules no longer became in direct contact with the rotating brusher. From the calculations of the cumulative wash-out of sludge it appeared that in experiment 2.6 g VSS/l of the sludge rinsed out from the reactor during 42 days of operation, which is relatively low compared to the initial concentration of 32.75 g VSS/l used at reactor start up. Also the intermittent instead of continuous operation of the brusher positively contributed to the better performance. A further factor of importance in this respect was the improved influent distribution system which gave less shear forces at the bottom of the reactor in the sludge bed, and therefore a lower damage to the sludge granule structure. Finally, also the choice of a granular sludge with a better settling velocity of 84.4 m/h compared to that of 13 m/h for the sludge from the first experiment obviously contributed to the better performance of the .system. Through all these factors sufficient time is left to the sludge to release adsorbed gas so that it is enabled to settle out in time back to the sludge bed.

Another important objective of the study was to get a better insight in the biodegradation of complex substrate components. The degradation of proteins forms a point of contradiction in the literature. Some researchers found a high removal efficiency of casein and gelatin in reactors supplied with sludge acclimatized to those type of proteins (Shulze *et al.*, 1988; Fang *et al,* 1994); but others observed a poor degradation of protein when sludges previously adapted to carbohydrates (Breure *et al.*, 1986; Perle *et al.*, 1995). The results of the batch tests conducted in our study indicate that the deamination of proteins generally proceeds well, viz. it reaches values readily exceeded 95 % of the N contained in the proteins. However the conversion of proteins into methane only ranged between 55 to 86 % within a period of 2 weeks of incubation. These results correspond with those found in batch experiments; of Moosbubrugger *et al.* (1990) using casein, Schulze *et al.* (1988) studying gelatin and Sarada and Joseph (1993) researching tomato proteins. All these findings indicate that the anaerobic treatment systems operating at a high hydraulic retention time should be capable to provide a 55-86 % conversion of proteins to methane under conditions of almost complete deamination. Apparently a rather high fraction of the proteins is converted into soluble or non soluble and relatively very poorly anaerobically biodegradable compounds.

In the continuous EGSB reactor, we indeed observed a deamination of gelatin-N viz. up to 90% at 40 °C, similar to observations reported by others (Breure *et al.*, 1985; Breure & van Andel, 1984; Alphenaar, 1994) in continuous reactors. But the conversion to methane remained very low in our experiment, viz. only 20 %. This very low methane yield compared to that obtained in the batch experiments, likely can be attributed to the imposed short hydraulic retention time of 4 hours in the EGSB-reactor. The higher levels of methane recovery from gelatin reported by Breure *et al.*(1985) and Breure and van Andel (1984) in their continuous reactor studies

presumably can be attributed to the better adaptation of the sludge to the proteinaceous substrates used! Apparently sufficient time has to be invested for adaptation.

The presence of sucrose in the feed following day 6 had a detrimental effect on deamination of gelatin. According to Breure *et al.* (1986) carbohydrates may substantially lower the hydrolysis of gelatin, and therefore sucrose could negatively affect the fermentation of protein. The presumed inhibition of protein fermentation may be associated with the increased hydrogen pressure due to the degradation of carbohydrates (Fox & Pohland, 1994; Hanaki *et al.*, 1987; Breure *et al.*, 1986).

Another factor to be considered is that the ammonium released from the degradation of proteins serves for the growth of acidifying biomass, e.g. growing on sucrose. The uptake of nitrogen can be estimated from the growth yield of the sucrose degrading consortium, viz. being 0.22 g COD of biomass according to (Pavlosthatis & Giraldo-Gomes, 1990) and the N-content of the biomass (COD), viz. 0.087 g N/g biomass COD (McCarty, 1990). Based on these figures and the average consumption of COD in the period 14 to 42 days, which was 1335 mg COD /l, we estimated that the nitrogen uptake would amount to 26 mg NH_4^+-N/l. This value represents 50 % of the unrecovered N (52 mg/l). Consequently taking the N-uptake by cells into account, the extent of protein degradation in the period between days 14 and 42 would amount to an average value of 84 %. This value is in close agreement with N-mineralization found in the batch biodegradation assays with various types of proteins and by adapted sludge.

With respect to the influence of lipids on the operation of the EGSB reactor the results reveal that at a low concentration (0.100 g/l) in the form of a milk-fat emulsion lipids do not seriously affect the COD removal efficiency of the system and the methane yield. A concentration of lipids of 100 g/l apparently is not toxic, which corresponds to observations made in other studies with lipids and LCFA (Koster & Cramer, 1987; Hanaki *et al.*, 1987; Perle *et al.*, 1995). From results of the analyses of lipids in the effluent we found a removal of 62 %. Since the addition lipids to the feed did not result in a clear extra methane production, the main removal mechanism probably is adsorption on the sludge, also found as previous EGSB experiment presented in Chapter 2.

The results obtained in experiment 2 reveal gradually drop in the COD elimination from approximately 60 % at day 24 to approximately 40 % around day 42, and also the conversion to methane of influent COD during period 14-42 continuously decreased. After the OLR was elevated from about 7 to 11 at day 13 a steep decrease in the COD-removal efficiency occurred, followed by a slight temporary recovery. It is obvious that the system could not accommodate the imposed load of 11.2 g COD/l.d and in fact even already was overloaded in the period 6-12, when the OLR was in the range 5-7. The results in Table 4 clearly reveal that the methanogenic activity was too low to eliminate the VFA-produced. The continuing deterioration in performance at least partially can be attributed to the overloading. Another reason for that poor performance likely can be attributed to a lack of nutrients. But an additional reason of the deterioration of the sludge very likely can be found in the accumulation of adsorbed substrate ingredients in/on the sludge. Part of these ingredients might be composed of degradation products of the proteins, but accumulating

lipids certainly contributed to major part! As also found by Sayed (1987), such a continuing accumulation alternatively will lead to a complete upset of the system!

The complexity of the processes proceeding in the reactor clearly manifests from the data shown in Fig 5! Apparently NAS can pass into colloidal matter and vice versa. The reason (s) why the phenomena occurs is (are) obscure. Very likely the fraction NAS consists -at least partially- of fine colloidal matter, which passes a 0.45μm membrane. Whatever the reasons for the increase in the size of particles may be, it is clear that the biodegradability of the components present in the fractions COD_{COLL} and NAS is very poor, and that these fractions together comprise approximately. 25-35 % of the influent COD during the period 15-42, the period where lipids were present in the feed. In the period prior to the feeding with a mixture of gelatin, sucrose and lipid, both fractions already were present but in a significantly lower amount, although a steep increase occurred at day 14 of NAS, consequently immediately following the elevation of the OLR. This is the more peculiar, because at day 13 the concentration of NAS and COD_{COLL}, but particularly that of NAS was low! From these observations it can be concluded that the formation of NAS and COD_{COLL} in the some way or another is associated with the extent of overloading of the system, and certainly not merely can be attributed to the presence of lipids in the feed.

Above we mentioned that the accumulation of adsorbed components very likely can be designated is a major reason for the gradual deterioration of the system. On the other hand it is clear that a substantial amount of poorly biodegradable fines (NAS + COD_{COLL}) is "produced" in the system. These fines presumably originate from some kind of "desorption" process proceeding in/on the sludge. Regarding the continuing deterioration of the system, this presumed dynamic sorption-desorption process insists in the system and a complete removal of VFA is not achieved. As a consequence it is doubtful if any recovery will occur when the OLR would be reduced to significantly lower values! It is needed to study the above mentioned dynamic process of sorption-desorption in more detail. Regarding the very complex character of the processes taking part in this "phenomena', we suggest to investigate this matter with a limited number of well defined compounds, e.g. solutions consisting of one LCFA and for instance with and in absence of the gelatin. But also more attention should be afforded to the phenomena occurring in the treatment of gelatin in absence and presence of the sucrose. And last but not least, much more attention should be afforded to the quality of the sludge, particularly the extend of adaptation.

Presence of a sufficient amount of all essential nutrients is a prerequisite for a good performance because the microbial regeneration strongly depends on that, while also rates of substrate metabolism are negatively affected in case of nutrient limitation. According to Speece (1983) the nutrients in decreasing order of importance for methanogens are: nitrogen, sulfur, phosphorous, iron, cobalt, nickel, molybdenum, selenium, riboflavin and vitamin B_{12}. In experiment 2, the amount of nitrogen was sufficient as a result of the degradation of gelatin, but the other nutrients likely have not been present in sufficient amount.

CONCLUDING REMARKS

The new sieve drum design proposed in the present investigation performed fairly well with respect to its ability to retain granular sludge in an EGSB reactor when treating complex wastewaters of the type studied. An important feature of the new design comprises the larger volume of quiescent zone in the GLS-device; which enables the buoying sludge granules time for releasing the gas entrapped, sothat they can settle back to the sludge bed without being damaged by mechanical forces of the brusher. Additionally the increased aperture area of the sieve drum reduces the risks of disruption of the granules because the liquid velocities in the apertures are substantially lower compared to the screen used in the reactor in experiment (at a similar hydraulic load). The EGSB reactor could be operated with complex wastewaters without major losses of the granular sludge.

The results obtained reveal that that hydrolysis of proteins proceeds well, but this certainly is not the case with respect to their conversion into methane, at least not by non-adapted sludge. Moreover, it was found that the presence of sucrose significantly decreased the deamination of gelatin, most likely can be due to a high uptake of mineralized N by newly ingrowing acidogenic organisms, particularly on sucrose.

The presence of a low concentration lipid in the influent did not affect the efficiency of the reactor. On the other hand it turned out that substrates like gelatin and sucrose (and possibly) milk-fats may lead, at least with non-adapted sludge and at conditions of overloading, to the formation of a soluble/colloidal COD-fraction which is poorly biodegradable. In the application of anaerobic treatment to complex wastewaters of the category studied here, it therefore is of crucial importance to address attention to a sufficient adaptation of the sludge. Additional research in this field clearly is needed.

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Chapter 4

THE ANAEROBIC TREATMENT OF GELATIN AND LIPIDS SUBSTRATES TOGETHER WITH DILUTED BEER IN EXPANDED GRANULAR SLUDGE BED REACTORS

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Abstract

Anaerobic treatment is a potential attractive option that can be applied to clean up wastewaters of the food industry. However the application of the upflow anaerobic sludge blanket (UASB) reactor concept gave some operational problems in previous applications to wastewaters composed of high concentrations of proteins and lipids. The expanded granular sludge bed (EGSB) concept provides an improved mixing between wastewater and biomass as a result of the applied increased liquid upflow velocities in the system. This study evaluates the application of the EGSB-reactor to complex synthetic wastewater composed of carbohydrates (beer), protein (gelatin) and lipids (milk-fat) emulsion. Upflow velocities of 6 m/h were applied in reactors seeded with granular sludge from a full scale UASB. Beer and gelatin were found to be readily removed at high COD removal efficiencies (90-95 %) and at a high COD-conversion to methane (85 %) at OLR's up to12 g COD/l.d. Gelatin-N was well mineralized to ammonium nitrogen (86 to 89 %) and the presence of lipids up to 0.260 g COD/l had no detrimental effect on the reactor operation, aside from a temporary partial decrease in methane production; but this recovered after 5 days. The degradation of lipids did not proceed satisfactory at conditions applied to the system and the main removal mechanism of lipids presumably was adsorption or precipitation. When one of the reactors was started up with a feed consisting of merely beer, after appr.16 days the granular sludge started to disintegrate heavily. However the disintegration of the granules ceased after gelatin was added to the feed. Granular sludge was successfully retained in the EGSB reactor, even after operation for over 100-days at an superficial velocity of 6 m/h.

Key words: EGSB reactor, lipids, gelatin, carbohydrates, beer, anaerobic treatment, granular sludge, fat, milk, emulsion.

INTRODUCTION

Due to rapid growing urbanization in developing countries, wastewater discharge in big cities is becoming a serious environmental and public health problem. Associated with urbanization, is the great number of food processing industries discharging waste effluents with soluble organic matter. These wastewaters frequently contain large amounts of carbohydrates, proteins and lipids, and gave a tremendous pollution potential. With increasing environmental awareness, many governments have set up strict environmental standards, viz. requiring treatment of food industrial effluents prior to their discharge. However, industries in developing countries frequently do not have the economic resources for costly aerobic activated sludge treatment systems.

Anaerobic treatment presents an attractive low cost treatment alternative. Compared to conventional aerobic treatment large cost savings with respect to aeration, nutrient supply and surplus sludge disposal can be realized. Furthermore, the main byproduct of anaerobic treatment, the biogas, can be used as a fuel source, displacing energy expenditures of the industry.

Based on the favorable perspectives that anaerobic treatment, Lettinga *et al.*(1980) conducted comprehensive investigation which resulted in the development of a new reactor concept, known as the Upflow Anaerobic Sludge Bed process (UASB). This UASB-concept is technologically a quite plain system; the wastewater is fed to the main reactor tank from below and leaves the system at the top via an internal baffle system for separation of the gas, sludge and liquid. In the period 1972-1974, the first 6m^3 pilot-scale UASB reactor was tested at the sugar beet factory of the Centrale Suiker Maatschappij (CSM) in Breda (The Netherlands). In the period 1974-1976 pilot-plants of 30 m^3 and of 200 m^3 were out in operation, followed in 1977 with the construction of the first full-scale plant of 1000 m^3 Pette *et al.*, 1980). Others full-scale plants treating relatively "easy" wastewaters, i.e. composed of soluble carbohydrates such as those of sugar beet processing effluents were installed in the late seventies at other sugar beet factories in the Netherlands, and also for other agro-industrial wastewaters like from potato processing. The number of UASB reactors in operation until the year of 1996 amounts to 914, treating in the meantime very different types of wastewaters.

It turned out that the treatment of more complex wastewaters, like those containing proteins and lipids, may be accompanied with serious operational problems. Typical problems that manifest are flotation of granular sludge and foaming, which may cause wash-out of granules and clogging of gas lines (Lettinga & Hulshoff Pol, 1986; Hulshoff Pol & Lettinga, 1986; Fang *et al.*, 1994; Rinzema, 1988; Andersen & Schimid, 1985; Samson *et al.*, 1985; Öztürk *et al.*, 1993). The precipitation (or sorption) of protein and lipids, under conditions of overloading, resulted in serious problems in the release of gas from the sludge bed present in the reactor, which enhanced the flotation of granular sludge (Rinzema, 1988; Shin & Paik, 1990). A satisfactory biodegradation of proteins has been found, viz. proceeding with high efficiency, when using adapted sludge (Chapter III, Fang *et al.*, 1994; Sarada & Joseph, 1993; Thaveersi *et al.*, 1990; Alphenaar, 1994; Schulze *et al.*, 1988, Moosbrugger *et al.*, 1990, Petruy *et al.*, 1997). However according to experimental results Breure *et al.* (1986), Sarada & Joseph (1993), Morgan *et al.*(1990) and Perle *et al.* (1995) the biodegradation of proteins did not occur satisfactorily, i.e. at a low efficiency, when the feed solution also contains carbohydrate and/or when sludge was poorly adapted to proteins. Biodegradation of lipids to methane proceeds very slowly and the conversion of lipids into methane also can proceed only partially (Petruy & Lettinga, 1997; Rinzema, 1988). Serious inhibition of methanogenesis has been observed when treating wastewaters containing lipids by Perle *et al.*(1995), Koster (1987) and Koster and Cramer (1987).

In experiments using UASB reactors of 0.12 and 6 m^3 containing granular sludge treating raw domestic wastewater it was found that at low temperatures, below 10 °C, accumulation of suspended solids occurred due to very low hydrolysis of the entrapped solids (van der Last & Lettinga, 1992). Results obtained in 6 and 20 m^3 reactors, with granular sludge, even gave lower efficiencies, which could be attributed to a poor sludge-wastewater contact and decreased removal

of suspended solids (de Man, 1988). In order to solve these problems, the UASB reactor was modified to the so called expanded granular sludge bed (EGSB) system. In this reactor concept higher superficial velocity are applied to cause granular sludge expansion, resulting in better sludge-wastewater contact and less accumulation of flocculent excess sludge between the granules of the bed. The applied superficial liquid velocities in these systems generally exceed 6 m/h in order to achieve the required good contact. However under these conditions the removal of suspended solids is poor, so that the system has to be combined with pre- or post-settling. The higher superficial liquid velocities can be accomplished by designing the reactors with a higher height /diameter and/or by applying effluent recycling (van der Last, 1992).

The objective of the present investigation is to generate better information about the operation of EGSB-systems, including also the type of granular sludge to be used, the applicable upflow velocities, temperatures, organic loading rates, lipids concentration and hydraulic retention time. Therefore, emphasis was put on the feasibility of the EGSB-reactor for treating more complex wastewaters such as those containing lipids and proteins. In this respect a better understanding is needed with respect the removal mechanisms of these complex ingredients.

METHODS

Analysis

The samples analysis for effluent and influent COD (COD_{EFF}, COD_{PF}, COD_{SOL}, and COD_{IN}), volatile fatty acids (VFA), total volatile solids (TSS) and volatile suspended solids (VSS), Gerber test (gravimetric) and ammonium concentration in the effluent were made according the procedures described in Chapter 3. The gas production and flow rates were measured daily.

Preparation of the wastewater

The wastewater used in reactor R1 was composed of beer, gelatin and a milk-fat lipid emulsion in a COD ratio of 1.0; 1.0; 0.13 g COD/l, respectively, according to Table 1. As shown in the Table, 0.26 and 0.52 g COD/l lipids were used in final stages of the reactor operation. In reactor R2, the wastewater used was composed of beer and gelatin in a COD ratio of 1.0:1.0 according to Table 1.

The beer used was commercially available low alcohol beer (Brouw Meester 1.5 % vol. alcohol, Brouwerij B.V., West, The Netherlands) with a COD of 80 g/l. The concentration of nitrogen-beer was 8 mg/l, which was considered to be negligible to effect of calculations.

The gelatin used was technical grade obtained from Boom B.V., Meppel, The Netherlands. It contained 1.15 g COD/g of gelatin. The amount of gelatin-nitrogen (gelatin-N) was 0.157 g N/g gelatin (0.137 g gelatin-N/g COD).

The lipid emulsion was prepared according to the procedure described in Chapter 2 with pure milk-fat (99.8%) which was kindly provided by the Dept. of Food Technology (Wageningen Agricultural University, Wageningen, The Netherlands). The milk-fat lipid contained 2.61 g COD/g of lipid .

Sodium bicarbonate, commercial grade was added to the influent in R1 and R2 as a buffer at a rate of 1 g of bicarbonate for each g influent COD applied. Nutrients (Brons *et al.*, 1985) were not added neither R1 nor R2.

The substrate was prepared in containers of 10 l by diluting beer, gelatin and lipid emulsion with hot (60-70 °C) tap water and was homogenized before entrance into the reactor. The container was equipped with bag containing nitrogen to maintain anaerobic conditions to prevent fast utilization at the substrates aerobically. The flow of substrate was measured by monitoring the weight of these containers (Figure 1).

Table 1. Scheme of feeding of reactor R-1 and R-2

R-1					
Scheme of feeding			Infl. conc.	OLR [1]	SLR [2]
Period (number)	Period (days)	Composition	g COD/l	g COD/l.d	g COD/ g VSS.d
1st	0-6	B	1 *	3.2	0.06
2nd	6-18	B+G	1+1 **	6.6	0.13
3rd	18-31	B+G+L1	1+1+0.13 ***	8.6	0.16
4th	31-75	B+G+L2	1+1+0.26	11.8	0.4
5th	75-103	B+G+L3	1+1+0.52	16	0.32
R-2					
Scheme of feeding			Infl. conc.	OLR [1]	SLR [2]
Period (number)	Period (days)	Composition	g COD/l	g COD/l.d	g COD/ g VSS/l
1st	0-16	B	1 *	4.4	0.1
2nd	16-41	B+G	1+1 **	7. 8	0.16
3rd	42-45	B	1	3. 9	0.08
4th	45-84	B+G	1+1	12	0.22

B - beer
G - gelatin
L - lipids (milk fat emulsion)
1g of lipid= 2.61 g COD
*= g COD of beer
**= g COD of gelatin
***= g COD of lipids
OLR[1] = organic loading rate (period average)
SLR[2]= sludge loading rate (period average)

Biomass

The anaerobic granular sludge used in this experiment was obtained from a full-scale UASB reactor treating recycled paper manufacturing wastewater (Industriewater, Eerbeek, The Netherlands) which had a sedimentation velocity of 84 m/h. The sludge was elutriated to remove the fines and than stored one month at ambient temperature before it was used. The maximum specific methanogenic activities of the Eerbeek sludge at 40 °C (simulating the temperature conditions in the continuous reactor) using either 4 g COD/l of a VFA-mixture or acetate as substrates, were 0.495 and 0.627 g COD/g VSS.d; respectively.

The assessments of the maximum specific activities of the sludge were performed according to the procedures presented in Chapter 2.

Approximately 450 g wet sludge was supplied to each reactor R1 and R2, at start up, which corresponds to a sludge concentration in the reactor of 49.5g VSS/l.

EGSB Reactors

The continuous experiments were performed using two 1.6 liter EGSB reactors (R1 and R2) placed in a temperature controlled box at 40 °C.

The reactors (see Fig. 1) were constructed of polyvinyl chloride and were 5.8 cm in diameter and 61 cm tall, with a total volume of 1.6 l and a working liquid volume of 1.43 l. For the gas-liquid-solid separator (GLSS) device, a simple plate was placed in front of the effluent line with

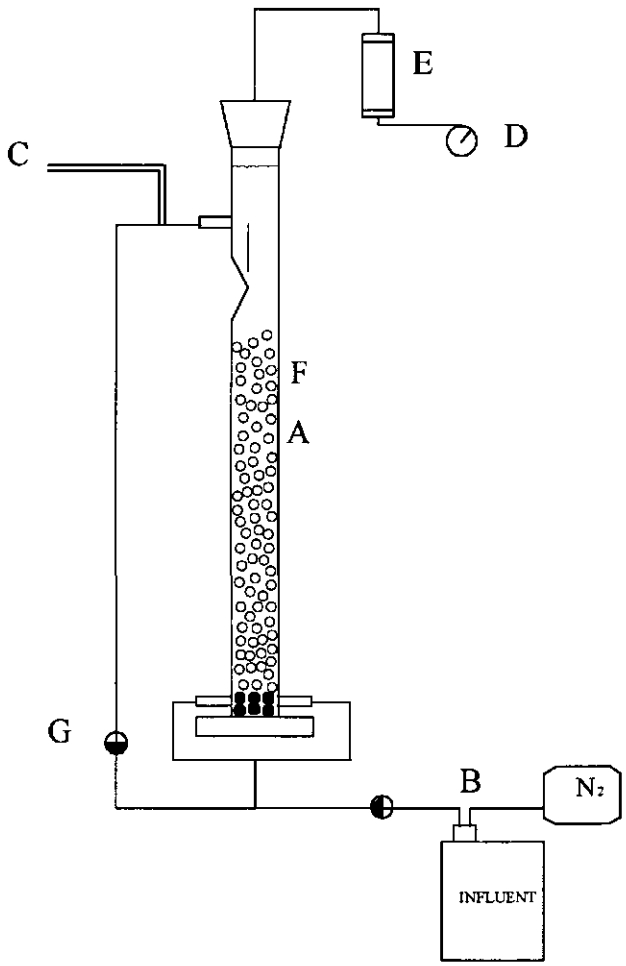

Fig. 1. Schematic diagram of reactors R1 and R2 used in this study.
(A) EGSB reactor. (B) Influent. (C) Effluent. (D) Wet test gas meter. (E) Soda lime pellets. (F) Granular sludge. (G) Recirculation of effluent.

the objective of deflecting the gas and creating a settling zone for the granular sludge.

The gas produced escaped from the top of reactor and passed through of a cylinder packed with pellets of soda lime with the objective of removing CO_2 from CH_4 in the biogas.

The required effluent recycling was achieved using a peristaltic pump (Heidolph, Kelheim, Germany) placed in the effluent line. For the influent supply, another peristaltic pump (101 U, Watson-Marlow, Wilmington, Massachusetts, USA) was used.

A bed of glass balls at the bottom of reactor was used to improve the flow distribution of the incoming influent in order to diminish channeling.

The two reactors were operated, one with a feed containing beer and gelatin plus lipids (R1) and the another one (R2) with a feed merely containing beer and gelatin for comparison.

Calculations

The performance of reactors R1 and R2 was evaluated using the parameters discussed in Chapter 3.

RESULTS

Reactor 1

In this experiment, the two laboratory-scale EGSB reactors were operated with a mixture of beer and gelatin as main substrate. The first experiment conducted in reactor R1 was intended to study the effect of adding lipids on the performance of the system. The second experiment in reactor R2 was conducted with only beer and gelatin as substrate as a reference experiment.

The results of experiment R1 are shown in Fig. 2. During the first period (days 0-6), when the reactor was fed with only beer as a substrate, the COD removal efficiency amounted to approximately 90 % (Fig. 2A). The effluent VFA concentration only accounted for 1.1 % of COD_{IN} (Fig. 2A). The conversion of COD into methane amounted to approximately 86 % (Fig. 2A). In the second period (days 6-18), when the reactor feed consisted of a mixture of beer and gelatin, the COD removal efficiency still remained at a value up to 90 %, an accordingly the effluent VFA-concentration remained low, viz. at 1 % of the COD. However, the conversion of the COD_{IN} into methane decreased significantly, viz. to values of approximately 65 %. The OLR applied in this period was approximately 7 g COD/l.d, which corresponded to a sludge loading rate (SLR) of 0.13 g COD/gVSS.d (Fig 2B and Table 1). In the third period (days 18-31) in addition also lipids were added to the beer and gelatin feed mixture, i.e. at a concentration of approximately 0.05 g/l (0.130 g COD/l). The operation of the reactor was not affected by the lipids addition, viz. the COD removal efficiency remained at approximately 90 % and also the effluent VFA remained low (1.8 % of the COD_{IN}). Moreover, during this period the conversion of the COD to methane slightly recovered and increased to 80 %. The imposed OLR was 9 g COD/l.d, corresponding to SLR of 0.16 g COD/gVSS.

In the fourth period (days 31-75), the lipids concentration in the feed was doubled to 0.100 mg/l (0.260 g COD/l). The performance of the reactor continued to be good with COD removal efficiencies of approximately 90 % (Fig. 2A). The conversion of COD into methane averaged 70 % (Fig 2A). The effluent VFA was still low (1.2 % of COD_{IN}). Therefore the OLR imposed to the reactor then was increased to approximately 12 g COD/l.d (SLR to 0.4 g COD/gVSS.d.).

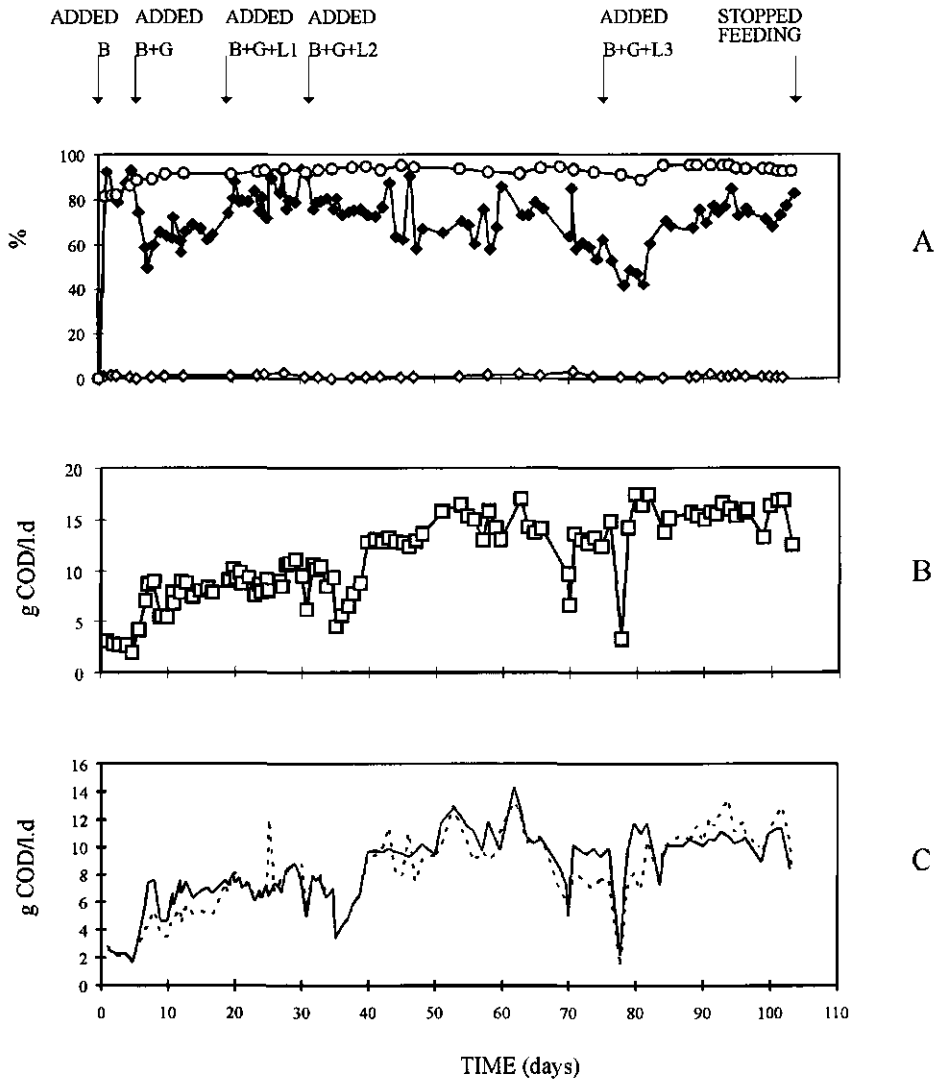

Fig. 2. Results of performance of reactor R1.
Painel A: o COD removal based on COD_{PF} , ◊ VFA, ◆ conversion to methane.
Painel B: □ OLR applied.
Painel C: —— 0.85 X OLR due to beer and gelatin, ····· methane measured.

In the fifth period (days 75-103) the lipid concentration was elevated once again, viz. up to 0.200 g/l (0.520 g COD/l), and even then the reactor treatment efficiency remained at a value around 90 %. However, the conversion of COD to methane was affected, although temporary, it

decreased to 40 % for a few days and then recovered to a value of 70 % at day 80 (Fig 2A). The effluent VFA remained low, despite the decrease in methane production. The imposed OLR amounted to up to approximately 16 g COD/l.d by the end of the period (Fig. 2B) and the SLR then amounted to 0.32 g COD/gVSS.d.

In order to evaluate if any methane production could be attributed to the presence of lipids, the curve of 85% of the OLR due to beer and gelatin (15 % accounted for cell yield) is plotted in panel C of Figure 2. For comparison, the specific volumetric production of methane (in COD) per liter of reactor is plotted. This comparison reveals that the volumetric production of methane coincides with that expected from the beer and gelatin OLR. This suggest that any significant additional methane production as a result of the presence of lipids in the feed is unlikely, even though during the fifth period, lipids accounted for approximately 20 % of the influent COD.

In reactor R1, the mineralization of gelatin-N was followed by measuring the ammonium concentration in the effluent (Fig. 3). The results obtained indicate that on the average 89 % of the influent gelatin-N became deaminated (122 mg NH_4^+-N/l measured in the effluent). Therefore apparently the lipids added, did not exert any drastic inhibition on the degradation of proteins under

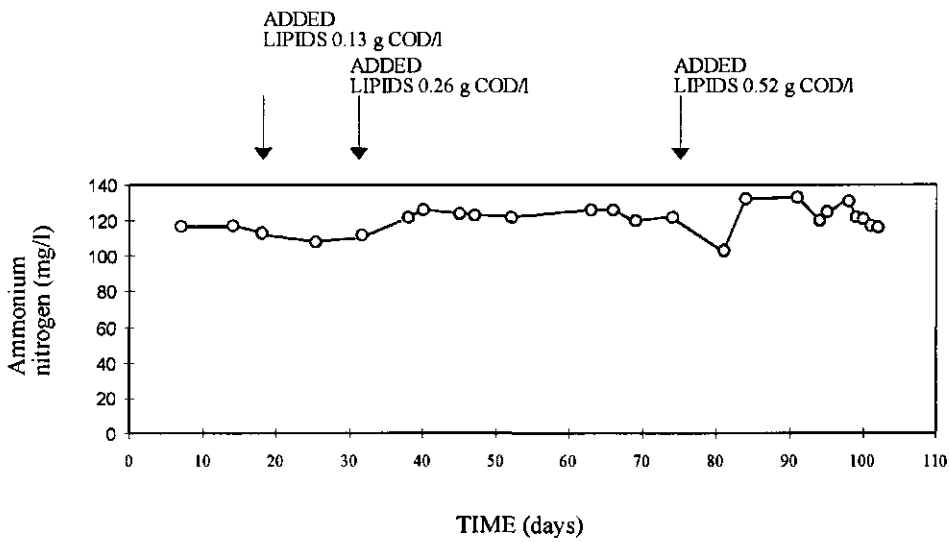

Fig. 3. Concentration of effluent NH_4-N in R1.

the conditions applied.

The COD_{COLL} in the effluent in R1 averaged 90 mg/l, in the first and second period. However in later periods the COD_{COLL} increased to 119 mg/l due to the increase of the lipid concentration in the influent. The non-acidified COD of the effluent remained low with an average value of 45 mg/l.

The performance of the EGSB system as far as the results of reactor R1 concerned, is rather satisfactory despite the complex character of the feed. The granular seed sludge used

could accommodate a superficial velocity of 6 m/h quite well: any significant disintegration did not occur, while the wash-out of sludge was relatively low (despite the simple design of the GLS-device). The amount of sludge present after termination of the operation of the reactor at day 103 amounted to 46.2 g VSS/l compared to 49.5 g VSS/l at the start of the experiment. The amount of sludge lost is not really dramatic.

Reactor 2

The intention was to operate R2 similarly to that of reactor R1, except that no lipids would be supplied to the feed. However, we were unable to accomplish this objective, due to some specific phenomena that manifested in the beginning of the experiment. Since these phenomena were considered of big practical interest, we continued the experiment and did not attempt to begin the experiment once again as a reference for experiment in reactor R1 (same feed, but without lipids!). The results are shown in Figure 4.

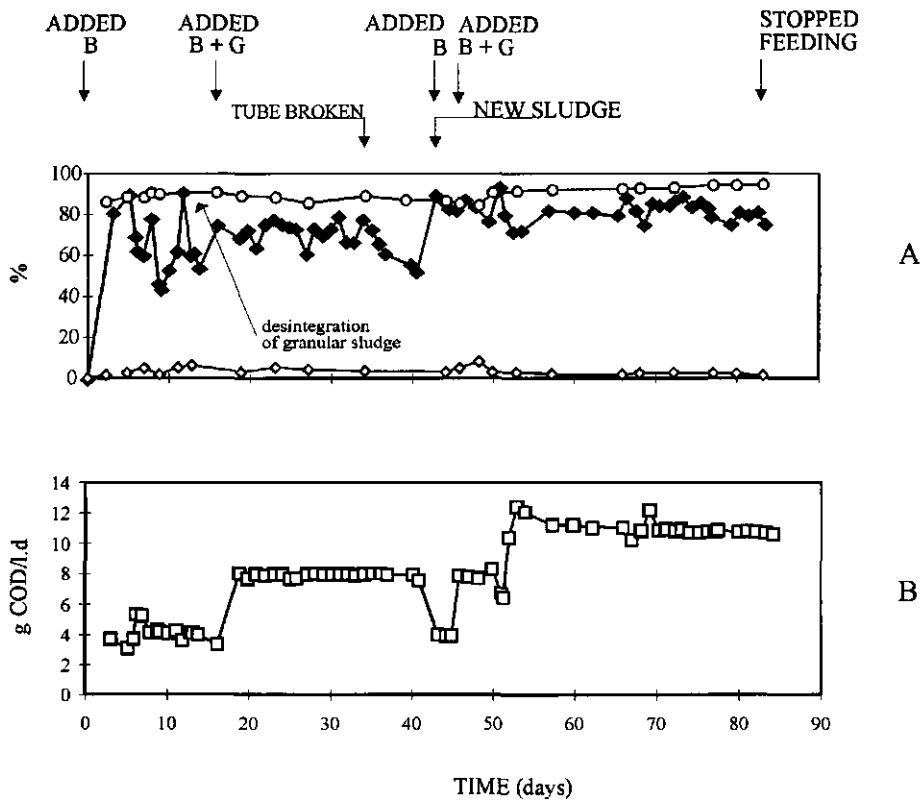

Fig. 4. Results of performance of reactor R2.
Painel A: o COD removal based on COD_{PF}, ◊ VFA, ◆ conversion to methane.
Painel B: □ OLR applied.

During the first period (days 0-16), when only beer was added, the COD removal efficiency came up to a value of 90 % (Fig. 4A), and accordingly the effluent VFA concentration was low, viz. only accounting for 4 % of COD (Fig. 4A). The conversion of COD to methane initially was 80-90 %, but later it dropped to a value of only 65 % (Fig. 4A), despite the moderate OLR of approximately 4 g COD/l.d (Fig. 4B). On day 16, suddenly a significant disintegration of part of the granular sludge in the reactor occurred, resulting in the formation of a distinct amount of dispersed sludge flocs, which gave a black appearance to the liquid phase of the reactor. Immediately following this observation, gelatin was supplied to the feed together with beer. In this second period (days 16-41) the COD removal efficiency remained at 90 % and a slight increase of the COD conversion to methane occurred, viz. up to 70 %. The effluent VFA concentration increased slightly, i.e. to a value accounting for 5 % of the incoming COD. The average OLR applied in this period was approximately 8 g COD/l.d. Very interestingly, immediately after adding gelatin to the feed, the disintegration of granular sludge ceased.

At day 34, the influent tube accidentally broke and due to that the reactor mixed liquor leaked out of the system, and the granular was exposed to air. Upon resuming reactor operation, this probably accounted for the dramatic decrease in the COD conversion to methane, viz. down to 50 %, although surprisingly the COD removal efficiency remained at appr. 90 %.

In view of the poor performance, the sludge bed was replaced at day 42 with a new batch of seed sludge (approximately 450 g of wet sludge), which gave reactor sludge concentration of 49.5 g VSS/l. During the third period with this fresh seed sludge (days 42-45), the feed switched once again to merely beer at a COD-concentration of 1 g COD/l. During period 3, at an imposed OLR of 4 g COD/l/d (SLR of 0.08 g COD/gVSS.d), the COD removal efficiency amounted to approximately 85 %, while 80 % of the COD was converted to methane (Figure 4A). The effluent VFA concentration was low only accounting for 3 % of the COD.

Gelatin was added once again together with beer in the fourth period (days 45-84) resulting in a OLR of 12 g COD/l.d (SLR: 0.22 g COD/gVSS.d). The COD removal efficiency gradually increased to 95 % and consequently the effluent VFA concentration continued to be low (only 3.1 % of incoming COD). The conversion to methane was 85 %.

The mineralization of gelatin-N was calculated from the measurements of effluent ammonium analyses (Fig. 5). The results show that the average effluent NH_4^+-N concentration was

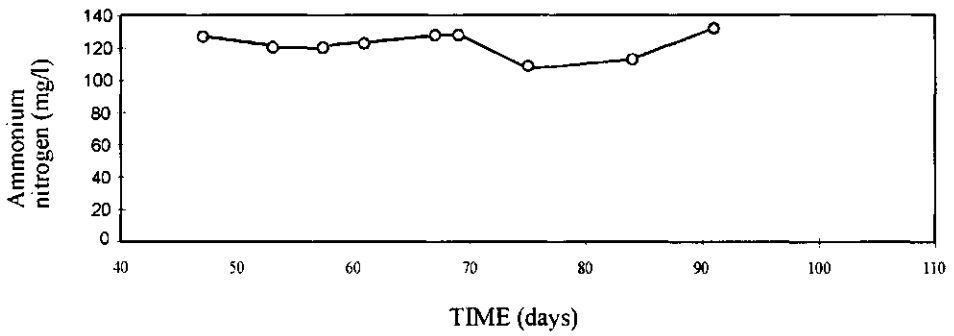

Fig. 5. Concentration of effluent NH_4-N in reactor R2.

120 mg/l, indicating that 88 % of the gelatin-N was mineralized.

The measurements of the COD_{COLL} gave an average value of 56 mg/l, and that of the non-acidified soluble COD (NAS) amounted to 60 mg/l on the average.

The reactor R2 performed quite well with the freshly added seed sludge. The granular sludge was well retained in the system during the period day 42-84, when the imposed superficial velocity amounted to 6 m/h. At the termination of the experiment the amount of sludge present in the reactor was 46 g VSS/l, which means a loss of 3.5 g compared to the start of the experiment with the new seed. This loss of sludge is moderate, taking into account that the granular seed sludge always contains a certain amount of fines, which easily rinse out from the system.

DISCUSSION

The objective of the investigations was to assess the feasibility of an EGSB reactor for treating complex wastewater containing components like lipids, and proteins. An additional objective of this research was to verify the long term behavior of granular sludge (settling velocity of grains: 84 m/h) at an imposed superficial velocities of 6 m/h, and at operational temperatures up to 40 °C and 6 hours of hydraulic retention time.

The results of the experiments demonstrate that a component like gelatin is quite well mineralized when present in a feed together with beer. In experiments conducted by Breure *et al.*(1986) using cultures adapted to gelatin it was found that after addition of glucose to a feed consisting of gelatin as substrate, the gelatin still was well hydrolyzed but not or very poorly fermented. According to Fox and Pohland (1994) and Hanaki *et al* (1981) the detrimental effect of glucose might be due to the high concentration of hydrogen resulting from the degradation of glucose; the fermentation of amino acids formed in the hydrolysis of gelatin would become inhibited. The results of our present investigations show an excellent conversion of gelatin to methane and ammonium; this also was the case when the feed to the anaerobic reactor contained beer.

Lipids were found to contribute not significantly to the gas production in experiment R1, indicating that their degradation was poor. Probably the main removal mechanism of lipids resulted from adsorption as found earlier by Sayed *et al.* (1987), Rinzema (1988) and Petruy and Lettinga (1997). The observed increases in effluent colloidal COD concentration probably mainly can be due to wash-out of dispersed lipids from the reactor; it responded to increased concentrations of the lipid in the influent. A relatively low lipid influent concentration of 0.05 g/l did not affect the methanogenesis, but at concentrations as high as 0.200 g/l at least temporarily a slight inhibition of methanogenesis manifested; however the system recovered after approximately 5 days.

Aerobic treatment with EGSB-systems for wastewaters containing relatively low concentrations of lipids (between 0.05-0.100 g/l) looks possible, although the use of a proper GLS-device to prevent the loss of floating granular sludge looks necessary. Sludge flotation is highly enhanced by adsorption of lipids in/on granules (Rinzema, 1988), and therefore the system should be equipped with a device that effectively retains buoying granules.

The results obtained with reactor 1 clearly illustrate the promising potentials of EGSB

systems for complex wastewaters. A satisfactory retention of granular sludge was obtained in the reactor at an superficial velocity of 6 m/h and at hydraulic retention time of 6 hours at an OLR of approximately 15 g COD/l.d. This despite the quite simple design of the GLS-device in this reactor.

The results on the other hand also reveal that the degradation of lipids does not proceed satisfactorily in an EGSB-system, despite the fact that part of these ingredients are removed as a result of a sorption/ or precipitation mechanisms. Future studies should be carried out to assess the proper design criteria for scaled up EGSB-systems.

The results obtained during period 1 in reactor 2 using merely beer as substrate revealed two interesting phenomena, which for the practical application for UASB/EGSB systems are of big importance, i.e.

- the occurrence of a sudden disintegration of the granular sludge after it has been fed with diluted beer for a period of 16 days. It resulted in a high concentration of finely dispersed particles in the reactor liquid, and consequently in the effluent,
- the immediate cessation of the sludge disintegration once gelatin was supplied to the feed.

The reason (s) and/or mechanisms underlying these phenomena are completely obscure so far. Presumably the sludge disintegration can be related with the rather poor performance of the system, particularly the process of methanogenesis. Despite the relatively low OLR imposed during the first period, the system apparently was insufficiently capable, at least beyond day 5, to convert the removed COD into methane. Possibly some essential ingredient might have been lacking in the feed. The disintegration of the granules in some way or an other is related with a breakdown of links/bridges, presumably consisting of polymers, responsible for sludge matrix structure. Very interesting is the observation that the 'falling apart' process ceased immediately following the addition of gelatin to the feed. Results of experiments with solutions of proteins, carbohydrates and lipids presented in Chapter 3 also revealed the occurrence of a transformation of 'colloidal COD' into 'non-acidified COD' (NAS). Although the original ingredients in the feed are quite well biodegradable, the compounds present in these 'colloidal-COD' and 'non-acidified COD' fraction produced in the system were very poorly biodegradable. Possibly there is a "link" between these phenomena of 'colloidal-COD' and 'non-acidified COD' formation/transformation and that of the sludge disintegration of the present experiments. But at yet it is still unclear.

CONCLUSIONS

The EGSB system offers attractive potentials for application to more complex wastewaters, although for lipid containing wastewaters the reactor should be equipped with a improved gas-liquid-solid separator device in order to retain buoying granular sludge. Due to the inevitable adsorption of lipids on the granules and the fact that lipids are only degraded very slowly, i.e. particularly sorbed/precipitated lipids (or higher fatty acids) sludge flotation is difficult to avoid.

High values of protein mineralization were obtained, also in the presence of a carbohydrate containing substrates such as beer.

Lipids temporally negatively affected the process of methanogenesis once their

concentration was elevated up to 0.200 g/l; however the system was capable to recover within a few days, and therefore it can be concluded that wastewaters with lipid concentrations in the range of 0.05-0.100 g/l can be well accommodated in an EGSB system, at least at moderate OLR; any lasting detrimental effect on the process of methanogenesis did not manifest, even although apparently the degradation of the lipids did not proceed quite satisfactorily. Evidence was obtained that lipids are removed from liquid by an adsorption mechanism, but so far insufficient information is available about the rate limiting factors of their degradation.

For some - yet unknown - reason(s) a sudden and quite heavy disintegration of granular sludge may in a - relatively poorly performing - system fed with diluted beer. This disintegration of the granules ceases when gelatin is supplied to the feed. Regarding the big importance of granular sludge stability for practice - particularly for EGSB-systems - in future research should be carried out to elucidate the reasons for granular sludge deterioration.

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Chapter 5

THE APPLICATION OF PROTEIN AND CARBOHYDRATE IN EXPANDED GRANULAR SLUDGE BED REACTORS

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Abstract

Anaerobic treatment offers excellent potentials for the treatment of agro-industrial wastewater's which are produced in huge amounts and - regarding their composition and strength - in enormous varieties. Proteins and carbohydrates, frequently constitute a considerable fraction in these wastewaters. This paper presents the results of a feasibility study evaluating the application of the anaerobic Expanded Granular Sludge Bed (EGSB) reactor concept for the degradation of mixtures consisting of gelatin (protein) and sucrose (carbohydrate). Using an undated granular sludge and conducting the experiment in absence of nutrients, gelatin when present as mere substrate, will be deaminated for 70-90 % at space loads up to 7 g COD/l.d. However under these conditions its acidification and consequently conversion to methane was found to proceed poorly, i.e. only for 65 % even although COD-removal efficiencies ranged up to 85-90 %.. Addition of sucrose to the gelatin resulted in a slight improvement of the deamination, but at the same time in a decrease of the fermentation of gelatin. Latter phenomenon very likely can be attributed to a lack in phosphate. However, after starting supplying nutrients together with the gelatin and sucrose to the feed storage vessel, pre-acidification of substrates proceeded already well in the storage vessel, which resulted in an improved conversion of gelatin. When hampering the pre-acidification in the storage vessel by supplying gelatin and sucrose separately, the conversion of gelatin once again dropped sharply. Apparently the presence of sucrose seriously represses the conversion of gelatin. Curiously enough the COD removal efficiency remained up to 90 % under these conditions; this indicates that the mechanism of protein removal originates from a sorption or precipitation process. Although the COD-removal efficiency remained high, the sorption of the substrate ingredients - or possible polymeric products formed from the gelatin and sucrose - lead to a deterioration of granular sludge characteristics. Particularly the release of gas bubbles from the sludge aggregates became seriously hampered, which led to problems due to sludge flotation. The pre-acidification in the storage vessel could be restored by adding the substrates (gelatin, sucrose) and nutrients simultaneously to the vessel; after the recovery of the pre-acidification, also the conversion of gelatin resumed.

The EGSB reactor was found to represent an attractive concept, particularly the sieve-drum GLS-device looks promising.

Key words: EGSB, gelatin, carbohydrate, sucrose, protein, deamination, sieve-drum, granular sludge, anaerobic.

INTRODUCTION

In various types of wastewaters from agricultural industries such as those of food processing industries, like dairy industry, slaughterhouse, gelatin and meat packing industries, proteins constitute an important fraction of the pollution load. The protein in the wastewater of these industries can be degraded to volatile fatty acids and subsequently to methane in anaerobic wastewater treatment systems. From the literature it is known, that many anaerobic bacteria have the ability to hydrolyze proteins (Buchanan & Gibson, 1975). The presence of proteases in anaerobic sludge was confirmed by Siebert and Toerien (1969) and van Assche (1982). During anaerobic digestion, bacteria degrade protein first to amino acids which are subsequently degraded to volatile fatty acids via Stickland reactions (Nisman, 1954), where oxidative and reductive deamination then results in the conversion of amino acids to volatile fatty acids.

Other components frequently present in agro-industrial wastewaters are carbohydrates, which generally are quite rapidly fermented in the anaerobic digestion process to volatile fatty acids. According to Glenn (1976), Pansare *et al.* (1985), Wiersma and Hander (1978) and Whooley *et al.* (1983) the presence of glucose and other easily fermentable compounds can repress the synthesis of protease's, enzymes responsible for the hydrolysis of proteins, in pure cultures.

In studies of Breure *et al.* (1986*a*), a poor degradation of gelatin was observed, using in the experiments a mixed anaerobic culture previously adapted to glucose. On the other hand they found that a mixed culture, when adapted to gelatin, was well capable to hydrolyze protein. However, in presence of a second substrate, such as for instance glucose, the hydrolysis of gelatin occurred, but the fermentation of the amino acids produced did not proceed smoothly. In an other study of Breure *et al.* (1986*b*), the protease activity of a mixed culture adapted to gelatin was found to be severely inhibited by increasing concentrations of carbohydrates in the feed, such as glucose and lactose

In continuos anaerobic chemostat experiments, Breure *et al.* (1984), observed that 78 % of the gelatin was hydrolyzed and that 79 % of the hydrolyzed protein was fermented. In an experiment carried in a lab-scale up-flow reactor, Breure *et al.* (1985) found that, 84 % of the gelatin was hydrolyzed at hydraulic retention time (HRT) as low as 30 minutes and 85 % of the hydrolyzed product was fermented.

Since proteins fed to UASB treatment system may cause granular sludge flotation, which may result in a poor sludge retention, it is important to improve the understanding of the degradation of these compounds (Lettinga & Hulshoff Pol, 1986; Hulshoff Pol & Lettinga, 1986; Fang *et al.*, 1994).

In the present experiments, we used a lab-scale expanded granular sludge (EGSB) reactor to study the degradation of gelatin, as a model for proteins and also to assess the effect of sucrose (as a model for carbohydrates) on its degradation. A second objective was to test the feasibility of the EGSB concept to protein containing wastewaters. The EGSB reactor uses upflow velocities exceeding appr. 6 m/h, which can be accomplished for instance by applying effluent recycling. These high superficial velocities are applied in order to obtain expansion of granular sludge bed, ad herewith the required good contact between sludge and wastewater (van der Last *et al.*, 1992; de Man *et al.*, 1988).

METHODS

Analyses

Influent and effluent samples for chemical oxygen demand (COD), VFA, ammonium concentration and analysis in sludge for total suspended solids (TSS), volatile suspended solids (VSS) were made according to methods described in Chapter 3.

The EGSB Reactor

The reactor (Fig. 1) used in the experiments consisted of a double wall glass column 173 cm height, 5.16 cm internal diameter and a total working volume of 4.44 l. In the top the reactor, a sieve drum, gas-liquid-solid separator (GLSS) was installed. It was constructed from a screen cylinder (7 cm height x 6 cm of diameter) with aperture area of 40 mm^2 with holes of 2.0 mm in diameter. The purpose of the sieve drum was to retain the buoying granular sludge in the reactor and test its practical application. The flow in the sieve drum is 12.7 $m^3/m^2.h$. The effluent leaves the reactor after passing the screen via the top of the reactor. The biogas was collected at top of the reactor and from an external settler. The amount of CH_4 produced was measured using a wet test gas meter, after the CO_2 was removed using a scrubber filled with 3% NaOH and dried in a column filled with pellets of soda lime.

The effluent was recycled by a Heidolph peristaltic pump (Kelheim, Germany). The reactor system was continuously fed from the bottom with stock solutions of influent substrate via a peristaltic pump model 503 U Watson-Marlow pump Wilmington, Massachusetts, USA. (Fig. 1). Glass beads (approximately 1 cm of diameter) and stones (1-2 cm of diameter) were used as flow distributor in the bottom of reactor.

The operational temperature maintained during the experiments was 40°C, which was accomplished by recycling hot water via a thermostat through the jacket.

Preparation of the wastewater

The synthetic wastewater used in the experiment was composed of gelatin and sucrose in COD ratio according to the figures presented in Table 1. The gelatin was of technical grade and obtained from Boom B.V., Meppel, The Netherlands. It contained 1.15 g COD/g of gelatin. The N-content of gelatin (gelatin-N) amounted to 0.157 g N/g gelatin (0.137 g N/g COD). The sucrose was a product of Suiker Unie, Breda, The Netherlands.

Sodium bicarbonate, a commercial grade quality, was added to the influent as a buffer at a concentration of 1 g of bicarbonate for each g influent COD applied. Nutrients, as a basal medium (Brons *et al.*, 1985) (except NH_4Cl), were added as a freshly prepared stock solution, viz. in an amount of 1 ml per liter of wastewater.

The substrate solution present in a container of 100 l was prepared by diluting gelatin and sucrose stock solutions with hot tap water. The mixture was homogenized before supplying it to the reactor. Every four days a fresh substrate solution was prepared.

During specific periods of the experiment gelatin and sucrose were added separately to the reactor using two containers of 10 l in that case, one containing the solution of gelatin with nutrients and the other sucrose + sodium bicarbonate. Both containers were locked with bags

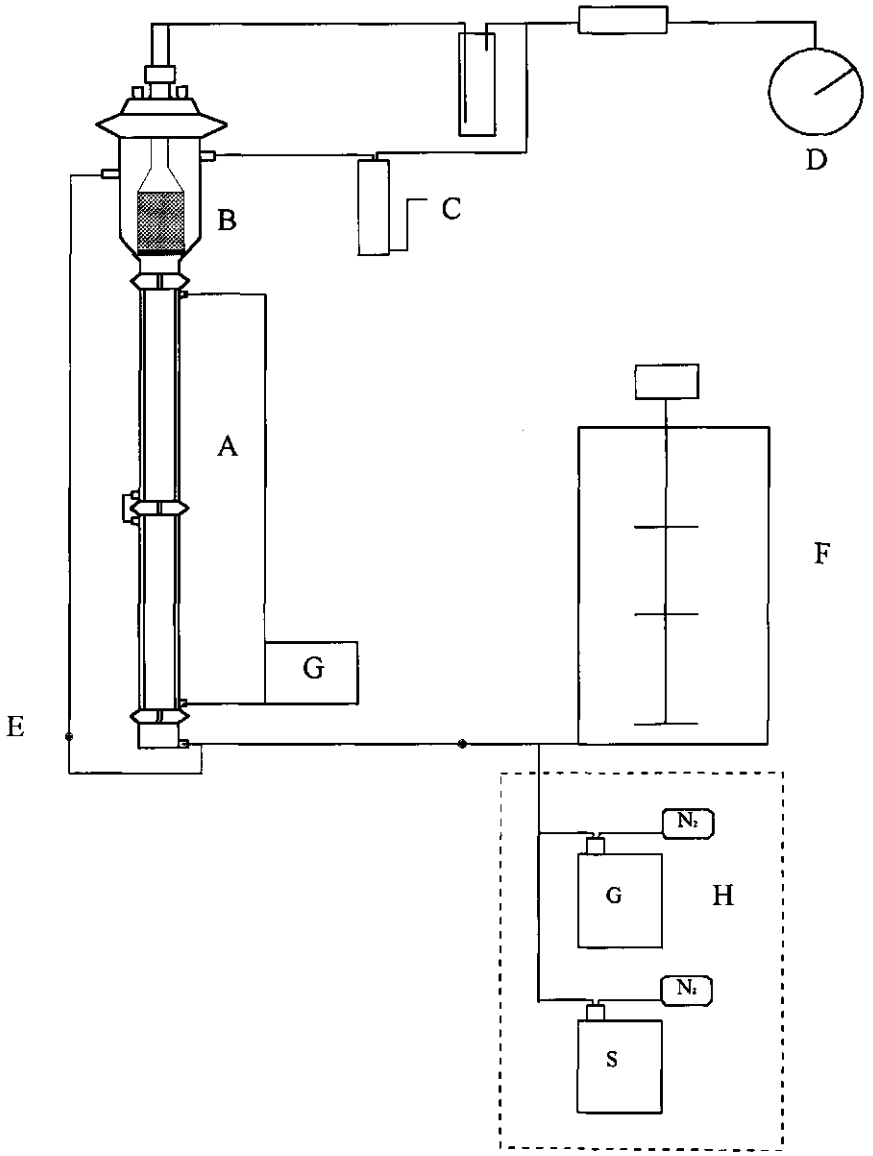

Fig. 1. Schematic presentation of experimental reactor.
(A) reactor. (B) sieve-drum. (C) effluent. (D) biogas measurement (wet gas meter).
(E) recirculation. (F) influent vessel. (G) heater. (H) gelatin and sucrose added separately.

containing nitrogen, to prevent growth that air comes in the containers, consequently the growth of aerobic bacteria on the substrates. The substrates were prepared daily. Therefore, during these specific periods the gelatin and sucrose were only mixed up inside the reactor: therefore the extent of pre-acidification of the substrates was very low.

During specific other periods, the feed ingredient gelatin, sucrose and nutrients were mixed up in the same feed container. As a result in this case, the substrates already became (at least partially) pre-acidified in this storage vessel prior, and consequently then a pre-acidified feed was introduced in the reactor.

Table 1. Scheme of feeding of experiment

Scheme of feeding			Influent Conc.	OLR
Period number	Period (days)	Composition.	(g COD/l)	(g COD/l.d)
1st	0-36	G	0.5 - 1.75	1.9-7.4
2nd	36-77	G + S	1.75 *+ (0.5-1.75)**	4.8-15.5
3rd	77-84	G + S	1.75 + 1.75	13.8
4th	84-92	G + S	1.75 + 1.75	14.8

G = gelatin
S = sucrose
* = g COD of gelatin
** = g COD of sucrose
OLR = organic loading rate (period average)

Biomass

The anaerobic granular sludge used in this experiment was obtained from a full-scale UASB reactor treating paper manufacturing water, Industriewater, Eerbeek (The Netherlands). This seed sludge consequently was not adapted to proteins. Before putting the sludge into the reactor, it had been elutriated to remove the fines and stored at ambient temperature until it used. The maximum specific methanogenic activity (assessed according the method described in Chapter 3) of the Eerbeek sludge at 40 °C (simulating the temperature conditions in the continuous reactor) using 4 g COD/l of a VFA-mixture as substrate, amounted to 0.5 g COD/g VSS.d, consequently relatively very low for a granular sludge at this temperature.

Approximately 1140 g wet sludge was supplied to the reactor, which corresponds to an initial sludge concentration of 40.5 g VSS/l in the reactor.

Calculations

The calculations are made according to procedures explained in Chapter 3.

RESULTS

In order to assess the effect of the presence/absence of nutrients on the performance of the EGSB reactor treating a relatively complex, mainly soluble substrate, in the first experiments gelatin was used as the sole substrate and without any nutrients. In the next period, sucrose was added to the gelatin feed, first likewise in the absence of any nutrients but later in presence of nutrients. Supply of nutrients directly to the influent storage container resulted in a distinct pre-acidification of the substrates in the feed storage vessel. Consequently during this period in fact we were dealing with a two step (phase separation!) reactor system.

We also investigated the supply of nutrients and the gelatin and sucrose via separate lines, with the objective to avoid pre-acidification of the substrate.

The results are depicted in Fig. 2. During the first period (days 0-36, merely gelatin and no nutrient supplied) the COD removal efficiency amounted to 85 % and the deamination (mineralization) of gelatin to approximately 90 % when fed at an influent concentration of 0.5 g COD/l. However, the deamination dropped to approximately 70 %, when the concentration of the feed was increased to 1 g COD/l (and the OLR doubled from 2 to 4 g COD/l.d). The extent of acidification of the gelatin amounted only to 50 % at OLR = 2 g COD/l.d, but it increased up to 75 % at OLR = 4 g COD/l.d. The conversion of gelatin-COD into methane-COD amounted to 50 % at OLR = 2 g COD/l.d and it improved to approximately 65 % at OLR = 4 g COD/l.d, but it slightly decrease after elevating the OLR to 8 g COD/l.d during this period.

During the second period (days 36-77), when in addition to gelatin also sucrose was added to the feed. In the beginning the feed concentration amounted to 0.5 g COD/l (day 36) and at day 38 it was increased to 1.75 g COD/l. The results indicate that the deamination of gelatin tended to improve, viz. from 70 to 80 %. However, on the other hand a significant drop in the acidification occurred, i.e. from 70 % to 40 %, while also the conversion into methane and the COD removal efficiency dropped sharply, viz. both to round 20 %. When starting at day 44 the direct supply of nutrients to the influent storage vessel, a clear pre-acidification of the substrate was obtained here, as evidenced by the increased concentrations of VFA and ammonium in the influent solution fed to the reactor (data not shown). The acidification improved from 40 to 70 %, resulting in a decrease of the pH in the reactor from 7.8 to 6.3 on day 47. As the VFA concentration of the solution in the reactor was high, starting from day 49 extra sodium bicarbonate was added to the influent container, i.e.increased from 42 to 162 meq/l. The measured VFA-concentrations inside the reactor at day 50 amounted to 2.2 g COD/l, and both the COD removal efficiency and methanogenesis declined to 10 %. However, after increasing the addition of sodium bicarbonate and lowering the OLR from 14 to 4 g COD/l.d, at day 52, the effluent VFA values decreased, and both COD the removal efficiency and the methanogenesis improved significantly. And this situation remained unchanged after increasing the OLR again increased at day 60 up to 14 g COD/l.d. From day 60 until day 77, the degradation of the substrate proceeded well. This satisfactory performance apparently can be attributed to the pre-acidification occurring in the storage vessel; both sucrose and gelatin were acidified, at least partially, before introduced in the reactor. The COD removal efficiency amounted up to approximately 90%, the deamination of gelatin and the acidification of both substrates were approximately 80 % and also the conversion to methane amounted to 80 %.

During the third period (77-84) the influent substrate components remained separated by using two feed containers, one containing gelatin plus nutrients and the other sucrose plus bicarbonate (freshly prepared every day). In this way pre-acidification of the substrates is greatly prevented. The response of the reactor was immediate and quite dramatic. So, the gelatin deamination dropped to only approximately 40 %, and the acidification from 80 to 40 %, and consequently also the conversion to methane, viz. to only 40 %. Also the pH dropped, although the solution remained neutral. However, surprisingly the COD removal efficiency remained high with a value up to 90 %. Apparently an adsorption or precipitation of unmethabolized gelatin prevailed in

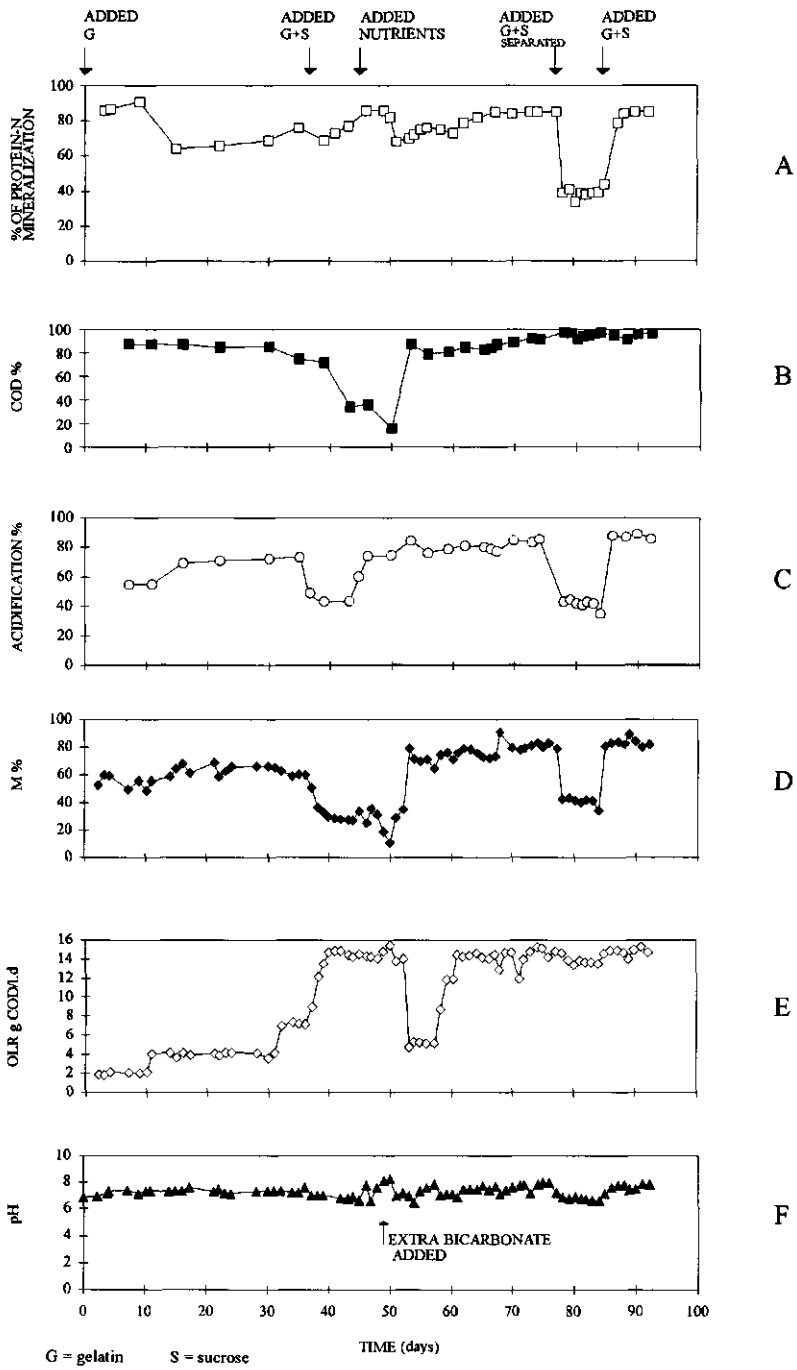

Fig. 2. Results of experiment. (A) % of mineralization of gelatin. (B) % COD removal efficiency. (C) % acidification. (D) % conversion to methane. (E) OLR, (F) pH.

the system during this period. Moreover, also a dramatic deterioration of the sludge characteristics manifested. We observed that the granular sludge strongly tended to float during this period, mainly due to the fact that gas produced gas in the granules could not escape sufficiently rapid. On the other hand, we also observed that the installed GLS-device was quite efficient in retaining the buoying granules within the reactor.

During the fourth period (days 84-92), gelatin and sucrose again were prepared in the same influent storage vessel and the system then recovered completely, clearly demonstrating the importance of applying (a certain) pre-acidification in the influent vessel. The reactor functioned with the same performance as before, indicating that the system had not been seriously detrimentally affected.

As mentioned above, the reactor system used in the experiment performed satisfactorily in terms of granular sludge retention as a result of the installed sieve drum GLSS. At the end of experiment, the system contained 36.4 g VSS/l in comparison to the starting concentration of 40.5 g VSS/l. The structure of the granules and their settleability were still very good and the granules did not show any slimy appearance.

DISCUSSION

The results obtained provide useful information about the treatment of complex soluble types of wastewaters, viz. composed of gelatin and sugars and in absence and presence of nutrients. Also the importance of a certain extent of pre-acidification was demonstrated. When supplied as sole substrate, gelatin was reasonably well deaminised by "the non-adapted" granular sludge present in the reactor, viz. for 90 % at an influent concentration of 0.5 g/l and 70 % at influent COD 1.75 g/l. The COD removal efficiency with values between 85-90 % also was reasonable, but clearly the conversion into VFA and consequently into methane did not proceed very satisfactory, viz. it only amounted to 65 % and it did not improve rapidly, at least not when a sufficient amount PO_4^{-3} was not present. Within 4 weeks the deamination, A and M only improved slightly. Apparently part of the gelatin COD is removed by an adsorption or precipitation mechanism. The relatively poor degradation very likely can be attributed to a lack of nutrients, presumably of phosphate, and possibly of trace elements. When sucrose was added as a co-substrate, the hydrolysis of gelatin slightly improved, but it is questionable whether this was also the case for its acidification and for the methane production. The performance of the system in terms of treatment efficiency (E), A and M deteriorated, which very likely can be attributed to a deficiency in one or more nutrients, presumably PO_4^{-3}. The addition of nutrients to the influent storage vessel stimulated pre-acidification of the substrates in the feed, but whether this was followed with a clear improvement in the fermentation of the protein is highly questionable, because E and M all dropped significantly. Only after the OLR was decreased to 4 g COD/l.d a high COD removal efficiency was achieved mainly because both the process of acidogenesis and methanogenesis improved After the recovery, the system could accommodate an OLR up to 14 g COD/l.d, i.e. imposed to the system following day 58. However, when interrupting the pre-acidification process in the storage vessel by supplying the two substrates and the nutrients separately to this vessel (at day 77), the process deteriorated

once again, as appears from the drop in the deamination of the proteins and of conversion into methane. Apparently the presence of sucrose in the reactor liquid represses the microorganisms from producing proteolytic enzymes. Hydrolysis of the protein and its fermentation to methane restored upon resuming the preacidification of the combined and nutrients in the influent storage vessel. But the results also reveal that the removal efficiency remained well, despite the poor deamination, performance of the acidogenesis and methanogenesis. This can be attributed to sorption or precipitation of either soluble substrate ingredients or of polymeric products formed from it.

Previously Breure *et al.* (1986*a*) observed that feeding gelatin to continuously mixed anaerobic cultures degrading glucose resulted in poor protein degradation (< 30 %). In another study where the feed contained glucose to culture Breure *et al.* (1986*b*) found that degradation of gelatin was retarded. This presumably occurred at day 36 in our experiment, i.e. after sucrose was added together gelatin with to the substrate solution. This effect may has been aggravated by the lack of nutrients. Although deamination of gelatin occurred, its acidification did not proceed satisfactorily. Also in the study of Breure, a progressive retardation of gelatin degradation was observed at elevated concentrations of carbohydrates. According to Breure and co-workers, carbohydrates are preferentially degraded when mixture of protein and carbohydrate is used as substrate. This phenomenon presumably prevailed in the period, following that in which the reactor was operated with pre-acidified influent; it then received a mixture of gelatin and sucrose as substrate, supplied separately though with nutrients and trace elements, but without pre-acidification (day 77). The sludge possibly lost its ability to degrade proteins sufficiently well. However, by allowing the sucrose to become pre-acidified prior to feeding it to the reactor, any serious problems in protein degradation were not observed (period 60-77). Apparently the presence of sucrose in the reactor liquid (period 77-84) acts repressively towards the proteolytic metabolism. In previous studies conducted with pure cultures, similar effects of repression of the proteolytic metabolism were observed upon the addition of glucose and other easily fermentable substrate (Glenn, 1976; Pansare *et al.*, 1985; Wiersma & Hander, 1978; Whooley *et al.*, 1983). The explanation for the repression of the protein degradation in heterotrophic cultures with might therefore be that the carbohydrates are the preferential substrates for these organisms (Beure *et al.*, 1986*b*). A likely explanation for the retarded acidification of proteins could be the elevated partial pressure of hydrogen, resulting from the rapid degradation of carbohydrates. Hydrogen may act inhibitory for the degradation of amino acids, produced in the hydrolysis of proteins (Fox & Pohland, 1994). In Chapter 4 we found a good degradation of gelatin and beer (co-substrates) and the conversion into methane proceeded well. Apparently beer did not compete with gelatin in these experiments, or - possibly - the partial pressure of hydrogen generated by beer degradation remained sufficiently low.

In an investigation conducted using a UASB reactor fed with gelatin, Breure *et al.* (1985) observed, that the granular seed sludge used in the reactor, attained a slimy appearance. The formation of this slimy matter could be responsible for the observed poor release of gas bubbles from the granules, and consequently for the sludge flotation occurring in the UASB-reactor. In our experiment, granular sludge flotation only occurred, under conditions where gelatin, or gelatin +

sucrose, were satisfactorily well removed from the solution, but the degradation (mineralization) remained poor. This leads to the conclusion that the reasons for the 'slime formation' in/on granular sludge as observed by Breure *et al.* (1986*b*) can be attributed to adsorption or precipitation of the gelatin ingredients, or of polymeric products formed from gelatin (+ sucrose).

It is obvious from the results obtained in our present investigations that a certain pre-acidification of substrate ingredients like soluble proteins and carbohydrates is a prerequisite for the proper performance of the EGSB reactor.

With respect to the EGSB-reactor system investigated in the investigation in the present experiments, it can be concluded that a sieve drum gas-liquid-solid separator in principle represents a proper device, because - when well designed - it efficiently can prevent floating granules from washing out. More research is needed to assess the design criteria.

CONCLUSIONS

When treating soluble 'more or less complex', but basically well biodegradable substrate consisting if mixtures of gelatin and carbohydrates, in an EGSB-type reactor, it is necessary to use an well adapted type of granular sludge as seed in order to be capable to accomplish a stable and satisfactory performance. Moreover, for a satisfactory performance of the EGSB-system, the substrate should be sufficiently pre-acidified prior to introducing it into the reactor. In order to achieve this, nutrients like phosphate and ammonia should be present in sufficient amounts. For NH_4^+-N this obviously always will the case, because it will be generated in sufficient amounts from the deamination of the proteins.

When using poorly adapted granular sludge, a satisfactory performance of EGSB-reactors at OLR in terms of acidogenesis and methanogenesis will hardly be possible at loads exceeding 7.5 g COD/l.d, despite the fact that the COD-removal - as a result of sorption of substrate ingredients - may look satisfactory.

In case the pre-acidification remains insufficient, the performance of the EGSB-reactor will deteriorate. This even will be the case when sufficient nutrients are supplied to the influent of the reactor. Both, the process of deamination and of acidification, will not proceed sufficiently well in that case. Moreover due to the occurrence of a significant sorption of substrate ingredients - or possible polymeric products formed from the gelatin and sucrose - the granular sludge will become slimy, resulting in problems with the release of gas bubbles from the sludge aggregates and consequently problems with sludge flotation. Furthermore the sludge yield will increase sharply as well its substrate content.

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Chapter 6

GENERAL DISCUSSION

The work described in this thesis deals with the feasibility of anaerobic treatment of complex types of wastewaters containing mixtures of lipids, proteins and/or carbohydrates. In view of specific problems manifesting in some of the experiments with mixed components, occasionally separate experiments were conducted with solutions containing merely one of these components. In the investigations particular emphasis was afforded to the application of Expanded Granular Sludge Bed (EGSB) reactors, because results obtained in earlier research indicted that the EGSB-systems might represent particular promise for the treatment of lipid containing wastewaters.

Chapter 1 provides a brief literature survey of some relevant literature reports dealing with the production of complex industrial wastewaters and with available information dealing with the feasibility of anaerobic treatment systems like the EGSB-reactor concept.

Chapter 2 deals with investigations concerning the degradation of a milk-fat emulsion using a closed circuit with an EGSB reactor as treatment system. The results of the experiment show that the major part of lipids present in the emulsion adsorb to the sludge granules. This adsorbed fraction remained non-degraded. In fact only the colloidal fraction was found to be degrade, although quite slowly. Latter could be expected regarding the very small value of the rate hydrolysis k_h, viz. amounting to approximately $0.01 d^{-1}$. The main mechanism for lipids removal in an EGSB-system apparently results from a sorption mechanism rather than from biological degradation.

The results in Chapter 3 concern investigations conducted with an expanded granular sludge bed (EGSB) reactor system equipped with a sieve drum as gas-liquid-solid separator device (GLSS). The sieve drum GLS-device was investigated in order to assess its feasibility to prevent the wash-out of floating granular sludge. Due to sorption of lipids in/on the granular sludge particles and the poor degradation of these sorbed lipids, a heavy flotation of granular sludge may prevail in the system. And consequently the reactor therefore then needs to be equipped with an appropriate sludge retention device. Two sieve drum designs were evaluated in experiments conducted with complex synthetic wastewaters composed of carbohydrates, proteins and lipids. One of these GLS-devices was capable to retain floating granular sludge effectively and without damaging the granular sludge structure. In this experiment it was observed that major part of effluent COD consisted of 'soluble' matter passing a membrane of 0.45 µm, viz. up to 85 %. The hydrolysis and/or acidification of this fraction proceeded very slowly. Furthermore a peculiar phenomenon was observed, i.e. that drops in the content of non-acidified soluble COD (NAS) in the effluent always coincided with an increase in the content of colloidal COD (COD_{COLL}) and vice versa The biodegradability of this particular matter is very poor. The gradual deterioration in the treatment performance found in the experiments mainly can be attributed to the formation of this 'soluble/colloidal' poorly biodegradable COD-fraction. In this Chapter also results were presented dealing with the batch biodegradation experiments (during 2 weeks) for assessment of the biodegradation of different types of proteins, viz. originating from potato, corn, milk and egg, together with gelatin and bovine. The experiments were conducted with granular sludge. Although

all the proteins investigated were well deaminated the conversion into methane-COD was relatively poor.

The research presented in Chapter 4 deals with the application of the EGSB-system to complex synthetic wastewater composed of carbohydrates and ethyl alcohol, protein (gelatin) and a milk-fat lipid emulsion. Like in the other EGSB-experiments liquid upflow velocities applied amounted to 6 m/h; the reactors were seeded with granular sludge from a full scale UASB-reactor. It was found that the organic pollutants from beer and gelatin were rather well removed at a high COD removal efficiency (90-95 %) and also the COD-conversion to methane was as expected, viz. up to 85 % at imposed OLR's up to 12 g COD/l.d, consequently moderate loading rates. Gelatin-N was deaminated up to 86-89 %. The presence of lipids up to concentrations of 0.260 g COD/l did not detrimentally affect the reactor performance, although temporary a slight decrease in methane production manifested, the methane production recovered after 5 days. However, the degradation of lipids did not proceed satisfactorily under the conditions imposed to the system; the main removal mechanism of lipids presumable was adsorption and/or precipitation. In experiments conducted in one of the reactors with merely beer as substrate, a peculiar observation was made with respect to the granular sludge stability. After 16 days of continuous feeding, the granular sludge suddenly started to disintegrate, a phenomenon which obviously can not be tolerated in an EGSB-system. The mechanism(s) underlying this granular sludge deterioration phenomenon are unknown yet, but regarding the fact that a high granular sludge stability is a factor of crucial importance for the feasibility of EGSB-systems, it is essential to continue research in this field. In our experiments we observed that granular sludge disintegration ceased almost immediately after supplying gelatin to the fed, an observation which clearly deserves elucidation.

Chapter 5 presents the results of investigations dealing with the applicability of the EGSB-system to wastewaters composed of mixtures of gelatin and sucrose. An non-adapted granular sludge was used in the experiments. The results obtained once again demonstrate the complexity of phenomena which can occur in an anaerobic treatment systems under specific 'feed' conditions, such as manifesting when the system is fed with solutions not containing the required growth macro-nutrients (not supplied). Under such conditions little if any growth can occur, although in case of protein degradation (deamination) proceeds, generally sufficient ammonia is set free for growth. Indeed, with gelatin as mere substrate, 70-90 % deamination was found at space loads up to 7 g COD/l.d. However, under these conditions the acidification remained poor, and consequently the conversion to methane, viz. it only amounted up to approximately 65 %. However, at the same time the assessed COD-removal efficiency ranged up to 85-90 %, indicating that a substantial fraction of the gelatin, or at least intermediates (regarding the relatively good deamination) either sorbed to the sludge surface or precipitated in the sludge. Addition of sucrose to the gelatin feed resulted in a slight improved deamination, but at the same time in a further decline of the fermentation of gelatin. When however nutrients supplied, i.e. simultaneously with the gelatin and sucrose in the feed storage vessel, pre-acidification of both these substrates appeared to proceed already well in the storage vessel. This resulted in a significant improved fermentation of gelatin. However, when the solutions of gelatin and sucrose are introduced separately into the reactor, the extent of the fermentation of gelatin once again dropped sharply. Apparently the presence of non-

acidified sucrose seriously depresses the fermentation of gelatin. But curiously enough the COD removal efficiency remained up to 90 % under these conditions, indicating that the mechanism of protein removal originates from a sorption or precipitation mechanism. Although the COD-removal efficiency remained high, the sorption of substrate ingredients - or possibly of some polymeric products formed from the gelatin and sucrose (or possibly specific intermediate degradation products) - lead to a deterioration of granular sludge characteristics. Particularly the release of gas bubbles from the sludge aggregates became seriously hampered, leading to problems with sludge flotation. By recovering the pre-acidification of gelatin and sucrose in the pre-acidification tank, the performance of the system improved again.

The scientific importance of the investigations comprises a number of interesting observations related to phenomena prevailing in EGSB treatment processes with respect to the behavior of granular sludge. These phenomena are of big importance considering the feasibility of these systems in treating more complex types of wastewaters, and they therefore deserve serious attention in future research. Important issues in this respect are the following:

a. The mechanism(s) prevailing in lipid removal particularly adsorption processes and the factors controlling the degradation of the sorbed lipids. In various of the experiments conducted we found that a substantial fraction of lipids from milk-fat emulsions was sorbed in/on the sludge granules, which induced serious flotation of the sludge granules. The flotation of the granules can result from difficulties in the release of gas bubbles but also can be due to the strong buoying characteristics of lipids. Granular sludge flotation can clearly detrimentally affect the performance of an EGSB-reactor, particularly the retention of granular sludge.

b. More information is needed about the rate of hydrolysis (k_h= 0.01 d^{-1}) of dispersed lipids present in milk-fat emulsion, because the results indicate that the liquefaction step of milk-fat proceeds very slowly and it would be big importance to assess whether or not the liquefaction rate can be enhanced, e.g. by imposing higher temperatures in some additional granular sludge recuperation reactor.

c. The reasons for deterioration in COD-treatment performance of an EGSB-reactor when fed with a mixture of gelatin, sucrose and milk-fat needs to be elucidated. In this respect particularly attention should be afforded to the poor biodegradability of 'soluble' COD-ingredients proceed in the system, i.e. including the nature and the reasons(s) of the formation of these ingredients. The formation of these 'recalcitrant' ingredients is the more striking, regarding the rather good biodegradability of the separated feed constituents. Of particular interest is also the observed relatively easy conversion of non-acidified 'soluble' COD into colloidal-COD and vice versa.

d. Although the EGSB-system was capable to treat a mixture of a milk-fats, gelatin and beer rather satisfactorily, even in presence of lipid concentrations up to 0.260 g COD/l, the stability of the treatment system looks questionable. This like particularly is the case at higher lipid concentrations, ultimately serious operational problems may manifest and therefore specific measures should be developed in order to prevent such problem. So far to little reliable quantitative information is available about this matter. As mentioned above, a possible attractive solution to prevent such operational problems could be found in combining the EGSB-reactor with a separate digester for

granular sludge recuperation; in this digester the adsorbed lipids in/on granules are allowed to became sufficiently degraded.

e. The reasons for the disintegration of granular sludge manifesting upon feeding the system with merely diluted beer, in fact at first sight a not too complex and quite well biodegradable soluble substrate, should be elucidate. And this also applies for the observation that the disintegration ceases almost instantaneously after supplying gelatin to the feed.

f. The detrimental effect of the presence of sucrose on the fermentation of gelatin needs elucidation, and also why concomitantly an improved COD-removal is found. The observed improved COD-removal presumable can be attributed to a sorption or precipitation process. The question is why and how.

g. The reasons of occurrence of granular sludge flotation when the system is fed with solutions containing gelatin and sucrose as substrate. Indications were obtained that flotation may be due to the formation of layers/films of sorbed of some substrate ingredients (or intermediates) on the sludge surface. These layers likely obstruct the easy release of gas produced inside the aggregates, leading to the entrapment of gas bubbles. More information is needed about the type of ingredients responsible for this 'film' formation.

EGSB-reactor equipped with a well designed and situated sieve drum GLS-device look attractive for the treatment of wastewaters containing lipids in concentrations in the range 0.05-0.100 g/l like dairy wastewaters. The sieve should be offering a sufficient open surface area to accommodate the relatively hydraulic loads applied in EGSB-reactors. Although already useful information has been obtained with respect to the required number of apertures per unit surface area, and about the dimensions of the apertures as well, still more research is need in this field. The particularly applies with respect to factors like the achievable surface load, the effect wastewater composition, imposed COD-load, temperature and treatment efficiencies. However, the observations made in our investigation indicate that a sieve drum type of GLS-device represents a quiet promising means in retaining buoying sludge grains in EGSB-reactors treating lipid containing wastewaters. With respect to the position of the sieve in the GLS-device clear evidence was obtained that it is beneficial to place it in the lower part of the device, sothat in the top of the device a zone is left for a layer of buoying granular sludge. As found in early research by Rinzema *et al.* at our laboratory the superficial liquid velocities of 6 m/h generally applied in EGSB provide a sufficient contact between substrate and granular sludge to guarantee a good degradation of capric and lauric acids at very high organic space load (exceeding 30 kg COD/m^3.day). However, despite the good contact apparently still a rapid accumulation of sorbed layers of lipids will occurs when treating milk-fat emulsions even at relatively moderate loading rates, similarly as made by Rinzema in experiments with emulsions of triglycerides. The results in Chapter 2 revealed a rather poor biodegradability of milk-fat under conditions prevailing in the EGSB reactor. A large amount of the lipids is eliminated by adsorption, while the rate of liquefaction of the colloidal matter left in liquid phase was low. Only a part of the liquefied lipids was found to be converted into methane, the remaining part for some reason is not or extremely poorly biodegradable under the conditions prevailing in an EGSB-reactor. This also is an issue that deserves attention in future research. The results in Chapter 4 revealed that lipids are mainly removed by an adsorption (or precipitation)

mechanism; so far insufficient information is available about the degradation rate of this 'matter' and factors which are rate limiting in their degradation. Although lipid concentrations in the range of 0.05-0.100 g/l can be accommodated in an EGSB-system without a clear detrimental effect on the methanogenesis, it remain questionable whether or not we are dealing with a stable process, because the degradation of the accumulating lipids clearly did not proceed satisfactorily. The results in Chapter 3 show that proteins are easily hydrolyzed, but also that non-adapted sludge is unable to convert the products of the hydrolysis process rapidly to methane. This is in fact very similar as the observations made in Chapter 5 with gelatin and sucrose as main constituents of soluble COD. Their biodegradation proceeded slowly and therefore attention should be afforded to the adaptation aspect of granular sludge in order to prevent overloading of the sludge, viz. how to proceed in the adaptation process, how much time is needed, how can the process be speeded up. As found in Chapter 5 for a satisfactory performance of the EGSB-system it is needed, at least very beneficial, to apply a pre-acidification step for both the substrates (protein and carbohydrate). The very clear overall conclusion of the investigations is that the main mechanism for lipids removal comprises either precipitation or adsorption on granular sludge, and that the degradation of lipids proceeds very slowly, particularly of sorbed lipids. Lower lipid concentrations presumably can be accommodated by EGSB-systems, at least for prolonged periods of time. However, when using non-adapted granular sludge for treating complex types of wastewater the system easily passes in situation of serious overloading. With respect to the EGSB-reactor concept, the reactor equipped with a sieve drum GLS-device show a quite promising performed with respect to granular sludge retention when treating complex wastewater. Therefore the EGSB concept certainly offers attractive potentials for full scale application for treating complex types of wastewaters, but in case lipids are present the EGSB-reactor should be combined with a digester for granular sludge recuperation.

The recommendations we want to make on the basis of the observations in our investigations are:

1. For wastewaters containing lipids we recommend to install a first EGSB reactor. When serious flotation of granules occurs, these buoying granules can be conveyed to a parallel EGSB-reactor, which acts as a secondary contact process for sludge recuperation. This second EGSB-reactor is operated at a relatively high hydraulic retention time and optimal temperature. The reconditioned sludge from this second reactor (i.e. after sufficient degradation of the sorbed ingredients) can be returned to the main reactor, if needed with new (fresh) granular sludge. This likely will improve the lipid degradation capacity of the main reactor, e.g. as a result of the specialized consortia which possibly developed in the granular sludge in the second (contact) reactor. The LCFA set free by hydrolysis in the recuperation reactor presumably will be well degraded, particularly when the reactor is operated at optimal temperatures.
2. Complex wastewaters composed of carbohydrates and proteins should be sufficiently pre-acidified in a pre-acidification reactor in order to achieve a stable and efficient performance in the high rate EGSB-system.

3. Always sufficient time should be invested for adaptation of granular sludge when treating a wastewater containing proteins, carbohydrates and lipids in order to achieve stable performance conditions (e,g. no granular sludge deterioration) at sufficient high loading rate.

DISCUSSÃO GERAL

O trabalho descrito nesta tese trata da possiblidade de tratamento anaeróbio de águas residuárias tipo complexas contendo misturas de lipídios, proteínas e carboidratos. Em vista de específicos problemas manifestados em alguns experimentos com componentes misturados, ocasionalmente experimentos individuais (separados) foram executados com soluções contendo apenas um desses componentes. Nas investigações, particular ênfase foi dada para a aplicação do Reator de Leito Granular Expandido (RELGE), porque resultados obtidos em pesquisa anterior indicou que o sistema RELGE pode representar particular promessa para tratamento de águas residuárias contendo lipídios.

O Capítulo 1 fornece um levantamento resumido de literatura com alguns relevantes relatos científicos tratando da produção de águas residuárias complexas e com acessível informação que trata da aplicabilidade de sistemas de tratamento anaeróbio semelhante ao conceito RELGE.

O Capítulo 2 trata da investigação a respeito da degradação de emulsão de gordura de leite usando um reator RELGE em circuito fechado como sistema de tratamento. Os resultados do experimento mostram que a maior parte dos lipídios presentes na emulsão adsorvem nos grânulos de lôdo. Essa fração adsorvida permaneceu sem degradar-se. De fato somente a fração coloidal degradou, embora muito lenta. Por último, poderia ser esperado um valor relativamente menor da taxa de hidrólise, k_h, como de fato foi encontrado, de aproximadamente 0.01 d^{-1}.

O resultado no Capítulo 3 diz respeito a pesquisa realizada com um sistema RELGE equipado com uma peneira cilídrica como dispositivo separador de gás, líquido e sólido. O dispositivo peneira cilídrica separador de gás, líquido e sólido foi investigado para avaliar sua praticabilidade em prevenir o escape de lôdo granular flutuante. Devido a adsorção de lipídios nas partículas de lôdo granular e a pobre degradação desses lipídios adsorvidos, uma forte flutuação de lôdo granular pode predominar no sistema. Consequentemente, o reator necessita portanto ser equipado com um dispositivo adequado para retenção de lôdo. Dois desenhos de peneira cilíndrica foram avaliados no experimento realizado com águas residuárias complexas sintéticas compostas de carboidratos, proteínas e lipídios. Um desses dispositivos separador de gás, líquido e sólido foi capaz de reter lôdo granular flutuante sem prejuízo da estrutura do lôdo granular. Neste experimento foi observado que a maior parte do efluente DQO (Demanda Química de Oxigênio) consiste de matéria solúvel, mais de 85 % da matéria passando numa membrana porosa de 0,45 µm. A hidrólise e/ou acidificação desta fração se realiza muito lentamente. Além disso, um peculiar fenômeno foi observado, queda no conteúdo de DQO solúvel não acidificada no efluente sempre coincide com um aumento no conteúdo de DQO coloidal e vice-versa. A biodegradabilidade desta matéria particular é muito pobre. A deterioração gradual no desempenho do tratamento achada no experimento principalmente pode ser astribuida para a formação desta fração coloidal/solúvel da DQO pobremente biodegradável. Neste Capítulo, os resultados também são apresentados referentes

a experimentos de biodegradação em batelada (durante 2 semanas) para avaliação da biodegradação de diferentes tipos de proteínas, isto é, originadas de batata, milho, leite e ôvo, junto com gelatina e albumina sérica bovina. Os experimentos são realizados com lôdo granular. Embora todas as proteínas investigadas fossem bem deaminada, a conversão para DQO-metano foi relativamente pobre.

A pesquisa presentada no Capítulo 4 trata da aplicação do sistema RELGE para água residuária complexa sintética composta de carboidratos, álcool etílico, proteína (gelatina) e uma emulsão de lipídio de gordura de leite. De forma semelhante como em outros experimentos, as velocidades de ascenção do líquido aplicado equivaleu a 6 m/h. Os reatores foram inoculados com lôdo granular de um Reator Anaeróbio de Fluxo Ascendente de Leito de Lôdo (UASB) em escala industrial. Foi constatado que os poluentes orgânicos da cerveja e gelatina foram bem melhor removidos para uma alta eficiência de remoção de DQO (90-95 %) e também a conversão de DQO para metano foi como esperado, isto é até 85 % para carga orgânica até 12 g DQO/l.d, consequentemente uma taxa de carga orgânica moderada. Nitrogênio da gelatina foi deaminado até 89 %. A presença de lipídios em concentrações até 0.260 g DQO/l prejudicialmente não afetou o desempenho, embora um insignificante decrécimo na produção de metano manifestou-se. A produção de metano recuperou-se depois de 5 dias. Embora a degradação de lipídios não ocorreu satisfatoriamente sob as condições impostas para o sistema, o principal provável mecanismo de remoção de lipídios foi a adsorção e/ou a pecipitação. No experimento conduzido em um dos reatores com apenas cerveja como substrato, uma observação peculiar foi feita com respeito a estabilidade do lôdo granular. Depois de 16 dias de alimentação contínua, o lôdo granular de repente começou a desintegrar-se, um fenômeno que obviamente, não pode ser tolerado em um sistema RELGE. O(s) mecanismo(s) fundamental(ais) deste fenômeno de deterioração do lôdo granular é ainda desconhecido, mas tendo em vista que uma alta estabilidade do lôdo granular é fator de crucial importância para a praticabilidade do sistema RELGE, é essencial continuar a pesquisa neste campo. Em nossos experimentos observamos que a desintegração de lôdo granular cessou quase imediatamente depois do suprimento de gelatina para a alimentação, uma observação que nitidamente merece elucidação.

O Capítulo 5 apresenta os resultados das investigações tratando da aplicabilidade do sistema RELGE para águas residuárias compostas de misturas de gelatina e sacarose. Um lôdo granular não adaptado foi usado no experimento. Os resultados obtidos mais uma vez demonstraram a complexidade do fenômeno, o qual pode ocorrer em um sistema de tratamento anaeróbio sob especificas condições de 'alimentação', como manifestada quando o sistema é alimentado com soluções sem os requeridos macro-nutrientes de crescimento. Sob tais condições pouco ou nenhum crescimento pode ocorrer, embora no caso de ocorrência de degradação de proteína (deaminação) geralmente suficiente anônia é liberada para o crescimento. Realmente, com gelatina como único substrato, 70-90 % de deaminação foi achada para cargas espaciais até 7 g DQO/l.d. Entretanto, sob estas condições a acidificação permanece pobre e consequentemente, a conversão para metano somente alcançou até 65 %. Entretanto, para o mesmo tempo, a avaliada eficiência de Remoção de DQO variou até 85-90

%, indicando que uma fração substancial de gelatina, ou pelo menos intermediários (com respeito a relativa boa deaminação) ou adsorve para a suferfície do lôdo ou precipita no lôdo. A adição de sacarose para a alimentação de gelatina resultou em uma insignificante melhora na deaminação, mas ao mesmo tempo em um adicional declínio da fermentação da gelatina. Quando, entretanto, nutrientes são supridos, isto é, simultanemente com a gelatina e sacarose no tanque de alimentação, uma pré-acidificação de ambos substratos surgiu prontamente no tanque de alimentação. Isto resultou em significativo melhoramento na fermentação da gelatina. Entretanto, quando as soluções de gelatina e de sacarose são introduzidos separadamente no reator, a extensão da fermentação da gelatina mais uma vez caiu abruptamente. Aparentemente, a presença de sacarose não acidificada faz baixar seriamente a fermentação da gelatina. Mas curiosamente permaneceu bastante eficiente a remoção da DQO até 90 % sob estas condições, indicando que o mecanismo de remoção de proteínas originou de mecanismo de adsorção ou precipitação. Embora a eficiência de remoção de DQO permaneceu alta, a adsorção de ingredientes dos substratos - ou possivelmente adsorção de alguns produtos poliméricos formados da gelatina e sacarose (ou possivelmente específicos produtos de degradação intermediária) - resultou na deterioração das características do lôdo granular. Particularmente, a liberação de bolhas de gás dos agregados do lôdo é seriamente dificultada, levando a problemas com flutuação de lôdo. Em consequência da recuperação da pré-acidificação da gelatina e sacarose no tanque de pré-acidificação, o desempenho do sistema melhorou novamente.

A importância científica da investigação resume um número de interessantes observações relacionadas com o fenômeno predominante em processos de tratamento RELGE com respeito ao comportamento do lôdo granular. Estes fenômenos são de grande importância considerando a possibilidade destes sistemas no tratamento de tipos mais complexos de águas residuárias. Eles portanto, merecem séria atenção em futuras pesquisas. Importantes questões a este respeito são as seguintes:

a. O(s) mecanismo(s) predominante(s) em remoção de lipídios, particularmente o processo de adsorção e os fatores controladores da degradação de lipídios adsorbidos. Em vários dos experimentos conduzidos constatamos que uma substancial fração de lipídios foram adsorbidos nos grânulos de lôdo, os quais induzem sérias flutuações de grânulos de lôdo. A flutuação de grânulos pode ser devida a dificuldades na liberação de bolhas de gás mas também pode ser resultado de fortes características flutuantes dos lipídios. Flutuação de lôdo granular pode claramente afetar o desempenho de um reator RELGE, particularmente a retenção de lôdo granular.

b. É nescessário obter mais informação sobre a taxa de hidrólise (k_h=0.01 d^{-1}) dos lipídios dispersados presentes na emulsão de gordura de leite, porque os resultados indicam que a etapa da liquefação dos lipídios da gordura de leite se realiza muito lenta. É de grande importância avaliar se a taxa de liquefação pode ou não ser acentuada, por exemplo impondo altas temperaturas em algum reator adicional de recuperação de lôdo.

c. As razões para deterioração no desempenho do tratamento da DQO de um reator RELGE quando alimentado com uma mistura de gelatina, sacarose e gordura de leite necessita ser

elucidado. Neste aspecto, particular atenção deve ser dada para a pobre biodegradação de 'solúveis' ingredientes da DQO produzidos no sistema, isto é incluindo a natureza e a(s) razão(ões) da formação destes ingredientes. A formação destes ingredientes 'recalcitrantes' é o mais surpreendente, relativamente a melhor biodegradabilidade dos constituintes da alimentação em separados. De particular interesse é também a observação da relativamente fácil conversão de DQO solúvel não acidificada para DQO coloidal e vice-versa.

d. Embora o sistema RELGE seja capaz de tratar uma mistura de gordura de leite, gelatina e cerveja satisfatoriamente melhor, mesmo em presença de concentrações de lipídio até 0.260 g DQO/l, a estabilidade do sistema de tratamento parece questionável. Este situação é particularmente provável no caso de alta concentrações de lipídio; fundamentalmente, sérios problemas operacionais podem se manifestar e, portanto, medidas específicas devem ser desenvolvidas para prevenir problemas semelhantes. Até aqui pouca quantidade de informação segura está disponínivel sobre esta matéria. Como mencionado acima, uma atrativa e possível solução para prevenir semelhantes problemas operacionais deveria ser a colocação de um RELGE combinado com um digestor separado para recuperação do lôdo granular; neste digestor os lipídios adsorvidos nos grânulos tornam-se suficientemente degradados.

e. As razões para a desintegração do lôdo granular manifestada na alimentação do sistema com apenas cerveja diluida. De fato, à primeira vista, um não tão complexo e completamente bem biodegradável substrato solúvel, deveria ser elucidado. E isto também se aplica para a observação que a desintegração cessa quase instantaneamente depois de suprir gelatina para a alimentação.

f. O efeito prejudicial da presença de sacarose na degradação da gelatina necessita elucidação. E também porque concomitantemente uma improvável remoção de DQO foi encontrada. A observação da improvável remoção de DQO possivelmente pode ser atribuida para a adsorção ou processo de precipitação. A pergunta é porque e como.

g. A razão da ocorrência da flutuação de lôdo granular é alimentação quando o sistema é alimentado com soluções contendo gelatina e sacarose como substrato. Indicações foram obtidas de que a flutuação pode ser devido à formação de camadas/filmes de ingredientes (ou intermediários) de substrato adsorvidos na superfície do lôdo. Estas camadas provavelmente obstruem a fácil liberação de gás produzido dentro dos agregados, levando para o conglomeração de bolhas de gás. É necessário mais informação sobre os tipos de ingredientes responsáveis pela formação deste filme.

Reatores RELGE equipados com um dispositivo com peneira cilíndrica separadora de gás, líquido e sólido bem desenhado e situado parece atrativo para tratamento de águas residuárias contendo lipídios em concentrações variando de 0.05-0.100 g/l semelhante a águas residuárias de leiterias. A peneira deve oferecer uma suficiente área superficial aberta para acomodar a relativa carga hidraúlica aplicada no reator RELGE. Embora informação útil fosse obtida prontamente com respeito ao necessário número de aberturas por unidade de área superficial, bem como dimenssões sobre as aberturas, ainda mais pesquisa é necessária nesse campo. Esta particularidade aplica-se com respeito a fatores como alcance da carga superficial, o efeito da composição da água residuária, carga imposta de DQO, temperatura, e

eficiências de tratamento. Contudo as observações feitas em nossas investigações induzem que uma peneira cilídrica tipo dispositivo separador de gás, líquido e sólido representa um completo meio na retenção de grãos de lôdo flutuante em reatores RELGE tratando águas residuárias contendo lipídio. Com respeito a posição da peneira no dispositivo separador de gás, líquido e sólido clara evidência foi obtida que é benéfica situá-la na parte inferior do dispositivo, de modo que no tôpo do dispositivo uma zona é deixada para uma camada de lôdo boiante. Como constatado em pesquisa anterior por Rinzema *et al.*, em nossos laboratórios a velocidade líquida superficial de 6 m/h geralmente aplicada em RELGE fornece um suficiente contato entre substrato e lôdo granular para garantir uma boa degradação dos ácidos cáprico e láurico para uma alta carga orgânica espacial (excedendo 30 kg DQO/m^3.d.). Entretanto, apesar de aparentemente bom contato ainda uma rápida acumulação de camada de lipídios adsorvida poderá ocorrer quando se trata emulsão de gordura de leite mesmo para taxas de carga relativamente moderadas, similarmente como feito por Rinzema nos experimentos com emulsões de triglicerídeos. O resultados do Capítulo 2 revela uma mais pobre biodgradação de gordura de leite sob condições predominantes no reator RELGE. Uma grande quantidade de lipídios é eleminada por adsorção, enquanto a taxa de liquefação da matéria coloidal deixada na fase líquida foi baixa. Somente a parte dos lipídios liquifeita foi encontrada convertida para metano; a parte restante, por alguma razão, não é biodegradável ou é possue uma biodegradação extremamente pobre sob as condições predominantes no reator RELGE. Esta também é uma questão que merece atenção em futuras pesquisas. Os resultados do Capítulo 4 revelaram que lipídios são removidos principalmente por um mecanismo de adsorção (ou precipitação). Até aqui insuficientes informações são disponíveis sobre a taxa de degradação desta 'matéria' e quais fatores são limitantes nas suas degradações. Embora a concentração de lipídios na variação de 0.05-0.100 g/l possa ser ascomodada no sistema RELGE sem um claro efeito prejudicial na metanogênise, isto permanece questionável se nós estamos ou não tratando com um processo estável, porque a degradação dos lipídios acumulados claramente não se efetuou satisfatoriamente. Os resultados no Capítulo 3 mostraram que as proteínas são facilmente hidrolisadas, mas também que o lôdo não adaptado é incapaz para converter rapidamente os produtos do processo de hidrólise para metano. Este fato é muito similar com as observações feitas no Capítulo 5 com gelatina e sacarose como principais constituintes da DQO solúvel. Sua biodegradação procede muito lenta e portanto atenção deve ser dada para o aspecto da adaptação de lôdo granular para prevenir sobrecarga do lôdo, isto é, como proceder no processo de adaptação, quanto tempo é necessário, como o processo pode ser acelerado. Como vimos no Capítulo 5, para um satisfatório desempenho do sistema RELGE é necessário. Pelo menos muito benéfico aplicar uma etapa de pré-acidificação para ambos substratos (proteína e carboidrato). A clara conclusão geral da investigação é que o principal mecanismo para a remoção de lipídios compreende ou precipitação ou adsorção no lôdo granular e que a degradação de lipídios se realiza muito lentamente, particularmente de lipídios adsorvidos. Baixas concentrações de lipídios provavelmente podem ser acomodadas pelo sistema RELGE, pelo menos por prolongados períodos de tempo. Entretanto, quando usam lôdo granular não adaptado para tratamento de

águas residuárias do tipo complexa o sistema facilmente passa por situações de sérias sobrecargas. Com respeito ao conceito de reator RELGE, reatores equipados com dispositivos de peneira cilíndrica separadora de gás, líquido e sólido mostrou uma completa promessa de desempenho com respeito a rentenção de lôdo granular quando tratando águas residuárias complexas. Portanto o conceito RELGE certamente oferece atrativos potenciais para aplicação em grande escala para tratamento de tipos de águas residuárias complexas, mas no caso de lipídios estarem presentes o reator RELGE deve ser combinado com um digestor para recuperação de lôdo granular.

As recomendações que queremos fazer, baseados nas observações de nossa investigação são:

1-Para águas residuárias contendo lipídios recomendamos instalar primeiro um reator RELGE. Quando sérias flutuações de grânulos ocorre, estes grânulos boiantes podem ser transportados para um reator RELGE paralelo, o qual atua como um processo de contato secundário para recuperação de lôdo. Este segundo reator RELGE é operado para um tempo relativamente alto de retenção hidraúlico e ótima temperatura. O lôdo recondicionado do segundo reator (depois da degradação dos ingredentes adsorvidos) pode retornar para o reator principal, se necessario com novo (frêsco) lôdo granular. Isto provavelmente pode aumentar a capacidade de degradação de lipídios do reator principal, por exemplo como um resultado de consórcio especializado qual possibilita desenvolvimento no lôdo granular no segundo reator (contato). Os LCFA liberados pela hidrólise no reator de recuperação provavelmente poderão ser bem degradados, particularmente quando o reator estiver operando para ótimas temperaturas.

2- Águas residuárias complexas compostas de carboidratos e proteínas devem ser suficientemente pré-acidificadas em um reator de acidificação para encontrar um estável e eficiente desempenho em sistema RELGE de alta taxa.

3- Sempre deve ser investido um tempo suficiente para adaptação de lôdo granular no tratamento de águas residuárias contendo proteínas, carboidratos e lipídios no sentido de encontrar condições de desempenho estável (por exemplo não deterioração de lôdo granular) suficiente para suportar altas taxas de carga.

DISCUSSIE

Dit proefschrift beschrijft de resultaten van een onderzoek naar de haalbaarheid van de anaërobe zuivering van vet, eiwit en/of koolhydraat houdend complex afvalwater. Met het oog op specifieke problemen die ontstonden bij sommige experimenten met mengsels van bovengenoemde stoffen zijn een aantal experimenten uitgevoerd waarbij gebruik werd gemaakt van synthetisch afvalwater waarin slechts één van deze componenten was opgelost. De nadruk is in dit onderzoek gelegd op het 'expanded granular sludge bed' (EGSB) systeem omdat uit eerder onderzoek is gebleken dat dit systeem een veelbelovend alternatief vormt voor het zuiveren van vethoudend afvalwater.

Hoofdstuk 1 geeft een overzicht van relevante literatuur betreffende de produktie van complexe industriële afvalwaters en de haalbaarheid van de zuivering van dergelijke afvalwaters met anaërobe zuiveringssystemen zoals het EGSB-systeem.

In hoofdstuk 2 worden de resultaten van ladingsgewijs uitgevoerde recirculatie experimenten besproken waarmee de afbraak van een melk/vet emulsie in een EGSB-systeem is onderzocht. Hierbij is gebleken dat het grootste deel van de vetten in de emulsie adsorberen aan het korrelslib. Het geadsorbeerde deel wordt niet afgebroken. Uit de resultaten bleek tevens dat alleen het colloïdale materiaal in de vloeistof fase werd afgebroken. Deze afbraak verloopt echter zeer langzaam, hetgeen te verwachten was omdat de hydrolyseconstante (k_h) van dit materiaal erg laag is (ongeveer $0.01 d^{-1}$). Verwijdering van vetten berust klaarblijkelijk niet op biologische afbraak maar op een of ander sorptiemechanisme.

In hoofdstuk 3 worden de resultaten van het onderzoek met een EGSB reactor behandeld, de driefasenscheider in deze reactor is gemaakt van een trommelzeef om te onderzoeken of zo het uitspoelen van floterend korrelslib kan worden voorkomen. Door de sorptie van vetten in/aan het korrelslib en de slechte afbraak van deze vetten kan het korrelslib gaan floteren. Om uitspoeling van het van floterend korrelslib te voorkomen is een geschikt systeem voor de slibretentie nodig. De werking van twee typen trommelzeven is onderzocht tijdens experimenten met synthetisch afvalwater bestaand uit koolhydraten, vetten en eiwitten. Eén van deze driefasenscheiders was in staat het floterende korrelslib in de reactor te houden zonder de structuur van het slib aan te tasten. Het grootste deel, tot 85%, van het effluent bestaat uit opgelost CZV; de hydrolyse en verzuring van deze fractie bleek uiterst langzaam te verlopen. Tijdens het onderzoek werd een opmerkelijke relatie tussen de opgeloste niet verzuurde en de colloïdale CZV-fracties waargenomen. Wanneer het niet verzuurd opgelost CZV in het effluent afneemt werd er altijd een toename van het colloïdaal CZV in het effluent waargenomen en omgekeerd. De afbreekbaarheid van deze colloïdale deeltjes is gering. De geleidelijke vermindering van het zuiveringsrendement wordt voornamelijk toegeschreven aan de vorming van deze slecht afbreekbare opgeloste/colloïdale CZV fractie. In dit hoofdstuk zijn tevens de resultaten van twee weken durende ladingsgewijze proeven weergegeven waarmee de biologische afbreekbaarheid van verschillende eiwitten uit aardappelen, maïs, melk, ei en rundvlees is bepaald. De experimenten zijn uitgevoerd met korrelslib. Hoewel de eiwitten in dit onderzoek goed gedeamineerd worden, is de omzetting naar methaan-CZV gering.

Hoofdstuk 4 beschrijft de resultaten van onderzoek naar toepassing van een EGSB-systeem voor het zuiveren van een complex synthetisch afvalwater bestaande uit koolhydraten, ethyl alcohol, eiwit (gelatine) en een melk/vet emulsie. Evenals bij de andere EGSB experimenten werd een opstroomsnelheid van 6 m/h gebruikt, de reactor was geënt met korrelslib uit een praktijk UASB reactor. De organische verontreinigingen in het bier en gelatine afvalwater werden goed verwijderd (90-95 %), ook de CZV omzetting naar methaan (85 %) was zoals verwacht relatief goed bij de toegepaste matige organische belasting van 12 g CZV/l. d. De Gelatine-N werd voor 86-89 % gedeamineerd. De aanwezigheid van vetten in concentraties van 0.260 g CZV/l had geen nadelig effect op de prestaties van de reactor wel werd een tijdelijke vermindering van de methaanproductie waargenomen welke na 5 dagen herstelde. De afbraak van vetten was niet voldoende, onder deze omstandigheden is adsorptie en/of precipitatie waarschijnlijk het belangrijkste verwijderingsmechanisme. Bij experimenten met bier als substraat begon na 16 dagen continue voeden het korrelslib te desintegreren, hetgeen niet wenselijk is in een EGSB-syteem. Het mechanisme dat deze desintegratie veroorzaakt is niet bekend. Gezien het feit dat stabiel korrelslib essentieel is voor het kunnen toepassen van een EGSB-systeem is verder onderzoek naar de oorzaak van deze desintegratie van belang. De desintegratie stopt nagenoeg onmiddellijk wanneer gelatine aan het influent wordt toegevoegd, deze waarneming vraagt tevens om opheldering.

In hoofdstuk 5 worden de resultaten van onderzoek naar de toepassing van het EGSB-systeem voor de behandeling afvalwater dat bestaat uit mengsel van gelatine en sucrose behandeld. Voor dit onderzoek is niet geadapteerd slib gebruikt. De resultaten laten nogmaals zien hoe complex de processen zijn die zich kunnen voordoen bij anaërobe behandeling van een bepaald influent onder bepaalde condities. Condities zoals deze zich bijvoorbeeld voordoen in een systeem dat wordt gevoed met een oplossing welke niet de benodigde macro-nutriënten bevat. Onder dergelijke condities vindt nauwelijks groei van bacteriën plaats, de afbraak van eiwitten (deaminatie) gaat wel door en over het algemeen wordt voldoende ammonia vrijgemaakt voor bacteriegroei. Bij dit onderzoek werd met alleen gelatine als substraat bij volume belastingen tot 7 g CZV/l.d inderdaad een deaminatie van 70-90 % gevonden. Onder deze omstandigheden blijft de verzuring beperkt en als gevolg hiervan ook de vorming van methaan (in dit onderzoek tot 65%). Tegelijkertijd werd een CZV verwijdering van 85-90 % gevonden, hetgeen erop wijst dat een substantieel deel van de gelatine, of de intermediairen hiervan (met het oog op de relatief goede deaminatie), door sorptie mechanismen worden gebonden aan het slib oppervlak of precipiteren in het slib. Toevoegen van sucrose aan het gelatine influent resulteert in een iets betere deaminatie maar tegelijkertijd in een slechtere vergisting van de gelatine. Indien samen met de gelatine en sucrose nutriënten worden toegevoegd vindt in het influentvat voorverzuring plaats, hetgeen resulteert in een duidelijk verbeterde vergisting van gelatine. Daarentegen wanneer de gelatine en sucrose apart in de reactor worden gebracht vermindert de vergisting van gelatine sterk. Het niet verzuurde sucrose remt klaarblijkelijk de vergisting van gelatine. Het is opmerkelijk dat de CZV verwijdering onder deze omstandigheden 90 % blijft, hetgeen aangeeft dat eiwit wordt verwijderd door sorptie of precipitatie. Hoewel het CZV verwijderingsrendement hoog blijft verslechtert de sorptie van het substraat, of de polymeren die mogelijk worden gevormd uit sucrose en gelatine (of mogelijk speficfieke intermediairen die ontstaan bij de afbraak), de slibeigenschappen. Het ontsnappen van het

geproduceerde gas uit de slibkorrels werd belemmerd waardoor het slib ging floteren. Door de voorverzuring van gelatine en sucrose in de voorverzuringstank te herstellen verbeterden de prestaties van de reactor opnieuw.

Het wetenschappelijke belang van dit onderzoek bestaat uit een aantal interessante verschijnselen die zijn waargenomen in het EGSB-systeem m.b.t. het gedrag van korrelslib. Deze verschijnselen zijn met het oog op de toepasbaarheid van dergelijke systemen bij het behandelen van complexere afvalwaters van groot belang, en daarom is nader onderzoek op dit gebied zeer gewenst. Belangrijke aandachtspunten voor nader onderzoek zijn:

a. De verwijderingsmechanismen bij de behandeling van vethoudend afvalwater, met name adsorptie processen en de factoren die een rol spelen bij de afbraak van aan het slib gebonden vetten. Uit verschillende experimenten tijdens dit onderzoek bleek dat een belangrijk deel van de vetten in de melk/vet emulsie door sorptie processen gebonden wordt in/aan de slibkorrels waardoor ernstige flotatie van de slibkorrels optreedt. Doordat de afgifte van gas uit de korrels wordt belemmerd kan flotatie optreden. Tevens kan flotatie onstaan doordat vet de neiging heeft te drijven op water. Het floteren van het korrelslib kan de prestaties van de reactor, en met name de slibretentie, negatief beïnvloeden.

b. Meer gegevens over de hydrolysesnelheid (k_h= 0.01 d^{-1}) van gedispergeerde vetten in de melk/vet emulsie zijn nodig, omdat de resultaten suggereren dat de vervloeingsstap van melkvet zeer langzaam verloopt. Het is daarom van belang te onderzoeken of de hydrolyse van vetten versneld kan worden, bijvoorbeeld door het verhogen van de temperatuur in een aan het systeem toegevoegde vergistingsreactor waarin het korrelslib kan herstellen.

c. Voor het verminderde CZV verwijderingsrendement van een EGSB reactor wanneer deze wordt gevoed met een mengsel van gelatine, sucrose en melkvet moet een verklaring worden gevonden. Hierbij zou extra aandacht besteed moeten worden aan de slechte biologisch afbreekbaarheid van het in de reactor gevormde opgelost CZV en met name de aard van, en de reden voor, de vorming van deze stoffen. Het onstaan van deze moeilijk afbreekbare stoffen is opmerkelijk met het oog op de goede biologische afbreekbaarheid van de afzonderlijk influent componenten. Bijzonder interessant is de waargenomen relatief gemakkelijke omzetting van niet verzuurd opgelost CZV in colloïdaal CZV en omgekeerd.

d. Hoewel het EGSB-systeem zelfs bij een vetconcentratie van 0.260 g CZV/l in staat bleek een mengsel van melkvet, gelatine en bier voldoende te zuiveren, bestaan er twijfels over de stabiliteit van het systeem. Vooral bij hoge vetconcentraties kunnen problemen ontstaan, het is daarom van belang maatregelen te nemen om dit te voorkomen. Tot dusver zijn er weinig kwantitatieve gegevens hierover beschikbaar. Een mogelijke oplossing is het toepassen van een aparte vergister naast het EGSB-systeem, waarin het korrelslib de gelegenheid krijgt zich te herstellen. In deze vergister kunnen de aan/in het korrelslib geadsorbeerde vetten in voldoende mate worden afgebroken.

e. De desintegratie van het slib welke optreedt wanneer de reactor met verdund bier wordt gevoed, hetgeen op het eerste gezicht een substraat is dat goed biologisch afbreekbaar is, moet worden verklaard. Dit geldt tevens voor het onmiddellijke stoppen van deze desintegratie nadat gelatine wordt toegevoegd aan het influent.

f. Het nadelige effect van sucrose op de afbraak van gelatine en de reden(en) waarom tegelijkertijd een hogere CZV verwijdering wordt gevonden moet verder worden onderzocht. Deze verbeterde CZV verwijdering wordt mogelijk veroorzaakt door een sorptie of precipitatie proces, de vraag is waarom en hoe.

g. De oorzaken van korrelslibflotatie bij de behandeling van synthetisch afvalwater met gelatine en sucrose als substraat verdient nader onderzoek. Er zijn aanwijzingen dat flotatie veroorzaakt wordt door de vorming van lagen/films van geadsorbeerd substraat (of intermediairen) op het sliboppervlak. Deze lagen verhinderen waarschijnlijk dat de in het korrelslib geproduceerde gas gemakkelijk kan ontsnappen, met als gevolg insluiting van gasbellen in de korrels. Naar de aard van de stoffen welke deze lagen/films veroorzaken zou nader onderzoek moeten worden verricht.

Een EGSB reactor uitgerust met een goed ontworpen en correct geplaatste trommelzeef driefasenscheider lijkt aantrekkelijk voor de behandeling van vethoudend afvalwater met vetconcentraties van 0,05 tot 0,100 g/l., zoals bijvoorbeeld in het afvalwater van diverse zuivelindustrieƷn. Het 'filter' gedeelte van de driefasenscheider moet voldoende open zijn om de relatief hoge hydraulische belasting in een EGSB reactor te kunnen verwerken. Hoewel er inmiddels bruikbare informatie is over de afmetingen en het aantal openingen in het filter is extra onderzoek op dit gebied noodzakelijk. Het gaat dan vooral om het effect van factoren als de oppervlakte belasting, samenstelling van het afvalwater, toegepaste CZV belasting, temperatuur en het verwijderingsrendement. Uit dit onderzoek blijkt dat een trommelzeef driefasenscheider een veelbelovende manier is om de floterende slibkorrels die ontstaan bij de behandeling van vethoudend afvalwater in de EGSB reactor te houden. De zeef kan het best in het onderste gedeelte van driefasenscheider worden geplaatst zodat bovenin de driefasenscheider een gedeelte overblijft voor een laag drijvende slibkorrels. Uit onderzoek gedaan door Rinzema *et al.* is gebleken dat bij de opstroomsnelheid van 6 m/h, zoals deze over het algemeen wordt toegepast in EGSB-systemen, het kontakt tussen de slibkorrels en het substraat voldoende is om de afbraak van caprinezuur en laurierzuur bij hoge volumebelastingen (hoger dan 30 kg CZV/m^3.d) mogelijk te maken. Wanneer de reactor matig wordt belast met een melk/vet emulsie wordt ondanks het goede contact tussen het substraat en het slib snel een geadsorbeerde vetlaag rond de slibkorrels gevormd. Deze vetlaag is vergelijkbaar met de vetlaag die werd gevormd tijdens het onderzoek van Rinzema met een mengsel van triglycerides. Uit de resultaten in hoofdstuk 2 blijkt dat melkvet slecht afbreekbaar is onder EGSB condities. Een groot deel van de vetten wordt verwijderd door adsorptie, terwijl de hydrolysesnelheid van het colloïdale materiaal in de vloeistof fase laag is. Een klein deel van de gehydrolyseerde vetten wordt omgezet in methaan, het resterende deel is onder EGSB condities niet of slecht biologisch afbreekbaar. Dit is tevens een onderwerp voor verder onderzoek. De resultaten van hoofdstuk 4 laten zien dat vetten voornamelijk door adsorptie (of precipitatie) worden verwijderd, tot dusver is er te weinig bekend over de afbraaksnelheid van dit geadsorbeerde materiaal en de factoren die de afbraak remmen. Hoewel de verwijdering van vet in een EGSB-systeem bij concentraties van 0.05 tot 0.100 g/l zonder nadelig effect op de methanogenese kan plaatsvinden, blijft het echter onduidelijk of het hier gaat om een stabiel proces, dit omdat de afbraak van de geaccumuleerde vetten niet goed verloopt. De resultaten uit hoofdstuk 3 laten zien dat eiwitten gemakkelijk gehydrolyseerd kunnen worden, niet geadapteerd slib kan de hydrolyse

producten echter niet snel omzetten in methaan. Dit komt sterk overeen met de bevindingen in hoofdtuk 5 waar sucrose en gelatine de belangrijkste bestanddelen van het opgeloste CZV vormden, de afbraak van hiervan verloopt zeer langzaam. Het is van belang inzicht te krijgen in de adaptatie van korrelslib zodat overbelasting van het slib kan worden voorkomen. Met andere woorden hoe kan slib worden geadapteerd, hoeveel tijd is hiervoor nodig en hoe kan dit proces worden versneld. Voor bevredigende prestaties van het EGSB-systeem is het, zoals in hoofsdtuk 5 bleek, noodzakelijk of in ieder geval voordelig om beide substraten (koolhydraat en eiwit) voor te verzuren. De hoofdconclusie van het onderzoek is dat adsorptie of precipitatie aan het korrelslib het belangrijkste mechanisme is voor de vetverwijdering en dat de afbraak van de vetten, met name de geadsorbeerde vetten, zeer langzaam verloopt. Afvalwater met een lage vetconcentratie kan waarschijnlijk gedurende langere tijd behandeld worden in een EGSB reactor. Indien echter gebruik wordt gemaakt van niet geadapteerd slib kan het systeem gemakkelijk overbelast raken. Bij het behandelen van complexe afvalwater in EGSB reactoren, is met het oog op de slibretentie een EGSB reactor uitgerust met een trommelzeef driefasenscheider een veelbelovend alternatief. Het EGSB concept is hierdoor zeker aantrekkelijk voor het op praktijkschaal zuiveren van complex afvalwater. In het geval van de behandeling van vethoudend afvalwater zal een vergister voor het herstel van de korrelslibactiviteit aan het systeem moeten worden toegevoegd.

Aanbevelingen op basis van dit onderzoek zijn:

1. Voor het behandelen van vethoudend afvalwater wordt een EGSB-systeem aanbevolen. Indien ernstige korrelslibflotatie optreedt zullen de floterende slibkorrels naar een tweede, parallelle EGSB reactor moeten worden gebracht welke zal dienen als secundair contact proces waarin het slib zich kan herstellen. Deze tweede EGSB reactor wordt bedreven bij een relatief lange hydrausiche verblijftijd en een optimale temperatuur. Het herstelde slib uit de tweede reactor (d.w.z. na voldoende degradatie van het geadsorbeerde materiaal) kan, indien nodig met nieuw korrelslib, worden teruggevoerd in de reactor. Mogelijk wordt de vetverwijderingscapaciteit van het slib van de eerste reactor verbeterd als gevolg van de ingroei van bacteriën in het slib van de tweede (contact) reactor welke beter in staat zijn het vet af te breken. De hogere vetzuren die vrijkomen bij de afbraak in de tweede reactor worden waarschijnlijk goed omgezet, vooral wanneer de reactor bij een optimale temperatuur wordt bedreven.
2. Om een hoog belast EGSB-systeem stabiel en efficiënt te kunnen bedrijven met complex afvalwater bestaande uit eiwitten en koolhydraten moet het afvalwater voldoende zijn voorverzuurd, bijv. d.m.v. toepassing van een verzuringsreactor.
3. Bij de behandeling van afvalwater met eiwitten en koolhydraten moet voldoende tijd voor de adaptatie van het slib worden uitgetrokken om de reactor bij een voldoende hoge belasting stabiel (zonder dat de kwaliteit van het slib achteruit gaat) te kunnen bedrijven.

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