ANAEROBIC TREATMENT OF SLAUGHTERHOUSE WASTEWATER USING THE UASB PROCESS

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee,
in het openbaar te verdedigen
op vrijdag 11 december 1987
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen.

tellingen

. Bij de anaerobe zuivering in UASB reactoren van afvalwater dat hoge gehalten aan gesuspendeerde materiaal bevat is het niet zozeer de afbreekbaarheid van dit gesuspendeerd materiaal, die de belastbaarheid en de zuiveringsrendementen van het systeem bepaalt, als wel de concentraties en de verwijderingsmechanismen ervan.

Dit proefschrift.

2. De oplossing van de lichte slib problematiek in aerobe zuiveringsinstallaties gaat door een contact tank.

Rensink, J.H., Donker, H.J.G.W. & IJwema, T.S.J. (1982). Licht slib; voorkomen of bestrijden? H_2O, **10**, 241-245.

3. Bij het berekenen van de CZV afname beschouwen Nanninga & Gottschal verdunning ten onrechte als een vorm van zuivering.

Nanninga, H.J. & Gottschal, J.C. (1986). Anaerobic purification of waste water from a potato-starch producing factory. Water Research, **20**, (1), 97-103.

4. Op grond van de wet van behoud van ellende kan worden voorspeld dat massale toepassing van P-vrije wasmiddelen een nieuw milieuprobleem zullen veroorzaken.

5. De toepassing van het aktief slibproces voor de zuivering van huishoudelijk afvalwater volgens het A/B-proces leidt niet tot de gewenste resultaten die in de literatuur worden gesuggereerd.

Böhnke, B. (1978). Möglichkeiten der Abwasserreinigung durch das 'Adsorptions-Belebungsverfahren' - Verfahrenssystematik, Versuchsergebnisse - Schriftenreihe des Instituts für Siedlungswasserwissenschaft der RWTH Aachen, **25**, 437-465.

6. De verontrusting op het gebied van de bodemverontreiniging neemt toe met de afname van de detectiegrenzen.

7. Bemonsteringen van fytoplankton met planktonnetten (30 micro maaswijdte) kan een verlies geven van 80% van zowel de biomass als het soortenaantal.

Roijackers, R.M.M., 1985. Phytoplankton studies in a nymphaeid-dominated system. Ph.D. Thesis, Nijmegen

8. In de discussie over normstelling ten aanzien van desinfectie bi de lozing van rwzi's dient in aanmerking te worden genomen da er bij RWA meestal toch niet gezwommen wordt.

9. Het door Jannasch gestelde geval: 'The concentration of the growth-limiting substrate permits the inoculated population to double within one retention time and a steady state will be established' kan eenvoudig tot een niet beoogde evenwichtssituatie aanleiding geven.

Jannasch, H.W., (1969). Estimation of bacterial growth rates in natural waters. J. Bacteriol, **99**, 156-160.

10. De bedenkelijke kwaliteit van het afvalwater van slachthuizen kan worden opgevat als een argument voor het vegetarisme.

Dit proefschrift.

Stellingen behorende bij het proefschrift 'Anaerobic treatment of slaughterhouse wastewater using the UASB process' van Sameh K.I. Sayed.

Wageningen, 11 december 1987.

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VOORWOORD

Bij de totstandkoming van dit proefschrift wil ik gaarne allen bedanken die hieraan een bijdrage hebben geleverd.

Allereerst wil ik Bert Lijklema en Gatze Lettinga bedanken voor de mij geboden gelegenheid om het onderzoek af te ronden met een promotie.

Beste Gatze, ik ben je bijzonder erkentelijk voor je constructieve en stimulerende begeleiding, wat erin geresulteerd heeft dat het onderzoek in de juiste banen werd geleid.

Beste Willem de Zeeuw, hartelijk dank dat je - ondanks je drukke werkzaamheden - tijd wist te vinden voor talrijke discussies en waardevolle adviezen.

Lies van Campen, Jos van der Zanden en René Wijffels wil ik gaarne dank zeggen voor hun bijdrage die zij tijdens hun doctoraalonderzoek aan dit proefschrift hebben geleverd.

Arja Span ben ik veel dank verschuldigd voor haar onmisbare bijdrage op het gebied van de statistiek.

De nauwgezette wijze waarop Katja Grolle het gehele manuscript tekstueel heeft doorgenomen, heb ik zeer gewaardeerd.

Verder gaat mijn dank uit naar de heren Rienk van der Vegt en Willem Ekeris van de Technische Dienst Stroomberg, voor hun technische assistentie bij de bedrijfsvoering van de proefinstallatie in Ede.

Mijn bijzondere waardering gaat uit naar de Centrale Dienst Biotechnion, naar de heren C. Rijpma en M. Schimmel voor hun tekenwerk, en naar de heer W. Hof voor zijn reproduktiewerk.

Alle medewerk(st)ers van de vakgroep waterzuivering wil ik hartelijk bedanken voor de prettige samenwerking gedurende mijn onderzoeksperiode.

Beste Stiny, hartelijk dank voor je hulp, steun en enthousiasme.

Sameh

CHAPTER 1

INTRODUCTION

Wastewater characteristics and the pollution potential of the waste.

According to a survey paper by Ten Have (1976) concerning slaughterhouses and meat and poultry processing plants, it appears that the pollution capacity of these industries in The Netherlands exceeds 1,000,000 population equivalents. Slaughterhouses generally produce lower effluent and pollutant quantities per ton of liveweight killed (LWK) than meat packing plants, which perform more meat handling operations. Poultry processing plants usually generate greater pollutant and effluent quantities per ton LWK than either slaughterhouses or meat processing plants (Table 1). Wastewater from slaughterhouses and meat poultry processing plants varies widely in composition, in strength and in flow. Differences in the wastewater characteristics and quantity can be primarily attributed to differences in processing activities, animal species, employee habits and wastewater management.

Table 1. Slaughtering capacity of the various classes of animals and the pollution potential of the wastewater, according to Ten Have (1976).

classes	LWK [a] tons per year	P.E. [b] per ton LWK	Total P.E. [b]
pigs	116400	0.3	349000
cattle	500000	0.5	250000
calves	110000	0.2	22000
poultry	370000	0.7	259000
meat packing	245000	0.6	147000
Total			1027000

a. LWK liveweight killed .

b. PE population equivalents.

Slaughterhouse wastewater is a moderate to low strength complex-type wastewater. Of the pollutants, 40 - 50% are present as insoluble slowly biodegradable suspended solids (Table 2). These solids originate from the lipid and protein materials present in the wastewater and may also include lignocellulosic substances and bacterial cell walls if manure is a part of the wastewater. The complex suspended solids are therefore composed of polymeric substrate that must first undergo hydrolysis to become soluble (liquefaction) before they become available for biological consumption.

Table 2. The most important characteristics of the slaughterhouse wastewater.

COD_{total}	1500 - 2200 mg $litre^{-1}$
coarse suspended solids as a percentage of COD_{total}	40 - 50 %
BOD_{total}	490 - 650 mg $litre^{-1}$
VFA-COD (% of total COD)	12 - 15 %
pH	6.8 - 7.1

The slaughterhouse wastewater is comparable to other complex wastewaters like municipal wastewater and dilute manure effluents for their high fraction of slowly biodegradable suspended solids.

Wastewater treatment methods

- **Primary and secondary treatment.**

Most of the slaughterhouses and meat and poultry industries in The Netherlands discharge their wastewater to a municipal treatment system. In order to comply with water pollution control standards and to reduce costs on sewer surcharges, these industries have to apply an adequate primary and secondary treatment of their wastewater.

Primary treatment by physical and/or chemical methods is applied in these industries (Funke 1969). Frequently, for pretreating the wastes a combination of screening with static and vibrating screens, centrifugation, hydrocyclones, sedimentation, flocculation, precipitation and air flotation for grease recovery, is used (Witherow & Lammers 1976).

As secondary treatment processes for the bacterial decomposition of the organic pollutants and nitrogen removal have been investigated and applied in these industries. The biological processes applied include the conventional anaerobic processes, viz. a completely mixed tank system (Black & Brown 1974), the anaerobic contact process (Schroepfer et al. 1955), anaerobic ponds (Rotlag and Dornbush 1966, Oswald 1964) and the aerobic ponds (Steffen 1961). Combinations of these systems are required in case of discharge of the effluent to surface water since generally no single secondary treatment process will provide sufficient effluent.

All these secondary treatment methods have disadvantages. They are low rate treatment systems, and consequently, the area of land required is large. Investment and operating costs are high and odour nuisance problems are unavoidable, particularly with the anaerobic ponds.

- **High rate systems for anaerobic treatment.**

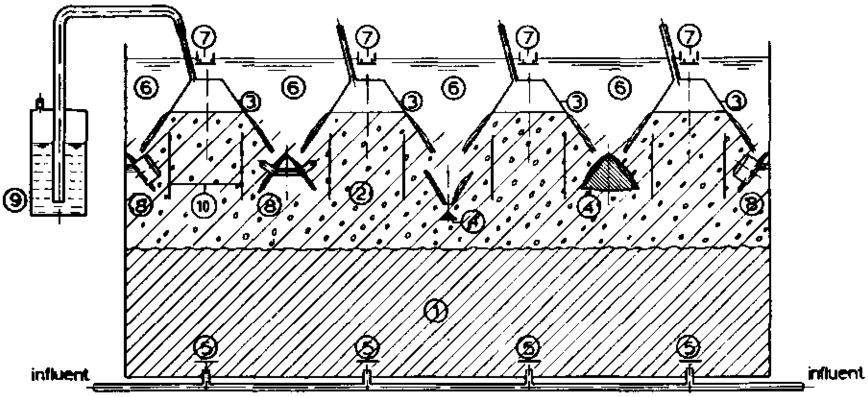
There is a considerable need for inexpensive, efficient high rate wastewater treatment systems. Therefore, efforts have been made in recent years towards the development of high rate anaerobic treatment systems. The important advantages of anaerobic wastewater treatment are that it couples the degradation of organic materials from waste with the production of energy in the form of methane, while the excess sludge production is low compared to aerobic processes. The excess sludge is highly stabilized and generally its dewaterability is excellent. Costs for aeration are eliminated, because oxygen is not required during the anaerobic treatment process as is the case with aerobic processes.

Modern high rate anaerobic treatment systems developed include the anaerobic filter (Young & McCarty 1969), the downflow stationary fixed film reactor (Van den Berg and Lentz 1979), the anaerobic attached film expanded bed (Switzenbaum and Jewell 1980), the fluidized bed reactor (Heijnen 1983, Jeris 1983). All these systems employ carrier materials for preventing biomass washout, i.e. they use bacterial attachment or entrapment of bacterial aggregates between packing materials.

The upflow anaerobic sludge blanket (UASB) process (Lettinga et al. 1980) uses no carrier material. The UASB process (Figure 1) does not apply a separate external settler.

All high rate anaerobic treatment processes are based on the achievement of a high retention of viable biomass while also a good contact between incoming wastewater with the sludge should be achieved. The importance of high rate anaerobic treatment systems for practice is considerable, because:

1. very high organic loading rates can be applied at optimal temperatures and for mainly soluble wastewaters, consequently small reactor volumes suffice;
2. unless designed at their maximum loading potentials, the stability of high rate systems to suboptimal conditions (lower temperatures, shock loads, presence of inhibitory compounds) is high,
3. they make anaerobic treatment economically feasible at low ambient temperature and for very low strength wastes as well.



1. sludge bed
2. bulk of the liquid with dispersed sludge forming a 'blanket'
3. gas bowl
4. gas seal
5. feed-inlet distribution system
6. settler
7. effluent launder
8. gas collector with exhaust pipe to (3)
9. water seal

Figure 1. Schematic diagram of a full scale UASB reactor.

Generally a mechanical mixing device is not installed in a UASB reactor. Usually the 'natural' mixing caused by the gas production is sufficient, provided the digestion process proceeds satisfactorily and the organic loading rates are sufficiently high. A gas-solids separator is installed in the most upper part of the reactor for collecting the produced biogas and for retaining settleable bacterial sludge aggregates. Generally, biomass aggregates with a sufficiently high settling velocity in relation to the average superficial velocity of wastewater will be retained in the reactor.

Applicability of high rate anaerobic systems for the treatment of slaughterhouse wastewater.

The high rate anaerobic systems were initially developed for the treatment of mainly soluble low and medium strength wastewaters and therefore, provide only a partial treatment in the case of complex wastes (i.e. partially soluble wastes), containing a high fraction of biodegradable suspended solids, such in the case of slaughterhouse wastewater.

The successful application of a UASB system for agro-industrial wastewater treatment has shifted the investigations towards more difficult to handle, complex wastewater, for instance those containing relatively high concentrations of biodegradable suspended solids. Domestic sewage wastewater, although containing a low concentration of COD, has a high fraction of suspended solids. Still, the UASB process seems well suited for the treatment of these wastewaters, as long as the temperature exceeds 12 °C (de Man et al. 1986, Grin 1985). From these studies the accumulation of slowly biodegradable suspended solids during the treatment of this complex wastewater in the UASB reactors particularly, those operating with flocculent sludge as seed material can be considered as an advantage because such solids accumulation will result in stabilization of the solids during the long retention time in the reactor.

Therefore, in the case of slaughterhouse wastewater the UASB-process can provide a complete treatment of both soluble and suspended solids fractions of the waste. Table 3 summarizes the results obtained with UASB reactors treating various types of complex wastewater.

Table 3. Results obtained with UASB-reactors treating various types of complex wastewater.

Type of wastewater		COD_{total} concentration ($kg\ m^{-3}$)	coarse suspended solids (% of COD_{total})	seed sludge inoculum	loading rate kg COD $m^{-3} day^{-1}$	COD removal (%)		Ref. [a]
						COD_{total}	$COD_{filt.}$	
Domestic sewage	(20 °C)	0.65	35 - 45	flocculent	2.0	60 - 70	40 - 80	1
Raw sewage	(20 °C)	0.55	55 - 65	granular	2.7	54 - 72	60 - 89	1
Liquid calf manure	(30 °C)	9.5	60	flocculent	4.0	90	93	2
Rendering wastewater	(30 °C)	3.5	25	granular	25 - 60	63	80	3

a. 1: Grin et al. 1983, 2: van Velsen et al. (1979), 3: Zeeuw, W.J. de. (1982).

The purpose of this study was to appraise the feasibility of the UASB process for complete treatment of slaughterhouse wastewater.

Problems expected in treating complex wastewaters

An important consequence of treating complex wastewaters (i.e. partially insoluble) is, that a significant decrease of the methanogenic capacity of the treatment system will occur due to the entrapment of non-biomass coarse suspended solids from the wastewater in the sludge. Such inert suspended solids entrapment will result in a dilution effect of the active biomass and consequently this may result ultimately in an severe decrease in the methanogenic bacterial concentration in the sludge. This phenomenon was observed with municipal wastewater (de Man 1986).

Adsorption of finely dispersed suspended solids (i.e. more or less colloidal organic matter) to the surface of the sludge may also lead to a significant decrease in the methanogenic capacity of the system, because it may lead to an entrapment of the sludge bacterial matter by a film of increasing thickness, which increasingly will hamper the supply of the substrate to the bacteria present in the sludge aggregate. This may ultimately result in a complete deterioration of the specific methanogenic activity of the sludge. The occurrence of adsorption of dispersed suspended solids was reported by Lettinga et al. 1983 and Grin et al. 1985 in experiments performed with raw domestic sewage.

The extent of the deterioration of the specific methanogenic activity of the sludge due to the entrapment of coarse suspended solids and the adsorption of the dispersed suspended solids as well will strongly depend on the biodegrability of these organic solids. The poorer the biodegrability of these organic solids, obviously the less complete will be the liquefaction of the biodegradable fraction of the organic solids, and consequently the greater the deterioration of the specific methanogenic activity of the sludge.

Another problem in treating complex wastewater may represent the continuous formation of a scum layer at the liquid air interface in the settler compartment. Lettinga et al. 1983 reported a distinct scum layer formation in experiments performed with raw sewage, particularly at lower ambient temperatures and higher loading rates. This scum layer contained a considerable fraction of lipids. It was concluded that the scum layer formation presumably depends on the concentration of compounds which easily float, such as lipids present in the wastewater.

Some aspects of the anaerobic digestion process

Anaerobic digestion is a biological process in which organic materials are converted into methane and carbon dioxide in the absence of oxygen. This process is widely used for the purification (stabilization) of wastes containing a high concentration of biodegradable organics, i.e. domestic sewage sludge, manure and to some extent also for high strength wastes from various industries.

Anaerobic digestion of organic materials to methane is brought about by the combined action of a wide variety of bacteria. According to the present knowledge, the complete degradation of complex organic matter to methane proceeds according to the multistep scheme depicted in Figure 2 (Gujer & Zehnder 1983).

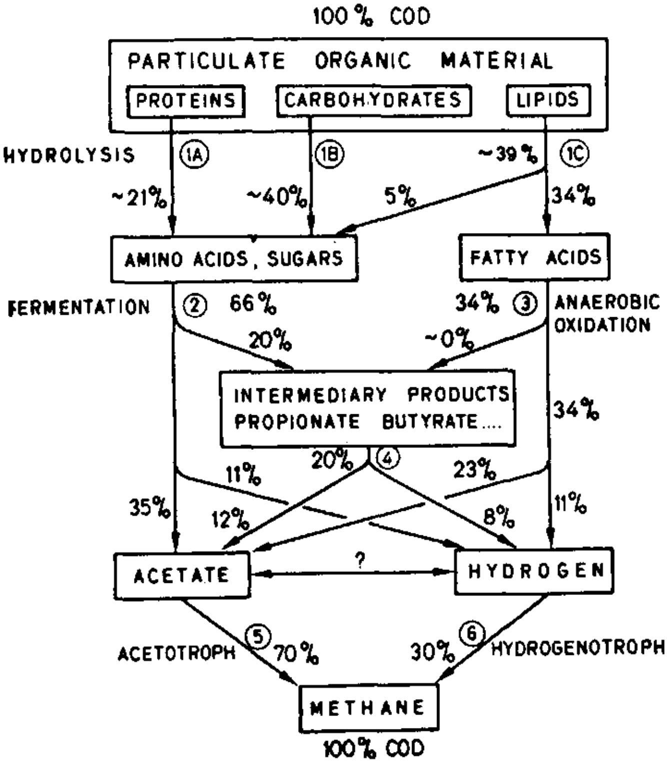


Figure 2. Four stages scheme for the anaerobic digestion.

Six processes are distinguished here, each of which is linked with part of the flow of the total carbon from the organic matter to methane:

1. hydrolysis of polymers (proteins, carbonhydrates and lipids),
2. fermentation of amino acids and sugars,
3. anaerobic oxidation of long-chain fatty acids,
4. anaerobic oxidation of volatile fatty acids to acetate,
5. formation of methane from acetate,
6. formation of methane from hydrogen and carbon dioxide.

However, the degradation process is not a sequence of independent reactions, but it is characterized by a complex of mutual interactions between different microbial species (Bryant 1979). These processes can be grouped into four stages which proceed consecutively during the anaerobic digestion of complex material, viz. hydrolysis, acidogenesis, acetogenesis and methanogenesis.

Hydrolysis

In general bacteria are unable to take up particulate organic material and therefore these organic pollutants first have to be liquefied into soluble polymers or monomers with low molecular weight which can cross the cell barrier. The liquefaction is the first step required for the microbial utilization of complex biopolymers.

The liquefaction of the complex biopolymers occurs through the action of exo-enzymes of the hydrolytic bacteria. According to the type of action of exo-enzymes hydrolytic bacteria isolated from anaerobic digesters as well as from rumen populations are classified in lipolytic, proteolytic and cellylytic bacteria (Hobson & Wallace 1982; Zeikus 1980, McInerney 1985, Gijzen 1987). Hydrolytic bacteria are generally associated with the insoluble substrate. Estimates of 10^8 and 10^9 hydrolytic bacteria per ml have been reported in mesophilic digestion of sewage sludge (Mah & Sussman 1967, Toerien & Siebert 1967).

In the digestion of complex wastes containing high amounts of insoluble substrate, such as liquid calf-fattening manure (Schomaker et al. 1986), piggery wastes (van Velsen 1981), packing house wastes (Schroepfer et al. 1955) and cellulosic waste (Gijzen 1987), the liquefaction step frequently is reported as being the rate limiting step in the overall process.

The rate of liquefaction is determined by the chemical composition of the substrate (i.e. the biodegrability of the substrate) and physical nature (e.g.

the size and porosity of the separate particles, viz. the accessibility for enzymes (Lin et al. 1985, Hungate et al. 1970) as well as of environmental factors such as particularly the temperature (O'Rourke 1968, Golueke 1958, Malina 1961, Pfeffer 1974).

Acidogenesis

The products of the hydrolysis viz.the long-chain fatty acids, amino acids, sugars and alcohols are metabolized intracellularly by hydrolytic and non-hydrolytic bacteria. The main end-products of acidogenesis by mixed cultures are acetate, propionate, butyrate and H_2/CO_2 (McInerney & Bryant 1981). Minor amounts of formate, lactate, valerate, methanol, ethanol, butanediol or acetone may be produced by fermentative bacteria. Since volatile fatty acids (VFA) are the main products of these bacteria, they are usually designated as acidifying or acidogenic bacteria. Acidogenic bacteria are resistant to low pH values, i.e. the formation of acids can proceed at pH values as low as pH 4.

Acetogenesis

The hydrogen-producing acetogenic bacteria are responsible for the oxidation of the end-products of the acidogenic stage to substrates suitable for methanogenesis (Bryant 1979, McInerney et al. 1979). Since the oxidation reactions of the H_2-producing acetogenic bacteria are thermodynamically unfavourable unless the partial pressure of hydrogen (PH_2) is kept below 10^{-3} atm., as illustrated by Gujer & Zehnder (1983), these bacteria are obligatorily coupled to hydrogen-utilizing bacteria such as methanogens and sulphate reducing bacteria (Hatchikian 1976, McInerney et al. 1981). The close partnership between hydrogen producing and hydrogen consuming species in synthropic association is termed 'interspecies hydrogen transfer'. McInerney et al. (1981) reported interspecies hydrogen transfer reactions in the methane formation from propionate and long-chain fatty acids.

Methanogenesis.

The final stage in the overall anaerobic conversion of organic materials into methane and CO_2 is catalized by methanogenic bacteria. Methanogens utilize only a limited number of simple substrates such as acetate or the C_1-compounds CO_2/H_2, formate methanol and CO. Methanogens are classified into two major groups: the acetate converting (also called acetoclastic) and the hydrogen utilizing (hydrogenotrophic) bacteria. The growth rates of the acetoclastic bacteria are low. This is the main reason for the fact that anaerobic treatment systems require a high biomass retention time. Generally, 70 - 80 % of the methane formed from the organic materials originates from acetate. The rest is mainly derived from H_2 and CO_2. Hydrogenotrophic bacteria have a much higher maximum growth rate than the acetoclastic bacteria. Therefore, the hydrogenotrophic bacteria are presumably not a critical group. However, the ability of these bacteria to maintain the PH_2 very low, forms the basis for thermodynamically favourable conditions for the preceeding essential pre-methanogenesis substrate conversion steps.

Proteins

• Proteins in anaerobic environment

The anaerobic degradation of proteins is important for the recycling of N and C in nature. Examples of waste materials containing considerable amounts of very different types of proteins, frequently used as substrates for methanogenic digestors are cattle manure (Varel et al. 1977), pig manure (Hobson et al. 1974), chicken manure (Huang & Shih 1981), domestic sewage (Heukelekian & Mueller 1958) and sewage sludge (Buswell & Neave 1930).

• Structure and classification

Proteins are the most abundant organic molecules in cells; they are fundamental in all aspects of cell structure and function. Proteins are the main constituents of animal tissues, viz. contributing to 50 per cent or more of their dry weight. All proteins contain carbon, hydrogen, oxygen, nitrogen and with a few exceptions, sulphur. The approximate values of these compounds are: carbon, 45 to 55%; hydrogen, 6 to 8%; oxygen, 19 to 25%; nitrogen, 14 to 21% and sulphur, 0 to 4%. The α-amino acids are the fundamental structural units of the proteins. These building-block molecules contain at least one carboxyl group and one α-amino group but differ from each other in the structure of their R groups, or side chains. Twenty different α-amino acids are commonly found as the building blocks of proteins (Table 4). In protein molecules, the amino acids are joined together by peptide bonds. The amino acid sequence in a protein refers to its primary structure. The secondary structure refers to the manner in which the polypeptide chains are arranged in space along one dimension. The tertiary structure refers to how the polypeptide chain is bent or folded in three dimensions, to form the compact, tightly folded structure of protein. The quaternary structure refers to how individual polypeptide chains of a protein having two or more chains are arranged in relation to each other.

Table 4. The most important building-block amino acids of proteins.

Amino acid	Abbreviation	Structure formula	molecular weight
Alanine[a]	Ala	$CH_3-CH(NH_2)-COOH$	89
Valine[a]	Val	$CH_3-CH(CH_3)-CH(NH_2)-COOH$	117
Leucine[a]	Leu	$CH_3-CH(CH_3)-CH_2-CH(NH_2)-COOH$	131
Isoleucine[a]	Ile	$CH_3-CH_2-CH(CH_3)-CH(NH_2)-COOH$	131
Methionine[a]	Met	$CH_3-S-CH_2-CH_2-CH(NH_2)-COOH$	149
Glycine[b]	Gly	$CH_2(NH_2)-COOH$	75
Serine[b]	Ser	$CH_2(OH)-CH(NH_2)-COOH$	105
Cysteine[b]	Cys	$HS-CH_2-CH(NH_2)-COOH$	121
Threonine[b]	Thr	$CH_3-CH(OH)-CH(NH_2)-COOH$	119
Asparagine[c]	Asn	$0 = C(NH_2)-CH_2-CH(NH_2)-COOH$	132
Glutamine[c]	Gln	$0 = C(NH_2)-CH_2-CH_2-CH(NH_2)-COOH$	146
Lysine[d]	Lys	$CH_2(NH_2)-CH_2-CH_2-CH_2-CH(NH_2)-COOH$	146
Arginine[d]	Arg	$NH = C(NH_2)-NH-CH_2-CH_2-CH_2-CH(NH_2)-COOH$	174

a. Amino acids with nonpolar R groups.
b. Amino acids with polar R groups.
c. The acidic amino acids and their amides.
d. The basic amino acids.

In its native state each type of protein molecule has a characteristic three-dimensional shape, referred to as its conformation. Depending on their conformation, proteins are classified into three major classes, fibrous, globular and conjugated proteins.

The fibrous proteins are physically tough, insoluble in water or dilute salt solutions and are highly resistent to the action of proteolytic enzymes. Examples are collagen, α-elastin which is an important constituent of elastic connective tissue and α-keratin which is present in hair, skin, nails, horn, hoofs and bone. The globular proteins are soluble in aqueous systems. Most of the proteins in this class are subject to a breakdown by proteolytic enzymes yielding amino acids. Important categories of proteins like albumins, globulins, histones and prolamins are included in this class. The conjugated proteins are proteins combined with non-protein substances such as lipids, nucleic acids, carbohydrates and others. They are classified in terms of their non-protein substances and they have varying properties such as solubility and susceptibility to enzymatic breakdown and include the phosphoproteins, lipoproteins, nucleoproteins, chromoproteins and myosin which is an important structural element of meat.

- **Meat proteins**

Meat proteins consist of sarcoplasmic proteins , myofibrillar proteins and collagen protein.

The sarcoplasmic or muscle juice proteins are globular proteins, consisting of over fifty components, many of which are enzymes. They are mainly globulins and, essential to the utilization of energy in living muscle. The myofibrillar proteins are fibrous proteins and they are the working parts of the machinery of muscle and include two main proteins myosin and actine. The fibrous protein collagen is the major protein in the connective tissues of the meat. Collogen is tough, insoluble and inelastic, yet is readily converted into water-soluble gelatine on boiling.

- **Degradation of proteins**

In anaerobic digestion the biodegradable proteins are hydrolysed by two groups of enzymes called proteases and peptidases (Brock et al. 1982, Schlegel 1981) according to the general scheme for enzymatic breakdown of proteins : protein→ polypeptiedes→ peptiedes→ amino acids

These two categories of enzymes are partially cellwall-bounded and occur partially free in the reactor fluid (Glenn 1976).

The factors affecting the enzymatic hydrolysis of proteins have been reviewed by Kilara (1985) and are shown in Table 5 . The degradation of amino acids derived from protein hydrolysis is a complex process. A large complex number of amino acids and other nitrogenous compounds can serve as energy sources for anaerobic bacteria.

The anaerobic degradation of amino acids involves oxidation-reduction reactions between one or more amino acids or non-nitrogenous compounds derived from amino acids (Barker 1981).

The oxidative reactions are often similar to if not identical to those found in aerobic bacteria and include oxidative deamination, transamination and α-keto oxidations. The reductive reactions used by anaerobes are much more distinct. Amino acids, α and β -keto acids, α , β -unsaturated acids or their coenzyme A (CoA) derivatives and protons are the major electron acceptors used. The major end-products produced by these reactions include short-chain fatty acids, succinate, δ -aminovalerate and hydrogen.

Table 5. Factors affecting the enzymatic hydrolysis of proteins[a].

Factors	Comments
Enzyme specifity	no single proteolytic enzyme is able to hydrolyse a protein completely. Mixtures of proteolytic enzymes are used.
Protein conformation	globular and conjugated proteins are more susceptible to hydrolysis than fibrous proteins.
Substrate and enzyme concentration	should be controlled, Degree of Hydrolysis (DH) will depend on enzyme substrate (E/S) ratio.
pH	optimum varies with enzyme (range from 4.5 to 8.5).
Temperature	preferably higher than 30 °C
Inhibitory substances	should be absent

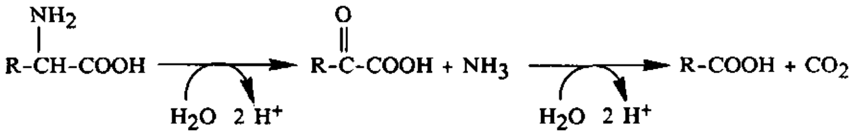
a. Data from Kilara (1985).

Each single amino acid released from the hydrolysis of proteins is degraded via a specific pathway and converted into various fatty acids. For example, alanine and glycine are degraded according to the reactions shown in the equations 1,2 (Massey et al. 1976, Stadtman 1980).

$$\underset{\text{alanine}}{3\ CH_3\text{-}CH(NH_2)\text{-}COOH} + 3\ H_2O \longrightarrow \underset{\text{propionate}}{2\ CH_3\text{-}CH_2\text{-}COOH} + \underset{\text{acetate}}{3\ CH_3\text{-}COOH} + H_2CO_3 + 2\ NH_3 \quad (1)$$

$$\underset{\text{glycine}}{4\ CH_2(NH_2)\text{-}COOH} + 4\ H_2O \longrightarrow \underset{\text{acetate}}{3\ CH_3\text{-}COOH} + 2\ H_2CO_3 + 4\ NH_3 \quad (2)$$

The oxidation of alanine and other amino acids such as valine, lecine proceeds via a α-keto acid as shown in equation 3 (Barker 1961).



$$\mathrm{R\text{-}\overset{\overset{NH_2}{|}}{C}H\text{-}COOH} \xrightarrow[H_2O\ \ 2\,H^+]{} \mathrm{R\text{-}\overset{\overset{O}{\|}}{C}\text{-}COOH + NH_3} \xrightarrow[H_2O\ \ 2\,H^+]{} \mathrm{R\text{-}COOH + CO_2} \qquad (3)$$

Many bacteria can obtain their energy by coupled oxidation-reduction reactions between pairs of amino acids. This coupled deamination of amino acids is designated as the Stickland reaction (Barker 1961, Nisman 1954). In the Stickland reaction, one member of the pair is oxidized (dehydrogenated) while the other is reduced (hydrogenated). An example of a Stickland reaction is shown in equation 4 for the oxidation of alanine with glycine serving as the electron acceptor.

$$\begin{array}{l}
3\ \underset{\textbf{alanine}}{\mathrm{CH_3\text{-}CH(NH_2)\text{-}COOH}} + 2\ \mathrm{H_2O} \rightarrow \underset{\textbf{acetate}}{\mathrm{CH_3\text{-}COOH}} + \mathrm{CO_2} + \mathrm{NH_3} + 4\ \mathrm{H^+} \\
2\ \underset{\textbf{glycine}}{\mathrm{CH_2(NH_2)\text{-}COOH}} + 4\ \mathrm{H^+} \longrightarrow \underset{\textbf{acetate}}{\mathrm{CH_3\text{-}COOH}} + 2\ \mathrm{NH_3} \\
\hline
3\ \mathrm{CH_3\text{-}CH(NH_2)\text{-}COOH} + 2\ \mathrm{CH_2(NH_2)\text{-}COOH} + 2\ \mathrm{H_2O} \longrightarrow 3\ \mathrm{CH_3\text{-}COOH} + 3\ \mathrm{NH_3} + \mathrm{CO_2}
\end{array} \qquad (4)$$

The end-products of the amino acids degradation can further be converted into methane by acetogenic and methanogenic bacteria.

- **Proteolytic bacteria**

Siebert & Toerien (1969) investigated the occurrence and identity as well of proteolytic bacteria in the anaerobic digestion of raw sewage sludge. They concluded that the clostridial species were the most prevalent proteolytic bacteria.

Mead (1971) divided the amino acid-fermenting clostridia into four major groups and several subgroups based on the patterns of amino acid used or formed (Table 6). Group I consists of species that carry out the Stickland reaction between pairs or amino acids. These bacteria reduce proline or arginine to δ-aminovalerate by oxidizing serine, phenylalanine and other amino acids. Group II bacteria use arginine and glycine. Group III bacteria use serine and histidine. Group IV contains only clostridium putrefaciens which readily use serine and threonine.

Table 6. Amino acid utilization patterns of clostridial species[a].

Group	Species	Characteristics [b]
I	Clostridium bifermentans C. sordellii C. botulinum types, A, B, F C. caloritolerans C. sporogenes C. cochlearium (one strain) C. difficile C. putrificum C. sticklandii C. ghoni C. mangenoti C. scatologenes [c] C. lituseburense [c]	Organisms that carry out Stickland reaction; proline reduced and δ-aminovalerate produced; serine, phenylalanine and other amino acids oxidized; α-aminobutyrate produced from threonine or methionine and γ-aminobutyrate produced from glutamate by most species.
II	C. botulinum types C, G C. histolyticum C. cochlearium (one strain) C. subterminale	δ-aminovalerate not produced. arginine and/or glycine used by all species.
III	C. cochlearium (one strain) C. tetani C. tetanomorphum C. microsporum C. lentoputrescens C. limosum C. malenomenatum	δ-aminovalerate not produced; glutamate, serine and histidine utilized.
IV	C. putrefaciens	δ-aminovalerate not produced; serine and threonine utilized.

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a. Classification of Mead (1971) with additional species added (Elsden 1980).

b. Major characteristics of each group are given. Other amino acids may be used by various species (Elsden et.al. 1979, 1978, 1976).

c. α-aminobutyrate and γ-aminobutyrate but not δ-aminovalerate are formed (Mead 1971).

Elsden et al. (1980) extended the studies of Mead by including additional species as well as the end-products by these bacteria. In general, their work confirms that of Mead and shows the importance of these kinds of analyses in clostridial taxonomy.

Lipids

• Lipids in anaerobic environment

In nature very considerable amounts of lipids are anaerobically converted. Typical examples of waste materials containing significant concentrations of very different types of lipids which are frequently stabilized are piggery wastes (Hobson et al. 1981), sewage sludge (Viswanathan et al. 1962), palm oil processing effluents (Ma & Ong, 1986) and wool scouring wastes (Genon et al. 1984).

• Structure and classification

Lipids include a large number of different types of substances. Their principal common characteristic is that they are generally insoluble in water but soluble in certain nonpolar solvents such as chloroform, alcohols and benzene. There are several different classes of lipids but all derive their distinct properties from the hydrocarbon nature of a major portion of their structure. Lipids have several important biological functions, viz. they serve (1) as structural components of membranes, (2) as storage and transport forms of metabolic fuel, (3) as protective coating on the surface of many microorganisms and (4) as cell-surface components. Lipids often occur with members of other classes of biomolecules to yield hybrid molecules such as glycolipids, which contain both carbohydrate and lipid groups and lipoproteins, which contain both lipids and proteins. Lipids have been classified in several different ways.

The most satisfactory classification is based on their backbone structure (Table 7). The complex lipids, which contain fatty acids as components, include the acylglycerols, the phosphoglycerides, the sphingolipids, and the waxes. They differ in their backbone structures to which the fatty acids are joined covalently. The complex lipids are also called saponifiable lipids since they yield soaps (salts of fatty acids) upon alkaline hydrolysis. The other large group of lipids consists of the simple lipids, which do not contain fatty acids and thus are nonsaponifiable.

Table 7. Classification of lipids.

Lipid type	Backbone
Complex (saponifiable)	
Acyglycerols	Glycerol
Phosphoglycerides	Glycerol 3-phosphate
Sphingolipids	Sphingosine
Waxes	Nonpolar alcohols of high molecular weight
Simple (nonsaponifiable)	
Terpeness	
Steroids	
Prostaglandins	

Up to 99% of the lipids in animals and microorganisms are complex lipids consisting of esters of fatty acids and glycerol, and are known as fats. In these fats, the fatty acids occur in large amounts as building-block molecules. All the fatty acids contain a long hydrocarbon chain and a terminal carboxyl group.

This hydrocarbon chain may be saturated, in which case the fatty acids are called saturated fatty acids, or unsaturated in which case they may contain one or more double bonds. Tables 8 and 9 show the common saturated and unsaturated fatty acids. Fatty acids differ from each other primarily in chain length and in number and in the position of their double bonds. The most abundant saturated fatty acids found in animal fats are palmitic acid (16:0) and stearic acid (18:0) and the most abundant unsaturated fatty acids found are palmitoleic (16:1), oleic (18:1) and linoleic (18:2)

• Fat depots in animals

The principle fat depots in animals are classified as follows:

1. subcutaneous: the fatty tissue found immediately under the skin.
2. perinephric: the fatty tissue surrounding the kidney.
3. intramuscular: the fatty tissue deposits along the line of the perimysium within the muscle, often referred to as marbling fat.
4. intermuscular: all the other fatty tissue.

Table 8. Saturated even and odd-carbon numbered fatty acids.

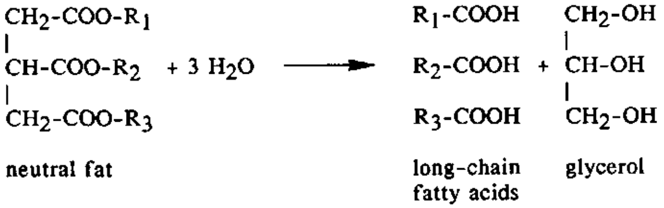
Systematic Name	Common Name	Structural Formula	Short-hand Description
n -Butanoic	Butyric	$CH_3.(CH_2)_2.COOH$	4 : 0
n -Hexanoic	Caproic	$CH_3.(CH_2)_4.COOH$	6 : 0
n -Octanoic	Caprylic	$CH_3.(CH_2)_6.COOH$	8 : 0
n -Denanoic	Capric	$CH_3.(CH_2)_8.COOH$	10 : 0
n -Dodecanoic	Lauric	$CH_3.(CH_2)_{10}.COOH$	12 : 0
n -Tetradecanoic	Myristic	$CH_3.(CH_2)_{12}.COOH$	14 : 0
n -Hexadecanoic	Palmitic	$CH_3.(CH_2)_{14}.COOH$	16 : 0
n -Octadecanoic	Stearic	$CH_3.(CH_2)_{16}.COOH$	18 : 0
n -Eicosanoic	Arachidic	$CH_3.(CH_2)_{18}.COOH$	20 : 0
n -Docosanoic	Behenic	$CH_3.(CH_2)_{20}.COOH$	22 : 0
n -Pentanoic	Valeric	$CH_3.(CH_2)_3.COOH$	5 : 0
n -Heptanoic	Enanthic	$CH_3.(CH_2)_5.COOH$	7 : 0
n -Nonanoic	Pelargonic	$CH_3.(CH_2)_7.COOH$	9 : 0
n -Undecanoic	-	$CH_3.(CH_2)_9.COOH$	11 : 0
n -Tridecanoic	-	$CH_3.(CH_2)_{11}.COOH$	13 : 0
n -Pentadecanoic	-	$CH_3.(CH_2)_{13}.COOH$	15 : 0
n -Heptadecanoic	Margaric	$CH_3.(CH_2)_{15}.COOH$	17 : 0

Table 9. Unsaturated fatty acids.

Systematic Name	Common Name	Structural Formula	Short-hand Description
Dec-9-enoic	-	$CH_3.CH.(CH_2)_7.COOH$	10 : 1
Dodec-9-enoic	-	$CH_3.CH_2.CH{=}CH.(CH_2)_7.COOH$	12 : 1
Tetradec-9-enoic	Myristoleic	$CH_3.(CH_2)_3.CH{=}CH.(CH_2)_7.COOH$	14 : 1
Hexadec-9-enoic	Palmitoleic	$CH_3.(CH_2)_5.CH{=}CH.(CH_2)_7.COOH$	16 : 1
Octadec-6-enoic	Petroselinic	$CH_3.(CH_2)_{10}.CH{=}CH.(CH_2)_4.COOH$	18 : 1
Octadec-9-enoic	Oleic	$CH_3.(CH_2)_7.CH{=}CH.(CH_2)_7.COOH$	18 : 1
Octadec-11-enoic	Vaccenic	$CH_3.(CH_2)_5.CH{=}CH.(CH_2)_9.COOH$	18 : 1
Octadeca-9:12:15-trienoic	Linolenic	$CH_3.CH_2.(CH{:}CH.CH_2)_3.(CH_2)_6.COOH$	18 : 3
Eicos-9-enoic	Gadoleic	$CH_3.(CH_2)_9.CH{:}CH.(CH_2)_7.COOH$	20 : 1
Docos-13-enoic	Erucic	$CH_3.(CH_2)_7.CH{:}CH.(CH_2)_{11}.COOH$	22 : 1

- **Degradation of lipids**

In anaerobic digestion of lipids the neutral fats are first hydrolysed by a group of enzymes called lipases (Codfrey & Reichelt 1983) to glycerol and long-chain fatty acids as shown in equation (1)



$$\begin{array}{l} CH_2\text{-}COO\text{-}R_1 \\ | \\ CH\text{-}COO\text{-}R_2 \\ | \\ CH_2\text{-}COO\text{-}R_3 \\ \text{neutral fat} \end{array} + 3\ H_2O \longrightarrow \begin{array}{l} R_1\text{-}COOH \\ \\ R_2\text{-}COOH \\ \\ R_3\text{-}COOH \\ \text{long-chain} \\ \text{fatty acids} \end{array} + \begin{array}{l} CH_2\text{-}OH \\ | \\ CH\text{-}OH \\ | \\ CH_2\text{-}OH \\ \text{glycerol} \end{array} \qquad (1)$$

in which R_1, R_2 and R_3 represent three molecules of either the same or different fatty acids. The released long-chain fatty acids are degraded via ß-oxidation in anaerobic digestion (Jeris & McCarty 1965). The basic scheme of ß-oxidation of the long-chain fatty acids in anaerobic digestion is outlined as follows:

$$CH_3\text{-}(CH_2)_{16}\text{-}COOH \xrightarrow[2\ H_2O]{4\ H^+} CH_3\text{-}(CH_2)_{14}\text{-}COOH + CH_3\text{-}COOH$$

This process is repeated $(\frac{n-2}{2})$ times (n = number of carbon atoms in the fatty acid molecule) until the acid is completely converted into acetate. These reactions are carried out by H_2-producing synthrophic acetogenic bacteria as was indicated by Bryant (1979).

For example, the degradation of palmitate proceeds according to the overall reaction

$$CH_3\text{-}(CH_2)_{14}\text{-}COO^- + 14\ H_2O \longrightarrow 8\ CH_3COO^- + 7\ H^+ + 14\ H_2$$

$(G^{0'} = +\ 345.6\ kJ.mol^{-1})$

where $G^{0'}$ is the standard free energy change.

The degradation of long-chain fatty acids is thermodynamically unfavourable unless the hydrogen partial pressure is maintained at an extremely low level (Gujer & Zehnder 1983). Hydrogenotrophic methanogenic bacteria generally utilize molecular hydrogen in a balanced anaerobic digester so rapidly that the hydrogen partial pressure can be kept low enough to ensure the active performance of H_2-producing acetogenic bacteria. This in fact means that the degradation of fatty acids via β-oxidation depends strongly on the available methanogenic activity. The acetate as the main end-product of the long-chain fatty acids degradation can further be converted into methane by acetoclastic methanogenic bacteria.

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Agricultural Wastes, 11 (1984) 197-226

CHAPTER 2

ANAEROBIC TREATMENT OF SLAUGHTERHOUSE WASTE USING A FLOCCULENT SLUDGE UASB REACTOR

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ABSTRACT

This study was carried out to assess the feasibility of using the upflow flocculent anaerobic sludge blanket (UASB) process for a one-stage anaerobic treatment of slaughterhouse wastewater which contains approximately 50% insoluble coarse suspended COD. Batch experiments, as well as continuous experiments, were conducted. The continuous experiments were carried out in a 25.3 m^3 *UASB pilot-plant, with digested sewage sludge which was used as seed from the municipal sewage treatment plant of Ede, The Netherlands. The UASB pilot-plant was operated under semi-continous conditions, viz. with an organic load varying between day and night (high organic load at daytime combined with low load at nighttime with weekend feed interruptions. Initially the UASB pilot-plant was operated at a temperature of 30* ^oC*, but, 20 weeks after the start up, the temperature was reduced to 20* ^oC *because application of the process at this lower temperature might be quite attractive for economic reasons.*

The process can be started up at an organic space load of 1 kg COD m^{-3}-day^{-1} *(sludge load, 0.11 kg COD* kg^{-1}*VSS* day^{-1}*) and at a liquid retention time of 35 h at a process temperature of 30* ^oC*. Once started up, the system* *can satisfactorily handle organic space loads up to 3.5 kg COD* $m^{-3}day^{-1}$ *at a liquid retention time of 8 h at temperatures as low as 20* ^{o}C. *A treatment efficiency up to 70% on a* COD_{total} *basis, 90% on a* $COD_{filtered}$ *basis and 95% on a* $BOD_{filtered}$ *basis was smoothly approached. Temporary shock loads up to 7.5 kg COD* $m^{-3}day^{-1}$ *during the daytime at a liquid retention time of 5 h can well be accommodated provided such a shock load is followed by a period of underloading, e.g. at night.*

The methane yield amounted to 0.28 Nm^{3} *per kilogram of COD removed; the methane content of the biogas from the wastewater varied between 65 and 75%.*

INTRODUCTION

The environmental protection authorities in The Netherlands require that the discharge of wastes to surface waters meets stringent effluent standards. These effluent standards have to comply with the basic quality for surface waters according to 1980-1984 water pollution control programmes and correspond to maximum pollutant concentration of 40 and 30 mg $litre^{-1}$, respectively for BOD_5 and Total Suspended Solids.

From a survey paper by Ten Have (1976) concerning meat-processing plants and slaughterhouses, it appears that the pollution capacity of these industries presently exceeds 1,000,000 population equivalents. Most of these plants discharge their wastewaters to a municipal treatment system. In order to comply with water pollution control standards and to save money on sewer surcharge, slaughterhouses and meat-processing plants are required to apply an adequate pretreatment to their discharges.

Primary treatment by physical and/or chemical methods can be used in both types of industry (Funke, 1969). Frequently, a combination of screening, centrifugation, sedimentation, air flotation and flocculation precipitation for pretreating the wastes is used. In addition, mechanical primary treatment devices, such as static and vibrating screens, hydrocyclones, air flotation units for grease recovery and clarifiers, are applied in these industries (Witherow & Lammers, 1976).

Secondary treatment processes for the bacterial decomposition of the organic pollutants and nitrogen removal have been investigated and applied in these industries. The biological processes applied include the conventional anaerobic processes, viz. a completely mixed tank system (Black & Brown, 1974), anaerobic ponds (Rotlag & Dornbush, 1966) and the aerobic ponds (Steffen, 1961). Combinations of these systems are needed since no single secondary treatment process will provide sufficient effluent. All these secondary treatment methods have distinct disadvantages, either in odour nuisance or in investment and operating costs.

Obviously, there is a considerable need for inexpensive, efficient wastewater treatment systems. Therefore, considerable efforts have been made in recent years in The Netherlands toward the development of a more sophisticated anaerobic treatment process, suitable for treating low strength wastes such as slaughterhouse and meat-processing wastes. These efforts have resulted in the development of a new anaerobic treatment process, now known as the Upflow Anaerobic Sludge Blanket (UASB) process (Lettinga & Van Velsen, 1974; Lettinga, 1978). The UASB process has been applied at full scale since 1977 in The Netherlands for the treatment of sugarbeet waste (Pette et al., 1979), maize-starch waste, potato-processing wastes (Versprille, 1979) and potato-starch wastewater.

The technology developed may be helpful in solving the problems encountered in the treatment of wastewaters from slaughterhouses and the meat-processing industry.

MATERIALS AND METHODS

Sampling and analyses

All the samples taken for chemical analysis were 24 h composites. Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Dry Suspended Solids (DSS), Volatile Suspended Solids (VSS), total nitrogen, and phosphate were determined according to standard methods (Standard Methods, 1975). The total Volatile Fatty Acids (VFA) content and the bicarbonate (alkalinity) were determined according to the Dutch Normalized Standard Methods (1969). Separate VFA analyses were determined by gas chromatography using a Packard Becker model 417 equipped with a 6 m x 2 mm glass column and a flame ionisation detector. The column was packed with Chromosorb 101 (60-80 mesh). Flow rate of the carrier gas, nitrogen saturated with formic acid, was 18 ml min^{-1}. Column temperature was 210 °C and the injection port temperature was 230 °C.

The COD for raw wastewater is expressed as $\text{COD}_{\text{total}}$. The coarse Suspended Solids in the raw wastewater were separated using a filter (Schwarzband black ribbon No. 589^{I} Ref.No. 300011) with a pore size of 7.4 µ m. The filtrate COD of the wastewater consists of colloidal, as well as soluble, fractions.

Gas samples removed from the gas sampling port were analysed immediately for the determination of methane, carbon dioxide, nitrogen and hydrogen content using a Packard Becker gas chromatograph model 409 equipped with two columns - a Porapak (2m x 3mm) and a stainless steel column with molecular sieve 5A (1m x 3mm). Helium was used as a carrier gas (20 ml min^{-1}). Oven temperature was 70 °C and column temperature, 75°C.

The methanogenic capacity of the sludge was either calculated from the methane production rate and the amount of sludge present in the reactor or from a determination of the amount of F-420 co-enzyme (de, Zeeuw et al., 1981). The amount of F-420 co-enzyme gives, for certain substrates, a good, rapid assessment of the potential methanogenic activity of anaerobic sludge and also indicates possible inhibition effects on the methanogenesis in the system.

Seed sludge

The UASB pilot-plant (25.3 m^3) was seeded with 13 m^3 of digested sewage sludge from the municipal sewage treatment plant at Ede, The Netherlands. The Total Suspended Solids (TSS) content of the sludge was 3.4% and the Volatile Suspended Solids (VSS) content 2.3%.

The maximum specific activity of the sludge for VFA decomposition as measured in a standard batch-fed test using a feed mixture of 600 mg litre^{-1} each of acetic acid, propionic acid and butyric acid, was 0.05 kg CH_4-COD kg^{-1} VSS day^{-1} at 30 °C.

Wastewater characteristics

Wastewater from slaughtering areas was collected after passing a screen installed to separate dispersed particles larger than 1 mm. The composition of the wastewater depends considerably on the production process and the type of animals slaughtered. The major waste load originates from the slaugh-

Table 1 The most important characteristics of the wastewater.

COD_{total}	1500 - 2200 mg $litre^{-1}$
Coarse suspended solids as a percentage of COD_{total}	40 - 50 %
BOD_{total}	490 - 650 mg $litre^{-1}$
VFA-COD (% of total COD)	12 - 15 %
Fats	50 - 100 mg $litre^{-1}$
	(5% of the total solids)
Nitrogen (N) Kjeldahl	120 - 180 mg $litre^{-1}$
Phosphate $(P)_{total}$	12 - 20 mg $litre^{-1}$
Temperature	20 °C
pH	6.8 - 7.1

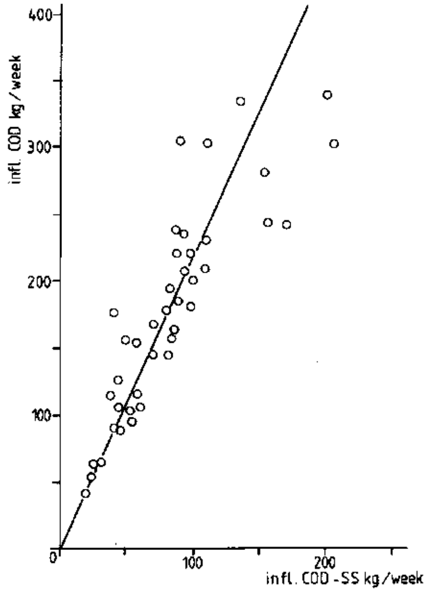


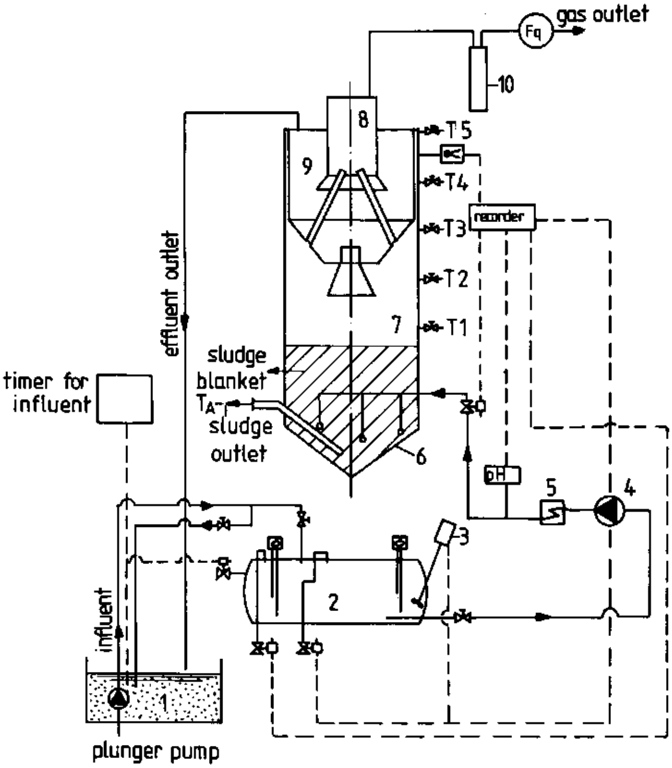
Figure 1. Relationship between the influent COD_{total} and the influent COD-coarse suspended solids during the entire experimental period.

tering, which is a one-shift operation from Monday through Friday. The most important characteristics of the wastewater are presented in Table 1.

The wastewater is an unsettled low-strength complex-type waste. Of the pollutants 40% - 50% is present as insoluble and slowly biodegradable-coarse suspended matter (Figure 1).

The 25.3 m^3 semi-technical experimental, pilot plant

A flow diagram of 25.3 m^3 semi-technical experimental pilot-plant is shown in Figure 2.



1. wastewater pit
2. equalizing tank
3. stirrer
4. influent pump
5. heat exchanger
6. reactor
7. digester compartment
8. gas collector
9. settler compartment
10. water seal
11. gas meter

Figure 2. Flow diagram of a 25.3 m^3 semi-technical, experimental pilot-plant.

The equalizing tank (15 m^3) installed provided a storage facility enabling the experiments to be carried out over a full 24 h period. The process procedure was according to the Upflow Anaerobic Sludge Blanket (UASB) process (Lettinga et al., 1980). Feed was introduced at the bottom of the reactor, forcing the wastewater upward through the sludge bed present in the lower part of the reactor. The reactor had an internal diameter of 2.6 m and was 6 m in height. The reactor was equipped in the upper part with a proper gas-solid separator (GSS) for separating and collecting the produced biogas. In this way, a quiescent zone settling compartment is created in the upper part of the reactor. This enabled sludge particles arriving there to flocculate, to settle out and return back into the digester compartment via the aperture between the gas collectors. No mechanical mixing or sludge recirculation was applied for the sake of sludge settleability.

RESULTS

Start up and operation

The 25.3 m^3 pilot-plant experiments with slaughterhouse wastes were seeded with digested sewage sludge from the municipal sewage treatment plant at Ede, The Netherlands. The pilot-plant experiments were operated at a temperature of 30 °C during the period 0-142 days and, from 159 to 296 days, at a temperature of 20 °C, because application of the process at this lower temperature would be quite attractive for economic reasons. The reactor was started up at an organic space load of 0.8 kg COD m^{-3} day^{-1} (sludge load 0.10 kg COD kg^{-1}VSS day^{-1}) and at a hydraulic retention time of 35 h. The load was increased stepwise, using the treatment efficiency, the sludge bed expansion and sludge wash out as guidelines. The reactor was operated in semi-continuous conditions, i.e. during the daytime for approximately 8h; higher feed rates were applied, in combination with lower feed rates, at night. No feed was applied at the weekend.

Table 2 summarizes the applied overall organic loading rates, the hydraulic retention times and the temperature history over the entire experimental period for the pilot-plant reactor.

Due to unforeseen technical problems, the experiments had to be interrupted during weeks 18 and 19, 33 and 41.

Table 2. The organic loading rates, the hydraulic retention times and the process temperature over the entire experimental period.

Phase	Number of days	Organic space load (kg COD $m^{-3}day^{-1}$)	Hydraulic retention time (h)	Temperature (^{o}C)
I	0-142	0.5-1.5	40-20	30
II	159-296	1.5-2.5	18-12	20
III	297-324	2.5-3.5	10	30

Loading rates and treatment efficiency

The results of the loading rates and treatment efficiencies are shown in Figure 3. Organic space loads up to 3.5 kg COD $m^{-3}day^{-1}$ and a hydraulic retention time of 7 h at temperatures as low as 20 ^{o}C were achieved at fairly satisfactory treatment efficiencies; after 3 weeks, up to 70% COD_{total} reduction (η_{total}) and 90% $COD_{filtered}$ reduction ($\eta_{filtered}$). The $BOD_{filtered}$ was reduced from an average value of 650 to 25 mg $litre^{-1}$, corresponding to a 96% treatment efficiency.

purification percentage = [1 - total C(B)OD (effluent) / total C(B)OD
(η) (influent)] x 100 and
[1 - filtered C(B)OD (effluent) / total C(B)OD
(influent)] x 100

As expected, the anaerobic digestion procedure was inefficient in nitrogen reduction, only approximately 24% of the N-total being eliminated.

Gas production

Tables 3 to 5 show the results of the gas production, together with the imposed applied organic space load and the calculated percentage of COD_{total} converted into methane at the two applied temperatures. The calculated ratio (M) between COD-methane/$COD_{filtered}$-removed during the 24 h loading rates are shown in Table 4. The calculated data are based on the 8 h loading rates, the mean average 24 h loading rates and the weekly loading rates. In addition, the course of the gas production has been plotted, versus the applied organic space load during the 24 h loading rates, in Figure 4 for the entire experimental period.

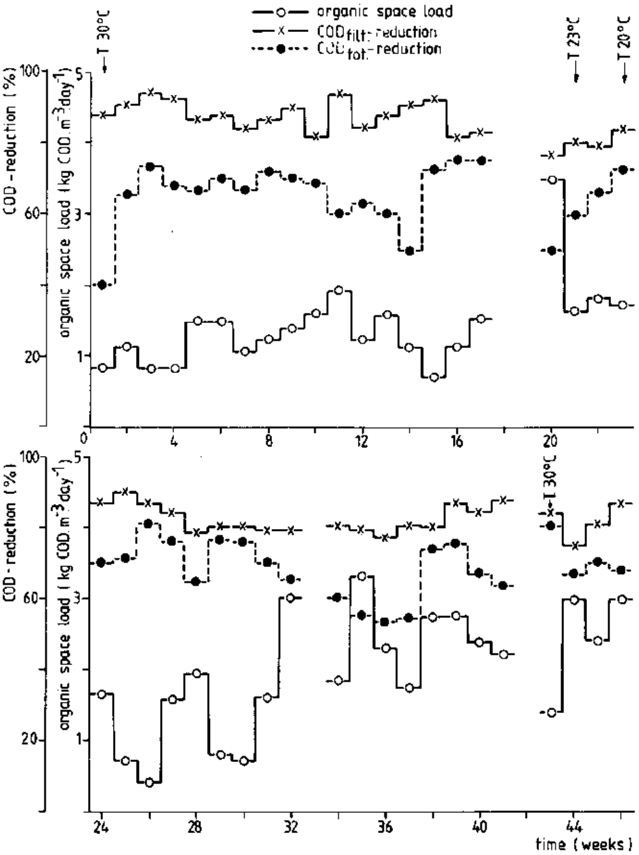


Figure 3. Progress of the organic space load and the reduction rates, during the entire experimental period.

The results in Tables 3 and 4 clearly indicate a higher conversion of the supplied COD_{total} to methane, at loading rates exceeding 1.5 kg COD $m^{-3}day^{-1}$, at a process temperature of 30 °C than at a process temperature of 20 °C. Moreover, at organic space load rates lower than 1.5 kg COD $m^{-3}day^{-1}$, the conversion of the supplied COD_{total} into methane at a process temperature of 30 °C is less complete than at a process temperature of 20 °C. It is also evident that there is a difference in the temperature dependency of the conversion of the COD_{total} into methane between the 24 h loading (Table 4) and the 8 h loading (Table 3).

According to the results summarized, in Table 5, it seems that the conversion of COD_{total} and $COD_{removed}$ to methane under operational conditions at a process temperature of 20 °C is always higher than at a temperature of 30 °C. This phenomenon can be attributed to the continuation of the fermentation under unfed conditions at weekends. Further explanation will be given below.

Table 3. The course of the gas production, the applied organic space load and the calculated percentage of COD_{total} converted into methane during the 8-h loading rate at the two different temperatures.

Organic space load ($kg\ COD\ m^{-3}\ day^{-1}$) Minimum	Maximum	verage	Days[a]	Gas production ($m^3\ day^{-1}$)	($m^3 h^{-1}$)	COD_{total} converted into methane (%)
At a process temperature of 30 °C						
0	0	0[b]	72	2.61	0.110	0
1	2	1.60	35	6.91	0.288	42.10
2	3	2.57	25	8.73	0.348	33.10
3	4	3.56	28	10.25	0.427	28.10
6	7	6.33	14	18.45	0.769	28.40
7	8	7.90	14	17.36	0.724	21.41
At a process temperature of 20 °C						
0	0	0[b]	66	3.32	0.138	0
2	3	2.66	13	8	0.333	30.91
3	4	3.73	21	10.0	0.416	27.54
6	7	6.23	9	12.0	0.500	19.80
7	8	7.56	19	14.20	0.589	19.30

a. Number of days for which loading was applied.

b. During weekends and weeks off when there was no loading.

Considerable emphasis has been placed on the response of the process (gas production) to feed interruptions. For this purpose, an experiment was set up at day 250 (process temperature, 20 °C) where the feed was shut down for a period of 16 h (period 1). This period was followed by one of 4 h with an average feed rate of 3 kg COD $m^{-3}day^{-1}$ (period 2) followed by a feed interruption for 8 h (period 3). The gas production measured under these circumstances is shown in Figure 5.

Table 4. The course of the gas production, the applied organic space load, the calculated percentage of COD_{total} converted into methane and the calculated M-factor, during the 24-h loading rates at the two applied temperatures.

Organic space load (kg COD $m^{-3}day^{-1}$)			Days[a]	Gas production		COD_{total} converted into methane (%)	Factor M[b]
Minimum	Maximum	Average		(m^3day^{-1})	(m^3h^{-1})		
At a process temperature of 30 °C							
0	0	0[c]	72	2.61	0.109	0	0
0.3	0.5	0.36	8	3.04	0.125	82.23	2.24
0.5	1.0	0.843	42	3.43	0.143	39.64	1.02
1	1.75	1.63	39	6.47	0.270	38.70	1.36
1.75	2.50	2.17	13	8.41	0.350	37.76	1.14
3	3.5	3.38	13	13.21	0.550	38.10	1.33
At a process temperature of 20 °C							
0	0	0[c]	66	3.32	0.138	0	0
0.3	0.5	0.37	5	4.00	0.166	111	3.90
0.5	1.0	0.58	9	5.24	0.220	92.8	3.39
1.5	2.0	1.78	21	7.46	0.311	43.1	0.99
2	3.0	2.39	13	9.00	0.370	38.7	0.96
3	4.0	3.38	12	11.20	0.470	34.1	0.87
4	6	5.40	3	14.10	0.588	27	1.67

a. Number of days which loading was applied.

b. Factor M = COD - methane / $COD_{filtered}$ removed.

c. During the weekend when there was no loading and during weeks off.

These results show that, after resuming the feed after the first shutdown period, it takes approximately 4 h before the gas production reaches the expected level. It also appears that, during the second period of feed interruption, the gas production rate declines relatively slowly from 0.50 m^3h^{-1} to 0.16 m^3 h^{-1} after 4 h, which is about 25% higher than the 0.12 m^3 h^{-1} measured 16 h after the feedless period. The gas production continued and remained at the same level for at least the next 4 h.

The very slow decline of the gas production rate during the second feedless period is, in fact, not surprising considering the characteristics of the waste, which is high in slowly biodegradable Suspended Solids.

Table 5. Relationship between the course of the gas production (ΔCH_4-week) and the applied organic space load (Q-week) during the preceding week as calculated on the basis of the 24-h loading rate average over a week at the two different process temperatures.

Organic space load ($kg\ COD\ m^{-3} day^{-1}$) Minimum	 Maximum	Weeks [a]	COD_{total} converted into methane (%)	$COD_{removed}$ converted into methane (%)
At a process temperature of 30 °C				
0.4	0.8	8	65.30	70.30
0.8	0.9	5	52.22	59.70
1.0	1.4	6	41.93	45.84
1.5	2.0	3	45.92	47.33
At a process temperature of 20 ° C				
0.2	0.7	6	90.30	121
0.9	1.0	4	64.60	71.91
1.1	1.5	6	50.40	55.84
1.5	2.0	4	47.60	51.91

a. Number of weeks for which load was applied.

Figure 6 also shows the existence of an almost linear relationship between the gas production at the weekend (CH_4-weekend per 48 h) and the loading potentials (Q-week) applied during the preceding week, at process temperatures of 30 °C and 20 °C. These results clearly indicate that, at both temperatures, the gas production rates during the weekend are dictated by the loading rates applied during the preceding week. Obviously, at both temperatures the digestion process still continues under unfed circumstances (Q-week = o) to a considerable extent. In addition, it seems that both the gas production rate and the amount of gas produced under unfed circumstances are always higher at a process temperature of 20 °C than at a temperature of 30 °C. This phenomenon can be explained on the basis that, under loading conditions, the conversion of the supplied COD_{total} into methane at a process temperature of 20 °C is distinctly less complete than at 30 °C.

As a consequence, a considerable amount of substrate ingredients is left in the reactor for digestion during the weekend. Thus, approximately nearly the same degree of stabilization - and consequently conversion into methane - is achieved over longer periods (weeks) of time at the two different temperatures, mainly as a result of 'completion' of the fermentation during the feedless weekends.

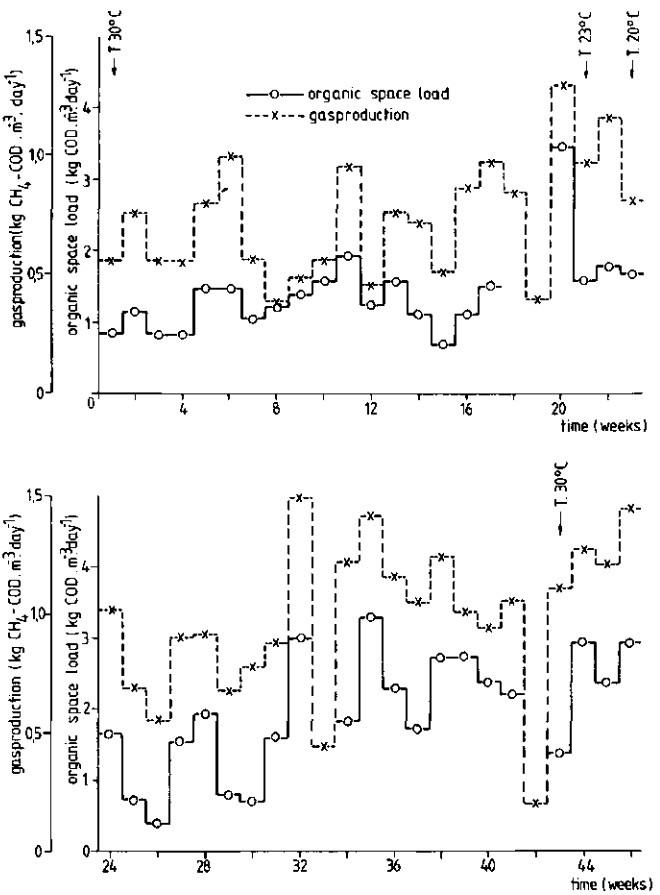


Figure 4. Applied organic space load and the course of the gas production, during the 24-h loading rate for the entire experimental period.

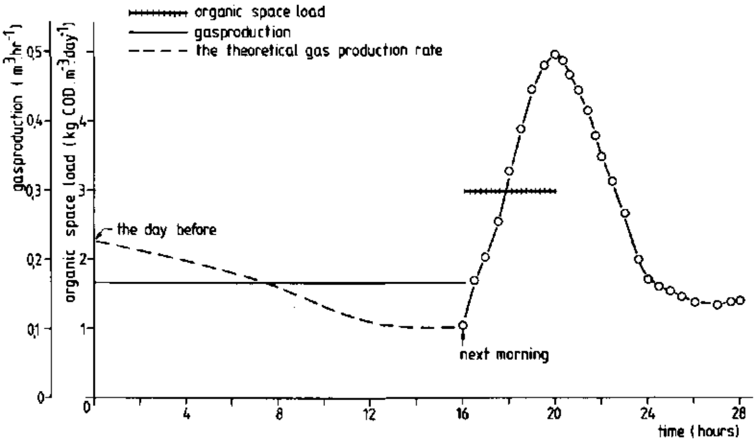


Figure 5. The course of the gas production during feed interruption periods: 0-16 h, the first feedless period; 16-20 h, feed applied and 20-28 h, the second feedless period. Theoretical gas production calculated from the average over 24-h of the day before (day 249), the start value, and the end value of the feedless period.

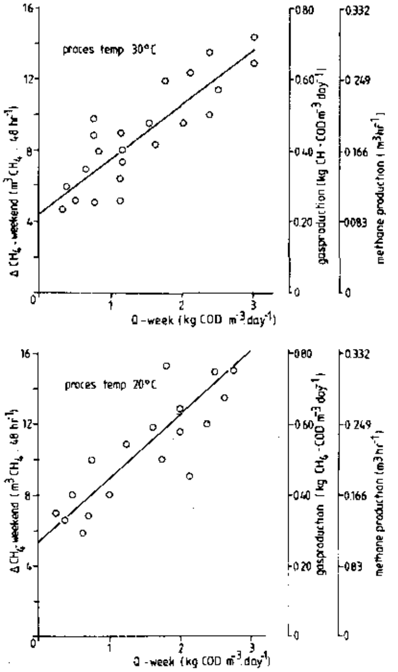


Figure 6. Relationship between the gas production at the weekend (ΔCH_4-weekend) and the organic space load (Q-week) applied during the preceding week.

Sludge wash out

The sludge wash out follows directly from the difference between the COD values of the filtered and unfiltered effluent samples. The results of the sludge wash out and the various loads applied are shown in Figure 7. At both 30 °C and 20 °C there exists a linear relationship between the applied organic loading rates and the extent of the sludge wash out. It appears that the sludge wash out at a temperature of 30 °C is slightly higher than at a temperature of 20 °C, but the differences between the two temperatures are not significant. Moreover, sludge washout does not occur at loading rates of 0.25 kg COD $m^{-3}day^{-1}$ at 30 °C and 0.1 kg COD $m^{-3}day^{-1}$ at 20 °C.

The sludge wash out apparently mainly occurs due to the increased gas production at higher loading rates. Under such circumstances, a significant expansion of the sludge blanket will come about, leading to a rather severe wash out of the finer dispersed sludge particles and also to an accumulation of sludge in the form of a scum layer in the settler.

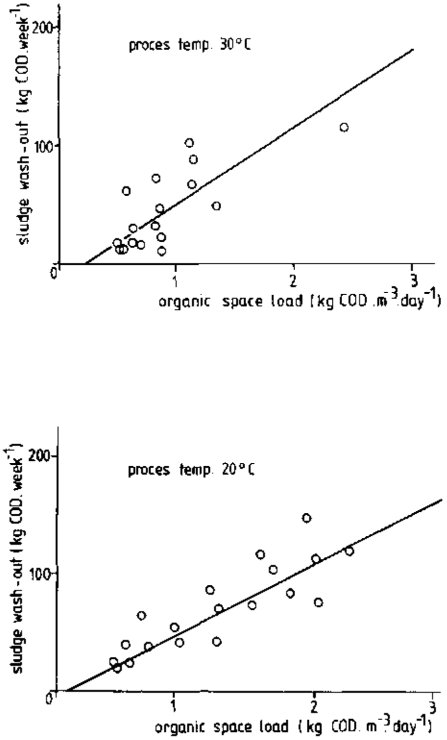


Figure 7. Relationship between the applied organic space load and the sludge washout, at process temperatures of 30 °C and 20 °C during the entire experimental period.

An additional reason for the sludge washout is the relatively high content of coarse Suspended Solids in the influent solution, even though most of the insoluble slowly biodegradable matter will be accumulated in the sludge bed.

Figure 8 shows the relationship between the influent COD-coarse Suspended Solids and the total weekly sludge wash out measured over the entire experimental period.

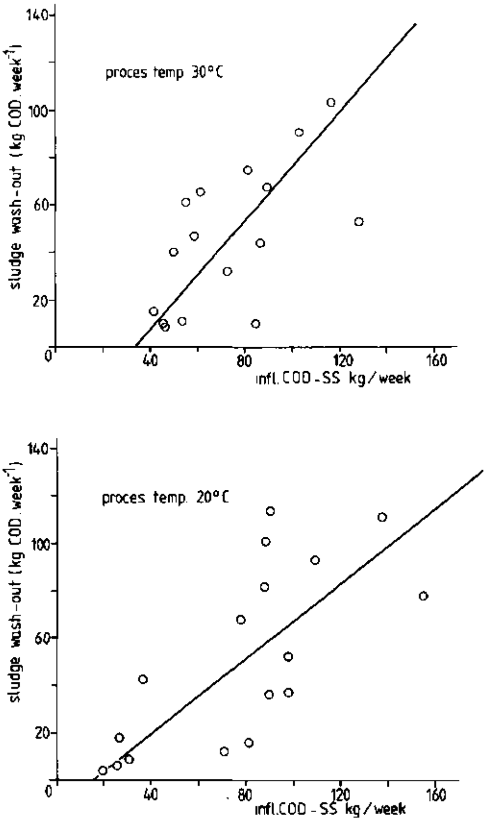


Figure 8. Relationship between the influent COD-coarse suspended solids and the total weekly sludge washout, during the entire experimental period.

COD balance and sludge accumulation

The COD balance, and the calculated sludge accumulation factor $(Y)_{acc.}$ during the entire experimental period at temperatures of 30 ^{o}C and 20 ^{o}C are summarized in Tables 6 and 7.

Table 6. COD balance, total sludge accumulation in the reactor at a process temperature of 30 °C, over the entire experimental period.

Week No.	kg COD $\text{infl.}_{\text{total}}$	kg CH_4-COD	(1) kg COD sludge washout	(2) kg COD removed	(3) sludge accumulation in the reactor as kg COD	(4) $(Y)_{\text{accumulation}}$
1	104.3	70.8	61.3	92.2	21.4	0.16
2	115.3	76.8	30.8	105.9	29.1	0.19
3	105.7	56.6	11.0	97.1	40.5	0.29
4	125.5	54.7	8.1	117.3	62.6	0.38
5	194.1	101.1	104.9	183.1	82.0	0.32
6	154.0	105.4	47.9	137.3	31.9	0.17
7	95.7	76.1	11.0	86.4	11.3	0.10
8	157.7	42.7	22.5	136.7	34.0	0.49
9	239.7	62.9	45.7	216.8	153.9	0.50
10	201.8	72.3	89.7	164.0	91.7	0.39
11	245.1	63.8	54.8	231.6	167.8	0.51
12	156.7	57.8	9.5	130.2	72.4	0.39
13	146.9	98.8	75.6	128.9	30.1	0.17
14	143.7	91.7	32.8	131.4	39.7	0.21
15	89.6	66.4	15.9	82.8	16.3	0.14
16	88.1	65.2	10.5	91.2	26.0	0.20
17	198.8	124.2	68.1	177.4	53.4	0.21
20	438.4	164.4	83.1	370.6	206.0	0.39
43	104.4	74.1	3.7	97.1	23.0	0.17
44	302.9	139.4	6.8	278.0	138.6	0.35
45	244.8	130.5	14.9	244.0	113.5	0.33
46	302.5	175.4	25.9	280.0	104.6	0.27
Total kg COD	3955.0	1971.4	823.6	3580.0	1581.5	

(1) kg COD sludge washout = kg COD $\text{effluent}_{\text{total}}$ - kg COD $\text{effl.}_{\text{filtered}}$.

(2) kg COD removed = kg COD $\text{influent}_{\text{total}}$ - kg COD $\text{effl.}_{\text{total}}$.

(3) sludge accumulation in the reactor = kg COD removed - kg CH_4-COD .

(4) $(Y)_{\text{acc.}}$ (sludge accumulation factor in the reactor) = kg COD sludge accumulation/ kg COD removed x 1/1.41 *

* 1 kg sludge VSS is equal to 1.41 kg COD.

The sludge accumulation factor does not distinguish between cell yield, entrapment and adsorption of the substrate ingredients.

According to the results in Tables 6 and 7 it appears that the calculated sludge accumulation factor $(Y)_{\text{acc.}}$ of 0.15 - 0.50 is significantly higher than the sludge accumulation factors mentioned in the literature (Versprille, 1979) for less complex, mainly soluble wastes; e.g. $Y_{\text{acc.}}$= 0.11 - 0.22 for potato-processing wastewater.

Table 7. COD balance, the total sludge accumulation in the reactor at a process temperature of 20 °C, over the entire experimental period.

Week No	kg COD infl.$_{total}$	kg CH_4-COD	(1) kg COD sludge washout	(2) kg COD removed	(3) sludge accumulation in the reactor as kg COD	(4) $(Y)_{accumulation}$
23	224.2	104.5	38.8	204.6	100.0	0.35
24	168.2	102.3	12.3	155.8	53.5	0.24
25	64.1	69.0	9.3	44.0	-25.0	-
26	34.2	55.6	4.3	32.8	-22.8	-
27	198.5	113.5	14.4	171.8	58.0	0.24
28	236.5	112.4	36.2	222.4	110.0	0.35
29	53.4	64.7	6.7	47.9	-16.8	-
30	62.2	54.9	19.7	54.2	-0.7	-
31	165.2	99.1	47.8	148.3	49.2	0.24
32	326.4	143.4	80.1	306.7	163.3	0.38
34	185.7	133.9	100.7	162.1	28.2	0.12
35	336.1	155.1	109.6	293.9	138.8	0.33
36	117.4	86.4	40.8	95.0	8.7	0.06
37	177.6	105.3	67.3	148.2	42.9	0.21
38	261.4	149.4	79.4	277.7	128.3	0.33
39	209.8	96.3	93.1	196.0	99.7	0.36
40	180.3	86.2	51.3	164.3	78.1	0.34
41	282.8	135.0	114.0	245.8	110.8	0.32
Total kg COD	3307.6	1866.9	925	2971.5	1017.7	

For footnotes - See Table 6.

Obviously, an important consequence of treating complex wastes such as slaughterhouse waste is the occurrence of a significant sludge accumulation of slowly biodegradable substrate ingredients. As a consequence, a considerable part of this sludge will consist of non-methanogenic sludge.

The data presented in Figure 9 also show that, at both 30 °C and 20 °C, a linear relationship always exists between the applied loadings and the sludge accumulation in the reactor. Apparently, any significant temperature dependency does not exist for the sludge accumulation, although at 30 °C, the excess sludge production is slightly higher at the loads applied. Under unfed circumstances a distinct degradation of the sludge occurs in the reactor and this will lead to a negative accumulation value.

The average drop in the retained sludge of the system appears to be about 30 kg of COD a week and 50 kg of COD a week at temperatures of 30 °C and 20 °C, respectively. These values could be expected on the basis of the gas production at the weekend, already mentioned.

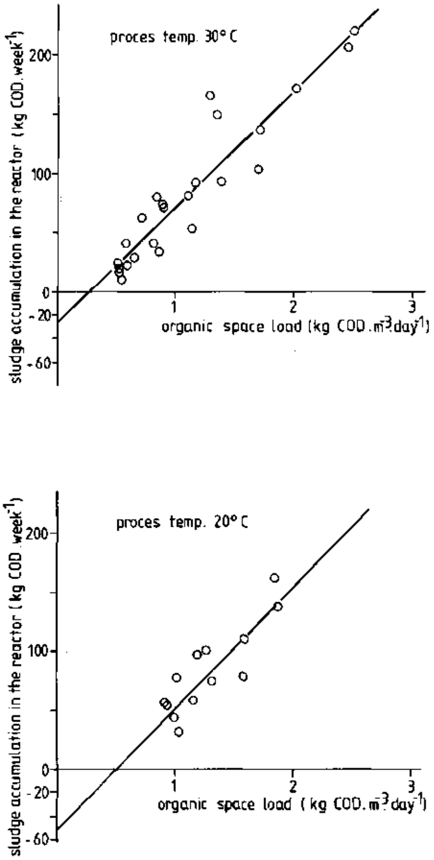


Figure 9. Relationship between the applied organic space load and the sludge accumulation in the reactor, at process temperatures of 30 °C and 20 °C during the entire experimental period.

Sludge retention and total methanogenic activity

The sludge development during the three phases over the entire experimental period is reflected in the results of the sludge profile measurements over the height of the reactor. Some profiles are shown in Figure 10. As almost the same pattern of sludge retention was obtained in all measurements, only a few figures are presented here for illustration. The results in Table 8 reveal the progress of the methanogenic capacity of the sludge in the reactor during the entire experimental period.

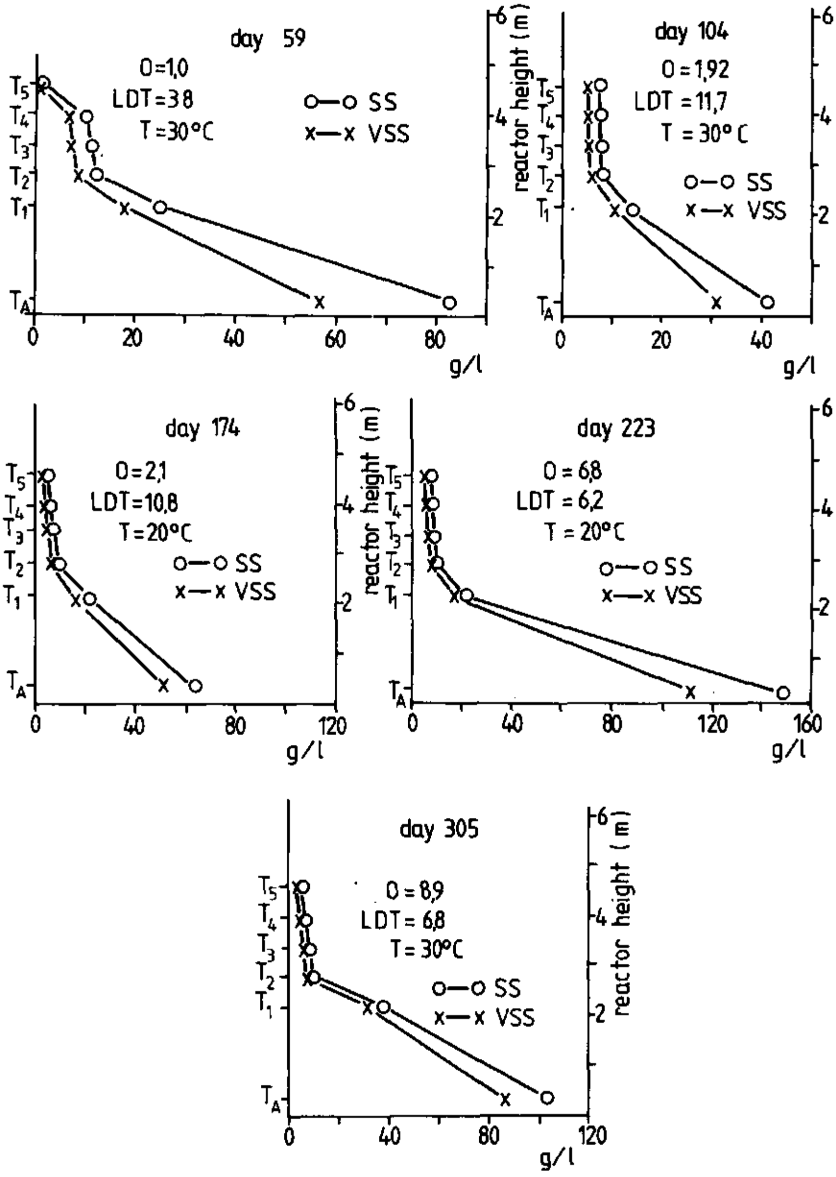


Figure 10. Sludge profiles as measured over the reactor height during various days of the experiment. O, organic space load (kg COD m^{-3} day^{-1}), HRT hydraulic retention time (h) and T, temperature (^{o}C). T_1, etc. taps shown in Fig. 2. T_A is the sludge-outlet pipe.

Table 8. Progress of the total amount of the sludge, the sludge concentration and the methanogenic capacity of the sludge during various days of the experiment.

Day No.	Organic space load (kg COD $m^{-3}day^{-1}$)	Methanogenic capacity (kg CH_4-COD kg^{-1}VSS day^{-1})	T (^oC)	HRT (h)	Total amount of sludge (kg TS)	Total amount of sludge (kg VSS)	Tap A[a] TSS (g $litre^{-1}$)	Tap A[a] Ash (%)	Tap 1[b] TSS (g $litre^{-1}$)	Tap 1[b] Ash (%)
49			30				44.1	44.2	14	32
56			30				41.2	28.9	15	31
59	1	0.05-0.07	30	38	524.1-671.7	296-464	82.5	31	25	29
70			30				60.5	25	17	22
96			30				43.8	22.6	16	24
104	1.92	0.10-0.14	30	11.7	252.7-368	187-277	41	23	14.2	27
117							40.2	25	16	21
154		0.15 [c]					61.6	21.1		
174	2.1	0.08-0.15	20	10.8	324.4-542.7	244-418	64.5	20	21	22
223	6.8	0.06-0.12	20	6.2	509-840	389-793	100	21.0	21.3	20.5
305	8.9	0.10-0.15	30	5.8	555-834	449-680	103	17	38.5	18

a. Tap A is 28 cm from the bottom of the reactor (See Figures 2 and 10).

b. Tap 1 is 219 cm from the bottom of the reactor.

c. Results obtained from batch experiments: sludge discharged from tap 1; feed, VFA mixture consisting of acetate, propionate and butyrate.

T Process temperature.

Apparently, according to the measured shapes of the sludge profiles, even at low loading rates the sludge bed is expanded over almost the entire reactor height, mainly as a result of the gas production. The flocculent sludge was quite satisfactorily retained in the system, even at a hydraulic retention time of less than 6 h. This is shown by the results summarized in Table 8. The total amount of sludge in the reactor increased during the relatively short period of the investigation from 275 kg organic sludge at the start of the experiment to 500-600 kg organic sludge at day 305. Moreover, from the TSS and VSS measurements of the sludge, it is evident that there is a gradual decline in ash content, from 44.2 % of the sludge present at the bottom of the reactor (Tap A) at the start of the investigation to 25% around day 70 and only 17% at day 305.

The activity of the sludge increased from the rather low specific methanogenic activity of 0.04 - 0.05 kg CH_4-COD kg^{-1}VSS day^{-1} (de, Zeeuw & Lettinga, 1980) at the start of the experiment to a constant value of 0.10 - 0.15 kg CH_4-COD kg^{-1}VSS day^{-1} at days 104-174. Also, the F-420 concentration measurements presented in Figure 11 show a fairly gradual increase of the F-420 content (mol of F-420 per gram VSS) of the anaerobic sludge.

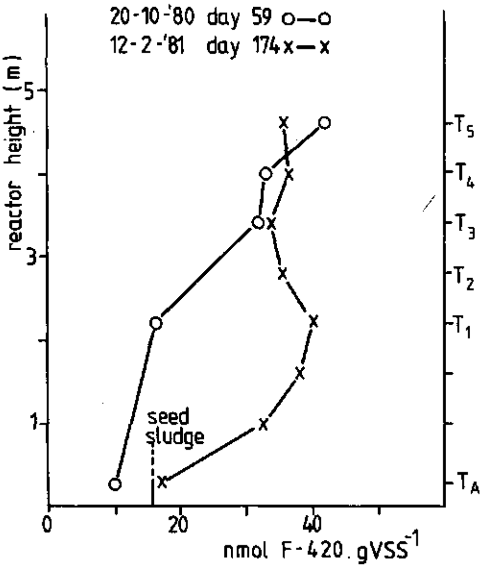


Figure 11. Progress of the methanogenic activity (F-420 profiles) over the reactor height during various days of the experiment. For T_1, etc., see Fig. 10.

The F-420 is exclusively a co-enzyme found in methanogens (Eirich et al., 1979) and, as an electron transfer co-enzyme, F-420 is involved in the hydrogenase system.

In spite of the fact that the sludge has a low specific activity in comparison with the high specific methanogenic activity of the granular sludge found in treating sugarbeet wastewater (de, Zeeuw et al., 1981), satisfactory treatment efficiencies were obtained during these experiments using digested sewage sludge as seed.

DISCUSSION

The performance of the reactor at an intermittent feeding

Considering the results obtained in the present investigation, a one-stage anaerobic treatment using UASB process seems feasible for complex low strength wastes like slaughterhouse wastewater. The method of operation chosen provides the opportunity for effective digestion of the accumulated substrate ingredients in the reactor during the feedless periods (weekend) and/or periods of decreased load at night.

In order to illustrate the feasibility of the method of operation chosen in this study, the data for the COD_{total} conversion percentages into methane are summarized in Table 9, together with the data concerning methane production during feedless periods (complete fermentation). The results in Table 9 show a lower COD_{total} conversion into methane of approximately 12-19%, during the high loads day time.

Table 9. The calculated percentages of the COD_{total} converted into methane and the complete fermentation during the feedless periods at various phases of the experiment.

Period	COD_{total} converted into methane (%)		Complete fermentation rate (kg CH_4-COD $m^{-3}day^{-1}$)	
	20 °C	30 °C	20 °C	30 °C
Daytime (8h)	12.0	19.0	0.53	0.39
Natural day (24 h)	20.5	34.5	0.40	0.10
Week (7 days)	32.5	36.0	0.25	0.16
Month	40.5	47.5	0.18	0.11
Entire experimental period	59.0	54.0		

However, the conversion increases considerably, to 20.5 - 34.5%, when calculated on a 24 h basis. The COD_{total} conversion percentages into methane are even higher when calculated on a weekly basis (including the weekend) and over the entire experimental period (including weekends and the long feedless periods).

No clearly defined start up difficulties were observed after feed interruptions, even not at interruptions of a few weeks. From this it may be concluded that the UASB process may become of great practical importance for situations where large daily and/or weekly variations occur in the pollution load, the more so because the installation of an equalization tank can be avoided, or at least its size can be reduced significantly.

Performance at process temperatures of 20 °C and 30 °C

Digested sewage sludge is a suitable seed material for treating low strength complex wastes, such as slaughterhouse waste, because organic space loads up to 3.5 kg COD $m^{-3}day^{-1}$ at a hydraulic retention time of 7 h are satisfactorily accommodated (up to 70% COD reduction) at temperatures as low as 20 °C. A COD_{total} reduction of approximately 70% is smoothly approached in the third week after the start of the pilot-plant (Figure 3). Methane formation starts immediately after the commencement of feeding with the slaughterhouse wastewater. This observation demonstrates that the anaerobic bacteria present in the digested sewage sludge are capable of metabolizing the digestible components of the waste without any preadaptation.

In spite of the fact that the η_{total} drops sharply, due to sludge wash out, in comparison with $\eta_{filtered}$ ($\eta_{filtered}$ 90%; η_{total} 70%), this lower treatment efficiency should not necessarily lead to the conclusion that the UASB process is less attractive for complex wastes treatment than conventional anaerobic treatment systems. Moreover, the relatively high settling properties of the excess sludge present in the effluent solution will make it possible to discharge the effluent under practical conditions via a final tank which can be used as a combined settling tank and digestion tank.

Sludge retention and sludge wash out

Although the sludge retention of the process was fairly satisfactory in these experiments, efforts should still be made to minimize the sludge wash out from the UASB reactor (kg VSS per m^{-3} reactor), because a great part (67%) of the excess sludge rinses from the reactor either via the effluent as Suspended Solids or in the form of increasing scum layers. The data presented in Table 10 summarize the percentage of influent COD_{total} converted into methane COD, the total excess sludge production as percentage of the supplied COD_{total} and the sludge wash out expressed as percentages of the total amount of excess sludge produced at process temperatures of 30 °C and of 20 °C.

Table 10. The percentage of influent COD_{total} converted into methane, the total excess sludge production as a percentage of the supplied COD_{total} and the sludge washout as a percentage of the total amount of the excess sludge at process temperatures of 30 °C and 20 °C.

Parameters		Process temperature 20 °C	Process temperature 30 °C		
		Weeks 23-41	Weeks 1-20	Weeks 43-46	Weeks (1-20)+(43-46)
Methane-COD	%	56.4	48.4	54.4	49.8
Total excess sludge production	%	31.0	40.7	37.8	40.0
Sludge wash out	%	91.0	63.3	14.2	52.1

It should be pointed out in this connection that, during the entire experimental period, no sludge was discharged from the reactor. As a result, the sludge bed volume gradually increased and sludge ultimately filled up the complete reactor. Consequently, the wash out of the sludge gradually approached the total excess sludge production. In order to avoid such a situation, from time to time a certain amount of the sludge should be discharged from the reactor. The data in Table 8 show that the sludge concentration at the bottom of the reactor (Tap A) is higher than at the top of the reactor (Tap 1); 85.5 g VSS $litre^{-1}$ versus 31.6 g VSS $litre^{-1}$, respectively. As the sludge at the top of the reactor is relatively thin, it is recommended that the excess sludge should be discharged here for the sake of achieving as high a sludge retention in the reactor as possible.

The rate of liquefaction in the reactor

Considering the results obtained so far it can be stated that the limiting factor for the application of a one-stage anaerobic (UASB) treatment for complex wastes such as slaughterhouse waste is the relatively low rate of liquefaction. This is particularly true for low temperatures such as 20 °C, as the rate of liquefaction drop sharply with the temperature. The effect of the slow rate of liquefaction is clearly reflected in the results obtained at the various loading rates applied, in the low gas production and the methanogenic capacity of the sludge, as well as the development of the scum layer. The effect of the low rate of liquefaction on the conversion of the substrate into methane is illustrated in Figures 12 and 13 where the available experiments data of the volumetric CH_4-COD gas production has been plotted in relation to the applied 8 h and 24 h organic space load for 30 °C and 20 °C, during the entire experimental period. It appears from these figures that a higher conversion of COD_{total} into methane is obtained at a temperature of 30 °C than at 20 °C for loading rates exceeding 1.5 kg COD $m^{-3}day^{-1}$. This phenomenon can mainly be attributed to the slower microbial rate of the hydrolytic enzymes at 20 °C than at 30 °C. Contrarily, at loading potentials below 1.5 kg COD $m^{-3}day^{-1}$ the conversion of COD_{total} into methane is less complete at a process temperature of 30 °C than at 20 °C. These observations are in accordance with the results concerning the amount of CH_4 gas produced (continuing fermentation) under unfed circumstances at the weekend. A smaller amount of accumulated substrate materials was left in the reactor for digestion at a temperature of 30 °C than at 20 °C. The effect of the low rate of liquefaction is also shown in the observed scum layer formation.

Over the whole 46 weeks of the experimental period, up to 4.2% of the supplied influent COD_{total} accumulated in a scum layer, of which up to 40%, on a Dry Suspended Solids (DSS) basis, consisted of lipids, which due to their strong tendency towards flotation, enhance the formation of scum layers. From these data it is also clear that the DSS of the scum layer are relatively poorly stabilized. In view of the scum layer formation, the settler compartment should be equipped with a skimmer for removing these materials to enable them to be digested in a separate sludge digester.

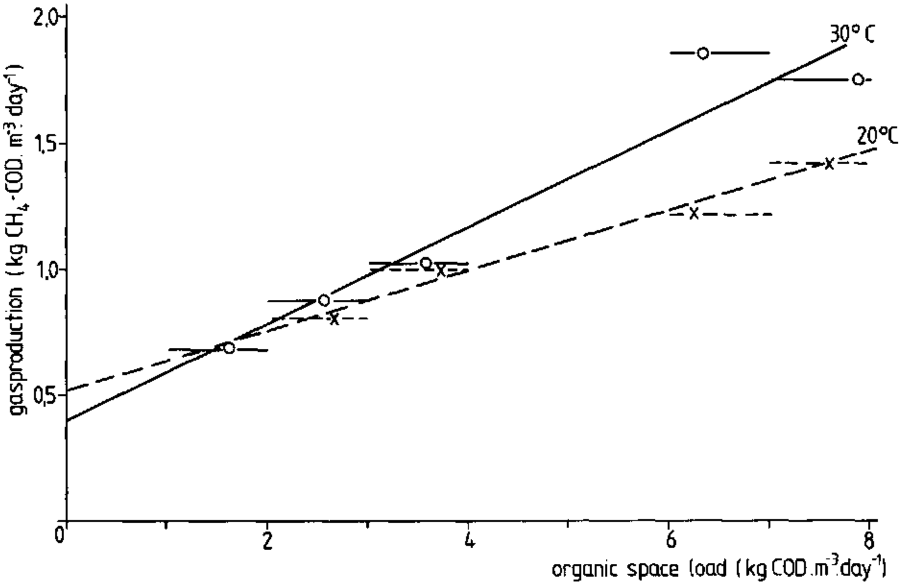


Figure 12. Relationship between the methane-COD production rate and the applied organic space load during the 8-h loading rate at process temperatures of 30 °C and 20 °C.

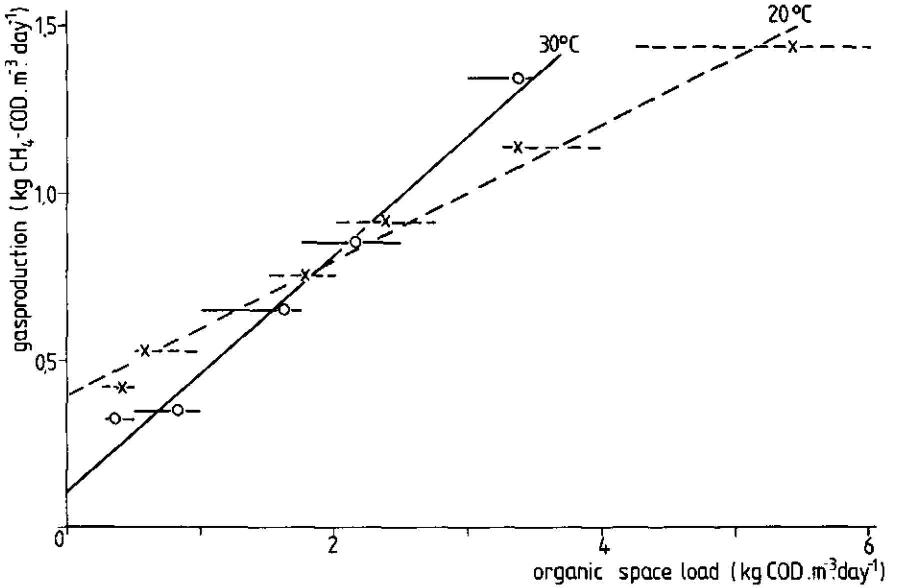


Figure 13. Relationship betwen the methane-COD production rate and the applied organic space load, for the main average 24-h loading rate, at process temperatures of 30 °C and 20 °C.

ACKNOWLEDGEMENT

This investigation was supported by a grant from the Dutch Ministry of Public Health and Environmental Affairs.

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Biological Wastes, 21 (1987) 11-28

CHAPTER 3

ANAEROBIC TREATMENT OF SLAUGHTERHOUSE WASTE USING A GRANULAR SLUDGE UASB REACTOR

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ABSTRACT

This study was carried out to assess the feasibility of the upflow granular anaerobic sludge blanket (UASB) process for one-stage anaerobic treatment of unsettled slaughterhouse wastewater, which contains approximately 50% insoluble coarse suspended COD. The experiments used continuous feeding with a constant organic load 24 h a day during the working days with a weekend feed interruption at 30 °C and 20 °C.

An organic space load up to 11 and 7 kg COD $m^{-3}day^{-1}$ *was satisfactorily accommodated at process temperatures of 30 °C and 20 °C, respectively, with a treatment efficiency up to 55% on* COD_{total} *basis and 85 % on* $COD_{filtered}$ *basis. The system was less effective in the removal of the coarse Suspended Solids than the removal of the colloidal and soluble fractions. The data indicate that a proper application of a one-stage granular UASB system treating unsettled wastewater, such as slaughterhouse waste, strongly depends on the processes involved in the removal of the colloidal and soluble fraction from the waste and on its conversion into methane. Under the optimal loading conditions of 11 kg COD* $m^{-3}day^{-1}$ *(30 °C) and 7 kg COD* $m^{-3}day^{-1}$ *(20 °C) the conversion of removed colloidal and soluble* *materials into methane was up to 87% and 82%. However, the system still performed very satisfactorily in the removal of the colloidal and soluble pollutants up to loading rates of 15 and 19 kg COD $m^{-3}day^{-1}$ at 30 °C and 20 °C, respectively, although the conversion of the removed organic matter into methane dropped dramatically, rendering the application of the process under these conditions unattractive, if not impossible.*

INTRODUCTION

The environmental protection authorities in The Netherlands require that the discharge of wastes to surface waters meets stringent effluent standards. These effluent standards have to comply with the basic quality for surface waters according to 1980-84 water pollution control programmes and correspond to maximum pollutant concentrations of 40 and 30 mg $litre^{-1}$, respectively, for BOD_5 and Total Suspended Solids.

From a survey paper by Ten Have (1976) concerning meat-processing plants and slaughterhouses, it appears that the pollution capacity of these industries presently exceeds 1,000,000 population equivalents. Most of these plants discharge their waste waters to a municipal treatment system. In order to comply with water pollution control standards and to save money on sewer discharge, slaughterhouses and meat-processing plants are required to apply an adequate pretreatment to their discharge.

Primary treatment by physical and/or chemical methods can be used in both types of industries (Funke, 1969). Frequently, a combination of screening, centrifugation, sedimentation, air flotation and flocculation precipitation for pretreating the wastes is used. In addition, mechanical primary treatment devices, such as static and vibrating screens, hydracyclones, air flotation units for grease recovery and clarifiers, can be applied in these industries (Witherow & Lammers, 1976).

Secondary treatment processes for the biological decomposition of the organic pollutants, nitrogen removal and solids settling system have been investigated. The biological processes include anaerobic lagoons (Rotlag & Dornbush, 1966), aerobic lagoons (Steffen, 1961) and anaerobic digestion (Black & Brown, 1974). Combinations of these systems are needed since no single secondary treatment process will provide an effluent suitable for discharge. All these treatment methods have distinct disadvantages, either in odour nuisance or in investment and operating costs. Obviously, there is a considerable need for simple, inexpensive and efficient wastewater treatment methods. Therefore, considerable efforts have been made in recent years in The Netherlands toward the development of a more sophisticated anaerobic treatment process suitable for treating low strength wastes such as slaughterhouse and meat-processing wastes.

These efforts have resulted in the application of the upflow anaerobic sludge blanket (UASB) process (Lettinga & Van Velsen, 1974; Lettinga, 1978). The technology developed may be helpful in solving the problems encountered with the treatment of wastewaters from slaughterhouses and the meat-processing industry.

In a previous paper (Sayed et al., 1984) we reported the results obtained in a one-stage flocculent sludge blanket reactor using a 25.3 m^3 pilot plant. Although the results were satisfactory in terms of treatment efficiency, it also turned out that a flocculent sludge UASB system can handle only low to moderate loading rates (up to 3.5 kg COD $m^{-3}day^{-1}$ at 30 °C) when applied during the working days and combined with weekend feed interruptions. A considerably higher loading potential may be expected in using granular sludge UASB reactors, because granular sludge cultivated on some types of mainly soluble industrial effluents generally exerts a significantly higher methanogenic activity than the digested municipal sewage sludge that was used for starting up the 25.3 m^3 pilot plant. As gradually more excess granular sludge becomes available from existing full-scale UASB plants, the application of a reactor seeded with granular sludge might become an attractive alternative for sludge blanket reactors. The main objectives of the present investigation therefore were to assess the loading potentials of granular sludge blanket reactors (at 30 °C and 20 °C) and the limiting factors, as well as to assess whether or not the granular character of the sludge can be maintained with unsettled slaughterhouse wastewater as feed and to what extent flocculent excess sludge will segregate from the granular seed sludge.

METHODS

Sampling and analysis

All the samples taken for chemical analysis were 24 h composites. Dry Suspended Solids (DSS), Volatile Suspended Solids (VSS), total nitrogen, phosphate, Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were determined according to standard methods (Standard Methods, 1980). Volatile Fatty Acids (VFA) analyses were carried out gas chromatographically using a Packard Becker Model 417 equipped with a 6 m x 2 mm glass column and a flame ionisation detector. The glass column was packed with Chromosorb 101 (60-80 mesh). Flow rate of the carrier gas, nitrogen saturated with formic acid, was 18 ml min^{-1}. Column temperature was 210 ^{o}C and the injection port temperature was 230 ^{o}C. The COD for the raw wastewater is expressed as COD_{total}. The coarse Suspended Solids in the raw wastewater were separated using a filter (Schwarzband black ribbon No. 589^{1} Ref.No. 300011) with a pore size of 7.4 μm. The filtrate COD of the wastewater consists of colloidal, as well as soluble, fractions. Gas samples removed from the gas sampling port were analysed immediately for CH_4 content using a Packard Becker gas chromatograph Model 409 equipped with two columns - a Porapak (2m x 3mm) and a stainless steel column with molecular sieve 5A (1m x 3mm) for the determination of methane, carbon dioxide, nitrogen and hydrogen. Helium was used as a carrier gas (20 ml min^{-1}). Oven temperature was 70 ^{o}C and column temperature 75 ^{o}C. The methanogenic capacity of the sludge was calculated from the methane production and the amount of sludge present in the reactor and expressed as kg CH_4-COD per kilogram of VSS per day.

Seed sludge

The granular sludge used in these experiments was a sugar-beet wastewater cultivated sludge obtained from a UASB pilot plant of the Centrale Suiker Maatschappij (CSM) (sugar factory) at Breda, The Netherlands. The Total Suspended Solids (TSS) content of the sludge was 10.2 % and the VSS content 8.5 %. The maximum specific activity of the sludge for VFA decomposition, as measured in a standard batch-fed experiment using a feed mixture of 600 mg $litre^{-1}$ each of acetic acid, propionic acid and butyric acid, was 0.56 kilogram of CH_4-COD per kilogram of VSS per day at 30 ^{o}C.

Each reactor was seeded with 12 litres of granular sludge, i.e. 36.5 g TSS sludge per litre reactor volume.

Wastewater characteristics

Wastewater from slaughtering areas was collected after passing a screen installed to separate dispersed particles larger than 1 mm. The composition of the wastewater (a mixture of grease, protein, blood, intestinal contents and manure) depends considerably on the production process and the type of animals slaughtered. The major waste load originates from the slaughtering, which is a one-shift operation from Monday through Friday. The most important characteristics of the wastewater are presented in Table 1. The wastewater is an unsettled low-strength complex-type waste. Of the pollutants, 40%-50% is present as insoluble and slowly biodegradable coarse suspended matter (Fig. 1).

Table 1. Some characteristics of the wastewater.

COD_{total}	1500 - 2200 mg $litre^{-1}$
Coarse suspended solids as a percentage of COD_{total}	40 - 50 %
BOD_{total}	490 - 650 mg $litre^{-1}$
VFA-COD (% of total COD)	12 - 15 %
Fats	50 - 100 mg $litre^{-1}$ (5% of the total solids)
Nitrogen (N) Kjeldahl	120 - 180 mg $litre^{-1}$
Phosphate $(P)_{total}$	12 - 20 mg $litre^{-1}$
Temperature	20 °C
pH	6.8 - 7.1

Experimental

The experiments described here mainly concern those of two identical UASB reactors operated in parallel at two different process temperatures, viz. reactor A at a process temperature of 30 °C and reactor B at a process temperature of 20 °C. An operational temperature of 20 °C was chosen because it is the temperature at which the wastewater is discharged.

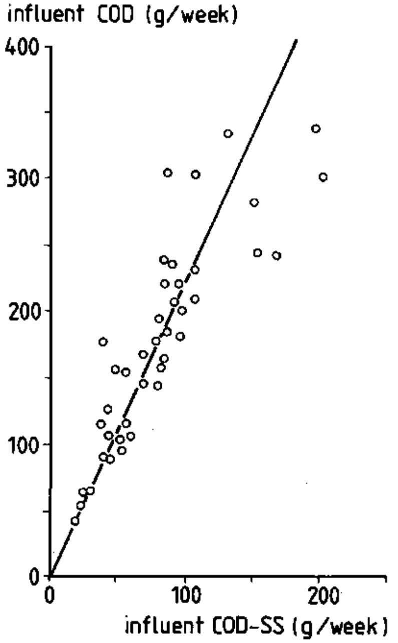


Figure 1. Relationship between the influent COD_{total} and the influent COD-coarse suspended solids during the entire experimental period.

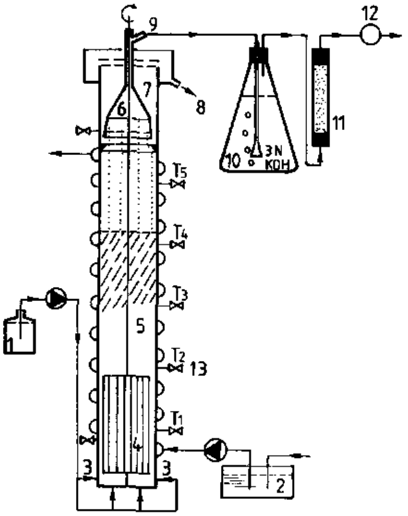
Both reactors were operated under semi-continuous conditions, viz. continuous feedings with a constant organic load 24 h a day during the working days combined with weekend feed interruption. A schematic diagram of the UASB reactor set-up used in this study is shown in Fig. 2.

The operational procedure was according to the upflow anaerobic sludge blanket (UASB) process (Lettinga et al., 1980). Feed was introduced at the bottom of the reactor, thus forcing the wastewater upward through the sludge bed, present in the lower part of the reactor. Both reactors had a total volume of 33.5 litres, an internal diameter of 0.19 m and were 1.30 m in height. The reactors were equipped with a stirring assembly of a radial flow impeller type. Mechanical mixing to prevent short-circuiting was done intermittently, usually one rotation per 15 min at 20-40 rpm. The reason for keeping the mechanical mixing at a minimum is that we consider high mixing intensities as detrimental for the sludge settleability. The 'natural' mixing caused by the gas production is sufficient, provided the digestion process proceeds satisfactorily and the organic load exceeds

5 kg COD m^{-3} day^{-1}. No sludge recirculation was applied.

Both reactors were equipped in the upper part with a proper gas-liquid-solid separator for separating and collecting the produced biogas. The installed 100-litre equalizing vessel provided a sufficient storage capacity to enable the experiments to be carried out over a full 24 h period, with the same feedstock solution. The wastewater in the equalizing vessel was continuously stirred. The temperature of both reactors was controlled with the aid of two thermostats; heated water was pumped through an external tubing placed around the reactor.

Due to unforeseen technical problems the experiments had to be interrupted during the weeks 4 and 5 for reactor A (30 °C) and during week 17 for reactor B (20 °C).



1. equalizing vessel of the influent
2. thermostat; temperature control influent
3. influent inlet points
4. stirrer
5. sludge bed (digester compartment; 33.5 litre)
6. gas collector
7. settler
8. effluent discharge
9. gas outlet
10. CO_2 (and H_2S) stripping device
11. granular soda lime
12. wet gasmeter
13. sampling taps.

Figure 2. Schematic diagram of the pilot plant UASB reactor.

RESULTS

Performance of the UASB reactors in terms of COD reduction

The experimental results obtained under the various loading conditions applied for both reactors are summarized in Table 2.

Table 2. The treatment efficiency of the total and filtered COD and the coarse suspended solids reduction during the various phases of the experiments.

Phase	Number of days [a]	Influent COD (mg litre^{-1})		Organic space load (kg COD m^{-3} day^{-1})	Hydraulic retention time (h)	% COD reduction [b]		Coarse % COD$_{SS}$ reduction [c]
		Total	Filtered			Total	Filtered	
Reactor A: At a process temperature of 30 °C								
I	13	1086	557	2.5- 4.0	9	55	85	50
II	7	1310	709	6.0- 6.2	5	53	83	54
III	8	2724	617	11.0- 16.0	5	55	82	54
IV	7	1270	695	7.0- 8.0	4.3	67	87	45
V	14	1412	765	10.0- 10.5	3.2	54	87	44
VI	14	1377	780	14.0- 15.0	2.2	53	82	30
VII	7	1380	780	19.0- 19.5	1.7	59	85	32
Reactor B: At a process temperature of 20 °C								
I	11	1337	735	3.0- 4.0	10	56	68	52
II	24	1478	697	4.0- 5.0	10-8	62	89	55
III [d]	25	1626	815	6	6-7	45[d]	91	45
IV	17	1750	1262	8.5	5	50	83	46
V	45	1546	727	7.0- 9.0	5.5-6	55	81	24
VI	18	1793	694	11.0- 12.0	5.5-5	40	79	23

a. Number of days for which loading was applied.

b. [1 - total COD (effluent)/total COD (influent)] x 100 and [1 - filtered COD (effluent)/total COD (influent)] x 100 .

c. [1 - COD_{SS} (effluent)/COD_{SS} (influent)] x 100 .

d. Due to technical problems this results is unreliable.

Both reactors were started at an organic space load of 3 kg COD m^{-3} day^{-1} corresponding to 0.08 kg of COD per kilogram of VSS per day and at a hydraulic retention time of 10 h. After a stable operation had been achieved the loading was increased stepwise. Both reactors performed quite satisfactorily in removing the $COD_{filtered}$ fraction, even at a space load up to 19 and 11 kg COD m^{-3} day^{-1} at process temperatures of 30 °C and 20 °C respectively. The average treatment efficiency of the $COD_{filtered}$ fraction for both reactors during the entire experimental period was 85%. With regard to the removal of the total COD, it appears that reactor A (30 °C) performed slightly better than reactor B (20 °C). However, for both systems the purification efficiency, based on COD_{total}, is significantly lower than the efficiency based on $COD_{filtered}$. As appears from Table 2, the relatively poor COD_{total} treatment efficiency is mainly due to the poor coarse Suspended Solids removal.

Gas production

Table 3 presents the results of the measured volumetric CH_4-COD gas production rate and the calculated percentage conversion of the imposed COD_{total} into methane over the entire experimental period for both reactors. In order to illustrate any possible effect of the space loading rate, the gas production rate has also been plotted versus the applied organic space loading rate in Fig. 3.

The plots in Fig. 3 reveal a linear relationship between the methane production rate and the applied organic loading rates in the lower range of the loading conditions for both temperatures. Under these conditions a slightly higher conversion of the imposed COD into methane was obtained at a process temperature of 30 °C as compared to 20 °C. However, at organic loads exceeding 11 and 7 kg COD $m^{-3}day^{-1}$ for process temperatures of 30 °C and 20 °C respectively, the conversion of influent COD into methane COD declines and even decreases at loading rates higher than 15-16 kg COD $m^{-3}day^{-1}$ and 8-9 kg COD $m^{-3}day^{-1}$ for 20 °C.

Table 3. The coarse of the volumetric methane production rate and the percentage of the imposed COD$_{total}$ converted into methane, as calculated on the basis of the average 24-h loading rate after grouping days of the entire experimental period at 30 °C and 20 °C according to the minimum and maximum limits given.

Organic space load (kg COD $m^{-3} day^{-1}$) Minimum	Maximum	Average	Volumetric methane production (kg CH_4-COD $m^{-3} day^{-1}$)	COD$_{total}$ converted into methane (%)
Reactor A : At a process temperature of 30 °C				
1	2	1.60	0.68	42.5
2	3	2.40	0.82	33.8
3	4	3.50	1.64	46.9
4	5	4.30	2.10	48.6
6	7	6.35	3.20	50.9
7	8	7.55	3.81	50.3
9	10	9.95	4.20	42.3
10	11	10.70	5.22	48.9
11	12	11.40	4.42	38.8
12	13	12.10	5.20	43.4
13	14	13.80	5.10	36.9
14	15	14.55	4.40	30.3
15	16	15.45	5.33	34.5
16	17	16.15	4.30	26.6
19	20	19.65	2.15	10.9
Reactor B : At a process temperature of 20 °C				
1	2	1.75	0.63	36.0
2	3	2.60	1.24	47.9
3	4	3.55	1.39	39.2
4	5	4.45	1.91	42.8
5	6	5.40	2.41	44.8
6	7	6.40	2.58	40.5
7	8	7.55	3.20	42.4
8	9	8.60	2.72	31.6
9	10	9.15	2.52	27.5
11	12	11.20	2.11	20.0
12	13	12.30	1.70	13.8
13	14	13.30	1.22	9.2

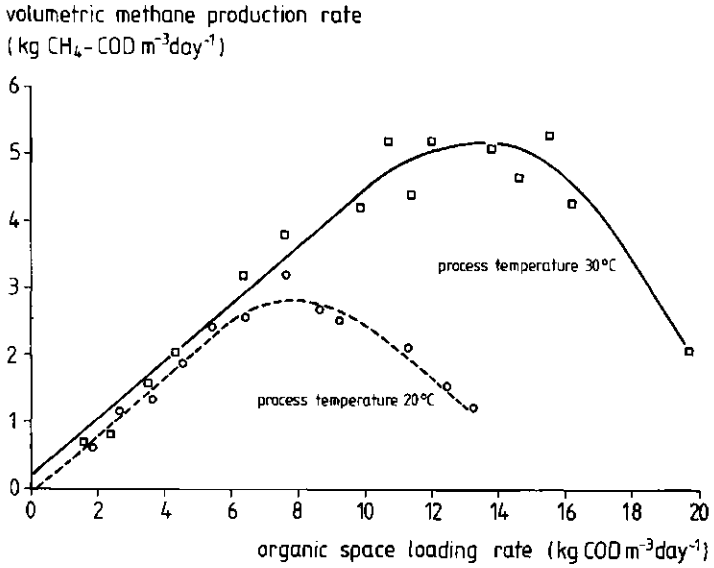


Figure 3. Applied organic space loading rate and the course of the volumetric methane production rate during the entire experimental period.

Table 4 presents the calculated percentages of the supplied COD_{total} and the $COD_{removed}$ converted into CH_4-COD, together with the calculated factor M of the $COD_{filtered}$ removed converted into methane for the various 24-h COD loading conditions applied. The data in Table 4 indicate that, under optimal loading conditions, most of the removed COD is converted into methane, viz. an average 87% for loading rates below 12 kg COD $m^{-3}day^{-1}$ at a process temperature of 30 ^{o}C and 82% for loading rates below 8 kg COD $m^{-3}day^{-1}$ for process temperature of 20 ^{o}C. The proportion of the removed COD converted into methane decreases sharply at higher loading rates.

The results of the measured average weekend methane production are presented in Fig. 4, together with 24-h loading rates (organic and hydraulic) applied the day preceding the weekend (Q-day). The measured weekend CH_4 production rates show a clear minimum at Q-day loads of approximately 11 and 7 kg COD m^{-3} day^{-1} at 30 ^{o}C and 20 ^{o}C process temperatures, respectively.

Table 4. The percentage of COD_{total} and $COD_{removed}$ converted into methane and the calculated factor M; the calculation is based on the average 24-h loading rate applied at the various phases of the experiments.

Phase	Number of days [a]	Organic space load (kg COD m^{-3} day^{-1})	Hydraulic retention time (h)	$COD_{removed}$ converted into methane (%)	COD_{total} converted into methane (%)	Factor M^b
Reactor A : at a process temperature of 30 °C						
I	13	2.5 - 4.0	9	73	40	1.03
II	7	6.0 - 6.2	5	123	65	1.48
III	8	11.0 - 16.0	5	74	41	2.90
IV	7	7.0 - 8.0	4.3	85	57	1.36
V	14	10.0 - 10.5	3.2	87	51	1.20
VI	14	14.0 - 15.0	2.2	68	37	0.83
VII	7	19.0 - 19.5	1.7	17	15	0.28
Reactor B : at a process temperature of 20 °C						
I	11	3.0 - 4.0	10	52	39	1.14
II	24	4.0 - 5.0	10-8	88	54	1.21
III	25	6	6-7	109	49	0.95
IV	17	8.5	5	82	41	0.74
V	45	7.0 - 9.0	5.5-6	75	44	1.16
VI	18	11.0 - 12.0	5.5-5	60	35	0.61

a . Number of days for which loading was applied.

b . Factor M = COD-methane/$COD_{filtered}$ removed.

The results of the cumulative gas production measured during the prolonged period of feed interruptions are presented in Fig. 5 for both reactors.

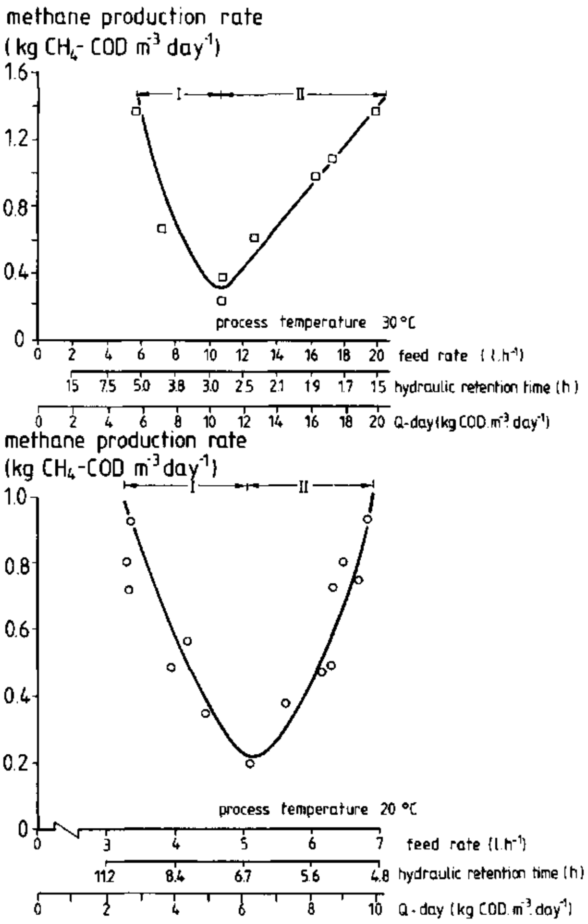


Figure 4. Relationship between the methane production rate at the weekend and the loading rates (organic and hydraulic) applied during the day preceding the weekend.

COD balances and sludge accumulation in the reactor.

The COD balance data are presented in Table 5, together with the calculated sludge accumulation factor $(Y)_{acc}$. The $(Y)_{acc}$ does not distinguish between cell yield, entrapment and adsorption of the substrate ingredients.

Sludge retention in the reactor

The total sludge retention of the reactors can be estimated from the sludge profiles measured over the height of the reactors. For illustration some of these profiles are presented in Fig. 6.

Table 5. COD balances, total sludge accumulation in the reactor and the coarse suspended solids removed at three different loading rate ranges for both reactors (30 °C and 20 °C).

Loading rate ranges (kg COD m^{-3} day^{-1})		g COD $infl._{total}$	g COD $effl._{total}$	g COD $effl._{filtered}$	(1) g COD sludge washout	(2) g COD removed	g CH_4-COD	(3) sludge accumul. in the reactor as g COD	(4) $(Y)_{acc.}$	(5) Coarse suspended solids removed (%)
Reactor A : At a process temperature of 30 °C										
4 - 5	(lower)	300	130	60	70	170	132	38	0.15	53.0
9 - 11	(optimal)	960	405	160	245	555	460	95	0.11	44.7
14 - 16	(higher)	1788	858	298	560	930	540	390	0.28	31.0
Reactor B : At a process temperature of 20 °C										
3 - 5	(lower)	245	115	55	60	130	98	32	0.16	53.0
6 - 8	(optimal)	565	258	100	158	307	254	53	0.11	45.0
11 - 13	(higher)	1600	1010	320	690	590	280	310	0.35	23.5

The above parameters were calculated using the following equations:

(1) g COD sludge washout = g COD $effl._{total}$ - g COD $effl._{filtered}$.

(2) g COD removed = g COD $infl._{total}$ - g COD $effl._{total}$.

(3) Sludge accumulation in the reactor = g COD removed - g CH_4-COD.

(4) $(Y)_{acc.}$ (sludge accumulation factor in the reactor) = g COD sludge accumulating/g COD removed x 1/1.51*

(5) Suspended Solids removed (%) = [1 - COD_{SS} (effluent)/COD_{SS} (influent)] x 100.

* 1 kg sludge VSS is equal to 1.51 kg COD.

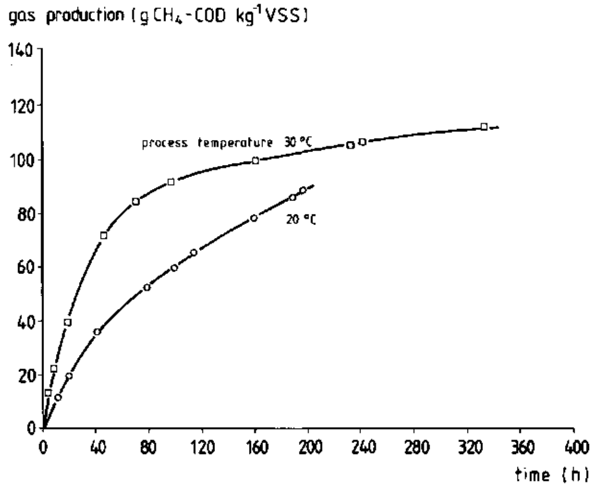


Figure 5. Cumulative gas production during the prolonged period of the feed interruption at process temperatures of 30 °C and 20 °C.

DISCUSSION

The results of our investigations (Table 2) demonstrate that one-stage granular sludge UASB reactors are quite effective in removing the $COD_{filtered}$ fraction from unsettled slaughterhouse wastewater over a wide range of imposed COD loading rates, both at a process temperature of 30 °C and 20 °C. On the other hand, it is also clear that the performance of granular sludge blanket reactors in terms of COD_{total} treatment efficiency is fairly moderate and, as a matter of fact, poorer than that of flocculent sludge blanket reactors. In the latter system (Sayed et al., 1984) COD_{total} removal efficiencies were accomplished ranging from 70% to 65% at imposed COD loading rates ranging from 2.5 to 3.5 kg COD $m^{-3}day^{-1}$ at 20 °C and 30 °C. The results in Table 2 reveal a COD_{total} treatment efficiency of 55% under comparable conditions. The coarse Suspended Solids present in the wastewater are better entrapped in flocculent sludge blanket reactors than in granular sludge blanket reactors. Granular sludge bed reactors perform quite satisfactorily with respect to the removal of the colloidal and soluble fractions of the organic pollutants present in the wastewater, even under high loading conditions, i.e. up to 19 kg COD $m^{-3}day^{-1}$ at 30 °C and 11 kg COD $m^{-3}day^{-1}$ at 20 °C (Table 2).

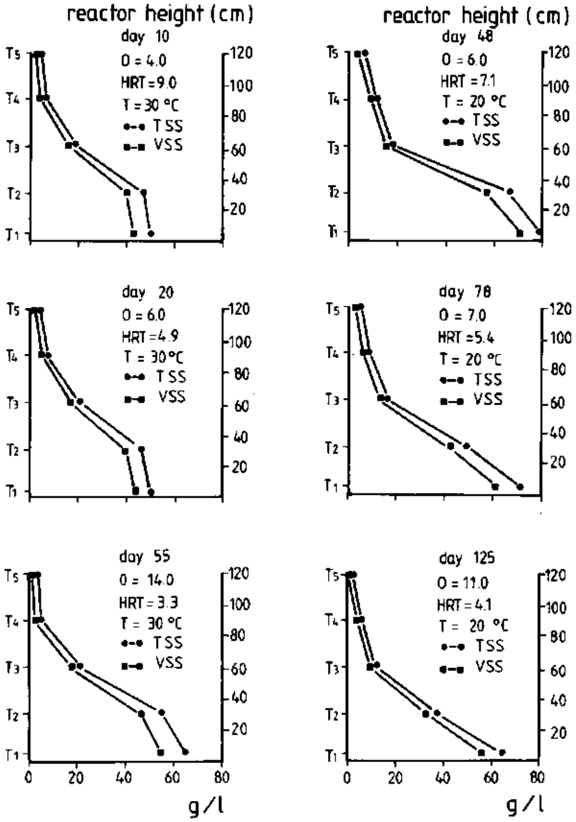


Figure 6. Sludge profiles as measured over the reactor height during various days of the experiments. O, organic space load (kg COD m^{-3} day^{-1}); HRT, hydraulic retention time (h); and T, temperature (°C). T_1, etc., taps shown in Fig. 2.

However, the results in Table 3 and Fig. 3 also reveal a dramatic deterioration of the system in its efficiency to convert the removed pollutants into methane under high loading conditions. This phenomenon certainly is a matter of considerable concern, because imposed loading rates for a long period will be irrevocably accompanied by a high accumulation of slowly biodegradable insoluble substrate ingredients in the sludge (bed). In due time this may result in an almost complete deterioration of the methanogenic activity of the system. For a proper application of granular sludge bed reactors for slaughterhouse wastes it is therefore crucial to elucidate the processes involved in the removal of the colloidal and soluble compounds from the wastewater and their conversion into methane. According to our opinion the mechanism underlying the removal of these compounds primarily has to be found both in a partial flocculation and in an adsorption to the available internal and external surfaces of the sludge grains. The appearance of flocculent sludge under high loading conditions has been observed and the occurence of adsorption was clear (Sayed, in press). It was not possible to assess the proportions of the removed fraction being adsorbed or flocculated.

However, both mechanisms may retard significantly the rate of liquefaction of these materials, and consequently their conversion into methane. The flocculation will lead to a decreasing accessibility of enzymes and bacteria to these materials, the more so because the flocs tend to accumulate above the dense granular sludge bed. The adsorption of the colloidal and soluble matter in and at the surface of the granular sludge may result in an entrapment of the granular sludge bacterial matter by a film of increasing thickness, and perhaps also density, which increasingly will hamper the supply of substrate to the bacteria present in the grains. The results in Table 4 show that the flocculated and adsorbed compounds, in principle, are easily biodegradable because, under lower loading conditions, the $\mathrm{COD}_{\mathrm{removed}}$ is efficiently converted into methane (viz. $\mathrm{COD}_{\mathrm{total}}$ removed is equal to 55% and $\mathrm{COD}_{\mathrm{total}}$ converted into methane 47%; and the calculated factor M is greater than one (Table 4).

It is well known that the rate limiting factor in the conversion of insoluble substrate ingredients is the liquefaction step (Gujer & Zehnder, 1983; Sayed et al., 1984). Apart from the chemical composition of the substrate, the rate of liquefaction strongly depends on its physical nature, e.g. the size and porosity of the separate particles, viz. the accessibility for enzymes and bacteria, and of environmental factors such as particularly the temperature. Results obtained by Coulter et al. (1957) with domestic sewage waste indicate that liquefaction of the insoluble fraction of the substrate in the waste is strongly dependent on the temperature, viz. temperatures exceeding 20 ^{o}C are essential for a more rapid liquefaction. Additionally, lower levels of liquefaction at higher loading rates may be attributed to the shorter retention time prevailing under these conditions, causing excreted enzymes to be washed out too rapidly from the system.

Obviously, too little information is available from the present investigation to speculate about the relative importance of the factors mentioned above. Results of ongoing, more fundamental, research will be published in the near future. However, what remains with respect to the practical application of a granular sludge UASB system for treating wastewaters of the type investigated in the present study certainly is that serious consideration should be paid to the achieved COD treatment efficiency as compared to the conversion of the removed COD into methane.

The dense granular sludge itself is satisfactorily retained in both systems over the entire range of loading conditions applied, especially in the lower part of the reactor (Fig. 6). The measured shape of the sludge profiles in both reactors illustrate that the sludge blanket is expanded over a considerable part of the height of the reactor for both process temperatures, probably mainly as a result of gas production, i.e. the organic loading rate and the conversion efficiency to methane and - although to a minor extent - of the hydraulic loading rate, i.e. superficial velocity.

As mentioned above, the granular UASB reactors perform poorly toward the removal of coarse Suspended Solids. This particularly is true at higher loading conditions (14 and 11 kg COD m^{-3} day^{-1}) as only 31% and 23.5% of the influent Suspended Solids are removed at process temperatures of 30 ^{o}C and 20 ^{o}C respectively (Tables 2 and 5). The systems perform better toward the removal of coarse Suspended Solids at the lower loading conditions. If coarse Suspended Solids are 45% of the total COD (Fig. 1) and consequently the colloidal and soluble fractions together make up 55% of the COD_{total}, then if 85% of the filtered COD is removed (Table 2), the total $COD_{removed}$ of 55% (Table 2) consists of (0.85 x 0.55/0.55) x 100 = 85% colloidal and soluble matter and 15% coarse Suspended Solids.

Considering the percentage of the $COD_{removed}$ which is converted into methane (Table 4), the contribution of the coarse Suspended Solids to the methane production remains uncertain. From the results in Fig. 4, however, it can be reasoned that the coarse Suspended Solids contribute to the gas production, especially at lower loading rates. Considering these results, the observed minima in the average weekend gas production rates can be explained as follows.

a. The decrease in the gas production in the lower range of Q-day loads (I) should be attributed to a decreasing accumulation (entrapment) of coarse Suspended Solids in the reactor at increasing Q-day loading rates, mainly due to higher gas production and the shorter hydraulic retention time. Less substrate is being left in the reactor for digestion during the weekend at increasing Q-day loading rates. The decreasing accumulation of coarse Suspended Solids at higher loading rates is illustrated clearly by the results shown in Table 5.

b. The increase in the weekend gas production occuring in the higher range of the Q-day loading rates (II) obviously should be attributed to an increasing accumulation of the colloidal and soluble fractions in the reactor, because the results in Fig. 3 and Table 4 show a very evident decrease in the volumetric methane production rate during week days at increasing COD loads while at the same time the efficiency of the system toward the removal of the filtered COD fraction remains satisfactory (Table 2). Thus, an increasing amount of flocculated and adsorbed COD is left for digestion during the weekend feed interruption.

The intermittent mode of operation chosen in this study apparently is appropiate for treating unsettled complex low-strength wastes like slaughterhouse wastes. Weekend feed interruptions enable the system to liquefy and to digest the accumulated coarse Suspended Solids as well as the flocculated or adsorbed colloidal and soluble material, consequently enabling the system to operate at higher loading rates during the week. Additionally, prolonged feed interruptions enable the system to get rid of accumulated substrate ingredients more completely (Fig. 5). The results in Fig. 5 show that within approximately 2 to 3 days up to 70% of the accumulated substrate is being converted at a 30 °C operational temperature, while considerable more time is involved at a 20 °C operational temperature. Considering the shape of the curve at 20 °C, after 7 days still a significant fraction of the accumulated substrate is left, while this is not the case for 30 °C.

In view of the insight obtained from the present investigation, we recommend a maximum COD load up to 11 kg COD $m^{-3}day^{-1}$ at 30 °C and 7 kg COD $m^{-3}day^{-1}$ at 20 °C for the practical application of granular sludge UASB systems with unsettled slaughterhouse waste. This corresponds to 5.2 and 2.8 kg CH_4-COD $m^{-3}day^{-1}$ in terms of methane production capacity. Under the above-mentioned conditions a stable operation of the system is guaranteed, i.e. at a maximum COD reduction, a high conversion of COD into methane, and a minimum excess sludge accumulation as well.

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Biological Wastes
Submitted May 1987, Accepted August 1987

CHAPTER 4

THE PERFORMANCE OF A CONTINUOUSLY OPERATED FLOCCULENT SLUDGE UASB REACTOR WITH SLAUGHTERHOUSE WASTEWATER

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ABSTRACT

This investigation was carried out to assess the performance of a one-stage flocculent sludge UASB-reactor treating slaughterhouse wastewater. The experiments deal with a continuously operated UASB-reactor at a process temperature of 30 ^{o}*C.*

The results of the present investigation indicate that the substrate composition: coarse suspended solids, colloidal and soluble compounds, affects the performance of the reactor due to different mechanisms involved in the removal of these substrate ingredients and their subsequent conversion into methane. Two different mechanisms are distinguished in the removal of substrate ingredients from the wastewater; entrapment mechanism and adsorption mechanism. The entrapment mechanism prevails regarding the elimination of coarse suspended solids while the adsorption mechanism is involved in the removal of the colloidal and the soluble fractions of the wastewater.

The results obtained lead to the conclusion that the system can satisfac-

torily handle organic space loads up to 5 kg COD $m^{-3}day^{-1}$ *at 30* ^{o}*C. The data indicate, however, that continuing heavy accumulation of substrate ingredients in the reactor is detrimental for the stability of the anaerobic treatment process, as the accumulation can lead to sludge flotation and consequently to a complete loss of the active biomass from the reactor.*

INTRODUCTION

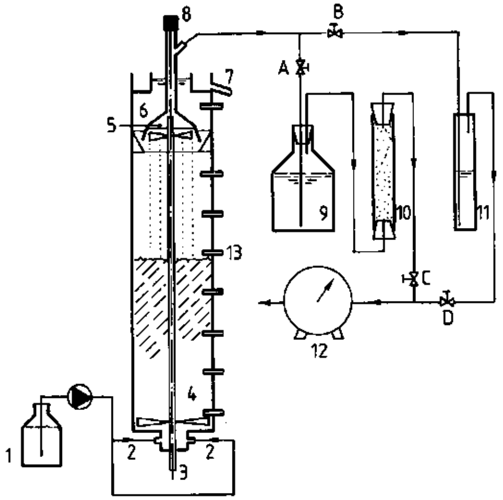
Anaerobic processes are attractive for the treatment of wastewaters varying widely in strength and composition (McCarty, 1981). The advantages of anaerobic treatment are that it couples the removal of organic pollutants of the waste with the production of energy in the form of methane while also its excess sludge production is low as compared to aerobic processes. Moreover the excess sludge is highly stabilized and generally its dewaterability is excellent.

Amongst the several anaerobic treatment processes, the upflow anaerobic sludge blanket (UASB) process appears to be an attractive system for the treatment of food industry wastes. Recent studies have also shown the feasibility of using the UASB process for a one-stage anaerobic treatment of complex wastewaters such as domestic sewage (Grin, et al., 1985) and slaughterhouse wastewater (Sayed et al., 1984; 1987), owing to the fact that the process is able to maintain a sufficient amount of viable sludge, thereby providing efficient and stable treatment. In a previous paper (Sayed et al., 1984) we reported that apart from the amount of sludge that can be retained in the reactor at the given temperatures, the performance of the reactor with respect to its loading potential, will strongly depend on the type of the organic pollutants present in the wastewater. The flocculent sludge UASB system demonstrated to be capable of removing the organic pollutants from the wastewater quite effectively, but the mechanisms involved in the removal of the different size fractions were not fully understood. Therefore, the objective of the present study is to elucidate further the way of elimination of the different substrate ingredients in flocculent sludge systems, particularly under continuous loading conditions.

MATERIALS AND METHODS

Experimental arrangement

The experiments were performed in a continuously operated one-stage flocculent sludge UASB-reactor at a process temperature of 30 °C. A flow diagram of the experimental arrangement is shown in Figure 1.



1. equalisation vessel for raw wastewater
2. influent inlet points
3. stirrer
4. sludge bed (digester compartment: 10.5 litres)
5. gas collector
6. settler
7. effluent discharge
8. gas outlet
9. CO_2 (and H_2S) stripping device
10. granular soda lime
11. water seal
12. wet gasmeter
13. sampling taps

Note: A, B, C & D are taps: B + D open; A + C closed: measuring total biogas; A + C open; B + D closed; measuring only CH_4.

Figure 1. Schematic diagram of the experimental arrangement used in the experiments.

The operational procedure was according to the upflow anaerobic sludge blanket (UASB) process (Lettinga et al., 1980). The reactor volume was 10.5 litres, its internal diameter 0.11 m and the height 1.12 m. The reactor was equipped with a radial flow impeller type stirrer. Mechanical mixing was done intermittently, usually one rotation per 15 minutes, at a speed of 20 - 40 rpm. The employed 100 litres equalisation vessel provided a storage facility enabling the experiments to be carried out over a full 24 h period. The wastewater in the equalisation vessel was continuously stirred.

Seed sludge

The UASB-reactor was seeded with 5 litres of sludge from the digester of the municipal sewage treatment plant at Ede, The Netherlands. The total suspended solids (TSS) content of the sludge was 3.4% and the volatile suspended solids (VSS) content was 2.3 %. At the start of the experiments, the sludge concentration in the reactor amounted to 16.2 g TSS sludge per litre reactor volume. The maximum specific activity of the sludge for VFA decomposition as measured in a standard batch-fed test using a feed mixture of 600 mg l^{-1} each of acetic acid, propionic acid and butyric acid, was 0.05 kg CH_4-COD kg^{-1}VSS day^{-1} at 30 °C.

Wastewater composition

Wastewater from the slaughtering areas was collected after passing a screen installed to remove the dispersed particles larger than 1 mm from the wastewater. The slaughterhouse wastewater varied widely in composition, strength and flow. Differences in wastewater composition can primarily be attributed to differences in processing activities, animal species, wastewater management methods and employee habits.

Slaughterhouse wastewater was collected during four distinct periods, and was fed separately to the reactor (See Table 1 and Figure 2).

Table 1. The main characteristics of the slaughterhouse wastewater used in the four different experimental periods.

exp. periods	days no	$COD_{tot.}$ [a] (mg l^{-1})	$COD_{filt.}$ [a] (mg l^{-1})	$(COD/BOD)_{tot.}$ [a] ratio	coarse [a] suspended solids as % of $COD_{tot.}$	VFA-COD [a] (mg l^{-1})	nitrogen [a] (N) - Kjeldahl (mg l^{-1})	phosphate [a] (P)-total (mg l^{-1})
I	0-17	1925	1597	2.1	17	120	140	17
II	21-39	2695	780	3.8	71.1	80	110	13
III	40-48	11,118	10,090	2.4	9.1	220	240	22
IV	49-56	6056	4500	2.9	25.6	180	160	20

a. average values.

Each period was distinct because it could be associated to different processing activities of the slaughterhouse, that varied from time to time. Each of these periods will be discussed separately.

The wastewater used in experimental period 1 contained a relatively low coarse suspended solids concentration, while during experimental period 2 the coarse suspended solids content was high i.e. 71.1 % of the COD_{total}. During experimental period 3 the wastewater was mainly soluble with relatively high COD_{total} concentration. During experimental period 4 the wastewater was a mixture of the three above mentioned types of waste.

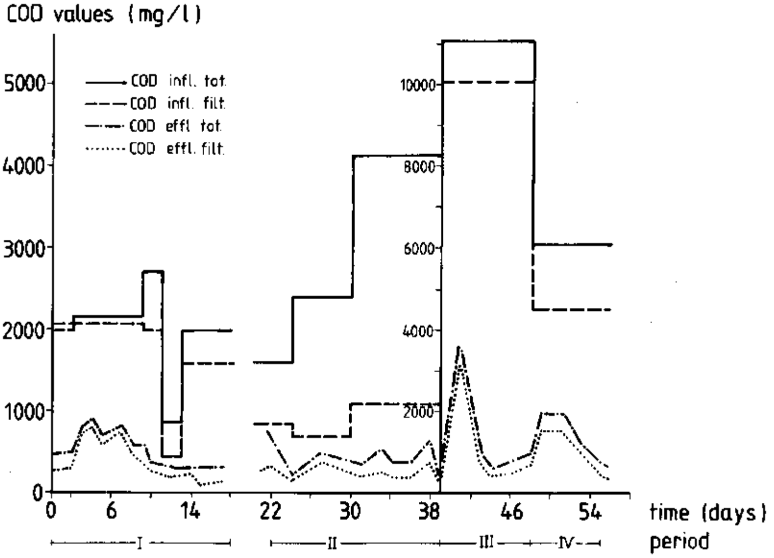


Figure 2. COD values of the influent and the daily effluent quality achieved during the four experimental periods.

Sampling and analyses

All samples taken for chemical analysis were 24 h composites. Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Dry Suspended Solids (DSS), Volatile Suspended Solids (VSS), total nitrogen and phosphate were determined according to standard methods (Standard Methods for the Examination of Water and Wastewater (1980)). Volatile Fatty Acids (VFA) analysis were carried out gas chromatographically using a Packard

Becker model 417 equipped with a 6 m x 2 mm glass column and a flame ionisation detector. The column was packed with chromosorb 101 (60 - 80 mesh). The flow rate of the carrier gas, nitrogen saturated with formic acid, was 18 ml min^{-1}. Column temperature was 210 °C and the injection port temperature was 230 °C.

The COD for the raw wastewater is expressed as COD_{total}. The coarse suspended solids in the raw wastewater were separated using a filter Schwarzband black ribbon no 589^{I} Ref. no 300011, pore size 7.4 µm. The filtrate COD of the wastewater is referred to as colloidal and soluble fractions. In this study the filtrate COD is presented as $COD_{filtered}$.

Gas samples removed from the gas sampling port were analyzed immediately after sampling for CH_4 content using a Packard Becker gas chromatograph model 409 equipped with two columns - a Porapak 2 m x 3 mm and a stainless steel column with molecular sieve 5A (1 m x 3 mm) for the determination of methane, carbon dioxide, nitrogen and hydrogen. Helium was used as a carrier gas (20 ml min^{-1}). Oven temperature was 70 °C and column temperature 75 °C.

The methanogenic capacity of the sludge was calculated from the methane production rate and the amount of the sludge in the reactor and expressed as kg CH_4-COD kg $^{-1}$VSS day^{-1}.

The slope of the regression line fitting the natural logarithm of the methane production versus time gives the net specific growth rate of the methanogenic bacteria of the sludge (μ). Mathematical details of this method are reported by Powell (1983).

The significance of the correlation and the regression coefficient (B) were estimated by the T-test. The T-test was also used to estimate the significance of the difference between the line based on the total data set (entire experimental period) and the lines obtained from the four different periods.

RESULTS

Operational parameters

The UASB-reactor was started at hydraulic retention times (HRT) ranging from 70 to 28 hours at an average of 40 hours (period 1) followed in period 2 at an average of 16 hours, and 14 and 12 hours during period 3 and 4 respectively. Mainly as a consequence of the wide variation in the type and concentration of substrate ingredients of the wastewater, different organic space loads were imposed during the four experimental periods. At day 57 a sudden and heavy sludge flotation and a complete loss of active biomass from the reactor occurred. The experiments were therefore terminated at day 57. Table 2 summarizes the operational data during the four different experimental periods.

Table 2. The average effluent VFA-COD, operational conditions and average treatment efficiencies achieved during the four experimental periods.

exp. periods	days[b]	operational parameters				effluent[a] VFA-COD (mg l^{-1})	treatment efficiency (η)[c]			
		organic space load (kg COD m^{-3} day^{-1})	hydraulic[a] retention time (h)	surface[a] load (mh^{-1})	sludge[a] bed expansion (l)		$COD_{tot.}$[a]	$COD_{filt.}$[a]	$BOD_{tot.}$[a]	$BOD_{filt.}$[a]
I	17	0.5 - 1.7	40	0.036	7.5	50	78.8	82.0	90	92
II	19	2.5 - 5.0	16	0.056	8.5	65	82.3	89.5	85	94
III	9	16 - 20	14	0.063	10	600	78.9	81.1	89	91
IV	8	10 - 12	12	0.090	8.5	420	68.4	75.5	n.m.	n.m.

a. average values.

b. number of days for which the load was applied.

c. $\eta_{C(B)OD}$ purification percentage - [1 - C(B)OD (total or filtered) / C(B)OD influent total] x 100.

n.m. not measured.

Performance of the UASB-reactor in terms of treatment efficiency.

Figure 2 presents the daily measured influent and effluent COD-values during the four periods. The calculated average treatment efficiencies achieved during the four experimental periods and the average values of effluent VFA-COD are summarized in Table 2.

Gasproduction

The CH_4 gas production rate as a function of time is shown in Figure 3 together with the imposed organic loading. Using the data from Figure 3 a plot of the natural logarithm of the methane production rate versus time has been made over the entire experimental period as well as for the four distinguished periods (Figures 4 and 5). These show a continuous linear increase of the gas production rate over the experimental periods. From the data in Figure 3 and the performance data of the reactor with respect to COD-removed (Table 2) we have calculated the average fractional conversion into methane-COD of the supplied COD_{total} and COD_{total}-removed in the four experimental periods. In addition we have also calculated the ratio (M) between COD-methane / $COD_{filtered}$- removed over the four periods. The results are presented in Table 3.

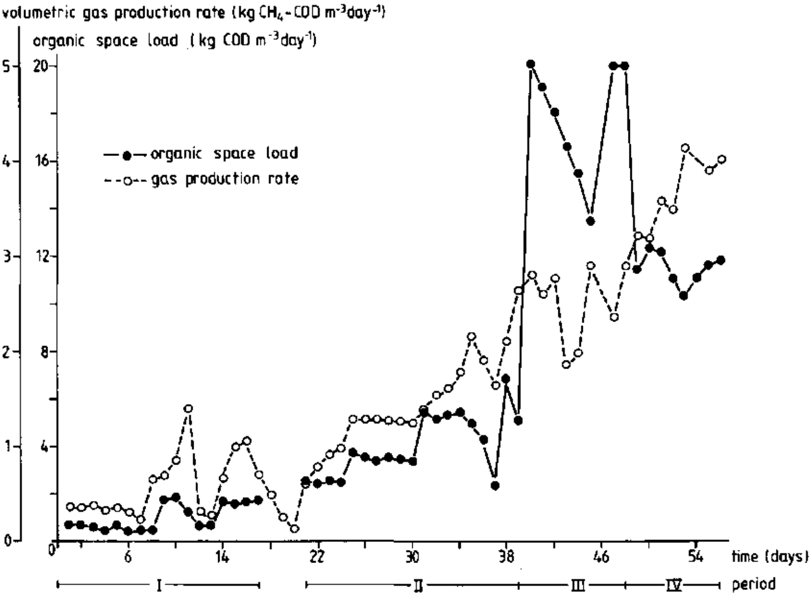
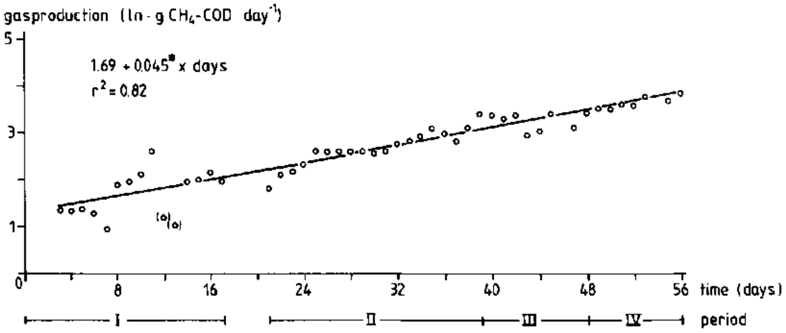


Figure 3. The applied volumetric organic loading and the volumetric methane production rate during the four different experimental periods.

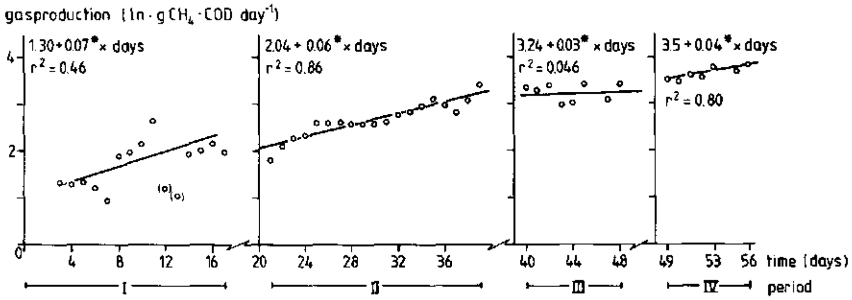


* significantly different from zero by slope at $p = 0.005$ level
(o) severely underloaded

Figure 4. The natural logarithm of the methane production rate as a function of time over the entire experimental period.

COD balances and sludge accumulation in the reactor

The COD balance data are presented in Table 4 together with the calculated sludge accumulation factor $(Y)_{accumulation}$. The $(Y)_{acc.}$ does not distinguish between cell yield, entrapment and adsorption of the substrate ingredients.The COD accumulation in the reactor as calculated according to equation 3 of Table 4 for the four experimental periods has been plotted in Figure 6 as a function of time. In addition Figure 6 also shows the theoretical maximum possible entrapment (100% entrapment) of the supplied influent COD_{SS} fed in the reactor.



* significantly different from zero by slope at p=0.005 level

(o) severely underloaded

Figure 5. The natural logarithm of the methane production rate in relation to the time during the four different experimental periods.

Table 3. The calculated percentages of COD-total and COD-removed converted into methane and the calculated value of the M-factor during the different experimental periods.

exp. periods	days [a]	coarse suspended solids as % of COD_{total}	COD_{total} converted into methane (%)	$COD_{removed}$ converted into methane (%)	factor M [b]
I	17	17	56.6	71.9	1.11
II	19	71.1	36.7	44.4	1.98
III	9	9.1	14.7	18.7	0.20
IV	8	25.6	32.1	47.4	0.64

a. number of days for which the load was applied.

b. Factor M = $\frac{\text{COD-methane}}{COD_{\text{filtered removed}}}$

Table 4. COD balances and total sludge accumulation in the reactor during the four experimental periods.

experimental periods	g COD infl.$_{tot.}$	g COD effl.$_{tot.}$	g COD effl.$_{filt.}$	(1) g COD sludge washout	(2) g COD removed	g CH_4-COD	(3) sludge acc. in the reactor as g COD	(4) $(Y)_{acc.}$	expected bacterial cell yield (g COD-cell$_{produced}$/ g COD$_{removed}$)
I	157	27.6	23.2	4.4	129.4	92.2	37.2	0.20	0.10
II	790.9	138	83.4	54.6	652.9	290	362.9	0.39	0.08
III	1878	397	354.4	42.6	1481	276.9	1204.1	0.58	0.04
IV	692.2	219	169.9	49.1	473.2	222.4	250.8	0.38	0.07
Total g COD	3518.1	781.6	630.9	150.7	2736.3	881.5	1854.0	-	-

The above mentioned parameters were calculated using the following equations:

(1) g COD sludge washout = g COD effluent$_{total}$ - g COD effluent$_{filtered}$.

(2) g COD removed = g COD influent$_{total}$ - g COD effluent$_{total}$.

(3) sludge accumulation in the reactor = g COD removed - g CH_4-COD.

(4) $(Y)_{acc.}$ (sludge accumulation factor in the reactor) = g COD sludge accumulation / g COD removed x 1/1.41 *.

* 1 kg sludge VSS is equal to 1.41 kg COD.

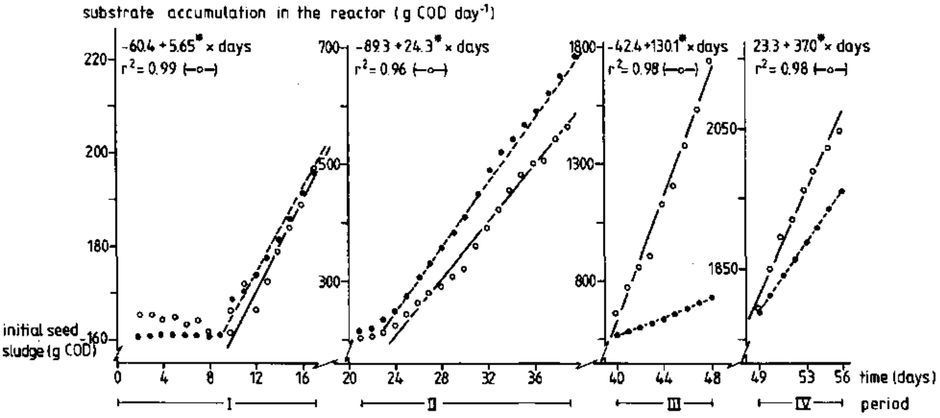


Figure 6. The calculated COD accumulation in the reactor according to equation 3 of Table 4 (o) and the theoretical maximum possible entrapment (100% entrapment) of the influent COD_{SS} (●) as a function of time, during the four different experimental periods.

* significantly different from zero by slope at p= 0.005 level

DISCUSSION

The experimental results shown in Figure 2 and Table 2 indicate that a continuously fed flocculent sludge UASB-reactor performs satisfactorily up to organic loading rates as high as 10 - 20 kg COD $m^{-3}day^{-1}$. The COD_{total}-reduction achieved ranges from 79 to 82% over the first three experimental periods and equals to 68% during the fourth experimental period. These results are very well comparable to the results obtained in an intermittently operated 25.3 m^3 flocculent sludge UASB-reactor (Sayed et al., 1984). In the latter experiments up to approximately 70% COD-reduction could be accomplished at 30 ^oC process temperature and at applied loading rates up to approximately 7.5 kg COD $m^{-3}day^{-1}$ during 8 hours per day. However, the results in Table 3 and Table 4 reveal very different conversion percentages for the removed COD into methane as well as different values for the sludge accumulation factor for the four experimental periods. This may lead to the conclusion that the type of substrate ingredients in the wastewater affects the performance of the reactor with regard to the prevailing mechanisms for the removal of the substrate ingredients and their subsequent conversion into methane. The overall treatment efficiency, however, is fairly constant.

In order to assess which mechanisms are involved in the removal of the different types of pollutants in the waste, the calculated COD accumulation in the reactor and the theoretical maximum possible entrapment of the supplied coarse suspended solids (COD_{SS}) are shown in Figure 6.

Regarding the mechanisms involved the removal of the substrate ingredients from the wastewater, we can distinguish three possible ways in which these substrates can accumulate as COD in the reactor viz. the bacterial growth, entrapment of COD-coarse suspended solids and the adsorption of colloidal and soluble COD-ingredients of the wastewater to the sludge of the reactor. If the COD accumulation is only the result of the bacterial cell yield at the maximum only 10% of the removed COD can be accumulated (see Table 4). If the COD accumulation results only from entrapment of coarse suspended solids the COD accumulation in the reactor cannot be greater than the supplied influent COD_{SS}. In case a large amount of COD accumulates due to the adsorption of the colloidal and soluble substrate ingredients of the wastewater, a greater COD accumulation can occur than can be expected from the sum of cell yield and entrapment influent COD_{SS}.

The results in Figure 6 clearly illustrate that during period 1, except for the first 8 days which will be discussed below, the calculated COD accumulation in the reactor almost coincides with the maximum COD_{SS}-entrapment-curve, indicating that most of the COD accumulation can be attributed to entrapment of influent COD_{SS}. In the first 8 days of period 1 the influent contained a very low percentage of coarse suspended solids (3% of the COD_{total}) and consequently the amount of COD-coarse suspended solids accumulated during these 8 days of the period is low. Also in period 2 the accumulation of COD does not deviate considerably from the maximum COD_{SS} entrapment curve, indicating a large entrapment percentage of the supplied influent COD_{SS} and relatively little conversion of these ingredients into methane. Assuming a maximum bacterial cell yield of 0.1 g $COD\text{-}cell_{produced}$ g^{-1} $COD_{removed}$, the maximum possible value of the M-factor will be 0.90. Values of the M-factor of 1.11 and 1.98 as found in period 1 and 2 (see Table 3) also indicate the occurrence of entrapment of influent COD_{SS} as well as their conversion into methane. These higher values of the M-factor are comparable with those found i.e. ranging from 2.24 to 1.33, in the intermittently operated 25.3 m^3 flocculent sludge UASB-reactor at loading rates ranging from 0.3 to 3.5 kg COD m^{-3} day^{-1} at a process temperature of 30 °C (Sayed et al., 1984). In period 3 the calculated COD accumulation in the reactor largely exceeds the maximum COD_{SS}-entrapment-curve (9.1 % of the influent COD_{total}), which clearly demonstrates that more than just the entrapment of coarse suspended solids was responsible for the COD accumulation found. As the expected cell yield during this period is low, viz. 0.041 g COD-cell produced - g^{-1} $COD_{removed}$ (Table 4), adsorption of colloidal and soluble substrate ingredients of the wastewater to the sludge is the only possibility that can explain this high COD accumulation in the reactor. The occurrence of an adsorption mechanism of the influent colloidal and soluble matter is confirmed by the low M-factor (i.e. 0.20) found during this period, as shown in Table 3. Such a low value of the M-factor was also found under condition of continuous feeding during working days combined with weekend feed interruptions in a granular sludge UASB-reactor at loading rates of 19 kg COD $m^{-3}day^{-1}$ (i.e. a value of 0.28 was found for the M-factor) at a process temperature of 30 °C (Sayed et al., 1987). The adsorption of the influent $COD_{filtered}$ to the sludge contributes to a significant decrease in substrate (VFA) accessibility to the methanogenic bacteria present in the sludge. Obviously, this can result in a lower specific growth rate.

In order to assess to what extent the type of substrate in the waste may affect the specific growth rate we have plotted the natural logarithm of the methane production rate versus time both over the entire experimental period and during the four distinguished periods in Figures 4 and 5 respectively.

The data in Figure 4 reveal a gradual linear increasing gasproduction rate over the entire experimental period. A good linear regression line with a high coefficient of determination (r^2) of 0.82 was obtained, except during period 3, when the methane production fluctuates sharply. The experimental results in Figure 5 reveal that wastewaters used in experimental period 1 and 2 give the highest specific growth rates, respectively 0.07 and 0.06 day^{-1}. Considering the complexity of the wastes used during those periods the obtained specific growth rates are high, because the maximum growth rate at 30 °C found with a standard VFA mixture (de Zeeuw, 1984) is 0.07 day^{-1}. Apparently, growth of the methanogenic bacteria proceeds almost uninhibited on the types of the slaughterhouse waste used during periods 1 and 2.

As shown in Figure 5 during period 3 the specific growth rate is clearly lower than in the preceding periods. Regarding the fact that during period 3 sufficient substrate (VFA) was present in the reactor (Table 2), the occurrence of adsorption of substrate ingredients indeed seems to result in a serious drop of the specific methanogenic growth rate.

In period 4 the calculated accumulation of COD exceeds also the supplied influent COD_{SS}, although relatively less than in period 3, which can be explained by the higher percentage of coarse suspended solids in the wastewater during this period as compared to the wastewater used in period 3 (see Table 1). The occurrence of the adsorption during period 4 is again confirmed by the low value of the M-factor (Table 3).

Like in period 3 the specific growth rate during period 4 was limited to 0.04 day^{-1} despite the fact that also here sufficient methanogenic substrate (VFA) was present in the reactor (Table 2).

A continuing accumulation of substrate ingredients either coarse suspended solids, colloidal matter or soluble ingredients of the wastewater may eventually lead to severe sludge flotation and consequently to a complete loss of the active biomass from the reactor. This phenomenon occurred on day 57 in this study. From these observations it is clear that a heavy accumulation of substrate ingredients from slaughterhouse wastewater is detrimental for the stability of the anaerobic treatment process. A significant drop in the specific activity of the sludge may occur, while ultimately also the sludge will be rinsed out of the reactor due to flotation problems. It is difficult to assess from the present investigations at what loading rate a continuously operated flocculent sludge UASB-reactor will deteriorate, because the high loading rates achieved in this study were applied using wastewaters which are far more concentrated than the COD-concentrations normally occurring in the investigated slaughterhouse wastewater (Sayed et al., 1984; 1987). However, regarding the very high value of the M-factor found during period 2 at space loads of 2.5 - 5 kg COD $m^{-3}day^{-1}$ and considering the fact that gradually volumetric gas production rates over 4 kg CH_4-COD $m^{-3}day^{-1}$ can be achieved (period 4), presumably organic space loads up to 5 kg COD m^{-3} day^{-1} can be satisfactorily accommodated at 30 °C under conditions of continuous operation with a flocculent sludge UASB-reactor.

In order to prevent process failure under circumstances where an excessive accumulation of organic substrate ingredients occurs, arrangements should be taken to allow that these materials are sufficiently converted into methane. This could be accomplished either by applying intermittently feeding as applied in the 25.3 m^3 UASB-experiments (Sayed et al., 1984) or by applying an occasional removal of part of the sludge from the reactor. The removed sludge might then be digested in a separate sludge digester and, after stabilization the sludge could be returned to the UASB-reactor.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the constructive comments on the manuscript by Dr G. Lettinga.

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CHAPTER 5

ANAEROBIC DEGRADATION OF THE VARIOUS FRACTIONS OF SLAUGHTERHOUSE WASTEWATER

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ABSTRACT

The main objectives of the present investigations were to determine the maximum extent of anaerobic biological degradation of the soluble, colloidal and coarse suspended solids fractions of slaughterhouse wastewater, in order to elucidate the mechanisms involved in the removal of these organic pollutant fractions and to determine the rate-limiting steps in the degradation of each fraction of the wastewater and to assess the effects of the fractions on the methanogenic activity of the sludge.

The experiments were performed with membrane-filtered wastewater (wastewater$_{mf}$*), paper-filtered wastewater (wastewater*$_{pf}$*) and the whole wastewater. The experimental arrangements consisted of a column containing granular sludge, through which the test wastewater was recirculated. The biodegrability tests with the coarse suspended-solids fraction of the wastewater were performed with granular and flocculent sludge in conventional stirred batch digesters. All experiments were performed at 30* ^{o}C *and 20* ^{o}C*.*

The maximum biodegrabilities (i.e. conversion into methane) found at 30 ^{o}C *were 75% for wastewater*$_{mf}$*, 61% for wastewater*$_{pf}$ *and 67% for* *water*$_{total}$, *while at 20 °C these values were 72%, 49% and 51% respectively. The maximum biodegrability of the coarse suspended-solids fraction of the waste was 50% at 30 °C and 45% at 20 °C.*

The results indicate that an important mechanism in the removal of the soluble, and especially the colloidal, fractions of the wastewater is adsorption. Particularly at imposed high sludge loads of approximately 0.17 g colloidal-COD g^{-1}*VSS the limiting rate of liquefaction of the absorbed compounds may result in a serious drop in the methanogenic activity of the sludge.*

The extent as well as the rate, of the liquefaction of the adsorbed insoluble substrate material of the wastewater are the controlling factors with respect to loading potentials of the process, and consequently temperature is a factor of predominant importance. The methanogenic activity of the granular sludge deteriorates because of the relatively high degree of adsorption of the colloidal fraction of the wastewater to the surface of the sludge and its high fat content.

INTRODUCTION

Anaerobic processes are attractive for the treatment of wastewaters varying widely in strength and composition (McCarty, 1981). The advantages of the anaerobic treatment are that it couples the removal of organic pollutants of the waste with the production of energy in the form of methane, while excess sludge production is low compared to aerobic processes. Moreover, the excess sludge is highly stabilized and generally its dewaterability is excellent.

The nature and composition of the wastewaters from the food industry have been discussed in detail by Isaac & Anderson (1974). They report that most effluents from food industries tend to be heavily polluted and contain a high concentration of biodegradable organic materials. Therefore, the discharge of such effluents to a sewer or a watercourse will exert a substantial oxygen demand.

For slaughterhouses, which constitute an important branch of the food industry, it is, of course, essential, and normal practice to purify effluents before discharge to a sewer or a watercourse. Amongst the several anaerobic treatment processes, the upflow anaerobic sludge blanket (UASB) process appears to be an attractive system for the treatment of food industry wastes (Pette & Versprille, 1981). Recent studies have shown the feasibility of using the UASB process for a one-stage anaerobic treatment of slaughterhouse wastewater (Sayed et al., 1984; 1987).

In a previous paper (Sayed et al., 1987) we reported that, apart from the poor removal of the coarse suspended solids fraction, the performance of a one-stage granular sludge UASB with respect to the removal of the soluble and colloidal COD of the wastewater was quite satisfactorily, even under high loading conditions (up to 19 kg COD m^{-3} day^{-1} at 30 °C and 11 kg COD $m^{-3}day^{-1}$ at 20 °C).

However, at the same time, there was a dramatic deterioration in the efficiency of the system in the conversion of the removed pollutants present in the filtrate into methane under the high loading conditions. Therefore, the mechanism of the removal of the organic pollutants from the filtrate fraction of the wastewater is a matter of concern. It is necessary to elucidate the mechanism involved in the removal of these compounds, particularly if it might induce significant retardation of the liquefaction of the materials (Sayed et al., 1987) and consequently of their conversion into methane. The liquefaction of insoluble biodegradable ingredients is generally the rate-limiting step in the digestion process (Gujer et al., 1983; Stafford, 1983). Therefore, the main objectives of the present investigations were to determine the maximum extent of the anaerobic degradation of the soluble, colloidal and coarse suspended-solids fractions of the slaughterhouse wastewater; to elucidate the mechanisms involved in the removal of the organic pollutants of the filtrate of the waste, and to assess the rate-limiting steps in the degradation of each fraction of the wastewater, as well as to assess the effects of the fractions on the methanogenic activity of the sludge.

METHODS

Sludge

Two different types of anaerobic sludge were used in these experiments, granular and flocculent. The granular sludge was obtained from a UASB-plant at a potato processing factory (AVIKO, Steenderen, The Netherlands).

The total suspended solids (TSS) content of this sludge was 9.2% and the volatile suspended solids (VSS) content 6.4%. The flocculent sludge was a digested sewage sludge, obtained from the municipal sewage treatment plant at Renkum, The Netherlands. The TSS content was 2.9% and the VSS content was 1.8%.

Analyses

Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), total nitrogen, phosphate, ammonia nitrogen (NH_4^+-N), Dry Suspended Solids (DSS) and Volatile Suspended Solids (VSS) were determined according to standard methods (Standard Methods, 1980). Protein was determined according to the methods described by Lowry (1951) and fats were determined according to the Dutch Normalized Standard Methods (1969). Volatile Fatty Acids (VFA) concentrations were determined by gas chromatography using a Packard Becker Model 417 equipped with a 6 m x 2 mm glass column and a flame ionisation detector. The column was packed with chromosorb 101 (60 - 80 mesh). Flow rate of the carrier gas, nitrogen saturated with formic acid, was 18 ml min^{-1}. The column was maintained at a temperature of 210 °C and the injection port was at 230 °C.

Preparation of the wastewater fractions

Wastewater originating from slaughtering areas was collected. The raw wastewater was separated into three different fractions according to the procedure presented in Table 1.

Table 1. Outline of the procedure applied in preparing the various waste fractions.

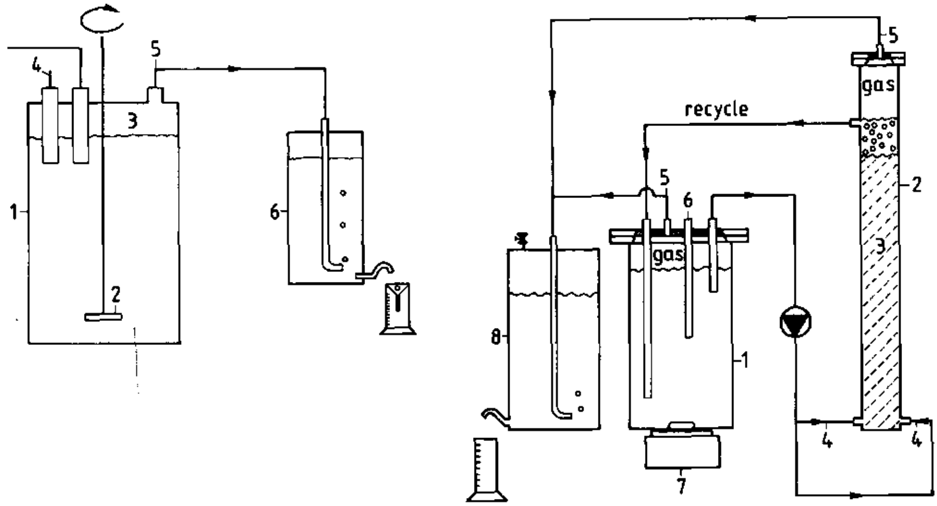
distinguished wastewater fractions	separation procedure	size limits of fraction removed
(raw wastewater)		
↓	screen	
$wastewater_{total}$ = coarse suspended solids + colloidal + soluble fraction		
↓	filtration paper filter	coarse suspended solids: 1 mm - 7.4 µm
$wastewater_{pf}$ [a] = colloidal + soluble fraction		
↓	filtration membrane filter	colloidal: 7.4 - 0.45 µm
$wastewater_{mf}$ [b] = soluble fraction		

a. $wastewater_{pf}$ = $wastewater_{paper\ filtered}$

b. $wastewater_{mf}$ = $wastewater_{membrane\ filtered}$; this fraction is arbitrarily defined as soluble.

Experimental set-up

All experiments were performed at temperatures of 30 °C and 20 °C. Two types of experiments were employed depending on the specific purpose of the experiment. Recirculation experiments with wastewater$_{mf}$, wastewater$_{pf}$ and with wastewater$_{total}$ were performed in the recirculated batch digester system shown in Figure 1. The experimental arrangement consisted of a column containing a granular sludge through which the 5.6 litres wastewater present in the container was recirculated at a superficial velocity of 3.5 m h^{-1}. The 5.3 cm diameter columns were 60 cm in height, the total volume of the digester column was 1.32 litres with a working volume of 1.20 litres.



1	digester
2	stirrer
3	head space
4	sampling points
5	gas outlet
6	Mariotte flask

1	influent vessel
2	digester column
3	granular sludge
4	influent inlet points
5	gas outlet
6	sampling points
7	magnetic stirring device
8	Mariotte flask

Figure 1. Schematic diagram of the conventional batch fed digester (left) and the recirculated batch digester (right), used in these experiments.

No mechanical mixing was applied. In all experiments 26.7 g VSS granular sludge per litre reactor volume was used in the digestion column.
The reasons for using this type of batch recirculation experiment instead of the conventional stirred-tank batch experiments were:

- little if any erosion of the granular sludge occurs,
- the practical operating conditions of granular sludge bed reactors are approached better in this experimental arrangement,
- a satisfactory contact between wastewater and sludge is provided in the column,
- information is obtained about the extent and the rate of elimination of the various wastewater fractions.

The recirculation experiments provide therefore realistic information about the maximum extent and rate of the degradation of the substrate materials and the mechanisms involved in the removal of the substrate materials.

The degradation experiments with the coarse suspended-solids fraction of the wastewater were performed in conventional stirred batch digesters (Figure 1) because recirculation experiments are less informative in this case, and it was difficult to perform with a suspension of coarse solids. All the conventional batch experiments were performed in intermittently stirred (30 sec at 140 rpm every 3 min) 3 litre digesters, using a working volume of 2.5 litres. Both types of sludge were investigated in these experiments using in all cases an initial sludge concentration of 5 g VSS per litre working volume.

The CH_4 gas production was measured using a liquid displacement system (Mariotte flask), carbon dioxide being removed from the biogas by having a 1% w/v NaOH solution in the vessels.
The degradation experiments were performed twice for wastewater$_{mf}$, four times for wastewater$_{pf}$ and once for wastewater$_{total}$ (Table 2). After each series of experiments, the column of the recirculated batch digester was seeded with a fresh amount (26.7 g l^{-1}) of the granular sludge.
The degradation experiments with the coarse suspended-solids fraction of the wastewater were performed once at 30 °C and once at 20 °C with granular sludge; with digested sewage sludge one series of experiments

Table 2. Recirculation batch experiments conducted with the various wastewater fractions and the performed sludge activity tests.

wastewater	exp.	type of experiments	
wastewater$_{mf}$	A	sludge activity measurements	(VFA-feed, Figure 3)
	B	recirculation experiments, test 1	
	C	recirculation experiments, test 2	(Figure 2)
	D	sludge activity measurements	(VFA-feed, Figure 3)
wastewater$_{pf}$	E	sludge activity measurements	(VFA-feed)
	F	recirculation experiments, test 1	
	G	recirculation experiments, test 2	(Figures 4 and 5)
	H	sludge activity measurements	(VFA-feed)
	I	recirculation experiments, test 3	
	J	recirculation experiments, test 4	
	K	sludge activity measurements	(VFA-feed)
wastewater$_{total}$	L	sludge activity measurements	(VFA-feed)
	M	recirculation experiments, test 1	(Figures 7, 8 and 9)
	N	sludge activity measurements	(VFA-feed)

Table 3. Conventional batch-fed degradation experiments conducted with the coarse suspended solid fraction of the wastewater and the performed sludge activity tests.

type of sludge	process temp. (°C)	sludge load (g COD_{SS} g^{-1} VSS)	exp.	type of experiments	remarks
granular	30		O	sludge activity measurements (VFA-feed)	
		0.5	P	batch exp. test 1	consecutive load (Fig. 10)
		0.5	Q	batch exp. test 2	"
		0.5	R	batch exp. test 3	"
			S	sludge activity measurements (VFA-feed)	
granular	20		T	sludge activity measurements (VFA-feed)	
		0.5	U	batch exp. test 1	consecutive load
		1.0	V	batch exp. test 2	"
			W	sludge activity measurements (VFA-feed)	
digested sewage sludge	30		O_1	sludge activity measurements (VFA-feed)	
		0.3	O_2	batch exp. test 1	consecutive load
		0.5	O_3	batch exp. test 2	"
		1.0	O_4	batch exp. test 3	"
			O_5	sludge activity measurements (VFA-feed)	

was performed (Table 3). All the batch experiments were performed at consecutive loads in order to assess if any sludge adaptation occurred as well as to determine the maximum extent of the substrate degradation in relation to the applied loads.
The digesters were seeded with a fresh batch of granular or digested sewage sludge after each series of experiments.

The experiments were terminated after the gas production ceased as this indicated the complete elimination of the biodegradable COD. The COD remaining in the system can be considered as non-biodegradable. The digestion process was followed by measuring the CH_4 production and by analyzing the liquid phase for VFA and COD.

The sludge-activity measurements were performed both in the recirculated batch experiments and in the conventional batch digester. A sludge concentration of 26.7 g l^{-1} was used in the recirculated batch experiments while 5 g l^{-1} was applied in all the conventional batch experiments. A mixture of acetate, propionate and butyrate at an initial concentration of 0.6 g l^{-1} each was used as substrate, the pH was between 6.5 and 7.5. In addition to the substrates the following nutrients were supplied: 174 mg NH_4Cl, 28.3 mg KH_2PO_4 and 530 mg Na_2SO_4 as well as 1 ml trace element solution according to Zehnder (1976), in all cases per litre working volume. Before starting the experiments the reactors were flushed with nitrogen gas in order to remove oxygen from the liquid and from the headspace.
The methane production was measured several times a day and also various samples were taken during the day for determining the VFA-concentrations. The maximum specific methanogenic activity of the sludge was calculated from the maximum slope of graph of the methane production with time. Generally the results of the second VFA-feed were used for calculation, because the methane production rate obtained in the second feed was significantly higher than in the first feed while it was close to that of the third feed. The specific activity is expressed as g CH_4-COD g^{-1} VSS day^{-1}.
The adsorption experiments were conducted as follows. A certain amount of granular sludge was rinsed with tap water saturated with oxygen in order to inhibit the methanogenic activity of the sludge and to remove

all the finely dispersed materials from the sludge. The adsorption experiment was performed using 0.5 litre sludge (64 g sludge-VSS per litre) with 2.5 litres wastewater$_{pf}$ in a flask. The wastewater$_{pf}$ consisted of 50% soluble and 50 % colloidal matter and its initial COD-value was 2.2 g COD l^{-1}, corresponding to a sludge load of 0.17 g COD g^{-1} VSS. Immediately after the addition of the sludge and wastewater to the flask, mechanical shaking of the mixture was started. After fixed time intervals the flask was taken from the shaking machine and the mixture was allowed to settle for 1 minute. Then a sample was drawn from the supernatant solution for determining the total COD and COD$_{mf}$.

Definitions and calculations

1. Definitions of the various fractions

For the purpose of the interpretation of the experimental results a number of fractional factors, all based on COD-equivalent, have been defined;

- Fraction Methanogenized (F_M) % = 100 x M/T_o
- Fraction Acidified (F_A) % = 100 x $(M + f_A) / T_o$
- Fraction Liquefied (F_L) % = 100 x $(M + f_A + f_{NAS}) / T_o$
- Fraction Colloidal (F_C) % = 100 x C / T_o
- Fraction Retained (F_R) % = 100 x $(1 - (M + T_x) / T_o)$

in which: M = COD removed via methane gas (g l^{-1})

1 litre CH_4 (30 °C and 720 mm Hg) is equal to 2.485 g CH_4-COD

1 litre CH_4 (20 °C and 720 mm Hg) is equal to 2.620 g CH_4-COD

f_A = COD corresponding to the VFA concentration (g l^{-1}) in the liquid phase

f_{NAS} = COD of non-acidified soluble fraction (g l^{-1}) in the liquid phase

C = COD of the colloidal fraction (g l^{-1}) in the liquid phase

T_o, T_x = total COD of the wastewater at time t=0 and time t=x (g l^{-1}) in the liquid phase.

2 Calculation of the contact time in the recirculation experiments.

$$\text{contact time (h)} = \frac{\text{working volume of the digester column (l)}}{\text{volume of the wastewater (l)}} \times \text{recirc.time (h)}$$

RESULTS

Wastewater composition

The composition of the slaughterhouse wastewater for the three fractions, expressed as a percentage of the total COD, is presented in Table 4.

Table 4. Wastewater composition.

fraction		as % of COD_{total}
coarse suspended solids		45 - 55
colloidal		20 - 30
soluble		20 - 30
Total	2870 mg COD l^{-1}	100

The results in Table 5 present the composition of the coarse suspended solids and colloidal fractions of the wastewater in terms of COD_{total} and COD_{fats} and $COD_{protein}$.

Table 5. The composition of the coarse suspended solids and colloidal fractions of the wastewater.

parameter			fractions			
			coarse SS	as % of COD	collod. SS	as % of COD
ash	%		18.3		17.2	
COD_{total}	g g^{-1} VSS	a	1.63	100	1.78	100
fats	g fats -COD g^{-1} VSS	b	1.10	67.5	1.20	67.4
protein	g protein COD g^{-1} VSS	b	0.30	18.4	0.40	22.5
rest	g COD g^{-1} VSS		0.23	14.1	0.18	10.1

1 g fats is equal to 2.91 g COD (Krol et.al., 1977)

1 g protein is equal to 1.15 g COD (Bungaard et.al., 1980)

a. Average of 8 analyses; standard deviation ± 0.10 g g^{-1} VSS

b. Average of 3 analyses; standard deviation ± 0.10 g fats COD per g^{-1} VSS, ± 0.02 g protein COD g^{-1} VSS.

The results in Table 5 indicate that the compositions of the coarse suspended solids and the colloidal fractions are similar, the fats concentration is significantly higher than the protein concentration for both fractions.

Experiments with wastewater$_{\text{membrane filtered}}$

The results of the recirculation experiments with wastewater$_{mf}$ (i.e. the soluble fraction of the wastewater) after 20 contact time) are presented in Table 6. Details of experiment C (Table 2) are shown in Figure 2. A similar figure was obtained for experiment B.

Table 6. Results of the recirculation experiments B & C after 20 h contact time.

Exp. No	a	Test No	Contact time (h)	Initital COD concentration (g l^{-1})	COD-reduction (%)	COD_{MF} converted into methane (%)
At a process temperature of 30 °C						
B		1	20.0	1.42	74	74
C		2	20.2	1.50	75	75
At a process temperature of 20 °C						
B		1	20.0	1.42	70	70
C		2	20.2	1.50	72	72

a. For explanation see Table 2.

The left hand axis of the figure represents the initial $COD_{sol.}$ concentration of the wastewater. The percentages shown in the centre column represent the maximum percentage conversion into methane-COD of the supplied COD (treatment efficiency) and COD removed.

The results of the sludge activity experiments A and D at 30 °C with the recirculated batch procedure are shown in Figure 3. A similar figure was obtained for the tests performed at 20 °C. The calculated specific sludge activities prior to and after the recirculation experiments (A, D) with wastewater$_{mf}$ are presented in Table 7.

Table 8 shows the COD values of the influent and the effluent solution in terms of COD_{total}, COD_{fats}, $COD_{protein}$ and $COD_{reduction}$ of

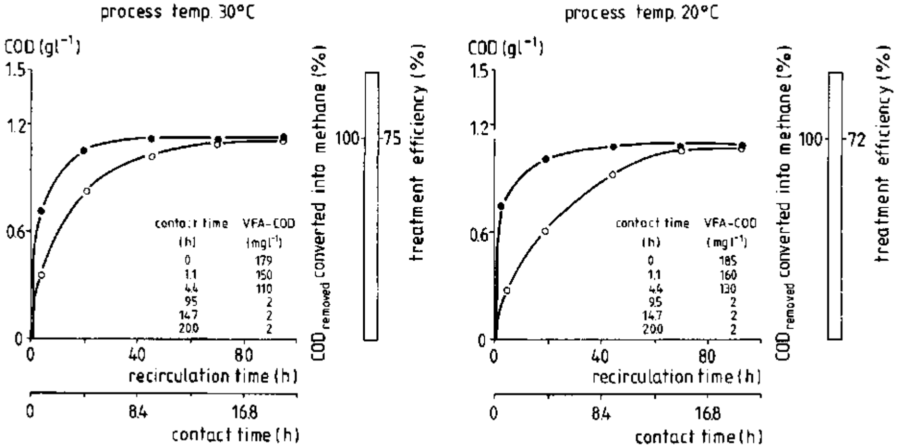


Figure 2. Results of recirculation experiment C with wastewater$_{mf}$ at 30 °C and 20 °C (●) COD removed; (o) COD converted into methane-COD ($COD_{t=o}$ = 1.50 g l^{-1}).

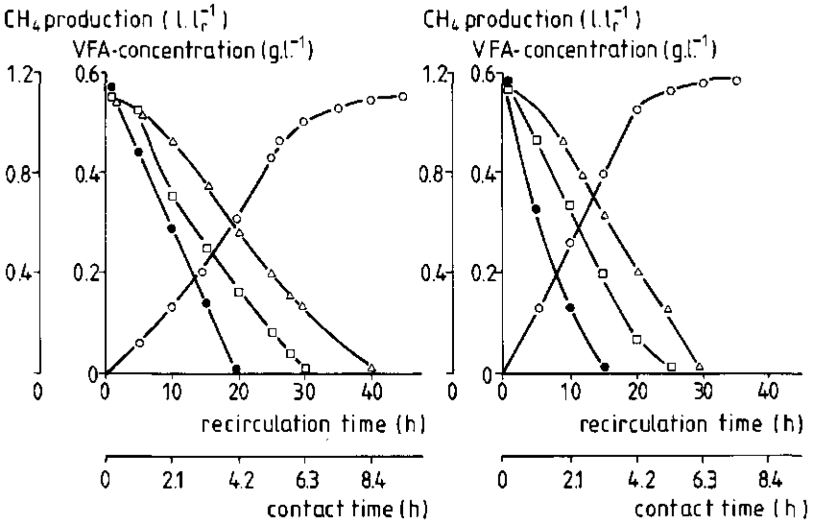


Figure 3. Results of the sludge activity experiments A (left) and D (right) (Table 2), using the recirculated batch digester at 30 °C.

o = CH_4, ● = C_2, □ = C_3, Δ = C_4 acids.

experiment C performed at 30 °C. The fat fraction of both the influent and effluent is insignificant while the protein fraction of both liquids is relatively high.

Table 7. The calculated specific methanogenic activity from the recirculation batch system with VFA-mixture (experiments A & D).

Temperature (°C)	Specific sludge activity (kg CH_4-COD kg^{-1} VSS day^{-1}) Exp. A [a]	Exp. D [a]
30	0.30	0.60
20	0.20	0.38

a. For explanation See Table 2.

Table 8. The composition of the influent and effluent in experiment C performed at 30 °C, and the calculated degradation percentages.

composition	influent		effluent		degradation
	mg l^{-1})	as % of COD	(mg l^{-1})	as % of COD	(%)
COD	1500	100	375	100	75
fats [a]	15	1.0	8	1.6	47
protein [b]	570	38.0	75	20	86.8
rest	915	61.0	292	78	68.1

a. 1 g fats is equal to 2.91 g COD (Krol et.al., 1977)

b. 1 g protein is equal to 1.15 g COD (Bungaard et.al., 1980)

Experiments with wastewater$_{\text{paper filtered}}$

Table 9 summarizes the results obtained from the recirculation experiments with wastewater$_{\text{pf}}$.

Table 9. Results of the recirculation experiments F, G, I and J after 20 hours contact time. The results indicate the COD-reduction and the calculated percentages conversion into methane-COD of the supplied COD and COD$_{\text{removed}}$ with wastewater$_{\text{pf}}$ at 30 °C and 20 °C.

Exp. No [a]	Test No	contact time (h)	initial COD concentration (g l^{-1})		COD-reduction [b] (%)		COD$_{\text{pf}}$ converted into methane (%)	COD$_{\text{pf}}$ removed converted into methane (%)
			COD$_{\text{pf}}$	COD$_{\text{mf}}$	COD$_{\text{pf}}$	COD$_{\text{mf}}$		
At a process temperature of 30 °C								
F	1	20.1	2.20	1.10	85	86	54	63.5
G	2	20.0	2.23	1.22	86	81	61	71
I	3	20.8	2.43	1.20	91	89	60	66
J	4	20.8	2.54	1.17	85	84	58	68
Average					87	85	58	67
At a process temperature of 20 °C								
F	1	20.1	2.20	1.10	78	86	42	54
G	2	20.0	2.23	1.22	80	86	49	61
I	3	20.8	2.43	1.20	88	87	47	53
J	4	20.8	2.54	1.17	82	82	45	55
Average					82	85	46	56

a. For explanation see Table 2.

b. [1-COD$_{\text{pf}}$ (effluent) / COD$_{\text{pf}}$ (influent)] x 100 and [1 - COD$_{\text{mf}}$ (effluent) / COD$_{\text{mf}}$ (influent)] x 100 .

More detailed results of experiment G (Table 2) are shown in Figure 4. Very similar figures were obtained for the other experiments. The results in Figure 4 indicate that at the termination of the experiments, after a contact time of 20 h, a part of the eliminated COD has not been converted into methane. This is represented by the area between the upper and the lower curves in the figure. The removal efficiency amounts to 86% at 30 °C; only 61% of the imposed COD load has been converted into methane after this 20 h contact time, corresponding to 71% of the COD$_{\text{removed}}$. The retained COD, comprising 29% of the COD$_{\text{removed}}$, apparently has been eliminated in a different way. At a process temperature of 20 °C 39% of the COD$_{\text{removed}}$ can not be accounted for as methane-COD. Additional information about the course of the degradation of the various waste-

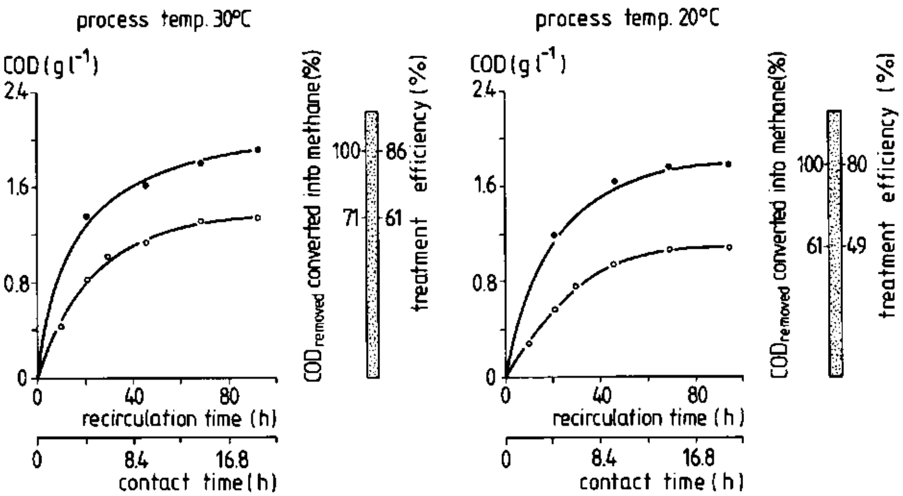


Figure 4. Results of recirculation experiment G with wastewater$_{pf}$ at 30 °C and 20 °C. (●) COD removed; (o) COD converted into methane-COD ($COD_{t=o} = 2.23\ g\ l^{-1}$).

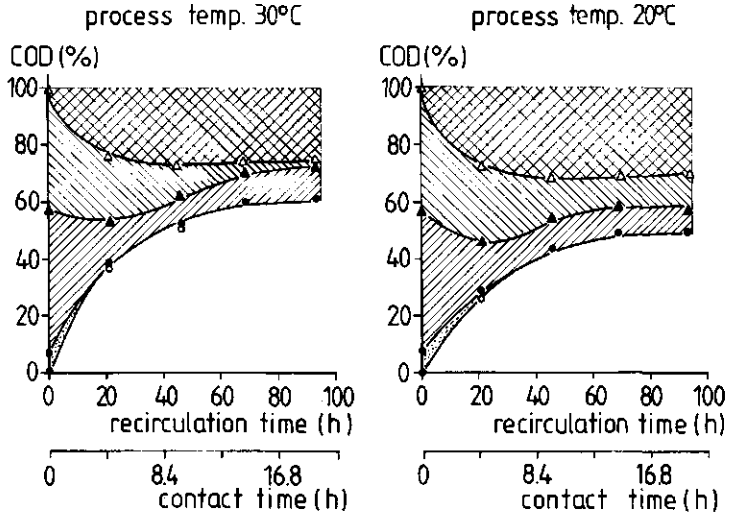


Figure 5. Development of the various f-factors in relation to the contact time for experiment G at 30 °C and 20 °C.

☐ = F_M, ▩ = f_A, ▨ = f_{NAS}, ▧ = F_C, ⊠ = F_R.

water fractions is provided by Figure 5, where the calculated values of the various f-factors have been plotted versus the digestion time. The figure shows the five f-factors, F_M, f_A, $f_{\text{non-acidified soluble}}$, $F_{\text{colloidal}}$ and F_{retained}. The results of the specific-sludge activity experiments E, H and K (see Table 2) are summarized in Table 10.

Table 10. The calculated specific methanogenic activity from the recirculation batch system with VFA-mixture (experiments E, H & K).

Temperature (°C)	specific sludge activity (kg CH_4-COD kg^{-1} VSS day^{-1})		
	exp.[a] E	exp.[a] H	exp.[a] K
30	0.30	0.26	0.25
20	0.20	0.17	0.15

a. For explanation See Table 2.

Adsorption experiments

The adsorption experiments were performed to assess to what extent the adsorption of soluble and colloidal fractions of the organic pollutants occurred. The data in Figure 6 reveal that 10% of the soluble-COD and 50% of the total COD of the $\text{wastewater}_{\text{pf}}$ have been adsorbed.

Experiments with the total wastewater

Figures 7 and 8 present the results of the recirculation experiment M (Table 2) performed with the total wastewater at 30 °C and 20 °C. The results in Figure 7 show that the treatment efficiency of the total wastewater is much higher at a process temperature of 30 ° C (92%) than at 20 °C (73%). Only 73% and 70% of the removed substrate materials are converted into methane-COD at process temperatures of 30 °C and 20 °C respectively. Figure 9 shows the development of the digestion process in terms of the various f-factors.

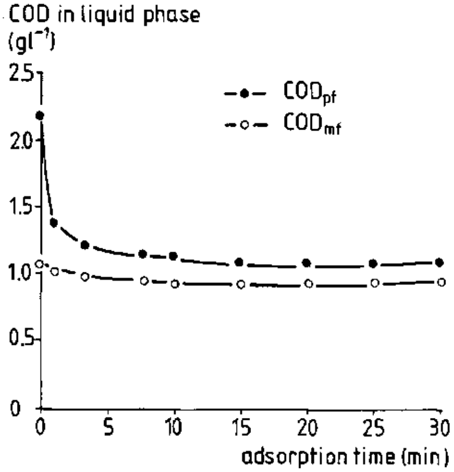


Figure 6. Results of the adsorption experiment with wastewater$_{pf}$ and granular sludge. COD-wastewater$_{pf}$ = 2.20 g l^{-1}, COD$^{pf}_{mf}$ = 1.10 g l^{-1}, sludge load 0.17 g COD g^{-1} VSS, temperature = 30 °C.

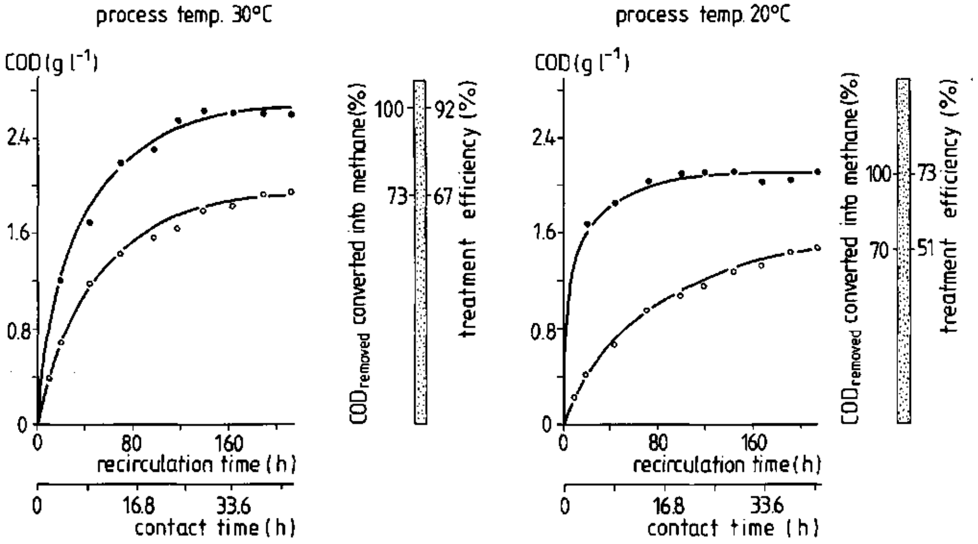


Figure 7. Results of the recirculation experiment M with the total wastewater at 30 °C and 20 °C. (●) COD removed, (o) COD converted into methane-COD (COD$_{t=o}$ = 2.87 g l^{-1}).

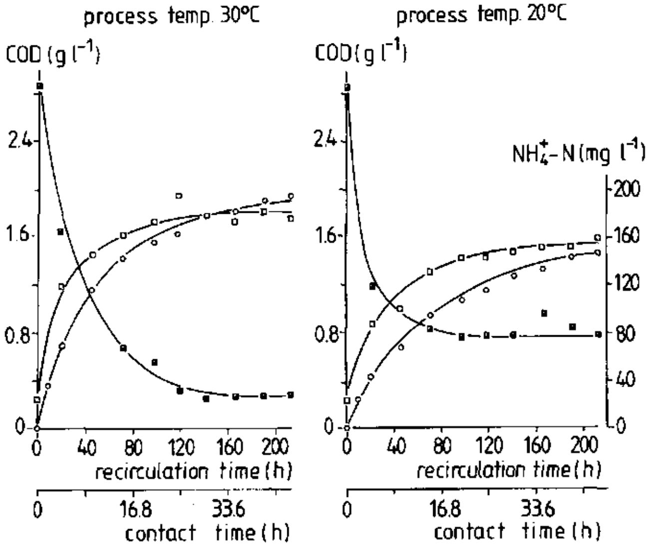


Figure 8. The COD_{total} concentration (◪) and the course of the methane (o) and the ammonia nitrogen (□) production during the degradation of the total wastewater.

Degradation of the coarse suspended solids of the wastewater

Table 11 summarizes the results of the conventional batch degradation experiments with the coarse suspended solids fraction of the wastewater after 260 h digestion time. More detailed results of the experiments P, Q and R with granular sludge performed at a sludge load of 0.5 g COD_{SS} $g^{-1}VSS$ and at a process temperature of 30 ^{o}C are shown in Figure 10. Similar figures were obtained for the other experiments. In all these experiments no differentiation was made between the soluble and colloidal COD fractions, because only the COD of the paper filtered samples was analyzed, so only four COD-fractions can be distinguished. In addition to Figure 10, the data in Table 12 provide information about the degradation of the various fractions of the coarse insoluble matter for the experiments P, Q and R. The results of the specific sludge activity experiments Q and S at 30 ^{o}C and T and W at 20 ^{o}C with granular sludge and experiments O_1 and O_5 at 30 ^{o}C with digested sewage sludge made prior to and after the coarse suspended solids digestion are summarized in Table 13.

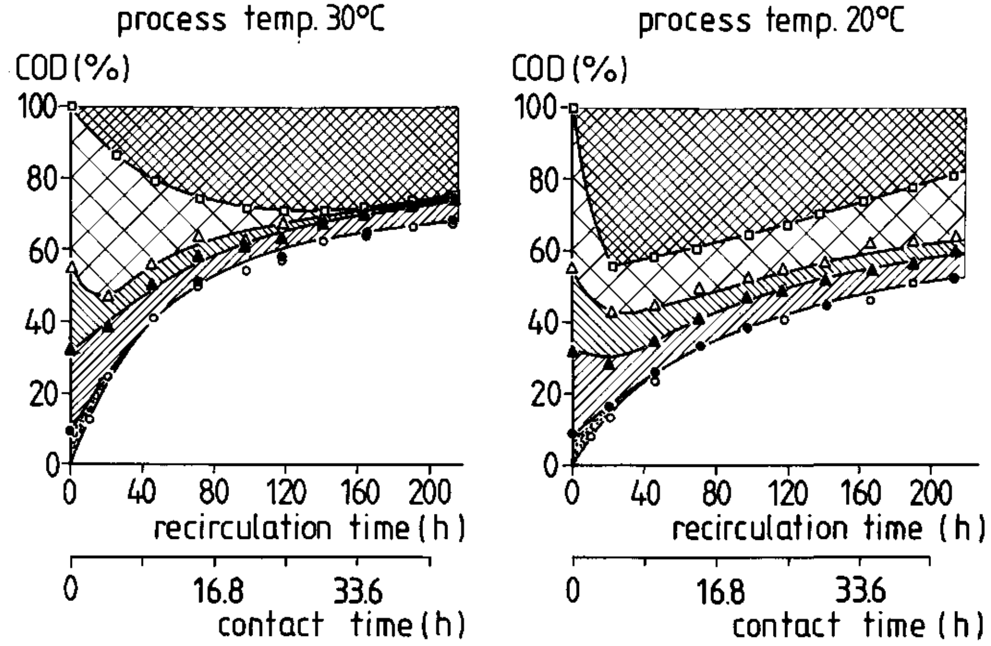


Figure 9. Development of the various f-factors in relation to the contact time for experiment M at 30 °C and 20 °C.

□ = F_M, ▦ = f_A, ▨ = f_{NAS}, ▧ = F_C,

▩ = $F_{\text{coarse suspended solids}}$, ■ = F_R.

DISCUSSION

Degradation percentages of the various fractions of slaughterhouse wastewater.

The experimental results of the recirculation tests obtained with wastewater$_{mf}$ (Table 6 and Figure 2) indicate that the COD reduction achieved, which corresponds in this case to COD soluble reduction after 20 h contact time, and the calculated percentage conversion of the supplied COD$_{soluble}$ into methane-COD, are almost equal for the two experiments; only a slightly higher COD reduction is achieved at 30 °C (74-75%) than at 20 °C (70-72%).

Table 11. Results of the conventional batch degradation experiments performed with granular sludge (experiments P,Q,R,U & V) and with digested sewage sludge (experiments O_2, O_3 & O_4) using the coarse suspended solids fraction of the slaughterhouse waste as a substrate.

type of sludge	exp.[a] no	process temp. (°C)	digestion time (h)	sludge load (g COD_{SS} g^{-1} VSS)	conversion into methane (%)	remarks
granular	P	30	260	0.5	43	consecutive load (Fig. 10)
	Q	30	260	0.5	40	"
	R	30	260	0.5	50	"
granular	U	20	260	0.5	45	consecutive load
	V	20	260	1.0	40	"
digested	O_2	30	260	0.3	50	consecutive load
sewage	O_3	30	260	0.5	47	"
sludge	O_4	30	260	1.0	41	"

a. For explanation see Table 3.

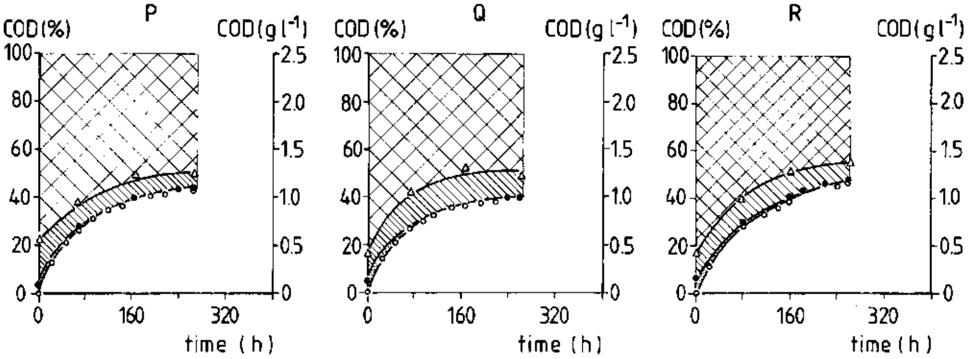


Figure 10. Results of the batch degradation experiments P,Q & R (Table 3) with the coarse suspended solids fraction, which was performed at 30 °C at consecutive sludge loads of (0.5 g COD_{SS} g^{-1} VSS) with granular sludge.

□ = F_M, ⊡ = f_A, ▧ = $F_C + f_{NAS}$, ▩ = $F_{\text{coarse suspend.solids}}$.

However, at both temperatures the removed COD was completely converted into methane-COD after 20 h contact time.

Table 12. The composition of the coarse suspended solids fraction in experiments P,Q & R (Table 3) performed at 30 °C and the calculated degradation percentages.

parameter		raw SS	as % of COD	digested SS	as % of COD	degradation (%)
ash	%	18.3	-	-	-	-
$COD_{(total)}$ [a]	g g^{-1} VSS	1.63	100	0.81	100	50.0
fats [b]	g fats COD g^{-1} VSS	1.10	67.5	0.60	74.1	45.5
protein [b]	g protein-COD g^{-1} VSS	0.30	18.4	0.04	4.9	86.7
rest	g COD g^{-1} VSS	0.23	14.1	0.17	21.0	26.1

1 g fats is equal to 2.91 g COD (Krol et.al., 1977).

1 g protein is equal to 1.15 g COD (Bungaard et.al., 1980).

a. Average of 8 analyses; standard deviation ± 0.10 g g^{-1} VSS.

b. Average of 3 analyses; standard deviation ± 0.10 g fats COD per g^{-1} VSS, ± 0.02 g protein COD g^{-1} VSS.

The results of the recirculation experiments with $wastewater_{pf}$ (Table 9) show that the COD_{pf}- and COD_{mf}- reductions, as well as the calculated conversion percentages of the supplied COD_{pf} and the removed COD into methane, are within a fairly narrow range. The amounts of the supplied COD converted into CH4-COD after a contact time of 20-21 h range from 54 - 61% (an average of 58%) at 30 °C, and 42-49%, with an average of 46%, at 20 °C. At 30 °C an average of 67% of the removed COD was converted into CH4-COD, while the value is 56% at 20 °C. Apparently, a considerable fraction of the removed COD is eliminated by some kind of adsorption, precipitation and/or flocculation mechanism. As the soluble COD eliminated from the system (compare results in Table 6 and Figure 2) is almost completely converted into CH4-COD, after 14 h contact, the relatively poor conversion of the eliminated COD found in the experiments with $wastewater_{pf}$ obviously should be attributed almost exclusively to the poor conversion of colloidal fraction of the wastewater.

The results in Figure 10 (Table 11) illustrate that at maximum, approximately 50 % of the coarse suspended solids is converted into methane-COD with granular sludge at 30 °C.

On the basis of the wastewater composition (Table 4) it is possible to estimate the contribution of each of the various fractions of the wastewater to the average conversion into CH_4 of the COD present in the total wastewater.

Table 13. Results of the specific methanogenic activity; experiments O & S at 30 °C and experiments T & W at 20 °C with granular sludge and with digested sewage sludge at 30 °C experiments O_1, O_5 made prior to and after the coarse suspended solids digestion.

type of sludge	process temp. (° C)	exp. no[a]	specific sludge activity ($kg\ CH_4 - COD\ kg^{-1}\ VSS\ day^{-1}$)
granular	30	O	0.30
	30	S	0.10
granular	20	T	0.20
	20	W	0.12
digested sewage sludge	30	O_1	0.12
	30	O_5	0.10

a. For explanation see Table 3.

At 30 °C the contribution of the soluble fraction of the wastewater (experiments with $wastewater_{mf}$, Table 6) will be 0.25 x 75 = 18.70%, while for the sum of the colloidal and soluble fractions ($wastewater_{pf}$) it is 0.5 x 58 = 29%. Consequently the contribution of the colloidal fraction separately is 29 - 18.70 = 10.3 %. The coarse suspended solids present in the wastewater contribute 0.50 x 45 = 22.5% to the amount of COD converted into methane-COD. On this basis, the average percentage of the total wastewater COD converted into methane-COD can be estimated as 18.75 + 10.25 + 22.5 = 51.5 %. In the recirculation experiments with the total wastewater (Figure 7), after a contact time of 20 h (the same as used with the separate fractions of the wastewater) a slightly higher conversion percentage, 57%, was found at 30 °C. The same calculation for a process temperature of 20 °C gives an average conversion value of 44.3 % on the basis of the contribution of the separate fractions of the wastewater, while in the experiment with the total wastewater 40% was found after 20 h contact time (Figure 7).

In conclusion it can be stated that the soluble fraction is better converted into methane (75% at 30 ^{o}C), than the colloidal fraction (i.e. wastewater$_{pf}$ minus wastewater$_{mf}$) and the coarse suspended solids (40 to 50% at 30 ^{o}C).

The removal mechanism of the organic pollutants fractions

The data in Figure 2 provide essential additional information, because they show that, temporarily, part of the removed soluble COD is present in, on, or between the sludge. The conversion of this part of the COD into methane-COD proceeds fairly slowly. Particularly at lower temperatures, a considerable fraction of the soluble COD accumulates temporarily in the sludge bed. It will be obvious that under conditions of continuous operations, as is the case in normal UASB-application, a considerable accumulation of the soluble substrate materials may occur depending on the imposed sludge load and the operational temperature. The sludge load applied in these experiments was equal to 0.263 g soluble-COD g^{-1}VSS. Consequently, despite the fact that the 'soluble pollutants' ultimately will be completely converted into CH_4-COD, in practice there may be insufficient time available to accomplish this complete conversion. The results in Figure 2 also show that at the termination of the experiments, after a contact time of 20 h, approximately 25% of the soluble fraction, corresponding to 6-7 % of the total COD of the wastewater, is still not converted into methane-COD.

Taking into account that the VFA-COD concentration was very low (2 mg l^{-1}) after a contact time of 9.5 h we can conclude that the acidification is the rate-limiting step in the conversion of the soluble fraction into methane-COD.

The experimental results obtained with the wastewater$_{pf}$ (Figure 5) and those with the total wastewater (Figure 9) are in accordance with the experiments with the soluble fraction of the wastewater (Figure 2) as far as the behaviour of the non-acidified fraction (f_{NAS}) is concerned. The results in Figures 5 and 9 indicate that after a contact time of approximately 4.2 h for the wastewater$_{pf}$ and 9 h for the total wastewater, all volatile fatty acids have been removed from the liquid phase. Furthermore, the data in both figures show that at the termination of the experiment a part of the non-acidified soluble, fraction is still not converted into methane. This also corresponds with the results in Figure 2. The amount of the non-acidified soluble COD remaining in the liquid phase of both the wastewater$_{pf}$ and the total wastewater are almost equal to that of the wastewater$_{mf}$. It can therefore be concluded that the remaining soluble COD is not anaerobically biodegradable. Figures 4 and 5 provide information about the experiments with wastewater$_{pf}$, consisting of soluble as well as colloidal fractions. At the termination of the experiments, after a contact time of 20 h, a part of the removed COD has still not been converted into methane. This retained COD is 29% at 30 ^{o}C and 39% at 20 ^{o}C.

In a previous paper (Sayed et al., 1987) we reported that the mechanism involved in the removal of the soluble and colloidal fractions of the wastewater presumably could be the adsorption of these fractions to the available surface of the sludge. In order to assess to what extent adsorption of these fraction occurs we performed adsorption experiments. The experimental results shown in Figure 6 show that at a sludge load of 0.17 g COD g^{-1}VSS, with wastewater$_{pf}$ (with equal quantities of the colloidal and the soluble fraction), 10% of the soluble COD and 50% of the total COD of the wastewater$_{pf}$ adsorbs in less than 10 minutes. As roughly 50% of the wastewater$_{pf}$ consists of soluble matter, the soluble fraction contributes only 0.1 x 50 (5%) of the total adsorption in terms of the total COD of the wastewater$_{pf}$. The contribution of the colloidal fraction therefore is 50 - 5 (45%) of the total COD of the wastewater$_{pf}$, corresponding to 90% of the colloidal COD fraction in the waste. The experimental results of the wastewater$_{pf}$ (Figure 5) indicate the adsorption of the non-acidified, soluble, fraction to the sludge. The experimental data in this figure, which show the elimination of the non-acidified, soluble, fraction as a function of time, show that at a contact time of 4.2 h at 30 ^{o}C, only 14% remained as non-acidified, soluble, fraction, compared to the initital soluble fraction of 56%. From the eliminated soluble fraction alone 55 - 14 (41%) CH_4-COD can be expected when completely converted. The amount of CH_4 produced was 37%. Similar dips in the upper boundary line of the non-acidified, soluble, fraction (f_{NAS}) pointing to adsorption occur at a process temperature of 20 ^{o}C in the experiments with total wastewater at the two applied temperatures (Figure 9).

In the adsorption experiments the adsorption of the colloidal matter to the sludge proceeds rapidly; 90% of the ultimately adsorbed amount is removed in the first 10 minutes at the imposed colloidal matter sludge load of 0.085 g colloidal-COD g^{-1} VSS (Figure 6). In the experiments in Figure 5, apparently the adsorption proceeds more slowly and is far from complete. This significant discrepancy can be explained on the basis of the different load of colloidal matter imposed on the sludge in these experiments. It should be taken into account that the sludge had been exposed to the wastewater already in previous runs (see Table 2). The colloidal matter sludge-load used in these experiments for each run was equal to 0.17 g colloidal- COD g^{-1} VSS, which is twice as high as the sludge load applied in the adsorption experiments. However, the above-mentioned significant discrepancy in the removal of the colloidal fraction of the wastewater confirms that the prevailing mechanism involved in the removal of this organic colloidal matter is the adsorption mechanism, because, as is known (Schroeder, 1977), with a fixed number of accessible sites available on the adsorbent (in this case the granular sludge) the quantity of the adsorbate (the colloidal matter) that can be taken up by an adsorbent is a function of the concentration of the adsorbate.

As far as the behaviour of the colloidal matter is concerned, the results with the total wastewater (Figure 9) are very similar to those of the experiments with wastewater$_{pf}$. At 30 °C the colloidal matter is completely eliminated after approximately 34 h contact time, while at 20 °C after 42 h contact time its concentration remains almost constant at 23% of the initital concentration.

Apparently the liquefaction of the colloidal matter proceeds at a low rate and is far from complete at 20 °C. Assuming that the extent of the adsorption will not be very different between 20 °C and 30 °C, the disappearance of the colloidal matter at 30 °C can only be attributed to liquefaction. The fact that at 20 °C a significant fraction of the colloidal matter is still present in the liquid phase points to a significantly lower rate of liquefaction at 20 °C than at 30 °C. This temperature dependency of the liquefaction of the colloidal matter is not unexpected, because it is well known that the liquefaction of the insoluble substrate ingredients is strongly dependent on the temperature (Pfeffer, 1974; Sayed et al., 1984). This is also the case with the coarse suspended solids of the wastewater (Table 11).

The results in Figure 9 show that the amount of retained COD at 20 °C increases very rapidly during the first contact hours, while at 30 °C the retained COD increases slowly during the whole experimental period. Apparently the entrapment mechanism of the coarse suspended-solids proceeds more effectively at 20 °C. This can probably be explained by the lower gas production (0.045 g CH_4-COD g^{-1} VSS day^{-1} at 20 °C and 0.081 g CH_4-COD g^{-1}VSS day^{-1} at 30 °C). Furthermore the results in Figure 9 also show a significant difference in the the completeness of the removal of the coarse suspended-solids fraction from the liquid phase. Contrary to removal at 30 °C the coarse suspended-solids are not completely removed from the liquid phase at 20 °C. This difference can be attributed to the poor retention of part of the (coarse) suspended solids at 20 °C compared to retention at 30 °C, presumably due to a difference in the physical nature of the (coarse) suspended solids. Also, the slower rate of liquefaction of the suspended solids at 20 °C than at 30 °C leads to an incomplete removal at the termination of the experiments. The decrease in $F_{retained}$ at 20 °C can be attributed to the conversion of the retained suspended-solids to methane-COD, because the results in Figure 9 show that from approximately 4.2 h contact time onwards, until the termination of the experiments, the amount of the coarse suspended-solids remaining in the liquid phase is almost constant.

Apart from the temperature, the rate of liquefaction depends greatly on the chemical composition of the substrate. Presumably the liquefaction of the coarse- and colloidal-matter in slaughterhouse wastewater will proceed in a similar way, because both fractions are very similar in composition (Table 5). In both fractions the fats concentration is significantly higher than the protein concentration. The results in Table 12 indicate that the protein fraction is degraded quite efficiently and considerably better than the fats fraction. Results obtained by Breure et al., (1986) in UASB-experiments with protein solutions as substrate and with granular sludge indicate that the degradation of the protein proceeds easily at 30 °C, similarly to the degradation of easily hydrolysable carbohydrates. Nagase & Matsuo (1982) also reported a high degradation-rate of various proteins in anaerobic batch cultures. The high degradation-rate of the proteins present in the slaughterhouse wastewater is also illustrated by the results in Figure 8. The proteins are fairly well degradaded, because the measured NH_4^+-N concentration at the termination of the experiments accounted for 85% and 76% of the total N-Kjeldahl at process temperatures of 30 °C and 20 °C respectively. The measured maximum degradation percentages of the fats fraction at 30 °C (Tables 8, 12) are comparable to those reported by Heukelekian & Mueller (1958) for the fats fraction in raw sewage sludge between 30 and 35 °C. According to Neave & Buswell (1928) the sewage fats will be degradaded anaerobically to a maximum extent varying between 40 and 49 % at temperatures from 25 °C to 35 °C. A low degree of anaerobic degradation of the fats fraction in sewage sludge, particularly at low temperatures (15 - 25 °C), was also reported by O'Rourke (1968).

The methanogenic activity of the sludge after exposing it to the various fractions of slaughterhouse wastewater.

From the results of this study the main reason for the deterioration of the methanogenic activity of the sludge can be attributed to the high adsorption of the colloidal fraction of the wastewater to the surface of the sludge and to the high fats content of this fraction (Table 10). Such a deterioration has not been found with the mainly-soluble fraction of the wastewater. In that case, the degree of adsorption remains low, while in the soluble fraction also the fats content is low. As a matter of fact, exposure of the sludge to the soluble fraction can have a positive effect on its methanogenic activity (Table 7). Considering the results of the experiments with the soluble fraction (Figure 2) one should take care that under conditions of continuous operation the sludge load remains low, because, as explained above, the conversion of part of the soluble matter proceeds via a temporary adsorption phase. The conversion of this adsorbed matter is a relatively slow process and consequently the sludge load should be kept low enough to avoid any accumulation of this matter. The adsorption of the colloidal materials, and possibly also of soluble matter, will result in a film surrounding the granular sludge of increasing thickness and perhaps also density which increasingly will hamper the supply of substrate to the bacteria present in the grains. This may finally lead to a complete deterioration of the methanogenesis of the granular sludge, as was previously observed in the UASB-experiments (Sayed et al., 1987). These phenomena will also most likely prevail in other high-rate systems such as the fluidized-bed and anaerobic fixed film systems.

The inhibitory effect of fats towards the anaerobic degradation can be attributed to long-chain fatty acids. Investigations by Hanaki et al., (1981) indicate that the anaerobic hydrolysis of neutral fats to glycerol and long-chain fatty acids proceeds easily. Their results also indicate that long fatty acids produced by the hydrolysis of the neutral fat inhibited their own β-oxidation and conversion into methane, although neutral fat itself was not inhibitory.

Recommendations for practical application

From this study and previous work (Sayed et al., 1984; 1987) we can make the following recommendations for the application of the UASB-system to the treatment of slaughterhouse wastewater.

A good fat separator should be installed to prevent excessive scum layers in the reactor (Sayed et al., 1984).

An UASB-reactor operated with granular sludge may be chosen when high loading rates (11 kg COD m^{-3} at 30 ^{o}C) are required. In this case, suspended solids comprising 50% of the COD will not be removed in the UASB-reactor and the waste should be treated by preflotation / presettling or postsettling. To prevent inhibition of the sludge, the sludge loading-rate should be kept below approximately 0.34 kg COD kg^{-1} VSS day^{-1} (Sayed et al., 1987). In our opinion the best results are achieved with an UASB-reactor, with flocculent sludge, operated semi-continuously e.g. with an organic load varying between day and night (7.5 - 3.5 kg COD m^{-3} day^{-1}) and with a weekend interruption, in order to provide periods of low or no loading which help to complete the liquifaction of complex organic matter which accumulates in the sludge bed (Sayed et al., 1984).

Preferably, during the weekend interruption of the load the reactor temperature should be increased to 30-35 ^{o}C from a lower operational temperature, in order to speed up the conversion of the accumulated organic pollutants into methane (Sayed et al., 1987; from this study).

In circumstances where an excess accumulation of organic pollutants occurs, we recommend occasional removal of part of the sludge. The removed sludge might then be digested in a separate sludge digester. The stabilized sludge could be returned as active sludge to the UASB-reactor (Sayed: in press).

ACKNOWLEDGEMENTS

Valuable advice and numerous discussions on the manuscript by Dr W. de Zeeuw are gratefully acknowledged.

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CHAPTER 6

SUMMARY

Effluents from the slaughterhouses, meat and poultry industries are heavily polluted and contain a high concentration of biodegradable organic materials. Therefore, the pollution capacity of these industries is high. Most of these industries discharge their effluents to a sewer or a watercourse.

In order to comply with water pollution control standards and to reduce costs on sewer surcharges, these industries have to apply an adequate treatment of their effluents.

Physical and chemical treatment methods as well as the conventional biological treatment processes are frequently applied in the treatment of these effluents. A combination of the methods are required where the effluent is to be discharged to surface waters, since no single treatment method will provide sufficient effluent.

In the last decade, the high rate anaerobic wastewater treatment systems have become a good alternative for conventional aerobic as well as anaerobic biological treatment methods. The high rate anaerobic treatment systems were initially developed for the treatment of highly soluble low and medium strength wastewaters. These systems provided only a partial treatment of complex wastewaters containing a high fraction of suspended solids such as slaughterhouse wastewater.

Investigations have shifted towards the application of high rate systems like the upflow anaerobic sludge blanket (UASB) for the complete treatment of agro-industrial wastewaters which are more difficult to handle, because they contain relatively high concentrations of suspended solids, i.e. complex wastewaters.

Presently, the UASB system is the most widely applied high rate anaerobic system for complete treatment of such complex wastes.

This thesis focuses on the question whether, and under which operational conditions and environmental circumstances a one stage UASB mesophilic anaerobic treatment system is suitable for a complete treatment of slaughterhouse wastewater in practice.

The feasibility of using the upflow flocculent anaerobic sludge blanket (UASB) process for a one stage anaerobic treatment of unsettled complex slaughterhouse wastewater, which contains approximately 50% of coarse insoluble COD, was investigated (Chapter 2). The continuous experiments were performed in a 25.3 m^3 UASB pilot-plant which was operated under semi-continuous conditions, viz. with a varying organic load over day and nighttime (i.e. high organic load during the daytime and with low organic load at night) and with weekend feed interruptions. The UASB pilot-plant was operated at a temperature of 30 °C. In order to assess the feasibility of the process under conditions of lower temperatures the temperature was reduced to 20 °C, 20 weeks after the start-up of the reactor.
The data indicated that the system can satisfactorily handle organic loads up to 3.5 kg COD m^{-3} day^{-1} at a liquid retention time of 8 h at temperatures as low as 20 °C. Temporary shock loads up to 7.5 kg COD m^{-3} day^{-1} during the day time at a liquid retention time of 5 h were accommodated satisfactorily provided such a shock load was followed by a period of low loading, e.g. at night.
A significant discrepancy was found between the treatment efficiency in terms of COD reduction and to the lower calculated percentage of supplied COD_{total} converted into methane-COD. This difference indicated that a significant portion of the achieved COD reduction was due to the accumulation of non- or slowly biodegradable substrate ingredients in the reactor. No differentiations could be made between the different types of substrate ingredients that accummulated in the reactor because the accumulated sludge was not characterized. However, a part of the accumulated substrate was converted to CH_4 in periods of feed interruptions.

In Chapter 3 the feasibility of the upflow granular anaerobic sludge blanket process for a one-stage anaerobic treatment of slaughterhouse wastewater was investigated. The experiments were performed under semi-continuous operational conditions viz. continuous feeding at a constant organic load (24 h day) during the working days but with weekend feed interruptions, and process temperatures of 30 °C and 20 °C. Under a stable operation of the system, i.e. at a maximum COD reduction and a high conversion of COD into methane, the optimal loading rates that could be applied were 11 kg COD m^{-3} day^{-1} and 7 kg COD m^{-3} day^{-1} at 30 ^{o}C and at 20 ^{o}C respectively.

The system was less effective in the removal of coarse suspended solids, compared to the removal of the colloidal and soluble fractions from the slaughterhouse wastewater.

The data obtained in these investigations indicate that imposed prolonged loadings exceed the optimal loading rates, lead to deterioration of the specific methanogenic activity of the sludge, due to the accumulation of colloidal and soluble fractions of the wastewater in the sludge bed. Therefore, it was concluded that the system stability strongly depends on the processes involved in the removal of the colloidal and soluble compounds from the wastewater and their conversion into methane. As the predominant - non-biological-mechanisms underlying the elimination of these wastewaters pollutants were considered the entrapment and the adsorption mechanisms. The effect of these mechanisms on the rate of the liquefaction of the accumulated substrate - which is the required first step in their conversion into methane - were discussed.

The different pollutant fractions of the wastewater, viz. the coarse suspended solids, the colloidal and the soluble compounds affect the performance of the UASB reactors because of the different mechanisms involved in the removal of these substrate ingredients and their subsequent conversion into methane. Therefore, these mechanisms were investigated in more detail. The results of these investigations are presented in Chapter 4. The experiments were performed in a one-stage flocculent sludge UASB-reactor under continuous operational conditions viz. continuous feeding at a constant organic load during 24 h a day and 7 days a week.

The COD removal efficiency of the UASB reactor exceeded the COD removal efficiency as expected from the observed CH_4 production, indicating once again that non-biological mechanisms are involved. Two different non-biological mechanisms were distinguished in the removal of substrate ingredients from the wastewater. The entrapment mechanism prevailed in the elimination of coarse suspended solids, while mainly adsorption mechanisms are involved in the removal of colloidal and the soluble fractions of the wastewater.

A continued accumulation of substrate ingredients in the reactor ultimately will become detrimental for the stability of the anaerobic treatment process, as it leads to sludge flotation and consequently could result in a complete loss of the active biomass from the reactor.

After having demonstrated the principle feasibility of the upflow anaerobic sludge blanket (UASB) process for a one-stage anaerobic treatment of the slaughterhouse wastewater, we decided to assess the maximum possible extent of anaerobic degradation of the soluble, colloidal and coarse suspended solids fractions of the slaughterhouse wastewater (Chapter 5). In this way we intended to get a better insight in the real limitations of the system. All the experiments were performed at process temperatures of 30 ^{o}C and 20 ^{o}C, using membrane filtered wastewater (wastewater$_{mf}$), paper filtered wastewater (wastewater$_{pf}$) and total wastewater. The experiments were performed in a recirculated batch digester system with granular sludge. The experiments with the coarse suspended solids separated from the wastewater were performed with granular sludge as well as with flocculent sludge using conventional batch-fed stirred digesters. The maximum biodegrability percentages (i.e. conversion into methane) found at 30 ^{o}C were 75% for wastewater$_{mf}$, 61% for wastewater$_{pf}$ and 67% for wastewater$_{total}$ while at 20 ^{o}C these values were 72%, 49% and 51% respectively. The maximum biodegrability of the coarse suspended solids fraction of the waste amounts to 50% at 30 ^{o}C and 45% at 20 ^{o}C.

The mechanisms involved in the removal of the soluble and colloidal fractions of the slaughterhouse wastewater were thoroughly studied and elucidated. The data obtained in these experiments indicate that the prevailing mechanism in the removal of the soluble but especially also the colloidal fraction of the wastewater is an adsorption mechanism. The relatively high degree of adsorption of the colloidal fraction of the wastewater to the surface of the sludge, in combination with its high fat content, will deteriorate the specific methanogenic activity of the sludge. The adsorption of the colloidal materials will ultimately result in an enclosure of the granular sludge bacterial matter with a film of increasing thickness, and perhaps also density, which increasingly will hamper the supply of substrate to the bacteria present in the grains. The deterioration effect of fats towards the methanogenic activity of the sludge was explained on the basis of the inhibitory effect of the long-chain fatty acids of the neutral fats.

As the extent of adsorption is very similar at lower and higher temperatures, but the rate of liquefaction of adsorbed compounds drops significantly at decreasing temperatures, it will be evident that the process can withstand considerably lower loading rates at 20 °C as compared to 30 °C.

Therefore, it is concluded that the rate of liquefaction of the adsorbed insoluble colloidal fraction of the wastewater is the controlling factor with respect to loading potentials of the process and consequently that the temperature is the factor of predominant importance.

SAMENVATTING

Afvalwater van slachthuizen, vlees- en pluimvee-verwerkende industrieën is zwaar vervuild en bevat een hoge concentratie biologisch afbreekbare organische stoffen. Dit afvalwater vormt een grote belasting voor het milieu. De meeste van deze industrieën lozen hun afvalwater op een riool of waterloop. Om te kunnen voldoen aan lozingsnormen en om kosten te drukken van milieuheffingen zullen vleesverwerkende bedrijven een adequate zuivering van hun afvalwaterstromen moeten toepassen. Zowel fysische, chemische als conventionele biologische zuiveringsmethoden worden veelvuldig toegepast op deze afvalwaterstromen. Daar waar op oppervlaktewater geloosd wordt is een combinatie van zuiveringsmethoden noodzakelijk, omdat geen enkel op zichzelf staand zuiveringssysteem een toelaatbaar effluent levert.

De hoogbelaste anaerobe zuiveringssystemen hebben zich in de afgelopen decade als een goed alternatief voor de conventionele aerobe zowel als anaerobe zuiveringen ontwikkeld. Oorspronkelijk zijn de hoogbelaste anaerobe zuiveringsmethoden ontwikkeld voor afvalwater waarvan de vervuilende verbindingen voornamelijk opgelost zijn. Deze methoden voorzien slechts in een gedeeltelijke zuivering van complex afvalwater dat een grote fractie gesuspendeerd materiaal bevat, zoals slachthuisafvalwater. De toepassing van hoogbelaste systemen zoals het 'upflow anaerobic sludge blanket' (UASB) systeem, voor de complete behandeling van agro-industrieel afvalwater is een zwaartepunt in het onderzoek geworden. Dit agro-industrieel afvalwater is moeilijk te zuiveren als gevolg van de hoge concentratie van niet-opgelost materiaal. Dergelijk afvalwater wordt wel 'complex' afvalwater genoemd.

Tegenwoordig is het UASB-systeem de meest toegepaste hoogbelaste anaerobe zuiveringsmethode voor de totale zuivering van dit complexe afvalwater.

Dit proefschrift spitst zich toe op de vraag of en onder welke bedrijfsvoering en procesomstandigheden een ééntraps mesofiel UASB-systeem toepasbaar is voor de complete zuivering van slachthuisafvalwater in de praktijk.

Onderzocht werd de toepasbaarheid van het 'upflow flocculent anaerobic sludge blanket '(UASB) proces voor een ééntraps-zuivering van niet-bezonken slachthuisafvalwater, dat ca. 50% grof gesuspendeerd materiaal bevat (Hoofdstuk 2). De continue experimenten werden in een 25,3 m^3 UASB pilot-installatie uitgevoerd. De procesvoering was semi-continu, te weten met variërende organische belastingen voor dag en nacht (hoge belastingen overdag, lage belastingen gedurende de nacht) en met een voedingsonderbreking in de weekenden. De installatie werd bij 30 °C bedreven. Om het functioneren van het systeem bij lagere temperaturen te onderzoeken werd na 20 weken bij 30 °C, de temperatuur verlaagd tot 20 °C.

Uit de resultaten bleek dat het systeem goed kan functioneren bij organische belastingen tot 3,5 kg CZV m^{-3} dag^{-1} met een hydraulische verblijftijd van 8 uur, terwijl de temperatuur slechts 20 °C bedroeg. Piekbelastingen tot 7,5 kg CZV m^{-3} dag^{-1} bij een hydraulische verblijftijd van 5 uur konden goed opgevangen worden mits deze gevolgd werden door een laagbelaste periode (b.v. bij nacht).

Een significante discrepantie werd gevonden tussen de rendementen berekend uit de CZV-verwijdering en het berekende (lagere) percentage influent totaal CZV dat omgezet werd in methaan-CZV. Dit verschil gaf een indicatie dat een significant gedeelte van de CZV-reductie verkregen was door accumulatie van niet- of slecht afbreekbare materiaal in de reactor. Een differentiatie in de verschillende fracties van het opgehoopte materiaal kon niet gemaakt worden omdat het geaccumuleerde slib niet gekarakteriseerd was. Een gedeelte van het opgehoopte slib werd gedurende de onbelaste perioden alsnog in CH_4 omgezet.

In Hoofdstuk 3 werd de toepasbaarheid onderzocht van het 'upflow granular anaerobic sludge blanket' proces voor de ééntraps-zuivering van slachthuisafvalwater. De experimenten werden uitgevoerd onder semi-technische omstandigheden, t.w. continue voeding bij een constante organische belasting (24 uur per dag) gedurende de werkdagen gecombineerd met voedingsonderbrekingen gedurende de weekenden bij procestemperaturen van 30 °C en 20 °C. Bij een stabiel functioneren van het systeem t.w. bij een maximale CZV-reductie en een hoge conversie van CZV in methaan, waren de optimale belastingen 11 kg CZV m^{-3} dag^{-1} en 7 kg CZV m^{-3} dag^{-1} respectievelijk bij 30 °C en 20 °C.

Het systeem was minder effektief in het verwijderen van grof gesuspendeerd materiaal dan in het verwijderen van de colloïdale en opgeloste fracties uit het afvalwater. De uit dit onderzoek verkregen resultaten geven aan dat een aanhoudende overbelasting tot een achteruitgang van de specifieke methanogene aktiviteit van het slib leidt, hetgeen te wijten is aan de ophoping van colloïdaal en opgelost materiaal in het slibbed. Hieruit kon geconcludeerd worden dat het proces sterk afhankelijk is van de verwijderingsprocessen van de colloïdale en opgeloste bestanddelen en vervolgens hun omzetting in methaan. Als voornaamste niet-biologische verwijderingsmechanisme werden invanging en adsorptie beschouwd. Het effect van deze mechanismen op de snelheid van hydrolyse van het geaccumuleerde substraat, de benodigde eerste stap tot omzetting in methaan, werd nagegaan.

De verschillende bestanddelen van het afvalwater: de grof gesuspendeerde, colloïdale en opgeloste ingrediënten beïnvloeden de prestaties van de UASB reactor gezien de verschillende mechanismen die betrokken zijn bij de verwijdering van deze ingrediënten en vervolgens hun omzetting in methaan. Daarom werden eerdergenoemde mechanismen nader onderzocht. De resultaten van dit onderzoek worden gepresenteerd in Hoofdstuk 4. De experimenten werden uitgevoerd in een éèntraps vlokkig slib UASB-reactor, die continu gevoed werd bij een constante organische belasting gedurende 24 uur per dag, 7 dagen per week.

De CZV-verwijderingsefficiëntie van de UASB overtrof het verwachte verwijderingsrendement, berekend uit de methaanproduktie; wederom een indicatie dat niet-biologische verwijderingsmechanismen betrokken zijn bij de verwijdering van substraat ingrediënten uit het afvalwater. Een onderscheid werd gemaakt tussen de twee verschillende niet-biologische verwijderingsmechanismen. Het invangsmechanisme overheerste in de eliminatie van het grof gesuspendeerd materiaal terwijl voornamelijk het adsorptiemechanisme debet is aan de verwijdering van de colloïdale en opgeloste fracties. Een gecontinueerde accumulatie van substraatingrediënten in de reactor kan uiteindelijk schadelijk zijn voor de stabiliteit van het proces, omdat het tot slibflotatie kan leiden en dientengevolge tot een totaal verlies van de aktieve biomassa uit de reactor.

Nadat de toepasbaarheid van het UASB-proces voor een éèntrapszuivering van slachthuisafvalwater werd aangetoond, werd de maximale afbreekbaarheid van de opgeloste, colloïdale en grof gesuspendeerd fracties onderzocht (Hoofdstuk 5). Op deze manier zou een beter inzicht verkregen kunnen worden in de beperkingen van het systeem. Alle experimenten werden bij 30 °C en 20 °C uitgevoerd, met gebruikmaking van membraan-gefiltreerd afvalwater, papier-gefiltreerd afvalwater en totaal afvalwater. De experimenten werden uitgevoerd in recirculatie-batch reaktoren met korrelslib. De experimenten met uit het afvalwater gescheiden grof gesuspendeerd materiaal werden zowel met korrel- als met vlokkig slib uitgevoerd, met gebruikmaking van conventionele batch-gewijs gevoed reaktoren. De maximale afbreekbaarheid (d.w.z. conversie naar methaan) bij 30 °C was 75% voor membraan-gefiltreerd afvalwater, 61% voor papier-gefiltreerd afvalwater en 67% voor totaal afvalwater; bij 20 °C waren deze waarden respectievelijk 72%, 49% en 51%.

De maximale afbreekbaarheid van grof gesuspendeerd materiaal bedraagt 50% bij 30 °C en 45% bij 20 °C.

De mechanismen die betrokken zijn bij de verwijdering van opgeloste en colloïdale fracties van het slachthuisafvalwater werden aan een nauwkeurig onderzoek onderworpen. De uit dit onderzoek verkregen resultaten tonen aan dat het overheersende verwijderingsmechanisme van de opgeloste, maar vooral van de colloïdale fraktie, adsorptie is. De relatief hoge mate van adsorptie van de colloïdale fractie aan de oppervlakte van het slib, in combinatie met het hoge vetgehalte van die fraktie, zal de specifieke methanogene aktiviteit van het slib verslechteren. De adsorptie van het colloïdaal materiaal zal uiteindelijk leiden tot een inkapseling van het korrelslib door een laag van toenemende dikte, die in toenemende mate de toevoer van substraat naar de bacteriën zal hinderen. Het negatieve effect van vetten op de methanogene aktiviteit kan worden verklaard door de remmende werking van de vetzuren met lange ketens van de neutrale vetten.

Terwijl de mate van adsorptie vrijwel gelijk is bij hogere en lagere temperaturen, gaat de hydrolysesnelheid van de geadsorbeerde materie significant achteruit bij afnemende temperaturen. Het zal duidelijk zijn dat het proces bij 20 °C lagere organische belastingen kan verwerken dan bij 30 °C.

Dientengevolge is geconcludeerd dat de hydrolysesnelheid van de geadsorbeerde niet-oplosbare colloïdale fractie de beperkende factor is voor wat betreft het belastingspotentieel van het proces en dat de temperatuur een faktor van overheersend belang is.

CURRICULUM VITAE

De auteur van dit proefschrift werd op 21 september 1946 geboren te El-Mini (Egypte). Hij behaalde in 1970 het diploma B.Sc. in Agricultural Sciences aan de Ain-Shams universiteit te Cairo. In september 1973 begon hij met de studie scheikunde aan de rijksuniversiteit Groningen, waar hij het kandidaatexamen in september 1975 behaalde. In datzelfde jaar begon hij met de studie aan de landbouwuniversiteit in Wageningen. Het doctoraal-examen, afgelegd in 1980, omvatte de vakken waterzuivering (hoofdvak), microbiologie (verzwaard hoofdvak) en bodemkunde (hoofdvak).

Vanaf mei 1980 was hij verbonden aan de vakgroep waterzuivering van de landbouwuniversiteit als onderzoeker. Zijn onderzoeksaktiviteiten concentreerden zich op de lichte slib problematiek, het A/B-proces en de anaerobe zuivering van industrieel afvalwater.

Sinds september 1987 is hij werkzaam bij de vakgroep afvalwatertechnologie van DHV Consulting Engineers, PO Box 85, 3800 AB Amersfoort, The Netherlands.