Pelletisation in a UASB system with protein (casein) as substrate

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Abstract

Biological pellet formation was readily established in a laboratory-scale upflow anaerobic sludge bed (UASB) reactor treating a proteinaceous substrate, casein. Pellets were small (< 2mm), fragile and black. To obtain information on bed behaviour, the bed was divided and operated as a 2 in-series reactor system, the first reactor operating in a high and the second in a low hydrogen partial pressure state. The first reactor produced (virtually) all the pellets with a sludge yield of 0,26 mgVSS/mgCOD removed (COD removal 3 500 mg/ℓ for influent COD 10 000 mg/ℓ). However, two thirds of the VSS generated was lost to the second reactor due to pellet break-up. Deamination generated ammonium in excess of nitrogen requirements for pellet formation, and generated sufficient alkalinity to buffer the minimum pH in the bed to ~ 6,2 without alkalinity supplemented in feed; maximum pH in bed ~ 7,6. With influent COD of 10 000 mg/ℓ maximum loading was achieved at 65 kgCOD/m³ pelletised bed.d (without signs of failure), with soluble COD removal 95 per cent of influent COD.

Introduction

Sam-Soon *et al.* (1987) showed that sufficient prerequisites for pelletisation are:

- a high hydrogen partial pressure;
- excess supply of free and saline ammonia;
- pH buffered to near neutrality; and
- limited source of the amino acid cysteine.

These prerequisites are satisfied in a UASB reactor receiving a carbonaceous substrate, provided adequate free and saline ammonia and adequate alkalinity are supplied to control the minimum pH to near neutrality. With carbohydrate substrate, hydrogen is generated during the acidogenic phase of anaerobic fermentation, when the carbonaceous molecule is broken down to hydrogen and short-chain fatty acids (SCFA). In the UASB system there is a partial phase separation of acidogenesis and acetogenesis due to the plug flow regime; this allows the partial pressure of hydrogen $\bar{p}H_2$ to build up to the high values that are necessary for biopellet formation (Sam-Soon *et al.*, 1987).

Gujer and Zehnder (1983) have indicated that in anaerobic fermentation, a proteinaceous substrate follows the same phases of breakdown as a carbohydrate substrate. In the acidogenic breakdown all amino acids yield ammonia and SCFA, and some yield hydrogen. Accordingly the possibility exists that in a UASB system treating such a waste a high $\bar{p}H_2$ zone may develop. In that event the prerequisites for pelletisation are:

(1) high $\bar{p}H_2$;
(2) adequate free and saline ammonia;
(3) adequate pH buffer may be satisfied; and
(4) with regard to the prerequisite cysteine deficiency, it is not possible to state *ab initio* whether this will be satisfied or not because most proteins contain cysteine.

Up to the present no study has been reported on the fermentation of a pure proteinaceous substrate in a UASB system. In this paper a study is presented on the response of a UASB system to a pure proteinaceous substrate, casein.

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Biochemical prognosis

Fermentation of amino acids follows the same phases as those for a carbohydrate i.e. acidogenesis, acetogenesis and methanogenesis (Gujer and Zehnder, 1983). The acetogenic and methanogenic pathways remain the same independent of the substrate, but the acidogenic pathway can differ greatly between substrate types and organic structures within a type. Take as an example the acidogenesis reactions of the substrate type casein. This protein consists of about 20 amino acids and these differ greatly in their chemical structure. Despite their different structures for most amino acids the acidogenic phase of fermentation takes place in two stages:

(1) a deamination stage with release of ammonia and various organic products depending on the amino acid deaminated; and
(2) an oxidation stage where the products from (1) are oxidised to short-chain fatty acids (SCFA) principally butyric, propionic and acetic acid (White *et al.*, 1973).

Deamination stage

Deamination occurs either via oxidative, reductive or oxidative/reductive coupled deamination reactions, all these reactions releasing ammonia.

Oxidative deamination: Examples of amino acids which undergo oxidative deamination are alanine, valine, leucine, and isoleucine. Considering alanine the oxidative deamination process is:

$$2\ \text{alanine} + 2\ NAD_{OX} \rightarrow 2\ \text{pyruvic acid} + 2\ NH_3 + 2\ NADH \quad (1)$$

(Formulation of Eq. (1), using 2 moles of alanine, becomes clear in discussing the oxidative stage below, under high $\bar{p}H_2$). This reaction terminates unless the NADH can be oxidised - see **oxidative stage** below.

Reductive deamination: Examples of amino acids that undergo reductive deamination glycine, proline, tryptophan. Considering glycine, the reductive deamination process is:

$$\text{glycine} + NADH \rightarrow \text{acetic acid} + NH_3 + NAD_{OX} \quad (2)$$

Coupled deamination: An amino acid undergoing oxidative deamination can be coupled with another undergoing reductive deamination giving rise to the so-called Stickland coupled deamination reaction. The NADH generated in the oxidative reaction forms the NADH source for the reductive reaction. The sum of Eqs. (1 and 2) gives the coupled reaction:

$$\text{glycine} + \text{alanine} \rightarrow \text{pyruvic acid} + \text{acetic acid} + 2\,NH_3 \quad (3)$$

This coupled reaction is independent of $\bar{p}H_2$.

Oxidative stage

The organic products and NADH generated in the oxidative deamination stage [e.g. Eq. (1)] can be oxidised via various pathways depending on the nature of the organic products and $\bar{p}H_2$. As an illustration consider the products pyruvate and NADH in Eq.(1) under low and high $\bar{p}H_2$.

Low $\bar{p}H_2$: Where $\bar{p}H_2$ is less than 10^{-6} atm oxidation of NADH to NAD_{OX} and H_2 is thermodynamically feasible. In Eq. (1) above the NADH generated during deamination is oxidised (dehydrogenated) as follows:

$$NADH \rightarrow NAD_{OX} + H_2 \quad (4)$$

The pyruvic acid is oxidised to acetyl CoA as follows:

$$\text{pyruvic acid} + NAD_{OX} \rightarrow \text{acetyl CoA} + CO_2 + NADH \quad (5)$$

Acetyl CoA is converted to acetic acid:

$$\text{acetyl CoA} + ADP + P \rightarrow \text{acetic acid} + ATP \quad (6)$$

Eq. (6) is a highly favourable reaction from a thermodynamic standpoint. Again the NADH formed in Eq. (5) is oxidised spontaneously to NAD_{ox} and H_2, as in Eq. (4). The net result of the reactions in Eqs. (4, 5 and 6) is that pyruvate is oxidised to acetic acid and hydrogen.

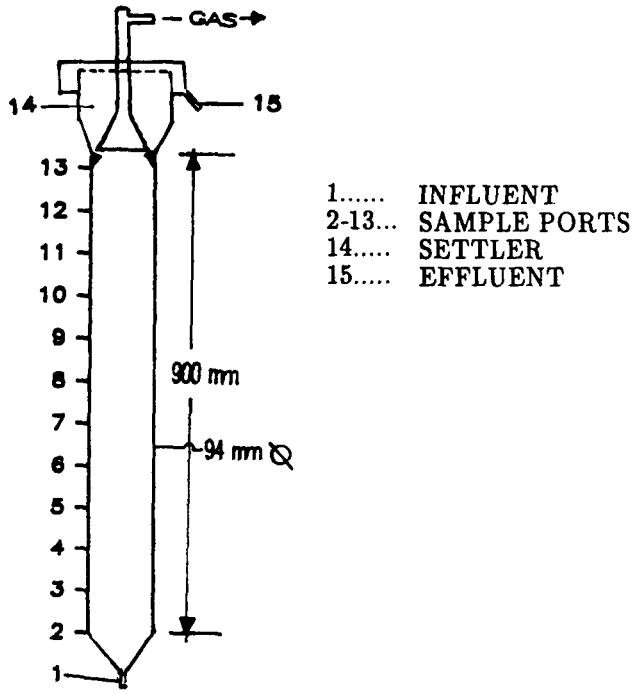


Figure 1a
Laboratory-scale UASB reactor used for high/low $\bar{p}H_2$ single reactor experiment

High $\bar{p}H_2$: In Eq. (4) as the $\bar{p}H_2$ rises, NADH oxidation is increasingly inhibited. When $\bar{p}H_2$ exceeds 10^{-6} atm, NADH oxidation is no longer thermodynamically feasible. With high $\bar{p}H_2$ $> 10^{-6}$ atm oxidation of the 2 mol of NADH, generated in Eq. (1), takes place by reducing 1 mol of pyruvic acid to propionic acid:

$$\text{pyruvic acid} + 2\,NADH \rightarrow \text{propionic acid} + 2\,NAD_{OX} \quad (7)$$

and the remaining mole of pyruvic acid to acetyl CoA and NADH:

$$\text{pyruvic acid} + CoA \rightarrow \text{acetyl CoA} + CO_2 + NADH \quad (8)$$

In Eq. (8) energy is required to oxidise NADH to NAD_{OX} and H_2; this is achieved by coupling into the energetically favourable reaction Eq. (6) to give:

$$\text{acetyl CoA} + NADH + ADP + P \rightarrow \text{acetic acid} + NAD_{OX} + CoA + H_2 + ATP \quad (9)$$

The net result of the reactions shown in Eqs. (7, 8 and 9) is that pyruvate is oxidised to propionic acid, acetic acid and hydrogen.

The description above applies to amino acids in which pyruvate and hydrogen is a product in the acidogenesis pathway. Now pyruvate and hydrogen are the common intermediates in the acidogenic breakdown of carbohydrates. Further breakdown of pyruvate will be the same irrespective of whether it originates from carbohydrates or amino acids. Hence if pyruvate and hydrogen are products in the acidogenic breakdown of proteins, development of a high $\bar{p}H_2$ is as likely with amino acid as with carbohydrate fermentation. Should a high $\bar{p}H_2$ develop, prerequisite (1) for pelletisation would be satisfied; production of NH_3 in the acidogenic fermentation of proteins should assist in satisfying prerequisite (2). The amino acid cysteine present in the influent protein may be deaminated or be so low, to be insufficient to satisfy the requirements for balanced hydrogenotrophic organism growth, in which event prerequisite (4) would be satisfied. Provided the pH remains near neutral it would seem that, from a biochemical point of view, it is possible that pelletisation in a UASB system could take place with a protein type substrate.

Experimental methods

Apparatus

To investigate the response of the UASB system to a proteinaceous substrate a laboratory-scale, steady state study was undertaken using two types of UASB systems:

- ***High/low $\bar{p}H_2$ system:*** This system comprised a single reactor with a sufficiently large bed to incorporate both high and low $\bar{p}H_2$ zones. The unit comprised a vertical 100 mm diameter perspex cylinder with a conically shaped inlet at the bottom and a solid liquid separation unit at the top (Fig. 1a); twelve ports were installed up the wall of the reactor for sampling along the line of flow. The total volume of the reactor was 10,5 ℓ. Temperature was maintained at 30°C by a thermostat controlled electrical heating tape wrapped around the reactor.
- ***Two in-series reactor system:*** In this system the first reactor was operated with a sludge volume sufficiently low to

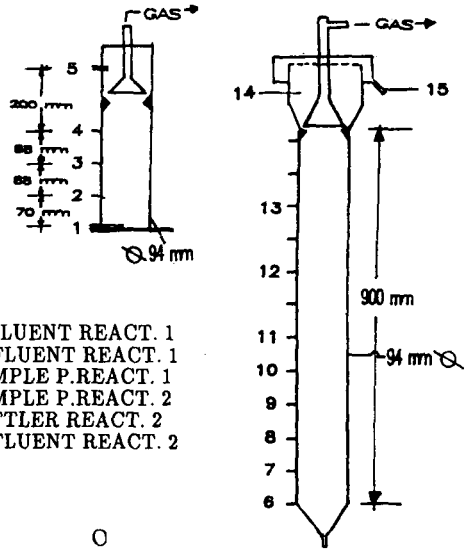
TABLE 1
APPROXIMATE AMINO ACID COMPOSITION OF CASEIN CALCULATED TO 16 PER CENT NITROGEN

Amino acids	per cent
Arginine	4,1
Histidine	2,5
Lysine	6,9
Tyrosine	6,4
Tryptophan	1,8
Phenylalanine	5,2
Cysteine	0,36
Methionine	3,5
Serine	6,7
Threonine	3,9
Leucine	12,1
Isoleucine	6,5
Valine	7,0
Glutamic acid	22,8
Aspartic acid	6,3
Glycine	0,5
Alanine	5,6
Proline	8,2
Hydroxyproline	2,0

TABLE 2
TRACE ELEMENT AND NUTRIENT SOLUTION

Trace element	Concentration g/ℓ
H_3BO_3	0,05
$FeCl_2.2H_2O$	2,00
$ZnCl_2$	0,05
$MnSO_4$	0,50
$CuCl_2.2H_2O$	0,03
$(NH_4)_6\ MO_7O_{24}.4\ H_2O$	0,05
$AlCl_3.6H_2O$	0,05
$CoCl_2.6H_2O$	2,00
$MnCl_2$	0,25
$MgCl_2$	1,00
EDTA	0,05
$Ni\ Cl_2.6H_2O$	0,25
HCl	1 mℓ
Nutrients	**Concentration g/ℓ**
NH_4Cl	10,00
K_2HPO	4,00

isolate the high $\bar{p}H_2$ zone i.e. operated at such a volume that the propionate profile showed a steady increase throughout the bed (Sam-Soon *et al.*, 1987). The second reactor received the effluent discharged from the first reactor and operated principally under a low $\bar{p}H_2$. The first reactor had a volume of 3,5 ℓ



1..... INFLUENT REACT. 1
5..... EFFLUENT REACT. 1
2-4.... SAMPLE P.REACT. 1
6-13... SAMPLE P.REACT. 2
14..... SETTLER REACT. 2
15..... EFFLUENT REACT. 2

Figure 1b
Laboratory-scale UASB reactors used for two reactor in-series experiment

and the second a volume the same as the high/low $\bar{p}H_2$ reactor, 10,5 ℓ (Fig. 1b). Both reactors were controlled at 30°C.

Substrate feed

During the starting-up period, with the high/low $\bar{p}H_2$ reactor, the feed was a mixture of diluted apple juice concentrate and peptone (casein). Over a period of about 50 d the feed was changed gradually from a mixture of peptone-apple juice (50:50 with regard to COD) to 100 per cent peptone. The peptone, supplied by Merck, consisted of nearly 100 per cent casein with a TKN/COD ratio of 0,12 and a NH_3-N/COD ratio of 0,02; the various amino acids contained in casein are listed in Table 1.

Nutrients and trace elements

To ensure adequate nutrients and trace elements for growth, solutions of these were made up, following the recipe of Zehnder and Wuhrmann (1977), see Table 2, except that the NH_3-N content was higher - in the inital stage of the study there was no certainty that sufficient NH_3-N would become available from deamination of amino acids to provide an excess of NH_3-N for pellet formation. To ensure adequate buffering against pH change, 6,5 g $NaHCO_3$ per litre influent was added.

Parameters measured

The following analyses were performed on the systems at 1 to 2-day intervals:

- unfiltered influent and filtered and unfiltered effluent COD;
- unfiltered influent and filtered effluent TKN and NH_3-N;
- pH in the influent, settler and effluent; and
- substrate flow rate.

In addition, at appropriate times, profiles of COD, TKN, NH_3-N, the short-chain fatty acids (SCFA) propionic (HPr) and acetic (HAc) were measured along the axis of the reactors. Samples were taken at each sample port, starting at the top. Samples were filtered using ordinary filter paper, Schleicher and Schuell 595. For the SCFA, samples were refiltered through a 0,45 micron filter paper

(millipore) and the SCFA measured by gas chromatography using a 60/80 Carbo pack C/0,3% Carbo wax packing. COD, TKN and NH_3-N were measured in accordance with Standard Methods (1988). Total alkalinity, more precisely H_2CO_3/ $H_2PO_4^-$/HAc /HPr/NH_4^+ alkalinity, was determined using the titration procedure for a mixture of weak acid/base systems as set out by Loewenthal *et al.* (1989).

High/low pH_2 reactor system

Starting up procedure

Starting up procedure can be divided into two periods of 20 and 30 d. During the first period the sludge was adapted from a substrate of pure carbohydrate waste to one containing both carbohydrate and casein. During the second period the sludge was adapted to a casein substrate only.

First period: The reactor was seeded with approximately 1,5 ℓ of pelletised sludge from a UASB reactor treating diluted apple juice concentrate as substrate. A constant substrate mix of apple juice concentrate and casein (50:50 with respect to COD) was fed. The flow rate was kept constant at 5 ℓ/d but the influent COD concentration of the mix was increased in increments from 800 to 3 500 mgCOD/ℓ. Throughout this period 25 mℓ of nutrient solution and 2 mℓ of trace element solution per litre of influent were added to the feed. The sludge bed volume remained virtually unchanged at 1,5 ℓ during the first 20 d, giving a COD load of 2,7 kgCOD/m³ pelletised bed.d at the beginning and 11,7 kgCOD/m³.d at the end of the first period. Despite the increasing COD load the filtered effluent COD decreased, indicating that the sludge was adapting well. The filtered effluent COD eventually stabilised at about 300 mg/ℓ for a 3 500 mg/ℓ influent COD (50 per cent casein, 50 per cent apple juice concentrate).

Second period: In the second period, to adapt the sludge to a 100 per cent casein substrate, the flow was maintained at 5 ℓ/d and the influent COD remained at approximately 3 500 mgCOD/ℓ. Over the next 30 d the casein fraction was increased incrementally from 50 to 100 per cent. Alkalinity (6,5 g $NaHCO_3$), trace element (2 mℓ) and nutrient (25 mℓ) additions per litre of influent remained as before except for the NH_3-N. After 18 d into the second period the NH_3-N (70 mg/ℓ) was left out of the nutrient solution - as the casein fraction of the influent feed increased, the effluent concentration of ammonia did likewise, so that ammonia deficiency was no longer likely. COD removal remained above 90 per cent throughout the second period, indicating that the sludge was adapting well to the changing substrate.

After 30 d the sludge mass appeared to have been fully adapted to a 100 per cent casein substrate, thereafter the load was progressively increased from 17,5 gCOD/d (3 500 mgCOD/ℓ at 5 ℓ/d) to 135 gCOD/d (5 200 mgCOD/ℓ at 26 ℓ/d) over a period of 81 d. Alkalinity supplementation remained at 6,5 g $NaHCO_3$/ℓ influent. Despite this significant increase in load the COD concentration in the effluent remained virtually unchanged even at the highest load, indicating that the system was not overloaded. At the end of this period the sludge bed mass had increased from 1,5 ℓ to 2,8 ℓ giving a bed depth of 230 mm and a loading rate of 135/2,8 = 48 kgCOD/m³ pelletised bed.d. Because the increase in bed mass indicated that pelletisation was active up to the maximum loading imposed, it was decided to establish steady state conditions at this loading and investigate the system's response.

Steady state behaviour

At the steady state loading of 48 kgCOD/m³ pelletised bed.d, with no free and saline ammonia addition, the system responses with respect to COD, TKN, NH_3-N and org-N were the following:

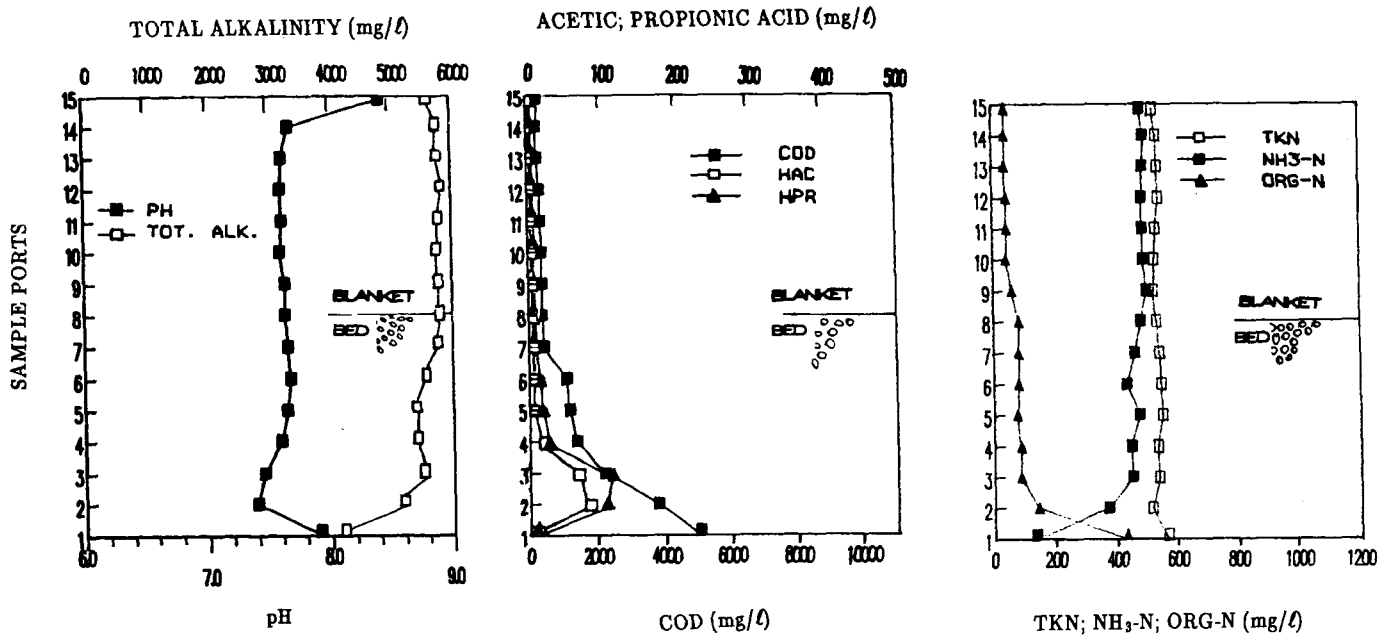


Figure 2

High/low pH_2 single reactor system: pH, total alkalinity, org-N, NH_3-N, TKN, COD, acetic (HAc) and propionic acid (HPr) profiles (addition of 6,5 g $NaHCO_3$ per litre influent; flow rate 25 ℓ/d; COD: 5 320 mg/ℓ influent, COD load on pelletised sludge: 48 kg COD/m³.d)

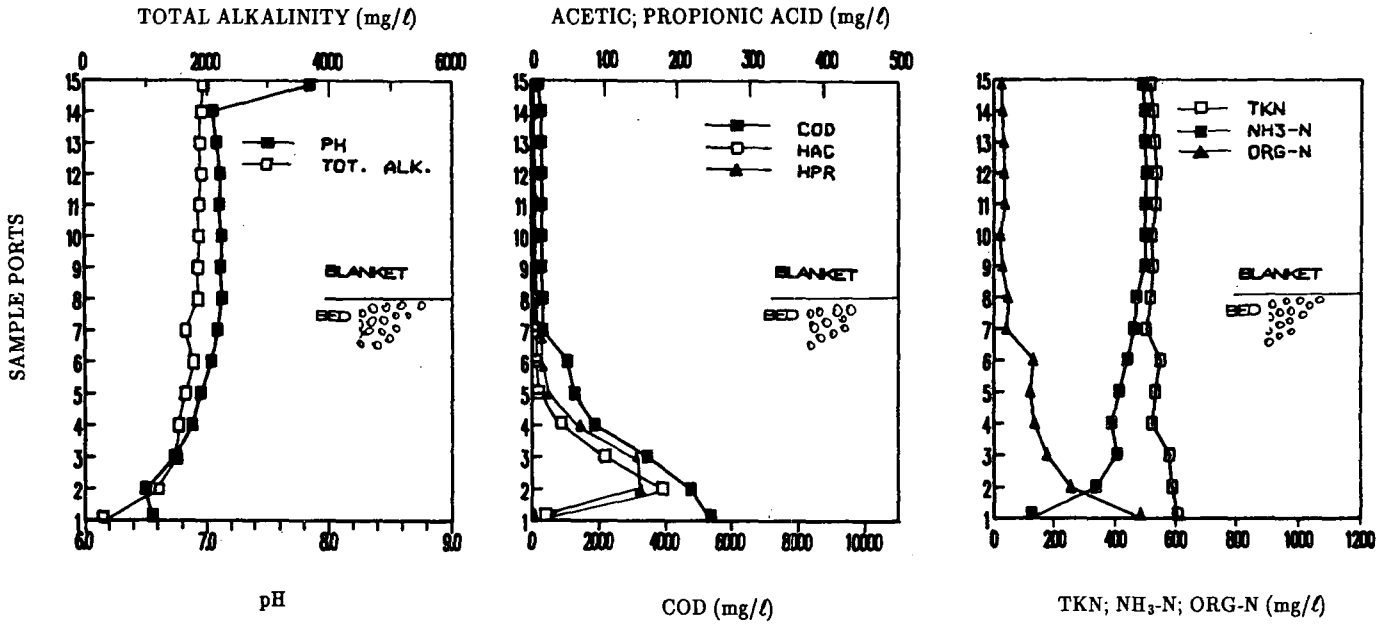


Figure 3

High/low $\overline{p}H_2$ single reactor system: pH total alkalinity, org-N, NH_3-N, TKN, COD, acetic (HAc) and propionic acid (HPr) profiles (no addition of $NaHCO_3$ to feed; flow rate 25 ℓ/d; COD: 5 300 mg/ℓ influent, COD load on pelletised sludge; 48 kg COD/m³.d)

COD removal: Average COD removal was 95 per cent (5 029 mgCOD/ℓ removed). The pelletised sludge volume remained constant at approximately 2,8 ℓ.

TKN uptake: Uptake of nitrogen, calculated from the difference in the TKN concentration in the influent and filtered effluent, was 72 mgN/ℓ. In a "normal" anaerobic process there are approximately constant ratios for the utilisation of nitrogen, COD and associated VSS generation, as follows:

TKN/COD ratio for protoplasm = 0,086 mgN/mgCOD
COD/VSS ratio for protoplasm = 1,42 mgCOD/mgVSS
i.e. TKN/VSS = 0,086 x 1,42 = 0,122 mgN/mgVSS
Biomass yield = 0,03 VSS/COD removed (Ten Brummeler *et al.,* 1985).

Thus in a "normal" anaerobic system the protoplasm generated would be 0,03 x 5029 mgVSS/ℓ influent; with a TKN/VSS ratio of 0,122 the TKN removal for synthesis would be 0,03 x 5029 x 0,122 = 13,4 mgN/ℓ influent. The observed nitrogen removal, however, was 72 mgN/ℓ; this is more than 5 times higher than that normally expected - the disappearance of nitrogen cannot be explained only by protoplasmic mass generation.

Free and saline ammonia: Org-N in the influent was 466 mgN/ℓ and in the effluent 21 mgN/ℓ, that is, about 95 per cent of the org-N was converted to NH_3-N (this aspect is discussed in greater detail later).

Profiles: To obtain detailed information on the system's response, profiles of total alkalinity, acetic acid (HAc), propionic acid (HPr), TKN, NH_3-N and pH were measured, shown in Fig. 2. For the moment the most significant profile is the pH. The pH was relatively high throughout the reactor, with the lowest pH 7,4 near the bottom of the bed at sample port No. 2, and the highest pH 7,7 in the upper zone of the bed. Methanogens operate optimally around pH 7,0 and acidogens below pH 7. To lower the pH to these more favourable values, $NaHCO_3$ addition was terminated. After the system had stabilised another profile was measured (Fig. 3). There was now a significantly lower pH throughout the bed with a minimum pH 6,6 at sample port No. 2. The general drop in pH had little effect on the pattern of COD removal, TKN uptake and organic nitrogen conversion. There was a slight increase in the concentrations of HAc and HPr, most likely due to increased activity of the acidogens at the lowered pH. HAc and HPr profiles indicated complete conversion to methane after passing through 70 per cent of the bed - the system was still underloaded.

High loading system response

To obtain information under yet higher COD loadings, the load was increased in two steps, by setting the flow rate at 28 ℓ/d and increasing the COD concentration to 7 500 and 10 500 mgCOD/ℓ (50 and 65 kgCOD/m³ pelletised bed.d) respectively. There was no alkalinity supplementation resulting in an influent pH of 6,6. At each load the sludge mass was allowed to increase. When the effluent COD indicated steady state, profiles were measured. Both profile sets show similar trends, hence, only the set representing the higher load (sludge bed volume 4,5 ℓ, loading rate 65 kgCOD/m³ pelletised bed.d), is shown in Fig. 4, and discussed in detail below:

pH: Within the bed the pH now showed a fall in the lower bed zone to 6,2 and thereafter an increase in the upper bed zone to 7,0 stabilising at 7,6 in the sludge blanket above the bed and in the settler (Fig. 4). The causes giving rise to the behaviour pattern were similar to those in UASB systems treating carbohydrate substrate. In the lower bed zone the fall in pH was due to the high SCFA concentration and the CO_2 generated (HAc and HPr 500 mg/ℓ and 420 mg/ℓ maximum respectively). The fall in pH was moderated by the conversion of org-N to NH_4^+ during deamination. In the upper bed zone the rise in pH was principally due to the reduction in the SCFA concentration. In the effluent bucket the pH rose to about 8,2 due to loss of CO_2.

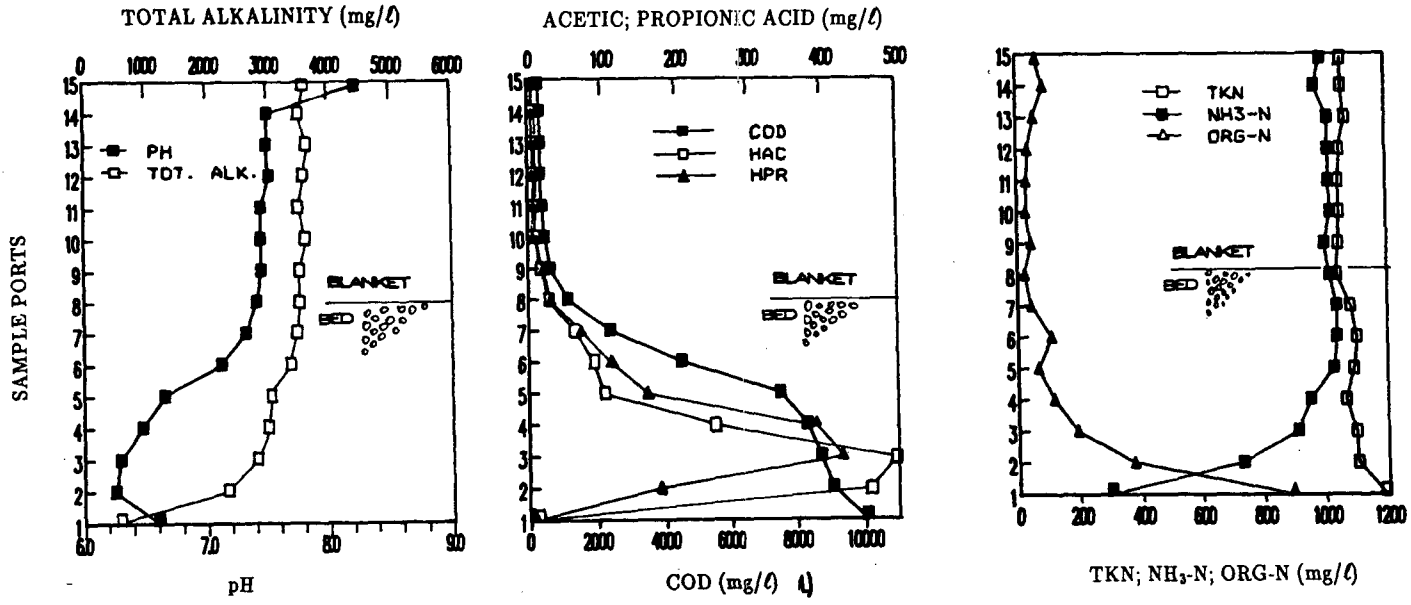


Figure 4

High/low $\bar{p}H_2$ single reactor system: pH, total alkalinity, org-N, NH_3-N, TKN, COD, acetic (HAc) and propionic acid (HPr) profiles (no addition of $NaHCO_3$ to feed; COD load on pelletised sludge: 65 kg COD/m^3.d)

Alkalinity: Referring to Fig. 4, the $H_2CO_3^-/H_2PO_4^-$ /HAc/HPr/NH_4^+ alkalinity increased rapidly from the influent value (12 mmol/ℓ, 600 mg/ℓ as $CaCO_3$) to a relatively stable higher level in the upper part of the bed (72 mmol/ℓ, 3 600 mg/ℓ as $CaCO_3$). Comparing the alkalinity increase with the org-N conversion to NH_3-N, Fig. 4, about 60 mmol orgN/ℓ influent were converted to NH_3-N and there was a corresponding increase of 60 mmol of alkalinity per litre influent; this confirms the theoretical expectation that the conversion of 1 mol org-N produces 1 mol alkalinity. The shape of the NH_3-N curve was virtually identical with that of the alkalinity indicating that generation of alkalinity was due to conversion of org-N to N-NH_3.

Nitrogen conversion and uptake: Org-N and NH_3-N profiles are shown in Fig. 4. Conversion of org-N took place primarily in the lower zone of the bed - 50 per cent conversion prior to sample port No. 2. The conversion rate decreased continuously up the bed, eventually to zero at the top of the bed (port No. 6), with a minimum org-N of about 20 mg/ℓ; this concentration did not change in the sludge blanket. The TKN (org-N + NH_3-N) profile shows an uptake of about 90 mgN/ℓ, mainly in the bottom part of the bed. In the section "TKN uptake" above, nitrogen requirements of about 0,03 x 0,122 ~ 0,0037 mgN/mgCOD utilised were predicted for normal anaerobic digestion. Accepting that (10 000-500) mgCOD/ℓ was processed in the bed the nitrogen requirements would have been 9 500 x 0,0037 ~ 35 mgN/ℓ. Thus the nitrogen utilisation was in excess of the requirements for protoplasmic growth and may be accounted for by accepting polypeptide formation.

COD: The COD profile, Fig. 4, indicates a relatively low COD removal rate up to sample port No. 5 and thereafter a rapid increase in this rate. The lower COD removal in the bottom part of the bed was probably due to the relatively low pH in that region (Fig. 4) which would have inhibited methanogenesis. Once the pH attained a value above 6,6 the COD removal rate appeared to improve significantly.

Propionate and acetate: The profiles (Fig. 4) indicate a lower zone, in which HAc and HPr increased monotonically up to sample port No. 3, and an upper zone in which these decreased to a minimum value, thereafter remaining constant. Generation of HPr occurs only where the $\bar{p}H_2$ is relatively high ($>10^{-4,1}$) so that a rising HPr profile defines the high $\bar{p}H_2$ zone in the system with which is associated pellet generation (Sam-Soon *et al.*, 1987); oxidation of HPr indicates a low $\bar{p}H_2$, so that the declining HPr profile defines the low $\bar{p}H_2$ zone. Hence according to Sam-Soon *et al.* (1987) pellet generation was confined to the high $\bar{p}H_2$ zone, up to port No. 3.

HPr conversion to HAc was virtually complete just below the top of the bed. At higher loading rates it is likely that oxidation would have been incomplete at the top of the bed and HPr discharged to the sludge blanket. This would indicate that the system was near its maximum loading capacity for methane production; at higher loading the system very likely would have shown signs of "failure", by increased HPr in the blanket.

Two in-series UASB system

In the high/low $\bar{p}H_2$ single reactor study above it was evident that the high $\bar{p}H_2$ zone, as indicated by the increasing HPr profile extended only up to about port No. 3. Sam Soon *et al.* (1987) showed that pellet formation takes place in the high $\bar{p}H_2$ zone and observed pellet break-up in the low $\bar{p}H_2$ zone in the upper region of the sludge bed. To obtain data on the VSS yield in the high $\bar{p}H_2$ zone the system was operated as two in-series reactors with the sludge volume in the first reactor limited to represent the high $\bar{p}H_2$ zone only. The units are shown in Fig. 1(b). The high $\bar{p}H_2$ zone in the high/low $\bar{p}H_2$ reactor extended up to between port 3 and 4 (volume ~ 1,5 ℓ) and thus 1,5 ℓ of sludge from this zone was transferred to the first in-series reactor. The previous high/low $\bar{p}H_2$ reactor served as the second in-series reactor and contained the remaining 3 ℓ of pelletised sludge. COD load on the system was set at 210 gCOD/d (flow rate 20 ℓ/d, influent COD 10 500 mgCOD/ℓ). This load was lower than the near maximum load on the high/low $\bar{p}H_2$ system to give greater surety that the system would operate in a stable fashion. Trace element and nutrient solution addition remained the same as for the high/low $\bar{p}H_2$ system experiment. No alkalinity ($NaHCO_3$) nor NH_3-N were added to the feed.

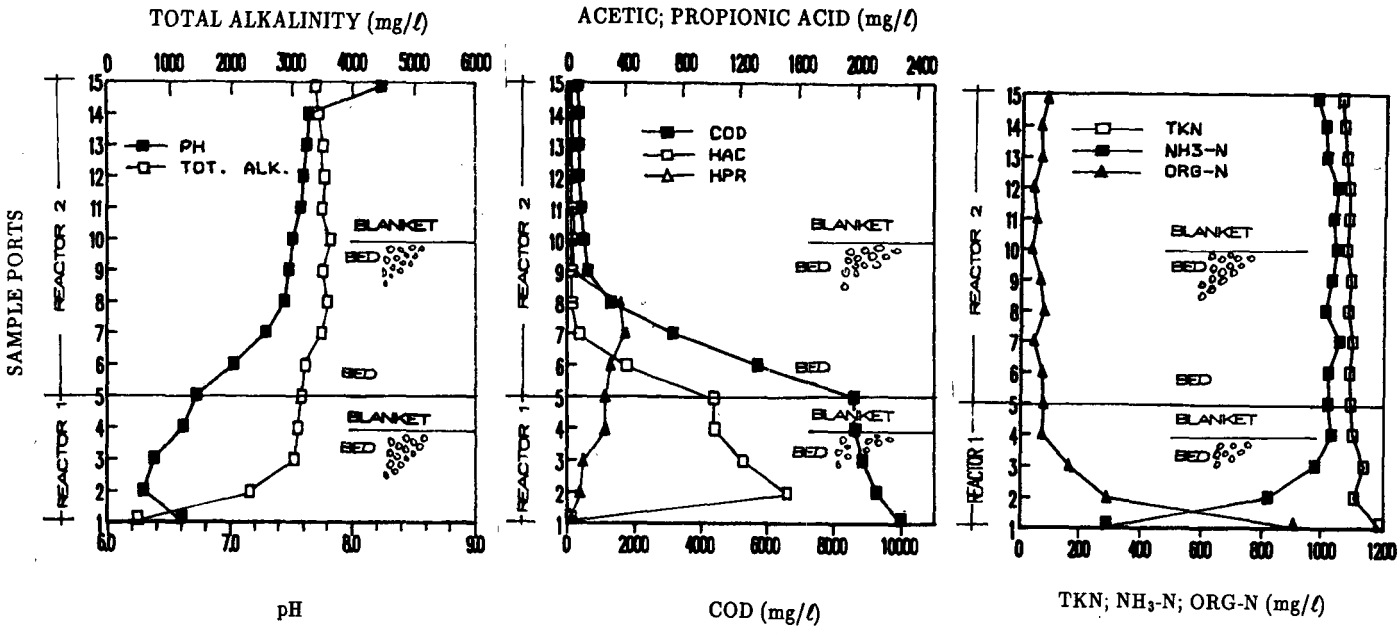


Figure 5

Two in-series reactor system: pH, total alkalinity, org-N, NH_3-N, TKN, COD, acetic (HAc) and propionic acid (HPr) profiles (no addition of $NaHCO_3$ to feed; COD load on pelletised bed: 36 kg COD/m^3.d)

The system was run for about 15 d, to attain a steady state, and thereafter for 40 d. During the initial period tests were performed on both reactors with the following objectives in mind:

- Daily testing of org-N, NH_3-N and COD on unfiltered influent and filtered and unfiltered effluents in both reactors, to determine when steady state was established.
- Sludge mass generation in the high $\bar{p}H_2$ reactor, to determine the sludge age and the specific yield of sludge per COD utilised.
- Profiles of pH, COD, TKN, org-N, NH_3-N, total alkalinity, HAc and HPr in the two reactors, to detail product generation.

After 15 d from the start of the experiment the filtered effluent COD, org-N, NH_3-N and pH from both reactors indicated that steady state had been attained, and attention was focused on the first (high $\bar{p}H_2$) reactor to determine the VSS yield.

Sludge mass generation in the high $\bar{p}H_2$ reactor: The sludge bed mass was controlled to port No. 4 (1,7 ℓ). To determine the yield the sludge mass was allowed to increase above sample port No. 4 for periods of 5 days. At the end of each period the sludge generated (i.e. pellets and suspended solids in the bulk liquid above sample port No. 4) was drained into a measuring cylinder, thoroughly mixed and a sample taken to determine the mass of VSS. Every day the VSS in the effluent from the high $\bar{p}H_2$ reactor was determined by subtracting the filtered COD (ordinary filter paper) from unfiltered COD and calculating the effluent VSS via the mean measured COD/VSS ratio of the sludge (1,38 mgCOD/mgVSS). Multiplying this value by the flow over the 5-day period (100 ℓ) gave the mass of VSS loss in the effluent. Total mass of VSS produced over the 5-day period was the sum of the masses of VSS increase in the sludge and the blanket, and the mass of VSS loss in the effluent. The concentration of COD utilised for metabolic purposes in the first reactor was obtained daily by subtracting the filtered effluent COD from the soluble influent COD. The average difference in concentration multiplied by the flow over the 5-day period (100 ℓ) gave the mass COD removed: Average influent COD = 10 500 mg/ℓ, average filtered effluent COD of the high $\bar{p}H_2$ reactor = 6 850 mgCOD/ℓ, i.e. COD utilised in the bed = 3 650 mg/ℓ, hence mass of COD removed /d = 20 x 3 650 = 73 gCOD/d. On an average 19 gVSS/d were generated. The specific yield, Y, is given by:

$$Y = \frac{\text{mass of VSS generated/d}}{\text{mass of COD utilised/d}} = \frac{19}{73} = 0{,}26 \text{ gVSS/gCOD}$$

Of the total mass of sludge (19 gVSS/d) generated, 38 per cent was retained in the pelletised bed, the rest was lost to the blanket above the bed and eventually discharged to the second reactor in the series.

The specific yield in the high $\bar{p}H_2$ reactor was lower than that obtained by Sam-Soon *et al.* (1987) treating apple juice waste and the pellets were smaller and appeared to be less compact. In the high $\bar{p}H_2$ reactor study Sam-Soon *et al.* (1987) reported no sludge debris discharge from the bed to the liquid above, whereas with the proteinaceous substrate large quantities of sludge debris discharged from the bed, to form a blanket above, and were discharged in the effluent. However, the two substrates are so dissimilar that one can only conjecture as to the differences in the specific yield. Possibly the specific hydrogen production per COD and the presence of cysteine in casein were contributory factors.

Sludge mass generation in the low $\bar{p}H_2$ (second in-series) reactor: In the two in-series reactor system sludge production was measured only in the first (high $\bar{p}H_2$) reactor. To get an estimate on the yield of VSS in the second reactor the following assumption is made: Assume that the second in-series reactor operated under low $\bar{p}H_2$ conditions, that is no pellet production took place (this is not unreasonable because little nitrogen was removed in the second reactor, see Fig. 5). Sludge production was mainly due to conversion of H_2 (under low pH_2) and acetate to methane. Under those conditions a yield value of 0,03 mgVSS/mgCOD removed is applicable (Ten Brummeler *et al.*, 1985). It was stated above that 3 650 mgCOD/ℓ were removed in the first reactor. The effluent COD concentration was 500 mgCOD/ℓ. Hence the COD removal in the second in-series reactor can be calculated: 10 500 - 3 650 -

500 = 6 350 mgCOD/ℓ. Thus the VSS generation per day in the second reactor was 6 350 x 0,03 x 20 = 3,8 gVSS/d. This value may be combined with the VSS yield in the first in-series reactor to give the overall yield of the system.

Overall sludge generation in two in-series reactor system: Combining the sludge generation per day of the two individual reactors gives: (19+3,8) = 22,8 gVSS/d. Hence the overall yield = 22,8/(20x10 000/1 000) = 0,114 gVSS/gCOD removed.

Sludge age: The sludge age, R_s, in the high pH_2 reactor (in days) is defined by:

$$R_s = \frac{\text{mass of sludge in the bed}}{\text{mass of sludge removed per day}}$$

The mass of sludge in the high $\overline{p}H_2$ reactor was determined from the bed volume (1,7 ℓ) and the sludge density. Draining the sludge bed and measuring the VSS gave a density of 37,5 gVSS/ℓ. Hence: R_s = 1,7 x 37,5/19 = 3,4 days.

Profiles: After 55 d from the start of the two in-series experiment, profiles of pH, COD, total alkalinity, NH_3-N, org-N, HAc and HPr were measured on the two in-series reactor system (Fig. 5).

The pH, COD, total alkalinity, NH_3-N and org-N profiles were similar to those observed in the high/low $\overline{p}H_2$ single reactor system (c.f. Fig. 4) indicating that the separation of the high $\overline{p}H_2$ zone (first reactor) from the remaining sludge bed did not significantly affect the system's response pattern with respect to these parameters. However, the HAc and HPr profiles differed in that in the two in-series reactor system the maximum value of the HAc in the high $\overline{p}H_2$ reactor exceeded that in the high/low $\overline{p}H_2$ single reactor system about 3 times. The HPr profile of the two in-series reactors reached its maximum at a higher point in the bed (in the second reactor) but was less in the lower zone of the bed (in the first reactor), than in the high/low $\overline{p}H_2$ single reactor system. The different behaviour pattern might be due to the absence of any intermixing of sludge from the low $\overline{p}H_2$ reactor with the sludge of the high $\overline{p}H_2$ reactor. Sam-Soon (1989) indicates that there is a measure of intermixing between the layers in a UASB reactor. This lack of intermixing might very likely give rise to selective pressure on the bacterial population in the high $\overline{p}H_2$ reactor as a result of the relatively short sludge age (3,4 d). The selection pressure would be particularly pronounced with a proteinaceous substrate because of the differences in acidogenic pathways for the various amino acids.

At the maximum loading rate (65 kgCOD/m³ pelletised bed.d) the effluent ammonium concentration was about 1 100 mgN/ℓ. In the high $\overline{p}H_2$ zone the pH declined to about 6,2 and in the low $\overline{p}H_2$ zone it rose to 7,6. Preferably the pH should be > 6,6 in the high $\overline{p}H_2$ zone. This can be done by adding alkalinity to the influent. However in that event the pH in the low pH_2 zone would increase above 7,6. McCarty (1964) reports that the NH_4^+ ion has inhibitory effects on methanogenesis in the range of 1 500 to 3 000 mg/ℓ and becomes toxic at concentrations > 3 000 mg/ℓ which would imply that NH_4^+ was unlikely to have had toxic effects on the systems. With regard to NH_3 an inhibitory concentration level of about 80 mg/ℓ (Koster, 1986) has been observed. Hence at the high free and saline ammonia concentrations of the experiment above, inhibition of methanogenesis could be expected if the pH in the reactors increased above about 8. The consequences of alkalinity augmentation on the pH profile and the resulting NH_3 inhibitory effects will be discussed in a further paper.

Conclusions

From the study on a UASB reactor system treating a proteinaceous substrate, casein, the following conclusions are pertinent:

- Pelletisation took place; hence the substrate casein is suitable for anaerobic fermentation in a UASB system.
- The response of the system treating proteinaceous substrate was similar to the same system treating carbohydrate substrate in that in the high hydrogen partial pressure zone an uptake of nitrogen in excess of that required for protoplasmic growth and a high specific yield of volatile suspended solids occurred in both systems.
- Casein substrate could be treated without addition of alkalinity - sufficient alkalinity was generated from conversion of org-N to NH_3-N.
- Maximum COD load of 65 kg/m³ pelletised sludge bed.d. could be imposed on the system before signs of failure becoming evident.
- COD removal remained at 95 per cent, up to the highest load applied.

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